EXPRESSIÓ DE LES MOLÈCULES D'ADHESIÓ CEL·LULAR EN LES VASCULITIS DE VAS GRAN I VAS MITJÀ

Tesi presentada per **Blanca Coll-Vinent i Puig**

per aspirar al grau de Doctor en Medicina.

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UNIVERSITAT DE BARCELONA

ia, novembre de 1998

Al Xavi

Al Martí, el Dídac i l'Alba

AGRAÏMENTS

- Als doctors Maria Cinta Cid i Josep Maria Grau per la direcció d'aquesta tesi i pel seu constant estímul a realitzar-la.

- Al Professor Álvaro Urbano-Márquez, Cap de Servei de Medicina Interna General, pel seu recolzament al treball realitzat.

- A tots els companys i amics del grup de recerca en Malalties Musculars, i molt especialment al Dr Ferran Masanés, pel seu important ajut i recolzament en l'elaboració d'aquesta tesi.

- A tots els companys i amics del Servei de Medicina Interna General de l'Hospital Clínic, i especialment al doctor Alejandro de la Sierra pels seus consells i ensenyaments.

- Als companys del Servei d'Immunologia de l'Hospital Clínic, especialment als doctors Jordi Yagüe i Carme Vilardell, per la seva col·laboració i suport.

- Al doctor Joaquim Oristrell pel seu interès i col·laboració en la obtenció del material.

- Al Dr. Eduard Mirapeix per la seva amable col·laboració en la obtenció del material.

- A la Mireia Cebrián pel seu valuós i imprescindible ajut en la realització dels treballs.

- A la Carme Carbonell pel seu inestimable ajut en les feines de secretariat.

- A tots els malalts afectats per poliarteritis nudosa i arteritis de cèl·lules gegants, que constitueixen el material humà d'aquesta tesi.

- Al Dr. Antoni Borràs, que m'ha permès treballar a l'Hospital Clínic mentre estava realitzant els treballs que constitueixen aquesta tesi.

- A l'Hospital Clínic de Barcelona i al Fondo de Investigaciones Sanitarias del Ministeri de Sanitat i Consum pel seu ajut econòmic mitjançant la concessió de beques per a la realització d'aquesta tesi doctoral.

- Al Xavier Surís i Armangué, pel seu constant suport moral, pels seus valuosos consells i per aguantar-me durant tot el temps que ha durat l'elaboració d'aquesta tesi.

- Als meus pares, Carmen Puig i Tintoré i Roberto Coll Vinent, pel seu suport moral i pel seu ajut real.

- Als meus sogres i la resta de la família per ajudar-me a tenir el temps suficient per a realitzar aqusta tesi.

A tots ells, el meu agraïment més sincer.

ABREVIATURES

Abreviatures utilitzades en la taula 2 i en el text

PAN: poliarteritis nudosa

ACG: arteritis de cèl·lules gegants

VLA-4: very late activation antigen-4

LFA-1, -2, -3: leukocyte function-associated antigen-1, -2, -3

ICAM-1, -2, -3: intercellular cell adhesion molecule-1, -2, -3

VCAM-1: vascular cell adhesion molecule

PECAM-1: platelet-endothelial cell adhesion molecule

CLA: cutaneous lymphocyte antigen

PSGL-1: P-selectin glycoprotein ligand

GlyCAM-1, -2: glycosilation-dependent cell adhesion molecule-1, -2

MadCAM: mucosal addressin cell adhesion molecule

CE: cèl·lules endotelials

NK: cèl·lules *natural killer*

ARN: àcid ribonucleic

NFκB: nuclear factor kappa B

ELISA: enzyme-linked immunossorbent assay

cols.: col·laboradors

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INTRODUCCIÓ

Les vasculitis són processos clínico-patològics caracteritzats per la inflamació dels vasos sanguinis. Poden presentar-se com una entitat primària o complicar el curs d'una altra malaltia, especialment infeccions, neoplàsies o malalties del teixit connectiu (artritis reumatoide, lupus eritematós sistèmic, malaltia de Sjögren). Com a conseqüència de la lesió dels vasos sanguinis pot aparèixer isquèmia i/o hemorràgia en els òrgans irrigats pels vasos afectats. L'espectre clínic de les vasculitis és molt ampli, ja que depèn del teixit o òrgan afectat i de l'extensió del procés. Aquesta varietat clínica comporta dificultats en el diagnòstic, amb el subsegüent retard en la instauració del tractament. De la mateixa manera, el pronòstic també és molt variable, tant entre els diferents tipus de vasculitis com a nivell individual (1).

La poliarteritis nudosa (PAN) va ser descrita per primera vegada el 1866 per Kussmaul i Maier (2). Des de llavors s'han descrit moltes altres vasculitis, i també s'han fet múltiples intents per classificar-les atenent a les manifestacions clíniques, al tipus o calibre dels vasos afectats, a troballes histopatològiques o a combinacions d'aquests criteris (1,3-8). Atès que no hi ha manifestacions clíniques patognomòniques, proves de laboratori diagnòstiques ni troballes histopatològiques uniformes, establir una classificació de vasculitis d'acord amb un esquema acceptat universalment és difícil. De totes maneres, és necessària una certa estandardització per a facilitar el diagnòstic i el maneig dels pacients afectats de vasculitis, per a comparar els resultats de diferents tractaments, i per a unificar criteris a l'hora de realitzar estudis destinats a millorar el nostre coneixement sobre l'etiopatogènia i la història natural de les diferents vasculitis. La classificació de Fauci (1) va ser la més utilitzada fins que es va fer la classificació de l'American College of Rheumatology (ACR) de 1990 (9-11) (taula 1). Aquestes classificacions no són oposades, ja que Fauci fa una descripció nosològica i l'ACR descriu criteris per a la classificació de set entitats ja prèviament descrites (púrpura de Henoch-Schönlein, granulomatosi de Wegener, síndrome de Churg-Strauss, vasculitis per hipersensibilitat, poliarteritis nudosa,

arteritis de cèl·lules gegants, arteritis de Takayasu). Donada la superposició que es produeix entre diferents vasculitis i l'existència de casos de difícil classificació, aquests criteris permeten, amb una elevada especificitat i sensibilitat, adscriure els pacients amb diagnòstic de vasculitis a entitats clínico-patològiques concretes.

La púrpura de Henoch-Schönlein és una vasculitis que afecta vasos petits, de presentació típica en la infància, que clínicament es caracteritza per l'inici brusc de febre alta, púrpura vasculítica en extremitats inferiors i natges, artràlgies i, sovint, dolor abdominal. En la meitat dels casos hi ha afectació renal, habitualment en forma de glomerulonefritis focal que es manifesta per hematúria microscòpica. L'evolució acostuma a ser favorable en pocs dies, fins i tot sense tractament, encara que són freqüents les recidives.

La granulomatosi de Wegener és una vasculitis necrotitzant granulomatosa caracteritzada per afectar les vies aèries superiors i inferiors i el glomèrul, però que també pot afectar vasos petits i mitjans en qualsevol altre territori (articulacions, pell, ulls). Sense tractament, la supervivència és pràcticament nul·la. Amb corticoids i immunosupressors ha millorat molt el pronòstic però és freqüent l'aparició de recidives.

La síndrome de Churg-Strauss és un procés caracteritzat per la presència d'eosinofília, formació de granulomes perivasculars, afectació pulmonar i manifestacions clíniques d'asma bronquial i rinitis al·lèrgica, encara que es poden afectar altres òrgans. Les troballes histològiques típiques són els granulomes perivasculars, la infiltració hística per eosinòfils i la necrosi fibrinoide. Normalment s'afecten vasos petits, encara que també es poden afectar els vasos de calibre mitjà. És característic observar lesions en diferent estadi evolutiu. L'evolució sense tractament és fatal, però amb corticoids la supervivència és d'un 60-70 % als 5 anys. L'afectació cardíaca i la del tub digestiu són els principals determinants de mal pronòstic.

La vasculitis per hipersensibilitat és una de les formes més freqüents de vasculitis. S'anomena així per la presència, en el sèrum, de

reaccions antigen-anticòs que donen lloc a la formació d'immunocomplexes. Afecta vasos de petit calibre (fonamentalment vènules postcapil·lars). La troballa anatomo-patològica característica és la presència de l'anomenada leucocitoclàsia ("pols nuclear") o presència de restes de nuclis de leucòcits polimorfonuclears. El principal òrgan afectat és la pell, amb l'aparició d'una púrpura palpable. El pronòstic és molt bo, sobretot si s'identifica i es retira l'agent causal.

La PAN és una vasculitis sistèmica que es caracteritza des d'un punt de vista histopatològic per l'afectació segmentària i irregular d'artèries de calibre petit i mitjà en forma d'infiltració leucocitària i necrosi fibrinoide en absència de granulomes demostrables. Com a conseqüència, s'observen àrees d'infart i hemorràgies. Poden observar-se petits aneurismes que ocasionalment es poden trencar. Progressivament, les zones afectades mostren lesions cicatricials, amb fibrosi periarterial que porta a una oclusió parcial o total de la llum del vas. Una característica de la malaltia és la coexistència de lesions en diferent estadi evolutiu que afecten segmentàriament la paret vascular. La PAN es pot presentar a qualsevol edat, amb una major incidència en la quinta i sisena dècades. Afecta més sovint homes que dones. La simptomatologia de la malaltia depèn del procés inflamatori vascular i de les lesions isquèmiques en cada un dels òrgans afectats. Normalment les manifestacions inicials són inespecífiques: febre, astènia, artromiàlgies i pèrdua de pes. Els síntomes de localització depenen dels òrgans afectats. Els més freqüents són hipertensió arterial i els secundaris a lesió del sistema nerviós perifèric (mononeuritis múltiple, polineuritis sensitivo-motriu difusa), del tub digestiu (dolor abdominal, hemorràgia digestiva alta i baixa, perforació intestinal, infart mesentèric, i, fins i tot, ruptura d'aneurisma mesentèric amb xoc hipovolèmic) i, menys freqüentment, el cor i la pell. Com comentarem més endavant, actualment es considera que l'afectació renal i pulmonar són característiques de la PAN microscòpica. L'evolució natural de la malaltia és fatal en la majoria dels casos. Amb el tractament amb corticoids i immunosupressors,

s'aconsegueix una remissió clínica en la majoria dels pacients, encara que no és rara l'aparició de recidives ni la presència de complicacions associades al tractament.

L'arteritis de cèl·lules gegants (ACG) (o arteritis de la temporal o arteritis de Horton) és una vasculitis granulomatosa que afecta vasos de mitjà i gran calibre. És relativament freqüent, amb una incidència de 15-30 casos per 100.000 habitants/any en persones majors de 50 anys, però la seva màxima incidència és en la vuitena dècada de la vida, i afecta més dones que homes. Encara que qualsevol artèria de l'organisme pot estar involucrada, els vasos més freqüentment afectats són els tributaris dels troncs supraaòrtics, especialment l'artèria temporal. En les fases inicials, s'observa una infiltració leucocitària en les làmines elàstica interna i externa i en la capa adventícia. Posteriorment, hi ha una afectació de totes les capes de la paret vascular (panarteritis) amb formació de granulomes constituïts per macròfags, cèl·lules gegants multinucleades, limfòcits i fibroblasts. Es produeix un engruiximent fibrós de l'íntima, que produeix una oclusió de la llum arterial i afavoreix l'aparició de fenòmens trombòtics afegits. La làmina elàstica interna està fragmentada i disgregada. És característica l'afectació segmentària del vas. La manifestació clínica més freqüent és la cefalea, que sovint és el síntoma inicial i es pot associar a la presència d'una artèria engruixida, nodular, dolorosa al tacte i amb un pols disminuït o absent. Altres manifestacions cranials freqüents són el dolor en el cuir cabellut i la claudicació mandibular i/o lingual. Una complicació temuda i freqüent, especialment en els pacients no tractats, és l'afectació ocular amb neuritis òptica isquèmica, que fins i tot pot produir ceguera. La simptomatologia focal no cranial és més infreqüent. L'ACG s'associa sovint a la polimiàlgia reumàtica, síndrome caracteritzada per dolor en músculs del coll, espatlles, part baixa de l'esquena, malucs i cuixes. També es pot presentar amb febre i altres símptomes sistèmics inespecífics. L'elevació de la velocitat de sedimentació globular (VSG) i l'anèmia són troballes de laboratori característiques. L'ACG respon

ràpidament al tractament amb corticoids, amb un bon pronòstic si no hi ha complicacions isquèmiques irreversibles abans de la instauració del tractament, però sovint apareixen recidives.

L'arteritis de Takayasu és una vasculitis que afecta vasos de gran calibre i que presenta algunes característiques en comú amb l'AGC. En les dues entitats, l'examen histopatològic demostra afectació de vasos mitjans i grans amb infiltració per macròfags i sovint un patró granulomatós amb formació de cèl·lules gegants. Els símptomes sistèmics són freqüents en les dues vasculitis, i també en les dues les manifestacions clíniques resulten del diferent grau d'isquèmia secundària a l'afectació cranial, ocular i de les extremitats. A diferència de l'ACG, l'arteritis de Takayasu afecta típicament dones joves del sudest asiàtic o d'origen hispànic. Les seves manifestacions clíniques més freqüents són conseqüència d'estenosis de l'aorta i de les seves branques principals. La resposta als corticoids no és tan espectacular com en l'ACG i, malgrat associar-hi immunosupressors o cirurgia, les recidives són freqüents i sovint difícils de controlar.

Taula 1. Criteris per a la classificació de les vasculitis segons l'American College of Rheumatology 1990 (9-11)

Condició	Criteri	Nª criteris	Sensibilitat (%)	Especificitat (%)
Púrpura de Henoch-Schönlein	Púrpura palpable Inici anterior als 20 anys Angor intestinal Granulòcits en la paret vascular (en la biòpsia)	≥ 2	87,8	87,7
Granulomatosi de Wegener	Inflamació nasal o oral Radiografia de tòrax anormal (nòduls, infiltrats o cavitats) Microhematúria o cilindres hemàtics Inflamació granulomatosa en la biòpsia	≥ 2	88,2	92
Síndrome de Churg-Strauss	Asma Eosinofília > 10% Neuropatia Infiltrats pulmonars (no fixes) Anormalitat dels sinus paranasals Eosinòfils extravasculars	≥ 4	85	99,7
Vasculitis per hipersensibilitat	Inici posterior als 16 anys Púrpura palpable Erupció maculopapular Granulòcits perivasculars o extravasculars (arterioles o vènules)	≥ 3	71	83,9
Poliarteritis nudosa	Pèrdua de pes ≥ 4 Kg *Livedo reticularis* Dolor a la palpació testicular Miàlgia o debilitat Mono o polineuropatia Pressió arterial diastòlica ≥ 90 mmHg Elevació del BUN o la creatinina Virus de l'hepatitis B Anormalitat arteriogràfica Biòpsia d'artèries petites o mitjanes que continguin granulòcits	≥ 3	82,2	86,6
Arteritis temporal o de cèl·lules gegants	Inici superior als 50 anys Cefalea d'aparició recent o de característiques diferents Dolor a la palpació de l'artèria temporal o pols disminuït Velocitat de sedimentació globular ≥ 50 Biòpsia amb infiltrats de cèl·lules mononucleades o inflamació granulomatosa	≥ 3	93,5	91,2
Arteritis de Takayasu	Inici anterior als 40 anys Claudicació d'extremitats Pols de l'artèria braquial disminuït Diferència de pressió arterial entre els dos braços > 10 mmHg Buf sobre les artèries subclàvies o l'aorta Anormalitat arteriogràfica (estretament o oclusió de l'aorta o de les seves branques principals)	≥ 3	90,5	97,8

En els darrers anys s'ha emfatitzat sobre el concepte de poliangitis microscòpica com a entitat separada de la PAN. En la conferència de consens de Chapel Hill (12) es defineixen les característiques clínico-patològiques de 10 entitats seleccionades de vasculitis, que s'agrupen segons el calibre dels vasos més afectats. La principal diferència respecte anteriors classificacions és la importància donada a la poliangitis microscòpica, que es descriu com una vasculitis necrotitzant amb absència o mínima presència d'immunocomplexes i que afecta vasos microscòpics, és a dir arterioles, capil·lars i/o vènules. En aquesta vasculitis, la glomerulonefritis necrotitzant i la capil·laritis pulmonar són freqüents. En la poliangitis microscòpica també poden estar afectats vasos de petit i mitjà calibre; en canvi, la presència de glomerulonefritis o l'afectació d'arterioles, capil·lars o vènules exclou el diagnòstic de PAN clàssica (taula 2). Donat que molts autors adopten els criteris d'aquesta classificació i per tal d'universalitzar el diagnòstic, en referir-nos a la PAN en els treballs presentats hem aplicat les dues classificacions. És a dir, tots els malalts inclosos, acompleixen els criteris diagnòstics de PAN de l'ACR i cap d'ells es pot considerar afecte de poliangitis microscòpica. Per a les altres vasculitis estudiades, concretament l'arteritis de cèl·lules gegants (ACG), hem aplicat els criteris de classificació de l'ACR. En ambdues vasculitis hem considerat la positivitat de la biòpsia un criteri obligatori per a la inclusió dels malalts.

Taula 2. Noms i definicions de les vasculitis acceptats en la Conferència de consens de Chapel Hill sobre la nomenclatura de les vasculitis sistèmiques (12)

Vasculitis de vas gran	
Arteritis de cèl·lules gegants (temporal)	Arteritis granulomatosa de l'aorta i les seves branques principals amb afectació preferent de les branques extracranials de l'artèria caròtida. *Sovint afecta l'artèria temporal. Habitualment es presenta en pacients més grans de 50 anys i s'associa freqüentment a la presència de polimiàlgia reumàtica*
Arteritis de Takayasu	Inflamació granulomatosa de l'aorta i les seves branques principals. *Habitualment es presenta en pacients menors de 50 anys.*
Vasculitis de vas mitjà	
Poliarteritis nodosa clàssica	Inflamació necrotitzant d'artèries petites o mitjanes sense glomerulonefritis o vasculitis en arterioles, capil·lars o vènules
Malaltia de Kawasaki	Arteritis d'artèries grans, mitjanes i petites amb síndrome ganglionar mucocutani associat. *Les artèries coronàries estan afectades sovint. Habitualment es presenta en nens.*
Vasculitis de vas petit	
Granulomatosi de Wegener	Inflamació granulomatosa en el tracte respiratori i vasculitis necrotitzant en vasos petits i mitjans (artèries, arterioles, capil·lars i vènules). *És freqüent la presència de glomerulonefritis necrotitzant.*
Síndrome de Churg-Strauss	Inflamació granulomatosa rica en eosinòfils en el tracte respiratori amb vasculitis necrotitzant en vasos mitjans i petits en pacients amb asma i eosinofília
Poliangitis microscòpica	Vasculitis necrotitzant amb absència o presència insignificant de dipòsits de complexes immunitaris que afecta vasos petits (arterioles, capil·lars i vènules). *Pot existir vasculitis necrotitzant en artèries de calibre petit o mitjà. La glomerulonefritis necrotitzant és freqüent. Sovint hi ha capil·laritis pulmonar.*
Púrpura de Henoch-Shönlein	Vasculitis amb dipòsits predominants d'IgA que afecta vasos petits (arterioles, capil·lars i vènules). *Típicament s'afecta la pell, l'intestí i el glomèrul renal. És molt freqüent la presència d'artràlgies o artritis.*
Crioglobulinèmia	Vasculitis amb dipòsits de crioglobulines que afecta vasos petits (arterioles, vènules i capil·lars) amb presència de crioglobulines sèriques. *La pell i el glomèrul renal s'afecten freqüentment.*
Angitis leucocitoclàstica cutània	Angitis leucocitoclàstica cutània sense vasculitis sistèmica o glomerulonefritis

El maneig de les vasculitis és complex, ja que el tractament que actualment s'utilitza és agressiu i inespecífic i s'ha de valorar el risc d'efectes indesitjats i el benefici que es pot obtenir. Tot i que els corticoids i els fàrmacs immunosupressors han suposat una gran millora en el pronòstic d'aquests malalts, les complicacions del tractament, bàsicament infeccions, neoplàsies, osteoporosi, diabetis i hipertensió arterial, comporten una morbilitat important. A més, els malalts no sempre responen al tractament, i tal com hem comentat en explicar les diferents vasculitis, sovint apareixen recidives en abandonar-lo. També cal considerar l'existència de casos amb una evolució més favorable que podrien respondre amb menys fàrmacs o amb dosis inferiors de tractament, amb la reducció subsegüent dels efectes secundaris (13-16). Per tot això, el que es vol aconseguir és un tractament el màxim d'específic i individualitzat en cada cas. Però això no és possible sense un coneixement precís i profund de la patogènia d'aquestes malalties. L'objectiu de la present tesi ha estat el d'aportar nous coneixements sobre els mecanismes que contribueixen al desenvolupament d'infiltrats inflamatoris en les vasculitis, amb la idea que en un futur contribueixin a millorar el tractament i el pronòstic dels malalts afectats per aquestes malalties. L'estudi està centrat en la PAN i l'AGG perquè són representatives de dos tipus diferents de vasculitis (de vas mitjà i vas gran respectivament) i perquè l'expressió de les molècules d'adhesió cel·lular, que és el que s'estudia en la present tesi, mai s'ha investigat anteriorment en grups grans i homogenis d'aquestes vasculitis.

1. Etiopatogènia de les vasculitis

Les vasculitis constitueixen un grup molt variat de malalties i, per tant, els mecanismes patogenètics que condueixen a la lesió vascular són heterogenis. Diferents agents etiològics, entre els quals cal considerar virus, fàrmacs i altres agents ambientals, desencadenen una cascada de mecanismes patogenètics immediats capaços de lesionar la paret dels vasos. Entre aquests mecanismes destaquen el dipòsit de complexes immunitaris, els anticossos anti-citoplasma de neutròfil, els anticossos anti-cèl·lula endotelial, i una resposta immunològica mitjançada per cèl·lules T enfront d'antígens presents en la paret arterial. Aquests mecanismes patogenètics no són mútuament excloents sinó que probablement actuen de manera combinada, amb un protagonisme variable segons la malaltia o el moment evolutiu. Sobre ells actuarien en alguns casos altres factors moduladors, com les hormones sexuals i el substrat genètic (17-19) (figura 1).

1.1. Agents desencadenants

Actualment, es coneix poc sobre els agents etiològics que desencadenen la cascada de fenòmens immunitaris que produeixen la lesió vascular. En un 53 % de pacients amb vasculitis leucocitoclàstica es recull l'antecedent d'ingesta d'algun fàrmac (20). S'han descrit vasculitis en les lesions pròpies de diverses malalties bacterianes (tuberculosi, sífilis, rickettsiosis) i fúngiques (aspergilosi, candidiasi) (21).

Diversos autors han suggerit que algunes infeccions víriques podrien jugar un paper important en l'etiopatogènia de les vasculitis. Entre aquestes infeccions, cal destacar la clàssicament coneguda associació entre la PAN i la infecció pel virus de l'hepatitis B (VHB), que es detecta en un 20-40 % de casos en algunes sèries (22). La infecció pel virus de l'hepatitis C (VHC) en els pacients amb crioglobulinèmia mixta encara és més prevalent, i arriba a ser el 96 % en algunes sèries (23). El

paper etiopatogènic d'aquests virus en el desenvolupament de les vasculitis no solament es basa en la seva estreta associació epidemiològica sinó també en el fet que sovint milloren les manifestacions clíniques amb tractaments que aconsegueixen una remissió virològica (24-26). També s'han descrit diversos tipus de vasculitis (vasculitis leucocitoclàstica, PAN, vasculitis aïllada del sistema nerviós central) en pacients infectats pel virus de la immunodeficiència humana (VIH) (27-29), sobretot en pacients amb una immunitat encara preservada (30). Altres virus descrits possiblement implicats en el desenvolupament de vasculitis són el parvovirus B19, el virus de la varicel·la-zóster i el citomegalovirus (21,31). La identificació de virus com a agents etiològics o desencadenants de les vasculitis té importants implicacions terapèutiques, ja que el tractament antiviral podria ser una alternativa al tractament immunosupressor.

1.2. Mecanismes patogenètics immediats

Dipòsit d'immunocomplexes circulants

Els complexes immunitaris circulants formats en presència d'un excés d'antigen poden no ser eliminats totalment pel sistema mononuclear fagocític i dipositar-se en la paret vascular. També poden originar-se complexes immunitaris directament en els vasos. Allà, activarien la cascada del complement, i els productes d'activació atraurien els neutròfils, que destruirien la paret vascular amb els seus enzims lisosòmics (1). Les vasculitis leucocitoclàstiques, la púrpura de Henoch-Schönlein, la crioglobulinèmia mixta, i, probablement, les vasculitis necrosants del grup de la PAN són les entitats que s'adapten millor a aquest model patogenètic (32,33). Però aquest mecanisme no explica satisfactòriament les lesions objectivades en altres vasculitis, com l'ACG, la granulomatosi de Wegener o la malaltia de Takayasu.

Anticossos anti-citoplasma dels neutròfils (ANCA)

Els ANCA reconeixen constituents del citoplasma dels neutròfils. S'han identificat dos constituents principals en els gànuls atzuròfils reconeguts pels ANCA: la proteïnasa 3 (PR3) i la mieloperoxidasa (MPO). Els ANCA-PR3 tenen una sensibilitat i especificitat molt importants per la granulomatosi de Wegener, i els ANCA-MPO es troben en una proporció variable de pacients amb poliarteritis microscòpica i amb glomerulonefritis. Els ANCA també poden reconèixer altres constituents dels gànuls dels neutròfils, com l'elastasa, la catepsina G, la lactoferrina, i la lisozima, entre altres. El significat biològic d'aquests nous ANCA encara es desconeix (19,34).

L'estreta associació entre els ANCA-PR3 i la granulomatosi de Wegener i, en menor grau, dels ANCA-MPO i la poliangitis microscòpica ha suggerit que aquests anticossos tenen un paper patogenètic en la lesió vascular. En l'actualitat, està demostrat que els ANCA contribueixen a l'activació dels neutròfils induïda per altres estímuls i poden augmentar el seu potencial lesiu, però per sí sols no semblen capaços d'induir lesions inflamatòries, i és prematur assumir que juguen un paper primari en la patogènesi de les vasculitis.

Anticossos anti-cèl·lula endotelial (ACE)

S'han detectat ACE en el sèrum de pacients afectats per una àmplia varietat de malalties que cursen amb afectació vascular, com el lupus eritematós sistèmic i l'esclerosi sistèmica progressiva, entre altres, però també en altres processos on la participació endotelial és menys evident, com l'hiperparatiroidisme autoimmunitari. També s'ha demostrat la presència d'ACE en diferents vasculitis sistèmiques, com la malaltia de Kawasaki, la granulomatosi de Wegener, la poliangitis microscòpica i la vasculitis retiniana (35). La majoria d'aquests anticossos són capaços de fixar el complement i poden exercir citotoxicitat directa o mitjançar citotoxicitat cel·lular depenent d'anticòs i, per tant, tenen un potencial lesiu sobre la cèl·lula endotelial. S'ha demostrat que altres ACE són

capaços d'activar les cèl·lules endotelials i induir l'expressió de molècules d'adhesió (36). En la majoria de processos en els quals s'han descrit, els nivells d'ACE es correlacionen amb l'activitat clínica de la malaltia (35). Encara es desconeix si aquests anticossos tenen un paper patogenètic primari o es produeixen a conseqüència de la lesió endotelial produïda per altres mecanismes.

Resposta immunològica mitjançada per cèl·lules T

Diferents estudis immunopatològics han demostrat que en algunes vasculitis, com l'ACG, la granulomatosi de Wegener, la malaltia de Takayasu, la PAN i les lesions coronàries de la malaltia de Kawasaki, l'infiltrat inflamatori està format fonamentalment per macròfags i limfòcits T que expressen marcadors d'activació immunitària i proliferen activament en les lesions (18,37,38). En lesions precoces d'ACG i de PAN s'ha identificat cèl·lules dendrítiques que expressen antígens de classe II del sistema major d'histocompatibilitat que probablement actuin com a cèl·lules presentadores d'antígens (18,37,38). Aquestes observacions suggereixen que els limfòcits T activats i els macròfags juguen un paper important en el desenvolupament de la lesió vascular. Els mecanismes a partir dels quals els limfòcits T s'activen són, molt probablement, heterogenis. En algunes vasculitis, com la malaltia de Kawasaki, es detecten expansions policlonals de limfòcits T circulants que poden ser el resultat d'un estímul per superantígens. En l'ACG s'ha demostrat que una petita proporció dels limfòcits activats infiltrants sofreixen una expansió clonal suggerint una resposta immunitària específica enfront d'un antigen possiblement present en la paret arterial (39-41). En un model animal d'arteritis granulomatosa s'ha demostrat la participació de mecanismes de citotoxicitat restringida per antígens de classe I del sistema major d'histocompatibilitat (18). La presència de limfòcits CD8 citotòxics s'ha demostrat en diferents vasculitis , com l'ACG, la PAN i la malaltia de Takayasu (18,37,38). En aquesta, una població significativa de limfòcits infiltrants expressa cadenes gamma/delta del receptor T (42).

Un cop activats per diferents mecanismes patogenètics, els leucòcits envaeixen la paret vascular a través de complexes interaccions amb l'endoteli i les proteïnes de la matriu extracel·lular, que es produeixen a través d'uns receptors de membrana i dels seus contrareceptors, anomenats genèricament molècules d'adhesió.

1.3. Resposta vascular

Un cop en la paret dels vasos, els leucòcits segreguen citocines i factors de creixement, els quals generen una sèrie de canvis morfològics i funcionals en els vasos que són responsables de la majoria de les manifestacions clíniques i complicacions isquèmiques dels pacients amb vasculitis. Algunes citocines, com les interleucines 1 i 6 i el factor de necrosi tumoral, produïdes activament en les lesions de l'ACG, ocasionen febre i símptomes constitucionals freqüents en aquests malalts (43). Les manifestacions focals més específiques de les vasculitis deriven sovint de la disfunció orgànica determinada per la isquèmia i, ocasionalment, per l'hemorràgia en els òrgans afectats. L'oclusió vascular en les vasculitis pot tenir lloc per espasme, trombosi i hiperplàsia o fibrosi intimal (18). Les citocines proinflamatòries interleucina 1 i factor de necrosi tumoral alteren les propietats anticoagulants de l'endoteli. Les mateixes citocines regulen el to vascular. Altres citocines produïdes en la paret vascular tenen activitat fibrogènica. Citocines amb activitat quimiotàctica continuen atraient els leucòcits, que penetren en els vasos gràcies a la inducció de molècules d'adhesió endotelials per interleucina-1, factor de necrosi tumoral i interferó gamma. La producció d'aquest sembla essencial per al desenvolupament i manteniment de la reacció granulomatosa característica de l'ACG. Altres factors de creixement amb activitat angiogènica poden contribuir a la neovascularització que té lloc en el sí de la paret arterial en algunes vasculitis com la PAN i l'ACG (18,37,38). Els vasos neoformats proporcionarien nous punts a través dels quals els leucòcits podrien infiltrar la paret vascular i contribuir al manteniment de

la inflamació. El paper precís que les citocines i els factors de creixement juguen en la resposta vascular és objecte d'una activa investigació en l'actualitat (18).

1.4. Factors moduladors

Hormones sexuals

Algunes vasculitis, com la malaltia de Takayasu, afecten preferentment a dones en edat fèrtil. Aquesta distribució demogràfica suggereix que les hormones sexuals poden modular els mecanismes immunitaris que porten a la lesió vascular o la resposta del propi vas a la inflamació. Recentment s'ha demostrat que la cèl·lula endotelial té receptors estrogènics funcionals, i que els estròGens regulen les vies a través de les quals determinades citocines, com el factor de necrosi tumoral, indueixen l'expressió de molècules d'adhesió endotelials pels leucòcits (44).

Predisposició genètica

Des de fa alguns anys, se sap que individus portadors d'alels HLA-DRB1'04 del Sistema Major d'Histocompatibilitat tenen un major risc de patir ACG (45). També s'ha identificat una seqüència concreta (DRYF) relacionada amb la mateixa malaltia situada a la zona de la unió a l'antigen durant la presentació antigènica (46), troballa que reforça la hipòtesi que les lesions vasculars en l'ACG tenen lloc a conseqüència d'una resposta immunitària antigen-específica. La malaltia de Takayasu és més freqüent en dones joves d'origen hispànic o del sud-est asiàtic i en algunes ètnies s'ha associat la suceptibilitat a patir la malaltia amb alguns antígens HLA, concretament amb HLA-B5 a la Índia i a HLA-B52 i B39.2 al Japó (47,48).

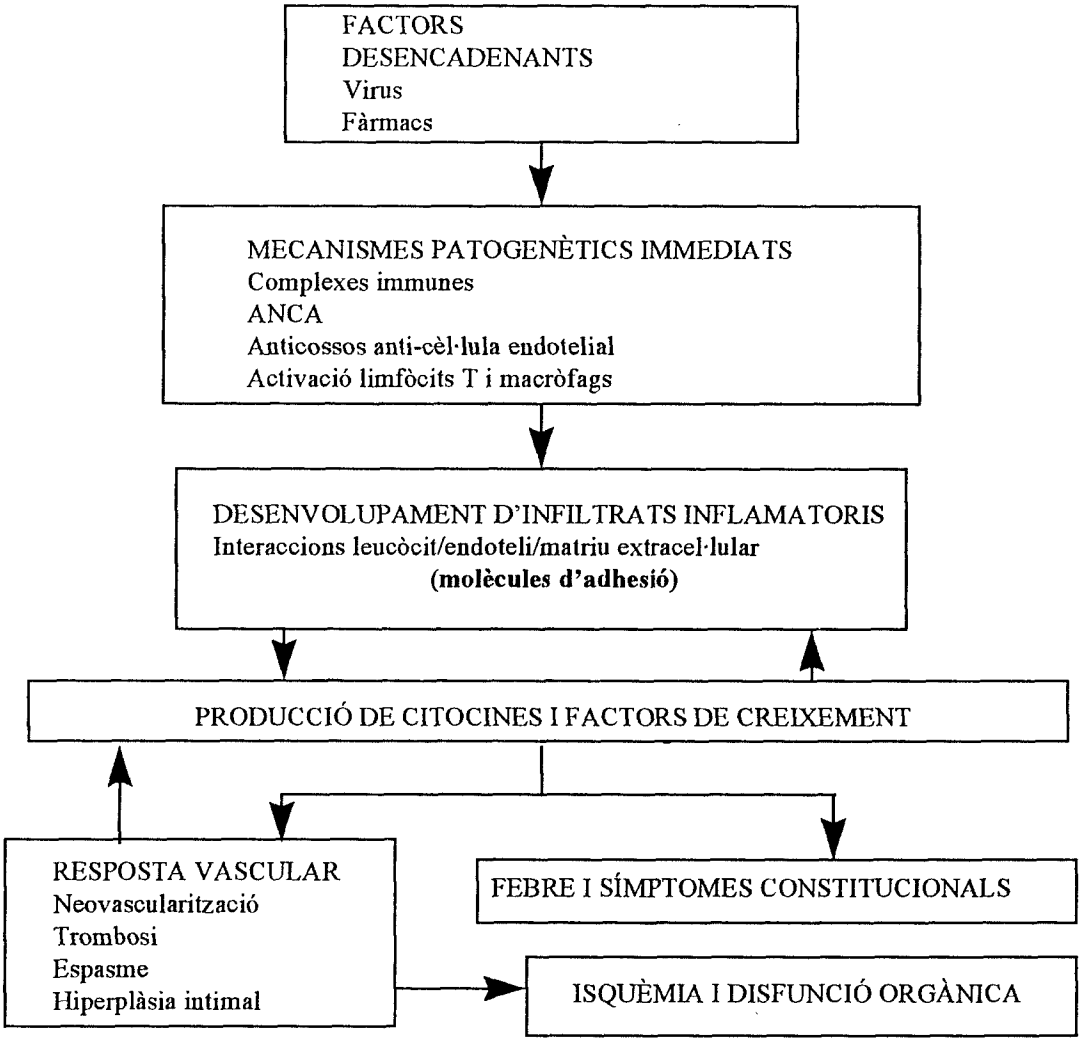

Figura 1. Etiopatogènia de les vasculitis

2. Molècules d'adhesió

2.1. Descripció

Tal com s'ha comentat, independentment dels agents etiològics o dels mecanismes patogenètics immediats, el desenvolupament dels infiltrats inflamatoris en els teixits es produeix a través d'interaccions dinàmiques entre leucòcits, cèl·lules endotelials i proteïnes de la matriu extracel·lular a través de les anomenades molècules d'adhesió. Aquestes es poden agrupar en tres famílies principals segons la seva estructura molecular: les *selectines*, les *integrines* i la *superfamília de les immunoglobulines* (49-52).

Les *selectines* són les molècules d'adhesió que vehiculitzen el contacte inicial en les interaccions leucòcit/endoteli. Són glucoproteïnes de membrana que comparteixen un domini aminoterminal tipus lectina per on s'uneixen als seus lligands. Fins ara es coneixen 3 selectines: selectina E, selectina P, i selectina L. La selectina E és induïda en la cèl·lula endotelial per diverses citocines (interleucina-1, factor de necrosi tumoral i lipopolissacàrid). La selectina P es sintetitza en les plaquetes i en l'endoteli. En les plaquetes s'emmagatzema en els gràmuls α i en la cèl·lula endotelial en els gràmuls de Weibel-Palade, des d'on és translocada a la superfície cel·lular sota l'influx de mediadors de la inflamació aguda (trombina, histamina, leucotriè, C4). Algunes citocines (factor de necrosi tumoral α) també indueixen la seva síntesi en la cèl·lula endotelial. La selectina L s'expressa en tots els leucòcits circulants excepte algunes subpoblacions de limfòcits T de memòria i, després de l'activació limfocitària, es desprèn de la membrana cel·lular. Els lligands de les selectines són hidrats de carboni específics, dels quals els més coneguts són formes sialilades dels antígens Lewis^x i Lewis^a. Altres lligands identificats fins ara són l'hidrat de carboni CLA (*cutaneous lymphocyte* antigen, per la selectina E), i les mucines PSGL-1 (*P-selectin*

glycoprotein ligand, per la selectina P) i GlyCAM-1 i GlyCAM-2 (*glycosylation-dependent cell adhesion molecule-1,-2*, per la selectina L).

Les *integrines* són proteïnes heterodimèriques formades per una cadena α i una altra β presents en una àmplia varietat d'estirps cel·lulars. Constitueixen els principals receptors que interaccionen amb proteïnes de la matriu extracel·lular. Algunes d'elles també participen en interaccions intercel·lulars. Les integrines es classifiquen en famílies la identitat de les quals s'estableix per la cadena β. S'ha identificat un mínim de 9 cadenes β que defineixen les corresponents famílies. Cada cadena β pot unir-se a diferents cadenes α. Fins ara es coneixen unes 20 combinacions diferents. Els limfòcits utilitzen fonamentalment integrines de les famílies β_1 (especialment $\alpha_4\beta_1$ o VLA-4 -*very late antigen-4*, que interacciona amb la immunoglobulina VCAM-1 - *vascular cell adhesion molecule-1*) i β_2 (sobretot $\alpha_L\beta_2$ o LFA-1 - *leukocyte function-associated antigen-1*, que interacciona amb les immunoglobulines ICAM-1, ICAM-2 i ICAM-3 (*intercellular adhesion molecule-1,-2,-3*), i $\alpha_M\beta_2$ o Mac-1, que interacciona amb ICAM-1) per unir-se a l'endoteli i a la matriu extracel·lular . La integrina $\alpha_V\beta_3$ també intervé en l'adhesió del leucòcit a l'endoteli mitjançant la seva interacció amb PECAM-1 (*platelet endothelial cell adhesion molecule-1*) (53). Les integrines s'expressen constitutivament en els leucòcits i, encara que la seva expressió pot ser modulada, la seva regulació és principalment funcional. La seva expressió no és uniforme en tots els leucòcits. Les integrines consoliden les interaccions iniciades per altres molècules d'adhesió, principalment selectines. També són molècules transductores de senyals i en unir-se als seus contrareceptors produeixen importants canvis funcionals en els leucòcits. La seva connexió al citoesquelet cel·lular fa que tinguin un paper important en el procés de migració.

Els membres de la *superfamília de les immunoglobulines* es caracteritzen per contenir els anomenats dominis immunoglobulínics, que consisteixen en dues làmines peptídiques en estructura β-plegada i en disposició antiparal·lela estabilitzades per ponts disulfur. Aquestes

característiques estructurals són compartides per molècules que participen en el reconeixement antigènic i en l'activació limfocitària, com els anticossos, el receptor T, els antígens de classes I i II del sistema major d'histocompatibilitat i els seus contrareceptors CD8 i CD4. Les principals molècules d'adhesió que pertanyen a aquesta família són ICAM-1, ICAM-2 i ICAM-3 (contrareceptors de la integrina LFA-1), VCAM-1 (que interacciona amb les integrines VLA-4 i $\alpha_4\beta_7$), LFA-3 (que interacciona amb la immunoglobulina CD2), i PECAM-1 (que interacciona de manera homofílica amb ella mateixa, i també amb la integrina $\alpha_V\beta_3$). Totes elles, excepte ICAM-3, s'expressen en la cèl·lula endotelial basalment o després de la inducció amb citocines, i participen en les interaccions leucòcit/endoteli. ICAM-3 s'expressa constitutivament en limfòcits no estimulats i en cèl·lules presentadores d'antígens (54). S'han descrit altres molècules d'adhesió, com CD44 o VAP-1, que pertanyerien a altres famílies (52,55).

Les principals molècules d'adhesió conegudes, així com la seva distribució cel·lular, es recullen a la taula 3.

Taula 3. Principals molècules d'adhesió involucrades en la migració dels leucòcits cap els teixits

Molècula	CD	Lligand/Contrareceptor	Distribució	Funció biològica
Selectines				
Selectina E	CD62E	Syalil Le^x o Le^a, CLA	CE	rodament, activació d'integrines
Selectina P	CD62P	PSGL-1*	CE, plaquetes	activació d'integrines
Selectina L	CD62L	Syalil Le^x o Le^a GlyCAM-1, GlyCAM-2**	leucòcits	rodament, "homing" a ganglis
Integrines				
$\alpha_4\beta_1$ (VLA-4)**	CD49d/CD29	VCAM-1, fibronectina	limfòcits, monòcits, eosinòfils	adhesió forta
$\alpha_L\beta_2$ (LFA-1)	CD11a/CD18	ICAM-1, ICAM-2, ICAM-3	leucòcits, NK	adhesió forta, transmigració
$\alpha_M\beta_2$ (Mac-1)	CD11b/CD18	ICAM-1	monòcits, granulòcits, macròfags, NK	adhesió forta
$\alpha_X\beta_2$ gp (150,95)	CD11c/CD18	?	monòcits, granulòcits, macròfags, NK	adhesió forta
$\alpha_V\beta_3$	CD51/CD61	PECAM-1, col·lagen, fibronectina, vitronectina	CE, monòcits, limfòcits B activats	
$\alpha_4\beta_7$**	CD49d/-	MadCAM, VCAM-1, fibronectina	limfòcits (subtipus)	homing a mucoses
Superfamília de les immunoglobulines				
ICAM-1	CD54	LFA-1, Mac-1	leucòcits, CE, fibroblasts, cèl·lules dendrítiques, cèl·lules epitelials	adhesió forta, transmigració
ICAM-2	CD102	LFA-1	cèl·lules endotelials, (altres rarament)	adhesió forta
ICAM-3	CD50	LFA-1	leucòcits, cèl·lules dendrítiques	
VCAM-1**	CD106	VLA-4, $\alpha_4\beta_7$	cèl·lules endotelials, macròfags (altres menys freqüent)	adhesió forta
PECAM-1	CD31	PECAM-1, $\alpha_V\beta_3$	neutròfils, monòcits, CE, plaquetes	activació d'integrines, transmigració
LFA-3	CD58	LFA-2 (CD2)	la majoria de cèl·lules	activació de limfòcits i d"integrines

* intervé fonamentalment en les interaccions neutròfil/endoteli

** intervé fonamentalment en les interaccions limfòcit/endoteli

2.2. Paper de les molècules d'adhesió en el pas dels leucòcits cap els teixits

La transició des d'un leucòcit circulant a un leucòcit adherent que infiltra els teixits requereix una sèrie d'esdeveniments coordinats. Les interaccions entre les molècules d'adhesió leucocitàries i els seus lligands específics de la cèl·lula endotelial es produeix d'una manera seqüencial i regulada amb gran precisió (55-57). Primer, quan un estímul apropiat indueix l'expressió de determinades molècules en la superfície endotelial i leucocitària, els leucòcits circulants s'enlenteixen i roden sobre la superfície endotelial. Aquest fenomen de rodament està mitjançat sobretot per selectines i carbohidrats, encara que no són les úniques molècules implicades. Posteriorment, les integrines leucocitàries, basalment en un estat no adherent, són activades. Aquesta activació es produeix a través de factors solubles (citocines quimiotàctiques o quimiocines) o altres interaccions cel·lulars que impliquen molècules coestimulatòries (52,58-60). Els contrareceptors de les integrines leucocitàries poden ser expressats constitutivament per la cèl·lula endotelial (ICAM-2, PECAM-1), induïts (VCAM-1) o sobreexpressats (ICAM-1) per l'estímul de citocines, fonamentalment interleucina-1, factor de necrosi tumoral α i interferó γ (52,56,61). Mitjançant la interacció d'integrines i immunoglobulines, especialment LFA-1/ICAM-1 i PECAM-1 (important component de les unions intercel·lulars entre les cèl·lules endotelials) amb ella mateixa i/o amb $\alpha_V\beta_3$, es produeix l'adhesió forta, l'extensió dels leucòcits sobre l'endoteli i subsegüent transmigració a través de les unions intercel·lulars cap a l'estímul quimiotàctic. Finalment, els leucòcits interaccionen amb proteïnes de la matriu extracel·lular, fonamentalment a través d'integrines β_1, i també β_3 i β_5 que s'uneixen principalment a laminina, col·lagen o fibronectina.

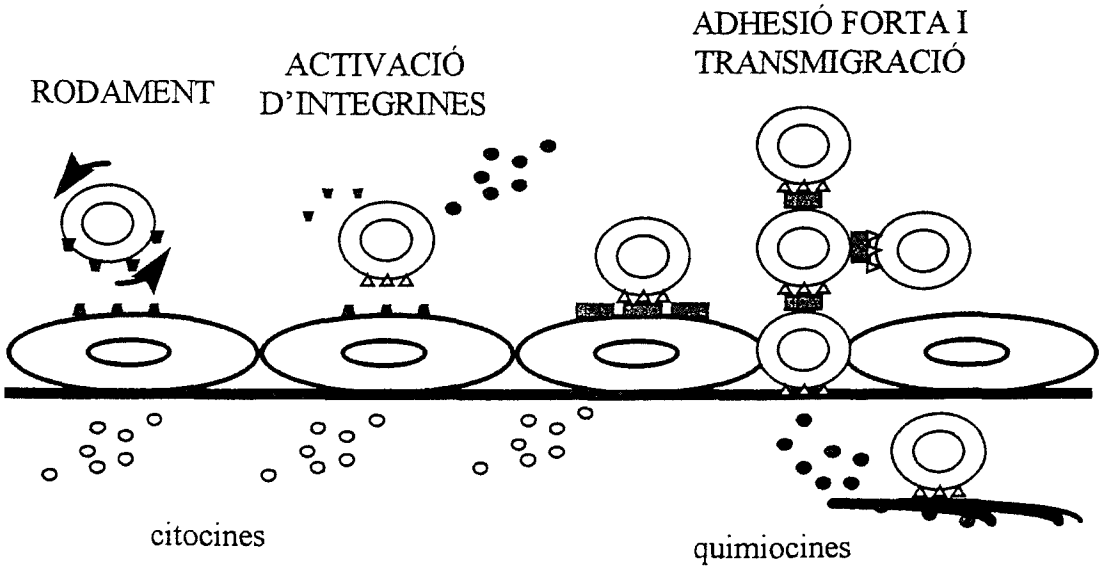

Figura 2. Interaccions entre molècules d'adhesió endotelials i leucocitàries

2.3. Molècules d'adhesió solubles

En el plasma humà i en altres líquids biològics s'han detectat selectines E, P i L i alguns membres de la superfamília de les immunoglobulines (ICAM-1, ICAM-3, VCAM-1, PECAM-1) en forma soluble (62-70). En alguns casos, com en el de la selectina P i VCAM-1, aquest fet es produeix a través d'un processament alternatiu de l'ARN que genera una variant sense el domini transmembrana. En altres casos, com en el de la selectina L i ICAM-3, la molècula es desprèn per proteòlisi. Estudis in vitro han demostrat que algunes molècules (selectina L, ICAM-3) es desprenen de la membrana leucocitària després de l'activació. Després de l'estímul amb citocines, la cèl·lula endotelial no solament augmenta l'expressió de membrana de molècules d'adhesió sinó també la seva alliberació al sobrenadant. Per aquest motiu,

l'increment en la concentració de molècules d'adhesió circulants es considera una evidència indirecta d'activació immunitària i/o endotelial.

Els efectes biològics de les molècules d'adhesió solubles no es coneixen amb exactitud. D'una banda, en conservar la seva capacitat d'unió al contrareceptor, poden bloquejar les unions intercel·lulars i tenir un efecte inhibidor de l'adhesió leucòcit/endoteli i posterior transmigració. D'altra banda, quan les molècules d'adhesió solubles s'uneixen al seu contrareceptor, poden transduir senyals i desencadenar una coestimulació efectiva de les cèl·lules amb les quals interaccionen (71).

2.4. Repercussió en patologia humana

Les molècules d'adhesió tenen una importància fonamental en la defensa de l'organisme enfront de la infecció. A més del seu paper en l'adhesió dels leucòcits a l'endoteli vascular i posterior transmigració cap els teixits, també participen en la recirculació limfocitària, especialment en el tràfic selectiu de limfòcits T de memòria cap a zones predeterminades (*homing*), i en la presentació antigènica i activació limfocitària (55). Però, per causes poc conegudes, les mateixes interaccions que poden beneficiar l'organisme contribueixen al desenvolupament dels fenòmens inflamatoris nocius que es produeixen en les malalties autoimmunitàries, en el rebuig de trasplantaments i en la lesió tissular causada en la reperfusió post-isquèmia. Algunes d'aquestes molècules també són utilitzades per les cèl·lules neoplàsiques per a penetrar en els teixits durant la disseminació metastàsica, i per les cèl·lules endotelials durant el procés d'angiogènesi (71-73). El coneixement dels mecanismes moleculars que regulen aquestes interaccions té importants repercussions en patologia humana i constitueix la base per a la recerca de nous mètodes d'intervenció terapèutica.

2.5. Molècules d'adhesió en les vasculitis

Per investigar la participació de les molècules d'adhesió en les malalties humanes, i, entre elles, les vasculitis, s'han utilitzat diferents mètodes: expressió de membrana i funcionalisme en leucòcits, expressió tissular en mostres biòpsiques, i detecció de formes solubles (71). Hi ha molt pocs estudis sobre l'expressió de les molècules d'adhesió en les vasculitis sistèmiques. Independentment del mètode utilitzat, els resultats obtinguts no són concloents perquè les mostres de població són petites i/o heterogènies i el nombre de molècules estudiades és reduït, amb el qual no es poden valorar les correlacions topogràfiques entre receptors i lligands. A més, els resultats obtinguts en els diferents treballs publicats fins ara no sempre són comparables degut a les diferències en els criteris d'inclusió de malalts, en l'especificitat dels anticossos i els estàndards utilitzats en les tècniques immunohistoquímiques, o en la terminologia utilitzada.

En general, els leucòcits circulants de pacients amb vasculitis, així com de pacients amb altres malalties inflamatòries cròniques, expressen més molècules d'adhesió i tenen una major adhesivitat que leucòcits de controls sense vasculitis (71). En la granulomatosi de Wegener, els limfòcits perifèrics tenen una major expressió d'ICAM-1 i de la subunitat β_1 de les integrines que en subjectes control (74). Els limfòcits circulants aïllats de pacients amb lupus eritematós sistèmic i vasculitis expressen més intensament la integrina VLA-4 en la seva superfície i tenen una major adhesivitat a cèl·lules endotelials que els obtinguts de pacients amb lupus sense vasculitis (75).

Les molècules d'adhesió més estudiades en mostres histològiques de pacients amb vasculitis són ICAM-1, VCAM-1, i selectina E. Aquestes s'han estudiat en diverses entitats, especialment en vasculitis renals i cutànies (76-83). En general i com comentarem posteriorment, s'observa una sobreexpressió de les molècules induïbles.

S'han realitzat nombrosos estudis per a detectar nivells de molècules d'adhesió solubles en malalts afectes de vasculitis (84-88). Les més estudiades també són ICAM-1, VCAM-1 i selectina E en la seva forma soluble. Normalment s'observa una elevació d'aquestes molècules en la fase activa de la malaltia, però pels motius anteriorment comentats, els resultats obtinguts són de difícil interpretació. Com comentarem més endavant, recentment s'han fet alguns treballs amb grups homogenis de vasculitis i amb seguiment longitudinal que intenten correlacionar els nivells de molècules d'adhesió circulants amb l'activitat de la malaltia i comparar-los amb concentracions en altres malalties diferents en les quals el sistema immunitari també pugui estar danyat (89-93).

2.6. Aplicacions terapèutiques potencials

Molts dels agents utilitzats habitualment pel tractament de les malalties autoimmunitàries i, entre elles, les vasculitis, exerceixen algun efecte sobre la funció i/o expressió de les molècules d'adhesió. Per exemple, l'acció antiinflamatòria dels corticoids es produeix principalment a través de la inhibició del factor nuclear κB (NF-κB), que és un factor de transcripció bàsic en l'activació de les cèl·lules endotelials i que regula l'expressió de les molècules d'adhesió (94). En estudis in vitro, Cronstein i cols. han demostrat que els corticoids disminueixen l'expressió endotelial d'ICAM-1 i selectina E induïda per lipopolissacàrid o interleukina-1 (95). Youssef i cols. van observar una disminució de l'expressió d'ICAM-1 i selectina E en la membrana sinovial de malalts amb artritis reumatoide després del tractament amb altes dosis de metilprednisolona (96). L'expressió d'ICAM-1, selectina E i selectina P disminueix en la membrana sinovial de pacients amb polimiàlgia reumàtica després d'alguns mesos de tractament amb dosis baixes de corticoids respecte a malalts no tractats (97). També s'han descrit efectes sobre les molècules d'adhesió en altres fàrmacs utilitzats en la pràctica clínica pel tractament de malalties autoimmunitàries: la colxicina i el

metotrexat disminueixen l'expressió de selectina L i la funció de selectina E; el metotrexat, a més, també disminueix l'adhesió mitjançada per integrines; l'or disminueix l'expressió de selectina E i VCAM-1 en l'endoteli sinovial; la ciclosporina A disminueix l'expressió d'ICAM-1; i els antiinflamatoris no esteroïdals inhibeixen l'adhesió dels neutròfils mitjançada per integrines β_2 (52).

El coneixement dels mecanismes moleculars implicats en les interaccions leucòcit/endoteli/matriu extracel·lular que condueixen a la formació dels infiltrats inflamatoris suggereix la possibilitat de noves vies d'intervenció terapèutica basades en el bloqueig d'aquestes interaccions. Amb aquesta finalitat, s'estan desenvolupant nombroses modalitats: anticossos monoclonals que bloquegen epítops funcionals de les molècules d'adhesió; carbohidrats que inhibeixen la funció de les selectines; pèptids sintètics no immunogènics que bloquegen competitivament les interaccions mitjançades per les molècules d'adhesió; molècules solubles recombinants, que també actuen per un bloqueig competitiu; oligonucleòtids antisentit capaços d'unir-se a l'ARN missatger de les molècules d'adhesió i inhibir la seva traducció a proteïna, evitant així la seva expressió; i fàrmacs capaços d'inhibir l'expressió o bloquejar funcionalment les molècules d'adhesió de manera selectiva i controlada (71,95,98-104).

Totes aquestes modalitats s'han estudiat exhaustivament en diferents models animals, alguns dels quals simulen malalties autoimmunitàries humanes. En ells s'ha demostrat que la inhibició de les interaccions leucòcit/endoteli/matriu extracel·lular ajuda a prevenir l'aparició de lesions inflamatòries o millora les ja existents (105-111). Actualment ja s'han realitzat alguns assaigs amb anticossos antimolècules d'adhesió en humans, amb resultats prometedors (112-114), però la tolerància i els efectes a llarg termini encara no s'han analitzat.

Bloquejar l'adhesivitat pot significar una nova opció terapèutica per les vasculitis. L'aplicació d'aquest concepte requereix una major

comprensió de la cronologia de l'expressió induïble de molècules d'adhesió en les vasculitis, les vies preferencials en síndromes particulars i l'estat d'expressió de les molècules d'adhesió en la remissió. L'objectiu fonamental dels treballs exposats a continuació és el de contribuir a què es puguin assolir aquests coneixements.

OBJECTIUS

1. Analitzar l'evolució morfològica de l'endoteli en les diferents fases del procés inflamatori en mostres histològiques (múscul i nervi) de pacients amb PAN clàssica.

2. Estudiar, en les mateixes mostres, el patró de l'expressió de les següents molècules d'adhesió: a) endotelials: selectina-E, selectina-P, ICAM-2, VCAM-1; b) leucocitàries: selectina-L, ICAM-3, CD18 (cadena β_2 de les integrines), LFA-1, VLA-4; i c) endotelials i leucocitàries: ICAM-1, PECAM-1.

3. Investigar la relació entre l'expressió de les molècules d'adhesió endotelials estudiades i les següents característiques clíniques dels pacients: edat, sexe, durada dels símptomes i resposta inflamatòria sistèmica (determinada per la presència de febre, pèrdua de pes i velocitat de sedimentació globular elevada).

4. Determinar l'efecte del tractament amb corticoids sobre l'expressió de les molècules d'adhesió endotelials.

5. Quantificar la concentració de les formes solubles de selectina E, selectina P, selectina L, ICAM-1, ICAM-3 i VCAM-1 en sèrum i plasma de grups homogenis de pacients amb PAN clàssica i de pacients amb ACG, i comparar-la amb la concentració en controls sans.

6. Correlacionar els nivells d'aquestes molècules amb les característiques clíniques anteriorment esmentades.

7. Investigar la relació entre els nivells de les molècules d'adhesió solubles i la resposta clínica i biològica al tractament.

INVESTIGACIÓ I RESULTATS

1. DYNAMIC PATTERN OF ENDOTHELIAL CELL ADHESION MOLECULE EXPRESSION IN MUSCLE AND PERINEURAL VESSELS FROM PATIENTS WITH CLASSIC POLYARTERITIS NODOSA

Patró dinàmic de l'expressió de les molècules d'adhesió endotelial en vasos musculars i perineurals de pacients amb poliarteritis nudosa clàssica

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Arthritis and Rheumatism 1998;41:435-444
(pàgines 41-50)

En aquest primer treball hem estudiat l'expressió de les molècules d'adhesió cel·lular en teixits (múscul i nervi) de malalts amb PAN, amb la idea d'assolir els quatre primers objectius que ens haviem proposat.

Per a la realització del treball hem obtingut mostres de múscul i de nervi sural de 30 malalts amb PAN i de 12 controls i hem aplicat la tècnica immunohistoquímica de la fosfatasa alcalina-antifosfatasa alcalina amb anticossos monoclonals dirigits contra les molècules d'adhesió següents: selectines E, P i L, ICAM-1, -2, i -3, VCAM-1, PECAM-1, CD18 (cadena comú de les integrines β_2), LFA-1 i VLA-4. Per a identificar l'endoteli hem aplicat la tècnica de l'avidina-biotina peroxidasa amb la lectina *Ulex europeus*, i per a detectar els neutròfils presents en les lesions vasculars hem utilitzat aquesta darrera tècnica amb l'elastasa neutrofílica.

ARTHRITIS & RHEUMATISM
Vol. 41, No. 3, March 1998, pp 435–444

DYNAMIC PATTERN OF ENDOTHELIAL CELL ADHESION MOLECULE EXPRESSION IN MUSCLE AND PERINEURAL VESSELS FROM PATIENTS WITH CLASSIC POLYARTERITIS NODOSA

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Objective. **To investigate endothelial cell adhesion molecule expression in vessels from patients with classic polyarteritis nodosa (PAN).**

Methods. **Frozen sections of 21 muscle and 16 nerve samples from 30 patients with biopsy-proven PAN and 12 histologically normal muscle and 2 histologically normal nerve samples from 12 controls were studied immunohistochemically, using specific monoclonal antibodies (MAb) that recognize adhesion molecules. Adhesion molecules identified were intercellular adhesion molecule 1 (ICAM-1), ICAM-2, ICAM-3, vascular cell adhesion molecule 1 (VCAM-1), platelet endothelial cell adhesion molecule 1 (PECAM-1), E-selectin, P-selectin, L-selectin, lymphocyte function–associated antigen 1 (LFA-1), and very late activation antigen 4 (VLA-4). Neutrophils were identified with a MAb recognizing neutrophil elastase. Endothelial cells were identified with the lectin ulex europaeus.**

Results. **In early lesions, expression of PECAM-1, ICAM-1, ICAM-2, and P-selectin was similar to that in control samples, and VCAM-1 and E-selectin were induced in vascular endothelium. In advanced lesions, immunostaining for adhesion molecules diminished or disappeared in luminal endothelium, whereas these molecules were clearly expressed in microvessels within and surrounding inflamed vessels. Staining in endothelia from vessels in a healing stage tended to be negative. A high proportion of infiltrating leukocytes expressed LFA-1 and VLA-4, and only a minority expressed L-selectin. No relationship between the expression pattern of adhesion molecules and clinical features, disease duration, or previous corticosteroid treatment was observed.**

Conclusion. **Endothelial adhesion molecule expression in PAN is a dynamic process that varies according to the histopathologic stage of the vascular lesions. The preferential expression of constitutive and inducible adhesion molecules in microvessels suggests that angiogenesis contributes to the persistence of inflammatory infiltration in PAN.**

Classic polyarteritis nodosa (PAN) is a systemic necrotizing vasculitis that involves medium-sized and small vessels. PAN can present as a primary disease, but it may occur during the course of hepatitis B and other viral infections (1,2), in association with hairy cell leukemia (3), or complicating the outcome of several autoimmune disorders such as Sjögren's syndrome or rheumatoid arthritis (4). Consequently, the immunopathogenic mechanisms leading to vascular injury are incompletely understood and are probably heterogeneous. Both immune complex–mediated vessel damage and T cell–mediated vascular injury appear to participate in the development of PAN lesions (5–8).

In recent years, the relevance of leukocyte–endothelial cell–extracellular matrix interactions in the development of inflammatory lesions has been increasingly recognized. Such interactions occur through a complex array of cell surface adhesion molecules. On the leukocyte membrane, molecules of the selectin (L-selectin) and integrin (CD18–CD11 complex, very late activation antigen 4 [VLA-4], $\alpha_v\beta_3$) families interact in a precisely regulated manner with specific counterrecep-

Supported in part by a grant from Fondo de Investigación Sanitaria (FIS 96/0347) and by a grant from Televisió de Catalunya (Marató TV3 95/3009). Dr. Coll-Vinent is the recipient of a research award from Hospital Clínic de Barcelona. Dr. Cebrián's work was supported by a grant from Ministerio de Educación y Ciencia.

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Submitted for publication June 10, 1997; accepted in revised form October 24, 1997.

tors on the endothelial cell surface. These include carbohydrates (sialylated forms of Lewis antigens) and members of the selectin (E-selectin, P-selectin) and immunoglobulin (intercellular adhesion molecule 1 [ICAM-1], ICAM-2, vascular cell adhesion molecule 1 [VCAM-1], and platelet endothelial cell adhesion molecule 1 [PECAM-1]) families. These receptors are induced, increased, or functionally regulated by cytokines, chemokines, steroid hormones, and by other cell–cell interplay. Such interactions have been demonstrated to be essential for the recruitment of leukocytes at the sites of injury (9–12).

Immunohistochemical studies have demonstrated that endothelial cell adhesion molecules are induced or up-regulated in a variety of chronic inflammatory diseases (9,13,14), and different patterns of adhesion molecule expression have been identified. E-selectin is usually expressed in acute inflammatory lesions (15,16). In addition, E-selectin is generally present in chronic inflammatory disorders involving the skin, where it binds to skin-homing T lymphocytes. Its detection is less frequent in chronic inflammatory conditions involving other tissues (17–19), where VCAM-1 or up-regulated ICAM-1 are more likely to be detected.

Experimental studies have shown that most of the primary immunopathogenic events that may participate in the pathogenesis of vasculitis, such as immune complex deposition, complement activation, antineutrophil cytoplasmic antibody expression, and T lymphocyte and macrophage activation, influence adhesion molecule expression or function (5). However, little is known about adhesion molecule expression in vasculitic lesions. Most of the previously published studies have addressed only a limited number of adhesion molecules or have included only small numbers of patients with a variety of vasculitides and heterogeneous characteristics (20–25). Preliminary studies have shown that E-selectin, ICAM-1, and VCAM-1 are up-regulated in endothelial cells from inflamed vessels. Studies of changes in adhesion molecule expression in relation to disease progression have not been reported. The present study was undertaken to investigate the expression of endothelial cell adhesion molecules and their leukocyte counterreceptors in muscle and nerve biopsy specimens from a highly homogeneous group of patients with classic PAN, in order to identify receptors potentially involved in the development of vascular inflammatory infiltration.

PATIENTS AND METHODS

Patients. The initial study group consisted of 46 patients in whom a muscle and/or sural nerve biopsy showed vasculitis in medium-sized or small vessels. All of these patients fulfilled the American College of Rheumatology classification criteria for PAN (26). Four patients were excluded because of suspicion of microscopic polyangiitis based on clinical or biologic findings, according to the specifications of the Chapel Hill International Consensus Conference (27). Three were excluded because they had an associated connective tissue disease (systemic lupus erythematosus). Nine additional patients could not be included because, given the segmentary nature of PAN lesions, subsequent biopsy specimens did not exhibit inflamed vessels. The final study group consisted of 30 patients (13 women and 17 men), with a mean age of 66 years (range 23–83 years). Hepatitis B surface antigen was tested in 25 patients and was negative in all of them. Clinical data retrieved included age and sex, number and type of disease manifestations, previous treatment with corticosteroids, disease duration, and the presence of a strong inflammatory response, defined as fever >38°C, weight loss >4 kg, and erythrocyte sedimentation rate >90 mm/hour (Table 1).

Table 1. Clinical features of the polyarteritis nodosa patients studied*

Age, mean (range) years	66 (23–83)
Male/female	17/13
Duration of symptoms prior to biopsy, mean (range)	4 months (2 weeks–2 years)
Previous treatment with corticosteroids	7
Fever >38°C	17
Weight loss >4 kg	15
Anemia (hemoglobin <10 gm/liter)	14
ESR >90 mm/hour	16
Hypertension	3
Arthralgia/arthritis	8
Cutaneous manifestations	10
Livedo reticularis	2
Other	7
Abdominal pain	1
Muscular involvement	14
Clinical	12
Electromyographic	5
Peripheral nerve involvement	23
Clinical	18
Electromyographic	17

* Except for age and symptom duration, values are the number of patients. ESR = erythrocyte sedimentation rate.

The mean disease duration prior to biopsy was 4 months (range 2 weeks–2 years). At the time of biopsy, all patients had clinical evidence of disease activity. Twenty-three patients were untreated, and 7 had been receiving prednisone at 10–60 mg/day, because of clinical suspicion of giant cell arteritis (2 patients), systemic vasculitis (2 patients), late-onset rheumatoid arthritis (2 patients), or dermatomyositis (1 patient). These patients had been treated for a period ranging from 1 week to 4 months.

The control group consisted of 5 women and 7 men (mean age 63 years, range 47–85 years), who underwent muscle and/or nerve biopsy for diagnostic purposes and had no vasculitis. Diagnoses in these patients were noninflammatory myopathy (n = 4), chronic alcoholism (n = 2), cutaneous sarcoidosis (n = 1), diabetic neuropathy (n = 1), lead poisoning (n = 1), olivopontocerebellar degeneration (n = 1), and

Table 2. Monoclonal antibodies used in this study*

Antigen*	CD designation	Clone†	Distribution (constitutive or induced)‡	Biologic function	Working concentration (μg/ml)
ICAM-1	CD54	RM3A5	EC, L, F, EpC, DC	Firm adhesion, transmigration	§
ICAM-2	CD102	CBR-IC2/2	EC (other cells infrequent)	Firm adhesion, transmigration	1
ICAM-3	CD50	152ID2	L	Stimulation of integrin function	§
VCAM-1	CD106	BBIG-V1	EC (other cells infrequent)	Firm adhesion, transmigration	5
E-selection	CD62E	1.2B6	EC	Rolling, integrin activation	4
L-selectin	CD62L	FMC46	L	Rolling	5
P-selectin	CD62P	AK6	EC, Pl	Rolling, integrin activation	0.5
β_2 integrin chain	CD18	MEM-48	L	Firm adhesion	§
LFA-1	CD11a	B-B15	L	Firm adhesion, transmigration	10
α_4 integrin	CD49d	HP2/1	Ly, M, Eo	Firm adhesion, transmigration	8
PECAM-1	CD31	JC70A	EC, L, Pl	Integrin activation, firm adhesion, transmigration	2.6

* ICAM-1 = intercellular adhesion molecule 1; VCAM-1 = vascular cell adhesion molecule 1; LFA-1 = lymphocyte function–associated antigen 1; PECAM-1 = platelet endothelial cell adhesion molecule 1.
† RM3A5 was kindly provided by Dr. Teresa Gallart (Immunology Department, Hospital Clínic, Barcelona); CBR-IC2/2 by Bender MedSystems (Vienna, Austria); 152ID2 and MEM-48 by Dr. Ramon Vilella (Immunology Department, Hospital Clínic); BBIG-V1 by R & D Systems Europe (Abingdon, UK); 1.2B6, FMC46, AK6, B-B15, and HP2/1 by Labgen (Barcelona); and JC70A by Dako (Carpinteria, CA).
‡ EC = endothelial cell; L = leukocyte; F = fibroblast; EpC = epithelial cell; DC = dendritic cell; Pl = platelet; Ly = lymphocyte; M = monocyte; Eo = eosinophil.
§ Used as crude hybridoma supernatant at a 1:2–1:5 dilution.

polymyalgia rheumatica (n = 1). One patient had a self-limited disease without a definite diagnosis.

Tissue samples. Twenty-one muscle and 16 sural nerve biopsy specimens were obtained from the PAN patients, and 12 histologically normal muscle and 2 histologically normal nerve specimens were obtained from the controls. Tissues were snap-frozen in isopentane that had been precooled in liquid nitrogen, and stored at −80°C. All PAN specimens exhibited a transmural infiltrate in at least 1 medium-sized or small vessel.

Antibodies. Endothelial cells were identified with the lectin ulex europaeus (Vector, Burlingame, CA), which binds fucose on endothelial cells and is considered to be an endothelial cell marker (28). Neutrophils were identified with a monoclonal antibody (MAb) recognizing neutrophil elastase (clone NP57; Dako, Carpinteria, CA). MAb used to detect adhesion molecules are shown in Table 2.

Immunostaining. Serial 4–8-μm frozen sections were fixed with cold acetone and incubated at room temperature with each primary antibody for 30 minutes. MAb immunoreactivity was detected using alkaline phosphatase–anti–alkaline phosphatase (Dako) (29). The immunoreactivity of biotinylated ulex europaeus and neutrophilic elastase was detected using avidin–biotin–peroxidase complex (Vector) (30). All sections were counterstained with hematoxylin.

Quantitative analysis. The stained sections were examined under a light microscope by 3 independent investigators (BC-V, JMG, and MCC) who were blinded to the clinical information. Staining intensity on endothelial cells was classified semiquantitatively on a 3-point scale (− = no immunoreactivity; + = moderate immunoreactivity; ++ = strong immunoreactivity). The endothelial staining pattern was classified qualitatively as sharp, blurry, or absent. In inflammatory infiltrates, the number of positively stained cells was expressed as a percentage of the total number of infiltrating cells visualized by hematoxylin counterstaining. Scores assigned by the 3 investigators varied by <10%.

Statistical analysis. Chi-square testing was used for statistical analysis.

Table 3. Adhesion molecule expression by endothelial cells*

Antigen	Control specimens, E	Early PAN lesions		Well-established PAN lesions		Healed PAN lesions	
		E	MV	E	MV	E	MV
Ulex europaeus	++	++	+/−	+	++	+/−	++
ICAM-1	++/+	++/+	−	+/−	+	+/−	+
ICAM-2	++/+	++	−	+	+/++	+/−	+/++
VCAM-1	−	+/−	−	+/−	+/−	−	−
PECAM-1	++	++	−	+/−	+	+/−	+
E-selectin	−	+/++	+/−	+/−	+/−	−	−
P-selectin	++/−	++	−	+/−	++	−	++
L-selectin	−	+/−	−	+/−	−	−	−

* In cases where the intensity varied among different samples, 2 values are given. See Patients and Methods for details on scoring. E = luminal endothelium; PAN = polyarteritis nodosa; MV = microvessels. See Table 2 for other definitions.

RESULTS

Endothelial expression of adhesion molecules. In control specimens, ICAM-1, ICAM-2, and PECAM-1 were universally expressed by vessels of any size.

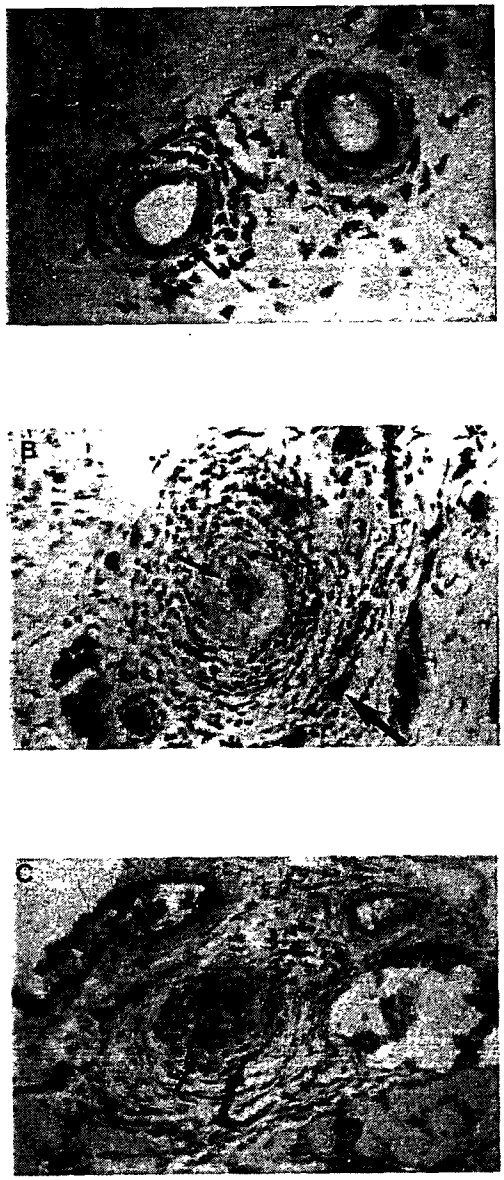

Figure 1. Ulex europaeus immunostaining pattern in polyarteritis nodosa lesions at different stages. **A**, Early lesion. The lumen is preserved and the endothelium is well defined (**arrow**) (similar to findings in an adjacent normal vessel). **B**, Well-established lesion. The luminal endothelium (**small arrow**) is slightly stained and blurry compared with normal vessels, and microvessels are strongly immunostained (**large arrow**). **C**, Healed lesion. The luminal endothelium is substituted by microvessels (**arrow**) and the vessel wall is thick, with scarce cellularity. Microvessels within and surrounding the vessel are well defined. (Original magnification × 400.)

P-selectin was positive in virtually all small and medium-sized vessels, but not in capillaries. VCAM-1, E-selectin, and L-selectin were negative (Table 3).

PAN specimens exhibited the classically described (31) inflamed vessels at different histologic stages (early lesions, with a slight inflammatory infiltrate [80–120 leukocytes per section], well-established lesions, with a strong inflammatory infiltrate [>400 leukocytes per section] and frequent fibrinoid necrosis, and healed [obsolescent] lesions, with scarce remaining inflammatory cells [<150 leukocytes per section] and prominent intimal hyperplasia obliterating the vessel lumen). In 26% of the samples studied, vessels at different histologic stages coexisted in the same section.

Staining with ulex europaeus lectin demonstrated dynamic participation of endothelial cells throughout the histologic stages of the vasculitic process. As shown in Figure 1, the luminal endothelium was morphologically preserved and microvascularization was minimal or absent in early lesions. In well-established lesions, the luminal endothelium appeared blurry or incomplete, the lumen was partially or totally occluded, and microvessels within and surrounding the inflammatory infiltrate were more evident, being the microvessels always more clearly defined and intense than the luminal endothelium. In healed (obsolescent) lesions, the luminal endothelium was negatively or only weakly stained, and the lumen was occluded and sometimes even substituted by microvessels, which were also evident within and surrounding the lesion. No microvessels were seen within or surrounding normal medium-sized or small vessels. Endothelial cell adhesion molecule expression by involved vessels evolved through the above-described stages in a very well-defined pattern (Table 3), and no relationship with other variables, such as disease duration, inflammatory response (as defined in Patients and Methods), tissue source (muscle versus nerve), or corticosteroid treatment was observed.

In early lesions, immunostaining of luminal ICAM-1, ICAM-2, PECAM-1, and P-selectin was similar to that seen in control samples. VCAM-1 was moderately expressed in only 2 vessels. E-selectin immunostaining was intense and generally well defined (Figure 2A). L-selectin was also expressed in luminal endothelium of 4 vessels with minimal inflammatory infiltration.

In well-established lesions, luminal ICAM-1, PECAM-1, and P-selectin expression decreased in intensity and definition in comparison with the findings in controls. In many cases (50%, 28%, and 73% for ICAM-1, PECAM-1, and P-selectin, respectively), luminal expression completely disappeared. This usually

occurred in vessels with a completely occluded lumen. In contrast, microvessel immunoreactivity was intense and well defined (Figures 2C and D). The expression pattern of ICAM-2 was similar, but it rarely disappeared completely from the luminal endothelium. VCAM-1 was expressed by the luminal endothelium of 15 vessels (45%) and, except in 1 case with very strong positivity, immunostaining was always moderate and blurry. Virtu-

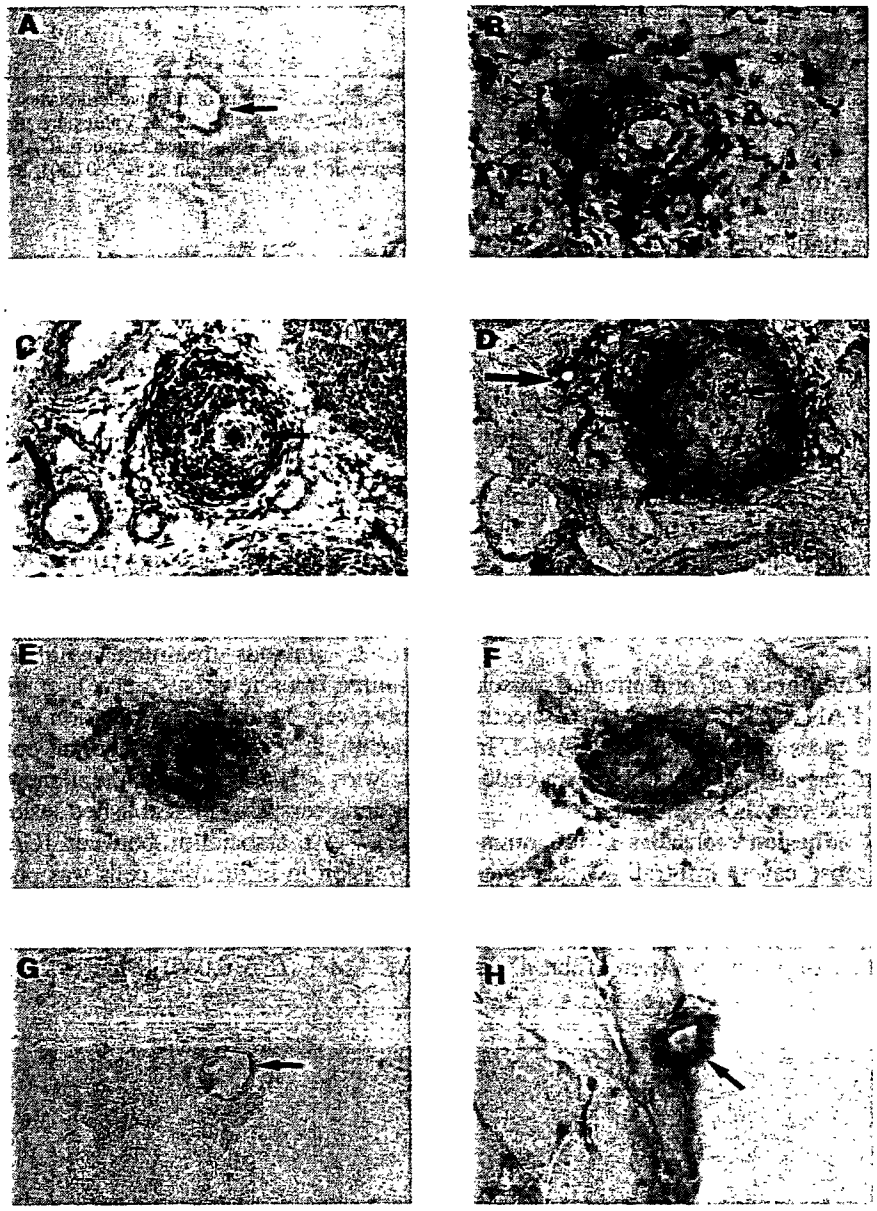

Figure 2. **A,** Endothelial E-selectin immunostaining (**arrow**) in an early polyarteritis nodosa (PAN) lesion, and **B,** corresponding cellular immunostaining for neutrophil elastase (serial sections). **C,** Intercellular adhesion molecule 1 (ICAM-1) immunostaining in a well-established lesion. Note that the high ICAM-1 expression on the luminal endothelium of an adjacent normal vessel (**large arrow**) is absent in the inflamed vessel (**small arrow**). Infiltrating leukocytes express ICAM-1. **D,** P-selectin immunostaining in a more highly magnified image of the same vessel seen in **C.** Note the negative staining in the obliterated luminal endothelium (**small arrow**), while the surrounding microvessels are clearly positive (**large arrow**). **E,** Endothelial expression of vascular cell adhesion molecule 1, and **F,** corresponding leukocyte very late activation antigen 4 immunostaining (serial sections). **G,** Expression of E-selectin and **H,** expression of L-selectin in the luminal endothelium of normal vessels (**arrows**) of PAN patients; such expression was observed in normal vessels of some, but not all, patients. (Original magnification × 400 in **A, B,** and **D–H;** × 200 in **C.**)

Table 4. Adhesion molecule expression by infiltrating leukocytes in polyarteritis nodosa patients*

CD18	53 ± 26
LFA-1	40 ± 27
VLA-4	35 ± 29
ICAM-1	34 ± 28
ICAM-3	35 ± 31

* Values are the mean ± SD %. VLA-4 = very late activation antigen 4. See Table 2 for other definitions.

Table 5. Association between the expression of leukocyte ICAM-1 and the expression of leukocyte LFA-1 in polyarteritis nodosa lesions*

	Leukocyte LFA-1			
ICAM-1	1	2	3	4
1	6	2	2	0
2	1	3	3	0
3	1	0	9	0
4	0	0	0	1

* Semiquantification of positive leukocytes: 1 = 0–10%; 2 = 10–40%; 3 = 40–70%; 4 = 70–100%. Values are the number of samples with each score. The association between ICAM-1 expression and LFA-1 expression was significant at $P < 0.0001$. See Table 2 for definitions.

ally all vessels positive for VCAM-1, even those with a large amount of inflammatory infiltration, had a still-preserved or only partially occluded lumen. VCAM-1 immunostaining in microvessels was rare (Figure 2E). E-selectin expression by the luminal endothelium was light and blurry or negative. Vessels positive for E-selectin also had a better-preserved lumen. In 6 inflamed vessels from different patients, E-selectin was positive in microvessels. In 2 vessels from a single sample, luminal endothelium expressed L-selectin.

In healed lesions, luminal endothelium stained negatively or very weakly for all constitutive and inducible endothelial adhesion molecules. Microvessels expressed ICAM-1, ICAM-2, PECAM-1, and P-selectin, but no immunoreactivity for VCAM-1 or E-selectin was detected.

In 10 cases, endothelia of noninflamed vessels from patients with PAN clearly expressed E-selectin (Figure 2G), and in 3 cases they expressed VCAM-1. In 7 samples, L-selectin was also clearly positive in endothelia from noninflamed vessels (Figure 2H).

Expression of adhesion molecules in inflammatory infiltrates. In most cases, infiltrating leukocytes strongly expressed β_2 (CD18 and lymphocyte function–associated antigen 1 [LFA-1]) and β_1 (VLA-4) integrins, although the percentage of positivity varied (Table 4). In addition, a notable, although variable, percentage of infiltrating cells were positive for the immunoglobulin superfamily members ICAM-1 and ICAM-3 (Table 4). In contrast, immunostaining for VCAM-1 and PECAM-1 was observed in only 9% and 36% of cases, respectively, and in a lower number of infiltrating cells (<10% for VCAM-1 and 5–30% for PECAM-1). CD18 was mainly expressed by central leukocytes, whereas ICAM-3 was clearly more positive on the periphery of the lesion, surrounding the adventitial microvessels when present (Figure 3). No relationship between the expression of any of these molecules and clinical features, previous treatment with corticosteroids, tissue source (muscle versus nerve), or histologic pattern was observed. Leukocyte expression of L-selectin was infrequent, and the percentage of positive cells was low (always <20%) and had a tendency to be higher in early lesions versus well-established lesions.

An association between ICAM-1 and LFA-1 expression in infiltrating cells was observed ($P < 0.0001$) (Table 5), as was an association between leukocyte expression of VLA-4 and endothelial expression of VCAM-1 ($P = 0.046$) (Figures 2E and F and Table 6).

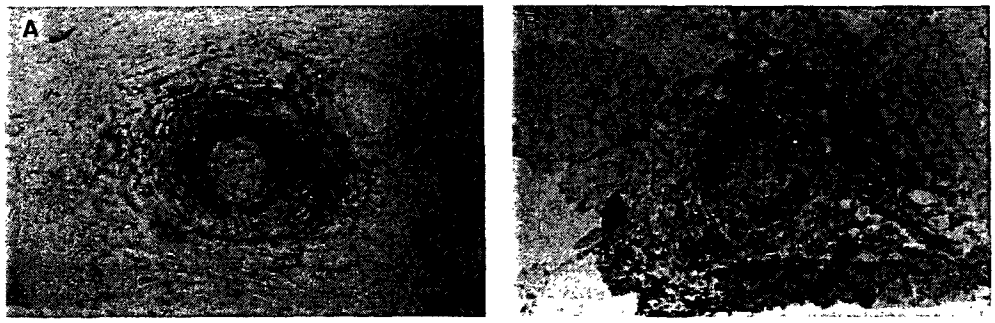

Figure 3. **A**, CD18 and **B**, intercellular adhesion molecule 3 (ICAM-3) immunostaining. Note that while CD18 is strongly expressed in the center of the lesion, ICAM-3 has a peripheral distribution. (Original magnification × 200.)

Table 6. Association between the expression of endothelial VCAM-1 and the expression of leukocyte VLA-4 in polyarteritis nodosa lesions*

	Cellular VLA-4			
VCAM-1	1	2	3	4
0	8	3	3	1
1	1	6	6	1
2	0	1	0	1

* Semiquantification of positive leukocytes: 1 = 0–10%; 2 = 10–40%; 3 = 40–70%; 4 = 70–100%. Semiquantification of endothelial immunostaining: 0 = −; 1 = +; 2 = ++. Values are the number of samples with each score. The association between vascular cell adhesion molecule 1 (VCAM-1) expression and very late activation antigen 4 (VLA-4) expression was significant at $P = 0.046$.

The presence of neutrophils, identified by neutrophil elastase, was variable, and correlated significantly with endothelial expression of E-selectin ($P = 0.009$) (Figures 2A and B and Table 7).

DISCUSSION

Little is known about adhesion molecule expression in systemic vasculitis. Previously published studies addressed only a limited number of adhesion molecules (mostly E-selectin, the immunoglobulins ICAM-1 and VCAM-1, and the integrins LFA-1 and VLA-4) in small and heterogeneous populations (13,20–25). Moreover, changes in the pattern of adhesion molecule expression in relation to disease duration and/or activity have been investigated only in cutaneous vasculitis (16). Wawryck et al observed strong ICAM-1 and LFA-1 expression in infiltrating leukocytes in 11 patients with giant cell arteritis (20). Endothelial cells also expressed ICAM-1, but the level of expression was not significantly different from that in controls. Panegyres et al demonstrated up-regulated ICAM-1 expression on endothelial cells in luminal endothelium of muscle and nerve samples from a heterogeneous group of patients with different vasculitides (21).

In the present study, we have shown that adhesion molecule expression followed a dynamic pattern throughout the previously defined histologic stages of PAN lesions. The expression of constitutive adhesion molecules, i.e., ICAM-1, ICAM-2, PECAM-1, and P-selectin, remained preserved in the luminal endothelium of early PAN lesions. In contrast, in well-established lesions, the intensity and definition of the immunostaining decreased in the luminal endothelium, but it was sharp and intense in microvessels within and surrounding the inflammatory infiltrates. In healed lesions, the luminal endothelial staining tended to become negative, and adhesion molecule expression decreased in microvessels. Since a similar pattern was observed with the endothelial cell marker ulex europaeus, we conclude that luminal endothelium becomes damaged throughout the vasculitic process and eventually disappears. The observation that microvessels were virtually absent from normal medium-sized and small vessels of PAN patients and were mostly present in well-established and healed (obsolescent) lesions indicates that microvessels present in PAN infiltrates are probably neovessels derived from inflammation-induced angiogenesis.

To our knowledge, there are no previous reports on the distribution of ICAM-2, PECAM-1, and P-selectin in inflammatory diseases and vasculitis. Focal absence of PECAM-1, which is constitutively expressed by healthy renal endothelial cells, has been observed in patients with renal allograft rejection, probably also reflecting endothelial injury (32).

E-selectin was expressed in luminal endothelium of early lesions as well as in endothelium of noninflamed vessels of PAN patients, but not in advanced lesions or in control specimens. These findings may reflect the transient nature of E-selectin expression, as demonstrated in cell culture systems (33). Therefore, the absence of staining does not necessarily exclude the possibility of an important role of this molecule in the earlier stages of vascular inflammation. Our observations support the hypothesis that E-selectin is expressed at the beginning of the inflammatory process, even in well-preserved vessels without inflammatory infiltrates, and subsequently disappears. Functional changes thus precede morphologic evidence of inflammation and endothelial damage. The notion of E-selectin involvement in the initial steps of the inflammatory process is also supported by the fact that there is a significant

Table 7. Association between the expression of endothelial E-selectin and the expression of neutrophils (neutrophil elastase) in polyarteritis nodosa lesions*

	Neutrophil elastase		
E-selectin	1	2	3
0	15	1	0
1	3	1	3
2	3	3	1

* Semiquantification of endothelial immunostaining: 0 = −; 1 = +; 2 = ++. Semiquantification of neutrophilic elastase–positive cells: 1 = 0–5%; 2 = 10–30%; 3 = >30%. Values are the number of samples with each score. The association between E-selectin expression and neutrophilic elastase expression was significant at $P = 0.009$.

association between the expression of this adhesion molecule and the presence of neutrophils in inflammatory infiltrates. This association was previously observed by Sais et al in patients with cutaneous leukocytoclastic vasculitis (16) and by Bradley et al in skin biopsy samples from a heterogeneous group of patients with cutaneous and systemic vasculitis (25). Our earlier observation that soluble E-selectin levels are elevated in PAN patients irrespective of disease duration or immunosuppressive drug treatment (34) may be due to the coexistence of lesions at different stages of evolution, which occurs almost invariably in this disease.

VCAM-1 was also expressed in endothelial cells of PAN lesions. Its expression varied among patients and even among different lesions in the same sample. It was mostly expressed in well-established lesions with a relatively well-preserved lumen. We also found a significant association between endothelial VCAM-1 immunostaining and leukocyte expression of α_4, the common α chain of VLA-4 and $\alpha_4\beta_7$ integrins, which act as VCAM-1 receptors.

In spite of the fact that regulation of adhesion molecule expression may vary among different vascular beds, we found similar endothelial immunostaining between muscle and nerve samples. Overall, our findings suggest that, except in early phases, luminal endothelium is unlikely to participate in leukocyte adhesion in PAN. Conversely, the remarkable expression of adhesion molecules by microvessels suggests that angiogenesis has an important role in leukocyte recruitment, and therefore in sustaining inflammation in PAN lesions.

A correlation between LFA-1 and ICAM-1 levels in infiltrating leukocytes was observed, suggesting that homotypic interactions may contribute to the consolidation of inflammatory infiltrates. Although virtually all circulating leukocytes express CD18, the common β chain of β_2 integrins, surprisingly, ~40% of infiltrating leukocytes were CD18 negative. We have observed a similar phenomenon in polymyositis and dermatomyositis infiltrates (19). Down-regulation of integrins in leukocytes after completion of the transmigration process might account for this observation, but, to our knowledge, this phenomenon has not been tested in functional studies. Alternatively, since leukocyte integrins undergo conformational changes upon activation, it may be possible that the CD18 MAb used in the present study failed to detect activation-related epitopes.

While CD18 was expressed mainly in the center of the lesion, ICAM-3 predominated at the periphery of vascular infiltrates, where macrophages are the main cell population (8), and surrounded microvessels. We have recently shown that signaling through ICAM-3 activates lymphocyte integrins and stimulates lymphocyte adhesion to endothelial cells (35). Strong ICAM-3 expression by leukocytes surrounding microvessels may then facilitate leukocyte adhesion and transmigration. It has been shown that ICAM-3 is shed from the lymphocyte membrane upon activation (36). Therefore, lymphocytes penetrating deeper in the vessel wall may have shed ICAM-3 from their surface. Recently, ICAM-3 expression by tumor-associated angiogenesis has been reported (37,38). However, we were not able to demonstrate endothelial ICAM-3 expression by luminal endothelia or by microvessels in PAN lesions. Endothelial ICAM-3 expression has only rarely been seen in angiogenesis associated with other chronic inflammatory diseases, such as rheumatoid arthritis (38).

L-selectin was expressed in only a small percentage of infiltrating leukocytes in early lesions. This molecule is expressed by most leukocytes but no other cell types, and is shed from the cell membrane upon leukocyte activation (39,40). This may explain the lack of L-selectin immunostaining of most tissue-infiltrating leukocytes in PAN lesions. Although increased levels of circulating L-selectin would then be expected in patients with PAN, in a previous study we observed diminished serum L-selectin levels in these patients (34). To explain this finding, it has been postulated that circulating L-selectin would bind to activated endothelial cells (41). Interestingly, we have now found, in some samples, positive immunostaining for L-selectin in endothelial cells from patients with PAN, even in noninflamed vessels.

Corticosteroid therapy may influence adhesion molecule expression or function in chronic inflammatory diseases. It is now well established that adhesion molecule expression is regulated by the transcription factor nuclear factor κB (NF-κB). NF-κB–activated transcription of genes involved in the inflammatory response is blocked by corticosteroids at different levels (42). Recently, in in vitro studies, Cronstein et al demonstrated that, indeed, corticosteroids decrease endothelial ICAM-1 and E-selectin expression induced by lipopolysaccharide or interleukin-1 (43). In addition, Youssef et al have observed decreased expression of E-selectin and ICAM-1 in synovium of patients with rheumatoid arthritis following treatment with high-dose methylprednisolone (44). After several months of low-dose corticosteroid treatment, adhesion molecules are down-regulated in synovial samples from polymyalgia rheumatica patients (45).

We did not observe any differences in adhesion

molecule expression between treated and untreated patients. However, our treated patients had received only moderate doses of corticosteroids, or high doses for only a short period of time, and at the time biopsies were performed all had clinical evidence of disease activity. Although, as we showed in a previous study (8), corticosteroid treatment at a similar dosage was able to down-regulate interleukin-2 receptor expression and decrease lymphocyte proliferation, it was probably not sufficient to down-regulate adhesion molecule expression. Interestingly, in PAN patients followed up longitudinally, circulating ICAM-1 and E-selectin levels remained elevated in spite of an appropriate clinical response to corticosteroid and immunosuppressive drug therapy (34). The persistence of inflammatory lesions despite treatment has also been observed in other vasculitides and suggests that, while some components of the disease are highly responsive to treatment, others are more refractory and probably contribute to subsequent relapses (46–50).

Monoclonal antibodies that block functional epitopes of adhesion molecules have efficiently prevented the development of inflammatory lesions and clinically apparent disease in animal models of several autoimmune disorders (51–55), and some of these antibodies have been investigated in phase I/II clinical trials (13,56,57). Although further studies are required, our results suggest that PAN might also be considered among the autoimmune diseases that could potentially benefit from anti-adhesion therapy.

ACKNOWLEDGMENTS

We thank Dr. Alex de la Sierra for advice on statistical analysis and Eva Sánchez for technical assistance.

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Síntesi dels resultats

1 - L'endoteli de les lesions vasculars dels pacients amb PAN clàssica sofreix modificacions al llarg del procés evolutiu d'aquestes: en les lesions incipients roman morfològicament preservat; en les lesions establertes l'endoteli luminal es destrueix parcialment o totalment i apareix microvascularització a l'entorn o enmig de l'infiltrat inflamatori; i en les lesions cicatricials, l'endoteli luminal desapareix i disminueix la microvasculatura neoformada.

2 - La intensitat de l'expressió de les molècules d'adhesió endotelials es correlaciona amb l'estadi histològic de la lesió vascular. Atès que en la PAN coexisteixen lesions en diferents estadis evolutius, l'expressió d'aquestes molècules pot variar fins i tot entre diferents lesions en un mateix pacient.

3 - Les molècules d'adhesió endotelials selectina-E i VCAM-1 són induïdes en l'endoteli luminal de lesions incipients de malalts amb PAN clàssica i en els vasos neoformats de les lesions establertes.

4 - L'expressió de les molècules d'adhesió constitutives de l'endoteli ICAM-1, ICAM-2, PECAM-1 i selectina-P disminueix en l'endoteli luminal de les lesions inflamatòries establertes i apareix en la neovascularització d'aquestes lesions. En les lesions cicatricials, també disminueix la seva expressió en els vasos neoformats.

5 - L'expressió endotelial de selectina-E es correlaciona amb el nombre de neutròfils presents en l'infiltrat inflamatori. L'expressió endotelial de VCAM-1 es correlaciona amb l'expressió leucocitària de VLA-4, i l'expressió leucocitària d'ICAM-1 es correlaciona amb l'expressió leucocitària de LFA-1.

6 - No s'observa cap correlació entre l'expressió de les molècules d'adhesió estudiades i les característiques clíniques següents: edat, sexe, temps d'evolució de la malaltia i resposta inflamatòria sistèmica (determinada per febre, pèrdua de pes i velocitat de sedimentació globular elevada).

7. El tractament amb corticoids a dosis baixes o durant un període curt de temps no modifica l'expressió de les molècules d'adhesió en les lesions vasculars de la PAN.

2. CIRCULATING SOLUBLE ADHESION MOLECULES IN PATIENTS WITH CLASSICAL POLYARTERITIS NODOSA

Molècules d'adhesió solubles circulants en pacients amb poliarteritis nudosa clàssica

Blanca Coll-Vinent, Josep M Grau, Alfons López-Soto, Joaquim Oristrell, Carme Font, Xavier Bosch, Eduard Mirapeix, Álvaro Urbano-Márquez, Maria C Cid

British Journal of Rheumatology 1997:36:1178-1183
(pàgines 57-62)

Donat que en el treball anteriorment presentat hem demostrat una participació activa de l'endoteli vascular en el desenvolupament dels infiltrats vasculars en la PAN, el pas següent ha estat investigar si aquesta participació es reflecteix en els nivells de les molècules d'adhesió solubles circulants en pacients afectes de la mateixa malaltia i si la instauració del tractament i la subsegüent remissió clínica tenen alguna traducció en canvis en els nivells circulants de les mateixes molècules.

Amb aquesta finalitat hem aplicat la tècnica immunohistoquímica d'ELISA (*enzyme-linked immunosorbent assay*) amb anticossos monoclonals específics per les selectines E, L i P i les immunoglobulines ICAM-1 i VCAM-1 en sèrums i plasmes de malalts amb PAN en activitat comparant-los amb controls sans, i posteriorment hem fet un seguiment en un subgrup d'aquests malalts.

British Journal of Rheumatology 1997;**36**:1178–1183

CIRCULATING SOLUBLE ADHESION MOLECULES IN PATIENTS WITH CLASSICAL POLYARTERITIS NODOSA

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SUMMARY

The objective was to evaluate whether changes in circulating soluble adhesion molecule levels reflect disease activity in patients with systemic polyarteritis nodosa (PAN). A sandwich ELISA was used to measure soluble (s) intercellular adhesion molecule 1 (sICAM-1), vascular cell adhesion molecule 1 (sVCAM-1), E-selectin, L-selectin and P-selectin in sera and plasma from 22 patients with active PAN, in sera from 13 of these patients taken serially during follow-up, and in sera from 13 healthy controls. At the time of diagnosis, sICAM-1, sVCAM-1 and sE-selectin levels (488.5 ± 201.3, 1176.5 ± 514.1 and 60.6 ± 27 ng/ml, respectively) were significantly higher in patients than in controls ($P < 0.0001$, $P = 0.001$ and $P = 0.003$, respectively). In contrast, sL-selectin levels tended to be lower in patients than in controls. Within the first 7 days after starting treatment, there was a significant increase in sICAM-1 concentrations, which fell thereafter, but did not completely reach normal levels when patients achieved clinical remission. sE-selectin also remained elevated during follow-up. Only sVCAM-1 decreased, tending to reach normal values in inactive disease. Increased levels of sICAM-1, sVCAM-1 and sE-selectin, and decreased levels of sL-selectin, in active PAN suggest immune and endothelial stimulation during disease activity. Abnormal levels of soluble adhesion molecules in clinically inactive patients might reflect persistence of immune activation and/or endothelial cell exposure to a remaining inflammatory microenvironment.

KEY WORDS: Polyarteritis nodosa, Adhesion molecules, Inflammation.

LONG-TERM follow-up of patients with systemic vasculitis has demonstrated that while immunosuppressive therapy has been life saving for most patients, it fails to induce a sustained remission in a remarkable proportion of individuals [1–3]. Subsequent relapses increase therapeutic requirements and, consequently, treatment-related morbidity and mortality. Clinical or laboratory findings identifying patients at high risk of relapse have not been recognized. In addition, our understanding of how immunosuppressive therapy influences vascular inflammation, injury and repair is still very limited, and accurate parameters discriminating persistent subclinical inflammatory activity from true remission have not been identified.

Over the past few years, cell–cell interactions critically involved in the development of inflammatory lesions have been discovered. Regardless of the primary immunopathogenic mechanisms, the development of vascular inflammatory infiltrates requires dynamic interactions between leucocyte surface receptors and their ligands on the endothelial cell surface. In order to infiltrate tissues, circulating leucocytes roll over the endothelial cell membrane through interactions between carbohydrates and selectins (L-selectin on leucocytes, P-selectin and E-selectin on the endothelial cells). Additional stimuli (chemokines and other co-stimulatory signals) activate leucocyte integrins, which bind tightly to their endothelial counter-receptors of the immunoglobulin superfamily: intercellular adhesion molecule 1 (ICAM-1), ICAM-2 and vascular cell adhesion molecule 1 (VCAM-1). These interactions are also involved in leucocyte transmigration through the endothelial layer [4–7].

Circulating forms of selectins and immunoglobulin superfamily members have been detected on human serum and plasma. These molecules are shed from the cell membrane or directly generated as splice variants lacking the transmembrane and cytoplasmic domain. Increased levels of circulating adhesion molecules have been detected in disorders where leucocyte/endothelial cell interactions play a significant role, namely infections, neoplasms and chronic inflammatory diseases [8–12]. *In vitro* studies have shown that these soluble forms appear in the supernatant of activated leucocytes or cytokine-stimulated endothelial cells [8, 13]. For that reason, elevated circulating levels of soluble adhesion molecules have been considered a consequence of endothelial and/or immune activation.

In this study, we measured circulating levels of soluble (s) ICAM-1, VCAM-1, E-selectin, L-selectin and P-selectin in patients with systemic polyarteritis nodosa (PAN), both at the time of diagnosis and during follow-up, in order to evaluate whether changes in soluble adhesion molecule levels may reflect disease activity.

PATIENTS AND METHODS

Patients

Twenty-two patients were included in the study. They all had a muscle and/or nerve biopsy showing

Submitted 18 November 1996; revised version accepted 21 April 1997.

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TABLE I
Clinical features of patients included

	n (%)
Sex (male/female)	11 11 (50/50)
Age (yr: mean, range)	63 (43–77)
Previous treatment (< 1 week)	3 (14)
Clinical manifestations	
General*	20 (91)
Neuromuscular	16 (73)
Other†	5 (23)

*Includes malaise, anorexia, weight loss (> 10% of initial weight) and/or fever.
†Includes abdominal pain, arthralgias and/or livedo reticularis.

vasculitis in medium-sized vessels and fulfilled the ACR classification criteria for PAN [14]. Twenty-one patients were HBsAg negative. The remaining patient was not tested. None of these patients had microscopic polyangiitis as defined by the Chapel Hill International Consensus Conference [15]. Clinical features are summarized in Table I. Serum from 18 patients and plasma from four patients with active PAN were obtained at the time of diagnosis, frozen at −70°C, and stored until analysis. None of these patients had received immunosuppressive therapy for more than 7 days. Sera from 10 and plasma from three of these patients were subsequently obtained at different times during follow-up (Table II). Whenever samples were drawn, the presence of any other disease, such as infection or neoplasia, was excluded. All these patients but one (who was treated with prednisone, 1 mg/kg/day) were or had been treated with prednisone (0.5–1 mg/kg/day) and cyclophosphamide (1–2 mg/kg/day), and followed during a median period of 15 months (range 1–36). None of these patients experienced relapses during the follow-up period. PAN was considered active when patients were clinically symptomatic and evaluated previously or within the first week after starting treatment. We considered a patient to be in remission when he/she fulfilled the following conditions: (a) none of the signs or symptoms present at the time of disgnosis was present anymore; (b) there were no new signs or symptoms attributable to PAN; (c) biological markers of disease activity (erythrocyte sedimentation rate, C-reactive protein and platelet count) were within the normal range. Persistent sequelae due to vasculitic lesions as defined by Bacon *et al.* [16] did not exclude remission. Accordingly, patients in groups 1 and 2 were considered active; patients in group 3 were judged as having low-grade clinical activity; and groups 4 and 5 were considered to be in remission.

TABLE II
Clinical status of the patients at the time when samples were obtained during follow-up

Group	Description
1	Active untreated patients
2	Patients at the beginning of treatment (1–7 days of treatment)
3	Patients with partial clinical remission between the first week and the first 3 months of treatment
4	Patients in remission still receiving immunosuppressive therapy
5	Patients in remission no longer receiving immunosuppressive therapy

All samples were obtained with the previous consent of the patients.

Control serum samples were obtained from 13 healthy volunteers, seven men and six women, aged 43–77 (mean 63.2).

Quantitation of soluble adhesion molecules

sICAM-1, sVCAM-1, sE-selectin, sL-selectin and sP-selectin levels were determined by a sandwich ELISA technique. We used commercially available kits from British Bio-technology Products, Abingdon, for sICAM-1, sVCAM-1 and sE-selectin, and kits from Bender MedSystems, Vienna, Austria, for sL-selectin and sP-selectin. The procedure was performed according to the manufacturer's instructions. Briefly, a horseradish peroxidase-conjugated monoclonal antibody against sICAM-1, sVCAM-1, sE-selectin, sL-selectin or sP-selectin was added to microtitre plates coated with a murine monoclonal IgG antibody recognizing a different epitope of the corresponding molecule. After incubation with patient and control samples or standards in appropriate dilution, the colour reaction was developed with tetramethylbenzidine, and the plates were read on an automated multiscanner at 450 nm wavelength and at 600 nm wavelength to correct for background signal. All measurements were performed in duplicate.

The calculated overall intra-assay coefficient of variation was 5.5% for sL-selectin and 2.8% for sP-selectin (Bender MedSystems). For sE-selectin, sICAM-1 and sVCAM-1, the intra-assay coefficient of variation was 4.8, 4.4 and 5%, respectively (R&D Systems).

The interassay coefficient of variation was 7.6% for sL-selectin, 6% for sP-selectin, 7.4% for sE-selectin, 7.4% for sICAM-1 and 9.2% for sVCAM-1.

Statistical analysis

Data presented are means ± S.D. For comparison between active patients and controls, Student's *t*-test was used. For comparison among groups in the longitudinal study, ANOVA was used. Bonferroni's method was applied to correct for multiple comparisons [17]. *P* values (two-tailed) < 0.05 were considered significant.

RESULTS

Circulating adhesion molecules in the control group

Mean ± S.D. values of control serum samples were as follows: sICAM-1 246.8 ± 65.8 ng/ml, sVCAM-1 717.3 ± 175 ng/ml, sE-selectin 34.1 ± 14.2 ng/ml, sL-selectin 847.9 ± 220.9 ng/ml and sP-selectin 314.8 ± 155.7 ng/ml.

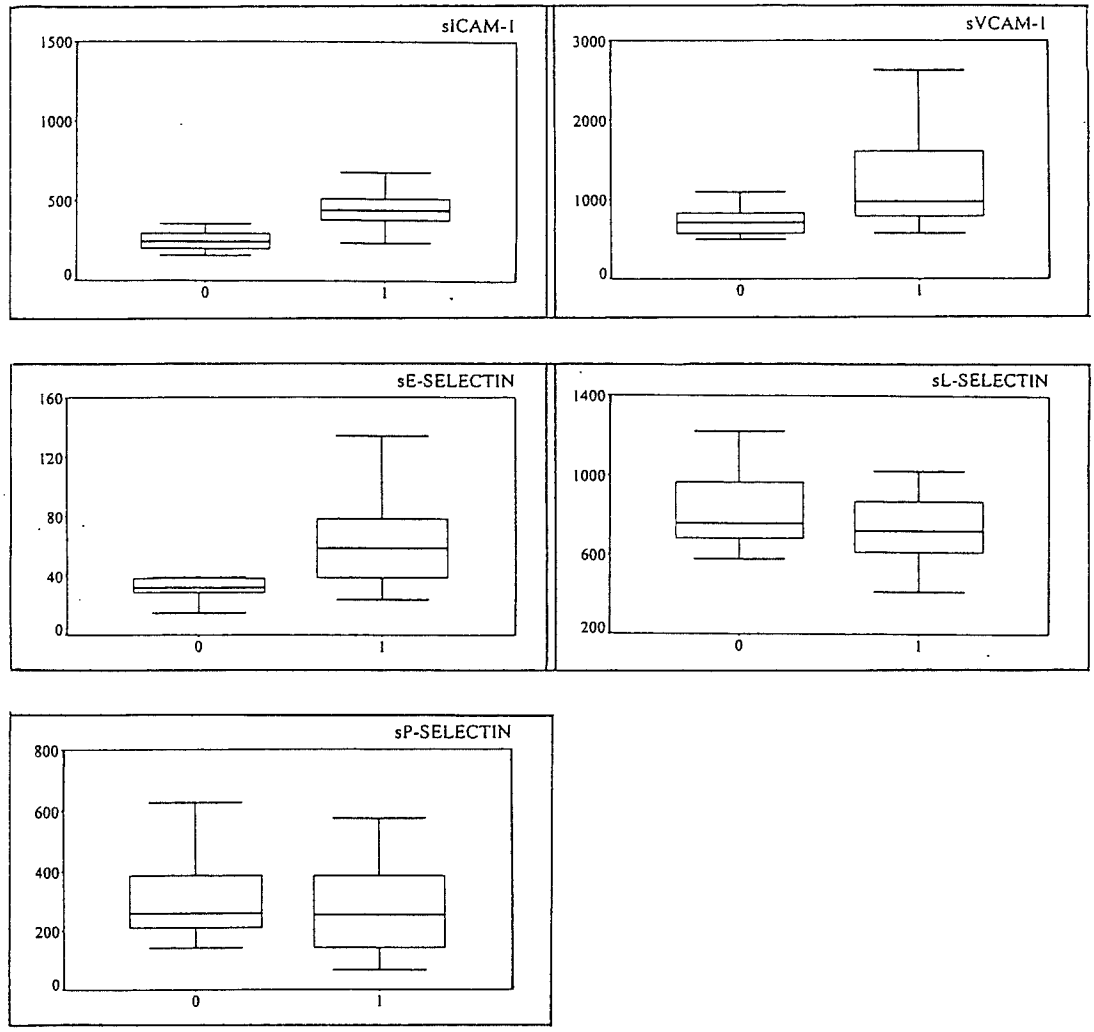

FIG. 1.—Box plots indicating range (error bars), 25–75% interval and median value (horizontal line) of levels of soluble adhesion molecules (sICAM-1, sVCAM-1, sE-selectin, sL-selectin and sP-selectin) in our patients at the time of maximal disease activity (1) and in 13 healthy controls (0).

Soluble adhesion molecule levels in active PAN patients

At the time of maximal disease activity, sICAM-1 (488.5 ± 201.3 ng/ml), sVCAM-1 (1176.5 ± 514.1 ng/ml) and sE-selectin levels (60.6 ± 27 ng/ml) were significantly higher than in controls ($P < 0.0001$, $P = 0.001$ and $P = 0.003$, respectively). sL-selectin levels (743.4 ± 182.5 ng/ml) were lower than in controls, but the difference was not statistically significant ($P = 0.139$). sP-selectin levels (275.4 ± 148.3 ng/ml) were not statistically different from those in controls ($P = 0.461$) (Fig. 1).

No differences were found between serum and plasma levels of sICAM-1, sVCAM-1, sL-selectin and sP-selectin ($P = 0.226$, 0.709, 0.406 and 0.572, respectively). sE-selectin was not tested in plasma samples.

Soluble adhesion molecules during follow-up (Fig. 2)

sICAM-1. At the time of maximal disease activity (group 1), sICAM-1 levels were significantly higher than in controls ($P < 0.0001$). At the beginning of treatment (group 2), a large increase in sICAM-1 was observed ($P = 0.0004$), which decreased thereafter (from group 3 on), although it never reached normal values (differences between group 5 and controls were still statistically significant; $P = 0.011$).

sVCAM-1. In active patients. sVCAM-1 levels were significantly higher than in controls ($P = 0.004$). After the beginning of treatment, there was a trend to a slight increase, and then levels tended to be similar to normal controls. Differences between samples obtained from patients in remission and controls were not statistically significant ($P = 0.536$).

sE-selectin. In active patients, E-selectin levels were higher than in controls and than in all the other groups ($P = 0.005$). Subsequently, they decreased but remained elevated, without reaching normal values ($P < 0.01$).

sL-selectin. In all groups, sL-selectin levels were lower than in controls. Although this difference did not reach statistical significance at the time of

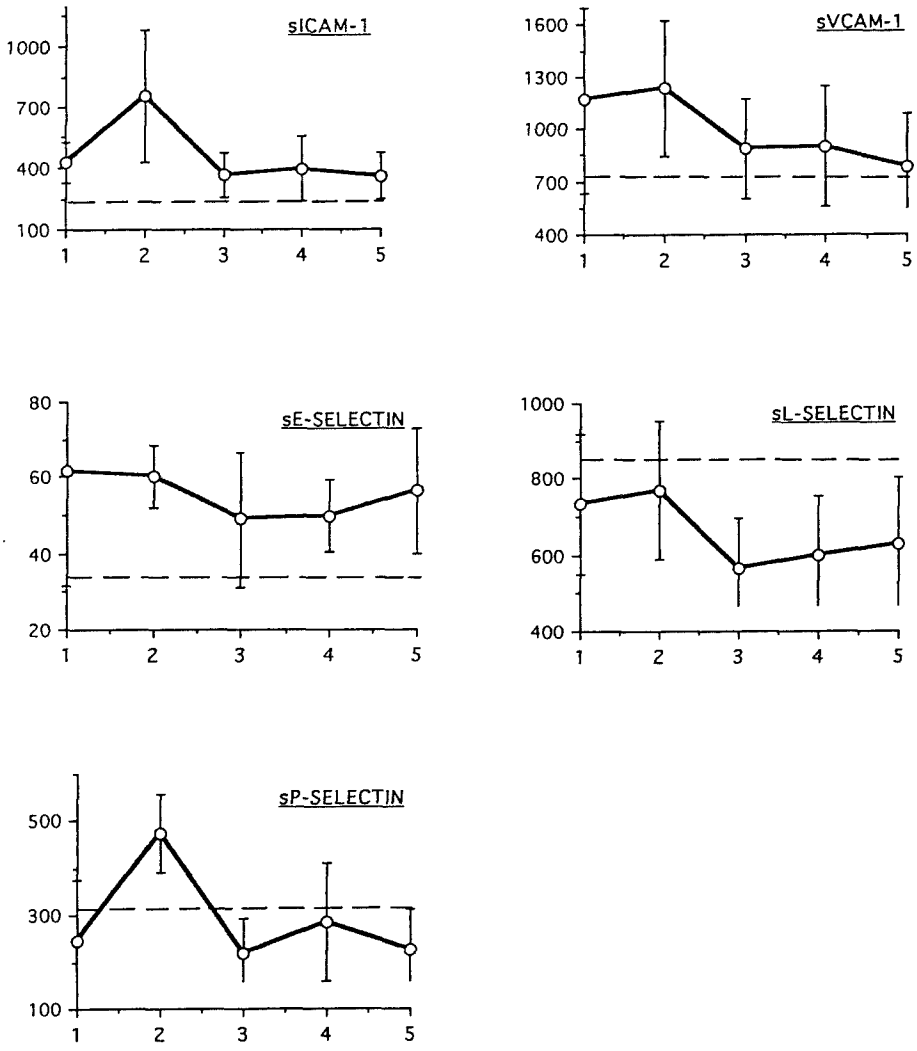

FIG. 2.—Mean and S.D. levels of soluble adhesion molecules (sICAM-1, sVCAM-1, sE-selectin, sL-selectin and sP-selectin) during follow-up. Numbers on the horizontal axis represent the groups described in Table II. The broken line stands for the mean level found in the control group.

maximal disease activity ($P = 0.327$), it became significant when remission began (from group 3 on; $P = 0.01$).

sP-selectin. A significant peak in sP-selectin concentration was observed in patients treated for < 1 week ($P = 0.006$), but none of the other groups differed significantly from controls.

DISCUSSION

Our results show that in patients with active PAN, circulating sICAM-1, sVCAM-1 and sE-selectin levels are elevated compared with those found in healthy matched controls. Given the widespread constitutive endothelial expression of ICAM-1 and its potential induction in a variety of cells under appropriate stimuli, the mechanisms leading to sICAM-1 increase are probably complex and heterogeneous. Elevated sICAM-1 may reflect both immune and endothelial activation [8]. Conversely, VCAM-1 has a more restricted distribution, mainly on the endothelium, and E-selectin is exclusively expressed by endothelial cells. Both VCAM-1 and E-selectin have a low or absent constitutive endothelial expression and are induced by proinflammatory cytokines [4, 18]. Accordingly, it is likely that the major source of these adhesion molecules is activated endothelial cells from inflamed vessels. Increased levels of circulating ICAM-1, VCAM-1 and E-selectin have been detected previously in other vasculitides. Most of these clinical studies, restricted to a single molecule, include miscellaneous patients with different vasculitis syndromes and, therefore, the results are difficult to interpret [9, 10, 19, 20]. Attempts to study the pattern of various circulating adhesion molecules in homogeneous groups of patients have found elevated concentrations of sICAM-1 and sVCAM-1 in Wegener's granulomatosis [11, 12], particularly in patients with extensive organ involvement compared with those with limited disease [11]. Increased levels of sICAM-1 and sE-selectin have also been shown in Kawasaki

disease [21,22], with higher sICAM-1 concentrations in patients with coronary artery lesions [21]. Raised levels of E-selectin were also found in a previous study performed on giant cell arteritis and polyarteritis nodosa patients [23]. While sICAM-1 and sVCAM-1 seem to be elevated in all the vasculitis studied, two independent groups have failed to demonstrate increased sE-selectin levels in Wegener's granulomatosis [9,11], and another one found elevated levels of sICAM-1, but not sE-selectin, in rheumatoid vasculitis [24]. These observations suggest a possible preferential use of particular adhesion pathways in different vasculitis syndromes.

Circulating P-selectin and L-selectin have not been thoroughly studied in vasculitis. In our patients, P-selectin levels were not significantly elevated. *In vitro* studies have shown that although P-selectin synthesis can be enhanced by cytokines [25], its translocation to the cell membrane from the granules where it is stored is a rapid process driven by acute inflammatory mediators [25,26]. Consequently, P-selectin probably plays a role at the very initial steps of leucocyte/endothelial cell interactions and, at the time our patients were diagnosed, they all had well-established lesions of several weeks duration.

Interestingly, circulating L-selectin, which is shed from the cell membrane upon leucocyte activation [25,26], tended to decrease in PAN patients. This apparent paradox has also been observed in Kawasaki disease [27]. Critically ill patients with low sL-selectin levels are at higher risk of developing adult respiratory distress syndrome [28]. It has been postulated that circulating L-selectin binds to diffusely activated endothelium in these conditions.

Curiously, in patients followed longitudinally, a transient increase in sICAM-1 and sP-selectin concentrations was observed during the first few days after starting therapy. This phenomenon might indicate a release of bound soluble adhesion molecules and suggests that treatment might modulate receptor/ligand affinity. Little is known about how corticosteroid or immunosuppressive therapy influences adhesion molecule expression or function. Corticosteroids decrease cytokine production [29] which, in turn, may reduce adhesion molecule production. In addition, in *in vitro* studies, Cronstein *et al.* [30] have demonstrated that corticosteroids directly decrease endothelial ICAM-1 and E-selectin expression induced by lipopolysaccharide or interleukin-1. However, these potentially downregulatory effects of corticosteroids on adhesion molecule synthesis were not observed in our patients with PAN. In patients followed longitudinally, ICAM-1 and E-selectin remained elevated in spite of an appropriate clinical response to corticosteroid and immunosuppressive therapy. Only VCAM-1 tended eventually to normalize. Although long-term follow-up studies are still not available, other authors have also noticed elevated levels of adhesion molecules in vasculitis patients in remission [11,23]. Corticosteroid and immunosuppressive agents often induce clinical remission in patients with PAN. The tendency of systemic vasculitis to relapse, and the histopathological evidence of persistent inflammatory lesions demonstrated in some treated patients [1–3,31], suggest that some components of the disease are highly responsive to current treatments, whereas other components are more refractory. Consequently, elevated levels of soluble adhesion molecules in patients apparently in remission might reflect a persistent exposure of endothelial cells to a mild remaining inflammatory microenvironment. Whether adhesion molecule detection may help in discriminating low-grade subclinical activity from true remission deserves further investigation in large, prospective, long-term follow-up studies.

ACKNOWLEDGEMENTS

We thank Dr Alex de la Sierra for his advice on statistical analysis. BC-V is a research award recipient from Hospital Clínic i Provincial de Barcelona. This work was partially supported by grants from FIS (96/0347) to MCC, and FIS (94/0953) to JO.

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Síntesi dels resultats

1 - En pacients amb PAN en activitat, els nivells de les formes solubles d'ICAM-1, VCAM-1 i selectina-E estan significativament elevats respecte els controls sans, els nivells de selectina L-s disminueixen i els de selectina P-s no es modifiquen significativament.

2 - Després d'un seguiment mig de 15 mesos (1-36 mesos), els nivells de les formes solubles d'ICAM-1 i selectina E persisteixen elevats en els pacients amb PAN, encara que estiguin en remissió clínica.

3. CIRCULATING SOLUBLE ADHESION MOLECULES IN PATIENTS WITH GIANT CELL ARTERITIS. CORRELATION BETWEEN SOLUBLE INTERCELLULAR ADHESION MOLECULE-1 (sICAM-1) LEVELS AND DISEASE ACTIVITY

Molècules d'adhesió solubles circulants en pacients amb arteritis de cèl·lules gegants. Correlació entre els nivells d'ICAM-1 soluble i l'activitat de la malaltia

Blanca Coll-Vinent, Carme Vilardell, Carme Font, Joaquim Oristrell, José Hernández-Rodriguez, Jordi Yagüe, Álvaro Urbano-Márquez, Josep M Grau, Maria C Cid

Annals of the Rheumatic Diseases. En premsa ,
(pàgines 69-86)

En l'anterior treball, no solament hem demostrat una activació immunològica i/o endotelial en la fase activa de la PAN, sinó també una persistència d'aquesta activació malgrat una resposta clínica i biològica al tractament instaurat (corticoids i immunosupressors), observació que per la seva possible trascendència per al seguiment dels malalts, prevenció de recidives i decisió sobre el moment idoni per a la retirada del tractament, dóna peu a prosseguir l'estudi en aquest camp. L'ACG és una vasculitis que presenta una ràpida resposta clínica i biològica als corticoids, però amb la retirada del tractament poden aparèixer recidives. Donat que la clínica, la histopatologia i la resposta al tractament és diferent en l'ACG respecte la PAN, en el tercer treball presentat hem volgut investigar si la variació en els nivells de les molècules d'adhesió solubles en l'ACG és similar al de la PAN o bé si reflecteix la diferent evolució clínica.

Per aquest estudi hem aplicat la tècnica d'ELISA amb anticossos monoclonals dirigits contra les selectines E i P, i les immunoglobulines ICAM-1 , ICAM-3 i VCAM-1 en sèrums i plasmes de malalts amb ACG en activitat i en l'evolució posterior al tractament, en dos estudis: un estudi transversal, en el qual també hi hem inclòs controls sans, i un estudi longitudinal.

ANNALS OF THE RHEUMATIC DISEASES

Published by the BMJ Publishing Group

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17 November 1998

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Dear Dr Cid,

ARD/1998/117 - CIRCULATING SOLUBLE ADHESION MOLECULES IN PATIENTS WITH GIANT CELL ARTERITIS. CORRELATION BETWEEN SOLUBLE INTERCELLULAR ADHESION MOLECULE-1 LEVELS AND DISEASE ACTIVITY

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ARD/1998/117 Revised manuscript (third revision)

CIRCULATING SOLUBLE ADHESION MOLECULES IN PATIENTS WITH GIANT CELL ARTERITIS. CORRELATION BETWEEN SOLUBLE INTERCELLULAR ADHESION MOLECULE-1 (sICAM-1) LEVELS AND DISEASE ACTIVITY

Concise report

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ABSTRACT

Objective. To evaluate whether changes in levels of circulating adhesion molecules are related to disease activity in patients with giant cell arteritis (GCA).

Methods. A sandwich ELISA was used to measure soluble intercellular adhesion molecule-1 (sICAM-1), sICAM-3, vascular cell adhesion molecule-1 (sVCAM-1), E-selectin (sE-selectin) and L-selectin (sL-selectin) in sera and plasma from patients with GCA. A cross-sectional study was performed on 64 GCA patients at different activity stages and on 35 age and sex-matched healthy donors. Thirteen of these patients were evaluated at the time of diagnosis and serially during follow-up.

Results. At the time of diagnosis, sICAM-1 levels were significantly higher in active GCA patients than in controls (360.55 ± 129.78 ng/mL versus 243.25 ± 47.43 ng/mL, $p<0.001$). In contrast, sICAM-3, sVCAM-1, sE-selectin and sL-selectin levels did not differ from those obtained in normal donors. With steroid administration, a decline in sICAM-1 levels was observed, reaching normal values when clinical remission was achieved (263.18 ± 92.7 ng/mL globally, 293.59 ± 108.39 ng/mL in the group of patients in recent remission, and 236.83 ± 70.02 ng /mL in those in long-term remission). In the thirteen patients followed longitudinally, sICAM-1 levels also normalized with clinical remission (225.87 ± 64.25 ng/mL in patients in recent remission, and 256.29 ± 75.15 ng/mL in those in long-term remission).

Conclusions. Circulating sICAM-1 levels clearly correlate with clinically apparent disease activity in GCA patients. Differences with results previously found in patients

with other vasculitides may indicate that different pathogenic mechanisms contribute to vascular inflammation in different disorders.

Key words: adhesion molecules, giant cell arteritis, inflammation

INTRODUCTION

Giant cell (temporal) arteritis (GCA) is a large-vessel vasculitis which affects mostly elderly people. Histologically, it is characterized by a lymphocyte and macrophage infiltration of large and medium-sized vessels which frequently exhibits a granulomatous pattern with giant cell formation. There is no specific treatment for this disease, although GCA patients present a favorable clinical and biological response to corticosteroids. However, relapses are not infrequent when corticosteroid are tapered or discontinued, and accurate parameters discriminating persistent subclinical inflammatory activity from true remission have not been identified [1].

Important contributions have currently improved our understanding of the immunopathogenic mechanisms involved in the development of GCA lesions. T lymphocytes infiltrating the temporal arteries seem to be activated by specific recognition of a putative antigen residing in the arterial wall and, subsequently, activate macrophages, which undergo a functional differentiation and contribute to vessel inflammation and damage through various pathways [reviewed in 1 and 2].

Independently of the primary immunopathogenic mechanisms, the development of inflammatory infiltrates requires dynamic interactions between leukocyte surface adhesion receptors and their ligands on the endothelial cell surface [reviewed in 3]. Circulating forms of these adhesion molecules have been detected in human serum and plasma and increased levels have been detected in disorders where leukocyte/endothelial cell interactions play a significant role, namely infections, neoplasms and chronic

inflammatory diseases [4]. The role that circulating adhesion molecules play in vivo is not well known. It has been suggested that elevated circulating levels of soluble adhesion molecules may reflect endothelial and/or leukocyte activation [5].

In this study, we measured circulating levels of soluble ICAM-1, ICAM-3, VCAM-1, E-selectin and L-selectin in a large and homogeneous series of patients with biopsy-proven GCA in order to define the pattern of circulating adhesion molecules in GCA patients and to evaluate whether changes in soluble adhesion molecule levels are related to disease activity.

PATIENTS AND METHODS

1. Patients.. The study group consisted of 64 biopsy-proven GCA patients (18 males and 46 females) aged 74 years (range 57- 88).

A cross-sectional study was performed, and patients were classified according to their disease activity. GCA was considered active when patients were clinically symptomatic and evaluated before starting treatment (prednisone 1 mg/Kg/day). Patients were considered to be in remission when they fulfilled all the following conditions: a) none of the GCA related signs or symptoms present at the time of diagnosis was present anymore, b) there were no new signs or symptoms attributable to GCA, and c) erythrocyte sedimentation rate -ESR- was within the normal range. Among them, we considered separately patients treated for one month to 2 years in whom remission was mantained with corticosteroid therapy (recent remission), and patients who, having been

treated for at least 2 years, no longer were receiving corticosteroids (long-term remission).

Data concerning clinical manifestations at diagnosis were obtained prospectively in patients with active disease and in those in recent remission. In individuals in long-term remission, clinical manifestations at presentation were obtained from their records. Disease activity was evaluated prospectively in all patients. Clinical features recorded were age, sex, duration of symptoms prior to biopsy, and the presence or absence of the following: polymyalgia rheumatica, cranial symptoms (hedache, scalp tenderness, jaw claudication), ischemic symptoms (amaurosis, diplopia, cerebrovascular accident), and systemic inflammatory response (fever, weight loss, ESR > 85, hemoglobin < 110 g/L).

The control group included 35 healthy blood donnors (10 male, 25 female) aged 73 years (range 60 - 88).

For the cross-sectional study, 73 determinations (49 sera, 24 plasma) were made: 45 in active GCA patients, and 28 in patients in remission (13 in recent remission and 15 in long-term remission).

A longitudinal substudy was done with 13 active patients who underwent serial determinations at diagnosis (with no treatment), when in complete remission one month to 2 years after starting corticosteroid therapy (recent remission), and when in long-term remission, no longer receiving therapy. None of these patients experienced significant

relapses during their follow-up. For that reason, months/years after the initiation of therapy were considered equivalent to months/years in clinical remission.

2. Soluble adhesion molecule detection. All samples were analyzed for sICAM-1, sICAM-3, sVCAM-1, sE-selectin and sL-selectin levels by sandwich ELISA according to the instructions of the manufacturer. We used commercially available kits from British Biotechnology Products, Abingdon, UK, for sICAM-1, sVCAM-1 and sE-selectin (BBE 1B, BBE 2B and BBE 3B respectively), and kits from Bender Medsystems, Vienna, Austria, for sICAM-3 and sL-selectin (BMS 218 and BMS 206 respectively).

3. Statistical analysis. Data are presented as means ± SD. For the cross-sectional study, a Kruskal-Wallis H test was used, correcting p values for multiple comparisons. For the longitudinal study, a Wilcoxon's rank sum test was employed, also correcting p values for multiple comparisons. Mann-Whitney U test was used for comparisons between two groups. Pearson correlation coefficient was used to correlate continuous variables. P-values (2-tailed) less than 0.05 were considered significant.

RESULTS

No differences were found between serum and plasma levels of sICAM-1, sICAM-3, sVCAM-1 nor sL-selectin ($p = 0.42$, 0.86, 0.17, and 0.35 respectively). Plasma sE-selectin levels were significantly lower than serum levels ($p < 0.01$). Therefore, for sE-selectin, only serum samples were considered.

Soluble ICAM-1 levels in active patients (360.55 ± 129.78 ng/mL) were elevated compared with controls (243.25 ± 47.43 ng/mL) ($p < 0.001$). Soluble ICAM-1 levels were higher in active patients than in patients in clinical remission, both when these were considered globally (263.18 ± 92.7 ng/mL, $p < 0.01$), and when only patients in long-term remission were considered (236.83 ± 70.02 ng/mL, $p < 0.01$). Circulating sICAM-1 values in patients in clinical remission did not differ from normal donors. No significant differences were observed, either, between patients in recent remission (293.59 ± 108.385 ng/mL) compared with those in long-term remission (table 1).

No statistical differences were found in sICAM-3, sVCAM-1, sE-selectin and sL-selectin levels between active patients and controls, nor between active patients and patients in remission (table 1).

A significant correlation was found between the number of inflammatory parameters, as defined in the methods section, and sICAM-1 levels ($p < 0.05$). No relationship was observed between circulating levels of any other adhesion molecule studied and the clinical features recorded.

Results from the longitudinal substudy are shown in figure 1. In keeping with the data obtained from the cross-sectional study, sICAM-1 levels decreased when clinical remission was achieved (from 369.63 ± 139.17 to 225.87 ± 64.25 ng/mL, $p < 0.01$), and remained at low levels when treatment was discontinued (256.29 ± 75.15 ng/mL). A

correlation was found between sICAM-1 levels and ESR values (Pearson's correlation coefficient, p = 0,034). No significant variation of any of the other adhesion molecules studied was observed in the longitudinal study.

DISCUSSION

Our results indicate that sICAM-1 levels were elevated in patients with active GCA compared with levels found in healthy matched controls. Subsequently, sICAM-1 returned to normal when clinical remission was achieved. In contrast, circulating sICAM-3, sVCAM-1, sE-selectin, and sL-selectin levels remained unmodified throughout the course of the disease.

Circulating adhesion molecules have been studied in a variety of vasculitis syndromes. Most of these studies include small series of miscellaneous patients with different vasculitides and, therefore, conclusions are difficult to draw. Studies performed in homogeneous groups of patients have demonstrated elevated levels of sICAM-1 and sVCAM-1, but not sE-selectin, in patients with active Wegener's granulomatosis [6]. Elevated sE-selectin and sICAM-1 levels have also been demonstrated in patients with Kawasaki disease [7]. We have previously demonstrated an increase in circulating sICAM-1, sVCAM-1, sE-selectin, and a decrease in sL-selectin concentrations in patients with classical polyarteritis nodosa [8]. Variations in the circulating pattern of soluble adhesion molecules in different vasculitides indicate a high complexity in the regulatory pathways involved in adhesion molecule expression and release and, probably, a diversity in the source of circulating adhesion molecules in each condition.

Immunopathologic studies performed on biopsy specimens obtained from homogeneous series of patients with GCA and patients with polyarteritis nodosa have demonstrated E-selectin, ICAM-1, and VCAM-1 expression in endothelia of inflammed vessels, particularly in the adventitial microvasculature and neovessels. Strong ICAM-1 and ICAM-3 expression as well as occasional VCAM-1 expression have also been observed in infiltrating leukocytes [9,10]. However, and in contrast with our previous findings in polyarteritis nodosa [8], only sICAM-1 was significantly elevated in sera from GCA patients. GCA involves large arteries whereas polyarteritis nodosa involves medium and small-sized vessels. Consequently, the endothelial surface contributing to the potential release of adhesion molecules is much wider in polyarteritis nodosa than in GCA. This fact could account for the elevated concentration of sE-selectin, sICAM-1, and sVCAM-1 found in polyarteritis nodosa and the lack of significantly elevated concentrations of adhesion molecules of endothelial origin in GCA.

Interestingly, the pattern of circulating adhesion molecules in our GCA patients is similar to that found by Macchioni et al. [11] and Meliconi et al. [12] in patients with polymyalgia rheumatica, a condition closely related to GCA. Patients with isolated polymyalgia rheumatica lack significant inflammatory vascular lesions in their arteries. Accordingly, it is not likely that elevated sICAM-1 found in both conditions is generated in inflammed arteries. Circulating activated monocytes are a characteristic feature in both GCA and polymyalgia rheumatica [13] and ICAM-1 is strongly expressed by activated cells of the monocytic lineage [3]. Activated circulating monocytes could be a likely source of elevated circulating sICAM-1 found in GCA and polymyalgia

rheumatica, given the absence of increase of circulating levels of other adhesion molecules of endothelial origin.

In vitro studies demonstrate that corticosteroid treatment down-regulates adhesion molecule expression [14]. According to this, we found that raised concentrations of circulating sICAM-1 returned to normal levels upon corticosteroid treatment in our GCA patients. The longitudinal study showed a good correlation between sICAM-1 levels and clinically apparent disease activity and acute phase response as assessed by ESR measurement. A decrease in sICAM-1 levels upon corticosteroid treatment was also observed in the cross-sectional study although, since different patients were included in each group, it was less apparent. In a previous study, we showed that elevated levels of circulating soluble endothelial adhesion molecules persist in patients with polyarteritis nodosa in spite of corticosteroid therapy [8]. We have also shown persistence of abnormally high levels of other products of endothelial origin such as vWFAg in GCA patients [15]. The clear decrease in circulating sICAM-1 induced by corticosteroid treatment further supports a role for activated circulating monocytes as a source of sICAM-1 in GCA patients since this cell subset is very sensitive to the down-regulatory effects of corticosteroids [13].

Changes in circulating adhesion molecules follow distinct patterns in different inflammatory diseases and show a certain level of specificity even in related syndromes such as the different vasculitides. Studying fluctuations of adhesion molecules along disease outcome may improve our understanding about the participation of different cell types in the pathogenesis of the inflammatory reaction in different disorders, the

assessment of subclinical disease activity and the effects of treatment on specific pathophysiologic mechanisms.

Abbreviations: GCA: giant cell arteritis, ICAM: intercellular adhesion molecule, VCAM-1: vascular cell adhesion molecule-1, PECAM-1: platelet endothelial cell adhesion molecule-1, ELISA: enzyme-linked immunossorbent assay.

ACKNOWLEDGEMENTS

We thank Dr. Àlex de la Sierra for his advise in statistical analysis. Blanca Coll-Vinent is a research award recipient from Hospital Clínic i Provincial de Barcelona. This work was partially supported by a grant from FIS n° 95/0860 and n° 98/0443.

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Table 1. Levels of the adhesion molecules studied in the cross-sectional study (ng/mL)*

Adhesion molecule	active patients	patients in remission			controls
		recent remission	long-term remission	total	
	n = 45	n = 13	n = 15	n = 28	n = 35
sICAM-1	360.55 ± 129.78 #, ϕ	293.59 ± 108.385	236.83 ± 70.02	263.18 ± 92.71	243.25 ± 47.43
sICAM-3	38.4 ± 20.6 $	44.64 ± 23	43.71 ± 20.829	44.14 ± 21.46	35.26 ± 24.674
sVCAM-1	705.21 ± 278.84 $	817.76 ± 508.69	622.34 ± 353.1	713.07 ± 435.32	661.19 ± 254.64
sE-selectin	44.46 ± 28.6 $	50.36 ± 33	39.84 ± 26.02	43 ± 27.82	38.33 ± 31.12
sL-selectin	540.13 ± 321.07 $	627.03 ± 407.68	652.63 ± 402.33	641.25 ± 397.04	467.34 ± 233.95

* Data are presented as means ± SD.

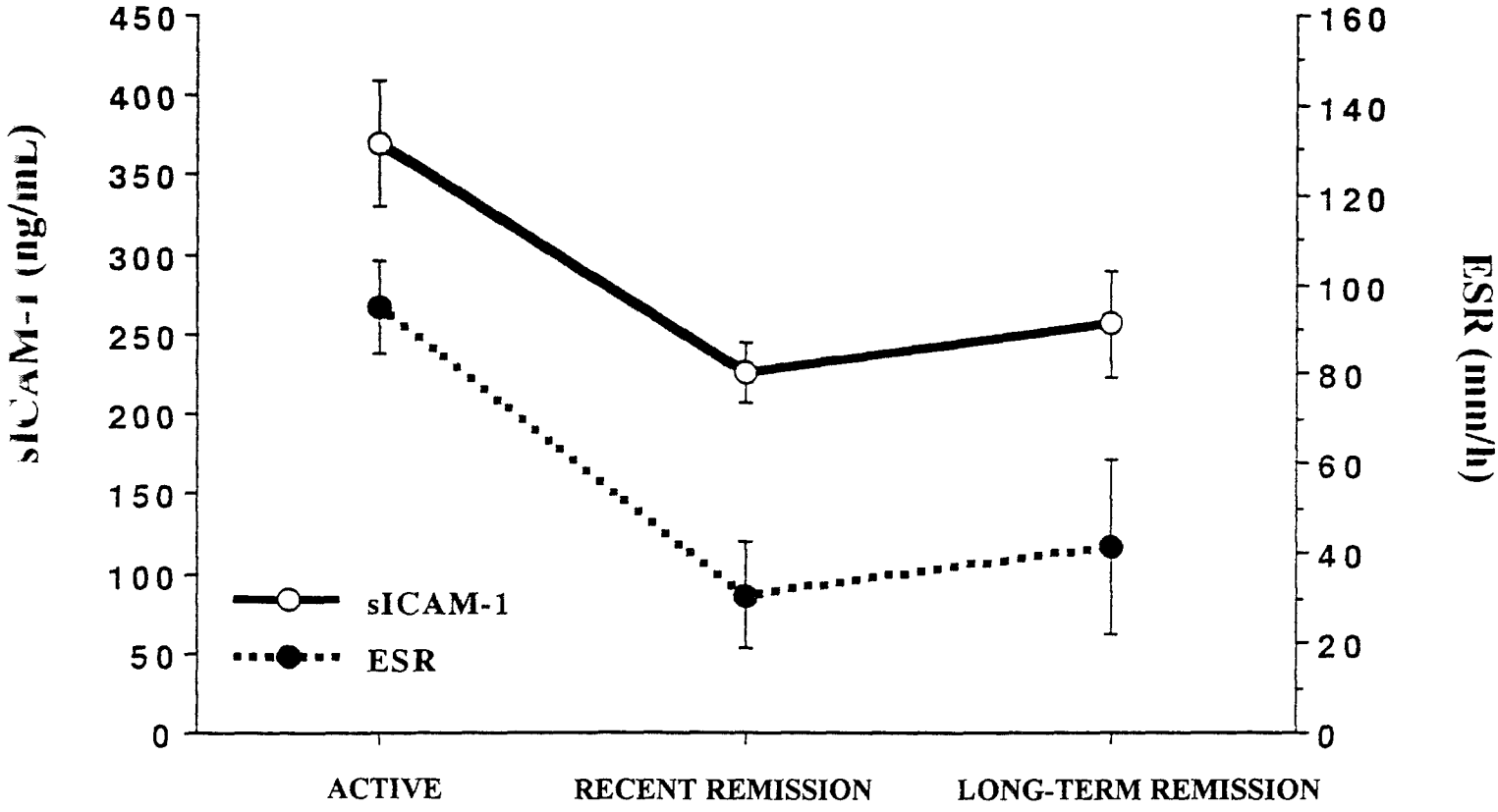
p compared with controls < 0.001

ϕ p compared with patients in remission < 0.01

$ p compared with patients in remission and p compared with controls not significative

LEGENDS

Figure 1. Soluble ICAM-1 levels and ESR values in the longitudinal study. Error bars indicate standard error of the mean.


sICAM-1 (ng/mL)
450
400
350
300
250
200
150
100
50
0
160
140
120
100
80
60
40
20
0
ESR (mm/h)
sICAM-1
ESR
ACTIVE
RECENT REMISSION
LONG-TERM REMISSION

Síntesi dels resultats

13 - En pacients afectes d'ACG en activitat, els nivells de la forma soluble d'ICAM-1 estan significativament elevats, però no els de les formes solubles de selectina E, selectina L, ICAM-3 ni VCAM-1.

14 - Els nivells d'ICAM-1 circulant disminueixen ràpidament després de la instauració del tractament amb corticoids en pacients afectes d'ACG.

DISCUSSIÓ CONJUNTA

En la present tesi, hem estudiat, mitjançant diferents treballs, l'expressió de molècules d'adhesió cel·lular en mostres histològiques o en la seva forma soluble, en sèrum i plasma de malalts amb dos tipus diferents de vasculitis, la PAN, que afecta a vasos de petit i mitjà calibre, i l'ACG, que afecta a vasos grans.

En l'estudi de l'expressió de molècules d'adhesió en biòpsies de múscul i nervi de malalts amb PAN, i tal com ja està descrit clàssicament (115), en els espècimens obtinguts hem objectivat vasos inflamats en diferents estadis histològics: lesions *incipients*, amb un petit infiltrat inflamatori (80-120 leucòcits per secció), lesions *establertes*, amb un important infiltrat inflamatori (més de 400 leucòcits per secció) i sovint amb necrosi fibrinoide, i lesions *cicatricials*, amb escàs infiltrat inflamatori residual (menys de 150 leucòcits per secció) i una hiperplàsia intimal prominent que oblitera la llum del vas. En força casos (26 % de les mostres estudiades) en una mateixa secció coexisteixen lesions en diferents estadis evolutius.

Mitjançant la tinció amb la lectina *Ulex europeus*, que s'uneix específicament a la fucosa de les cèl·lules endotelials i es considera un marcador específic de les mateixes (116), hem demostrat una participació dinàmica de les cèl·lules endotelials en els diferents estadis histològics del procés vasculític. En les lesions *incipients*, l'endoteli luminal està morfològicament preservat i pràcticament no hi ha microvasos envoltant la lesió. En les lesions *establertes*, l'endoteli luminal està mal definit o incomplet, i la llum parcialment o totalment ocluïda, i entre l'infiltrat inflamatori i envoltant-lo hi ha un nombre important de microvasos, que sempre estan més ben definits que l'endoteli luminal. En les lesions *cicatricials*, l'endoteli luminal no es tenyeix o ho fa molt tènuement, la llum està ocluïda i fins i tot a vegades substituïda per microvasos, que també estan presents entre i envoltant l'infiltrat inflamatori, encara que en un nombre inferior al de les lesions establertes. Atès que en vasos normals petits i mitjans no s'observen microvasos enmig o envoltant el vas principal, i que estan presents sobretot en lesions ben establertes, i en

lesions cicatricials en menor quantitat, hem assumit que els microvasos presents en les lesions vasculítiques són neovasos derivats de l'angiogènesi induïda per la inflamació.

L'expressió endotelial de les molècules d'adhesió segueix un patró dinàmic en les diferents fases de les lesions vasculítiques. Les molècules d'adhesió constitutives (ja presents en els endotelis dels controls normals), és a dir ICAM-1, ICAM-2, PECAM-1 i selectina P, romanen intactes en l'endoteli luminal de les lesions incipients de PAN. Contràriament, en lesions ben establertes, la intensitat i la definició de la tinció disminueix en l'endoteli luminal, i, en canvi, és intens i més ben definit en l'endoteli dels neovasos. En les lesions cicatricials, l'endoteli luminal és pràcticament sempre negatiu o molt tènue, i l'expressió en els vasos neoformats també disminueix. Donat que amb el marcador endotelial *Ulex europeus* s'objectiva un patró similar, es dedueix que l'endoteli luminal es lesiona al llarg del procés vasculític fins que desapareix.

Alguns estudis previs sobre l'expressió d'ICAM-1 en lesions vasculítiques són contradictoris. Wawrick i cols. van observar una important expressió d'ICAM-1 en l'infiltrat inflamatori de lesions d'ACG, i també en les cèl·lules musculars llises de la paret, però no especifiquen si en l'endoteli hi ha diferències rellevants respecte els controls (78). Pall i cols. no van objectivar diferències en la tinció endotelial d'ICAM-1 en biòpsies renals de pacients amb vasculitis respecte els controls (79). En canvi, Rastaldi i cols. van observar un augment de la tinció per ICAM-1 en vasos petits i capil·lars de pacients amb vasculitis renals associades a ANCA (80); i Flipo i cols. van descriure un augment de l'expressió endotelial d'ICAM-1 en mostres de glàndula salival de pacients amb vasculitis reumatoide, que desapareixia després del tractament (81). Els diferents resultats obtinguts en aquests estudis són poc comparables donat el reduït nombre de casos i/o l'heterogenicitat dels pacients inclosos. A més, no s'ha intentat correlacionar l'expressió de les molècules d'adhesió amb l'estadi histològic de la lesió. Fins ara, no tenim

informació sobre la distribució de les molècules d'adhesió constitutives de l'endoteli ICAM-2, PECAM-1 ni selectina P en vasculitis. En malalts amb rebuig de trasplantament renal, s'ha observat una absència focal de PECAM-1, molècula expressada constitutivament per les cèl·lules endotelials renals sanes, fet que, d'acord amb els nostres resultats, també reflectiria lesió endotelial (117).

La selectina E s'expressa en l'endoteli luminal de les lesions incipients i d'alguns endotelis de vasos no inflamats de malalts afectes de PAN. En les lesions establertes, s'expressa en alguns neovasos, però rarament en l'endoteli luminal, i en les lesions cicatricials tampoc s'expressa. Aquestes troballes poden reflectir la naturalesa transitòria de l'expressió de selectina E, tal com s'ha demostrat prèviament en cultius cel·lulars (118). Per tant, l'absència d'immunotinció no exclou necessàriament un paper important d'aquesta molècula en estadis més precoços de la inflamació vascular. Les nostres observacions donen suport a la hipòtesi que la selectina E s'expressa al principi del procés inflamatori, fins i tot en vasos preservats sense infiltrat inflamatori, i posteriorment desapareix. Els canvis funcionals, doncs, precedeixen l'evidència morfològica d'inflamació i dany endotelial. Aquesta interpretació també és avalada pel fet que hi ha una associació significativa entre l'expressió d'aquesta molècula i la presència de neutròfils en l'infiltrat inflamatori, ja que la presència de polimorfonuclears tendeix a ser més prominent en les lesions inicials (37,83). Aquesta associació ha estat també objectivada per Sais i cols. en vasculitis cutànies leucocitoclàstiques (83) i per Bradley i cols. en biòpsies cutànies d'un grup heterogeni de malalts amb vasculitis cutànies i sistèmiques (76).

VCAM-1, que, com la selectina E, és induïda en l'endoteli mitjançant l'estímul de citocines, s'expressa en l'endoteli luminal de vasos inflamats de malalts amb PAN. L'expressió d'aquesta molècula canvia d'un malalt a un altre i fins i tot entre diferents lesions en una mateixa mostra. S'expressa fonamentalment en l'endoteli luminal de lesions

establertes amb una llum encara relativament preservada, i ocasionalment en alguns vasos neoformats. Altres autors han observat un augment de l'expressió de VCAM-1 en alguns vasos de pacients amb vasculitis (79-81). En mostres d'artèria temporal de pacients amb ACG s'ha demostrat una important expressió de VCAM-1, i també de selectina E, en els vasos neoformats i la microvasculatura adventicial, mentre que la seva expressió en l'endoteli luminal és infreqüent, i en els controls és nul·la (119).

Globalment, doncs, les nostres troballes suggereixen que, excepte en les etapes més inicials, l'endoteli luminal probablement no participa en l'adhesió endotelial en la PAN. En canvi, la notable expressió de molècules d'adhesió en els neovasos suggereix que l'angiogènesi juga un paper important en el reclutament de leucòcits i, per tant, en el manteniment de la inflamació en els vasos afectats en la PAN i, probablement, també en altres tipus de vasculitis.

Malgrat que l'expressió de les molècules d'adhesió endotelials pot variar en la microvasculatura de diferents òrgans, nosaltres vam trobar una expressió similar en mostres de múscul i de nervi.

En l'estudi de les molècules d'adhesió solubles en malalts amb PAN, hem demostrat que en els malalts actius els nivells circulants de les formes solubles d'ICAM-1, VCAM-1 i selectina E estan augmentats respecte els controls sans. La selectina E només s'expressa en l'endoteli activat, i VCAM-1, malgrat que també està present en altres estirps cel·lulars, s'expressa fonamentalment en les cèl·lules endotelials quan és induïda per citocines proinflamatòries. Per tant, es pot suposar que la font principal d'aquestes molècules són cèl·lules endotelials activades dels vasos inflamats. En canvi, en l'ACG només augmenta ICAM-1-s, mentre que els nivells de VCAM-1-s i selectina E-s no es diferencien significativament dels controls.

En estudis anteriors s'han detectat nivells elevats d'ICAM-1-s, VCAM-1-s i selectina E-s circulants en diferents tipus de vasculitis (84-93). Stegeman i cols. van demostrar concentracions elevades d'ICAM-1-s

i VCAM-1-s però no selectina E-s en un grup homogeni de malalts amb granulomatosi de Wegener, especialment en malalts amb una afectació orgànica extensa respecte els pacients amb una malaltia limitada (90). En la malaltia de Kawasaki també s'han observat nivells elevats d'ICAM-1-s i selectina E-s (91-93), amb nivells més elevats d'ICAM-1-s en els pacients amb lesions coronàries (91,92), i amb una disminució ràpida dels nivells de selectina E-s amb el tractament (93).

Les diferències en el patró d'expressió de les molècules d'adhesió circulants en les diferents vasculitis demostra una elevada complexitat en els mecanismes reguladors de l'expressió i alliberació de les molècules d'adhesió i, probablement, una procedència diferent de les molècules d'adhesió en cada entitat. El fet que la selectina E-s estigui elevada en malalties caracteritzades per la presència de lesions agudes, com la malaltia de Kawasaki i la PAN (en la qual coexisteixen lesions en diferent estadi evolutiu), i no en vasculitis distingides per la presència de lesions cròniques, com l'ACG o la granulomatosi de Wegener, reforça la hipòtesi que la selectina E s'expressa a l'inici de l'infiltrat inflamatori i posteriorment desapareix.

L'ACG afecta a grans artèries, mentre que la PAN afecta vasos petits i mitjans. Per tant, la superfície endotelial que potencialment contribueix a l'alliberació de molècules d'adhesió circulants és molt més gran en la PAN que en l'ACG. Aquest fet podria explicar l'elevada concentració d'ICAM-1-s, VCAM-1-s i selectina E-s en la PAN i la manca d'augment significatiu de les molècules d'origen endotelial en l'ACG.

Curiosament, el patró d'expressió de les molècules d'adhesió circulants en els nostres malalts afectes d'ACG és igual que el que van observar Macchioni i cols. (120) i Meliconi i cols. (121) en malalts amb polimiàlgia reumàtica, entitat estretament relacionada amb l'ACG. Les artèries dels pacients amb polimiàlgia reumàtica aïllada no tenen lesions inflamatòries significatives. Per tant, és poc probable que l'elevació d'ICAM-1 present en ambdues entitats es generi en l'endoteli. Els monòcits circulants activats són característics tant de l'ACG com de la

polimiàlgia reumàtica i probablement contribueixen a la resposta de fase aguda present en les dues malalties (122). ICAM-1 és intensament expressada per cèl·lules activades de l'estirp monocítica (49). Per tant, els monòcits circulants podrien ser la font de l'augment dels nivells d'ICAM-1-s circulant observat en l'ACG i en la polimiàlgia reumàtica, donada l'absència d'augment d'altres molècules d'origen endotelial. A favor d'aquesta hipòtesi hi ha l'augment preferent o prominent d'ICAM-1-s observat en altres vasculitis granulomatoses en humans (90), així com en altres malalties granulomatoses com la sarcoidosi (123) o en altres entitats on s'han demostrat monòcits activats en sang perifèrica (91). D'acord amb això, en l'ACG, Wawrick i cols. van observar expressió d'ICAM-1 en cèl·lules mononucleades, fonamentalment macròfags, així com en cèl·lules epitelioides i granulomes (78). També s'ha demostrat expressió d'ICAM-1 en models animals experimentals de vasculitis granulomatoses (124,125).

Hem trobat una associació significativa entre la positivitat de VCAM-1 a l'endoteli i l'expressió leucocitària d'α_4, la cadena α comú de les integrines VLA-4 i $\alpha_4\beta_7$, que actuen de receptors per a VCAM-1. També hem observat una correlació entre l'expressió d'ICAM-1 i de LFA-1, ambdues en els leucòcits infiltrants. Aquest fet, també observat per Wawryck i cols. (78) suggereix que les interaccions homotípiques poden contribuir a consolidar els infiltrats inflamatoris.

CD18, la cadena β comú de les integrines β_2, s'expressa virtualment en tots els leucòcits circulants. Malgrat això, aproximadament un 40 % dels leucòcits infiltrants en la PAN no expressen aquesta molècula. Aquest fenomen ja l'havíem observat en les polimiositis i dermatomiositis (126), i podria ser explicat per una inhibició de l'expressió de les integrines leucocitàries després de la transmigració, però, fins ara, no coneixem estudis funcionals que hagin demostrat aquest fenomen. Una altra possible explicació seria que, donat que quan les integrines leucocitàries s'activen presenten canvis conformacionals, l'anticòs

monoclonal utilitzat en aquest estudi no detectés els epítops relacionats amb l'activació.

En les mostres de malalts amb PAN, mentre que CD18 s'expressa principalment en el centre de la lesió, ICAM-3 predomina a la perifèria dels infiltrats vasculars, envoltant neovasos, i on la principal població cel·lular són els macròfags (37). D'altra banda, senyals transduïts a través d'ICAM-3 activen les integrines limfocitàries i estimulen l'adhesió dels limfòcits a les cèl·lules endotelials (127). Una forta expressió d'ICAM-3 en els leucòcits que envolten els microvasos situats a la perifèria de l'infiltrat vascular podria, doncs, facilitar l'adhesió i transmigració leucocitària. D'altra banda, s'ha demostrat que, en activar-se, ICAM-3 es desprèn de la membrana limfocitària (68). Per tant, els limfòcits que penetren més profundament en la paret vascular podrien haver alliberat ICAM-3 de la seva superfície. Recentment, s'ha descrit expressió endotelial d'ICAM-3 en l'angiogènesi tumoral (128,129). Nosaltres no hem pogut demostrar expressió endotelial d'ICAM-3 en l'endoteli luminal ni en els neovasos dels malalts afectes de PAN. Tampoc s'ha demostrat expressió endotelial d'ICAM-3 en altres malalties inflamatòries cròniques, excepte en l'artritis reumatoide i en algun cas de malaltia granulomatosa crònica (129,130).

Excepte en la vasculitis reumatoide, en la qual s'hi ha detectat nivells elevats d'ICAM-3 circulant (87), no s'han fet estudis sobre possibles canvis en els nivells d'ICAM-3-s en vasculitis. En l'estudi de molècules d'adhesió circulant en l'ACG, les concentracions d'ICAM-3-s en aquests pacients són similars als dels controls sans. Els resultats obtinguts en el nostre estudi recolzen les observacions de Martin i cols. en diferents malalties autoimmunitàries (131) on no hi ha correlació entre les concentracions d'ICAM-3-s i ICAM-1-s. Aquestes troballes són sorprenents si, tal com hem postulat anteriorment, s'assumeix que els monòcits circulants activats contribueixen a l'augment d'ICAM-1-s, ja que els monòcits també expressen intensament ICAM-3.

La selectina L només s'expressa en un percentatge molt reduït de leucòcits infiltrants en lesions incipients. El fet que aquesta molècula es desprengui de la membrana cel·lular amb l'activació leucocitària podria explicar la manca d'immunotinció per aquesta molècula en la majoria dels leucòcits infiltrants en els vasos afectats. Així doncs, en els malalts amb PAN s'esperarien nivells elevats de selectina L circulant. Malgrat aquesta hipòtesi, en l'estudi de les molècules solubles circulants en malalts amb PAN, hem observat una disminució de la concentració de selectina L-s. En els malalts afectes d'ACG tampoc hem vist cap elevació dels nivells de selectina L-s. Altres autors també han descrit una disminució dels nivells de selectina L-s en pacients amb vasculitis (89,132). Per explicar aquestes troballes, s'ha postulat que la selectina L-s circulant s'uniria a cèl.lules endotelials activades (133). D'acord amb aquesta hipòtesi, és interessant la nostra troballa de positivitat per selectina L en alguns endotelis de pacients afectes de PAN, fins i tot en vasos no inflamats.

Malgrat que el tractament amb corticoids pot influenciar l'expressió o la funció de les molècules d'adhesió en les malalties inflamatòries cròniques, nosaltres no hem observat diferències en l'expressió tissular de les molècules d'adhesió entre malalts tractats i no tractats en la PAN. Els malalts tractats inclosos en el nostre estudi només havien rebut dosis baixes de corticosteroids o altes dosis però en un període curt de temps, i en tots ells existia activitat clínica de la malaltia. Malgrat que, tal com es va demostrar en un estudi previ, dosis similars de corticosteroids sí que van poder inhibir l'expressió del receptor d'interleukina-2 i disminuir la proliferació limfocitària (37), probablement no són suficients per inhibir l'expressió de molècules d'adhesió. D'acord amb això, en malalts amb PAN seguits longitudinalment, els nivells d'ICAM-1 i selectina E circulants s'han mantingut elevats malgrat una resposta clínica satisfactòria al tractament amb corticoids i immunosupressors. Aquestes troballes suggereixen que en la PAN la inhibició de les molècules d'adhesió endotelial requereix dosis superiors de corticoids o un tractament més prolongat. La persistència de lesions inflamatòries malgrat el tractament

també s'ha observat en altres vasculitis (48,134-138) i suggereix que, mentre alguns components de la malaltia són molt sensibles al tractament, altres components són més refractaris i probablement faciliten les recaigudes subsegüents.

Malgrat que la inhibició de les molècules d'adhesió endotelials sembla requerir un tractament més prolongat o a dosis més altes, i a diferència dels malalts amb PAN, en els malalts amb ACG els nivells d'ICAM-1-s tornen ràpidament als valors normals amb la instauració del tractament. Aquesta troballa és aparentment contradictòria amb la persistència de nivells anormalment elevats de productes d'origen endotelial, com el factor VonWillebrand, en l'ACG (139) i recolza la teoria que els monòcits circulants juguen un paper com a font d'ICAM-1-s en els pacients amb ACG, atès que aquesta població cel·lular és molt sensible a l'acció supressora dels corticoids. També recolzant aquesta hipòtesi, Roche i cols. han demostrat que els corticoids inhibeixen més l'activació dels monòcits circulants que la dels limfòcits T i, presumiblement, altres tipus cel·lulars (122).

En els primers dies després d'iniciar el tractament, hem observat un augment transitori dels nivells d'ICAM-1-s i selectina P-s en malalts amb PAN. Aquest fenomen podria indicar una alliberació de molècules solubles i suggereix que el tractament podria modular l'afinitat lligand/receptor.

Contràriament al que s'ha observat respecte l'estadi histològic, no hem demostrat cap relació entre les característiques clíniques dels malalts (edat, sexe, durada dels símptomes, resposta inflamatòria sistèmica) i l'expressió endotelial o cel·lular de cap de les molècules d'adhesió estudiades. En el cas de les molècules solubles, a part dels canvis objectivats amb el tractament, només hem observat una relació entre els nivells d'ICAM-1-s i el nombre de paràmetres inflamatoris en l'ACG. Aquesta manca de correlació amb la clínica contrasta amb els resultats obtinguts per altres autors, en els quals la severitat o extensió

de la malaltia es correlaciona amb l'augment de determinades molècules d'adhesió circulants (89,90,91).

Els canvis en les molècules d'adhesió circulants segueixen diferents patrons en diferents malalties inflamatòries i mostren un cert nivell d'especificitat fins i tot en síndromes relacionades, com són les diferents vasculitis. L'estudi de les fluctuacions de les molècules d'adhesió al llarg de l'evolució de la malaltia pot millorar la nostra comprensió sobre la participació de diferents tipus cel·lulars en la gènesi de la reacció inflamatòria en diferents malalties, la valoració de l'activitat subclínica de la malaltia, i els efectes del tractament en els mecanismes patofisiològics específics.

Amb anticossos monoclonals que bloquegen epítops funcionals de molècules d'adhesió s'ha evitat eficaçment el desenvolupament de lesions inflamatòries i de manifestacions clíniques en models animals de diverses malalties autoimmunitàries i alguns d'aquests anticossos ja s'han provat en estudis clínics en humans. Encara que cal fer més estudis, els nostres resultats suggereixen que la PAN, i probablement també l'ACG, podrien beneficiar-se d'una teràpia antiadhesiva.

CONCLUSIONS

1 - L'endoteli de les lesions vasculars dels pacients amb PAN clàssica sofreix modificacions al llarg del procés evolutiu d'aquestes: en les lesions incipients roman morfològicament preservat; en les lesions establertes l'endoteli luminal es destrueix parcialment o totalment i apareix microvascularització a l'entorn o enmig de l'infiltrat inflamatori; i en les lesions cicatricials, l'endoteli luminal desapareix i disminueix la microvasculatura neoformada.

2 - Les molècules d'adhesió endotelials selectina E i VCAM-1 són induïdes en els vasos de malalts afectats per PAN clàssica. La selectina E s'expressa en l'endoteli luminal de les lesions incipients i en algunes neollums de lesions establertes; i VCAM-1 s'expressa en l'endoteli luminal d'alguns vasos, fonamentalment lesions establertes amb llum encara preservada, i en alguns neovasos de lesions establertes.

3 - L'expressió de les molècules d'adhesió constitutives de l'endoteli ICAM-1, ICAM-2, PECAM-1 i selectina P disminueix en l'endoteli luminal de les lesions inflamatòries establertes i apareix en la neovascularització.

4 - La intensitat de l'expressió de les molècules d'adhesió endotelials es correlaciona amb l'estadi histològic de la lesió vascular i pot variar en diferents lesions d'un mateix pacient: en les lesions incipients l'expressió en l'endoteli luminal és intensa; en les lesions establertes l'expressió en l'endoteli luminal disminueix mentre que apareix en els vasos neoformats; i en les lesions cicatricials l'expressió en l'endoteli luminal és pràcticament nul.la i en les neollums disminueix.

5 - L'expressió endotelial de selectina E es correlaciona amb el nombre de neutròfils presents en l'infiltrat inflamatori.

6 - Hi ha una clara interacció entre diferents molècules d'adhesió: l'expressió endotelial de VCAM-1 es correlaciona topogràficament amb

l'expressió leucocitària de VLA-4, i l'expressió leucocitària d'ICAM-1 es correlaciona amb l'expressió leucocitària de LFA-1.

7 - No hi ha cap correlació entre l'expressió de les molècules d'adhesió estudiades en mostres histològiques de malalts amb PAN i les característiques clíniques dels pacients (edat, sexe, durada dels símptomes, resposta inflamatòria sistèmica).

8. El tractament amb corticoids a dosis baixes o durant un període curt de temps no modifica l'expressió de les molècules d'adhesió en les lesions vasculars de la PAN.

9 - En pacients amb PAN en activitat, els nivells de les formes solubles d'ICAM-1, VCAM-1 i selectina E circulants estan significativament elevats respecte els controls sans; els nivells de selectina L-s disminueixen, i els de selectina P-s no es modifiquen.

10 - Després d'un seguiment mig de 15 mesos (1-36 mesos), els nivells de les formes solubles d'ICAM-1 i selectina E persisteixen elevats en els pacients amb PAN encara que estiguin en remissió clínica.

11 - En pacients amb ACG en activitat, els nivells de la forma soluble d'ICAM-1 estan significativament elevats, però no els de les formes solubles d'ICAM-3, VCAM-1, selectina E ni selectina L.

12 - Els nivells d'ICAM-1 circulant disminueixen ràpidament després de la instauració del tractament en pacients amb ACG.

13 - El patró dels nivells de les molècules d'adhesió circulants varia entre les diferents vasculitis (PAN i ACG) tant en la malaltia en activitat com en l'evolució posterior al tractament instaurat.

EPÍLEG

En la present tesi s'ha estudiat l'expressió de les molècules d'adhesió cel·lular en dos tipus de vasculitis: la PAN, que es caracteritza per la coexistència de lesions inflamatòries en diferent estadi evolutiu en vasos de calibre petit i mitjà, i l'ACG, que afecta vasos grans, fonamentalment l'artèria temporal. Els resultats obtinguts permeten concloure el següent:

1 - L'expressió endotelial de molècules d'adhesió en les lesions de les vasculitis segueix un patró dinàmic durant els diferents estadis evolutius i es correlaciona topogràficament amb l'expressió dels respectius lligands pels leucòcits infiltrants, suggerint que les interaccions entre els leucòcits i l'endoteli mitjançades per les molècules estudiades juguen un paper fonamental en el desenvolupament dels infiltrats inflamatoris dels vasos.

2 - Els nivells de les molècules d'adhesió solubles circulants es modifiquen tant en la PAN com en l'ACG respecte els controls sans, però el patró que segueixen els nivells de les diferents molècules és diferent en les dues vasculitis estudiades tant en la malaltia en activitat com en l'evolució posterior a la instauració del tractament: en la PAN s'eleven els nivells de molècules d'origen endotelial i la majoria d'elles romanen elevades malgrat una remissió clínica i biològica, mentre que en l'ACG en activitat només s'eleven els nivells d'ICAM-1, que es normalitzen ràpidament amb la remissió clínica i biològica induïda pels corticoids. Aquestes diferències suggereixen un cert nivell d'especificitat entre diferents malalties.

Les nostres observacions han permès aprofundir en el coneixement de la patogènia de les vasculitis. Tot i que és molt prematur aventurar una utilitat concreta en el diagnòstic de les vasculitis estudiades, el control evolutiu o possibles nous tractaments, els resultats obtinguts sí que animen a continuar l'estudi en aquest sentit per aconseguir una millor comprensió de la patogènia d'aquestes i altres vasculitis enfocat a dissenyar millors estratègies terapèutiques.

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APÈNDIX

ORIGINALS

ARTHRITIS & RHEUMATISM Volume 37
Number 7, July 1994, pp 1055–1061

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF INFLAMMATORY CELLS AND IMMUNOLOGIC ACTIVATION MARKERS IN MUSCLE AND NERVE BIOPSY SPECIMENS FROM PATIENTS WITH SYSTEMIC POLYARTERITIS NODOSA

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Objective. To investigate the phenotype of infiltrating cells in classic lesions of polyarteritis nodosa (PAN).

Methods. Twenty-one muscle and 10 sural nerve biopsy samples from 24 patients with systemic PAN were studied using avidin–biotin–peroxidase and alkaline phosphatase–anti–alkaline phosphatase immunohistochemical techniques.

Results. The inflammatory infiltrates consisted mainly of macrophages (41%) and T lymphocytes (41%), particularly of the CD4+ subset. Granulocytes were present in varying quantities (0–45%) and were more abundant in heavily infiltrated vessels and in those with fibrinoid necrosis. Dendritic cells could be identified in 4 samples. Proliferating and interleukin-2 receptor–expressing cells, present in 71% and 79% of the patients, respectively, were more frequent in untreated patients.

Conclusion. T cell–mediated immune mechanisms may play a role in the development and perpetuation of PAN lesions.

Supported by grants from Fondo de Investigación Sanitaria (FIS 90/0638), CICYT (SAL 90/0408), and CIRIT (Generalitat de Catalunya).

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Submitted for publication July 9, 1993; accepted in revised form February 8, 1994.

The immunopathogenetic mechanisms leading to vascular injury in systemic necrotizing vasculitis of the polyarteritis nodosa (PAN) group are incompletely understood and are probably heterogeneous. PAN may occur during the course of hepatitis B and other viral infections (1,2), in association with hairy cell leukemia (3), or complicating the outcome of several autoimmune disorders such as Sjögren's syndrome or rheumatoid arthritis (4).

Irrespective of the triggering or causative agents or associated conditions, the precise mechanisms that ultimately cause damage to the vessel wall are not well understood. The potential of circulating immune complex deposition to damage the vessel wall, indicated on the basis of experimental animal models, provided important insights into pathogenetic mechanisms leading to vascular injury. According to these models, immune complexes would activate the complement cascade, whose activated products would in turn attract and activate neutrophils. For years, immune complex deposition has been the only proposed mechanism to explain vessel injury in PAN (5–7).

Immunohistochemical analysis of infiltrating cell phenotypes has provided interesting information regarding pathogenetic mechanisms involved in other immune-mediated diseases (8–11). However, this issue has remained virtually unexplored with regard to vascular lesions of systemic PAN, and only anecdotal cases or small series have been studied (12,13). Immunohistochemical studies performed on perineural and muscle vessels from heterogeneous groups of patients with peripheral nerve vasculitis have revealed that granulocytes are scarce in well-established lesions,

and T lymphocytes and macrophages have been identified as the predominant cell type (14–16). These observations suggest that other immunopathogenetic mechanisms might play an important role in the development or perpetuation of PAN lesions. In the present study, we further expanded this approach by investigating the immunophenotype of inflammatory cells, the expression of immunologic activation markers, and the effect of immunosuppressive therapy in muscle and nerve biopsy specimens from a homogeneous population of patients with classic PAN.

PATIENTS AND METHODS

Patients. The study group consisted of 24 patients (15 women and 9 men) with a histologic diagnosis of vasculitis. The average age was 66 years (range 23–77). All patients fulfilled the recently defined criteria for classification of systemic PAN (17). In all patients, PAN was considered to be a primary disease since no associated conditions could be identified.

The mean duration of disease prior to biopsy was 4.6 months (range 2 weeks–3 years). At the time biopsies were performed, all patients had clinical evidence of disease activity. Sixteen were untreated and 8 had received corticosteroid therapy. Two of these 8 patients had been treated for 2 weeks with prednisone (1 mg/kg/day) and cyclophosphamide (2 mg/kg/day) because of severe kidney involvement (creatinine >3 mg/100 ml) and a strongly suspected diagnosis of PAN. The remaining 6 were receiving prednisone at 10–60 mg/day because of clinical suspicion of giant cell arteritis (2 cases), systemic vasculitis (1 case), polymyalgia rheumatica (1 case), or late-onset rheumatoid arthritis (2 cases). These patients had been treated with adjusted steroid doses for a period ranging from 2 days to 3 years.

Tissue samples. Twenty-one muscle and 10 sural nerve biopsies were obtained from the patients for diagnostic purposes. In 7 patients, both muscle and nerve biopsies were performed simultaneously. Tissues were snap frozen in liquid nitrogen–prechilled methylbutane, and stored at −80°C.

All specimens exhibited at least 1 medium-sized or small vessel with a transmural infiltrate. In 10 samples, the inflammatory infiltrate was very dense and occupied the entire thickness of the vessel. In the remaining 21 samples, infiltrates were moderate and the structure of the vessel with its distinct layers was preserved. Seventeen specimens demonstrated fibrinoid necrosis.

Antibodies. Infiltrating cell phenotypes were determined with the following monoclonal antibodies: CD3 (Leu-4), CD4 (Leu-3a), CD8 (Leu-2a), CD22 (Leu-14) (all from Becton Dickinson Monoclonal Center, Mountain View, CA), anti–granulocytic elastase, and CD68 (EBM11) (both from Dako, Copenhagen, Denmark), recognizing T lymphocytes, T helper subset, T suppressor/cytotoxic subset, B lymphocytes, granulocytes, and macrophages, respectively. Interdigitating (dendritic) cells were detected by immunoreactivity to S-100 protein using a rabbit anti–S-100 polyclonal antiserum (Dako) (18). Endothelial cells were identified with the lectin *Ulex europaeus* (Dako), which binds fucose on endothelial cells and is considered to be an endothelial cell marker (19).

Infiltrating cell proliferative activity was assessed by detection of Ki-67 (Proliferating Ki67; Dako), a nuclear protein which is expressed in all but the G_0 phases of the mytotic cycle (20). The activation status of inflammatory cells was investigated by determining expression of interleukin-2 receptor (IL-2R) (CD25) (Becton Dickinson), transferrin receptor (CD71) (Dako), and class II antigens (HLA–DR) (Becton-Dickinson) of the major histocompatibility complex (MHC).

Immunostaining. For each sample, consecutive 4–8-μm cryostat sections were air dried overnight and fixed with cold acetone. Two to 4 serial sections per biopsy were incubated with each monoclonal antibody for 30 minutes at room temperature. Immunoreactivity was detected using the alkaline phosphatase–anti–alkaline phosphatase (APAAP) method (21).

S-100 protein immunoreactivity was detected using the avidin–biotin–peroxidase complex (ABC) method (Vector Laboratories, Burlingame, CA) (22). Binding of biotinylated *U europaeus* was also demonstrated with the ABC technique. In some cases, simultaneous detection of 2 different antigens in the same section was achieved by sequential immunostaining with both methods (APAAP and ABC).

Positively stained cells were counted by 2 independent investigators (M-CC and JMG) and expressed as the percentage of the total number of infiltrating cells visualized by hematoxylin counterstaining. When specimens contained more than one clearly involved vessel, the most strongly infiltrated vessel was evaluated. Variations in estimation between measurements made by the 2 investigators were <10%.

Statistical analysis. The Mann-Whitney U test, Student's *t*-test, Fisher's exact test, and Pearson's correlation coefficient were used for statistical analysis.

RESULTS

Inflammatory cell phenotypes. Table 1 shows the percentage of vessel-infiltrating cells found in all specimens examined. Macrophages and CD4+ T lymphocytes were the most predominant cell type. B lymphocytes were scarce and did not exceed 10% of cells. Interestingly, there were some differences in cell distribution between muscle and nerve biopsy samples. T lymphocytes were significantly more abundant in nerve than in muscle specimens (52% versus 35%; $P < 0.001$), whereas macrophages predominated in muscle specimens compared with nerve specimens (45% versus 33%; $P = 0.005$).

The presence of granulocytes was highly variable, ranging from 0% to 45% of inflammatory cells, and granulocytes were the most abundant cell type in only 2 samples from different patients. When possible

Table 1. Infiltrating cells in medium-sized and small vessels from patients with polyarteritis nodosa*

Cell type	All samples (mean ± SD)	Muscle samples (mean ± SD)	Nerve samples (mean ± SD)	P†
Macrophages	41 ± 11	45 ± 11	33 ± 9	0.005
T lymphocytes	41 ± 14	35 ± 12	52 ± 11	<0.001
Granulocytes	17 ± 13	20 ± 11	13 ± 14	NS
B lymphocytes	2 ± 3	1 ± 2	3 ± 3	NS
CD4:CD8 ratio	2.4 ± 1.6	2.9 ± 1.8	1.4 ± 0.5	0.001

* Except for the CD4:CD8 ratio, values are the percentage of infiltrating cells represented by the given cell type.
† Cell distribution in muscle versus nerve samples. NS = not significant.

variables influencing such distribution were evaluated, the percentage of granulocytes was found to be greater in heavily inflamed vessels (mean ± SD 24 ± 14%) than in those that were only moderately infiltrated (13 ± 12%) ($P = 0.046$). Granulocytes were also more abundant in vessels with fibrinoid necrosis (22 ± 13%) than in those without (12 ± 11%) ($P = 0.026$). Although granulocytes tended to be found more frequently in samples from patients whose clinical manifestations had been present for <3 months prior to biopsy (25 ± 10%) than in those whose samples were obtained later in the course of the disease (15 ± 12%), no significant correlation was observed between granulocyte percentage and disease duration. T lymphocytes were distributed all across the vessel wall, whereas macrophages tended to predominate in the periphery. Granulocytes, when present, were focally distributed in clusters (Figure 1).

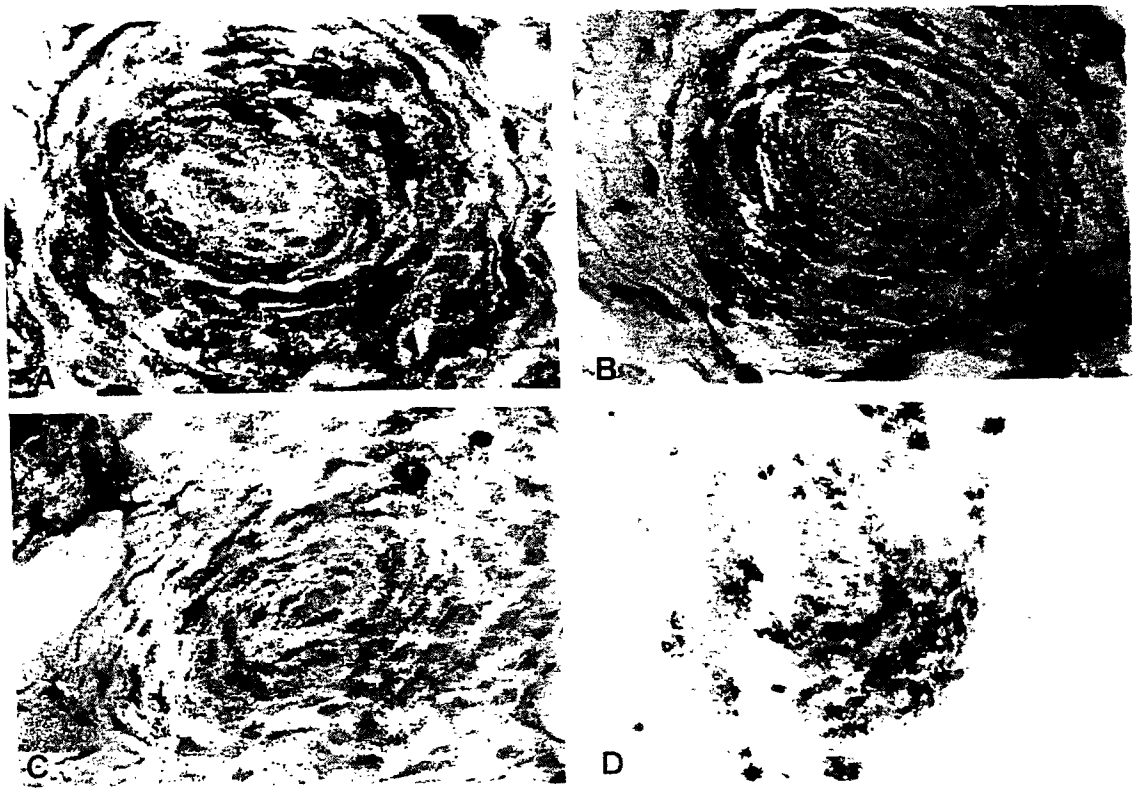

Figure 1. Distribution of A, macrophages (monoclonal antibody [MAb] EBM11), B, T lymphocytes (MAb CD3), and C, scarce granulocytes (MAb anti-granulocytic elastase) in serial sections of a typical perineural vessel with lesions of polyarteritis nodosa. D, Abundant granulocytes in a perineural vessel from a different patient. (Alkaline phosphatase–anti-alkaline phosphatase stained; hematoxylin counterstained.)

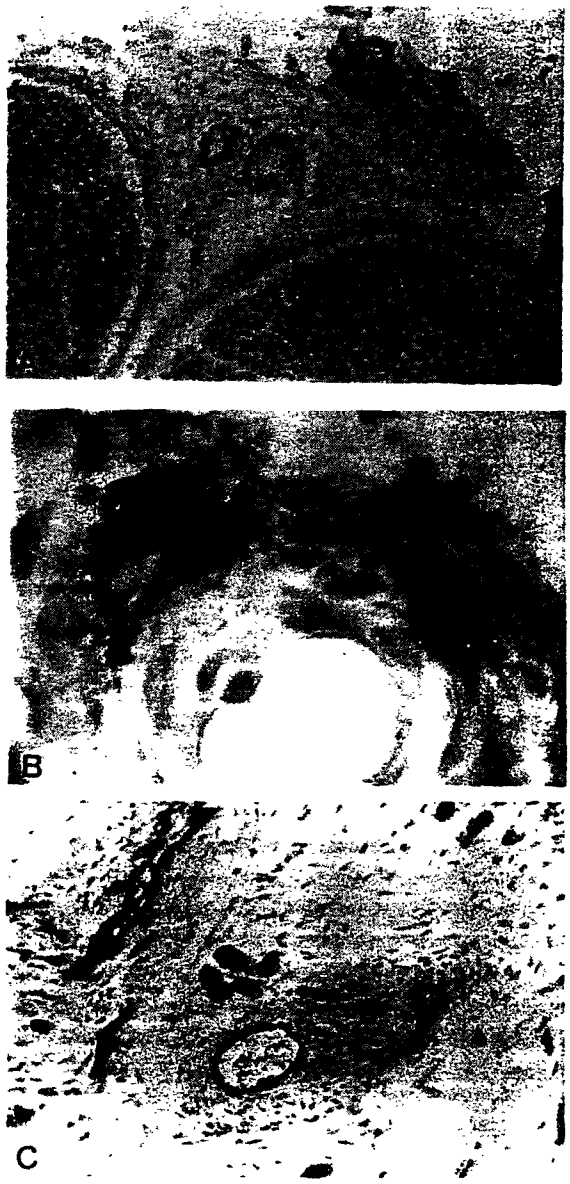

Figure 2. A, S-100–positive cells in a very slightly infiltrated perineural vessel. An S-100–negative artery with full-blown lesions is apparent in the upper part of the picture. B, Higher magnification view, showing dendritic prolongations of S-100–positive cells surrounding the scant lymphocytes present. C, Staining with *Ulex europaeus* reveals disappearance of the luminal endothelium in an obsolescent artery and clusters of newly formed capillaries within and surrounding the occluded vessel. (Avidin–biotin–peroxidase stained: hematoxylin counterstained.)

In 4 samples (1 nerve and 3 muscle biopsies from 4 different patients), S-100–positive cells were identified, always in slightly infiltrated, still well-preserved vessels. As shown in Figure 2, these cells exhibited a typical dendritic morphology with prolongations surrounding lymphocytes. Immunostaining of serial sections revealed that these cells strongly expressed DR antigens.

Activation/proliferation markers. IL-2R expression was demonstrated in samples from 19 (79%) of the patients. Positive cells were scarce and grouped in small clusters. They appeared to be lymphocytes by morphologic analysis and examination of CD3-stained serial sections. IL-2R expression was significantly more frequent in untreated patients (94%) than in specimens from treated individuals (50%) ($P = 0.027$) (Table 2).

Proliferating cells were also sparse. Typical nuclear Ki-67 immunoreactivity was observed in scattered cells from 17 patients (70%). Proliferating cells were also more frequent in untreated patients (88%) versus treated patients (38%) ($P = 0.021$) (Table 2). In 3 cases, double-staining with Ki-67 and CD3 was performed. Replicating cells belonged to 2 cell populations. Most cells were T lymphocytes, as indicated by concurrent CD3 expression. Some CD3-negative spindle-shaped cells in the thickened intimal layer were also found to be Ki-67 positive (Figure 3). These were probably myointimal cells contributing to intimal hyperplasia.

MHC class II DR expression was detected in 62 ± 24% of infiltrating cells (mean ± SD) and, when present, was always intensely positive. DR-expressing cells were mostly macrophages and T lymphocytes as assessed by examination of immunostained serial sections. No influence of therapy on DR expression was evident. In 2 cases the number of DR-positive cells greatly exceeded the number of macrophages and lymphocytes, and DR expression by smooth muscle cells was apparent. DR expression in the luminal endothelium of involved vessels was strongly positive in some vessels and faint and diffuse in others. Both patterns of DR expression frequently coexisted in the same sample. Endothelial cells from normal vessels were always DR positive. *U europaeus* stain on serial

Table 2. Relationship of interleukin-2 receptor (IL-2R) expression and Ki-67 expression to treatment in patients with polyarteritis nodosa

	IL-2R+	Ki-67+
Untreated patients (n = 16)	15	14
Treated patients (n = 8)	4*	3†

* $P = 0.027$ versus untreated patients.
† $P = 0.021$ versus untreated patients.

Figure 3. Proliferating T lymphocytes in lesions of polyarteritis nodosa. The specimen was double-stained with avidin–biotin–peroxidase (Ki-67; brown staining in the nuclear area) and alkaline phosphatase–anti–alkaline phosphatase (CD3; red staining in the cell membrane). Some CD3-negative, spindle-shaped cells in the intimal layer are also Ki-67 positive.

sections was imperceptible in DR-negative endothelial cells, or disclosed a slight, blurred positivity, probably indicating that endothelium was damaged. In contrast, clusters of newly formed capillaries were evident within inflammatory infiltrates or surrounding occluded vessels in an obsolescent stage (Figure 2).

Transferrin receptor expression was assessed in 15 samples. It was positive in 23 ± 12% of inflammatory cells, which included both lymphocytes and macrophages. Percentages of transferrin receptor–expressing cells showed some variability among samples. No differences in transferrin receptor expression were found between muscle and nerve samples, and no influence of treatment or disease duration could be demonstrated.

DISCUSSION

Inflammatory infiltrates in muscle and nerve vessels from our series of PAN patients consisted mainly of macrophages and T lymphocytes, particularly of the CD4+ subset. Infiltrating cells exhibited immunologic activation markers such as IL-2R, transferrin receptor, and MHC class II (DR) antigen expression.

As reported in other autoimmune diseases (9,23,24), IL-2R–expressing cells were scarce in these samples. IL-2R expression has a pivotal role in autocrine-stimulated T cell growth triggered by antigen recognition (25). Its expression is an early event after T cell activation, and it is subsequently downregulated (25). Consistent with the low percentage of IL-2R–expressing cells, there were only a few proliferating T lymphocytes in PAN lesions. Studies in animal models of autoimmune diseases have demonstrated that lymphocyte proliferation occurs early after antigen stimulation and subsequently decreases (26). Furthermore, most lymphocytes proliferate in lymphoid organs, and a lesser percentage replicate in target tissues (26).

In contrast, most of the infiltrating cells expressed DR antigens. DR-expressing cells essentially comprised macrophages and T lymphocytes. In 2 cases, smooth muscle cells were also DR-immunoreactive. Dissociation between IL-2R and DR expression has been observed in other autoimmune diseases (9,23), which is not surprising since they can be mediated by independent pathways (27). In addition, class II antigen expression appears later after T cell activation and remains longer than IL-2R (28).

Endothelium from both normal and involved vessels expressed DR antigens. DR immunoreactivity in the luminal endothelium from some strongly inflamed vessels was negative, and endothelial damage and even disappearance was demonstrated by staining of serial sections with *U europaeus*. Concurrently, clusters of newly formed capillaries were observed. This observation suggests that at some point in the inflammatory process, endothelial cells are injured and neovascularization occurs.

In a small percentage of patients, we were able to identify S100-positive dendritic cells in incipient lesions. Dendritic cells have a pivotal role in initiating the immune response, due to their antigen-presenting function and their ability to provide accessory signals for T cell activation (29,30). Dendritic cells have been identified in target tissues of several autoimmune diseases, including rheumatoid arthritis (31), primary biliary cirrhosis (32), inflammatory vasculopathy of chronic allograft rejection (33), giant cell arteritis (9), and experimental autoimmune diabetes (34). As observed in our samples, these cells are usually detected in incipient inflammatory infiltrates and become rare in well-established or scarring lesions (9,31,33).

Although corticosteroid dosage and duration of therapy were quite heterogeneous, a striking inhibitory effect of corticosteroids on IL-2R expression and on lymphocyte proliferation was observed. At the time of the biopsy all patients were symptomatic. This suggests that vessel damage and clinical manifestations do not depend solely on these initial events of T

cell activation in which only a small percentage of cells are participating in well-established lesions. In contrast, no effect of treatment was observed on cell density, distribution of cell subsets, or HLA–DR or transferrin receptor expression. Therefore, other effector mechanisms are crucial in the perpetuation of lesions and require higher steroid doses, more sustained therapy, or the complementary effects of another immunosuppressive agent.

Immune complex deposition with complement activation and subsequent neutrophil chemotaxis has been considered a crucial mechanism leading to vascular injury in PAN (5–7). More recently, antineutrophil cytoplasmic antibodies (ANCA) have received a great deal of attention as a possible pathogenetic factor in the development of endothelial damage in the so-called microscopic variant of PAN, where small vessel, capillary, and glomerular involvement have major relevance (35–37). However, granulocytes, which are leukocytes primarily attracted by complement activation products and the main target for ANCA, were not the predominant cell type in medium-sized and small vessels from patients with classic PAN. Granulocytes were found more consistently in heavily infiltrated vessels with fibrinoid necrosis. Conversely, activated macrophages and CD4+ T lymphocytes were the most abundant cells in our samples.

The phenotype of infiltrating cells and the pattern of activation found in PAN lesions closely resemble those found in organ-specific autoimmune disorders such as rheumatoid arthritis (8,23) or Sjögren's syndrome (24) and in experimental models of these and other autoimmune diseases (26,38,39). A similar profile has also been identified in other vasculitides involving medium-sized vessels, such as coronary arteries in Kawasaki disease (40), temporal arteries in giant cell arteritis (9), or vasculitis in autoimmune MRL-*lpr* mice (41).

Our findings suggest that T cell–mediated immune mechanisms may be very important in the development or perpetuation of PAN lesions in medium-sized and small vessels of muscle and nerve. A better understanding of specific immunopathogenetic mechanisms involved in the development of PAN is necessary to design more precise and efficient and less harmful therapeutic approaches than are currently available.

ACKNOWLEDGMENTS

We thank Dr. Gary S. Hoffman (Cleveland Clinic Foundation, Cleveland, OH) for carefully reviewing the manuscript and providing useful suggestions and comments, and Nuria Barros, Esther Tobías, Elena Gonzalbo, and Anna Picazo for excellent technical assistance.

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Clin Exp Immunol 1996, 104 467–472

Leucocyte/endothelial cell adhesion receptors in muscle biopsies from patients with idiopathic inflammatory myopathies (IIM)

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(Accepted for publication 28 February 1996)

SUMMARY

Interactions between leucocytes and endothelial cells through specific adhesion receptors play an increasingly recognized crucial role in the development of inflammatory infiltrates in chronic inflammatory diseases. In this study we investigated adhesion molecule expression in muscle biopsies from 18 dermatomyositis, six polymyositis, five inclusion-body myositis patients and from eight normal controls. Immunohistochemical detection of leucocyte integrins LFA-1 and VLA-4, their endothelial counter-receptors intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and the endothelial cell markers CD31 and von Willebrand factor-related antigen (vWFAg) was performed using specific MoAbs and an alkaline phosphatase anti-alkaline phosphatase technique. ICAM-1 expression was up-regulated and VCAM-1 induced in muscle capillaries of dermatomyositis samples. In both dermatomyositis and polymyositis, endothelial cells from vessels surrounded by inflammatory infiltrates strongly expressed ICAM-1 and VCAM-1. Infiltrating leucocytes were intensively LFA-1- and VLA-4-positive. These data suggest that leucocyte/endothelial cell interactions mediated by the receptor/ligand pairs LFA-1/ICAM-1 and VLA-4/VCAM-1 actively participate in the development of muscle inflammatory infiltrates in the major inflammatory myopathies. In addition, ICAM-1 and VCAM-1 over-expression by capillary endothelial cells in dermatomyositis supports the hypothesis that capillary activation and/or injury is a major feature in this disease.

Keywords adhesion molecules inflammation myopathies

INTRODUCTION

Autoimmune phenomena are considered to play a crucial role in the pathogenesis of the major IIM: dermatomyositis (DM), polymyositis (PM) and inclusion body myositis (IBM). Muscle inflammatory infiltrates, the hallmark of IIM, seem to be the consequence of different pathogenic mechanisms in each of these diseases [1–3]. On the basis of immunopathologic studies, muscle capillaries are considered a major target of immune reaction in DM. Indeed, typical focal histopathological features such as capillary loss with subsequent muscle ischaemia, and perifascicular atrophy, can be found early in the course of DM [4–7]. In addition, immunohistochemical studies have revealed deposition of the complement C5b-9 membrane attack complex in skin and muscle microvasculature of a significant number of DM biopsy samples, particularly in those from patients with recent onset of muscle weakness [8–11].

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On the other hand, antigen-specific, MHC I-restricted T cell-mediated cytotoxicity is considered a pivotal mechanism leading to muscle damage in PM, where $CD8^+$ T lymphocytes are the major infiltrating population [1–3]. Supporting this hypothesis, $CD8^+$ T lymphocytes from patients with IIM, particularly PM, exhibit cytotoxicity against autologous myotubes in culture [12]. The primary participation of autoimmune mechanisms in IBM is, at present, less clear.

Over the past few years the relevance of leucocyte/endothelial cell interactions in the development of inflammatory lesions has been increasingly recognized. Such interactions occur through a complex array of surface adhesion receptors. On the leucocyte membrane, molecules of the selectin (L-selectin), integrin (CD18–CD11 complex, VLA-4), and immunoglobulin (CD31) families interact, in a sequential and precisely regulated manner, with specific counter-receptors on the endothelial cell surface. These include carbohydrates (sialylated forms of Lewis antigens) and members of the selectin (P-selectin, E-selectin) and immunoglobulin families (intercellular adhesion molecule-1 (ICAM-1), ICAM-2, vascular cell adhesion molecule-1

(VCAM-1), and CD31) [13–16]. These receptors are induced, increased, or functionally regulated by cytokines, chemokines, steroid hormones, and by other cell–cell interplays [13–19]. Such interactions have been demonstrated to be essential for the recruitment of leucocytes in the sites of injury [20–22].

In the present study, we investigated the expression of endothelial cell adhesion molecules and their leucocyte counter-receptors in muscle biopsies from patients with IIM. The aims of our study were to identify receptors potentially involved in the development of inflammatory infiltrates, and to evaluate the activation status of the muscle vascular endothelium in different inflammatory myopathies.

PATIENTS AND METHODS

Patients

The study group consisted of 29 patients with IIM who underwent muscle biopsy for diagnostic purposes. The specimens showed characteristic changes of DM ($n = 18$), PM ($n = 6$) and IBM ($n = 5$) according to well defined clinical and histological criteria [4,23].

The age average was 57 years (range 3–80 years) in DM patients, 57 years (range 18–75 years) in PM, and 39 years (range 12–65 years) in IBM individuals. The male/female ratio was 3/15 in DM, 1/5 in PM and 1/4 in IBM. At the time when biopsies were obtained, all patients had active disease on clinical and histological grounds.

The control group included eight histologically normal muscle samples from normal volunteers (seven males and one female) aged 41 years (range 30–58 years).

Tissues were snap-frozen in isopenthane precooled in liquid nitrogen and stored at −80°C.

Antibodies

Endothelial cells were identified with a rabbit anti-human von Willebrand factor-related antigen (vWFAg) or with a CD31 (PECAM-1) MoAb (clone JC/70A) (Dako Corp., Carpinteria, CA). ICAM-1 (CD54) expression was determined using the MoAb RR 1/1, kindly provided by Timothy A. Springer (Harvard University, Boston, MA). VCAM-1 (CD106) was detected with the MoAb 4B9, a generous gift from Roy R. Lobb (Biogen Inc., Cambridge, MA). E-selectin (endothelial leucocyte adhesion molecule-1 (ELAM-1)) was identified with the MoAb H18 7 and a polyclonal rabbit anti-human E-selectin, kindly provided by Michael A. Gimbrone Jr (Harvard University) and Roy R. Lobb, respectively. The β common chain of the leucocyte β_2 integrins, CD18, was identified with the MoAb MHM23 (Dako). The $\alpha 4$ chain (CD49d) of the VLA-4 ($\beta 1\alpha 4$) receptor was identified with the MoAb HP2/1, a generous gift from Francisco Sánchez-Madrid (Hospital La Princesa, Madrid, Spain). MoAb HP 2/1, supplied as hybridoma supernatant, was employed at 1:5 dilution. RR 1/1 ascitis was tested at a range of dilution between 1 : 1000 and 1 : 40 000 as detailed below. The remaining (purified) MoAbs were used at 1 : 100 dilution (5–10 μg/ml).

Immunostaining

Serial 4–8 μm frozen sections were air-dried overnight, fixed with cold acetone, and incubated at room temperature with each primary antibody for 30 min. MoAb immunoreactivity was detected using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method [24] (Dako). For polyclonal rabbit anti-human antibodies, a polyclonal rabbit anti-mouse immunoglobulin bridge (Dako) was applied before the APAAP complex.

Quantification

For each condition, two to four consecutive sections were evaluated and a minimum area of 0·5 mm^2 was measured. When lesions were focal, involved and relatively preserved areas were considered together and independently. Since immunostaining detects molecules which may change depending on the biological satus of the cell, we quantified muscle microvessel content using antibodies raised against two different molecules: vWFAg and CD31. Microvessels immunostained for endothelial cell markers (vWFAg, CD31) were quantified using a computerized digital analyser (Microm Image Processing IMCO, Kontron Electronik, Munich, Germany) and expressed as average microvessel number per fibre as described [25]. E-selectin, ICAM-1 and VCAM-1 microvessel expression was evaluated as percentage of vWFAg or CD31-positive microvessels. Endothelial cell immunoreactivity in small/medium sized vessels either normal or surrounded by inflammatory infiltrates was estimated as positive or negative. Inflammatory infiltrates (perivascular or endomysial) were also evaluated for leucocyte expression of adhesion receptors. Positive leucocytes were visually counted by two independent investigators (J.-M.G. and M.-C.C.) and

Table 1. Microvessel number/muscle fibre in IIM as estimated by CD31 (PECAM-1) and von Willebrand factor-related antigen (vWFAg) immunostaining

	CD31			vWFAg		
	N	P	T	N	P	T
DM	0·89 ± 0·38*	0·75 ± 0·38**	0·86 ± 0·45*	0·54 ± 0·18**	0·42 ± 0·16***	0·47 ± 0·27***
PM	0·93 ± 0·21	0·97 ± 0·24	0·97 ± 0·20	0·72 ± 0·22	0·68 ± 0·16*	0·70 ± 0·20
IBM	0·78 ± 0·43	0·75 ± 0·34	0·75 ± 0·37*	0·56 ± 0·26*	0·48 ± 0·19**	0·52 ± 0·23*
C	1·23 ± 0·27		1·23 ± 0·27	0·96 ± 0·31		0·96 ± 0·31

C, Control; N, preserved (relatively normal) areas; P, involved (pathologic) areas; T, total; DM, dermatomyositis; PM, polymyositis; IBM, inclusion body myositis.

*$P < 0·05$ *versus* C; **$P < 0·01$ *versus* C; ***$P < 0·001$ *versus* C.

Table 2. Adhesion molecule expression by muscle microvasculature in IIM measured as percentage of von Willebrand factor-related antigen (vWFAg)-positive microvessels also expressing intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) or E-selectin

	ICAM-1			VCAM-1	E-selectin
	N	P	T	T	T
DM	105 ± 52	135 ± 44**	125 ± 53*	30 ± 30**	0
PM	108 ± 66	117 ± 52	112 ± 55	13 ± 9	0
IBM	74 ± 41	68 ± 26	68 ± 34	0 ± 1	0
C	78 ± 23		78 ± 23	4 ± 5	0

*$P < 0.05$; **$P < 0.001$.
DM, Dermatomyositis; PM, polymyositis; IBM, inclusion body myositis.

expressed as percentage of total infiltrating cells as assessed by haematoxylin counterstaining.

Non-parametric Mann–Whitney *U*-test was employed for statistical analysis.

RESULTS

Adhesion molecule expression in muscle microvessels

Microvasculature content was remarkably reduced in both preserved and involved areas of DM specimens compared with controls, as assessed by vWFAg and CD31 immunostaining (Table 1) (Fig. 1a,b). A slight reduction in microvessel number was also seen in PM and IBM (Table 1). vWFAg was more specific in detecting vessels in our samples, since CD31 was also expressed by leucocytes (Fig. 1a). Therefore, vWFAg immunoreactivity was subsequently employed to evaluate the percentage of adhesion molecule-expressing microvessels.

ICAM-1 was constitutively expressed by the endothelium of muscle vessels of all sizes. However, immunostaining was much more intense in DM microvessels than in PM or control biopsies. In order to quantify this observation a very high dilution of RR 1/1 ascitis (1:40 000) was employed. At this MoAb dilution, a significant proportion of endothelial cells in normal, PM and IBM biopsies were negative, whereas DM microvessels still showed intense immunoreactivity (Fig. 1c,d,e). The amount of ICAM-1-expressing microvessels (assessed as percentage of vWFAg-positive microvessels) was close to normal in relatively preserved areas of DM samples, but was significantly higher in involved regions where, in some cases, the number of ICAM-1-expressing microvessels was higher than the number of those immunoreactive for vWFAg (Table 2). ICAM-1-expressing microvessels in PM and IBM did not differ from the control population. VCAM-1 expression was negative in vessels of normal biopsies. Only a few scattered cells in the interstitium, probably resident macrophages, were VCAM-1-immunoreactive. VCAM-1 microvessel expression was significantly increased in DM, whereas in PM and IBM it did not differ from the control population (Table 2). No E-selectin expression was observed in muscle microvessels in normal or pathologic samples using two different antibodies at a range of dilutions that was effective in detecting cytokine-induced E-selectin in cultured endothelial cells [18].

Adhesion molecule expression in small and medium-sized vessels

Endothelium from small and medium-sized vessels constitutively expressed ICAM-1. VCAM-1 was only detected on endothelial cells of vessels surrounded by inflammatory infiltrates in both DM and PM (Fig. 1g). These endothelial cells also stained more intensively for ICAM-1. E-selectin expression could not be convincingly demonstrated in vessels of any size.

Adhesion receptors in inflammatory infiltrates

Dense inflammatory infiltrates were observed in 16 samples (14 DM and two PM). Infiltrating leucocytes strongly expressed β_2 (CD18) and β_1 (VLA-4) integrins (Fig. 1h). In addition, a notable percentage of infiltrating cells expressed ICAM-1, VCAM-1 and CD31. Although the number of available PM samples with dense inflammatory infiltrates was small and therefore no definitive conclusions can be drawn, no significant differences in adhesion receptor expression by inflammatory cells were found between DM and PM (Table 3). Nearly 100% of phagocytic cells invading necrotic muscle fibres intensively expressed the adhesion molecules tested. This was observed similarly in DM, PM, and IBM specimens.

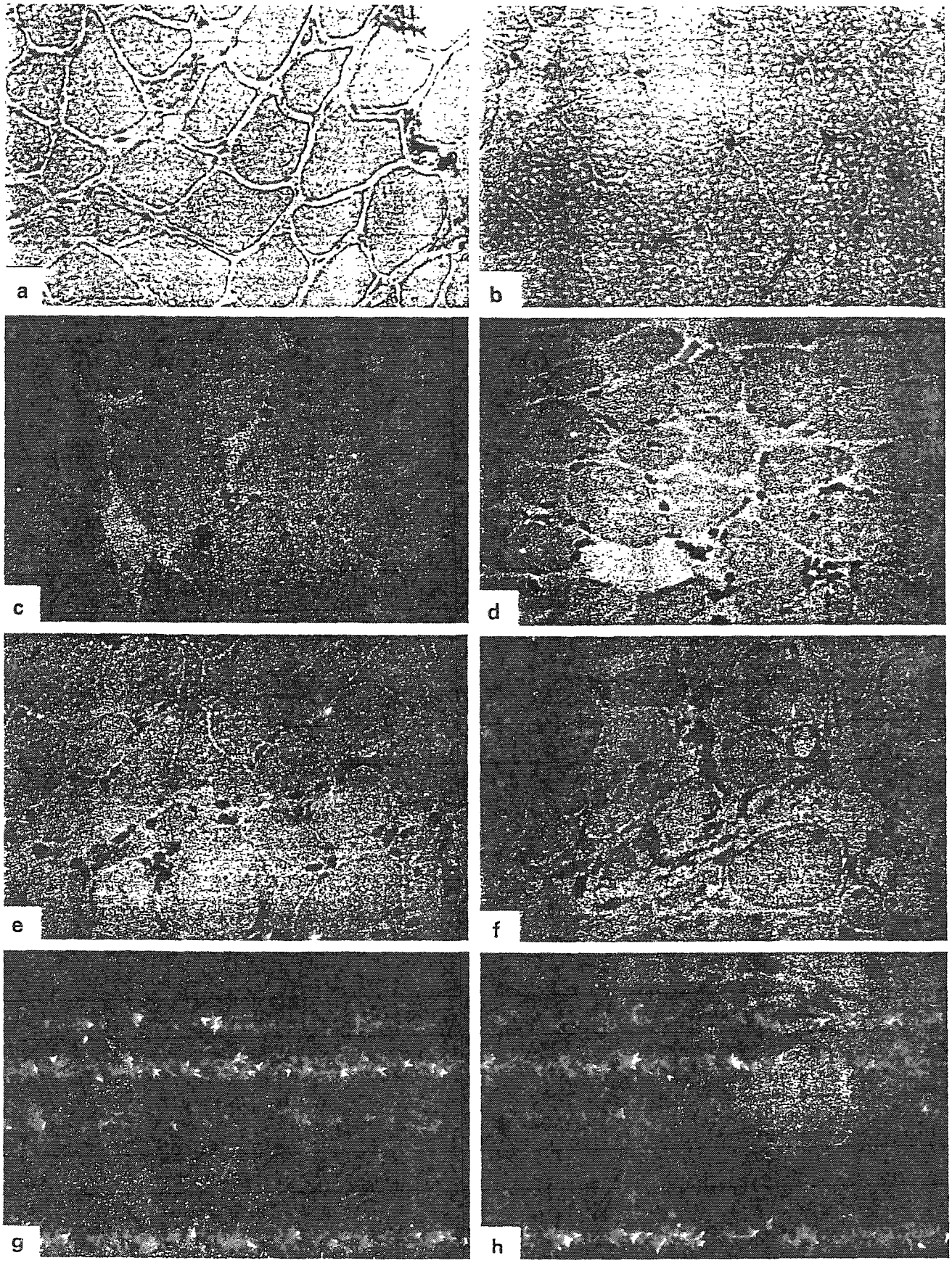
Adhesion molecule expression by muscle fibres

In nine DM biopsies (50%), ICAM-1 immunoreactivity was detected on the sarcolemma of scattered muscle cells which corresponded mostly to atrophic or regenerating fibres. ICAM-1 expression by muscle fibres normal in appearance was also noticed (Fig. 1f). In four DM specimens (22%), regenerating fibres also expressed VCAM-1. In two PM samples, ICAM-1 and VCAM-1 expression by partially invaded muscle fibres was evidenced (data not shown). No adhesion molecule expression by muscle cells was observed in IBM or control biopsies.

Table 3. Adhesion molecule expression by infiltrating leucocytes in IIM

	CD18 ($\beta 2$) (%)	CD49d ($\alpha 4$) (%)	ICAM-1 (%)	VCAM-1 (%)
DM	94 ± 5	66 ± 17	52 ± 24	43 ± 18
PM	80 ± 0	80 ± 0	30 ± 0	20 ± 14

ICAM-1, Intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; DM, dermatomyositis; PM, polymyositis.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DISCUSSION

Microvasculature content, as assessed by vWFAg or CD31 immunostaining, was significantly depleted in muscle samples from IIM patients. Similar observations were previously reported by our group and others using evaluation systems such as semithin section examination, *Ulex europaeus* lectin staining, and electron microscopy [4–7]. A slight decrease in microvessel content was also observed in PM and IBM, in agreement with previous findings [25]. Although endothelial expression of CD31 is more widely distributed than vWFAg [26], vWFAg was more accurate in detecting vessels in our system, since CD31, also expressed by leucocytes [27], stained resident macrophages, pericytes, and interstitial inflammatory cells.

The expression of ICAM-1 was up-regulated in DM microvessels, and VCAM-1, which was absent from normal muscle vessels, was present on a significant proportion of DM microvessels. Microvessels expressing ICAM-1 and VCAM-1 were not always in close proximity to clusters of inflammatory cells, suggesting that the expression of these adhesion molecules may not be induced exclusively in a paracrine fashion by cytokines released from inflammatory cells. Several investigators have demonstrated the presence of the final product of the complement activation cascade C5b-9 in muscle capillaries of DM patients, which implies that capillary endothelial cells are an early target of complement-mediated injury [8,9]. In view of the fact that other authors have demonstrated that C5a binds to endothelial cells and up-regulates adhesion molecule expression [28], it is possible that ICAM-1 and VCAM-1 expression by microvessels in DM may be induced by complement activation products or complement-mediated sublethal endothelial cell injury. Moreover, sublethal injury stimulates IL-1α production by endothelial cells and this cytokine induces ICAM-1 expression in an autocrine manner [29]. We could not satisfactorily detect the presence of E-selectin in any of the vessels present in our patient samples. Recently, other authors, using a different E-selectin MoAb, demonstrated E-selectin expression on endothelial cells in muscle biopsies from patients with systemic lupus erythematosus independently of the presence of inflammatory infiltrates [30]. Thus, the pattern of adhesion molecule expression may vary in different autoimmune diseases. Moreover, we have recently demonstrated that E-selectin is expressed in cutaneous vessels of skin biopsies from patients with DM [11]. E-selectin on endothelial cells binds to sialylated forms of carbohydrates related to Lewis x and Lewis a antigens [13,16,31]. It has also been shown that E-selectin can bind to cutaneous lymphocyte antigen (CLA), a related carbohydrate expressed by T lymphocytes with cutaneous tropism [32]. Lack of E-selectin expression in muscle vessels and its presence in simultaneously biopsied skin lesions from the same patients provide further evidence that the pattern of adhesion molecule expression contributes to tissue specificity in chronic inflammatory diseases.

In some instances ICAM-1 and VCAM-1 expression by muscle fibres was observed in both PM and DM, an observation that agrees with others [33,34]. Since both ICAM-1 and VCAM-1 are induced by interferon-gamma (IFN-γ) or tumour necrosis factor-alpha (TNF-α) on cultured myotubes [35], their expression by muscle cells could be a consequence of the inflammatory reaction of these diseases. The expression of ICAM-1 by target cells is necessary for T cell-mediated cytotoxicity [36] and, consequently, its expression by muscle cells could facilitate muscle damage. On the other hand, VCAM-1 is expressed during normal muscle development [37] and its presence on muscle fibres might represent a regenerative process.

In the present study, endothelial cells from small and medium-sized vessels that were surrounded by inflammatory infiltrates, strongly expressed VCAM-1 and ICAM-1. Perivascular leucocytes had a high expression of CD18 and CD49d. CD18 is the β-chain common to LFA-1 and Mac-1 [13–16]. CD49d is the α4 chain shared by VLA-4 (β1α4) and β7α4 integrins and recognizes VCAM-1 and fibronectin [13–16]. Lymphocytes and macrophages express CD18 and CD49d and strong integrin expression characterizes the phenotype of migrating cells [38]. Although virtually all circulating leucocytes express CD18, surprisingly, about 20% of infiltrating leucocytes, particularly in PM infiltrates, were $CD18^-$. We have observed a similar phenomenon in vasculitis infiltrates (unpublished observations). Since leucocyte integrins undergo conformation changes upon activation, it is possible that the anti-CD18 MoAb used in the present study does not recognize activated epitopes. Overall, our observations suggest that interactions between LFA-1/ICAM-1 and VLA-4 and potentially β7α4/VCAM-1 are important in the development of muscle inflammatory infiltrates in IIM. Even though the distribution of infiltrating lymphocytes is different in PM ($CD8^-$ T lymphocyte predominance) from that in DM (presence of B lymphocytes) [1,2,40], it appears that both disorders utilize similar adhesion/migration pathways.

Current evidence emphasizes the potential involvement of LFA/ICAM-1 and VLA-4/VCAM-1 interactions in the development of inflammatory lesions [20,21,41–45]. Moreover, MoAbs blocking functional epitopes of adhesion molecules have efficiently prevented the development of inflammatory lesions and clinically apparent disease in animal models of several autoimmune disorders [46–49], and some of these antibodies have entered phase I/II clinical trials [41,50]. Our results suggest that IIM, particularly DM and PM, might also be considered among the autoimmune diseases that could potentially benefit from anti-adhesion therapy.

Fig. 1. CD31 (PECAM-1)-expressing microvessels are depleted in dermatomyositis (DM) (a) compared with control (b) muscle biopsies. $CD31^-$ infiltrating leucocytes can also be seen in DM (a). At a very high dilution, intercellular adhesion molecule-1 (ICAM-1) expression is hardly apparent in muscle microvessels from control (c) and polymyositis (PM) (d) muscle specimens, whereas a remarkable immunostaining remains in DM samples (e). ICAM-1 expression by muscle fibres can be observed in biopsy specimens from DM patients (f). Vascular cell adhesion molecule-1 (VCAM-1) is intensively expressed by endothelial cells from vessels surrounded by inflammatory infiltrates in DM (g). In serial sections from the same specimen, strong VLA-4 (h) positivity in infiltrating leucocytes can be observed. (APAAP technique with the MoAbs described in Patients and Methods. Counterstaining with haematoxylin.)

ACKNOWLEDGMENTS

Supported by a grant from CICYT (SAL 90/0408) and FIS (95/0860). Results partially presented at the 11th International Meeting on

Neuromuscular Diseases, Marseille (France), September 1992, at the 45th Annual Meeting of the American Academy of Neurology, New York, NY, April 1993 (Neurology 1993; 43:A202-A203), and at the First Congress of European Federation of Neurological Societies, Marseille (France), September 1995 (Eur J Neurol 1995; 2:100). We are deeply indebted to Drs T. A. Springer, R. R. Lobb, M. A. Gimbrone Jr., and F. Sánchez-Madrid for kindly providing MoAbs, and to Dr J. M. Nicolás for his advice in statistical analysis.

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British Journal of Rheumatology 1996;35:796–799

CASE REPORT

POLYARTERITIS NODOSA IN HUMAN IMMUNODEFICIENCY VIRUS INFECTION: REPORT OF FOUR CASES AND REVIEW OF THE LITERATURE

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SUMMARY

Four patients with HIV infection were diagnosed as having panarteritis nodosa type vasculitis in muscle samples taken by biopsy. Fever, weight loss and neuromuscular symptoms were the major complaints. All but one were successfully treated with a low dose of prednisone. The other patient refused treatment, but her muscular complaints did not worsen during 9 months of follow-up. Previously reported cases in the literature are reviewed.

KEY WORDS: AIDS, HIV, Necrotizing arteritis, Rheumatic manifestations, Vasculitis, Panarteritis nodosa, Prednisone, Neuromuscular symptoms, Muscle biopsy, Muscle atrophy.

POLYARTERITIS nodosa (PAN) is a multisystem life-threatening disease characterized by inflammation and necrosis of small and medium-size arteries. Typically, it involves renal and other visceral arteries with sparing of the pulmonary circulation [1]. Nonetheless, in the last few years, limited forms of the disease localized to skin, muscle and other organ systems have been described, usually associated with a better prognosis and good response to relatively low doses of corticosteroids alone [1–3].

Although the aetiology remains unknown, in some instances, PAN may be associated with different infectious, collagen and neoplastic diseases that have a high incidence of circulating immune complexes [1]. Recently, cases of PAN associated with HIV infection have been described. However, there are no large series that allow evaluation of the clinical course, prognosis and management of these patients. We describe four new cases of PAN in this setting, and review previously reported cases in the literature. The present cases come from a cohort of 120 HIV patients who underwent muscle and/or nerve biopsy for several reasons in the past 5 yr. During this period, our hospital has looked after nearly 2000 HIV patients.

CASE REPORTS

Case 1

A 38-yr-old female with a past history of i.v. drug abuse and HIV infection was admitted because of a 3 day history of fever, chills and generalized arthromyalgias. She also referred with a weight loss of 10 kg for the last 3 weeks, and progressive weakness over the last 6 months. Physical examination was only remarkable for a temperature of 37.7°C and evident muscular atrophy. Laboratory data showed an erythrocyte sedimentation rate (ESR) of 104 mm/h, haemoglobin of 11.8 g/dl, leucocytes of 2310×10^6/l, with a normal differential count. CD4 lymphocyte count was 220×10^6/l. Hepatitis B surface antigen (HBsAg), hepatitis B core antibodies (HBcAb) and hepatitis C antibodies were all negative. After work-up to rule out opportunistic infection, a biopsy of her right gastrocnemius showed medium-sized necrotizing vasculitis of polyarteritis type. Prednisone was started at a dose of 0.5 mg/kg/day. Fever remitted within the next 3 days. Weakness decreased and her general condition improved within the next month. Five months later, prednisone was discontinued. The patient remained asymptomatic during 12 months of follow-up.

Case 2

A 37-yr-old male with a previous history of i.v. drug abuse and HIV infection was admitted because of a 10 day history of fever, night sweats and generalized myalgias. He also referred with progressive weakness and a 10 kg weight loss during the last year. He had started zidovudine therapy 18 months prior to admission because his CD4 lymphocyte count fell to 50×10^6/l. Physical examination revealed a cachectic chronically ill-appearing male with low-grade fever and generalized muscular atrophy. Osteotendinous reflexes were diminished in the lower limbs. Admission laboratory studies were remarkable only for ESR of 91 mm/h. Hepatitis C and HBcAb were both positive, but HBsAg was negative. Electromyography (EMG) revealed slight nerve motor conduction slowness and a myopathic pattern. A right gastrocnemius muscle biopsy was performed. Histopathological findings were consistent with PAN-type vasculitis. He was given prednisone 0.5 mg/kg/day, showing progressive recovery. Three months later, prednisone was tapered to 0.2 mg/kg/day. Treatment was discontinued by the sixth month. The patient remained symptom free 12 months later.

Submitted 12 October 1995; revised version accepted 2 February 1996.

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Case 3

A 27-yr-old male, a former i.v. drug abuser on methadone maintenance, was known to be HIV positive 8 yr ago when admitted because of lymphatic tuberculosis. He also had a history of chronic liver disease due to hepatitis C. HBsAg and HBcAb were both negative. CD4 lymphocyte count was 310×10^6/l. He was receiving antiretroviral therapy with didanosine. He was admitted because of a 3 day history of fever. He also referred with progressive weakness for the past 3 months. On examination, his temperature was 37.2 C, he had generalized muscular atrophy, and liver and spleen enlargement. The rest of the examination was unremarkable. Laboratory tests showed ESR 60 mm/h, platelets 99×10^9/l and total proteins 102 g/l, with polyclonal hypergammaglobulinaemia. Chest X-ray was normal. Blood and urine cultures were negative. Empirical antibiotic therapy was started. The patient remained feverish and his general condition worsened during the admission. A right gastrocnemius muscle biopsy was finally performed. Histopathological study showed several arteries with inflammatory infiltration of the wall and fibrinoid necrosis. He was given 0.5 mg/kg/day of prednisone. Fever remitted in 2 days and his general condition progressively improved. The patient died 4 months later at home. Autopsy studies were not authorized.

Case 4

A 27-yr-old woman, a former i.v. drug abuser, diagnosed with AIDS 2 yr before admission because of oesophageal candidiasis. Her CD4 lymphocyte count was 14×10^6 l. Hepatitis C and HBc antibodies were positive, but HBsAg was negative. She was receiving antiretroviral therapy with zidovudine. The patient was seen in the out-patient clinic where she complained of a 6 month history of progressive weakness and myalgias. Physical examination revealed generalized muscular atrophy. An EMG study showed a myopathic pattern in the lower limbs. A muscular biopsy in her right gastrocnemius was performed. Histopathological findings confirmed necrotizing vasculitis in several medium-sized arteries. Treatment with prednisone was proposed, but the patient refused it. She was followed-up during 9 months. Her weakness did not worsen, nor did she develop any systemic sign of vasculitis.

DISCUSSION

The co-occurrence of HIV infection and rheumatic manifestations, including arthropathies, Reiter's syndrome, myopathies and several forms of vasculitis, including necrotizing vasculitis type PAN, has been reported in recent years [4–7]. We present four new patients with advanced stage HIV infection diagnosed with PAN. Clinical, analytical and evolutive data from these patients are summarized in Table I. We have found 26 additional cases [5–26] of PAN in HIV-infected patients previously reported in the literature (MEDLINE 1987–1995). From the analysis of our patients and patients previously reported, one can see that the mean age is 44 yr (range 27–75) and 78% were males. PAN can appear in any stage of HIV disease and at any count of CD4 lymphocytes (range 14–540 $\times 10^6$/l). Symptoms suggestive of PAN in HIV-infected patients were predominantly related to the musculoskeletal (myalgias, polyarthralgia, muscular atrophy) and peripheral nervous systems (sensorimotor polyneuropathy and mononeuropathy), often as isolated manifestations. General symptoms like fever and weight loss were seen in one-third of the cases. Nevertheless, since these non-specific complaints are very common in patients with HIV infection, it is possible that some cases of vasculitis could remain underdiagnosed since histopathological studies are not always carried out.

Even though there was a lack of information about arteriographic studies and long-term prognosis in most reported cases, it seems that PAN in HIV patients rarely becomes a multisystemic and life-threatening disease. Some exceptions with heart [12], gut [8], kidney [12] or central nervous system [21, 24] involvement have been reported. Nevertheless, and in contrast to classical PAN in which systemic involvement is more frequently seen (Table II), the clinical behaviour of PAN in patients with HIV infection seems closer to previously described PAN limited to the peripheral nervous system [2] or muscle [3].

The aetiology of PAN remains obscure. HBsAg has been demonstrated in up to 30% of patients with classic PAN [1]. Although it is well known that hepatitis B virus infection is more prevalent in HIV-infected patients, positivity for HBsAg in patients suffering from HIV-related PAN occurred in one of the 21 cases in which information was available. This fact makes it unlikely that hepatitis B virus played a role in

TABLE I

Clinical and laboratory data from our four patients with polyarteritis nodosa and HIV infection

Case	Age	Sex	CDC class*	CD4	Clinical manifestations	Pathological study	HBsAg	HBcAb	Treatment outcome
1	38	F	IVC	220	Fever + weight loss + arthromyalgias + weakness + muscle atrophy	Muscle	–	–	Prednisone 0.5 mg kg day during 5 months asymptomatic 12 months of follow-up
2	37	M	IVC	50	Fever + weight loss + myalgia + peripheral neuropathy	Muscle	–	–	Prednisone 0.5 mg kg day for 3 months, 0.2 mg kg day for 3 months asymptomatic 12 months of follow-up
3	27	M	IVC	310	Fever + weakness + muscle atrophy	Muscle	–	–	Prednisone 0.5 mg day for 4 months dead 4 months later
4	27	F	IVC	14	Weakness + myalgia + muscle atrophy	Muscle	–	–	None no signs of systemic disease 9 months of follow-up

HIV, human immunodeficiency virus; M, male; F, female; HBsAg, hepatitis B surface antigen; HBcAb, hepatitis B core antibody.
*Staging of HIV infection according to the 1987 Criteria of the Centers for Disease Control and Prevention [31].

TABLE II
Clinical involvement in patients with classic and HIV-related PAN

Clinical involvement	HIV-related PAN per cent incidence (n = 30)*	Classic PAN per cent incidence (n = 50†)†
Peripheral neuropathy	60	51
Musculoskeletal system	43	64
Fever	37	71
Weight loss	37	54
Skin	16	43
Hypertension	10	54
Kidney	7	70
Central nervous system	7	23
Gastrointestinal tract	3	44
Cardiac	3	36
HBs antigenaemia	5	30

*From the present series and references [5–26].
†From Cupps and Fauci [1].

the development of PAN in these patients. On the other hand, Bardin *et al.* [9] demonstrated the presence of HIV capsid antigen in the vascular lesions from two patients with necrotizing vasculitis, suggesting that HIV itself may be involved in its pathogenesis.

The classic therapeutic approach for patients with severe and progressive PAN includes the use of immunosuppressive therapy with corticosteroids at high doses and cyclophosphamide [1]. On the other hand, other forms of PAN limited to the skin or muscle can be treated with corticosteroids alone without evidence of progression to more generalized forms. In these settings, the long-term prognosis, even in untreated patients, seems to be good [1, 2]. Currently, there is no consensus about the treatment and dose in HIV-infected patients who develop vasculitis, since the consequences of immunosuppressive therapy in HIV patients are controversial. Although an increase in opportunistic infections in HIV patients because of immunosuppressive therapy has been reported by some authors [27–29], recent studies suggest that corticosteroid therapy in asymptomatic and mildly symptomatic patients with HIV infection is safe, and can even lead to a sustained increase in CD4 cell counts [30]. The potential side-effects of immunosuppressive therapy, together with the clinical evidence that PAN in HIV-infected patients is less aggressive than classical PAN, led us to use a low dose of corticosteroids. In brief, three of our patients were successfully treated with prednisone at doses of 0.5 mg/kg/day. In addition, one of our patients who refused corticosteroid therapy had no worsening of weakness during the 9 months of follow-up, nor did she develop systemic symptoms of PAN. With regard to treatment in previously reported cases, data were available in only 13 of the 26 cases reviewed. A high dose of prednisone alone was instituted in six patients [7, 8, 11, 21, 23, 25] with a favourable outcome in five of them and no data about the outcome in the sixth [21]; another three patients [14, 15] were treated with corticosteroid therapy alone with unspecified dosage and the outcome was favourable as well. Three patients [6, 20, 26] were given prednisone and cyclophosphamide, one of them had a favourable response, one had no discernible effect and the last died 4 months later because of opportunistic infection. Finally, plasmapheresis associated with corticosteroid therapy was tried in another patient [16], but there were no data on outcome. No remarkable adverse effects have been described associated with this immunosuppressive therapy in HIV patients. We think that our successful experience with low-dose corticosteroids may benefit HIV patients with paucisymptomatic PAN. However, further prospective studies should be carried out in order to permit critical evaluation.

ACKNOWLEDGEMENTS

We are grateful to Dra. Elena Castellanos for helping us in the English editing of the manuscript. This work has been supported in part by grants from the Ministerio de Educación y Ciencia (FISS 92/0107 and 95/0554). FM was supported by a grant from Hospital Clínic i Provincial for 1994.

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British Journal of Rheumatology 1997;36:251–254

CLINICAL FEATURES IN PATIENTS WITH PERMANENT VISUAL LOSS DUE TO BIOPSY-PROVEN GIANT CELL ARTERITIS

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SUMMARY

The objective was to determine associated clinical findings in patients with visual loss due to giant cell arteritis (GCA) by means of a record review of 146 patients with biopsy-proven GCA. Twenty-three (15.75%) patients had lost vision. All of these patients complained of classical GCA cranial symptoms for an average of 1.3 months. 34.8% had an apparent isolated polymyalgia rheumatica for an average of 10.8 months and 65.2% had premonitory visual symptoms before visual loss for an average of 8.5 days. A clear delay in diagnosis and treatment was present in 15 patients (65.2%) who complained of at least two classical cranial symptoms for longer than 3 weeks and/or who had presented premonitory visual symptoms for longer than 72 h before blindness. Two additional patients lost vision while receiving standard steroid therapy. In conclusion, a high proportion of patients with permanent visual loss have a delayed diagnosis and treatment. A wider recognition of the disease would potentially reduce the prevalence of irreversible visual loss among GCA patients.

KEY WORDS: Giant cell arteritis, Horton's disease, Vasculitis, Visual loss, Blindness, Amaurosis, Amaurosis fugax, Diplopia, Corticosteroid therapy.

AMAUROSIS is the most frequent ischaemic complication and the main cause of permanent disability among giant cell arteritis (GCA) patients. It is generally believed that a prompt institution of corticosteroid therapy efficiently prevents blindness in this disease. This statement is based on the observation that since corticosteroid treatment was introduced, and with a progressively wider recognition of the disease, the incidence of permanent visual loss, which was ~60% in early series, has decreased over the years [1]. Moreover, corticosteroid therapy usually reverses premonitory symptoms such as amaurosis fugax, diplopia and blurry vision which, untreated, lead to permanent visual defects in a substantial proportion of individuals. However, visual impairment still affected 8–22% of patients in recent series [2–4]. This observation suggests that there is still an under-recognition of the disease or that certain patients are particularly prone to developing blindness early in the course of the disease before there is a feasible opportunity of them being appropriately diagnosed and treated.

In the present work, we reviewed clinical findings in patients with permanent visual loss due to biopsy-proven GCA. Special attention was paid to the number, nature and duration of classical disease manifestations at the moment of visual impairment. The aim of our study was to analyse whether patients with visual loss had enough typical clinical findings to allow an earlier diagnosis and treatment.

Submitted 3 June 1996; revised version accepted 31 July 1996.

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METHODS

We retrospectively reviewed the clinical records of 146 consecutive patients (102 females and 44 males) diagnosed at our institution over a 15 yr period (1980–1995). One hundred and twenty-four (85%) patients were directly evaluated by at least one of the authors at the time of diagnosis.

Recorded data included the medical history with particular emphasis on GCA-related clinical manifestations, findings at physical examination, ophthalmic evaluation, treatment received and outcome.

RESULTS

Characteristics of visual impairment

Twenty-three (15.75%) out of 146 patients evaluated (16 females and seven males) developed permanent visual loss. Twelve patients (52.1%) presented unilateral and seven patients (30.4%) bilateral amaurosis. In two patients from the latter group, blindness was simultaneous in both eyes. In the remaining five, loss of vision was sequential and the second eye was impaired within a 2 week interval. Four patients (17.4%) presented permanent visual field defects. In 13 patients (15 eyes), visual loss was sudden and in the remaining nine (14 eyes) progressive blurry vision or additive visual field defects led to complete blindness in less than 2 weeks (Table I).

Sixteen patients (65.2%) had premonitory visual symptoms an average of 8.5 days (range 0.5–60 days) before permanent visual loss appeared (Fig. 1A). Seven patients developed blurry vision and/or partial field defects, six amaurosis fugax, two diplopia and in one patient there were combinations of these (Table I).

Funduscopy revealed anterior ischaemic optic neuropathy (AION) in 17 patients (73.9%) which was bilateral in six of them. In an additional patient, AION

was associated with central retinal artery occlusion. One patient had an occlusion of a central retinal artery branch. In another patient, fundus examination was normal. Funduscopy was not performed in the remaining three patients.

Systemic symptoms and disease duration before visual loss

Constitutional symptoms were present in six (26%) patients, fever in two (8.7%), myalgia in three (13.04%) and polymyalgia rheumatica (PMR) in 12 (52.1%). At the time of visual impairment, all patients complained of cranial symptoms attributable to GCA (headache in 78.3%, jaw claudication in 56.5%, facial pain in 30.43%, scalp tenderness in 21.7%, odynophagia in 17.4%, tongue pain-ischaemia in 13.04%, otalgia in 8.7%, odontalgia in 4.3% and palate pain in 4.3% of patients). Twenty patients (86.9%) complained of more than one cranial symptom (Fig. 1B). The duration of cranial symptoms before blindness ranged from 1 week to 4 months (median 1.3 months) (Fig. 1C). In addition, abnormal findings at physical examination of the epicranial arteries were present in 19 (86.6%). These included absent or asymmetrical pulse, tenderness, hardness or nodules. In 18 patients, these abnormalities involved the temporal arteries and in one patient the occipital arteries.

Twelve patients had PMR. In four patients (17.4%), PMR co-existed with cranial symptoms at disease onset. Eight patients (34.8%) presented with an apparently isolated polymyalgia and later developed typical cranial symptoms, including blindness. The average duration of PMR before visual loss was 10.8 months (range 1 month–5 years). None of these patients were appropriately diagnosed during this period of time and received only symptomatic treatment with non-steroidal anti-inflammatory drugs.

TABLE I
Visual symptoms in patients with visual impairment due to GCA

Visual loss	*N*	Premonitory symptoms	(*N*)
Monocular	12		
Sudden	8	amaurosis fugax	4
		diplopia	1
Progressive	4	blurry vision	3
		inferior hemifield loss	2
Binocular	7		
Sudden	2	blurry vision	1
Progressive		blurry vision	4
		amaurosis fugax	1
		diplopia	1
		inferior hemifield loss	1
Visual fields defect	4		
Sudden	3	amaurosis fugax	2
		blurry vision	1

N, number of patients.

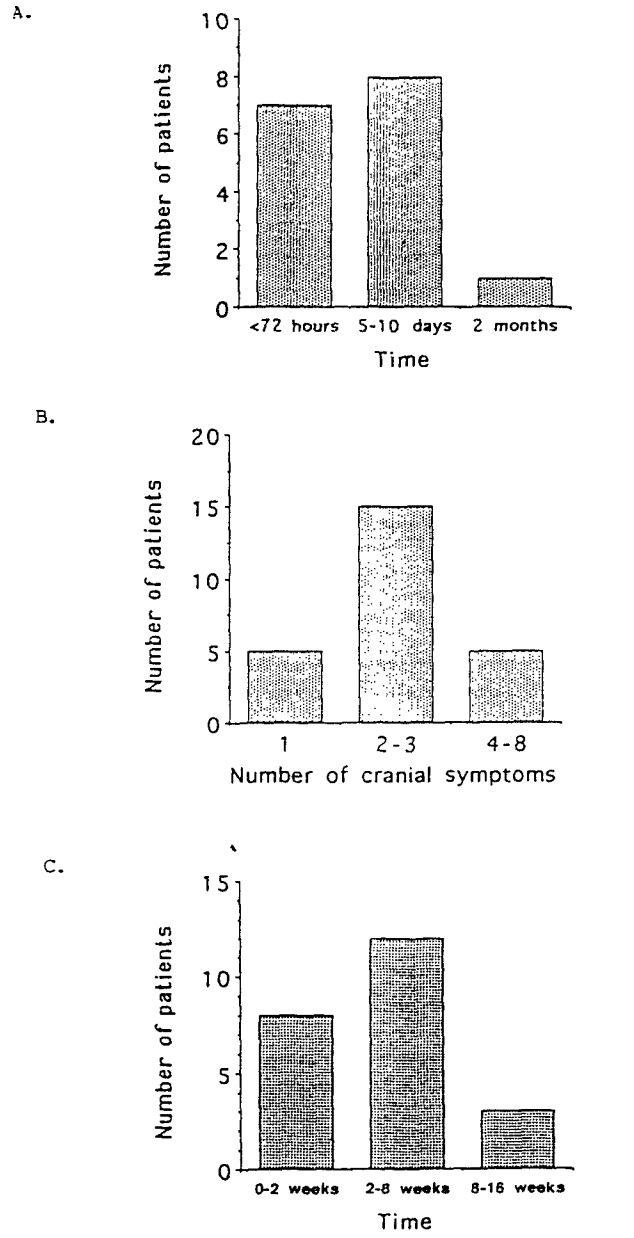

FIG. 1.—(A) Time with premonitory visual symptoms before blindness in patients with visual impairment due to GCA. (B) Number of cranial symptoms in patients with visual impairment due to GCA. (C) Time with cranial symptoms before visual impairment.

Response to treatment

Eighteen patients were treated with oral prednisone (1 mg/kg/day) and three patients received high i.v. doses of methylprednisolone (1 g/day) for 3–5 days. No patient felt significant improvement in their vision after treatment. However, two patients with permanent unilateral visual loss had amaurosis fugax and blurry vision in the remaining eye which completely reversed with treatment. The remaining two patients developed bilateral and unilateral blindness due to AION while taking 1 mg/kg/day of prednisone, 2 weeks and 5 months, respectively, after starting therapy.

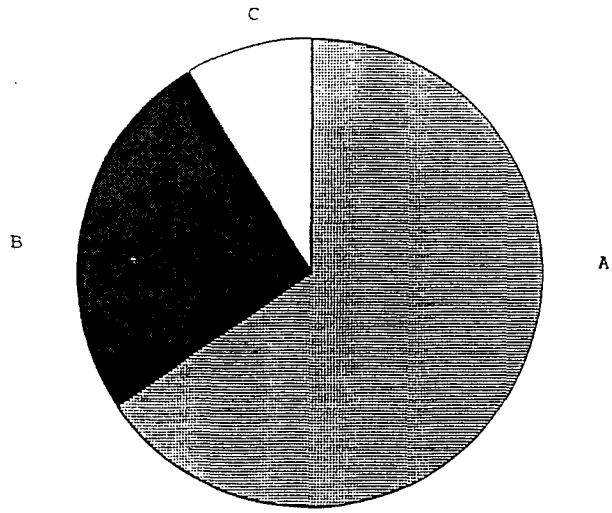

FIG. 2.—Proportion of patients in whom the diagnosis of GCA could have potentially been made before blindness (A), patients in whom blindness appeared very early in the course of GCA and/or when only mild cranial symptoms of GCA were present (B), and patients who presented permanent visual loss in the course of standard steroid therapy (C).

Delayed diagnosis and treatment

We considered that there had been a clear delay in diagnosis and treatment when patients had presented premonitory visual symptoms (amaurosis fugax, diplopia, sustained blurry vision or partial field defects) for longer than 72 h and or at least two classical cranial symptoms (new-onset headache, jaw claudication, facial pain or scalp tenderness) for longer than 3 weeks when visual loss occurred. According to these criteria, 15 (65.2%) patients had a delayed diagnosis and treatment. Conversely, in six patients (26.09%), permanent visual defects appeared either very early in the course of the disease or when only mild manifestations were present. In addition, as mentioned above, two (8.7%) additional patients became blind while receiving corticosteroid therapy (Fig. 2).

DISCUSSION

Even though corticosteroid treatment has clearly improved the visual prognosis of individuals with GCA, a substantial number of patients still suffer from permanent visual defects. In our series of 146 patients, diagnosed over the last 15 yr, 23 (15.75%) developed visual impairment, a similar pattern to that reported in recent reports [2–4]. Once amaurosis is established, the visual prognosis is known to be poor in patients with GCA [1, 5–7]. Although several cases of total or partial visual recovery following corticosteroid treatment have been reported [3, 5, 8–10], none of our patients experienced significant visual improvement. Moreover, two patients lost vision while receiving corticosteroid therapy.

A careful analysis of associated clinical findings and disease duration in patients with visual loss disclosed that in eight patients PMR preceded by an average of 10.8 months the onset of cranial symptoms, including amaurosis. Similar findings have been reported in other retrospective series [11]. None of our patients were previously diagnosed as having PMR, treated accordingly or instructed about the possibility of cranial symptom development. In prospective follow-up studies, only a minority of patients considered to have isolated PMR after a careful clinical evaluation and appropriately treated with low prednisone doses, eventually develop full-blown GCA [12, 13]. Therefore, our patients could theoretically have been diagnosed as having GCA if an appropriate work-up had been performed. However, in retrospect, we cannot evaluate whether at physical evaluation our patients had abnormalities of temporal arteries or other clinical findings which could have led to an earlier diagnosis of GCA during their phase of apparently isolated PMR.

At the time of visual loss, all our patients had cranial symptoms. Moreover, 16 (65.2%) of our patients had premonitory symptoms such as amaurosis fugax, diplopia, blurry vision or partial field defects which are considered alarming premonitory symptoms of permanent visual loss. This frequency is similar to previous reports [1, 14] and higher than reports from other recent series [2–4]. Fifteen (65.2%) of our patients had at least two classical cranial symptoms for longer than 3 weeks and/or classical premonitory ocular symptoms for longer than 72 h when ischaemic events occurred. These patients had sufficient clinical manifestations to allow an earlier diagnosis and treatment, and visual loss could potentially have been prevented. In contrast, in six (26.09%) patients, cranial symptoms had been present for <2 weeks or consisted of less classical manifestations, making the possibility of an early diagnosis and treatment less likely.

In summary, our results indicate that in a high proportion of patients, an early diagnosis and treatment could have been carried out, increasing the likelihood of preventing permanent visual loss, and suggest that a better awareness of disease manifestations at the primary care level would further reduce the frequency of blindness in GCA patients. However, in a remarkable proportion of patients (26.09%), visual impairment appeared very early in the course of the disease or when only atypical or mild symptoms were present without a feasible opportunity of them being appropriately diagnosed and treated. In addition, two patients developed bilateral amaurosis during the course of appropriate steroid therapy. These facts suggest that some patients are at special risk of developing ischaemic complications. We are currently trying to identify prospectively clinical or analytical data with good predictive value in delimiting patients at high risk of developing irreversible ischaemic events.

ACKNOWLEDGEMENT

Supported by a grant from Fondo de Investigación Sanitaria (FIS 95/0805).

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Reprinted from ARTHRITIS & RHEUMATISM
Lippincott-Raven Publishers
Vol. 41, No. 1, January 1998

ASSOCIATION BETWEEN STRONG INFLAMMATORY RESPONSE AND LOW RISK OF DEVELOPING VISUAL LOSS AND OTHER CRANIAL ISCHEMIC COMPLICATIONS IN GIANT CELL (TEMPORAL) ARTERITIS

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Objective. **To identify clinical and biochemical parameters that have good predictive value for identifying giant cell (temporal) arteritis (GCA) patients who are at high or low risk of developing cranial ischemic events.**

Methods. **In this multicenter study, records of patients at 3 university hospitals in Barcelona were reviewed retrospectively. Two hundred consecutive patients with biopsy-proven GCA were studied.**

Results. **Thirty-two patients developed irreversible cranial ischemic complications. The duration of clinical symptoms before diagnosis was similar in patients with and those without ischemic events. Patients with ischemic complications less frequently had fever (18.8% versus 56.9%) and weight loss (21.9% versus 62%) and more frequently had amaurosis fugax (32.3% versus 6%) and transient diplopia (15.6% versus 3.6%). Patients with ischemic events had lower erythrocyte sedimentation rates (ESR) (82.7 mm/hour versus 104.4 mm/hour) and higher concentrations of hemoglobin (12.2 gm/dl versus 10.9 gm/dl) and albumin (37.4 gm/liter versus 32.7 gm/liter). Clinical inflammatory status and biologic inflammatory status were defined empirically (clinical: fever and weight loss; biologic: ESR ≥85 mm/hour and hemoglobin <11.0 gm/dl). Patients not showing a clinical and biologic inflammatory response were at high risk of developing ischemic events (odds ratio [OR] 5, 95% confidence interval [95% CI] 2.05–12.2). The risk was greatly reduced among patients with either a clinical (OR 0.177, 95% CI 0.052–0.605) or a biologic (OR 0.226, 95% CI 0.076–0.675) inflammatory reaction. No patient with both a clinical and a biologic response developed ischemic events.**

Conclusion. **The presence of a strong acute-phase response defines a subgroup of patients at very low risk of developing cranial ischemic complications. Our findings provide a rationale for testing less aggressive treatment schedules in these individuals. Conversely, a low inflammatory response and the presence of transient cranial ischemic events provide a high risk of developing irreversible ischemic complications and require a prompt therapeutic intervention.**

Cranial ischemic events, particularly blindness, are the more common vascular complications and a major source of chronic disability among patients with giant cell (temporal) arteritis (GCA). The prevalence of irreversible visual complications, which was ~50–60% in early series (1,2), has decreased remarkably since the advent of widespread corticosteroid treatment, but partial or total irreversible visual loss was still noted in 8–22% of patients in recent series (3–5). Although some isolated cases of visual improvement following high-dose corticosteroid treatment have been reported (3,6,7), once established, amaurosis is usually irreversible (1,3, 4,8). Other cranial ischemic events such as cerebrovascular accidents are much less frequent (1,4,9), but are

Presented in part at the 60th National Scientific Meeting of the American College of Rheumatology, Orlando, FL, October 1996.

Supported by Fondo de Investigación Sanitaria (FIS) grants 95/0860 and 98/0443 to Dr. Cid and FIS grant 94/0953 to Dr. Oristrell.

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Submitted for publication January 27, 1997; accepted in revised form August 21, 1997.

among the leading causes of mortality directly related to GCA (10–12).

In a recent retrospective survey of 23 patients with visual loss due to GCA, we found that, while a delay in diagnosis and treatment could have been a factor in 15 patients (62.2%), in 6 (26%), visual loss occurred very shortly after the onset of cranial symptoms or when these symptoms were very mild or atypical. In addition, 2 patients lost vision after starting corticosteriod therapy. These observations suggest that some patients may be particularly predisposed to develop ischemic complications. Identification of patients at high or low risk for the development of ischemic events would be of great clinical value, allowing the prescription of more aggressive therapies when needed and less aggressive therapies in patients who are at low risk, thus decreasing the incidence of iatrogenic complications. In the present study, we retrieved clinical and biochemical data on 200 patients with biopsy-proven GCA in order to identify patients at high and low risk for development of cranial ischemic complications. Our results indicate that a strong acute-phase response, defined on clinical and biochemical grounds, is associated with a very low risk for development of irreversible cranial ischemic events in patients with GCA.

PATIENTS AND METHODS

The study population consisted of 200 consecutive patients with biopsy-proven GCA diagnosed at our institutions over a 16-year period. There were 59 men and 141 women, with a mean age of 74.5 years (range 57–92 years). Participating centers were 2 university referral tertiary hospitals (Hospital Clínic, University of Barcelona Medical School and Hospital Vall d'Hebró, Autonomic University of Barcelona Medical School) and 1 secondary hospital (Hospital de Sabadell, Autonomic University of Barcelona Medical School). These centers contributed 146 patients (73%), 24 patients (12%), and 30 patients (15%), respectively. Although the present study was retrospective in design, in 152 patients (76%), comprehensive clinical data were obtained by some of the authors (MCC, CF, JO, AL-S, and JV) at the time of diagnosis as part of other ongoing protocols (13–16).

Data recorded included age, sex, number and type of cranial symptoms, systemic manifestations such as polymyalgia rheumatica (PMR), fever (>37°C), and weight loss (> 5 kg), transitory or permanent visual impairment, ophthalmologic examination results, transient or irreversible cerebrovascular complications, and ischemic events in other vascular territories (angina, myocardial infarction, limb ischemia, intermittent claudication). For an ischemic event to be considered to be GCA-related, the following criteria were required: development concomitant with disease manifestations and absence of significant vascular risk factors such as heavy smoking, hypertension, hypercholesterolemia, or diabetes.

Laboratory parameters included erythrocyte sedimentation rate (ESR), protein electrophoresis results, levels of haptoglobin, C-reactive protein (CRP), von Willebrand factor–related antigen (vWFAg), fibrinogen, alkaline phosphatase, and hemoglobin, and platelet, red blood cell, and white blood cell counts. Complete clinical data and routine biochemical determinations were obtained for all patients, except that CRP, vWFAg, and haptoglobin levels were determined in only 35 patients (17.5%), 65 patients (32.5%), and 41 patients (20.5%), respectively.

Clinical and biologic variables in patients with versus those without irreversible cranial ischemic complications were compared by the nonparametric Mann-Whitney test for quantitative variables and the 2-tailed Fisher's exact test for qualitative variables. Estimated odds ratios (OR) with 95% confidence intervals (95% CI) were calculated using contingency tables. Statistical analysis was performed using BMDP (Biomedical Package, Berkeley, CA).

RESULTS

Characteristics of irreversible cranial ischemic complications in GCA patients. Thirty-two of the 200 GCA patients (16%) developed irreversible cranial ischemic complications (ICIC). These included total or partial visual loss (28 patients), persistent ophthalmoplegia (2 patients), stroke (3 patients), and scalp necrosis (1 patient). Detailed characteristics of these events are presented in Table 1.

Patients who experienced a permanent ischemic event were more prone to develop additional irreversible cranial ischemic complications. Fourteen of the 32 patients who had a cranial ischemic event (43.8%) developed an additional ischemic complication either simultaneously or consecutively within a 2-week period (second eye involvement, coexistence of anterior ischemic optic neuropathy and retinal central artery thrombosis, visual loss, and scalp necrosis or cerebrovascular accidents in areas supplied by different vascular beds), whereas the overall prevalence of permanent ischemic complications in the series as a whole was 16%.

Relationship of cranial ischemic complications to time of diagnosis and treatment. Presenting symptoms were systemic (fever, PMR, anorexia, and weight loss, either alone or in combination) in 77 patients (38.5%), cranial (headache, jaw claudication, visual symptoms) in 36 (18%), and simultaneous (cranial and systemic) in 87 (43.5%). The mean duration of clinically apparent disease (either systemic or cranial) before diagnosis was 21.4 weeks (range 0–240 weeks) in patients with ICIC, compared with 20.6 weeks (range 1–192 weeks) in patients without ICIC (P not significant). The duration of more specific cranial symptoms before diagnosis was shorter in patients with ICIC

Table 1. Irreversible cranial ischemic events in patients with giant cell arteritis (n = 200)

Event	No.	Clinical characteristics (no.)	Objective Abnormality (no.)*
Visual loss†	28	Monocular blindness (14)	AION (22)
		Binocular blindness (9)	CRA branch occlusion (1)
		Visual field defects (5)	AION and CRA occlusion (1)
			Normal fundoscopy results (1)
			Not explored (3)
III cranial nerve palsy	1	Diplopia	No MRI performed
VI cranial nerve palsy	1	Diplopia	Normal MRI results
Stroke	3	Hemiparesia and coma (1)	Internal capsula and cerebellum infarcts (1)
		Internuclear ophthalmoplegia (1)‡	Mesencephalus infarct (1)
		Lateral medullary syndrome and coma (1)§	Upper medulla infarct, cerebellum infarcts, temporoccipital infarct (1)
Scalp necrosis‡	1		

* AION = anterior ischemic optic neuritis; CRA = central retinal artery; MRI = magnetic resonance imaging.
† More detailed ocular findings on some of these patients have been reported previously (17).
‡ These patients also developed blindness (AION).
§ This patient has been reported previously (31).

compared with patients without ICIC (5.3 weeks versus 11 weeks; $P = 0.0214$) (Figure 1A) and compared with those who had only transient ischemic phenomena (5.3 weeks versus 17.3 weeks; $P = 0.004$) (Figure 1B).

Relationship of inflammatory response to the development of irreversible cranial ischemic complications in GCA patients. Table 2 shows the distribution of clinical manifestations in GCA patients with and those without ICIC. No significant differences in the frequency of classic cranial disease manifestations or PMR were found. Only transient ocular events such as amaurosis fugax (32.3% versus 6%; $P = 0.0001$) and diplopia (15.6% versus 3.6%; $P = 0.0179$) were significantly more frequent among patients who developed a permanent cranial ischemic defect. Notably, patients with ICIC less frequently had fever (18.8% versus 56.9%; $P = 0.0001$) and weight loss (21.9% versus 62%; $P = 0.0001$). Among the biologic parameters studied, the mean ESR and

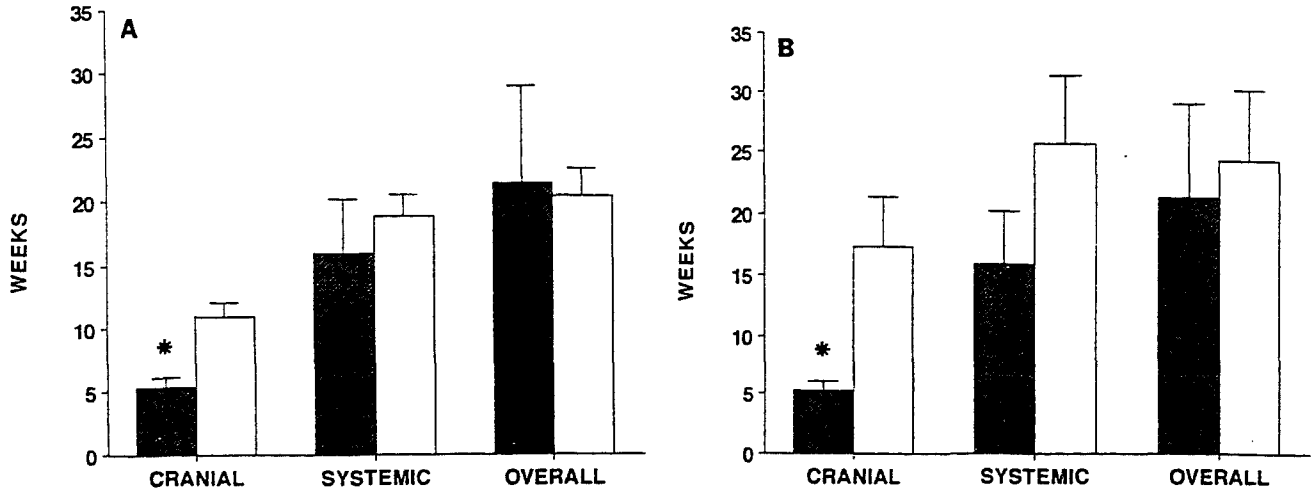

Figure 1. A, Duration of cranial symptoms, systemic symptoms, and overall disease in giant cell (temporal) arteritis (GCA) patients with (shaded bars) and without (open bars) irreversible cranial ischemic complications. * = $P = 0.0214$, patients with versus those without irreversible ischemic events. **B,** Duration of cranial symptoms, systemic symptoms, and overall disease in GCA patients with irreversible cranial ischemic complications (shaded bars) and those with transient cranial ischemic events (open bars). * = $P = 0.0008$, patients with irreversible versus those with transient ischemic events. Values are the mean and SEM.

Table 2. Clinical findings in giant cell arteritis patients with and those without irreversible cranial ischemic complications (ICIC)

	ICIC	No ICIC
General characteristics		
Age in years, mean (range)	76 (64–86)	73 (57–92)
Sex, no. male/no. female	10/22	49/119
Cranial symptoms, %		
Headache	81.2	75.4
Jaw claudication	53.1	44.9
Scalp tenderness	34.4	41
Facial pain/edema	25	16.2
Abnormal temporal arteries*	92.6	85.6
Amaurosis fugax	32.3†	6
Transient diplopia	15.6‡	3.6
Ocular pain	9.4	4.8
Tongue pain	9.4	4.8
Earache	9.4	20.4
Trismus	0	6
Odynophagia	15.6	15.6
Carotidynia	6.2	6.6
Toothache	6.2	3
Transient ischemic attack	6.3	2.4
Other vascular territories, %		
Lower limb gangrene	3.1	1.2
Intermittent claudication	3.1	4.2
Myocardial infarction	0	3.6
Angina	3.1	3.3
Systemic manifestations, %		
Polymyalgia rheumatica	46.9	49.7
Fever	18.8†	56.9
Weight loss	21.9§	62

* Hardness, pulse weak or absent, tenderness, or inflammatory signs.
† $P = 0.0001$ versus patients without ICIC.
‡ $P = 0.0179$ versus patients without ICIC.
§ $P < 0.0001$ versus patients without ICIC.

haptoglobin concentration were significantly lower in patients with ICIC (ESR 82.7 mm/hour versus 104.4 mm/hour; $P = 0.0001$; haptoglobin 297 mg/dl versus 499 mg/dl; $P = 0.026$). Conversely, mean levels of albumin (37.4 gm/liter versus 32.7 gm/liter; $P = 0.0024$) and hemoglobin (12.2 gm/dl versus 10.9 gm/dl; $P = 0.0001$) were significantly higher in patients with ICIC. No significant differences were found in platelet counts or in α_2-globulin, vWFAg, CRP, or alkaline phosphatase concentrations between patients with and those without ICIC (Table 3).

Since the above-mentioned discriminating parameters are known to be part of the nonspecific systemic inflammatory or acute-phase response, we empirically defined a group of patients with a strong clinical inflammatory response (defined as having both fever and weight loss) and a group with a high biochemical inflammatory reaction (defined as having both an ESR of ≥85 mm/hour and a hemoglobin level of <11.0 gm/dl). Albumin was not considered because it can be influenced by other factors such as nutritional status, particularly in elderly patients. Haptoglobin was not considered because of insufficient data. As shown in Figure 2, patients who exhibited neither a clinical nor a biochemical inflammatory response were at increased risk of developing ICIC (OR 5, 95% CI 2.05–12.2). The risk was significantly decreased for patients with either a clinical

Table 3. Blood chemistry and hematologic values in giant cell arteritis patients with and those without irreversible cranial ischemic complications (ICIC)*

Parameter	ICIC	No ICIC
ESR, mm/hour	82.7 (24–130)†	104.4 (22–148)
Albumin, gm/liter	37.4 (20–63)‡	32.7 (16–55)
α_2-globulin, gm/liter	9.7 (4–17)	10.2 (2–20)
Hemoglobin, gm/dl	12.2 (10–16)†	10.9 (7–15)
Platelets, × 10^9/liter	337 (49–571)	375 (163–785)
vWFAg, units/dl§	167 (105–220)	236 (96–720)
CRP, mg/dl§	3.5 (0.2–7)	10.8 (0–56)
Haptoglobin, mg/dl§	297 (213–370)¶	499 (187–936)
Alkaline phosphatase, units/liter	198 (127–394)#	268 (72–1,029)

* Values are the mean (range). ESR = erythrocyte sedimentation rate; vWFAg = von Willebrand factor–related antigen; CRP = C-reactive protein.
† $P = 0.0001$ versus patients without ICIC.
‡ $P = 0.0024$ versus patients without ICIC.
§ Data not available from all patients.
¶ $P = 0.026$ versus patients without ICIC.
# $P = 0.082$ versus patients without ICIC.

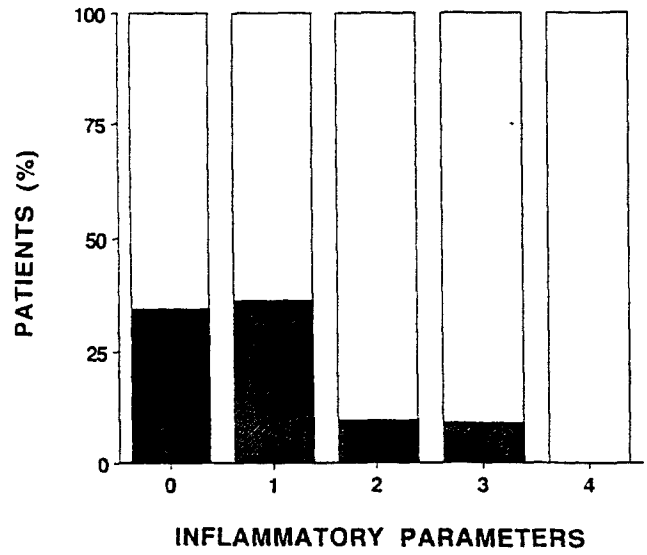

Figure 2. Prevalence of irreversible cranial ischemic complications among giant cell (temporal) arteritis (GCA) patients according to the number of clinical (fever or weight loss) and biochemical (hemoglobin <11.0 gm/dl or ESR ≥85 mm/hour) inflammation parameters as defined in Patients and Methods. Shaded portions of bars indicate percentage of patients with ischemic complications.

(OR 0.177, 95% CI 0.052–0.605) or a biochemical (OR 0.226, 95% CI 0.076–0.675) inflammatory reaction, whereas no patient who exhibited both a clinical and a biochemical inflammatory response developed ICIC.

DISCUSSION

Thirty-two individuals from our series of 200 GCA patients (16%) developed a permanent visual defect. This is similar to the percentage noted in other recent reports and appears to have remained stable over time in spite of increasing disease recognition and a widespread practice of prompt initiation of corticosteroid treatment when GCA is suspected (3–5). Moreover, 6 of our patients had cranial ischemic complications other than visual loss (Table 1). In a recent review of patients with ocular involvement due to GCA, we found that in 26% of patients, visual loss occurred very early after the onset of disease-related clinical manifestations or when only very mild classic cranial symptoms were present, suggesting that some patients were particularly predisposed to develop ischemic complications (17).

In the present study, designed to identify clinical or biochemical features with predictive value in identifying patients at high or low risk of ischemic events, we found that those with a given cranial ischemic complication were more prone to develop additional ischemic events. In this regard, it is noteworthy that many case reports and small published series of GCA patients with unusual cranial ischemic complications such as cerebrovascular accidents or tongue or scalp necrosis include a seemingly high proportion of individuals with visual loss (18–22). In addition, when we compared the overall duration of clinical symptoms prior to diagnosis and treatment, we found no significant differences between patients with and those without cranial ischemic complications. Furthermore, no significant differences in the mean duration of clinical manifestations were found between patients with transient ischemic events and those who developed irreversible complications. In fact, the duration of typical cranial symptoms at the time of diagnosis was even shorter in patients who experienced irreversible cranial ischemic events, probably due to earlier seeking of medical care or easier recognition of the disease by primary care physicians when ischemic events occurred. Taken together, these observations indicate that the development of ischemic complications is not mainly due to a delay in diagnosis and treatment and suggest that some patients with GCA are particularly predisposed to develop such complications.

Except for amaurosis fugax and diplopia, no differences in the prevalence of classic disease manifestations, such as headache, scalp tenderness, jaw claudication, facial pain, and PMR, were found between patients with and those without ICIC. Amaurosis fugax and diplopia have been recognized for many years as warning symptoms preceding, in most cases, a definitive visual loss (1,2,23,24). No other predictive or protective symptoms have been previously identified. Contrary to general belief, the presence of strong and manifest cranial symptoms was not associated with a high risk of ischemic complications in our series of patients. Moreover, disease presentation with only mild cranial symptoms or isolated PMR did not guarantee a low risk of ischemic events.

Besides amaurosis fugax and diplopia, the only discriminating feature between patients with and those without ICIC was the presence or the absence of a strong inflammatory response defined by clinical and biochemical parameters. We found a marked decrease in the prevalence of fever and weight loss among patients who experienced cranial ischemic events. Furthermore, patients with cranial ischemic complications had a lower ESR, higher albumin and hemoglobin levels, and lower haptoglobin concentrations. Together, these data suggest that a high inflammatory response is associated with a low risk of developing ischemic events in patients with GCA. Although this has never been clearly elucidated before, there is scattered information in the literature supporting our hypothesis. The term "occult" GCA, referring to patients without clinical evidence of systemic disease, has been repeatedly mentioned in reports of patient series studied by ophthalmologists, which, indeed, include a high proportion of patients with visual impairment (25–27). A trend toward a lower frequency of fever among patients with transient or definitive ocular involvement has been observed in smaller series (28,29). A substantial number of case reports of patients with cranial ischemic manifestations describe a close-to-normal ESR (5,18,20,21,30–34).

Vascular inflammation in vasculitis leads to vessel occlusion and, eventually, to organ ischemia through thrombosis, spasm, or intimal hyperplasia and fibrosis (35). The mechanisms through which a strong inflammatory response, as defined in our study, is associated with a low risk of developing ischemic complications are unknown. The acute-phase response is a nonspecific systemic reaction to injury, which includes fever, weight loss, and hepatic synthesis of acute-phase proteins (36). It is induced by a variety of cytokines, mostly interleukin-1, tumor necrosis factor α, and interleukin-6 (37,38). These cytokines, which in fact have been demonstrated to be

produced in inflamed arteries from GCA patients (39), have potent biologic effects on endothelial and smooth muscle cells, and consequently, are able to regulate biologic responses related to vessel occlusion, such as procoagulant activity, vascular tone, neovascularization, myointimal cell proliferation, and matrix deposition (40–42). Consequently, variations in the production of these and other cytokines or additional unrecognized factors might have an important impact on the ultimate degree of vessel occlusion and could determine high versus low risk for development of ischemic events. Recent work by Weyand et al demonstrates that GCA patients with prominent cranial symptoms produce more interferon-γ than patients who have only systemic manifestations (43). Therefore, variations in cytokine production may indeed account for different disease expression patterns in patients with GCA.

Endothelial cells are in constant and direct contact with serum proteins. Acute-phase proteins produced during the inflammatory response might also play a role in vessel remodeling and repair. The participation of acute-phase proteins in vascular biology is virtually unexplored, but preliminary evidence supports the notion that they may regulate endothelial cell functions. In this regard, we have demonstrated that haptoglobin, an acute-phase protein found in significantly reduced concentrations in patients with ischemic complications, promotes neovascularization (44).

In summary, our findings indicate that some patients with GCA are particularly predisposed to develop ischemic complications, and this predisposition is associated with a poor inflammatory response. GCA has been considered to be a self-limiting disease. Corticosteroids are administered to GCA patients because of the dramatic relief of clinical symptoms they induce and their ability to prevent ischemic events. Corticosteroids suppress proinflammatory cytokine production and reduce the acute-phase response, which, based on our results, is associated with a lower risk of ischemic events. This apparent paradox can be explained by the fact that corticosteroids influence virtually every step in immune activation and immune effector mechanisms (45), and the overall result in GCA patients seems to be beneficial. However, once clinical remission is achieved, potential corticosteroid-related morbidity highly outweighs the risk of ischemic complications (3,46,47). Besides opening new insights into the possible mechanisms implicated in vessel occlusion and organ ischemia in vasculitis, our findings allow the identification of patients who are at low risk for the development of ischemic events and provide a rationale for testing less aggressive treatment regimens in these individuals.

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INTERFERON-α MAY EXACERBATE CRYOGLOBULINEMIA-DERIVED ISCHEMIC MANIFESTATIONS. AN ADVERSE EFFECT POTENTIALLY RELATED TO ITS ANTI-ANGIOGENIC ACTIVITY

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Running title: Anti-angiogenic activity of interferon-α in mixed cryoglobulinemia.

This work was supported by a grant from Fondo de Investigación Sanitaria (FIS 98/0443).

Results partially presented at the 61th American College of Rheumatology meeting, Washington DC, November 1997 and at the British Society of Rheumatology meeting, Brighton, UK, April 1998.

Key words: Cryoglobulinemia, ischemia, interferon-α, vasculitis, angiogenesis, HCV

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ABSTRACT

The discovery of the strong association between hepatitis C virus (HCV) infection and the development of mixed cryoglobulinemia has motivated an active testing of anti-viral directed alternative therapies. Several trials have demonstrated that classical cryoglobulinemia-derived manifestations improve with interferon-α (IFNα) treatment. We report on 3 consecutive HCV-infected patients with severe cryoglobulinemia related ischemic manifestations who were closely followed during IFNα therapy. Clinical evaluation with special attention to ischemic lesions, liver function tests and cryocrit determinations was serially performed. In addition to prednisone and immunosuppressive agents, the three patients received IFNα at 3×10^6 units, 3 times/week for 2, 3, and 4 months respectively. In all patients, systemic complaints improved, liver function tests returned to normal, and cryocrits decreased. However, ischemic lesions became less vascularized and ischemia progressed leading to transmetatarsian and subcondilean amputation respectively in two of the patients and fingertip necrosis and ulcer enlargement in the remaining patient. Skin biopsies taken before and after two months of IFNα therapy in the third patient showed a significant decrease in subepidermal microvessels. When IFNα was discontinued, the lesions finally healed. Cryoglobulinemia-derived ischemic lesions may worsen during IFNα treatment, presumably through a decrease in inflammation-induced angiogenesis. The anti-angiogenic activity of IFNα may delay the appropriate healing of ischemic lesions.

INTRODUCTION

Cryoglobulinemia is a systemic disease associated with the presence of serum immunoglobulins able to reversibly precipitate at cold temperatures. Cryoglobulins may consist of a single monoclonal component, either IgG or IgM, (type I) or can be mixed. The latter are usually composed by polyclonal IgG complexed with monoclonal IgM rheumatoid factors (type II), or consist of polyclonal IgG or IgM complexes (type III) (1).

Classic cryoglobulinemia-related clinical manifestations include cutaneous vasculitic purpura, arthralgia and weakness. Glomerulonephritis and peripheral neuropathy are also common and cutaneous ulcers or Raynaud's phenomenon may also occur (1,2). Clinical findings in mixed cryoglobulinemia are due to direct occlusion of microvessels by precipitated cryoglobulins or from immune-complex deposition in the skin, renal glomeruli and perineural vessels (1). Although clinical manifestations and organ dysfunction are usually mild to moderate, some patients develop renal failure or present with widespread systemic necrotizing vasculitis with severe organ ischemia (1).

Over the past recent years it has been shown that up to 98% of patients with mixed cryoglobulinemia have antibodies to hepatitis C virus (HCV) (2). In addition, circulating HCV- RNA, can be detected in their plasma indicating active viral replication. Moreover, cryoprecipitates contain HCV antibodies and HCV-RNA 2-5 and 1,000 times more concentrated, respectively, than the corresponding sera (2). These observations suggest a role for HCV infection in the pathogenesis of mixed cryoglobulinemia.

Until recently, the treatment of mixed cryoglobulinemia has relied on corticosteroids, immunosuppressive agents and plasmapheresis, alone or in combination, with highly variable results (1). The recognition of the association of mixed cryoglobulinemia with

HCV infection has motivated an active search for antiviral-directed alternative therapies for this condition. Several prospective, randomized, controlled trials have demonstrated the efficacy of IFNα in inducing remission in patients with HCV-associated cryoglobulinemia (2,3). These studies include patients with mild to moderate clinical manifestations and a significant improvement is achieved in about 60-77% of individuals (2,3). In IFNα-responders clinical improvement usually correlates with disappearance of detectable plasma viral RNA providing further support to the pathogenic role of HCV in mixed cryoglobulinemia (2,3). Unfortunately, remissions are not sustained in most cases and, in a significant proportion of patients, symptoms recur after IFNα withdrawal after which HCV viremia becomes detectable again (2,3).

Although several reports indicate that patients with prominent systemic involvement related to cryoglobulinemia may also improve with IFNα therapy (4), the efficacy of IFNα in patients with severe ischemic manifestations has not been tested in therapeutic trials.

We report on three HCV-infected patients with significant cryoglobulinemia-related ischemic manifestations which substantially worsened with IFNα therapy. We provide evidence supporting a role for IFNα anti-angiogenic activity in impairing appropriate healing of ischemic lesions.

CASE REPORT

Three consecutive male patients, aged 58, 51, and 64 years respectively, all with prominent ischemic complications related to HCV-associated cryoglobulinemia, were evaluated before, during IFNα treatment, and after IFNα withdrawal.

Two patients had type II mixed cryoglobulinemia with a monoclonal component Ig M kappa (patients 1 and 3) with anti IgG activity. The nature of the cryoglobulins could not be determined in the remaining patient due to the scarce cryoprecipitate obtained. All patients tested positive for HCV antibodies by second generation ELISA. Plasma viral RNA was detected in two patients by RT-PCR (patient 1 and 2) and was not determined in the remaining.

The patients reported had prominent cryoglobulinemia-related ischemic manifestations including toes and fingertip necrosis in patients 1 and 2 respectively and ischemic leg ulcers in patient 2 and 3. Necrotizing vasculitis was observed in perineural and endoneural vessels in patient 1 and in dermal vessels in patients 2 and 3.

All patients were treated with prednisone at 0.5 -1 mg/Kg/day and IFNα at 3 x 10^6 units 3 times a week. In addition, 2 patients were treated with an immunosuppressive agent (patient 1 with cyclophosphamide and patient 3 initially with azathioprine and subsequently with cyclophosphamide) because of the severity of the lesions. IFNα treatment was discontinued when worsening of lesions was apparent. Corticosteroids and immunosuppressive agents were maintained when IFNα was withdrawn.

In addition to a complete clinical evaluation, cryoglobulins and liver function tests were determined serially during follow-up.

With corticosteroid and IFNα treatment general symptoms (fever, malaise, anorexia, and weakness) clearly ameliorated in all patients and no new cryoglobulinemia-derived

lesions appeared. Liver function tests also improved and cryocrits decreased. A remarkable rebound in these parameters was observed when IFNα was discontinued.

However, in spite of clinical, biochemical, and immunologic improvement achieved in all patients, established ischemic lesions worsened in all patients leading to amputations in 2 of them (transmetatarsian amputation in patient 1 and infracondileal amputation in patient 3) (figure 1). Table 1 shows the clinical course of the 3 patients throughout an 8-month follow-up period after the beginning of IFNα therapy. In all patients an appropriate healing of their lesions began shortly after IFNα was withdrawn and was virtually complete at the end of the follow-up period (figure 1).

A remarkable decrease of vascularization was apparent in ischemic lesions a few weeks after IFNα treatment (figure 1). Skin biopsies were taken from patient 3 near the edge of an ischemic ulcer before and during IFNα treatment and disclosed a remarkable decrease in subepidermic vascularization after 2 months of IFNα therapy (figure 2). Blood vessels were identified in the biopsy specimens with the lectin *Ulex Europaeus* (Dako, Copenhagen, Denmark) which recognizes fucose on endothelial cells. Vessel number in the subepidermic area was quantitated by a blinded investigator (J. H-R.) in random, non overlapping fields at 300x magnification. The number of vessels/field in the upper dermis was significantly reduced in the specimen taken during IFNα treatment. Specimens taken before IFNα administration showed a median of 11 (range 0-31) vessels/field whereas specimens obtained during IFNα treatment disclosed a median of 7 (range 1-13) vessels/field ($p=0.001$ by Mann Whitney U test).

DISCUSSION

The patients described in this report presented with few classical cryoglobulinemia-related manifestations and with unusually prominent ischemic lesions. Upon IFNα treatment, general symptoms remarkably improved, no new lesions or additional manifestations appeared, liver function tests returned to normal, and cryocrits decreased. The clear rebound in biochemical and immunological parameters observed in all patients when IFNα was discontinued as well as the development of a new ischemic lesion in patient 3 when IFNα was withdrawn, indicate that IFNα was able to suppress disease activity. However, preexisting ischemic lesions worsened and required amputations in two of the patients.

In spite of the clinical improvement achieved by a high proportion of patients with HCV-associated mixed cryoglobulinemia treated with IFNα, most series include a minority of patients in whom vasculitic manifestations worsen upon IFNα treatment (4). Moreover, the development of ischemic lesions during IFNα therapy for other conditions has also been reported (5,6). A careful follow-up of our patients revealed that cryoglobulinemic ulcers became progressively devoid of vascularization and granulation tissue with subsequent appearance of necrosis and bacterial colonization leading to amputation in two patients. In addition, in patient 3, a substantial decrease in subepidermal microvessels could be demonstrated . Although all patients concomitantly received prednisone and, some of them, immunosuppressive agents, and, consequently, the contribution of these medications in worsening ischemic lesions cannot be completely ruled out, IFNα had probably the main role since its withdrawal was followed by a dramatic healing in all cases, even though the remaining drugs were maintained.

IFNα may benefit patients with HCV-associated cryoglobulinemia through various pathways. Besides of its long-term recognized antiviral activity (2-4), IFNα has immunomodulatory properties which may directly influence cryoglobulin production and cryoglobulin-mediated organ damage (7). Its antiproliferative effect on lymphocytes, which has been demonstrated to be useful in treating some lymphoid malignancies, may limit the expansion of the clone producing the monoclonal component of type II cryoglobulins (3). Moreover, IFNα impairs B cell differentiation and decreases immunoglobulin synthesis (8). By enhancing macrophage functions, IFNα may potentiate the elimination of immune complexes (3). In addition, it has become apparent that IFNα is a pleiotropic cytokine and, besides its immunoregulatory properties, it has potent biologic effects on a variety of cells (7). Cumulated experience indicates that IFNα is an angiogenesis inhibitor (9) and delayes wound healing in animal models (10). The mechanisms through which IFNα inhibits blood vessel formation are poorly understood. Recent studies indicate that IFNα may down-regulate the synthesis of the angiogenic factor bFGF (11). The therapeutic efficacy of IFNα on disorders characterized by extensive vascular proliferation demonstrates its potent anti-angiogenic effects *in vivo*. IFNα has been succesfully used to treat life-threatening hemangiomas of infants , pulmonary hemangiomatosis , and both epidemic and AIDS-associated Kaposi sarcoma (12,13).

We have previously shown that angiogenesis is a prominent phenomenon in vasculitis (14,15). Newly formed microvessels may contribute to the development of vascular inflammatory infiltrates by recruiting leukocytes through adhesion molecule expression but may also prevent organ ischemia by providing new blood supply (14,15). Angiogenesis is crucial for wound healing and may also contribute to tissue repair in vasculitic lesions when the stimuli recruiting inflammatory cells in the vessel wall

cease or are therapeutically repressed. Our observations indicate that the deleterious effect of IFNα observed in some cryoglobulinemia patients may be related, at least in part, to its anti-angiogenic activity. IFNα impairs the appropriate revascularization and healing of ischemic organs and caution should be used for IFNα therapy in patients with prominent cryoglobulinemia-related ischemic manifestations.

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ACKNOWLEDGMENTS

We thank Mrs Margarita Mainar and Mrs Elena Gonzalbo for excellent technical assistance and Mr Albert Jordà for taking pictures of patients.

Tabla 1: Outcome of ischemic lesions after the initiation of IFNα therapy

	1 MONTH	2 MONTHS	3 MONTHS	4 MONTHS	8 MONTHS
ATIENT 1	Worsening of toes ischemia (left foot)	Worsening of toes ischemia (right foot)	Appropriate healing	Uneventful	Uneventful
	Transmetatarsian amputation	IFNα stopped	Right toes ischemia resolved		
ATIENT 2	Progression of fingertip ischemia and leg ulcers	Progression	Progression	IFNα stopped	Complete healing
ATIENT 3	Worsening of leg ulcers	Transmetatarsian amputation (left)	Ascending necrosis in left foot	Purpura	Appropriate healing of amputated leg
		Amputation of 2 right toes	Left infracondilean amputation	Increase in Proteinuria	Nearly complete healing of right foot ulcers.
		Increase in prednisone Azathioprine added	IFNα stopped	New ulcer Azathioprine changed to cyclophosphamide	

FIGURE LEGENDS

Figure 1: Evolution of ischemic lesions in patient 2 (A, B, and C) and in patient 3 (D, E, and F). A and D: ischemic lesions at first evaluation. B and D: Ulcer enlargement after 2 months of IFNα treatment with an apparent decrease in vascularization, particularly in patient 3 (D). D and F: Nearly complete healing of the ulcers 2 months (D) and 4 months (F) after IFNα withdrawal in patients 2 and 3 respectively.

Figure 2: Subepidermal blood vessels identified by staining with *Ulex Europaeus* lectin in a skin biopsy from patient 3 at baseline evaluation (A) and after being treated with IFNα for 2 months (B).

REVISIONS

MTA-Medicina Interna 1996; 14(1): 5-37

VASCULITIS SISTÉMICAS: NUEVOS CONCEPTOS

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INTRODUCCIÓN

Las vasculitis son procesos clicopatológicos caracterizados por la inflamación y/o necrosis de los vasos sanguíneos. Pueden presentarse como una entidad primaria o complicar el curso de otra enfermedad, como la artritis reumatoide o el lupus eritematoso sistémico (vasculitis secundarias). Como consecuencia de la lesión de los vasos sanguíneos puede aparecer isquemia y, en ocasiones, hemorragia en los órganos irrigados por los vasos afectados. El espectro clínico de las vasculitis es muy amplio, ya que depende del tejido u órgano afectado, así como de la gravedad del proceso. Del mismo modo, el pronóstico puede ser muy variable y el tratamiento debe individualizarse en cada caso. La variedad del espectro clínico también conlleva dificultades en el diagnóstico, con el consiguiente retraso en la instauración del tratamiento.

En los últimos años se han realizado importantes contribuciones al conocimiento de la patogénesis de estas enfermedades, así como a su diagnóstico y tratamiento, que han modificado las nomenclaturas y clasificaciones más clásicas. En esta revisión trataremos fundamentalmente sobre estos aspectos de las vasculitis.

CLASIFICACIÓN

Desde que en 1952 Zeek publicó la primera clasificación de las vasculitis (1) se han hecho numerosas clasificaciones de estas enfermedades, atendiendo a las manifestaciones clínicas, al tipo o calibre de los vasos afectados, a hallazgos histopatológicos o a combinaciones de estos criterios. Tal como se ha dicho, las vasculitis constituyen un grupo enormemente heterogéneo de enfermedades y carecen de manifestaciones clínicas patognomónicas, de pruebas de laboratorio diagnósticas o de una expresión histopatológica uniforme, además de tener una etiología y una patogénesis poco conocidas. Por todo ello, establecer una clasificación de las vasculitis de acuerdo con un esquema aceptado universalmente es difícil. Sin embargo, una cierta estandarización es sin duda necesaria para facilitar el diagnóstico y el manejo de los pacientes afectos de la enfermedad, para comparar los resultados de distintos tratamientos y para mejorar nuestra comprensión de la causa, la patogénesis y la historia natural de las distintas vasculitis.

En esta revisión vamos a comentar las clasificaciones más relevantes por las innovaciones que han aportado y su trascendencia en la evolución del conocimiento de las vasculitis.

El primer intento de clasificar las vasculitis lo realizó Zeek en 1952 (1). Zeek propone el término genérico de angitis necrosantes y las clasifica en cinco tipos distintos de vasculitis sistémicas: angitis por hipersensibilidad, angitis granulomatosa alérgica, arteritis reumática, periarteritis nodosa y arteritis de la temporal. La autora ya incluye el entonces recientemente descrito síndrome de Churg-Strauss (angitis granulomatosa alérgica), pero todavía no habla de la granulomatosis de Wegener ni de la arteritis de Takayasu. A pesar de sus limitaciones, esta clasificación tiene el mérito de haber servido de punto de partida para las clasificaciones posteriores.

La clasificación propuesta por Fauci y cols. en 1978 (2) es la primera que se aleja sustancialmente de la realizada por Zeek. En primer lugar, incluye más entidades, algunas ya conocidas (enfermedad de Buerger) y otras nuevas (enfermedad de Kawasaki). En segundo lugar, agrupa el síndrome de Churg-Strauss y la poliarteritis nodosa, junto con el llamado síndrome de solapamiento, en el grupo de vasculitis necrosantes sistémicas. La inclusión del síndrome de solapamiento en esta categoría puede ser confusa, ya que puede existir solapamiento entre todos los tipos de vasculitis, como posteriormente reconocieron los propios autores (3). En tercer lugar, incluye la granulomatosis linfomatoide, de la que poco después se conoció su naturaleza de linfoma angiodestructivo y angiocéntrico.

Lie (4-6) separa las vasculitis en infecciosas y no infecciosas, y divide esta última categoría de acuerdo con el tipo y el calibre predominantes de los vasos afectados (Tabla 1). Este esquema es útil para el clínico, pues ofrece directrices sobre cuándo sospechar, dónde biopsiar y qué esperar en la confirmación histológica del diagnóstico de vasculitis. Lie también enfatiza la observación de Gilliam y Smalley (7) sobre la naturaleza solapante del tamaño de las arterias afectas en los principales tipos de vasculitis (Fig. 1).

La clasificación del American College of Rheumatology (ACR) de 1990 (8-10) no es, en realidad, ningún intento de reclasificar las vasculitis, sino que describe criterios para la clasificación de siete entidades ya previamente seleccionadas (Tabla 2). Dado el solapamiento que se produce entre las vasculitis y la existencia de casos de difícil clasificación, estos criterios permiten, con una elevada sensibilidad y especificidad, adscribir a los pacientes con diagnóstico de vasculitis a entidades clinicopatológicas concretas. Aparte de las repercusiones terapéuticas

Tabla 1. Clasificación simplificada de las vasculitis según Lie, 1986-1991 (4-6).

Angitis infecciosas
Angitis no infecciosas
- A. Afectando vasos de gran, mediano y pequeño calibre
  1. Arteritis de Takayasu
  2. Arteritis de células gigantes (granulomatosas)
     - Arteritis de la temporal
     - Angitis granulomatosa diseminada
     - Angitis primaria del sistema nervioso central
  3. Aortitis de las enfermedades reumáticas del tejido conectivo
- B. Afectando predominantemente vasos de mediano y pequeño calibre
  1. Tromboangitis obliterante (enfermedad de Buerger)
  2. Grupo de la poliarteritis nodosa (PAN)
     - PAN clásica
     - PAN microscópica
     - PAN infantil (vasculitis de la enfermedad de Kawasaki)
  3. Angitis y granulomatosis patergicoalérgicas
     - Granulomatosis de Wegener
     - Síndrome de Churg-Strauss
  4. Vasculitis asociada a enfermedades sistémicas
- C. Afectando predominantemente vasos de pequeño calibre (angitis por hipersensibilidad, vasculitis leucocitoclástica)
  1. Enfermedad del suero o hipersensibilidad a fármacos
  2. Síndrome de Schönlein-Henoch (púrpura anafilactoide)
  3. Crioglobulinemia mixta esencial
  4. Vasculitis urticarial hipocomplementémica
  5. Vasculitis asociada a malignidad

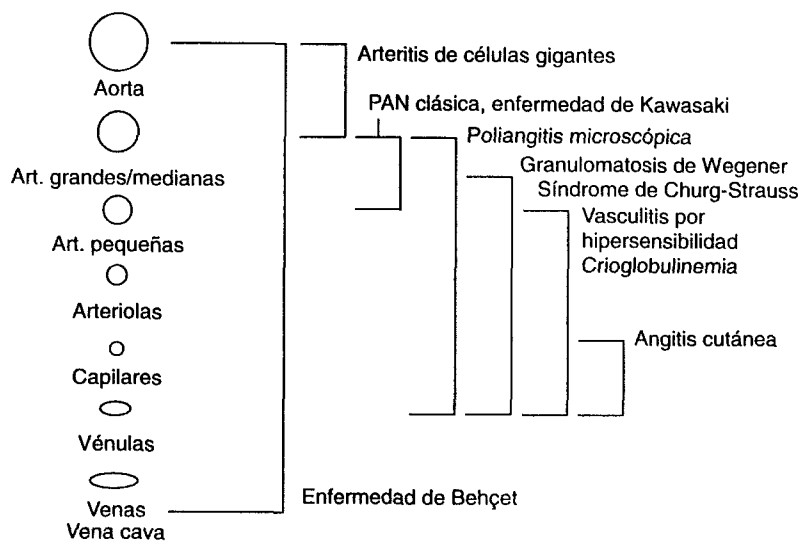

FIG. 1. Clasificación de las vasculitis según el calibre del principal vaso afectado. PAN: poliarteritis nodosa.

Tabla 2. Criterios para la clasificación de las vasculitis según el American College of Rheumatology, 1990 (8-10).

Condición	Criterio	Número de criterios necesario	Sensibilidad (%)	Especificidad (%)
Púrpura de Schönlein-Henoch	Púrpura palpable *Inicio anterior a los 20 años* Angor intestinal Granulocitos en la pared vascular (en la biopsia)	≥2	87,8	87,7
Granulomatosis de Wegener	Inflamación nasal u oral Radiografía de tórax anormal (nódulos, infiltrados o cavidades) Microhematuria o cilindros hemáticos Inflamación granulomatosa en la biopsia	≥2	88,2	92
Síndrome de Churg-Strauss	Asma Eosinofilia >10% Neuropatía Infiltrados pulmonares (no fijos) Anormalidad de los senos paranasales Eosinófilos extravasculares	≥4	85	99,7
Vasculitis por hipersensibilidad	Inicio posterior a los 16 años Púrpura palpable Erupción maculopapular Granulocitos perivasculares o extravasculares (arteriolas o vénulas)	≥3	71	83,9
Poliarteritis nodosa	Pérdida de peso igual o superior a 4 kg *Livedo reticularis* Dolor a la palpación testicular Mialgia o debilidad Mono o polineuropatía Presión arterial diastólica ≥90 Elevación del BUN o la creatinina Virus de la hepatitis B Anormalidad arteriográfica *Biopsia de arterias pequeñas o* medianas que contenga granulocitos	≥3	82,2	86,6
Arteritis de la temporal o de células gigantes	Inicio superior a los 50 años Cefalea de reciente aparición o de distinto tipo Dolor a la palpación de la arteria temporal o pulso disminuido Velocidad de sedimentación globular ≥50 Biopsia con infiltrados de células mononucleadas o inflamación granulomatosa	≥3	93,5	91,2
Arteritis de Takayasu	Inicio anterior a los 40 años Claudicación de extremidades Pulso de la arteria braquial disminuido Diferencia de presión arterial entre los dos brazos superior a 10 mmHg Soplo sobre las arterias subclavias o la aorta Anormalidad arteriográfica (estrechamiento u oclusión de la aorta o sus ramas principales)	≥3	90,5	97,8

de una clasificación adecuada, la aplicación de unos criterios estandarizados supone un consenso de cara a la realización de estudios clínicos.

En los últimos años se ha enfatizado sobre el concepto de poliangitis microscópica. Inicialmente fue descrita como una afectación glomerular aislada y llamada poliarteritis microscópica (11). Algunos autores piensan que es una variante de la poliarteritis nodosa, pero otros la consideran una entidad diferenciada (12). Independientemente de si la poliangitis microscópica se considera una entidad separada de la poliarteritis nodosa o no, lo cierto es que presenta algunas características histológicas y clínicas distintas (13). Desde el punto de vista histológico se caracteriza por la afectación de vasos de pequeño calibre (arteriolas, capilares o vénulas) y la ausencia de granulomas. La necrosis fibrinoide es rara y las lesiones vasculíticas tienden a estar en un mismo estadio evolutivo. Entre sus manifestaciones clínicas destaca la glomerulonefritis rápidamente progresiva y la hemorragia alveolar (14, 15), que cuando se presentan simultáneamente constituyen un síndrome pulmonar-renal similar al observado en el síndrome de Goodpasture o en la granulomatosis de Wegener. La hemorragia alveolar, que nunca se presenta en la poliarteritis nodosa clásica, es una manifestación grave que puede ser fatal. Aunque el curso inicial puede ser más indolente, el riñón se deteriora rápidamente y puede instaurarse una insuficiencia renal crónica. Las recidivas son más frecuentes que en la poliarteritis nodosa. El pronóstico es, pues, más sombrío, hecho que tiene implicaciones en el tratamiento, como se comentará más adelante. En la mitad de los casos se detecta la presencia de anticuerpos anticitoplasma del neutrófilo (ANCA), que suelen tener un patrón perinuclear y estar dirigidos contra la mieloperoxidasa (16-18). Por el contrario, no se ha descrito asociación al virus de la hepatitis B.

En la nomenclatura propuesta por la Conferencia de Chapel Hill (Chapel Hill International Consensus Conference, 1993) (12) se da una gran importancia a la poliangitis microscópica como entidad individualizada. En esta conferencia se definen las características clinicopatológicas de 10 entidades seleccionadas de vasculitis, las cuales se agrupan según el calibre de los vasos más afectados (Tabla 3). La principal diferencia respecto a clasificaciones previas es la importancia dada a la poliangitis microscópica. Según esta clasificación, en la poliangitis microscópica debe haber afectación de los vasos "microscópicos" (arteriolas, capilares y/o vénulas), aunque las arterias pequeñas o medianas también pueden estar afectadas. Por el contrario, en la poliarteritis nodosa no hay afectación de los vasos "microscópicos", ni, por tanto, glomerulonefritis. La poliarteritis nodosa es, pues, según este criterio, una entidad muy rara, mientras que la poliangitis microscópica es mucho más frecuente. Este concepto ha sido muy discutido por otros autores (19) e incluso es contradictorio respecto a afirmaciones hechas previamente por los autores que lo propugnan (20).

Así pues, aunque existen entidades bien diferenciadas con unas características clinicopatológicas bien definidas, no existe una clasificación universalmente aceptada.

En los aspectos concretos de esta revisión nos centraremos fundamentalmente en los tipos de vasculitis más claramente reconocidos y universalmente aceptados

Tabla 3. Nombres y definiciones de las vasculitis adoptados por la Conferencia Internacional de Chapel Hill sobre la nomenclatura de las vasculitis sistémicas*.

Vasculitis de gran vaso	
Arteritis de la temporal o de células gigantes	Arteritis granulomatosa de la aorta y sus ramas principales, con predilección por las ramas extracraneales de la arteria carótida. La arteria temporal frecuentemente se afecta. Suele aparecer en pacientes mayores de 50 años y a a menudo se asocia a polimialgia reumática.
Arteritis de Takayasu	Inflamación granulomatosa de la aorta y sus ramas principales. Normalmente aparece en pacientes con menos de 50 años.
Vasculitis de vaso mediano	
Poliarteritis nodosa (clásica)	Inflamación necrosante de arterias pequeñas o medianas, sin glomerulonefritis ni vasculitis de arteriolas, capilares o vénulas.
Enfermedad de Kawasaki	Arteritis que afecta arterias grandes, medianas y pequeñas y que se asocia al síndrome linfático mucocutáneo. Las arterias coronarias están afectadas frecuentemente. La aorta y las venas pueden estar afectadas. Suele aparecer en niños.
Vasculitis de pequeño vaso	
Granulomatosis de Wegener	Inflamación granulomatosa que afecta el traco respiratorio y vasculitis necrosante que afecta vasos de calibre pequeño a mediano (p. ej., capilares, vénulas, arteriolas y arterias). La glomerulonefritis necrosante es frecuente.
Síndrome de Churg-Strauss	Inflamación granulomatosa y rica en eosinófilos que afecta el tracto respiratorio, y vasculitis necrosante que afecta vasos de calibre pequeño a mediano, y asociada con asma y eosinofilia.
Poliangitis o poliarteritis microscópica	Vasculitis necrosante con pocos o sin depósitos inmunitarios, que afecta vasos pequeños (p. ej., arteriolas, capilares o vénulas). Puede haber arteritis necrosante en arterias pequeñas o medianas. Suele aparecer glomerulonefritis necrosante. La capilaritis pulmonar es frecuente.
Púrpura de Schönlein-Henoch	Vasculitis con depósitos inmunitarios con predominio de IgA, que afecta vasos pequeños (p. ej., arteriolas, capilares o vénulas). Típicamente afecta la piel, el intestino y el glomérulo, y se asocia a artralgias o artritis.
Vasculitis de la crioglobulinemia mixta	Vasculitis con depósitos inmunitarios de crioglobulinas, que afecta vasos pequeños (p. ej., arteriolas, capilares o vénulas) y asociada con crioglobulinas en suero. A menudo se afecta la piel y el glomérulo.
Angitis cutánea leucocitoclástica	Angitis cutánea leucocitoclástica aislada sin vasculitis sistémica ni glomerulonefritis.

*Gran vaso se refiere a la aorta y sus principales ramas, dirigidas hacia las extremidades, la cabeza y el cuello; vasos medianos son las principales arterias viscerales (p. ej., renales, hepáticas, mesentéricas, coronarias); vasos pequeños son vénulas, capilares, arteriolas y las arterias intraparenquimatosas distales que conectan con las arteriolas.

como síndromes clínicos diferenciados. Concretamente, nos referiremos a las vasculitis descritas en la clasificación de 1990 del ACR, haciendo mención especial a la poliangitis microscópica.

ETIOPATOGENIA

Las vasculitis constituyen un grupo de enfermedades muy heterogéneas con un sustrato histopatológico común, la inflamación de la pared vascular, que puede afectar a vasos de cualquier tamaño en todo el sistema vascular. Los primeros estudios sobre el posible mecanismo patogenético apuntaron hacia el depósito de inmunocomplejos circulantes y la activación del complemento. En los últimos años se han propuesto numerosos mecanismos patogenéticos potenciales, principalmente el papel de los ANCA, los anticuerpos anticélula endotelial y los mecanismos patogenéticos mediados por células T. Estudios moleculares han demostrado una asociación de la arteritis de la temporal o de células gigantes y la polimialgia reumática con alelos HLA-DRB1*04 del sistema mayor de histocompatibilidad (SMH) y, en cambio, no han demostrado ninguna relación entre la granulomatosis de Wegener o la poliarteritis microscópica y alelos HLA. Estos y otros mecanismos patogenéticos potenciales, revisados recientemente en una excelente actualización (21), no son mutuamente exclusivos y puede que actúen conjuntamente para mantener y reforzar el daño vascular desencadenado por agentes etiológicos todavía desconocidos y probablemente heterogéneos.

Independientemente de los mecanismos inmunopatogenéticos primarios que llevan a la inflamación vascular, los leucocitos son reclutados en los focos inflamatorios a través de una compleja interrelación con el endotelio y la matriz extracelular. En los últimos años se están llevando a cabo numerosos estudios para definir el papel que tienen las moléculas de adhesión celular en el desarrollo de los infiltrados vasculares. Una vez localizados en la pared vascular, los leucocitos activados secretan distintas citocinas y factores de crecimiento. La gran importancia de estos mediadores en la génesis del daño y la oclusión vascular también está empezando a ser objeto de interés.

Depósito de inmunocomplejos circulantes

Gracias a distintos modelos experimentales (reacción de Arthus, enfermedad del suero) se ha podido comprobar que inmunocomplejos circulantes, formados con exceso de antígeno, no son eliminados de la circulación por el sistema mononuclear fagocítico y se depositan en la pared vascular. Los vasodilatadores y el flujo turbulento aumentan estos efectos. La cascada del complemento, que puede ser activada por estos inmunocomplejos, produce factores quimiotácticos para los neutrófilos, factores que también pueden activar los leucocitos e inducir la liberación de enzimas lisosomales capaces de dañar la pared vascular (2, 22, 23).

Las vasculitis por hipersensibilidad, la púrpura de Schönlein-Henoch y algunas formas de vasculitis necrosantes sistémicas, como las asociadas al virus de la hepatitis B, son las que parecen adecuarse más a este modelo patogenético. Sin embargo, este modelo no explica satisfactoriamente la patogénesis de la arteritis de la temporal, el síndrome de Churg-Strauss, la granulomatosis de Wegener u otros procesos en los cuales los depósitos tisulares de inmunocomplejos son infrecuentes.

Anticuerpos anticitoplasma de neutrófilo (ANCA)

La asociación entre distintas vasculitis y la presencia de ANCA ha sido uno de los descubrimientos más relevantes en el campo de las vasculitis. Los ANCA reconocen componentes del citoplasma del neutrófilo. Los principales autoantígenos identificados en los gránulos azurófilos son la proteinasa 3 (PR3) y la mieloperoxidasa (MPO). Los ANCA-PR3 tienen una gran sensibilidad y especificidad por la granulomatosis de Wegener, y los ANCA-MPO están presentes en una proporción variable de pacientes con poliarteritis microscópica y glomerulonefritis rápidamente progresiva (24, 25). Los ANCA también pueden reconocer otros componentes de los gránulos de los neutrófilos, como elastasa, catepsina G, lactoferrina y lisozima. El número de antígenos reconocidos por los ANCA así como el número de enfermedades en las cuales pueden detectarse ANCA va creciendo a medida que avanzan los estudios sobre estos anticuerpos (26). El significado biológico de estos nuevos ANCA todavía se desconoce.

La intensa asociación existente entre los ANCA-PR3 y la granulomatosis de Wegener y, en menor grado, entre los ANCA-MPO y la poliarteritis microscópica y la glomerulonefritis necrosante han llevado a la hipótesis de que los ANCA participan directamente en la patogénesis de la inflamación y el daño vasculares. Sin embargo, aunque parece claro que los ANCA son capaces de potenciar respuestas de los neutrófilos desencadenadas por otros mediadores de la inflamación, asumir que desempeñan un papel principal en la patogénesis de las vasculitis asociadas a ANCA es todavía prematuro.

Anticuerpos anticélula endotelial

En algunas enfermedades autoinmunitarias, entre las cuales se encuentran la granulomatosis de Wegener, la poliangitis microscópica, la enfermedad de Kawasaki, la enfermedad de Behçet y la vasculitis reumatoide, se han descrito anticuerpos dirigidos contra las células endoteliales (27-30). En algunos pacientes se ha demostrado que los niveles de estos anticuerpos reflejan la actividad de la enfermedad (29, 31). A raíz de estas observaciones clínicas se ha formulado la hipótesis de que los anticuerpos anticélula endotelial podrían contribuir a la lesión vascular. La procedencia de estos anticuerpos, su especificidad, su significado biológico y su posible papel patogenético son todavía objeto de investigación.

Daño vascular mediado por células T

Algunos estudios inmunopatológicos han demostrado que en distintas vasculitis el infiltrado inflamatorio está compuesto básicamente por linfocitos T activados y macrófagos. Concretamente, se ha observado en la poliarteritis nodosa, la enfermedad de Kawasaki, la arteritis de la temporal, la arteritis de Takayasu y en los granulomas de la granulomatosis de Wegener (32-38). Incluso en los infiltrados

de las vasculitis por hipersensibilidad y la poliangitis microscópica en algún momento de la evolución de la enfermedad se halla cierto porcentaje de células mononucleares (36). Además, en los infiltrados inflamatorios de la arteritis de la temporal y la poliarteritis nodosa se han identificado células dendríticas con la capacidad de actuar como presentadoras y que expresan antígenos de SMH clase II (32, 34). En la arteritis de la temporal se ha demostrado recientemente una expansión clonal en una minoría de los linfocitos T infiltrantes, posiblemente como respuesta específica a un antígeno desencadenante de la enfermedad (37). Estos hallazgos sugieren que los mecanismos inmunitarios mediados por células T desempeñan un papel importante en la patogénesis de distintas vasculitis. Parece que los mecanismos a través de los cuales se activan los linfocitos T para producir daño vascular son heterogéneos y pueden cambiar entre las diferentes vasculitis (35, 37, 39).

Papel de las moléculas de adhesión en el desarrollo de los infiltrados inflamatorios vasculares

Independientemente de los agentes etiológicos o de los mecanismos patogénicos inmediatos, está bien establecido que el desarrollo de los infiltrados inflamatorios en los tejidos se produce a través de interacciones dinámicas entre leucocitos, células endoteliales y proteínas de la matriz extracelular (40-43). Estas interacciones se producen a través de una compleja gama de receptores de membrana llamados moléculas de adhesión. En la membrana leucocitaria, ciertos carbohidratos (formas sialiladas de los antígenos Lewis x y Lewis a), selectinas (selectina L), integrinas (LFA-1, Mac-1 y gp 150,95 de la subfamilia ß2 y VLA-4 de la subfamilia ß1) y miembros de la superfamilia de las inmunoglobulinas (CD31 o PECAM, CD2 o LFA-2) interaccionan de una manera secuencial y regulada con gran precisión con ligandos específicos de la célula endotelial. Estos ligandos son carbohidratos, selectinas (selectinas P y E) y otros miembros de la superfamilia de las inmunoglobulinas (ICAM-1, ICAM-2, VCAM-1, LFA-3, CD31) (Tabla 4). La transición desde un leucocito circulante a un leucocito adherente que infiltra los tejidos requiere una serie de eventos coordinados (41-44). Primero, cuando un estímulo apropiado induce la expresión endotelial de selectinas y de receptores para las selectinas leucocitarias, los leucocitos circulantes de las vénulas postcapilares se enlentecen y ruedan sobre la superficie endotelial. Este fenómeno está mediado fundamentalmente por interacciones entre carbohidratos y selectinas. Posteriormente, las integrinas leucocitarias, que suelen estar en un estado no adherente, son activadas. Esta activación ocurre a través de factores solubles (quimiocinas) u otras interacciones intercelulares que implican moléculas coestimulatorias (45-47). Los contrarreceptores de las integrinas leucocitarias pueden estar expresados de forma constitutiva por la célula endotelial (ICAM-2), o inducidos (VCAM-1) o sobreexpresados (ICAM-1) por el estímulo de citocinas, fundamentalmente IL-1, TNFα e IFNγ (43, 44). Mediante la interacción de integrinas e inmunoglobulinas se produce la adhesión fuerte y la extensión de los leucocitos sobre el endotelio y subsiguiente transmigración a través de las uniones intercelulares. Finalmente, los leucocitos interaccionan con proteínas de la matriz extracelular, fundamentalmente a través de integrinas ß1, que se unen principalmente a laminina, colágeno o fibronectina.

Tabla 4. Moléculas de adhesión que intervienen en las interacciones entre leucocitos y endotelio*.

Leucocito	Endotelio
Sialyl Lewis x	Selectina E, selectina P
Sialyl Lexis a	Selectina E
Selectina L	Adresinas
LFA-1 (αLß2)	ICAM-1, ICAM-2
Mac-1 (αMß2)	ICAM-1
gp 150,95 (αXß2)	?
VLA-4 (α4ß1)	VCAM-1

*Lista resumida y esquemática.

Las interacciones mediadas por las moléculas de adhesión forman parte de la respuesta inflamatoria fisiológica a la lesión. Sin embargo, en distintas enfermedades inflamatorias crónicas se puede observar la expresión persistente de receptores de la célula endotelial (ICAM-1 y VCAM-1) o la sobreactivación de integrinas leucocitarias, hecho que probablemente contribuya al continuado reclutamiento leucocitario en los tejidos afectados (48).

Se conoce muy poco sobre la expresión de las moléculas de adhesión en las vasculitis. Distintos trabajos demuestran una mayor expresión de algunas de estas moléculas en la superficie de las células endoteliales de los leucocitos infiltrantes o incluso de otros subtipos celulares (49-52) en pacientes afectos de vasculitis, pero los resultados son todavía preliminares, ya que los trabajos no son uniformes, la población estudiada suele ser pequeña y no se han hecho estudios sobre la actividad funcional de las moléculas expresadas.

Aunque la información específica es todavía escasa, las interacciones mediadas por moléculas de adhesión pueden ser muy importantes en el desarrollo final de los infiltrados inflamatorios en las vasculitis. Varios de los mecanismos patogénicos de los que se ha dicho que participan en la inflamación y el daño vasculares en algún punto implican la expresión o función de las moléculas de adhesión:

a) Las citocinas inductoras, producidas por linfocitos activados y macrófagos durante la respuesta inmunitaria específica, pueden ser detectadas en lesiones vasculíticas y coexisten con una mayor expresión de moléculas de adhesión por la célula endotelial (37).
b) Los ANCA, al unirse a la célula endotelial, incrementan la expresión de moléculas de adhesión (53).
c) El daño vascular mediado por inmunocomplejos y complemento también requiere la expresión y función de las moléculas de adhesión (54, 55). Algunos modelos experimentales apoyan también este supuesto (56-58).

Dado que la regulación de la expresión de las moléculas de adhesión puede cambiar entre distintos lechos vasculares, es razonable especular que la expresión diferencial de las moléculas de adhesión puede explicar en parte los distintos patrones específicos de afectación orgánica en algunas vasculitis.

La inhibición de las interacciones de las moléculas de adhesión por anticuerpos monoclonales o ligandos solubles ha ayudado a prevenir lesiones en distintos mo-

delos animales de enfermedades autoinmunes humanas (43, 55, 59). Bloquear eventos adhesivos puede llevar a nuevas opciones terapéuticas para enfermedades inflamatorias crónicas, entre ellas las vasculitis. La aplicación de este concepto requiere una mayor comprensión de la cronología de la expresión inducible de moléculas de adhesión en las vasculitis, las vías preferenciales en síndromes particulares y el estado de la expresión de las moléculas de adhesión en la remisión.

En el plasma humano y otros líquidos biológicos se han detectado selectinas y algunos miembros de la superfamilia de las inmunoglobulinas en forma circulante. Estas formas solubles son separadas de la membrana celular por lisis enzimática o son generadas directamente por un procesamiento alternativo de su RNA como variantes carentes de las porciones citoplasmática y transmembranosa (41, 60). Probablemente estas moléculas circulantes tengan una función reguladora, pero su significado biológico no es aún bien conocido.

Mediante la técnica de ELISA se han demostrado niveles elevados de moléculas de adhesión circulantes en distintas vasculitis. Algunos estudios incluyen vasculitis diferentes (61-65), con lo que los resultados son de difícil interpretación. Recientemente se han hecho algunos trabajos con grupos homogéneos de vasculitis y con seguimiento longitudinal que aportan resultados interesantes pero aislados (66-69). En algunos estudios las moléculas de adhesión circulantes se correlacionan con niveles de citocinas proinflamatorias, como TNFα e IL-6, y con proteínas de fase aguda (63, 68, 69). Resultados preliminares sugieren que, aunque los niveles de moléculas de adhesión solubles disminuyen cuando los pacientes logran la remisión clínica, pueden no volver a la normalidad. Estos hallazgos pueden reflejar una exposición persistente de las células endoteliales a un microentorno moderadamente inflamatorio. Observaciones recientes sobre el alto porcentaje de recidivas de muchas vasculitis (70-72) y hallazgos histopatológicos de inflamación vascular en pacientes supuestamente en remisión (71, 73) plantean la posibilidad de que la detección de niveles persistentemente altos de moléculas de adhesión solubles distinga entre una actividad inflamatoria subclínica y una verdadera remisión. La utilidad de los niveles de moléculas de adhesión circulantes de cara a tomar decisiones clínicas, como ajustar el tratamiento o suspenderlo, requiere ser investigada en estudios prospectivos amplios con seguimiento longitudinal.

Producción de citocinas y factores de crecimiento

Las células inflamatorias activadas y reclutadas en los vasos sanguíneos liberan una diversidad de citocinas y factores de crecimiento, cuyos efectos generales y sobre las células endoteliales y musculares lisas pueden explicar la mayoría de los síntomas y las complicaciones de las vasculitis. La fiebre, el mal estado general y la pérdida de peso son frecuentes en las vasculitis y pueden atribuirse a los efectos sistémicos de las citocinas proinflamatorias (IL-I, IL-6 y TNF) (74, 75). Las manifestaciones clínicas focales a menudo son secundarias a la disfunción de los órganos isquémicos, deficientemente irrigados por los vasos afectados. La oclusión vascular puede estar producida por espasmo, trombosis y/o, con más frecuencia,

por hiperplasia intimal y fibrosis. En los últimos años se ha demostrado que distintos mediadores liberados durante la inflamación poseen actividades vasoactivas, protrombóticas y fibrogénicas (76-78). Otras citocinas contribuyen a mantener el infiltrado inflamatorio. Las citocinas con actividad quimiotáctica (quimiocinas) continúan atrayendo leucocitos (45). IL-1, IL-4, IFNγ y TNFα sobreexpresan las moléculas de adhesión, hecho que permite que más leucocitos se adhieran y migren a través del endotelio (76, 77). Las citocinas y factores de crecimiento angiogénicos (IL-8, factores de crecimiento de los fibroblastos y de las células endoteliales) pueden contribuir a la neovascularización en el seno de la pared vascular de los grandes vasos, proporcionando así nuevos lugares a través de los cuales los leucocitos pueden migrar. Además, otras citocinas prolongan la vida media de los leucocitos en el infiltrado inflamatorio (77). Nuestro conocimiento del papel específico que todos estos factores desempeñan en las vasculitis todavía es muy limitado, pero basándonos en sus bien reconocidas propiedades biológicas, podemos sugerir que es muy probable que estos mediadores participen activamente en la patogénesis de las vasculitis.

Algunos investigadores han cuantificado los niveles de citocinas circulantes en pacientes afectos de vasculitis, e incluso su relación con el tiempo de evolución de la enfermedad y el tratamiento instaurado (79-83). Los resultados obtenidos apoyan la hipótesis de la participación activa de las citocinas en la patogénesis de las vasculitis, pero todavía son preliminares.

Dado que las citocinas actúan de manera autocrina/paracrina, el hecho de detectarlas en lesiones tiene, probablemente, una gran relevancia fisiopatológica. En algunos estudios se ha detectado la producción de distintas citocinas proinflamatorias y fibrogénicas en lesiones vasculíticas (84-86).

Otros factores patogénicos

Agentes infecciosos, principalmente virus, han sido implicados en un subgrupo de pacientes con poliarteritis nodosa, púrpura de Schönlein-Henoch, crioglobulinemia mixta y, en menor grado, otras vasculitis. La poliarteritis nodosa se asocia a la infección por el virus de la hepatitis B hasta en una tercera parte de los casos (87-89). El virus de la hepatitis C se ha descrito asociado a la poliarteritis nodosa (90-92) y a la crioglobulinemia mixta (93). Otros virus descritos asociados a las vasculitis son el parvovirus B19 (94) y el virus de la inmunodeficiencia humana (95, 96). Distintos estudios sugieren que estos virus participan en la patogénesis de las vasculitis a las cuales se asocian (89, 92, 93, 95). Esta posible implicación de los virus en la patogénesis de las vasculitis puede tener importancia en el tratamiento, como se comentará más adelante.

Los efectos inmunomoduladores de los esteroides sexuales han sido estudiados con detalle y revisados recientemente (97). Ante la predominancia del sexo femenino en algunas enfermedades autoinmunes (lupus eritematoso sistémico, arteritis de Takayasu) se ha sugerido un papel patogenético importante de los esteroides sexuales en estas enfermedades.

DIAGNÓSTICO

El diagnóstico de vasculitis requiere un alto grado de sospecha, ya que son enfermedades que, por la multiplicidad de órganos que pueden afectar, pueden, por un lado, simular muchos otros procesos patológicos (infecciones, neoplasias) y, por otro, un mismo proceso puede adoptar formas muy distintas. El reconocimiento de un proceso específico implica un conocimiento general de todas las vasculitis y de las características de cada una de ellas, así como del espectro de presentaciones clínicas (22, 98).

El diagnóstico definitivo de vasculitis se puede separar en tres estadios conceptuales: a) diagnóstico genérico de vasculitis; b) estimación de la gravedad y distribución anatómica de la enfermedad, y c) identificación, si es posible, de la entidad diagnóstica específica (98).

Diagnóstico de vasculitis

El diagnóstico de vasculitis requiere una confirmación histológica y/o una demostración angiográfica de lesión vascular. Es preferible la prueba histológica, pero en caso de enfermedad de vasos de gran calibre (p. ej. aorta y tributarias) es difícil acceder a ellos por biopsia. Las biopsias deberían tomarse de una zona u órgano clínicamente afectado. Dado que en todas las vasculitis la afectación es segmentaria, las muestras deben ser generosas y debe obtenerse un gran número de secciones seriadas para evitar falsos negativos (6, 10).

En la granulomatosis de Wegener, la biopsia pulmonar abierta es la que proporciona una mayor rentabilidad diagnóstica. El tejido obtenido suele demostrar la tríada característica de necrosis, vasculitis y formación de granulomas (10, 22). Biopsias de otras localizaciones más frecuentes pueden demostrar también lesiones características, pero en la mitad de los casos revelan lesiones inflamatorias crónicas inespecíficas (99).

Para el diagnóstico de arteritis de la temporal, la mejor localización para biopsiar es la arteria temporal superficial, ya que con frecuencia está afectada y es fácilmente accesible. Si se selecciona un vaso sintomático, se obtiene un segmento de arteria de 2-3 cm, se realiza un seccionamiento seriado amplio y, en el caso de obtener una biopsia negativa, se repite el proceso en la arteria contralateral, la sensibilidad diagnóstica es elevada (100).

En la poliarteritis nodosa, la gran diversidad de órganos potencialmente afectados requiere una individualización de la aproximación diagnóstica. Albert y cols., en una extensa revisión (101, 102), propusieron una estrategia útil: en los pacientes con evidencia clínica o electromiográfica de afectación muscular o de nervio periférico se deberían biopsiar estas zonas (con una sensibilidad diagnóstica del 70%). En caso de síntomas o signos de afectación visceral (hipertensión arterial, dolor abdominal, hemorragia digestiva o enfermedad hepática) debería practicarse una arteriografía (sensibilidad del 61%). En caso de negatividad de la misma se podría intentar la biopsia de una zona asintomática (músculo), aunque con una

sensibilidad mucho menor (29%). Cuando se practicaban biopsia y arteriografía, la sensibilidad era del 85% y la especificidad superior al 95%.

Dado que en la enfermedad de Takayasu se afectan fundamentalmente vasos de gran calibre, la posibilidad de obtener una muestra histológica se reduce a las intervenciones vasculares derivativas y, aun así, a menudo sólo se observan cambios reparativos o secundarios a enfermedad ateromatosa, por lo que el procedimiento de elección es la arteriografía. Los hallazgos más frecuentes son aneurismas segmentarios y estenosis de la aorta y sus ramas principales (98).

En el diagnóstico de la vasculitis aislada del sistema nervioso central la arteriografía también desempeña un papel importante (22). Sin embargo, carece de especificidad absoluta, por lo que es preferible una biopsia de la leptomeninge para la confirmación diagnóstica (103).

Algunas vasculitis se presentan de una manera tan característica que pueden ser diagnosticadas sólo por datos clínicos. Algunos ejemplos serían la vasculitis por hipersensibilidad con púrpura palpable, el síndrome de Schönlein-Henoch y la enfermedad de Behçet (104-106). En ocasiones, el diagnóstico de arteritis de la temporal puede establecerse clínicamente, cuando las manifestaciones craneales (cefalea, claudicación mandibular, dolor o ausencia de pulso temporal y neuritis óptica) son evidentes (107), pero dada la simplicidad de la biopsia de la temporal y el hecho de que un 15% de los casos se presentan con síntomas constitucionales sin apenas manifestaciones características de la enfermedad, es preferible la demostración histológica (108).

Extensión de la enfermedad

La evaluación de la extensión y la distribución de la enfermedad es necesaria para un diagnóstico preciso y es uno de los determinantes principales de la agresividad del tratamiento. Por ejemplo, una vasculitis por hipersensibilidad de localización exclusivamente cutánea no requiere la misma agresividad terapéutica que la afectación cutánea de una granulomatosis de Wegener, que puede afectar además pulmones y riñón. Debemos, pues, hacer hincapié en el hecho de que lesiones cutáneas idénticas a las halladas en vasculitis limitadas a la piel pueden presentarse en otras vasculitis sistémicas (poliarteritis nodosa, granulomatosis de Wegener, púrpura de Schönlein-Henoch, síndrome de Churg-Strauss) (22) y pueden anteceder o coexistlr con neoplasias, especialmente hematológicas (109). Las vasculitis asociadas a otras enfermedades autoinmunes, como el lupus eritematoso sistémico o la artritis reumatoide, suelen mostrar una afectación principalmente cutánea, pero pueden afectar también vasos de mayor calibre (6). Por tanto, el diagnóstico de vasculitis cutánea aislada requiere una evaluación extensa que excluya una enfermedad sistémica.

También se debe tener en cuenta que, si bien existen formas limitadas de vasculitis con afectación de un órgano aislado (6, 110, 111), algunos casos considerados inicialmente como vasculitis aisladas son en realidad manifestaciones de un

Tabla 5. Escala de actividad de las vasculitis sistémicas de Birmingham (BVAS).

Deben marcarse las alteraciones sólo si son nuevas o han empeorado en el último mes y si son atribuibles a vasculitis.

1. SISTÉMICOS	(máximo 3)
Ninguno	0
Afectación del estado general	1
Mialgia	1
Artralgia/artritis	1
Fiebre (<38,5 °C)	1
Fiebre (>38,5 °C)	2
Pérdida de peso (1-2 kg) en un mes	2
Pérdida de peso (>2 kg) en un mes	3
2. CUTÁNEOS	(máximo 6)
Ninguno	0
Infarto	2
Púrpura	2
Otras vasculitis cutáneas	2
Úlcera	4
Gangrena	6
Gangrena digital múltiple	6
3. MEMBRANAS MUCOSAS/OJOS	(máximo 6)
Ninguno	0
Úlceras orales	1
Úlceras genitales	1
Conjuntivitis	1
Epi/escleritis	2
Uveítis	6
Exudados retinianos	6
Hemorragia retiniana	6
4. OTORRINOLARINGOLOGÍA	(máximo 6)
Ninguno	0
Obstrucción nasal/rinorrea	2
Sinusitis	2
Epistaxis	4
Costras	4
Otorrea	4
Otitis media	4
Sordera nueva	6
Laringitis	2
Afectación subglótica	6
5. TÓRAX	(máximo 6)
Ninguno	0
Disnea	2
Nódulos o fibrosis	2
Derrame pleural	4
Infiltrado	4
Hemoptisis	4
Hemoptisis masiva	6
6. CARDIOVASCULAR	(máximo 6)
Ninguno	0
Soplos	2
Pérdida de pulsos	4
Insuficiencia aórtica	4
Pericarditis	4
Infarto de miocardio nuevo	6
Miocardiopatía	6
7. ABDOMINAL	(máximo 9)
Ninguno	0
Dolor abdominal	3
Diarrea sanguinolenta	6
Perforación de vesícula biliar	9
Infarto intestinal	9
Pancreatitis	9
8. RIÑÓN	(máximo 12)
Ninguno	0
Hipertensión arterial (diastólica >90)	4
Proteinuria (>1+ o >0,2 g/24 h)	4
Hematuria (>1+ o >10 hematíes x ml)	8
Creatinina 125-249 µmol/l	8
Creatinina 250-499 µmol/l	10
Creatinina >500 µmol/l	12
Aumento de creatinina >10%	12
9. SISTEMA NERVIOSO	(máximo 9)
Ninguno	0
Confusión/demencia orgánica	3
Convulsiones (no hipertensivas)	9
Accidente vascular cerebral	9
Lesión espinal	9
Neuropatía periférica	6
Mononeuritis múltiple motora	9
PUNTUACIÓN MÁXIMA	63

proceso multisistémico que incluye localizaciones asintomáticas. También puede ocurrir que una vasculitis aislada sea la manifestación inicial de una vasculitis multisistémica (6).

Luqmani y cols. enfatizaron la importancia de evaluar la extensión de las vasculitis en un índice de actividad de la enfermedad, realizado según el número de órganos afectados y la importancia vital de los mismos (Tabla 5) (112). En este trabajo los autores demuestran un peor pronóstico en pacientes con un mayor índice de actividad.

Diagnóstico del tipo específico de vasculitis

Una vez establecido el diagnóstico de vasculitis, la adscripción a una entidad clinicopatológica concreta se basa en la combinación de manifestaciones clínicas, hallazgos de laboratorio, distribución anatómica y características histopatológicas, ya que no existen signos patognomónicos ni exploraciones complementarias definitivas para el diagnóstico de ninguna vasculitis (98).

Dado que tanto la obtención de una muestra histológica diagnóstica como la práctica de una arteriografía son procedimientos invasivos, se han hecho numerosos estudios en un intento de encontrar algún parámetro biológico específico de vasculitis o de tipos de las mismas. Aunque hasta la fecha no se ha encontrado ningún marcador patognomónico de una vasculitis concreta, cabe destacar la importancia que los ANCA tienen en el diagnóstico de algunas de ellas.

Como se ha mencionado anteriormente, existe una intensa asociación entre los c-ANCA y la granulomatosis de Wegener. Numerosos estudios han confirmado una alta especificidad de los c-ANCA por la granulomatosis de Wegener (113-116), hasta el punto de que se ha cuestionado la necesidad de una confirmación histológica en el caso de una fuerte sospecha clínica si el título de c-ANCA es alto y se ha excluido la posibilidad de un proceso infeccioso (112, 116). Sin embargo, la negatividad de los c-ANCA no excluye la presencia de una granulomatosis de Wegener. La sensibilidad es limitada y depende de la actividad y la extensión de la enfermedad. Puede llegar a ser superior al 90% durante la fase sistémica activa, pero disminuye mucho en formas limitadas y con el tratamiento previo con corticoides (25, 118). En el único estudio prospectivo realizado hasta ahora, la sensibilidad total sobre 25 pacientes fue del 28% (119).

Se han descrito p-ANCA dirigidos contra distintos antígenos en un amplio espectro de enfermedades, la mayoría de ellas autoinmunes, como la enfermedad inflamatoria intestinal o la artritis reumatoide (25, 120). Por ello, la determinación indiscriminada de p-ANCA por microscopía de inmunofluorescencia tiene baja sensibilidad y especificidad y, por tanto, una utilidad clínica muy limitada. Sin embargo, la presencia de p-ANCA con especificidad para la MPO se ha demostrado estrechamente asociada a la poliangitis microscópica, aunque su sensibilidad es inferior al 50% (120). Síndromes que cursan con hemorragia alveolar, glomerulonefritis rápidamente progresiva idiopática o ambas también se asocian frecuentemente a

p-ANCA anti-MPO, por lo que se han considerado variantes limitadas de la poliangitis microscópica (121-123). Algunos trabajos sugieren una asociación del síndrome de Churg-Strauss con ANCA anti-MPO (18, 120). La sensibilidad y especificidad de los ANCA anti-MPO por este grupo de enfermedades es lo suficientemente significativa como para que el hallazgo de ANCA anti-MPO obligue a una extensa búsqueda de síntomas o signos que sugieran una vasculitis sistémica de pequeño vaso.

La determinación de los niveles de ANCA se ha intentado utlilizar para la monitorización de la actividad de las vasculitis y para la detección de recurrencias. Aunque los títulos de c-ANCA disminuyen paralelos a la actividad de la enfermedad en más de un 85% de los casos (115, 124), el valor predictivo de los c-ANCA para una recidiva inminente es controvertido (113, 125). En un estudio prospectivo realizado por Cohen Trevaert y cols. (115) se objetivó una elevación de los c-ANCA precediendo a todas las recidivas de granulomatosis de Wegener, pero este incremento puede producirse meses antes de la recidiva, y algunas de estas elevaciones no fueron seguidas de recidiva. Aunque en un pequeño grupo de pacientes se evitó la recidiva instaurando tratamiento inmunosupresor después de un incremento en los niveles de c-ANCA (126), la recomendación de esta medida terapéutica es arriesgada, especialmente teniendo en cuenta los efectos secundarios del tratamiento inmunosupresor. Los niveles de ANCA anti-MPO también parecen reflejar la actividad de la enfermedad (127), pero no se han realizado etudios prospectivos sobre el tema.

Se ha intentado buscar otros parámetros para la monitorización de la actividad de las vasculitis, como la interleucina-6 o las moléculas de adhesión circulantes, pero los reultados obtenidos todavía son preliminares (128).

TRATAMIENTO

Para instaurar un tratamiento eficaz en las vaculitis se requiere establecer un diagnóstico adecuado lo más precozmente posible, evaluar la extensión de la enfermedad, su gravedad y la rapidez de progresión de la misma. La agresividad de las vasculitis se halla determinada fundamentalmente por la afección renal, gastrointestinal, cardiaca y del sistema nervioso central. La hemorragia pulmonar contituye otra manifestación grave. Estas complicaciones determinan el pronóstico vital de las vascultitis (129). Tan importante es instaurar un tratamiento agresivo en los casos graves como evitarlo en los pacientes con enfermedad limitada o poco activa, ya que los glucocorticoides y los inmunosupresores generan una importante yatrogenia. El hecho de que las vasculitis sistémicas sean poco frecuentes implica que no se hayan podido realizar estudios controlados en grupos amplios de pacientes. Por tanto, no existen pruebas fehacientes de que los tratamientos propugnados sean realmente los más eficaces y menos nocivos.

Opciones de tratamiento

Glucocorticoides

Los glucocorticoides constituyen el primer tratamiento que se demostró eficaz para reducir la mortalidad y la morbididad en las vasculitis (130). Como se precisa más adelante, todavía siguen siendo el tratamiento de elección en muchas vasculitis (131, 132), ya sea solos (arteritis de la temporal, arteritis de Takayasu, síndrome de Churg-Strauss) o en combinación con otros agentes inmunosupresores (poliarteritis nodosa, granulomatosis de Wegener). Sin embargo, tras un seguimiento prolongado de los pacientes tratados únicamente con glucocorticoides se ha visto que las recidivas son cada vez más frecuentes, sobre todo al abandonar el tratamiento (70). Además, el tratamiento prolongado con glucocorticoides ocasiona una yatrogenia importante, que en ocasiones llega a ser más agresiva que la propia enfermedad (70, 132-134). Los efectos secundarios de los corticoides dependen de la dosis, el tiempo y el ritmo de administración, y los pacientes afectos de vasculitis sistémicas requieren tratamientos prolongados y a altas dosis. Por este motivo se han ensayado distintas pautas de administración (tratamiento a días alternos, pulsos de altas dosis intravenosas), así como intentos de reducir las dosis seleccionando grupos concretos de enfermos o mediante la combinación con otros fármacos, fundamentalmente inmunosupresores (135).

Al iniciar el tratamiento con glucocorticoides, se deben tener en cuenta sus efectos sobre el metabolismo glucídico, el equilibrio hidroelectrolítico, la cicatrización de las heridas y las infecciones oportunistas (131). Esto es especialmente importante en los pacientes de edad avanzada y en aquellos con hipertensión arterial, cardiopatía o diabetes mellitus preexistente. Las complicaciones más graves suelen ser las infecciones oportunistas, y en particular las neumonías. En una vasculitis con posible afectación pulmonar la aparición de una neumonía puede confundir al clínico, con el subsiguiente retraso en la instauración del tratamiento antibiótico, e incluso motivar un aumento de las dosis de glucocorticoides. La gravedad del problema aumenta con la creciente frecuencia de infecciones debidas a gérmenes virulentos, como bacterias gramnegativas, *Staphylococcus aureus, Pneumocystis carinii,* micobacterias y hongos oportunistas. Dado que el tratamiento inmunosupresor limita la capacidad de contener las infecciones, se produce sepsis y muerte secundaria a neumonía en hasta un 40% de los casos (134). Es importante tener en cuenta que el tratamiento con glucocorticoides puede enmascarar la fiebre, el mal estado general o la anorexia, y hacer que las infecciones cursen de manera silente (131).

Inmunosupresores

Los agentes inmunosupresores empezaron a probarse en vasculitis que no respondían al tratamiento con glucocorticoides. Además, en algunas vasculitis que sí responden las recidivas son tan frecuentes que el tratamiento prolongado con prednisona puede llegar a producir más morbididad que la propia enfermedad. En este contexto, el tratamiento inmunosupresor debe ser considerado tanto para aumentar la posibilidad de remisión como para reducir el tiempo y/o las dosis de glucocorticoides, disminuyendo así sus efectos secundarios. Las indicaciones concretas para añadir un agente citotóxico se comentan más adelante.

La ciclofosfamida ha sido el agente citotóxico más estudiado para el tratamiento de las vasculitis (130, 135, 136). A pesar de ser el mejor tratamiento para lograr la remisión en algunas vasculitis sistémicas graves, haber mejorado el pronóstico vital de algunas vasculitis de evolución previamente fatal y reducir sustancialmente la yatrogenia asociada a los glucocorticoides, la administración de ciclofosfamida no está exenta de morbididad e incluso mortalidad (70). Puede producir cistitis hemorrágica (43%), cáncer de vejiga (2,8%, 33 veces superior a la tasa esperada), linfoma (1,5%, 11 veces superior a la tasa esperada), mielodisplasia (2%), infertilidad (57% de las mujeres) y una mayor frecuencia de infecciones, especialmente cuando se usa en combinación con glucocorticoides. También se han probado los pulsos intravenosos de ciclofosfamida para reducir la toxicidad del fármaco, pero aunque suelen ser bien tolerados, no son tan eficaces en mantener la remisión como las dosis bajas diarias (137).

Otros agentes citotóxicos, en especial el metotrexato, están empezando a ser ensayados en algunas vasculitis sistémicas con resultados esperanzadores (138, 139), pero siguen siendo tratamientos ampliamente inmunosupresores, con las complicaciones que ello conlleva. También se ha probado la ciclosporina, con peores resultados (140).

Inmunoterapia

Bajo la hipótesis de la participación del depósito de inmunocomplejos circulantes en la patogénesis de las vasculitis, en un modelo animal de daño vascular mediado por complemento se utilizó inmunoglobulina intravenosa en un intento de bloquear los receptores tisulares Fc para que no se unieran a los inmunocomplejos y evitar el depósito de C3 y C4 activados en la pared vascular (141). La eficacia de la inmunoglobulina en este modelo apoyó inicialmente la hipótesis citada, aunque estudios posteriores evidencian que el tratamiento puede influir sobre otros mecanismos inmunorreguladores probablemente de igual importancia (142). En la práctica clínica, la capacidad de altas dosis de inmunoglobulina intravenosa para disminuir las manifestaciones inflamatorias se ha descrito en la enfermedad de Kawasaki (143) y más recientemente en vasculitis asociadas a ANCA (144-146). Richter y cols. demostraron mejoría en un 60% de los pacientes afectos de granulomatosis de Wegener que no habían respondido al tratamiento estándar, pero en ninguno de ellos se logró la remisión completa (147). Excepto en la enfermedad de Kawasaki, en ninguna otra vasculitis se ha demostrado una eficacia superior del tratamiento con inmunoglobulina intravenosa respecto al tratamiento estándar que justifique el alto coste que ésta implica.

En 1990 se probó por vez primera el tratamiento con anticuerpos monoclonales en un caso de vasculitis grave. La administración de un anticuerpo anti-CDw52 indujo una respuesta favorable pero transitoria. Si el tratamiento con el anticuerpo citado se seguía de una inyección de anti-CD4, la remisión llegaba a durar más de 2 años (148). Posteriormente, otros estudios han demostrado beneficios de esta terapia en distintas vasculitis: poliarteritis microscópica, síndrome de Sjögren, enfermedad de Behçet (149) y policondritis recidivante con vasculitis (150). La eficacia potencial del tratamiento con anticuerpos monoclonales se ve ensombrecida por el desarrollo en el receptor de anticuerpos dirigidos contra el anticuerpo monoclonal.

Tal como se ha comentado anteriormente, las citocinas proinflamatorias desempeñan un papel primordial en el desarrollo de las vasculitis. En algún caso de vasculitis cutánea se ha probado la eficacia de la pentoxifilina, un potente inhibidor de estas citocinas, con buenos resultados (151).

La plasmaféresis se ha demostrado eficaz en algunos tipos de vasculitis y en situaciones concretas. Es claramente eficaz en algunas situaciones urgentes, como en la reducción de títulos altos de crioglobulinas (152). En pacientes con glomerulonefritis necrosante (poliarteritis microscópica, granulomatosis de Wegener) en régimen de diálisis y en caso de hemorragia alveolar, la plasmaféresis es eficaz en combinación con corticoides e inmunosupresores (153, 154). Distintos estudios han probado la eficacia de la plasmaféresis, sola o como tratamiento adyuvante, en la poliarteritis nodosa asociada al virus de la hepatitis B (155-158). En un estudio de Quint y cols., la combinación de plasmaféresis con vidarabina fue eficaz en el tratamiento de la poliarteritis nodosa asociada al virus de la hepatitis C (91).

Agentes antimicrobianos

Ya se ha comentado anteriormente la asociación de algunas vasculitis con agentes infecciosos, fundamentalmente virus, y la posible participación de éstos en la patogénesis de las vasculitis a las cuales se asocian. Con estas bases, algunos grupos han ensayado terapéuticas antimicrobianas y especialmente antivirales para el tratamiento de las vasculitis. Guillevin y cols. demostraron en dos excelentes trabajos la eficacia de la vidarabina (157) y el interferón $\alpha 2\beta$ (158), ambos en combinación con plasmaféresis y siguiendo a un corto periodo de tratamiento con glucocorticoides, en pacientes afectos de poliarteritis nodosa asociada al virus de la hepatitis B. Los autores demostraron la eficacia del tratamiento no sólo para el control de la vasculitis sino también para el control de la infección. Aunque estos resultados no son concluyentes y serían necesarios estudios controlados, son muy esperanzadores por la eficacia obtenida, los mínimos efectos secundarios y la probable relación con la etiología o la patogénesis de la enfermedad. El interferón $\alpha 2\beta$ también ha sido probado con buenos resultados en el tratamiento de la crioglobulinemia mixta esencial asociada al virus de la hepatitis C (159) y con algún beneficio en la enfermedad de Behçet (160). Otros antivirales también se han probado con resultados bastante satisfactorios en la crioglobulinemia mixta esencial (161).

Se han probado otros agentes antimicrobianos en el tratamiento de las vasculitis, aunque con una base más empírica. Los resultados obtenidos en el tratamiento de la granulomatosis de Wegener con trimetoprima-sulfametoxazol son controvertidos (162, 163). La dapsona es eficaz en el tratamiento de las vasculitis leucocitoclásticas, la vasculitis cutánea asociada a la artritis reumatoide, la vasculitis asociada a un déficit de complemento y la púrpura de Schönlein-Henoch (164).

Tratamiento específico

En este apartado describiremos resumidamente las directrices básicas del tratamiento de cada vasculitis en particular. Para ello hemos escogido las vasculitis más frecuentes y mejor reconocidas como entidades clinicopatológicas. Sin embargo, insistimos en que cada paciente debe considerarse individualmente, ya que según la gravedad, los órganos afectados o la respuesta al tratamiento inicial, éste puede requerir modificaciones.

Tratamiento inicial

Poliarteritis nodosa

La mortalidad estimada de la poliarteritis nodosa dejada a su libre evolución es superior al 85% a los 5 años (130, 165). El tratamiento con altas dosis de glucocorticoides disminuyó esta cifra a un 30%-45% (130, 135, 166, 167). Cuando se asociaron glucocorticoides con inmunosupresores, la mortalidad a los 5 años disminuyó al 20%-25% (10, 166) y las recidivas fueron menos frecuentes (135). Las manifestaciones clínicas que afectan de una manera más adversa a la supervivencia son la isquemia intestinal, la afectación cardiaca y la insuficiencia renal. Si la función renal es normal y no hay evidencia de isquemia crítica en ningún órgano, el tratamiento inicial más beneficioso sería la corticoterapia sola (p. ej., prednisona a dosis de mg/kg/día). Si a los pocos días no hubiera mejoría, hubiera un empeoramiento clínico o se hiciese aparente una afectación renal, cardiaca, intestinal o del sistema nervioso central se debería añadir un tratamiento citotóxico en régimen diario. En los casos de poliarteritis nodosa asociada al virus de la hepatitis B podría instaurarse el tratamiento antiviral, tal como Guillevin ha propuesto en sus esperanzadores estudios (157, 158).

La gravedad de la poliangitis microscópica justifica un tratamiento intensivo con pulsos de corticoides, ciclofosfamida oral diaria o en pulsos y, en caso de hemorragia pulmonar, plasmaféresis (13, 154). En algunos casos en los que el tratamiento convencional ha fracasado se han descrito buenos resultados con dosis altas de inmunoglobulinas (144, 145) o con anticuerpos monoclonales (150).

Síndrome de Churg-Strauss

Suele responder mejor que la poliarteritis nodosa al tratamiento con corticoides solos. Si apareciera alguna de las complicaciones anteriormente citadas debería añadirse un agente citotóxico, pero estos casos son poco frecuentes.

Granulomatosis de Wegener

Las dramáticas estadísticas sobre la mortalidad de esta enfermedad dejada a su libre evolución (supervivencia media de 5 meses) (168) y tratada únicamente con glucocorticoides (supervivencia media de 12 meses) (169) se han modificado drásticamente gracias a la combinación de corticoides con ciclofosfamida. En el caso de enfermedad evolucionada y con riesgo vital, existe consenso sobre la combinación de glucocorticoides y dosis bajas diarias de ciclofosfamida (2 mg/kg hasta 4 mg/kg en situaciones extremas) como tratamiento de primera elección (70, 137, 170). Sin embargo, el grado de toxicidad de esta combinación es alarmante y ha llevado a estudiar otras opciones de tratamiento, como altas dosis intravenosas de ciclofosfamida (pulsos) u otros agentes citotóxicos, como el metotrexato. Ya se ha comentado la menor eficacia de la ciclofosfamida en pulsos respecto a la ciclofosfamida oral diaria. Estudios preliminares demuestran una buena tolerancia al metotrexato (0,15-0,30 mg/kg semanalmente por vía oral o intramuscular), con remisión en el 75% de los casos, incluso después de suprimir el tratamiento con glucocorticoides y con una media de seguimiento de 1,5 años (131). Estos resultados son alentadores, pero todavía no concluyentes.

Un avance importante en el tratamiento de la granulomatosis de Wegener ha sido el reconocimiento de formas de la enfermedad menos agresivas y sin afectación renal. La terapia combinada, aunque haya sido vital para muchos pacientes, puede suponer un riesgo excesivo para estas formas limitadas. Los glucocorticoides solos inducen la remisión en una parte importante de estos pacientes, pero al cabo de un tiempo variable la mayoría recae (70, 131). Se han hecho otros intentos para tratar menos agresivamente estas formas más indolentes de la enfermedad, incluyendo algunos ensayos anecdóticos con trimetoprima-sulfametoxazol (162, 163), pero todavía no se ha llegado a una solución convincente.

Además del tratamiento farmacológico específico de la enfermedad, hay que abordar al paciente de una manera individualizada e interdisciplinaria para tratar las complicaciones concretas de cada caso y obtener así mejores resultados, sobre todo con la finalidad de disminuir la morbilidad para el paciente. Por ejemplo, la otitis media crónica puede requerir timpanostomía y tubos de drenaje; en la estenosis subglótica se debe dilatar, resecar, practicar traqueostomía o incluso cirugía reconstructiva extensa; el tratamiento inmunosupresor puede llevar a complicaciones infecciosas para cuyo diagnóstico es necesaria una fibrobroncoscopia, o a complicaciones urológicas (cistitis hemorrágica, cáncer de vejiga) que requieren la intervención del urólogo.

Vasculitis por hipersensibilidad

Dado que en estos casos la afectación visceral es infrecuente y en general menos agresiva que en las vasculitis anteriores, se debería ser más cauto al instaurar un tratamiento. Si la enfermedad es leve y la causa desencadenante es identificable, lo aconsejable es tratar la causa (eliminar drogas, tratar infecciones). Si hay glomerulonefritis o isquemia visceral es más prudente iniciar un tratamiento con corticoides diarios. Si la remisión del cuadro persistiera debería iniciarse una reducción de las dosis a las 4-8 semanas.

Púrpura de Schönlein-Henoch

El tratamiento es esencialmente el mismo que el de las vasculitis por hipersensibilidad. Los corticoides deberían añadirse en caso de afectación gastrointestinal grave y/o afectación renal.

Arteritis de Takayasu

Aproximadamente dos terceras partes de los pacientes con esta vasculitis presentan síntomas y/o signos que sugieren inflamación activa. La mitad de estos pacientes responden al tratamiento con glucocorticoides y no presentan recidivas después de abandonar la medicación. Sin embargo, la otra mitad es resistente al tratamiento con glucocorticoides o no puede dejar el tratamiento sin recidivar. Este grupo de pacientes pueden mejorar y necesitar una dosis inferior de glucocorticoides si se añade ciclofosfamida diaria o metotrexato semanal (139, 171, 172). Pero los pacientes más difíciles de manejar son los que padecen una enfermedad clínicamente silente, sin síntomas constitucionales ni elevacion de la velocidad de sedimentación globular (VSG) pero desarrollan estenosis vasculares progresivas. Además del tratamiento médico de la enfermedad, que debe instaurarse inmediatamente, estos pacientes requieren la corrección quirúrgica de las estenosis irreversibles mediante dilatación o cirugía derivativa. En muchos casos los resultados no son los deseados, probablemente porque los tejidos están afectados por la enfermedad subyacente. La corrección quirúrgica debe realizarse en ausencia de actividad de la enfermedad, ya que en caso contrario el riesgo de reestenosis es elevado (24%) (71).

Arteritis de la temporal o de células gigantes

El tratamiento con glucocorticoides sigue siendo el más eficaz. La prednisona (1 mg/kg/día) mejora los síntomas en pocos días y suele eliminarlos en una semana. Un mes después de la normalización clínica y de los parámetros de laboratorio (VSG) se puede empezar a disminuir la dosis de glucocorticoides. Sin embargo, las recidivas son frecuentes y algunos pacientes requieren un tratamiento prolongado con glucocorticoides (173).

La eterna discusión sobre si la polimialgia reumática es una manifestación de la arteritis de la temporal o es un proceso distinto tiene implicaciones terapéuticas, ya que responde muy bien a dosis muy inferiores de prednisona (0,2-0,3 mg/kg/día). Los pacientes con polimialgia reumática aislada en quienes se ha descartado razonablemente, tras una cuidadosa evaluación clínica, la presencia de arteritis de la temporal, responden bien a dosis inferiores de glucocorticoides.

Síndromes de solapamiento

Bajo este epígrafe se agrupan los pacientes que presentan manifestaciones comunes a distintas vasculitis. Algunos acaban evolucionando a un tipo de vasculitis más definido. Dado que es un grupo muy heterogéneo, no se puede dogmatizar acerca de su manejo. La agresividad del tratamiento debería estar determinada por una correcta valoración de la gravedad de la enfermedad, su distribución anatómica (especialmente por la existencia o no de afectación renal, gastrointestinal, cardiaca o del sistema nervioso central), grado de progresión y enfermedades asociadas.

Tratamiento crónico

Después de años de tratamiento y seguimiento de las vasculitis sistémicas, el concepto inicial de enfermedades agudas y potencialmente mortales ha evolucionado hacia el de enfermedades crónicas potencialmente invalidantes.

Algunas vaculitis, como las vasculitis por hipersensibilidad o la púrpura de Schönlein-Henoch, pueden ser monofásicas, autolimitadas o no requerir tratamiento crónico. Algunos pacientes con poliarteritis nodosa o arteritis de Takayasu pueden ser también afortunados en este sentido. Sin embargo, la mayoría de los sujetos con poliarteritis nodosa, granulomatosis de Wegener, arteritis de la temporal o arteritis de Takayasu eventualmente recidivan, incluso años después de haber conseguido una remisión completa, y deben ser tratados de nuevo. Este grupo de pacientes no posee ningún marcador clínico o serológico que indique su propensión a recidivar, hecho que obliga al clínico a mantener una vigilancia indefinida y cuidadosa sobre estos pacientes aunque su evolución sea favorable. Esto es especialmente importante para los órganos en los cuales la recidiva puede ser clínicamente asintomática hasta que las lesiones son extensas o irreversibles (p. ej. el riñón en la granulomatosis de Wegener).

Cuando se ha indicado el tratamiento con glucocorticoides, las dosis altas deben mantenerse hasta aproximadamente un mes después de que desaparezcan las manifestaciones de actividad de la enfermedad. No hay consenso sobre la pauta de descenso de los glucocorticoides. Una aproximación que se ha demostrado eficaz sería la siguiente (131): disminuir la prednisona hasta dosis de miligramos por kilogramo de peso a días alternos en 2-3 meses, en ausencia de recidivas o exacerbaciones. Posteriormente ir reduciendo la dosis hasta que en 2-3 meses más quede suspendido el tratamiento. En general, el tratamiento con glucocorticoides suele mantenerse un mínimo de un año.

Para los pacientes que han recibido tratamiento con corticoides y/o inmunosupresores, que son la mayoría, el control también debe incluir la evaluación de síntomas que sugieran toxicidad, como infecciones oportunistas o cistitis hemorrágica. Debemos recordar que las complicaciones malignas pueden ser silentes y/o aparecer al cabo de años de haber finalizado el tratamiento, por lo que estos pacientes deben ser sometidos periódicamente a una exploración clínica, análisis de sangre y orina y otras exploraciones (p. ej., cistoscopia) si estuviera indicado.

Aparte de las posibles recidivas y complicaciones del tratamiento, se debe tener en cuenta que la morbididad de las vasculitis sistémicas no incluye sólo las lesiones inflamatorias e isquémicas de la fase activa y las complicaciones del

tratamiento, sino también las secuelas que permanecen incluso en los periodos inactivos de la enfermedad, como polineuropatía en la poliarteritis nodosa, insuficiencia renal crónica en la granulomatosis de Wegener o ceguera en la arteritis de la temporal, así como complicaciones permanentes asociadas al tratamiento (osteoporosis, infertilidad, vejiga desfuncionalizada). Estas secuelas son las que más demuestran el concepto anteriormente citado de enfermedad crónica invalidante. Bacon y cols. realizaron una escala para evaluar globalmente la morbididad de las vasculitis sistémicas (174) y en ella, además de las complicaciones de la fase activa (BVAS), valoran las secuelas de la enfermedad o el tratamiento, la capacidad funcional del paciente y la apreciación subjetiva de éste. Con todo ello se obtiene una idea mucho mas real de la discapacidad generada por las vasculitis sistémicas. En el seguimiento de los pacientes afectos de estas enfermedades será también muy importante el tratamiento, aunque sea paliativo, de estas complicaciones crónicas: tratamiento analgésico, hemodiálisis (o incluso trasplante renal), intervenciones quirúrgicas paliativas, etc.

CONCLUSIÓN

Las vasculitis sistémicas son enfermedades muy heterogéneas y con características clinicopatológicas comunes, por lo que es difícil conseguir que una clasificación sea universalmente aceptada. Sin embargo, es importante obtener el máximo consenso en la nomenclatura y la clasificación de estas enfermedades para conseguir resultados comparables, avanzar en el estudio de su etiopatogenia y mejorar su tratamiento y pronóstico. En los últimos años se ha desarrollado una amplia gama de nuevos conceptos que mejoran nuestra comprensión de los mecanismos patogenéticos capaces de producir lesión vascular. Nuestro conocimiento de cómo estas vías de lesión e inflamación vascular descubiertas recientemente actúan en enfermedades concretas es todavía preliminar. Investigar estos avances puede proporcionar nuevas herramientas diagnósticas, marcadores de actividad fiables y en un futuro conducir a nuevas intervenciones terapéuticas para disminuir la todavía importante morbididad y mortalidad asociadas a la evolución y/o al tratamiento de unas enfermedades cada vez más crónicas e invalidantes.

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RHEUMATOL

Vasculitis: definición, clasificación y etiopatogenia

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Definición

Las vasculitis constituyen un grupo ampliamente heterogeneo de entidades clinicopatológicas con un sustrato morfológico común: la inflamación de los vasos sanguíneos.

Dada la distribución universal de los vasos por todo el organismo, el espectro de manifestaciones clínicas determinadas por las vasculitis es marcadamente pleomórfico. Las formas de presentación de estas enfermedades pueden ser muy variadas debido a la afectación preferente de distintos órganos y sistemas en cada entidad, y en cada individuo en particular. Por la misma razón el pronóstico vital y funcional de las vasculitis es tambien muy heterogéneo: unos procesos siguen un curso benigno y autolimitado mientras otros pueden seguir una evolución fulminante y fatal.

Clasificación

Clasificaciones iniciales

La clasificación de las vasculitis entraña especiales dificultades debido a la existencia de numerosas formas de solapamiento y a la existencia no excepcional de casos que no encajan adecuadamente en ninguna de las categorías admitidas[1].

La clasificación de las vasculitis no es una mera necesidad académica, sino que tiene implicaciones terapéuticas importantes como la instauración precoz de un tratamiento inmunodepresor en entidades con un potencial conocido de desarrollar complicaciones graves como la enfermedad de Wegener y la poliangeítis microscópica, la opción de un tratamiento antivírico en vasculitis asociadas a infecciones víricas, o simplemente evitar un tratamiento inmunodepresor con elevado potencial yatrogénico en entidades de curso benigno como el síndrome de Henoch-Shönlein[1,2].

El concepto de vasculitis como grupo de enfermedades relacionadas y el primer intento de clasificación fue propuesto por Zeek en 1952, e incluía cinco grandes entidades que habían sido descritas de manera aislada: angeítis por hipersensibilidad, angeítis granulomatosas alérgicas, vasculitis reumatoide, poliarteritis nudosa y arteritis temporal[3]. En la clasificación de Zeek existen algunas omisiones importantes, ya que algunas enfermedades como la granulomatosis de Wegener o la arteritis de Takayasu no se conocían o no habían sido todavía descritas en la literatura anglosajona. Sin embargo, esta clasificación tiene el mérito de agrupar genéricamente por primera vez, y definir como angeítis las enfermedades que cursan con inflamación de los vasos sanguíneos. La clasificación de Zeek fue el punto de partida para clasificaciones posteriores.

La clasificación propuesta por Fauci en 1978 (tabla 1) amplió considerablemente el espectro de enfermedades incluidas[1]. Además de criterios morfológicos, esta clasificación tuvo en consideración implicaciones terapéuticas al distinguir entre vasculitis sistémicas con riesgo inherente de lesión irreversible de órganos vitales, y procesos localizados de curso más benigno y, en ocasiones, autolimitado. Por esta razón la clasificación de Fauci ha sido la más utilizada por clínicos y patólogos durante más de una década. Sin embargo, esta clasificación tiene algunos inconvenientes importantes como la inclusión de la granulomatosis linfomatoide, que es un síndrome linfoproliferativo de distribución angiocéntrica, y la reducción de las formas de solapamiento al grupo de las vasculitis necrosantes tipo poliarteritis nudosa. Estas limitaciones han sido reconocidas y superadas por los propios autores en publicaciones posteriores[2,4,5].

Criterios de clasificación del *American College of Rheumatology*

Excepto en aquellos casos en que la enfermedad afecta preferentemente a grandes vasos como la enfermedad de Takayasu, el diagnóstico de vasculitis es esencialmente histológico, y se basa en la observación de un infiltrado leucocitario en la pared de los vasos. Sin embargo, este hallazgo es altamente inespecífico. Algunos rasgos como la presencia de necrosis, el tipo de infiltrado (granulocítico o linfomonocitario), la presencia de granulomas y el tamaño de los vasos afectados permiten orientar hacia entidades concretas, pero cada uno de estos datos puede existir en distintos procesos. Además, el patrón histológico de las vasculitis es dinámico y puede evolucionar con el tiempo[6].

El diagnóstico específico de cada tipo de vasculitis es fundamentalmente clínico-patológico. Por esta razón, el *American College of Rheumatology* (ACR) elaboró en 1990 unos criterios de clasificación que permiten, una vez establecido el diagnóstico genérico de vasculitis, adscribir con elevada sensibilidad y especificidad un paciente determinado a una entidad concreta. Los criterios de clasificación del ACR se refieren únicamente a las siete entidades clínico-patológicas más frecuentes y mejor caracterizadas: vasculitis por hipersensibilidad, púrpura de Henoch-Schönlein, granulomatosis de Wegener, síndro-

TABLA 1
Clasificación de las vasculitis según A.S. Fauci (1978)[1]

Vasculitis necrosantes sistémicas del grupo de la PAN
- Poliarteritis nudosa clásica
- Angeítis alérgica granulomatosa (síndrome de Churg-Strauss)
- Síndrome de solapamiento

Vasculitis por hipersensibilidad
- Púrpura de Henoch-Schönlein
- Enfermedad del suero y reacciones afines
- Vasculitis desencadenadas por fármacos
- Vasculitis asociadas a enfermedades infecciosas
- Vasculitis asociadas a neoplasias (principalmente linfomas)
- Vasculitis asociadas a enfermedades del colágeno
- Vasculitis asociadas a otras enfermedades
- Deficiencias congénitas de complemento
- Eritema *elevatum diutinum*

Granulomatosis de Wegener

Arteritis de células gigantes
- Arteritis temporal
- Arteritis de Takayasu

Otros síndromes vasculíticos
- Síndrome ganglionar mucocutáneo (enfermedad de Kawasaki)
- Enfermedad de Behçet
- Tromboangeítis obliterante (enfermedad de Buerger)
- Miscelánea

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me de Churg-Strauss, poliarteritis nudosa, arteritis temporal y arteritis de Takayasu[7] (tabla 2).

Nomenclatura consensuada propuesta en la Conferencia de Chapel Hill

En 1948 Davson describió el substrato histológico del síndrome nefrítico que aparecía en algunos pacientes con vasculitis necrosante sistémica como una glomerulonefritis necrosante focal[8]. Esta lesión es indistinguible de la afectación renal propia de la granulomatosis de Wegener. Posteriormente se observó que pueden aparecer lesiones idénticas en algunos pacientes de manera aislada, sin afectación aparente de otros órganos. Para describir esta situación se acuñó el término poliarteritis microscópica. En los últimos años se ha considerado que la poliarteritis microscópica es una entidad distinta de la poliarteritis nudosa clásica[9,10]. En esta última se afectan arterias de calibre pequeño y mediano, mientras que la poliarteritis microscópica se caracteriza por la afectación preferente de capilares, arteriolas y vénulas, aunque también pueden resultar afectados vasos de mayor calibre[9], por esta razón, algunos autores prefieren el término poliangeítis microscópica. Las manifestaciones más características de la poliangeítis microscópica y que no se dan en la poliarteritis nudosa clásica son la glomerulonefritis necrosante que clínicamente suele manifestarse como una glomerulonefritis rápidamente evolutiva, y la capilaritis pulmonar que puede cursar con hemorragia alveolar. Este hecho hace que, en ocasiones, sea muy difícil el diagnóstico diferencial entre la poliangeítis microscópica y la enfermedad de Wegener cuando el componente granulomatoso de esta última resulta poco evidente[9]. Así pues, si bien los casos extremos de poliarteritis nudosa clásica, poliangeítis microscópica y granulomatosis de Wegener son de fácil identificación y caracterización, existe un espectro de situaciones no excepcionales de difícil clasificación.

En 1993 se celebró en Chapel Hill (North Carolina, EE.UU.) una reunión de expertos de distintas especialidades y nacionalidades con demostrada experiencia en el diagnóstico y seguimiento de pacientes con vasculitis sistémicas, con el objetivo de definir una nomenclatura aceptada internacionalmente. Los nombres y definiciones adoptados en esta conferencia no suponen ninguna innovación conceptual respecto a definiciones previas, pero suponen un esfuerzo de consenso de cara a adoptar un lenguaje común. Las conclusiones más importantes de la Conferencia de Chapel Hill consisten en la definición de la poliangeítis microscópica como entidad distinta, y su delimitación de la poliarteritis nudosa y de la enfermedad de Wegener (tabla 3).

TABLA 2
Criterios del *American College of Rheumatology* para la clasificación de las vasculitis (1990)[7]

Entidad	Criterios	N.º	SB(%)	SP(%)
Púrpura de Henoch-Schönlein	Púrpura palpable Edad inferior a los 20 años Dolor abdominal Granulocitos en los vasos	≥ 2	87,8	87,7
Granulomatosis de Wegener	Inflamación nasal u oral Rx de tórax con infiltrados, cavidades o nódulos Microhematuria o cilindros hemáticos Inflamación granulomatosa	≥ 2	88,2	92
Síndrome de Churg-Strauss	Asma Eosinofilia > 10% Neuropatía Infiltrados pulmonares cambiantes Afectación de senos paranasales Eosinófilos extravasculares	≥ 4	85	99,7
Vasculitis por hipersensibilidad	Edad > 16 años Púrpura palpable *Rash* maculopapular Granulocitos perivasculares o extravasculares (arteriola o vénula)	≥ 3	71	83,9
Poliarteritis nudosa	Pérdida de peso > 4Kg *Livedo reticularis* Dolor testicular Mialgias o debilidad muscular Mononeuropatía o polineuropatía TA diastólica > 90 mmHg BUN o creatinina elevados Virus de la hepatitis B Anomalías arteriográficas Biopsia de una arteria de tamaño pequeño o mediano con granulocitos	≥ 3	82,2	86,6
Arteritis temporal	Edad > 50 años Cefalea de inicio reciente o características nuevas Dolor/mimiento o pulso disminuido en la arteria temporal VSG > 50 Inflamación granulomatosa o infiltrados linfomonocitarios	≥ 3	93,5	91,2
Arteritis de Takayasu	Edad < 40 años Claudicación de extremidades Pulso braquial disminuido Diferencia entre la TA de ambos brazos > 10 mmHg Soplos arteriales (subclavias o aorta) Anomalías arteriográficas (estenosis a nivel de aorta o ramas principales)	≥ 3	90,5	97,8

N.º: número de criterios requeridos; SB: sensibilidad; SP: especificidad; Rx: radiografía; TA: tensión arterial; VSG: velocidad de sedimentación globular.

Impacto de los anticuerpos anticitoplasma del neutrófilo en la clasificación y diagnóstico de las vasculitis

El descubrimiento de la presencia de anticuerpos anticitoplasma del neutrófilo (ANCA) en las denominadas vasculitis asociadas a ANCA (característicamente la poliangeítis microscópica y la granulomatosis de Wegener) ha sido una interesante aportación de cara a la clasificación y diagnóstico de los pacientes con vasculitis[11,12]. Los ANCA reconocen fundamentalmente enzimas contenidas en los gránulos de los neutrófilos. Al ser detectados mediante inmunofluorescencia indirecta tras fijación con etanol, los ANCA pueden

adoptar fundamentalmente un patrón perinuclear (pANCA) o un patrón citoplasmático (cANCA). Los ANCA con patrón citoplasmático reconocen casi invariablemente a la proteinasa 3 (PR3)[15]. Aunque ocasionalmente se han descrito en asociación con otras enfermedades (amebiasis invasiva, endocarditis, etc.), los ANCA PR3 son muy característicos de la granulomatosis de Wegener y se hallan presentes en más del 90% de los casos floridos[14,15]. Los ANCA con patrón perinuclear tienen una especificidad más variada. La enzima reconocida con mayor frecuencia es la mieloperoxidasa (MPO)[11]. Pueden reconocer enzimas de los gránulos, enzimas lisosomales, enzimas citosólicas y otras proteínas. Los pANCA pueden detectarse en un amplio abanico de enfermedades, muchas de ellas de base autoinmune[14]. Los pANCA asociados a vasculitis reconocen fundamentalmente la MPO y se detectan en el 70% de los pacientes con poliangeítis microscópica[14,15]. El descubrimiento de los ANCA ha contribuido a afianzar el concepto de poliangeítis microscópica (frecuentemente ANCA positiva) como entidad distinta de la poliarteritis nudosa clásica (habitualmente ANCA negativa). La positividad de los ANCA-PR3 o de los ANCA-MPO en pacientes con un cuadro clínico sugestivo de granulomatosis de Wegener o poliangeítis microscópica, respectivamente, refuerza fuertemente el diagnóstico[14-17]. Sin embargo, estudios recientes han demostrado que la sensibilidad y especificidad de los ANCA de cara al diagnóstico de granulomatosis de Wegener o poliangeítis microscópica en pacientes con síntomas inespecíficos o incompletos es limitada. Así pues, la presencia de ANCA en pacientes con algún rasgo de estas enfermedades (hemorragia alveolar aislada, conjuntivitis granulomatosa, vasculitis demostrada en una biopsia de piel, músculo o nervio, glomerulonefritis rápidamente evolutiva, etc.), fundamenta la sospecha clínica pero obliga a confirmar el diagnóstico por otros procedimientos[18]. Del mismo modo, la ausencia de ANCA en estos pacientes no descarta la presencia de estas enfermedades, puesto que su prevalencia en casos clínicamente poco expresivos desciende considerablemente. La presencia de una u otra especificidad (PR3 o MPO) es un dato adicional para el diagnóstico diferencial entre la granulomatosis de Wegener y la poliangeítis microscópica en los casos con expresividad clínica incompleta (ej. hemorragia alveolar aislada, glomerulonefritis rápidamente evolutiva con o sin hemorragia alveolar) pero no suple la realización de exploraciones o controles adicionales de cara a precisar el diagnóstico[9,14-16,18]. De hecho en la conferencia de consenso de Chapel Hill no se tuvo en cuenta la positividad o negatividad de los ANCA como elemento decisivo para definir los distintos tipos de vasculitis[16].

Clasificación de J.T. Lie

La clasificación propuesta por Lie es posiblemente la más completa en la actualidad (tabla 4). Basada esencialmente en criterios morfológicos (tamaño de los vasos) subraya el carácter solapante de las lesiones histológicas, y orienta al clínico sobre los diagnósticos que debe considerar ante un hallazgo histopatológico concreto. Asimismo Lie incluye un apartado de síndromes pseudovasculíticos útiles para el diagnóstico diferencial de los pacientes con sospecha clínica de estas enfermedades. Los síndromes pseudovasculíticos son entidades que pueden simular una vasculitis desde el punto de vista clínico, angiográfico e incluso histológico[19] (tabla 5).

Etiopatogenia

Las vasculitis constituyen un grupo muy diverso de enfermedades y, por tanto, los mecanismos patogenéticos que conducen a la lesión vascular son probablemente heterogéneos. Distintos agentes etiológicos, entre los que cabe considerar virus, fármacos y otros agentes ambientales, desencadenan una cascada de mecanismos

TABLA 3

Nombres y definiciones de las vasculitis aceptados en la Conferencia de consenso de Chapel Hill sobre la nomenclatura de las vasculitis sistémicas[9]

Vasculitis de gran vaso	
Arteritis de células gigantes (temporal)	Arteritis granulomatosa de la aorta y sus ramas principales con afectación preferente de las ramas extracraneales de la arteria carótida. *A menudo afecta la arteria temporal. Habitualmente ocurre en enfermos mayores de 50 años y se asocia con frecuencia a la presencia de polimialgia reumática*
Arteritis de Takayasu	Inflamación granulomatosa de la aorta y sus ramas principales. *Habitualmente ocurre en pacientes menores de 50 años*
Vasculitis de vaso mediano	
Poliarteritis nudosa clásica	Inflamación necrosante de arterias de tamaño pequeño o mediano sin glomerulonefritis o vasculitis en arteriolas, capilares o vénulas
Enfermedad de Kawasaki	Arteritis en arterias de tamaño grande, mediano y pequeño con síndrome ganglionar mucocutáneo asociado. *Las arterias coronarias están a menudo afectadas. Habitualmente ocurre en niños.*
Vasculitis de vaso pequeño	
Granulomatosis de Wegener	Inflamación granulomatosa en el tracto respiratorio y vasculitis necrosante en vasos de tamaño mediano y pequeño (arterias, arteriolas, capilares y vénulas). *Es frecuente la presencia de glomerulonefritis necrosante*
Síndrome de Churg-Strauss	Inflamación granulomatosa rica en eosinófilos en el tracto respiratorio con vasculitis necrosante en vasos de tamaño mediano y pequeño en pacientes con asma y eosinofilia
Poliangeítis microscópica	Vasculitis necrosante con ausencia o presencia insignificante de depósitos de complejos inmunes que afecta vasos pequeños (arteriolas, capilares y vénulas). *Puede existir vasculitis necrosante en arterias de calibre pequeño o mediano. La glomerulonefritis necrosante es común. A menudo existe capilaritis pulmonar*
Púrpura de Henoch-Schönlein	Vasculitis con depósitos predominantes de IgA que afecta vasos pequeños (arteriolas, capilares y vénulas). *Típicamente se afecta la piel, el intestino y el glomérulo renal. Es muy frecuente la presencia de artralgias o artritis*
Crioglobulinemia	Vasculitis con depósitos de crioglobulinas que afecta vasos pequeños (arteriolas, vénulas y capilares) con presencia de crioglobulinas séricas. *La piel y el glomérulo renal se afectan con frecuencia*
Angeítis leucocitoclástica cutánea	Angeítis leucocitoclástica cutánea sin vasculitis sistémica o glomerulonefritis

Los componentes en cursiva son frecuentes pero no son esenciales para la definición de la entidad correspondiente.

TABLA 4
Clasificación de las vasculitis según J.T. Lie (1991)[19]

Vasculitis infecciosas
- Enfermedades causadas por espiroquetas (sífilis, enfermedad de Lyme)
- Enfermedades originadas por micobacterias
- Infecciones piógenas causadas por bacterias u hongos
- Rickettsiosis
- Enfermedades causadas por Mycoplasmas o virus
- Enfermedades producidas por protozoos

Vasculitis no infecciosas
- Vasculitis que afectan a vasos de calibre grande, mediano y pequeño
  - Arteritis de Takayasu
  - Arteritis granulomatosas (arteritis de células gigantes)
    - Arteritis craneal (temporal) y arteritis de células gigantes extracraneal
    - Angeitis granulomatosa visceral diseminada
    - Angeitis granulomatosa del sistema nervioso central
  - Arteritis de las enfermedades reumáticas y espondiloartropatías
- Vasculitis que afectan predominantemente vasos de calibre mediano y pequeño
  - Tromboangeitis obliterante (enfermedad de Buerger)
  - Poliarteritis nudosa
    - Poliarteritis nudosa clásica
    - Poliangeitis microscópica
    - Poliarteritis nudosa infantil
    - Enfermedad de Kawasaki
  - Angeitis granulomatosas
    - Granulomatosis de Wegener
    - Síndrome de Churg-Strauss
    - Granulomatosis necrosante sarcoidea
  - Vasculitis asociadas a enfermedades autoinmunes
    - Fiebre reumática
    - Artritis reumatoidea
    - Espondiloartropatías
    - Lupus eritematoso sistémico
    - Dermatomiositis
    - Policondritis recidivante
    - Esclerosis sistémica progresiva
    - Enfermedad de Sjögren
    - Enfermedad de Behçet
    - Síndrome de Cogan
- Vasculitis que afectan preferentemente a vasos de pequeño tamaño (vasculitis por hipersensibilidad o vasculitis leucocitoclástica)
  - Enfermedad del suero
  - Púrpura de Henoch-Schönlein
  - Vasculitis inducida por fármacos
  - Crioglobulinemia mixta
  - Vasculitis hipocomplementémica
  - Fiebre mediterránea familiar
  - Vasculitis asociada a neoplasia
  - Fibrosis retroperitoneal
  - Enfermedad inflamatoria intestinal
  - Cirrosis biliar primaria
  - Síndrome de Goodpasture
  - Vasculitis del órgano transplantado

TABLA 5
Síndromes pseudovasculíticos

Enfermedades que causan síntomas constitucionales y fenómenos isquémicos
- Embolismos de colesterol
- Endocarditis bacteriana
- Mixoma cardíaco
- Vasculopatía del síndrome antifosfolípido
- Amiloidosis sistémica
- Linfomas extranodales y otras neoplasias

Procesos que cursan con lesiones cutáneas necróticas o de aspecto inflamatorio
- Calcifilaxis
- Enfermedad de Köhlmeier-Degos
- Síndrome de Sweet
- Paniculitis
- Necrosis cutánea por cumarínicos
- Dermatosis pigmentaria purpúrica
- Perniosis
- Escorbuto

Enfermedades que producen afectación estructural o funcional de los vasos
- Mediolisis arterial/displasia fibromuscular
- Ergotismo
- Vasoespasmo por cocaína
- Calcificación vascular idiopática de la infancia

Enfermedades hereditarias con posible compromiso vascular
- Neurofibromatosis
- Seudoxantoma elástico
- Enfermedad de Fabry
- Síndrome de Ehler-Danlos

Modificada y ampliada de J.T. Lie.

patogenéticos inmediatos capaces de lesionar la pared de los vasos. Entre ellos cabe considerar el depósito de complejos inmunes, los anticuerpos anticitoplasma de los neutrófilos, anticuerpos anticélula endotelial, y una respuesta inmunológica mediada por células T frente a antígenos presentes en la pared arterial. Estos mecanismos patogenéticos no son mutuamente excluyentes sino que probablemente actúan de manera combinada con un protagonismo variable según la enfermedad o según el momento evolutivo. Sobre ellos actuarían en algunos casos otros factores moduladores como las hormonas sexuales y el sustrato genético[20] (fig. 1).

Agentes desencadenantes

Poco se conoce acerca de los agentes etiológicos que desencadenan la cascada de fenómenos inmunológicos que producen la lesión vascular. En un 53% de pacientes con vasculitis leucocitoclástica se recoge el antecedente de ingesta de algún fármaco[21]. Se han descrito vasculitis en las lesiones propias de diversas enfermedades bacterianas (tuberculosis, sífilis, rickettsiosis) y fúngicas (aspergilosis, candidiasis)[22]. Recientemente distintos investigadores han llamado la atención sobre el papel de algunas infecciones víricas en la etiopatogenia de las vasculitis. Es ya clásicamente conocida la asociación entre la poliarteritis nudosa y la infección por el virus de la hepatitis B (VHB), que se detecta hasta en un 20%-40% de casos en algunas series[23]. Más prevalente es todavía la infección por el virus de la hepatitis C (VHC) en los pacientes con crioglobulinemia mixta, que alcanza el 96% en algunas series[24] (fig. 2). El papel etiopatogénico de estos virus en el desarrollo de estas formas de vasculitis se basa no sólo en su estrecha asociación epidemiológica, sino en que la remisión de las manifestaciones clínicas se alcanza con tratamientos que consiguen una remisión virológica, como el interferón α2a en la crioglobulinemia asociada al VHC[25] y la vidarabina o interferón α2a en la poliarteritis nudosa asociada al VHB[26,27]. Se han descrito también distintos tipos de vasculitis (poliarteritis nudosa, vasculitis leucocitoclástica, vasculitis aislada del sistema nervioso central) en pacientes infectados por el virus de la inmunodeficiencia humana (VIH) (fig. 3)[28,29]. Con frecuencia estas complicaciones aparecen en pacientes que no están todavía en una fase de profunda inmunodepresión[30]. A diferencia de las vasculitis idiopáticas, donde suele

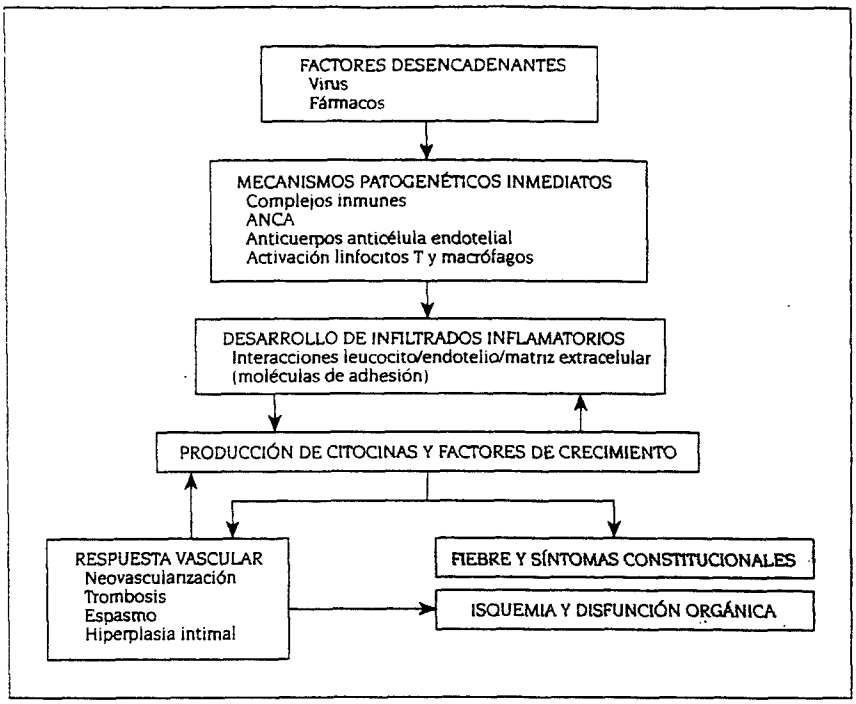

Fig. 1. Mecanismos patogéneticos involucrados en el desarrollo de inflamación y lesión vascular. ANCA: anticuerpos anticitoplasma del neutrófilo.

existir un predominio de linfocitos CD4, en las vasculitis asociadas a VIH el infiltrado inflamatorio suele estar constituido fundamentalmente por linfocitos CD8. La participación de infecciones oportunistas por otros virus o la infección por otros virus de transmisión similar al VIH no se ha excluido por completo. Otros virus descritos presuntamente implicados son el parvovirus B19, el virus de la varicela-zóster y el citomegalovirus[22]. La identificación de virus como agentes etiológicos ò desencadenantes de las vasculitis tiene importantes implicaciones terapéuticas. Los resultados preliminares obtenidos con las vasculitis asociadas al VHB y VHC permiten vislumbrar alternativas al tratamiento inmunodepresor cuyo potencial yatrogénico resulta cada vez más evidente.

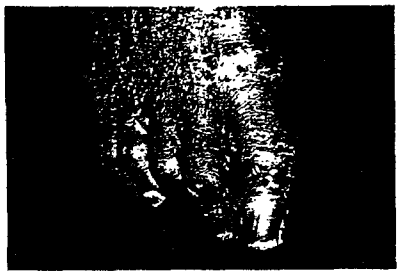

Fig. 2. Lesiones necróticas distales en un paciente con crioglobulinemia mixta asociada al virus de la hepatitis C.

Fig. 3. Vasculitis necrosante en la biopsia muscular de un paciente infectado por el virus de la inmunodeficiencia humana.

Mecanismos patogenéticos inmediatos

Depósito de complejos inmunes circulantes

A partir de modelos experimentales del fenómeno de Arthus y de la enfermedad del suero se conoció, hace más de dos décadas, que los complejos inmunes circulantes formados en presencia de un exceso de antígeno pueden no ser totalmente eliminados por el sistema mononuclear fagocítico y depositarse en la pared vascular. Asimismo pueden originarse complejos inmunes directamente en los vasos. Allí, activarían la cascada del complemento cuyos productos de activación atraerían a los neutrófilos que con sus enzimas lisosómicas destruirían la pared vascular[1]. Las vasculitis leucocitoclásticas, la púrpura de Henoch-Schönlein y, probablemente, las vasculitis necrosantes del grupo de la poliarteritis nudosa son las entidades que mejor se adaptan a este modelo patogenético. Las lesiones vasculíticas de los pacientes con púrpura de Henoch-Schönlein contienen típicamente depósitos de inmunoglobulinas, especialmente IgA. El complejo de ataque de membrana (C5b-9), producto final de la activación del complemento, se ha podido objetivar en algunas vasculitis tipo poliarteritis nudosa (fig. 4) y en las vasculitis asociadas al síndrome de Sjögren[2,31]. Sin embargo, el depósito de complejos inmunes, principal mecanismo capaz de producir daño vascular conocido durante muchos años, no explica de modo satisfactorio las lesiones objetivadas en otras vasculitis, como la arteritis de células gigantes, la granulomatosis de Wegener o la enfermedad de Takayasu.

Anticuerpos anticitoplasma de los neutrófilos

La estrecha asociación entre los ANCA PR3 y la granulomatosis de Wegener y, en menor grado, entre ANCA MPO y poliangeítis microscópica sugiere que estos anticuerpos tienen un papel patogenético en el desarrollo de inflamación vascular[14]. Las enzimas reconocidas por los ANCA tienen un elevado potencial destructivo, y su actividad fisiológica se halla estrictamente regulada. Por un lado, los ANCA son capaces de inhibir la actividad enzimática de la mieloperoxidasa o la proteinasa 3. Por otro lado, la unión de los ANCA a la proteinasa 3 evita la inactivación por su inhibidor natural, la α1 antitripsina. No se conoce el balance neto de estas interacciones aparentemente opuestas *in vivo*[14,20]. Tanto la mieloperoxidasa como la proteinasa 3 se translocan desde los gránulos a

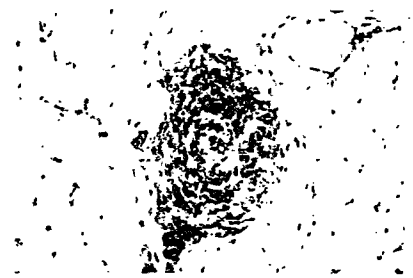

Fig. 4. El depósito del complejo de ataque de membrana C5b-9 (en rojo) puede objetivarse en las lesiones propias de la poliarteritis nudosa clásica. Tinción inmunohistoquímica (inmunofosfatasa alcalina). El C5b-9 se ha identificado con el anticuerpo monoclonal aE11 (Dako).

la membrana de los neutrófilos tras estímulo con citocinas, fundamentalmente el factor de necrosis tumoral (FNT) y la interleucina-8[14,20]. Su translocación y liberación forma parte de la respuesta fisiológica de los neutrófilos a estímulos inflamatorios. La unión de los ANCA a la mieloperoxidasa y a la proteinasa 3 produce señales coestimuladoras que aumentan el estado de activación de los polimorfonucleares: estimulan su degranulación, la producción de óxido nítrico, la liberación de radicales superóxido, la actividad quimiotáctica y la adhesión al endotelio. Estudios experimentales demuestran que la activación del neutrófilo potenciada por los ANCA es capaz de dañar el endotelio. Al parecer, tanto el reconocimiento específico de la PR3 o MPO asociados a membrana como la unión de los ANCA a los receptores Fc de los neutrófilos participan en su efecto coestimulador[14,20]. Algunos autores han inducido la producción de ANCA en modelos animales. Estos animales pueden desarrollar glomerulonefritis o capilaritis pulmonar pero precisan la perfusión local del órgano diana con estímulos adicionales como H_2O_2 o extractos de los gránulos de los neutrófilos[32]. Por otro lado, distintos modelos animales de autoinmunidad producen espontáneamente ANCA y nunca desarrollan las lesiones características de las vasculitis asociadas a éstos. En el momento actual, está demostrado que los ANCA contribuyen a la activación de los neutrófilos inducida por otros estímulos y pueden aumentar su potencial lesivo sobre el endotelio, pero no parecen por sí solos capaces de inducir lesiones inflamatorias, y es prematuro asumir que los ANCA juegan un papel primario en la patogénesis de las vasculitis[14,20].

Anticuerpos anticélula endotelial

Se han detectado anticuerpos anticélula endotelial en el suero de pacientes con una amplia variedad de enfermedades que cursan con afección vascular como el lupus eritematoso sistémico y la esclerosis sistémica progresiva entre otras, pero también en procesos como el hiperparatiroidismo autoinmune donde la participación endotelial es menos aparente. La presencia de anticuerpos anticélula endotelial se ha demostrado en diversas vasculitis sistémicas como la enfermedad de Kawasaki, la granulomatosis de Wegener, la poliangeítis microscópica, la vasculitis retiniana y la enfermedad de Behçet[33,34]. Algunos de estos anticuerpos, como los detectados en la enfermedad de Kawasaki, se unen únicamente a células endoteliales activadas por citocinas, por lo que presumiblemente reconocen antígenos inducibles en un contexto inflamatorio[33]. Otros, como los detectados en la granulomatosis de Wegener y la poliangeítis microscópica reconocen, por el contrario, antígenos expresados por las células endoteliales en reposo cuya expresión no es aparentemente regulable por citocinas[33]. La mayor parte de estos anticuerpos son capaces de fijar el complemento y pueden ejercer citotoxicidad directa o mediar citotoxicidad celular dependiente de anticuerpo y, por tanto, tienen un potencial lesivo sobre la célula endotelial. Las especificidades reconocidas por estos anticuerpos parecen ser muy heterogéneas, y los antígenos que reconocen no han sido todavía caracterizados. En la mayor parte de los procesos los niveles de anticuerpos anticélula endotelial se correlacionan con la actividad clínica de la enfermedad[33]. No se conoce todavía si estos anticuerpos tienen un papel patogenético primario o se producen a consecuencia de la lesión endotelial producida por otros mecanismos.

Respuesta inmunológica mediada por células T

Estudios inmunopatológicos han demostrado que en algunas vasculitis como la arteritis temporal, la granulomatosis de Wegener, la enfermedad de Takayasu, la poliarteritis nudosa y las lesiones coronarias en la enfermedad de Kawasaki, el infiltrado inflamatorio está compuesto fundamentalmente por macrófagos y linfocitos T que expresan marcadores de activación inmunológica y proliferan activamente en las lesiones[20,35] (fig. 5). Asimismo, en lesiones precoces de arteritis temporal y poliarteritis nudosa se han identificado células dendríticas que expresan antígenos de clase II del sistema mayor de histocompatibilidad que actuarían probablemente como células presentadoras de antígenos[2,20,35] (fig. 6). Estas observaciones sugieren que los linfocitos T activados y los macrófagos juegan un papel importante en el desarrollo de la lesión vascular. Los mecanismos a partir de los cuales los linfocitos T se activan parecen ser heterogéneos. En algunas vasculitis como la enfermedad de Kawasaki, se detectan expansiones policlonales de linfocitos T circulantes que pueden ser el resultado de un estímulo por superantígenos. En un modelo animal de arteritis granulomatosa se ha demostrado la participación de mecanismos de citotoxicidad restringida por antígenos de clase I del sistema mayor de histocompatibilidad[20]. La presencia de linfocitos CD8 se ha demostrado, efectivamente, en distintas vasculitis como la arteritis temporal, la poliarteritis nudosa y la enfermedad de Takayasu[2,20,35,36]. En esta última se ha podido demostrar la producción de perforina por parte de los linfocitos citotóxicos. En la arteritis temporal se ha demostrado que una pequeña proporción de los linfocitos activados infiltrantes sufren una expansión clonal sugiriendo, en efecto, una respuesta inmune específica frente a un antígeno posiblemente presente en la pared arterial[37]. En la enfermedad de Takayasu una población significativa de linfocitos infiltrantes expresa cadenas gamma/delta del receptor T. La expresión concomitante de proteínas *heat-shock* sugiere que además de los mecanismos de citotoxicidad antígeno-específica el reconocimiento de estas proteínas de estrés celular por los linfocitos

Fig. 5. Los linfocitos T CD4 (tinción de membrana en rojo) constituyen un importante porcentaje de los leucocitos infiltrantes en las lesiones de la poliarteritis nudosa. Tinción inmunohistoquímica con inmunofosfatasa alcalina (anticuerpo monoclonal leu 3a [CD4] [Becton-Dickinson]).

Fig. 6. En lesiones incipientes de poliarteritis nudosa pueden objetivarse células interdigitantes (células presentadoras de antígenos) que rodean con sus prolongaciones dendríticas a los linfocitos.

gamma/delta podría también contribuir y amplificar al daño vascular[30].

Una vez activados por distintos mecanismos patogenéticos, los leucocitos invaden la pared vascular a través de complejas interacciones con el endotelio y las proteínas de matriz extracelular que tienen lugar a través de unos receptores de membrana, y sus contrarreceptores denominados genéricamente moléculas de adhesión. El endotelio de los vasos inflamados en las vasculitis expresa moléculas de adhesión para los leucocitos, y las formas solubles de estas moléculas pueden detectarse en el suero o plasma de los pacientes con vasculitis. El patrón de expresión de estas moléculas y las fluctuaciones de sus niveles séricos en relación con la actividad de la enfermedad son objeto de investigación en la actualidad[20].

Respuesta vascular

Una vez en la pared de los vasos, los leucocitos segregan citocinas y factores de crecimiento. Estos mediadores generan una serie de cambios morfológicos y funcionales en los vasos que son responsables de la mayor parte de las manifestaciones clínicas, y complicaciones isquémicas de los pacientes con vasculitis. Algunas citocinas como la interleucina 1 (IL-1), el FNT y la interleucina 6, producidas activamente en las lesiones de la arteritis de células gigantes[35], producen fiebre y síntomas constitucionales frecuentes en estos pacientes. Las manifestaciones focales más específicas de las vasculitis derivan con frecuencia de la disfunción orgánica determinada por la isquemia y, en ocasiones, por la hemorragia en los órganos afectados. La oclusión vascular en las vasculitis puede ocurrir por espasmo, trombosis e hiperplasia o fibrosis intimal[20]. Las citocinas proinflamatorias IL-1 y FNT alteran las propiedades anticoagulantes del endotelio. Las mismas citocinas regulan el tono vascular a través de la inducción de sintasa de óxido nítrico cuya producción se ha demostrado en algunas vasculitis. Otras citocinas producidas en la pared vascular como el factor de crecimiento transformador β (TGFβ) tienen actividad fibrogénica. Otras citocinas contribuyen a mantener los infiltrados inflamatorios. Citocinas con actividad quimiotáctica continúan atrayendo a los leucocitos que penetran en los vasos gracias a la inducción de moléculas de adhesión endoteliales inducidas por IL-1, FNT e interferón gamma (IFNgamma). La producción de IFN-gamma parece esencial para el desarrollo y mantenimiento de la reacción granulomatosa característica de la arteritis temporal. Otros factores de crecimiento con actividad angiogénica pueden contribuir a la neovascularización que ocurre en el seno de la pared arterial en algunas vasculitis como la poliarteritis nudosa y la arteritis temporal (fig. 7)[20,35]. Los vasos neoformados proporcionarían nuevos puntos a través de los cuales los leucocitos pueden infiltrar la pared vascular y contribuir al mantenimiento de la inflamación. El papel preciso que las citocinas y factores de crecimiento desempeñan en la respuesta vascular es objeto de una investigación activa en la actualidad[20].

Factores moduladores

Hormonas sexuales

Algunas vasculitis como la enfermedad de Takayasu afectan de manera preferente a mujeres en edad fértil. Esta distribución demográfica sugiere que las hormonas sexuales pueden modular los mecanismos inmunológicos que conducen a la lesión vascular o la respuesta del propio vaso a la inflamación. Recientemente se ha demostrado que la célula endotelial tiene receptores estrogénicos funcionales y que los estrógenos regulan las vías a través de las cuales determinadas citocinas como el FNT inducen la expresión de moléculas de adhesión endoteliales para los leucocitos[39].

Predisposición genética

Desde hace algunos años se conoce que individuos portadores de alelos HLA-DRB1*04 del Sistema Mayor de Histocompatibilidad tienen un riesgo relativo incrementado de padecer arteritis temporal[40]. Recientemente Weyand et al han identificado una secuencia concreta (DRYF) relacionada con la enfermedad a nivel de la segunda región hipervariable de la cadena HLA-DRβ. Esta secuencia estaría situada en la zona de unión al antígeno durante la presentación antigénica, y refuerza la hipótesis de que las lesiones vasculares en la arteritis de células gigantes ocurren a consecuencia de una respuesta inmune antígeno específica. En la enfermedad de Behcet parece también existir una predisposición genética asociada al sistema mayor de histocompatibilidad y sería más frecuente en individuos portadores del HLA B51[41].

Fig. 7. En el seno de los infiltrados inflamatorios propios de la arteritis temporal tiene lugar una intensa neovascularización. Las células endoteliales (color pardo) se han identificado con la lectina Ulex Europaeus *(Dako).*

Conclusiones

En los últimos años se han producido muchos avances en el conocimiento de los mecanismos inmunológicos capaces de producir daño vascular. Al mismo tiempo se ha hecho evidente que la respuesta del propio vaso juega un papel importante en el mantenimiento de la inflamación y en la oclusión vascular y, por tanto, en las principales complicaciones de las vasculitis. Es de esperar que el progreso continuado en el conocimiento de los mecanismos patogenéticos implicados en las vasculitis nos lleve a disponer de mejores criterios de clasificación, mejores procedimientos diagnósticos, marcadores de actividad más útiles y, especialmente, mejores opciones terapéuticas que constituyan una alternativa a los tratamientos inmunodepresores con elevado potencial yatrogénico del que disponemos en la actualidad.

Agradecimientos

Este trabajo ha sido financiado por FIS 95/0860 y FIS 96/0347.

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Moléculas de adhesión en las interacciones entre los leucocitos, el endotelio y la matriz extracelular (II). Relevancia en clínica humana y aplicaciones terapéuticas potenciales

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Las moléculas de adhesión que median las interacciones entre los leucocitos, el endotelio y la matriz extracelular, descritas en la primera parte de esta revisión[1], tienen una importancia fundamental en la defensa del organismo frente a la infección a través de su participación en la respuesta inmunológica y en la migración de los leucocitos hacia los tejidos. Sin embargo, las mismas interacciones contribuyen al desarrollo de los fenómenos inflamatorios nocivos que ocurren en las enfermedades autoinmunes, en la lesión tisular causada por la reperfusión postisquemia y en el rechazo de trasplantes[2-4]. Algunos de estos mecanismos son utilizados también por las células neoplásicas para penetrar en los tejidos durante la diseminación metastásica. El conocimiento de los mecanismos moleculares que regulan estas interacciones tiene importantes repercusiones en patología humana y constituye la base para la búsqueda de nuevos métodos de intervención terapéutica.

Metodología utilizada para el estudio de la función in vivo de las moléculas de adhesión

Las moléculas de adhesión se han identificado y se han caracterizado funcionalmente in vitro mediante anticuerpos monoclonales (AcMo) capaces de bloquear interacciones adhesivas intercelulares o entre las células y la matriz extracelular y mediante AcMo capaces de transducir señales a través de la molécula reconocida, simulando la acción de su ligando natural. La relevancia funcional de estos sistemas in vivo se ha investigado mediante distintas aproximaciones que se detallan a continuación.

Abreviaturas utilizadas
AcMo: anticuerpo monoclonal.
ICAM: molécula de adhesión intercelular.
VCAM: molécula de adhesión vascular.
FNT: factor de necrosis tumoral.
IL-1: interleucina 1.
LPS: endotoxina, lipopolisacárido bacteriano.
IFN-γ: interferón gamma.
LAD: defecto en la adhesión leucocitaria.
VLA: antígeno de activación de aparición tardía.
LES: lupus eritematoso sistémico.
AR: artritis reumatoide.
CLA: antígeno linfocitario cutáneo.
Sida: síndrome de inmunodeficiencia adquirida.
VIH: virus de la inmunodeficiencia humana.
LFA: antígeno funcional de los linfocitos.

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Manuscrito aceptado el 13-2-1996

Med Clin (Barc) 1997; 108: 503-511

Expresión tisular tras inyección subcutánea de citocinas

La regulación de la expresión de moléculas de adhesión endoteliales mediante citocinas se conoce a través de estudios realizados en células endoteliales en cultivo. El papel que las citocinas desempeñan in vivo en la inducción/regulación de la expresión de estas moléculas se ha investigado mediante la inyección subcutánea de citocinas a animales de experimentación o a voluntarios humanos seguida del estudio inmunohistoquímico de biopsias cutáneas obtenidas de la zona inyectada. A partir de análisis inmunohistoquímicos se conoce que ICAM-1 se expresa constitutivamente por el endotelio sano in vivo[5], mientras que la selectina E y VCAM-1 no aparecen en las células endoteliales en condiciones normales[6]. Al igual que en las células endoteliales en cultivo, la inyección subcutánea de factor de necrosis tumoral α (FNT-α), interferón gamma (IFN-γ), endotoxina (LPS) o combinaciones de las mismas a primates o a voluntarios humanos induce la expresión endotelial de selectina E y VCAM-1 y produce un incremento en la expresión de ICAM-1 con una especificidad y cronología muy similares a las observadas in vitro[7,8]. En estos sistemas, la inducción de selectina E se correlaciona con una acumulación de neutrófilos y la de ICAM-1 con un aflujo, posterior en el tiempo, de células mononucleadas. Las moléculas de adhesión endoteliales tienen, pues, un papel crucial en el desarrollo de lesiones inflamatorias in vivo y su regulación es muy parecida a la observada en sistemas in vitro.

Animales knock-out

Los ratones *knock-out* son animales conseguidos mediante recombinación homóloga que presentan el gen que codifica una determinada proteína mutado, de manera que dicho gen no genera un producto funcionalmente adecuado. Se han conseguido animales *knock-out* para CD18, cadena común de las integrinas β2, para las selectinas L, E y P y para las inmunoglobulinas ICAM-1 y VCAM-1[3,9,10]. Los ratones *knock-out* para CD18 tienen respuestas inflamatorias defectuosas frente a estímulos químicos y una mayor tolerancia a los aloinjertos. Se observan anomalías similares en los animales sin ICAM-1, aunque menos llamativas. Curiosamente los ratones *knock-out* para VCAM-1 no son viables por anomalías cardíacas, de lo que se deduce que VCAM-1 tiene funciones cruciales durante el desarrollo embrionario[3]. Estos modelos permiten, por tanto, el conocimiento de otras funciones biológicas insospechadas para las moléculas de adhesión.

Anticuerpos monoclonales bloqueadores

La función de las moléculas de adhesión in vivo se ha investigado también mediante la inyección de AcMo bloqueado-

res a animales de experimentación. Se ha estudiado en animales sanos la respuesta inflamatoria frente a distintos estímulos en presencia del anticuerpo bloqueador. En modelos animales de enfermedades humanas se ha investigado la repercusión de la inhibición funcional de estas moléculas sobre el desarrollo del proceso patológico. Esta aproximación no es siempre fácil debido a que muchos de los AcMo disponibles son dirigidos hacia moléculas humanas y no tienen reacción cruzada con sus equivalentes animales. Además de la valoración de la existencia de reacción cruzada en los AcMo disponibles se requiere la generación de AcMo frente a moléculas de adhesión de otras especies, la aplicación de AcMo a ratones con inmunodeficiencia severa combinada trasplantados con tejidos humanos, o la inmunización de ratones *knock-out* para que generen anticuerpos frente a la molécula de que carecen[3,11].

Moléculas de adhesión en patología humana

Inmunodeficiencias

La importancia funcional de las interacciones mediadas por las moléculas de adhesión en generar una respuesta inmune y una reacción inflamatoria eficaz se conoce desde la pasada década cuando se identificó la enfermedad por deficiencia de adhesión de los leucocitos (LAD por *leukocyte adhesion defficiency*) tipo I[12]. La LAD tipo I se debe a distintas mutaciones en la cadena común de las integrinas β2 (CD18) y se caracteriza por un defecto grave en la migración de neutrófilos. Clínicamente cursa con neutrofilia crónica e infecciones recurrentes sin formación de pus. En esta enfermedad los neutrófilos tienen un rodamiento normal sobre el endotelio pero son incapaces de adherirse y transmigrar[13]. Estos pacientes, al igual que los animales *knock-out* para CD18, pueden desarrollar infiltrados linfocitarios puesto que la vía VLA-4/VCAM-1 se halla preservada. Recientemente, se ha identificado la LAD tipo II que consiste en un defecto en el metabolismo de la fucosa que condiciona una incapacidad para sintetizar adecuadamente moléculas fucosiladas, tales como los oligosacáridos sialil-Lewis^x y Lewis^a. En estos pacientes, las interacciones mediadas por selectinas son defectuosas[14]. Los leucocitos aislados de estos enfermos son capaces de unirse a las células endoteliales activadas en condiciones estáticas pero no experimentan un rodamiento adecuado y no son capaces de desarrollar una adhesión firme en condiciones de flujo[13].

Enfermedades inflamatorias crónicas

Para investigar la participación de las moléculas de adhesión en enfermedades inflamatorias crónicas humanas se han utilizado distintas aproximaciones que se detallan a continuación.

Expresión de membrana y funcionalismo en leucocitos. Se ha estudiado la expresión de superficie de estas moléculas en leucocitos circulantes procedentes de pacientes, así como en leucocitos que han migrado ya a compartimientos específicos (p. ej., líquido sinovial o líquido cefalorraquídeo). Así, los linfocitos circulantes aislados de pacientes con lupus eritematoso sistémico (LES) que desarrollan vasculitis expresan más intensamente VLA-4 en su superficie y tienen una mayor adhesividad a células endoteliales en cultivo y a proteínas de matriz extracelular que los obtenidos de pacientes con LES sin vasculitis[15]. Del mismo modo, linfocitos extraídos del líquido sinovial de pacientes con artritis reumatoide (AR) tienen mayor densidad de α4 en su membrana y muestran una adhesividad mayor a VCAM-1 recombinante y a fibronectina que los linfocitos de sangre periférica de los propios pacientes[16]. Así mismo, neutrófilos aislados del líquido sinovial de enfermos con AR tienen un incremento en la expresión de la integrina β2 Mac-1. Por el contrario, la expresión de CD44, CD43 y selectina L se encuentra reducida como corresponde al fenotipo de los neutrófilos activados in vitro[17]. Las células mononucleadas aisladas de pacientes con enfermedad de Crohn tienen una mayor agregación dependiente de CD2, LFA-3 y Mac-1 que las aisladas de sujetos control[18]. Así pues, los leucocitos obtenidos de pacientes con distintas enfermedades inflamatorias crónicas tienen un estado de adhesividad incrementado, especialmente aquellos que han migrado ya a los lugares afectados.

Expresión tisular en muestras biópsicas. La expresión de moléculas de adhesión endoteliales y sus receptores leucocitarios se ha estudiado mediante técnicas inmunohistoquímicas en una gran variedad de procesos entre los que destaca la AR, diversos tipos de glomerulonefritis, enfermedad de Sjögren o sarcoidosis, entre otros[19-23]. En ellos se ha objetivado, casi invariablemente, sobreexpresión de moléculas de adhesión endoteliales inducibles (selectina E, ICAM-1 y VCAM-1). Así, la expresión endotelial de selectina E se observa asiduamente en lesiones inflamatorias agudas como amigdalitis, apendicitis, glomerulonefritis agudas, rechazo agudo de riñón trasplantado y reacciones alérgicas cutáneas[6,8,24]. Más aisladamente puede detectarse también en lesiones bien establecidas de una gran variedad de enfermedades inflamatorias crónicas (fig. 1).

La expresión de ICAM-1 es más difícil de valorar dada su expresión constitutiva. La ICAM-1 aparece claramente sobreexpresada en los vasos de un gran número de enfermedades como la nefritis lúpica, arteritis de células gigantes, dermatomiositis, reacciones de hipersensibilidad retardada, sinovial de la AR, esclerosis múltiple, rechazo de trasplante renal y hepático[25-27] (fig. 2). Resulta interesante que su expresión disminuya cuando las lesiones progresan a un estado fibrótico como la esclerosis glomerular a la que evolucionan las glomerulonefritis crónicas[21,22]. Junto a su función como molécula de adhesión en las interacciones linfocito/endotelio, ICAM-1 tiene un importante papel como molécula accesoria en la activación del linfocito T y su expresión por la célula diana es necesaria para que los linfocitos CD8 puedan ejercer su acción citotóxica. Por ello, en un contexto inflamatorio aparece expresión de ICAM-1 en otras

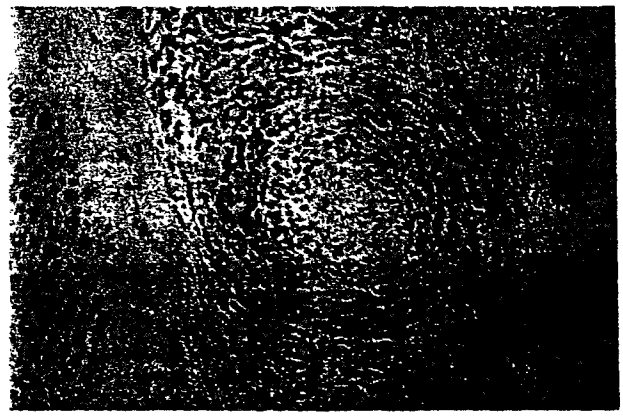
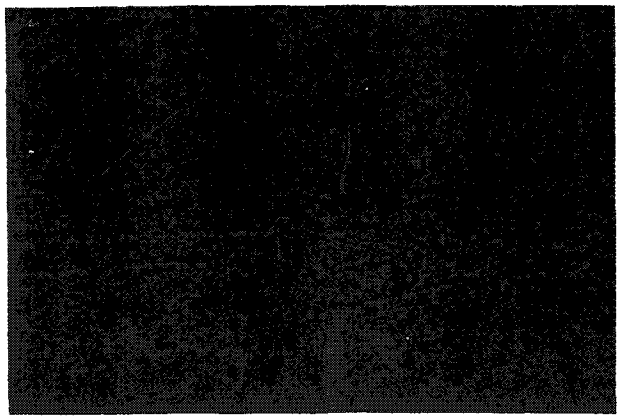
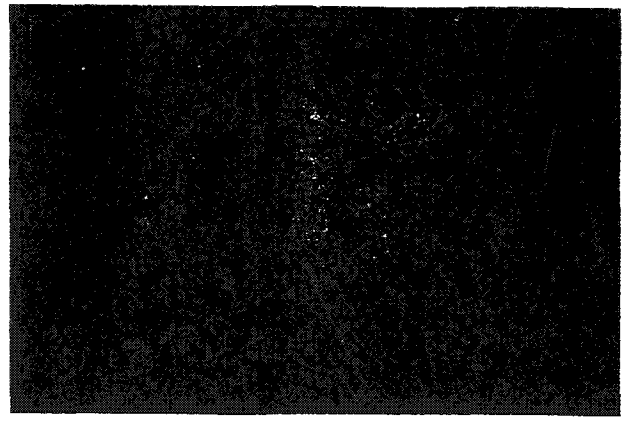


Fig. 1. Expresión de selectina E limitada a escasos capilares adventiciales en lesiones bien establecidas de poliarteritis nudosa. Tinción inmunohistoquímica mediante la técnica fosfatasa alcalina-antifosfatasa alcalina (PAAFA) con el AcMo H18/7 × 250





estirpes celulares como las células epiteliales del túbulo renal en las glomerulonefritis y rechazo de trasplante y las células musculares en las miopatías inflamatorias[21,22,25] (fig. 2). La VCAM-1 se expresa en los vasos rodeados por infiltrados inflamatorios en distintas enfermedades inflamatorias crónicas[6,20,23] (fig. 3). Al igual que ICAM-1, la expresión de VCAM-1 no se halla limitada a las células endoteliales, aunque es mucho más restringida[21].

La ICAM-2 tiene una expresión intensa constitutiva en las células endoteliales y no se ha demostrado su regulación in vitro mediante citocinas[28]. Sin embargo, se ha objetivado sobreexpresión en los vasos de algunos linfomas[29]. La ICAM-3 no se expresa en el endotelio en reposo ni se ha podido demostrar in vitro su inducción por citocinas[30]. Se ha objetivado expresión de ICAM-3 en la vasculatura de algunos linfomas de Hodgkin y no hodgkinianos[31] y en la neovascularización que acompaña a algunos tumores sólidos (Terol y Campo, observaciones no publicadas).

La participación de estas moléculas en el desarrollo de lesiones inflamatorias, aunque decisiva, parece ser bastante similar en la mayor parte de enfermedades. Existen, sin embargo, rasgos distintivos en algunas de ellas. Así, la selectina E, con una inducción rápida y transitoria y una adhesión preferente a neutrófilos, se expresa preferentemente en lesiones agudas con abundancia de granulocitos[6,24,32]. En cambio, la expresión de VCAM-1 se hace más evidente en lesiones crónicas con infiltrados linfomonocitarios[6]. Parece existir también una cierta especificidad tisular. La selectina E se expresa intensamente en los vasos de enfermedades inflamatorias crónicas cutáneas (psoriasis, dermatitis de contacto, dermatomiositis o enfermedad de Kawasaki)[8,33], de acuerdo con su capacidad para unirse a linfocitos con tropismo cutáneo que expresan el CLA *(cutaneous lymphocyte antigen)*[34,35]. Así, la selectina E se expresa en los vasos de las lesiones cutáneas de la dermatomiositis[33] y no en los vasos de los infiltrados musculares producidos por la misma enfermedad[36]. Del mismo modo, en la enfermedad del injerto contra el huésped, únicamente expresan CLA los linfocitos que infiltran la piel y no aquellos que infiltran otros órganos como, por ejemplo, el intestino[37]. También existen algunas diferencias entre enfermedades. Así, en el LES los pequeños vasos musculares expresan selectina E independientemente de la proximidad a infiltrados leucocitarios[38]. En cambio, la expresión endotelial de VCAM-1 se relaciona siempre con la existencia de infiltrados inflamatorios. Por el contrario, en la dermatomiositis los capilares no expresan selectina E y muestran una sobreexpresión de ICAM-1 y VCAM-1, aparentemente sin clara relación con la proximidad de infiltrados inflamatorios[36].

Fig. 2. A: leve expresión endotelial de ICAM-1 en los capilares musculares de la polimiositis; B: expresión de ICAM-1 en prácticamente la totalidad de los capilares musculares en la dermatomiositis. Se observa, así mismo, expresión de ICAM-1 en el sarcolema de algunas fibras musculares, y C: a mayor aumento puede observarse como los capilares lesionados y edematosos, típicos de la dermatomiositis, son intensamente positivos para ICAM-1. Tinción inmunohistoquímica mediante la técnica FAAFA con el AcMo RR1/1 a ×100 (A y B) y ×250 (C).

En algunas enfermedades se ha analizado la expresión tisular de moléculas de adhesión antes y después del tratamiento. Así, en los vasos de las lesiones cutáneas de la enfermedad de Kawasaki existe inducción de selectina E y sobreexpresión de ICAM-1 que se negativiza o disminuye tras el tratamiento con inmunoglobulina intravenosa[39]. La expresión de selectina E en la sinovial de la artritis reumatoide disminuye tras el tratamiento con corticoides y sales de oro[40]. Del mismo modo, la expresión de selectina E e ICAM-1 en la piel no afectada de LES disminuye al alcanzar la remisión[41].

Detección de formas solubles. Se ha demostrado que las selectinas y varios miembros de la superfamilia de inmunoglobulinas existen en forma soluble en plasma y otros líquidos biológicos[42]. En algunos casos, como la selectina P y VCAM-1, este hecho ocurre a través de un procesamiento alternativo del ARN que genera una variante sin el dominio transmembrana[42]. En otros casos, como ocurre con la selectina L e ICAM-3, la molécula se desprende por proteólisis[42,43]. Estudios in vitro han demostrado que algunas de las moléculas (p. ej., selectina L o ICAM-3) se desprenden de la membrana leucocitaria tras la activación[43,44]. Tras el

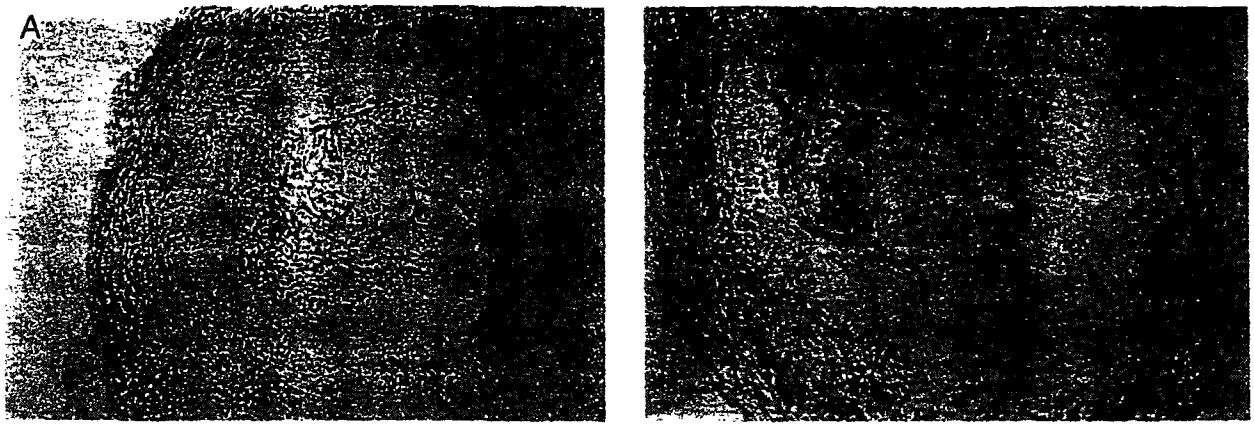


Fig. 3. A: expresión de VCAM-1 en los vasa vasorum *adventiciales, en la neovascularización que acompaña a los focos inflamatorios y en el endotelio luminal de la arteria temporal en la arteritis de células gigantes, y B: corte seriado que demuestra intensa expresión de VLA-4 en los linfocitos y macrófagos que rodean los vasos positivos para VCAM-1.*
Tinción inmunohistoquímica mediante la técnica FAAFA con los AcMo 4B9 (anti-VCAM-1) y HP2/1 (anti-α4) ×100.

estímulo con citocinas, la célula endotelial no sólo aumenta la expresión de membrana de estas moléculas sino también su liberación al sobrenadante[45]. Por este motivo el incremento en la concentración de moléculas de adhesión circulantes se considera una evidencia indirecta de activación inmunológica y/o endotelial. Los efectos biológicos de las moléculas de adhesión solubles no se conocen con exactitud. Por un lado, al conservar su capacidad de unión al contrarreceptor pueden bloquear las uniones intercelulares y tener un efecto inhibidor de la adhesión leucocito/endotelio y posterior transmigración. Por otro lado, las moléculas de adhesión solubles son capaces, al unirse a sus receptores, de transducir señales y desencadenar una coestimulación efectiva de las células con las que interaccionan. Las concentraciones circulantes detectables en distintas enfermedades no reflejan necesariamente la cantidad producida y/o la liberación de éstas. Por un lado, la regulación de su expresión está muy determinada por el microentorno. Por otro lado, al conservar su capacidad de unión al receptor, las concentraciones circulantes no valoran el tanto por ciento de moléculas solubles unidas a sus receptores celulares[42]. Se han detectado concentraciones de moléculas de adhesión solubles elevadas (selectina E, ICAM-1 o VCAM-1) en diversas enfermedades infecciosas (p. ej., sida o shock séptico)[42], inflamatorias (LES, AR o vasculitis)[46-49] y en neoplasias. También se han descrito incrementos en compartimientos específicos. Así, en el líquido sinovial de la AR se han observado concentraciones elevadas de CD44 soluble, molécula implicada en los mecanismos de migración transendotelial[50]. En cambio, las concentraciones de selectina L que se desprende de la membrana leucocitaria cuando ocurre la adhesión firme son similares a las encontradas en sangre periférica[50].

En general, en las enfermedades inflamatorias crónicas como la granulomatosis de Wegener, la enfermedad de Kawasaki, la poliarteritis nudosa y la AR, sus concentraciones se correlacionan, aunque no invariablemente, con la actividad de la enfermedad. Sin embargo, en el síndrome de distrés respiratorio del adulto las concentraciones de selectina L se correlacionan inversamente con la gravedad de la afección[51]. En esta situación se ha postulado que las concentraciones bajas de selectina L representarían un secuestro de la selectina L soluble por el endotelio activado, particularmente en el lecho vascular pulmonar. Resulta interesante que las concentraciones de ICAM-1 soluble se incrementan en el rechazo del trasplante renal y no en la nefropatía por ciclosporina, de manera que su detección podría ser de utilidad clínica para distinguir entre estas situaciones en el paciente trasplantado[21].

Estas observaciones ponen de manifiesto la relevancia funcional de las moléculas de adhesión en circunstancias patológicas in vivo. Sin embargo, la utilidad clínica de la detección de moléculas de adhesión solubles como determinantes pronósticos o como marcadores de actividad en el seguimiento evolutivo, queda aún por determinar.

Enfermedades infecciosas

Moléculas de adhesión y receptores virales. Las moléculas de adhesión involucradas en las relaciones entre leucocitos, endotelio y matriz extracelular son utilizadas como receptores por algunos virus. Un ejemplo ya clásico es CD4. Esta molécula, que interacciona con los antígenos de clase II del sistema mayor de histocompatibilidad durante el reconocimiento antigénico, es también el receptor por excelencia para el virus de la inmunodeficiencia humana (VIH). Se han identificado otras moléculas de adhesión como receptores virales. Así, ICAM-1 es receptor para el rinovirus A a través de un lugar de unión distinto al que interacciona con LFA-1. Algunos virus como el virus de la glosopeda, el adenovirus 2 y el ecovirus 1 contienen secuencias RGD, lo que sugiere que interaccionan con integrinas expresadas en la superficie de las células diana. Se ha demostrado que la integrina $\beta 1\alpha 2$ es, efectivamente, un receptor para el ecovirus 1. La proteína tat del VIH posee una secuencia RGD[52] y es capaz de interaccionar con las integrinas celulares $\beta 1\alpha 5$ y $\beta 3\alpha V$[53]. A través de estas y otras interacciones con receptores celulares esta proteína es importante en la patogenia de algunas de las manifestaciones de la infección por VIH: tat estimula la proliferación de las células del sarcoma de Kaposi[54] y es neurotóxica[55].

Otros agentes infecciosos. La integrina plaquetaria $\alpha IIb\beta 3$ se une a *Borrelia burgdorferi*, el agente causal de la enfermedad de Lyme y podría, de este modo, contribuir a la diseminación del germen por el organismo[53]. Así mismo, *Bordetella pertussis* interacciona con la integrina leucocitaria Mac-1 (CD11b-CD18)[56]. Algunas especies de *Candida* pue-

den unirse a ICAM-1[57]. ICAM-1, selectina E y VCAM-1 interaccionan con los hematíes parasitados por *Plasmodium falciparum*[57,58]. Tal interacción contribuye, probablemente, al secuestro vascular, sustrato de algunas de las complicaciones más graves de la enfermedad como la malaria cerebral.

Neoplasias

El proceso de invasión y metástasis, principal determinante del pronóstico de los tumores malignos, presenta una gran similitud con el desarrollo de lesiones inflamatorias. Con un estrecho paralelismo con los patrones de circulación linfocitaria, las células neoplásicas deben abandonar el tumor primario, degradar la membrana basal, invadir la estroma circundante, penetrar en los vasos linfáticos y sanguíneos y abandonarlos de nuevo para colonizar los ganglios linfáticos y órganos a distancia. Durante este proceso, las células tumorales utilizan unas vías de interacción con las células endoteliales y la matriz extracelular muy similares a las utilizadas por los leucocitos: interacciones hidratos de carbono/selectina, integrina/inmunoglobulina, inmunoglobulina/inmunoglobulina, integrina/matriz extracelular, junto a la participación de otras moléculas[19,59,60].

Interacciones hidratos de carbono/selectina. Este mecanismo parece intervenir en la diseminación del carcinoma de colon. Las células del cáncer de colon poseen actividad fucosiltransferasa y sintetizan oligosacáridos tipo sialil-Lewis^x y Lewis^a que son capaces de interaccionar con la selectina E de las células endoteliales y facilitar su penetración en los tejidos[19,60].

Interacciones integrina/inmunoglobulina. La interacción LFA-1/ICAM-1 únicamente es utilizada por las neoplasias hematológicas, particularmente linfomas, debido a la expresión exclusiva de las integrinas β2 en leucocitos[61,62]. Las interacciones VLA-4/VCAM-1 tienen importancia en la diseminación del melanoma maligno y de los osteosarcomas[59]. De hecho, VCAM-1 fue descrita originariamente como una molécula que mediaba la unión de células de melanoma a células endoteliales[63].
Los ligandos endoteliales (selectina E o VCAM-1) para las moléculas de adhesión tumorales serían inducidos en el microentorno por citocinas producidas por el propio tumor o por las células inflamatorias que constituyen la respuesta del huésped frente a la neoplasia. De hecho, en modelos experimentales, la inyección de IL-1 o FNT favorece la diseminación tumoral[60,64].

Interacciones integrina/matriz extracelular. La expresión de integrinas por líneas celulares transformadas y por tumores humanos es muy variable. De la experiencia acumulada parede deducirse que, dado que el proceso de invasión y metástasis requiere fenómenos de adhesión y liberación coordinada, la sobreexpresión o reducción de integrinas favorecería o dificultaría etapas concretas del proceso completo. En determinadas fases, como el abandono del tumor primario por las células metastatizantes y su paso al torrente circulatorio las células neoplásicas deben pasar de un estado tisular a un estado circulante y la disminución de integrinas favorece probablemente esta transición. Así, el receptor de fibronectina (β1α5) se pierde en fibroblastos transformados y otras integrinas, receptores de laminina y colágeno (β1α2, β1α3 y β1α6), se pierden en tumores epiteliales invasivos[59]. Sin embargo, la transfección de α2 confiere mayor poder metastatizante a rabdomiosarcomas y la expresión de β3αV (receptor de vitronectina) se encuentra aumentada en el melanoma maligno metastatizante[60]. Del mismo modo, la inyección del péptido RGD junto a células de melanoma inhibe el desarrollo de metástasis en modelos experimentales[59,60,65]. Estas observaciones sugieren que las integrinas participan activamente en la colonización metastásica de los tejidos.

Interacciones inmunoglobulina/inmunoglobulina. Entre los miembros de esta familia que participan en interacciones homofílicas se conoce fundamentalmente NCAM como implicada en los mecanismos de progresión tumoral. La NCAM se expresa en condiciones normales en las células de estirpe neural. Se expresa también en tumores de origen neuroendocrino. Señales transducidas a través de NCAM parecen regular el crecimiento tumoral. Su falta de expresión se asocia, en líneas celulares, a una pérdida de la inhibición por contacto[59]. La expresión de NCAM por algunos linfomas T parece determinar una elevada incidencia de infiltración del sistema nervioso central[66].

Otras moléculas

1. CD44. La CD44 es una glucoproteína de membrana que participa en el *homing* de los linfocitos a los ganglios linfáticos. Existen múltiples variantes de esta molécula generadas mediante procesamiento alternativo de su ARN. La forma de 85 kD se expresa en una gran variedad de estirpes celulares. Señales vehiculizadas por esta proteína, tras interaccionar con uno de sus ligandos, el ácido hialurónico, facilitan el crecimiento tumoral. Las variantes que contienen la secuencia codificada por el exón 6 confieren un elevado potencial metastatizante[67]. La expresión de estas isoformas de CD44 es necesaria para la colonización tumoral de los ganglios linfáticos, probablemente a través de mecanismos similares a los del *homing* linfocitario. El microentorno ganglionar sería favorable al crecimiento tumoral y facilitaría su diseminación posterior[67].
2. Cadherinas. Las cadherinas son moléculas dependientes de calcio que se unen unas a otras de manera homofílica y participan en interacciones intercelulares homotípicas, es decir, entre células de la misma estirpe. Se hallan involucradas en el mantenimiento de las conexiones intercelulares que generan la arquitectura tisular. Se agrupan por familias estructurales y la lista de los miembros de cada grupo se halla en expansión. Las más estudiadas son las cadherinas E y P que se expresan en epitelios y las cadherinas N que se expresan en el tejido neural y muscular[67].
Un gran número de líneas celulares neoplásicas expresa cadherinas E y P. El nivel de expresión es, sin embargo, muy variable. La participación de las cadherinas en la biología tumoral es muy interesante. En general, las células con baja expresión tienen mayor potencial invasivo in vitro. In vivo, la expresión de cadherinas se relaciona inversamente con el grado de diferenciación de los tumores. Neoplasias con baja expresión de cadherinas presentan una capacidad invasiva superior, aunque no una mayor capacidad metastatizante. El potencial metastatizante parece relacionarse más con la existencia de una expresión heterogénea de cadherinas en el tumor primario. Esta observación, junto a estudios funcionales, sugiere que la expresión irregular y la actividad aberrante de cadherinas conduce a una asociación intercelular inestable y, por consiguiente, facilita fenómenos secuenciales de adhesión/dispersión que favorecen la diseminación tumoral[68].

Expresión endotelial de contrarreceptores. La expresión selectiva por parte de las células endoteliales de algunos tejidos para receptores tumorales todavía no bien identificados constituye una hipótesis atractiva para explicar la preferencia de determinados tumores para metastatizar en órganos

concretos. Así, se ha identificado una molécula que se expresa en el endotelio de los vasos pulmonares a la que se adhieren las células de melanoma maligno[69].

Aplicaciones terapéuticas potenciales

La disfunción orgánica en la mayor parte de enfermedades autoinmunes se halla determinada por la infiltración leucocitaria de los tejidos, los productos liberados por los leucocitos y, en ocasiones, las consecuencias reparativas de estos procesos. El conocimiento de los mecanismos moleculares implicados en las interacciones leucocito/endotelio/matriz extracelular que conducen al desarrollo de infiltrados inflamatorios hace vislumbrar la posibilidad de nuevas vías de actuación terapéutica basadas en el bloqueo de estas interacciones.

Experiencias en modelos animales

Inhibición del desarrollo de lesiones mediante AcMo bloqueadores. La inhibición de las interacciones leucocito/endotelio mediante la administración de AcMo que bloquean epítopos funcionales de las moléculas de adhesión ha evitado el desarrollo de lesiones inflamatorias en una gran variedad de modelos animales. La administración de AcMo bloqueadores no sólo ha demostrado su potencial eficacia terapéutica sino que ha proporcionado importante información sobre la relevancia relativa de estas interacciones en distintos procesos patológicos[2,3,21] (tabla 1).

1. Inhibición de interacciones mediadas por selectinas. El bloqueo de la selectina L evita significativamente el desarrollo de infiltrados inflamatorios en la mayor parte de modelos de lesión aguda como el daño vascular y tisular mediado por complejos inmunes y las lesiones consecutivas a reperfusión postisquemia[70]. La inhibición de las interacciones mediadas por la selectina E ha sido eficaz en mejorar la obstrucción de las vías aéreas en un modelo de asma en primates[71]. El bloqueo de la selectina P ha sido efectivo fundamentalmente en modelos de daño secundario a reperfusión postisquemia[3,70]. En cambio, el bloqueo de las selectinas E o P no es tan efectivo en otras situaciones.

2. Bloqueo de las interacciones entre integrinas y miembros de la superfamilia de las inmunoglobulinas. Para inhibir estas interacciones se ha investigado preferentemente el bloqueo de CD18, la cadena común a las integrinas β2 (LFA-1, Mac-1 y gp150,95), y el bloqueo de ICAM-1. El bloqueo de CD18 ha sido el más eficaz en todos los modelos testados[3,21]. Los AcMo anti-CD18 inhiben el influjo de neutrófilos en prácticamente todos los órganos estudiados: piel, peritoneo, sinovial o pulmón. Anticuerpos anti-CD18 han evitado el rechazo de trasplantes, el fallo multiorgánico secundario al shock hemorrágico o endotóxico[72], reacciones de hipersensibilidad y el daño tisular secundario a reperfusión postisquemia[2,3,73]. El bloqueo de ICAM-1 también ha sido útil en modelos de asma, hipersensibilidad retardada, reperfusión postisquemia, daño tisular por complejos inmunes y rechazo de trasplantes[2,3,21,25]. El bloqueo de ICAM-1 se ha testado también en modelos de artritis por adyuvante o artritis inducida por colágeno[74,75], malaria cerebral[76] y encefalomielitis alérgica experimental[77]. En esta última situación el bloqueo de ICAM-1 ha sido más eficaz en el modelo obtenido por inmunización activa que en el modelo obtenido por transferencia pasiva de linfocitos sensibilizados. Este hecho sugiere que la unión LFA-1/ICAM-1 tiene una función más relevante como interacción coestimuladora en la generación de la respuesta inmune que en los fenómenos de adhesión endotelial y transmigración leucocitaria, donde probablemente sean tanto o más cruciales las interacciones VLA-4/VCAM-1. Se ha demostrado, en efecto, que las interacciones mediadas por VLA-4 son fundamentales para la entrada de los linfocitos en el sistema nervioso central[78]. Para la total inhibición de las reacciones de hipersensibilidad, se requiere el bloqueo simultáneo de LFA-1/ICAM-1 y de VLA-4/VCAM-1[79]. El bloqueo de VLA-4 ha sido altamente eficaz en prevenir el desarrollo de infiltrados y alteraciones neurológicas en la encefalomielitis experimental[80] y en reducir la obstrucción de vías aéreas en un modelo de asma bronquial[81].

TABLA 1

Eficacia relativa del bloqueo de distintas moléculas de adhesión en diversos modelos animales de enfermedades humanas

Modelo animal	Sel P	Sel E	CD18	ICAM-1	VLA-4
Lesión pulmonar por IC (IgG)	0	+++	++	++	+
Lesión pulmonar por C	+	0	++	++	0
Glomerulonefritis por IC	0	0	++	++	+
Isquemia/reperfusión	++		+++		
Asma		++		++	+++
Encefalitis experimental				++	+++
Rechazo trasplante			+++	+++	
Artritis por adyuvante			++	++	

IC: complejos inmunes; C. activación sistémica del complemento.

Inhibición mediante moleculas solubles. El bloqueo por competición mediante moléculas solubles recombinantes puede ser una alternativa al empleo de AcMo que conllevan el riesgo de sensibilización frente a proteínas heterólogas. Esta aproximación se ha utilizado en modelos animales para inhibir interacciones mediadas por selectinas. Para la selectina L y la selectina P se han obtenido resultados similares a los obtenidos con AcMo[70].

Péptidos sintéticos. La utilización de pequeños péptidos sintéticos no inmunogénicos para bloquear competitivamente las interacciones mediadas por moléculas de adhesión constituye otra posibilidad interesante. Esta aproximación requiere un conocimiento preciso de las secuencias específicas que participan en la unión a los correspondientes contrarreceptores y de las regiones implicadas en las distintas acciones biológicas en moléculas capaces de unirse a más de un ligando (p. ej., ICAM-1 como contrarreceptor de LFA-1 o como receptor de rinovirus)[82]. Se han diseñado péptidos basados en secuencias de ICAM-1[83], selectina P y CD31[3], capaces de bloquear fenómenos adhesivos in vitro.

Oligosacaridos. La identificación de los ligandos de las selectinas como oligosacáridos relativamente simples ha motivado una intensa investigación de cara a obtener análogos activos y estables de sialil-Lewis[x] y sialil-Lewis[a]. La administración de sialil-Lewis[x] ha evitado el daño pulmonar mediado por neutrófilos consecutivo a la activación sistémica del complemento o al depósito de complejos inmunes en distintos modelos animales[84-86]. Dado que las interacciones mediadas por selectinas intervienen en la agregación plaquetaria y en las interacciones entre células tumorales y endotelio, tales oligosacáridos tendrían asimismo un uso potencial no sólo como antiinflamatorios sino como antiagregantes y como inhibidores del desarrollo de metástasis[87].

Oligonucleótidos antisentido. El diseño de oligonucleótidos *antisense*, capaces de unirse al ARN mensajero de las moléculas de adhesión e inhibir su traducción a proteína y, por tanto, evitar su expresión, constituye otra elegante aproximación. Se han utilizado oligonucleotidos *antisense* con exi-

to para inhibir in vitro la expresión de moléculas de adhesión endoteliales (selectina E, ICAM-1 y VCAM-1). Estudios preliminares han demostrado su capacidad para inhibir el desarrollo de inflamación en distintos modelos murinos[3].

Fármacos inhibidores. El diseño de nuevos fármacos capaces de inhibir la expresión o bloquear funcionalmente las moléculas de adhesión de manera selectiva y controlada constituye actualmente un objetivo importante dentro de la investigación farmacológica. Algunos de los agentes terapéuticos clásicamente utilizados para el tratamiento de las enfermedades inflamatorias crónicas incluyen dentro de sus acciones farmacológicas algún efecto sobre la expresión y/o función de las moléculas de adhesión ya sea directamente o indirectamente a través de la inhibición de alguno de los estímulos inductores. Así, la dexametasona inhibe la expresión de selectina E e ICAM-1 inducidas por IL-1 o LPS[88]. La dapsona[89] y la pentoxifilina[90] inhiben la adhesividad de las integrinas leucocitarias a través de mecanismos desconocidos. La 3'deazaadenosina inhibe la expresión de ICAM-1 y la adhesión a leucocitos en las células endoteliales estimuladas con FNTα[91]. Las leumedinas, utilizadas con éxito en algunos modelos animales, constituyen una nueva familia de fármacos antiinflamatorios potenciales cuyo único mecanismo de acción conocido hasta el momento es el bloqueo funcional de las integrinas $\beta 2$[92,93].

Primeras experiencias en humanos

El uso terapéutico de AcMo se enfrenta con las limitaciones derivadas del desarrollo de anticuerpos por parte del receptor frente a inmunoglobulinas de ratón. Este problema puede solventarse parcialmente con la generación de AcMo humanizados que consisten en inmunoglobulinas humanas quiméricas donde únicamente la región hipervariable es murina. La aplicación de AcMo parece especialmente prometedora en procesos agudos como la prevención del daño por reperfusión postisquemia, del fracaso multiorgánico consecutivo al shock o del rechazo agudo de injertos. En estas situaciones, que no requieren una administración reiterada, el desarrollo de anticuerpos por parte del receptor no sería tan problemático. Resulta tranquilizador el hecho de que, en modelos animales similares, no se ha observado un incremento en la incidencia de complicaciones infecciosas durante el tratamiento[3].

En la actualidad se han realizado ya algunos ensayos en fase I-II con AcMo antimoléculas de adhesión en humanos. Resultados preliminares sugieren que AcMo anti-ICAM-1 pueden evitar el rechazo en trasplantes renales de alto riesgo[94]. En un estudio realizado en 32 pacientes con artritis reumatoide refractaria a otros tratamientos a los que se aplicó AcMo anti-ICAM-1 se obtuvo mejoría objetiva y subjetiva en el 60% de pacientes[95]. Esta mejoría se mantuvo durante 2 meses en el 40% de los mismos[96]. Estudios preliminares sugieren que en pacientes con enfermedad de inicio reciente los resultados podrían ser mucho mejores[96].

Conclusiones

El conocimiento de los mecanismos moleculares que median las interacciones intercelulares y las interacciones entre las células y la matriz extracelular ha supuesto un avance considerable en múltiples campos de la biología y la medicina. La posibilidad de manipulación farmacológica y/o biológica de las interacciones involucradas en estos procesos hace prever un considerable avance en el tratamiento de algunas enfermedades infecciosas, de las enfermedades inflamatorias crónicas, de los procesos trombóticos y de la diseminación neoplásica en los próximos años. Sin embargo, el bloqueo terapéutico de las moléculas de adhesión se enfrenta con la extraordinaria complejidad y redundancia de los fenómenos biológicos. La mayor parte de las interacciones involucradas en procesos patológicos reconocidas hasta el momento tienen una importante función fisiológica. La identificación de moléculas de adhesión específicas de tejido o específicas de enfermedad y el desarrollo de métodos de aplicación selectiva (directa o dirigida) de los agentes bloqueadores a los órganos afectados constituyen algunos hitos importantes a alcanzar en el progreso de la terapia antiadhesiva.

Agradecimiento

Financiado por FIS 95/0860.
B. Coll-Vinent recibe financiación de una beca del Hospital Clínic i Provincial de Barcelona.
Las técnicas inmunohistoquímicas de las figuras han sido realizadas por Esther Tobías.

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AIDS PATIENT CARE and STDs
Volume 11, Number 4, 1997
Mary Ann Liebert, Inc.

HIV-Related Vasculitis

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INTRODUCTION

SINCE THE BEGINNING of the HIV-pandemic, an increasing number of case reports of vasculitis and HIV infection have been published. Frequently, the vasculitis features have been assessed in terms of resemblance to those of one of the major vasculitides that may occur in HIV-negative individuals (Table 1). Six years ago, L.H. Calabrese[1] reviewed all vasculitis cases reported in HIV-infected patients. As Calabrese stated, diagnosis of vasculitis may not be easy, specially for the nonhypersensitivity vasculitic group, which is often not associated with dermatologic signs of vasculitis. In this setting, clinicians should suspect systemic vasculitis under the following clinical situations: (1) fever of unknown origin; (2) unexplained multisystem disease; (3) unexplained arthritis or myositis; (4) glomerulonephritis; (5) peripheral neuropathies; or (6) unexplained gastrointestinal, cardiac or central nervous system (CNS) involvement.

However, the lack of a large series does not allow the clinical picture and evolution of the vasculitis to be known, making it difficult to establish an accurate prognosis or to make a treatment decision. Therefore, data coming from vasculitis seen in non-HIV-infected patients must sometimes be extrapolated to HIV patients. Nevertheless, the clinical picture and the evolution are not always the same in both settings. In this sense, we recently published our experience in HIV-related polyarteritis nodosa (PAN).[2] From our cases and those reported in the previous literature, we demonstrated that PAN affecting HIV-infected individuals has a less aggressive clinical course, with more limitted organ dysfunction and better prognosis than classical PAN.

On the other hand the etiopathogenic relationship between HIV infection and the development of some vasculitic syndromes has not been fully elucidated. Some reports, based on the presence of viral antigens in pathological studies, have suggested that vasculitis could be caused by HIV infection itself.[3,4] However, it has not been possible to ascertain whether the coexisting diseases are causally or merely coincidentally due to the absence of controlled epidemiologic investigations and the abovementioned scarcity of clinical reports.

In the present study, we reviewed the spectrum of vasculitis that can affect HIV patients, with particular attention to those data regarding the clinical characteristics and therapeutical possibilities. We follow the clinical classification of vasculitic syndromes seen in the general population proposed by Fauci.[5] Since, in some cases, vasculitis in HIV-infected patients can be paucisymptomatic, and then mimic other HIV-infectious complications, we will also focus on differential diagnosis.

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TABLE 1. CLINICAL CLASSIFICATION OF VASCULITIS PROPOSED BY AS FAUCI[a]

1. Systemic necrotizing vasculitis
   - Classic polyarteritis nodosa
   - Allergic angitis and granulomatosis of Churg-Strauss
   - Polyangitis overlap syndrome
2. Hypersensitivity vasculitis group
   - Exogenous stimuli (Schönlein-Henoch purpura, serum-sickness reaction, drug, infection)
   - Endogenous antigens (neoplasms, connective tissue disease, congenital deficiencies of complement)
3. Wegener's granulomatosis
4. Giant cell arteritis
   - Horton's arteritis
   - Takayasu's arteritis
5. Other vasculitic syndromes
   - Kawasaki disease
   - Behçet's syndrome
   - Buerger's disease
   - Isolated central nervous system vasculitis
   - Miscellaneous vasculitis

[a]Reference 5.

SYSTEMIC NECROTIZING VASCULITIS

Polyarteritis nodosa

Polyarteritis nodosa is a life-threatening multisystem disease characterized by inflammation and necrosis of small and medium-sized arteries (Figure 1) that typically involves renal and other visceral arteries, sparing pulmonary circulation.[6] At the time of this report, 32 HIV-infected individuals with well-documented PAN had been reported in the literature,[2,3,7-28] and their clinical and evolutive data are presented in Table 2.

From an analysis of these cases, three issues deserve special comment. First, PAN in HIV-infected patients is paucisymptomatic, and the patients' main complaints are constitutional symptoms and those involving the neuromuscular system. Systemic disease involving kidney, skin, heart, central nervous system, or gut are infrequent, and account for less than 25% of the cases. This percentage is clearly in opposition to that obtained from other series of classical PAN,[6,29] in which more than 75% of the patients develop some systemic involvement (Table 3). Given both the high prevalence of constitutional symptoms in HIV-positive patients and the scarcity of typical symptoms of PAN, a high degree of suspicion of vasculitis is warranted. In this setting, muscle biopsy may represent a very useful tool in distinguishing both situations. Indeed, we have observed that nearly 20% of HIV patients fulfilling the wasting syndrome CDC criteria[30] are actually affected by a treatable inflammatory condition (either myositis or vasculitis) that can be diagnosed only if muscle biopsy is performed.[31] Second, despite the high prevalence of chronic hepatitis B virus (HBV) infection among HIV-infected patients, this infection only accounts for 4% of the HIV patients developing PAN, a figure lower than that observed in classical PAN, where the prevalence of HBV infection reaches up to 30%.[6] Similarly, hepatitis C virus (HCV) antibodies are present in 35% of patients with HIV infection suffering from PAN, a figure similar to that usually observed in all the HIV-infected population.[32] This fact makes the relationship of HBV or HCV with PAN in HIV-infected patients unlikely. And third, it is possible that this paucisymptomatic form of PAN could be satisfactorily treated with lower doses of corticosteroids than classical PAN. This is of special interest in patients in whom the use of corticosteroids may have a harmful effect.[33,34] However, the number of reported cases with a detailed treatment and outcome are so few that a comprehensive therapeutic schedule of this clinical entity has not been possible. Therefore, if there are not any systemic clinical manifestations other than neuromuscular, we recommend a starting dose of prednisone lower than that generally used in classical PAN (0.5 mg/kg

FIG. 1. Severe inflammatory infiltrate through the entire wall of a medium-sized vessel (PAN) in sural nerve biopsy from an HIV-infected patient with multineuritis.

body weight/day), and to modify subsequent doses in accordance with clinical response.

Churg-Strauss disease

Churg-Strauss disease is a systemic necrotizing vasculitis characterized by the clinicopathologic triad of asthma, tissue and blood eosinophilia, and granulomatous vasculitis of multiple organ systems, particularly the lung. Clinically, this disease is quite similar to PAN except for the high incidence of lung involvement with asthmatic crisis and pulmonary infiltrates, and less commonly, severe renal disease.[5] As far as we know, there is a only one case report of Churg-Strauss disease affecting an HIV-infected patient,[35] and therefore, the relationship between both processes must be considered anecdotal. The patient was a 24-year-old man with asymptomatic infection by HIV and a CD4 lymphocyte count greater than 1500/μl. His clinical picture did not differ from that described in the general population. He was treated with prednisone, 60 mg/6 h for 2 days, followed by 60 mg/day with improvement, and remained disease free 4 months after discharge from the hospital without infectious or neoplastic complications.

HYPERSENSITIVITY VASCULITIS GROUP

Hypersensitivity vasculitis delineates a heterogeneous group of disorders characterized by a vasculitic syndrome presumably associated with a hypersensitivity reaction following exposure to an exogenous (infectious agent, drug, foreign substance) or endogenous (mainly neoplastic or connective-tissue diseases) antigen. The common denominator of the hypersensitivity vasculitis group is the involvement of small vessels and the presence of leukocytoclastia (Figure 2). It is more frequently seen in clinical practice than systemic vasculitis. Clinically, skin involvement usually dominates the disease, and extracutaneous involvement, although possible, is generally less severe than that of the other vasculitis disorders.[5,36–38] Further discussion of those cases reported in HIV-infected patients will include separate consideration of Schönlein-Henoch purpura and cryoglobulinemia. More than 30 cases have been reported in the literature, with an extensive clinical and evolutive description of those published being summarized in Table 4.

Hypersensitivity vasculitis

Hypersensitivity vasculitis has been reported in several cases of HIV-infected patients.[14,39–49] Since, in most of such cases, an etiological agent (drug or infection) could not be identified,[39,42,46] some authors have hypothesized about the certitude of a direct pathogenic role of HIV in the development of vasculitis.[42] In fact, in some cases of other systemic vasculitis in HIV-infected individuals, HIV has been cultured from the affected tissues, such as peripheral nerves[50] or brain. Nevertheless, the attempts to demonstrate the presence of HIV in leukocytoclastic vasculitic lesions have failed. Interestingly, in some of these cases, infectious agents potentially causing leukocytoclastic vasculitis other than HIV were isolated,[43,44] minimizing the role of HIV. Indeed, it is generally accepted that other infectious agents such as cytomegalovirus, Epstein-Barr virus, or HBV and HCV are involved in the generation of immunocomplexes and the development of hypersensitivity vasculitis in the general population.[5]

The clinical picture of hypersensitivity vasculitis in HIV patients does not essentially differ from that seen in non-HIV-infected individuals (Table 5). It is characterized by cutaneous vasculitic purpura, mainly affecting the legs (Figure 3) and only exceptionally involving other systems. Similar to classic hypersensitivity vasculitis, vasculitis seen in some HIV-infected patients could be related to a drug. In fact, Coopman et al.[51] demonstrate in an extensive review that hypersensitivity reactions to drugs may account for nearly 20% of HIV-infected patients, being the most common morbilliform eruption, frequently associated with sulfonamides and/or trimethoprim use. However, few cases of drug-related hypersensitivity vasculitis have been reported in this population, and thus in the aforementioned report of Coopman et al., cutaneous vasculitis was only found in one out of 125 patients with cutaneous drug reactions. On the other hand,

TABLE 2. CLINICAL AND LABORATORY DATA FROM REPORTED PATIENTS WITH PAN AND HIV INFECTION[a]

Case	*Ref.*	*Age*	*Sex*	*CEC class*[b]	*CD4*[c]	*Clinical manifestations*	*Pathologic study*	*HBsAg/ HBcAb*	*Treatment/ Outcome*
1	3	74	M	?	?	Polyneuropathy	n	?/?	?/?
2	3	63	M	?	?	Polyneuropathy	n	?/?	?/?
3	7	33	M	II	480	Polyneuropathy + weight loss	n, m	−/+	Prednisone 1 mg/kg/day for 7 months/ Asymptomatic for 10 months
4	8	34	M	III	?	Spontaneous perineal hematoma + fever + weight loss + arterial hypertension	ag	−/+	Prednisone 60 mg/day/Favorable
5	9	36	M	III	?	Sensitive polyneuropathy + motor mononeuritis + weight loss + fever + arterial hypertension + skin lesions + gut involvement	r	−/−	Prednisone 60/mg/Asymptomatic after 9 months of follow-up
6	10	72	M	II	?	Sensorimotor polyneuropathy	m	−/−	?/?
7	10	75	F	II	?	Sensorimotor polyneuropathy	n, m	−/−	?/?
8	11	37	M	III	?	Sensitive polyneuopathy + weight loss + fever + bilateral hip synovitis	n	−/+	?/?
9	12	29	M	II	540	Skin lesions + fever + polyarthralgia	s	−/−	Methylprednisolone 1 mg/kg/day/ Marked improvement in few days. 12 months of follow-up
10	13	42	M	?	?	Sensorimotor polyneuropathy + renal insufficiency + congestive heart failure	n, m	?/?	?/?
11	14	33	M	III	?	Polineuropathy + weight loss	m	−/?	?/?
12	14	55	?	IVC	30	Monoarthritis + sicca syndrome	n, m	−/?	?/?
13	15	?	M	?	?	Sensitive polyneuropathy + mononeuritis	n, m	−/−	Corticosteroids/Favorable
14	15	?	?	?	?	Sensorimotor polyneuropathy	n, m	−/−	Corticosteroids/Favorable
15	16	?	?	?	?	?	n	?/?	Corticosteroids/Favorable
16	17	62	F	?	?	Polineuropathy + myalgia +skin lesions + sicca syndrome	n, m, s	−/−	Corticosteroids and plasmapheresis/?
17	18	27	M	IV	?	Sensorimotor polyneuropathy + myalgia + weight loss + fever + exanthema	n, m, s	−/+	?/?
18	19	32	F	IV	?	Polyneuropathy + skin lesions + arthritis + arterial hypertension	n, m, s	−/−	?/?
19	20	29	M	IV	?	Sensitive polyneuropathy + fever + weight loss + feet ischemic gangrene	m, s	−/+	?/Transverse myelitis and herpes zoster
20	21	?	?	?	?	Sensorimotor polyneuropathy + multiple mononeuritis + weight loss + fever	?	?/?	Prednisone and cyclophosphamide/ Dead 4 months later because of opportunistic infection.
21	22	65	F	?	?	Polyneuropathy + central nervous system	n, m	+/?	Methylprednisolone 12 mg/day/?
22	23	32	M	IV	273	Fever + weight loss + myalgia + arthralgia + spleen infarcts + ischemia in the lower limbs.	spleen, ag	−/−	?/?
23	24	72	?	?	?	Polyneuropathy + radiculopathy + myalgia	n, m	−/?	Prednisone 1 mg/kd/day and cyclophosphamide 100 mg/da/Favorable
24	25	?	?	III	?	Central nervous system	Autopsy	?/?	?/Dead
25	26	28	M	I	?	Ulcer on the posterior soft palate	Ulcer biopsy	?/?	High-dose prednisone/Favorable

26	27	?	M	I	?	Peripheral neuropathy	n, m	−/?	Prednisone and cyclophosphamide/ Little discernible effect
27	2	38	F	IVC	220	Fever + weight loss + arthromyalgias + weakness + muscle atrophy	m	−/−	Prednisone 0.5 mg/kg/day during 5 months/Asymptomatic 12 months of follow-up
28	2	37	M	IVC	50	Fever + weight loss + myalgia + peripheral neuropathy	m	−/+	Prednisone 0.5 mg/kg/day for 3 months, 0.2 mg/kg/day for 3 months/Asymptomatic 12 months of follow-up
29	2	27	M	IVC	310	Fever + weakness + muscle atrophy	m	−/−	Prednisone 0.5 mg/day for 4 months/ Dead 4 months later
30	2	27	F	IVC	14	Weakness + muscle atrophy	m	−/+	None/No signs of systemic disease 9 months of follow-up
31	28	32	M	IVD	191	Fever + Raynaud's phenomenon + skin gangrene	ag	−/+	Prednisone 60 mg/day/Favorable after 1 month of follow-up
32	28	31	F	II	2357	Fever + Weight loss + vasculitic skin lesions + polyneuropathy	ag	−/+	Methyl prednisolone pulses (1 g/day/ during 3 days) followed by 48 mg/ day + intravenous gammaglobulin (0.4 g/kg/d during 5 days)/Evolutive well-delimited digital gangrene

[a]Ref.: reference. M: male. F: female. HBsAg: hepatitis B surface antigen. HBcAb: hepatitis B core antibody. n: nerve. m: muscle. s: skin. r: rectal. ag: angiography. ?: data not available.

[b] Staging of HIV infection according to the 1987 Criteria of the Centers for Diseases Control and Prevention, Reference 101.

[c] CD4 lymphocyte count in cells/μl.

TABLE 3. COMPARATIVE CLINICAL PARAMETERS IN PATIENTS WITH CLASSIC AND HIV-RELATED PAN

Clinical involvement	*HIV-related PAN incidence (%) (n = 32)*	*Classic PAN incidence (%) (n = 53)*[a]	*Classic PAN incidence (%) (n = 507)*[b]
Peripheral neuropathy	59	60	51
Musculoskeletal system	44	55	64
Fever	41	58	71
Weight loss	38	30	54
Skin	22	58	43
Hypertension	9	26	54
Kidney	6	66	70
Central nervous system	6	NS[c]	23
Gastrointestinal tract	3	25	44
Cardiac	3	4	36
Hepatitis B surface antigenemia	4	11	30

[a]From Cohen et al.[29]
[b]From Cupps and Fauci.[6]
[c]NS: not specified.

Gherardi and co-workers[14] classified skin, muscle, and nerve biopsies of 148 symptomatic HIV-infected patients according to the American College of Rheumatology (ACR) criteria[52] for vasculitis. Six of the 34 patients with inflammatory vascular disease could be diagnosed with hypersensitivity vasculitis, and four of these patients had been treated with penicillin, cotrimoxazole, amytriptiline, and griseofulvin, respectively, within 15 days before biopsy. Additionally, Torres et al.[40] reported two cases of zidovudine (AZT)-induced leukocytoclastic vasculitis. Both cases presented with fever and pruriginous rash that appeared within 1 month after the beginning of AZT therapy. In one of these cases, the etiologic role of the drug in the induction of vasculitis was strengthened by the reappearance of the clinical picture after AZT rechallenge. Finally, Herranz et al.[41] reported a patient with cutaneous leukocytoclastic vasculitis 4 days after the beginning of didanosine (ddI) therapy, and they also assessed the recurrence of purpura when ddI was reintroduced.

In those cases without other concurrent life-threatening conditions, drug-related hypersensitivity vasculitis had an excellent outcome, sometimes spontaneously,[41] sometimes after the administration of antihistamines with or without prednisone.[40,42,49] Obviously, the discontinuation of the suspected drug causing vasculitis is mandatory.

Schönlein-Henoch purpura

Schönlein-Henoch purpura constitutes the most distinctive subgroup of hypersensitivity vasculitides, usually seen in children, that typically affects the skin, gut, and kidney. At least six cases have been reported in which this vasculitis developed during the course of HIV infection.[14,53–57] In two additional cases, it occurred in the seroconversion phase.[58,59] From the analysis of these cases, one can see that Schönlein-Henoch syndrome affects individuals older than the classical ones (range 20–61 years). However, the clinical picture remained unchanged. Usually, the treatment included prednisone with good response in almost all cases. Only one patient died due to progression

FIG. 2. Polymorphonuclear infiltrate with nuclear debris (leukocytoclasis) in a postcapillary venule from skin biopsy in an HIV-infected patient with hypersensitivity vasculitis.

TABLE 4. CLINICAL CHARACTERISTICS OF PATIENTS AFFECTED BY HYPERSENSITIVITY VASCULITIS AND HIV INFECTION[a]

Case	*Age*	*Sex*	*Ref.*	*CDC class*[b]	*CD4*[c]	*Etiology*[d]	*Clinical manifestations*	*Treatment/Outcome*
1	9	F	42	II	257	Idiopathic	Purpura (legs, trunk, neck)	Topical steroids + systemic antihistaminics/Resolved in 6 months
2	43	M	39	IVC	?	Idiopathic	Widespread purpura	?/Dead due to *P. carinii* and CMV pulmonary infection
3	?	M	39	IVC	?	Secondary to HIV	Purpura (feet)	?/Dead due to *P. carinii* and CMV pulmonary infection
4	20	M	46	?	?	Idiopathic	Purpura (legs, arms, back)	?/?
5	27	M	44	?	?	*N. gonorrhoeae*	Purpura (legs) + arthritis + gastrointestinal involvement	Ampicillin + probenecid + oxytetracycline/Resolved after 6 weeks
6	26	M	45	II	449	Venereal infection	Purpura (legs, scrotum)	Doxycycoline/Several recurrences
7	?	?	43	?	?	Secondary to CMV	Purpura (legs)	?/?
8	26	M	41	IVC	171	Drug-related (didanosine)	Purpura (legs)	Discontinuation of didanosine/Resolution
9	34	M	40	IVC-D	?	Drug-related (zidovudine)	Fever + headache + diarrhea + purpura (hands, arms, torso)	Discontinuation of zidovudine + antihistaminics/Resolution
10	33	M	40	?	15	Drug-related (zidovudine)	Fever + headache + purpura (legs)	Discontinuation of zidovudine + prednisone + antihistaminics/Resolution
11	18	F	49	I	?	Postviral	Encephalitis + purpura (legs)	Prednisone 1 mg/kg/d/Resolution
12	43	M	54	II	279	Idiopathic (SHP)	Purpura (legs) + hematuria + abdominal pain + bloody diarrhea + arthralgia	High doses of prednisone/Died after 21 days due to vasculitis progression
13	28	M	58	I	228	Postviral (SHP)	Purpura (legs) + arthritis + abdominal pain	Dapsone 100 mg/d/Resolution
14	30	M	55	II	60	Postviral (SHP)	Fever + purpura (legs) + arthromyalgia + abdominal pain	Non-steroidal antiinflammatories + prednisone 30 mg/day/Resolution
15	42	M	56	?	?	Idiopathic (SHP)	Purpura (legs) + abdominal pain + acute renal failure	Prednisone 60 mg/d during 3 weeks/Recovered, alive after 2 years
16	33	M	57	?	413	Idiopathic (SHP)	Purpura (legs, trunk and buttocks) + arthralgia	Steroids during 2 months/Initially recovered, but progressing to acute renal failure
17	20	M	59	I	?	Postviral (SHP)	Purpura (legs) + abdominal pain + diarrhea + arthritis	None/Spontaneous resolution
18	56	M	14	III	?	Idiopathic (SHP)	Purpura	?/?
19	42	M	49	II	?	Idiopathic (CRYO)	Arthralgia + purpura (legs)	?/?
20	61	M	53	?	?	Idiopathic (SHP)	Fever + purpura (legs) + hematuria + epistaxis	Prednisone 60 mg/d/Relapse after prednisone withdrawal, afterwards asymptomatic during subsequent 18 months

[a]Ref.: reference. M: male. F: female. ?: data not available.
[b]Staging of HIV infection according to the 1987 Criteria of the Centers for Diseases Control and Prevention.[101]
[c]CD4 lymphocyte count in cells/μl.
[d]SHP: Schoenlein-Henoch purpura. CRYO: Cryoglobulinemia.

TABLE 5. CLINICAL PARAMETERS IN PATIENTS WITH HYPERSENSITIVITY VASCULITIS WITH AND WITHOUT HIV-INFECTION

	HIV-positive patients (%)[a] (n = 20)	*HIV-negative patients (%)[b] (n = 106)*	*HIV-negative patients (%)[c] (n = 82)*	*HIV-negative patients (%)[d] (n = 72)*
Age (years)	32 ± 13	38 ± 19	45 ± ?	6–74 (range)
Sex (male)	89	58	38	47
Suspected etiology				
Infection	40	22	10	14
Drug	15	12	10	11
Infection + drug	0	28	3	17
Connective tissue disease	0	10	21	17
Other (neoplasm, liver disease)	0	7	0	7
Idiopathic	45	21	54	35
Clinical manifestations				
Cutaneous	100	100	100	86
Legs	90	88	NS	NS
Arms	10	21	NS	NS
Trunk	20	15	NS	NS
Head and neck	5	1	NS	NS
Diffuse	5	5	NS	NS
Articular	35	59	43	44
Renal	15	44	12	33
Gastrointestinal	35	41	10	12
Other	5	7	30	NS

[a]In addition to patients compiled in Table 4, we have included six cases from Gherardi et al.[3]
[b]From San José et al.[38]
[c]From Af Ekenstam and Callen[37]. NS: not specified.
[d]From McCombs.[36]

of vasculitis.[54] Conversely, spontaneous resolution is also possible, especially in those cases occurring in the seroconversion phase.[59]

Cryoglobulinemia

Idiopathic cryoglobulinemia is a disorder in which the cardinal clinical manifestations are vasculitic purpura, arthralgia, neuralgia, and/or glomerulonephritis. It is frequently associated with infectious pathogens such as cytomegalovirus, Epstein-Barr virus, and hepatitis virus, specially HCV.[60–62] For the last years, the latter have been suspected as the leading cause of essential type II mixed cryoglobulinemia.[62] Since HIV-infected patients have a high frequency of co-infection with these viruses,[32] the pathogenic role of HIV in cryoglobulinemia remains unknown. In spite of some authors having reported the presence of cryoglobulins in HIV-infected patients, even in the absence of HCV infection,[63,64] in a recent study carried out by Matsuda et al.,[65] the investigators failed to detect the p24 antigen in any cryoglobulin. Interestingly, some HIV-infected patients, in addition to having cryoglobulins present in serum,[10,49,63–66] have developed symptomatic cryoglobulinemia,[49,63,64,66] although the vasculitic lesion was histologically demonstrated in only three cases.[14,49] In contrast to cryoglobulinemia seen in HIV-negative individuals, in whom vasculitic purpura is nearly always pre-

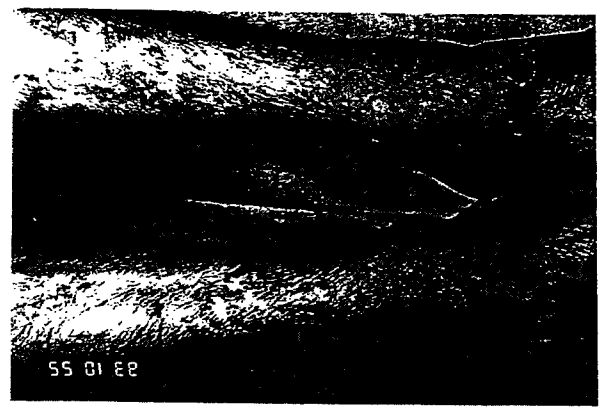

FIG. 3. Some palpable purpuric lesions on the legs of an HIV-infected patient (hypersensitivity vasculitis). Courtesy of Dr. M. Alsina (Dermatology Department, Hospital Clínic, Barcelona).

sent,[62] the clinical picture in HIV-infected patients is predominantly characterized by the development of polyneuropathy and mononeuritis multiplex.[63,64,66] Awareness of this association is important, since it constitutes a treatable cause of neuropathy, and a high degree of suspicion must be emphasized, even in the absence of cutaneous vasculitis.[64,66] In fact, when cutaneous purpura is absent, differential diagnosis with other causes of neuropathy affecting HIV-infected patients, mainly with those that are drug-related (ddI, vincristine, pentamidine), must be carefully considered.

Usual treatments for cryoglobulinemia include corticosteroids and cytotoxic agents, but these drugs are not recommended in HIV infection,[64] and then, plasma exchanges, occasionally combined with intravenous gammaglobulins, seem to be a more adequate approach in such patients.[66] Interestingly, improvement has been reported in a patient suffering from peripheral neuropathy secondary to cryoglobulinemia with the initiation of AZT therapy alone.[64]

WEGENER'S GRANULOMATOSIS

The diagnosis of the vasculitis Wegener's granulomatosis has undergone an important advance with the characterization of antineutrophil cytoplasmic autoantibodies (ANCA). By indirect immunofluorescence, two types of ANCA pattern are seen on ethanol-fixed neutrophils: the cytoplasmic and the perinuclear. The former is the most commonly found in HIV patients with active Wegener's granulomatosis. There are no reported cases of Wegener's granulomatosis affecting HIV-infected patients. However, Li et al.[67] screened 672 sera for ANCA, and found three false-positive patients having cytoplasmatic ANCA. Interestingly, one of these patients was a carrier of HIV, raising the possibility of false-positive ANCA tests and suggesting equivocal Wegener's granulomatosis diagnosis in patients infected by HIV.

GIANT CELL ARTERITIS

There are no reported cases of patients with either Horton's or Takayasu's disease during HIV infection. Only one single case of atypical temporal arteritis has been previously reported.[68] Although the clinical course showed a typical temporal arteritis clinical picture (generalized weakness, multiple episodes of amaurosis fugax, and accelerated erythrocyte sedimentation rate), histopathology of temporal artery biopsy specimen showed eosinophilic vasculitis inside of the giant cell granulomatosis classically observed in Horton's disease. The patient was treated with subcutaneous heparin and prednisone (80 mg/d) with prompt resolution of the episodes of amaurosis fugax, allowing the prednisone to be tapered without recurrence of symptomatology by 4 months of follow-up.

OTHER VASCULITIC SYNDROMES

Kawasaki Disease

Kawasaki disease, also known as mucocutaneous lymph node syndrome, is now considered an acute febrile vasculitic disease seen almost exclusively in children <5 years. It is characterized by fever for several days and a minimum of four of the following five signs: conjunctivitis, oropharyngeal changes (strawberry tongue, fissuring of the lips), extremity changes (skin desquamation, peripheral edema), rash, and cervical lymphadenopathy.[69] Although it is generally benign and self-limited, 25% of the patients develop coronary artery aneurysms, with an overall case fatality rate of 0.5–2.8%.[5] Its etiology remains unknown. Early findings of reverse transcriptase in specimens from patients with Kawasaki disease suggested that retroviruses were involved,[70] but later studies failed to confirm this hypothesis. Additionally, there are only a very few number of cases in which the disease occurred in HIV-infected patients, making the relationship with the etiopathogenesis of the disease unlikely. As far as we know, only four adults[71–74] and one child[75] infected by HIV and suffering from Kawasaki disease have been reported. The clinical picture did not differ from the classical Kawasaki disease. Treatment was based on aspirin plus intravenous gammaglobulin in all adult cases, and was always satisfactory, with no complications after a follow-

up period ranging from 3 days to 6 months. Conversely, the child presented a poor evolution with relapse (treatment was not mentioned), and he died 14 months later from systemic complications.

Behçet's Syndrome

Behçet's syndrome is an infrequent multisystem disease characterized by recurrent episodes of oral and genital ulcers, iritis, and cutaneous lesions. The underlying pathology is a leukocytoclastic venulitis, although vessels of any size and any organ can be involved.[5] Its etiopathogenesis remains unknown, and diagnosis is based on the criteria proposed by the International Study Group for Behçet's disease.[76] As far as we know, only five cases have been reported in association with HIV infection.[77–81] For this reason, it is possible that such an association was merely fortuitous. However, many of the clinical features of Behçet's disease may occur in HIV infection, such as arthritis, painful oral aphtous ulcers, recurrent genital ulceration, or folliculitis; and in this setting it is possible for a certain number of patients suffering from Behçet's disease to remain undiagnosed.[79] From the patients reported, it seems that Behçet's syndrome has a torpid evolution, with irregular response to commonly used drugs (colchicine, thalidomide, prednisone, dapsone, or methotrexate). To this point, three out of five aforementioned patients died during the course of the disease.[79–81]

Buerger's Disease

Only one HIV-positive man with a past history of Buerger's disease has been reported; however, the patient's reported current problem is a stroke related to circulating anticardiolipin antibodies.[82] In fact, the authors were doubtful about the diagnosis of Buerger's disease, and think the patient was actually affected by hypercoagulable state.

Isolated vasculitis of the Central Nervous System

Several cases of isolated vasculitis of the CNS in HIV-infected patients have been reported,[4,83–86] most of them with histological confirmation by necropsic studies.[4,83] Because some cases of vasculitis of CNS have been associated with cytomegalovirus, syphilis, varicella-zoster, piogenic bacteria, Hodgkin's disease and amphetamine abuse, HIV have been evoked as an additional cause leading to this type of vasculitis. In one case, HIV could be demonstrated in CNS, but not in any other site of the organism, suggesting that the HIV infection may have been causally related to the arteritis.[4] However, it is important to confirm the diagnosis pathologically since patients infected with HIV are at increased risk for cerebrovascular diseases,[87] not only due to cerebral vasculitis, but also to ischemic infarcts or hemorrhages related to nonbacterial thrombotic endocarditis,[88] paradoxal embolisms,[89] or circulating coagulation inhibitors.[82,90]

Miscellaneous vasculitis

Sometimes, vascular inflammatory cell infiltrates are observed in several tissues from HIV-infected patients, but they cannot be accurately defined according to the accepted histological criteria for vasculitis. In this sense, only 11 of 34 (32%) HIV patients with inflammatory vascular disease studied by Gherardi and co-workers had a distinct category of vasculitis, whereas the remaining two-thirds were classified in the group of "other vasculitis, type unspecified."[14] One of the tissues in which this situation is most frequently seen is the muscle. Indeed, it is well known that muscle can be affected by several pathological entities in HIV-infected patients, related[91–93] or not[94–98] to AZT therapy, and HIV-microvasculitis is one of the most conspicuous. Mononuclear inflammatory cell infiltrates surrounding small vessels in the endomysium and perimysium have been observed in more than 50% of muscle biopsy specimens of HIV patients with neuromuscular symptoms.[99] We have reported similar features, although our figures of frequency are less than 20% (Figure 4).[100] We agree with others[14] that microvascular inflammation may be asymptomatic in muscle, raising the question of the necessity of treatment. Our experience with such patients suggests that this inflammatory cell infiltrate must be interpreted more as an epiphenomenon related to HIV infection

FIG. 4. Perivascular mononuclear cell collection in a muscle biopsy from an HIV-infected patient with mild weakness (microvasculitis).

itself, rather than a true clinicopathologically treatable condition. In most of such cases, vasculitis does not progress despite therapeutic abstention.

ACKNOWLEDGMENTS

This work has been supported in part by a grant from FISS (Fondo de Investigación Sanitaria) (95/0554).

Mireia Cebrián received grants from the Ministerio de Educación y Ciencia (Spain). Òscar Miró, Carme Font, and Blanca Coll-Vinent received grants from Hospital Clinic i Provincial for 1996, 1997, and 1995, respectively.

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Large vessel vasculitides

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During the past few years remarkable progress has been achieved in the understanding of the pathogenic mechanisms leading to vascular inflammation and injury in giant cell (temporal) arteritis. T lymphocytes are activated by specific recognition of a putative antigen residing in the arterial wall and, subsequently, activate macrophages that undergo a functional differentiation and contribute to vessel damage through various pathways. Vascular response to inflammation amplifies the inflammatory response through neovascularization and adhesion molecule expression. We are beginning to appreciate that products released by infiltrating inflammatory cells may play an important role in vessel occlusion and resulting ischemic complications. Concomitantly, synovitis underlying polymyalgia rheumatica musculoskeletal symptoms has been immunopathologically characterized and the nature of its relationship to giant cell arteritis is discussed. Although some components of the disease are highly corticosteroid responsive, other underlying pathogenic mechanisms may remain active. Long-term outcome is heterogeneous in patients with giant cell arteritis. Much less is known about the pathogenesis of Takayasu's arteritis. Recent work supports its association with HLA class I antigens, which may differ in different geographic areas or ethnic groups. Because Takayasu's disease expression may vary in different ethnic settings, this possibility has led to the proposal of new diagnostic criteria. Finally, the role of new imaging techniques in diagnosis and assessment of disease activity is discussed.

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Current Opinion in Rheumatology 1998, 10:18–28

Abbreviations

GCA	giant cell arteritis
PMR	polymyalgia rheumatica
TA	Takayasu's arteritis
TGF	transforming growth factor

ISSN 1040-8711

Large-vessel vasculitides include two related but clearly distinct disorders: giant cell (temporal) arteritis (GCA) of the elderly and Takayasu's arteritis (TA). These entities share a number of clinical and pathologic features. In both diseases, histopathologic examination reveals lymphocyte and macrophage infiltration of large and medium-sized vessels, which frequently exhibits a granulomatous pattern with giant cell formation [1]. Systemic symptoms (fever, malaise, weight loss) are common in both disorders and, in both conditions, clinical manifestations result from different degrees of cranial, ocular, and limb ischemia.

Despite the clinical and pathologic similarities, various long-term recognized differences exist between GCA and TA. Ethnic background and age at presentation clearly differentiate these disorders: whereas TA typically occurs in young women of Southeast Asian or Hispanic origin [2], GCA is virtually restricted to elderly whites [3••]. Although necropsy studies have shown aortic involvement in most GCA cases [4,5], clinical symptoms are usually due to the inflammation of the extracranial branches of the carotid arteries. By contrast, although TA may involve distal branches of pulmonary and renal arteries, its major clinical manifestations occur as a consequence of stenoses of the aorta and its major tributaries [2]. Moreover, when apparent, aortic involvement in GCA usually results in aneurysm formation, whereas aortic stenoses are the more frequent consequence of aortic inflammation in TA [6,7].

Differentiating giant cell arteritis from Takayasu's arteritis

Takayasu's arteritis is increasingly recognized in white Western populations and, as will be discussed later, sometimes occurs in people older than 40 years of age [2,8••]. In addition, the striking female predominance observed in large series in Japan [9,10], the United States, and Mexico [11,12] may not be so obvious in other ethnic settings such as Southeast Asia [13,14••]. Whereas symptoms from large vessel disease occur in almost all patients with TA, symptomatic involvement from large vessel occurs in 10% to 15% of patients with GCA [7].

In some individuals, several of the previously described unusual features converge; thus it may be difficult to distinguish TA from GCA in these patients. Most patients with GCA have a satisfactory response to corticosteroid therapy, whereas the course of TA is more unpredictable and it is not unusual to observe failure of therapy in these patients [2]. Because differentiating those diseases may

have therapeutic and prognostic implications, Michel *et al.* [15•] defined discriminating features between both disorders by analyzing prospectively recorded data from the American College of Rheumatology vasculitis criteria data bank. In this study, comparing 217 patients with GCA with 63 with TA, the major discriminating features were, as expected, age and ethnic background. Additional differentiating data pointing toward diagnosis of TA were upper limb vascular involvement (asymmetry in blood pressure, decreased pulses or bruits) and angiographic evidence of renal artery stenoses. Significant features favoring a diagnosis of GCA were signs and symptoms of extracranial artery involvement (scalp tenderness, temporal artery abnormalities at physical examination) and polymyalgia rheumatica (PMR)-related symptoms such as shoulder stiffness [15•].

Giant cell (temporal) arteritis

Giant cell arteritis is not an uncommon disease. Recent epidemiologic studies performed in northern Europe and North America disclose an annual incidence rate of 19.1 to 27 cases per 100,000 population older than 50 years [16–19], which may reach 49 per 100,000 in individuals aged 80 years and older [19]. It appears that the incidence of GCA is increasing in different countries [19,20]. Although the occurrence of GCA may be in fact higher, other factors, such as a longer life expectancy and a better awareness of the atypical or less classic disease presentation forms by attending physicians, may contribute to the increase in reported incidences. Comprehensive reviews of the more remarkable aspects of the disease have been recently published [3••,21,22]. Important contributions have improved our understanding of the immunopathogenic mechanisms involved in the development of GCA, its relationship to PMR, and its long-term outcome.

Pathogenesis

Genetic predisposition

The existence of a genetic component in the pathogenesis of GCA is supported by epidemiologic data showing a clear predominance of the disease in whites, a higher prevalence in certain geographic areas such as northern Europe and in populations with similar ethnic background, and the sporadic occurrence of familial cases of GCA or GCA and isolated PMR. During the past decade, using standard microlymphocytotoxicity techniques, several investigators reported the association of GCA and PMR with the HLA class II antigen DR4. This association was observed in North America, northern Europe, and in Mediterranean countries [23–26]. Interestingly, patients with PMR alone or in combination with GCA have a much stronger association with the HLA-DR4 in most reports [24,26,27]. With the availability of new molecular techniques, Weyand *et al.* [28,29] identified the allelic variants of DR4 predominating in individuals with GCA and PMR. HLA DRB1*0401 was the most frequent allele in patients with GCA. The nonrandom distribution of DRB alleles in patients who were DRB1*04 negative led to the search of a shared HLA amino acid sequence. A four-amino-acid motif (DRYF) at the second hypervariable region of the polymorphic HLA-DRβ chain has been proposed to be a disease-related sequence [28]. This disease-associated motif can be mapped to the antigen-binding area of the HLA-DR molecule and is different from the sequences associated with rheumatoid arthritis (QKRAA/QRRDA), which are located at the third hypervariable region [28].

Giant cell arteritis as an antigen-driven disease

A number of nonspecific immunologic abnormalities have been described in patients with GCA, which support the concept that immune mechanisms play a role in pathogenesis. These include decreased CD8 lymphocytes [30,31], increased circulating soluble interleukin-2 receptors [32,33], circulating soluble adhesion molecules [34] (Coll-Vinent *et al.*, Unpublished data), and cytokines such as interleukin-6 [35]. Oligoclonal expansion of circulating T lymphocytes are often demonstrated in these patients [36]. Disease association with HLA-DRB1*0401 and excellent response to corticosteroid therapy provide additional evidence supporting the participation of immune mechanisms in disease pathogenesis.

Immunopathologic studies have shown that activated macrophages and CD4 T lymphocytes are the major constituents of GCA inflammatory infiltrates in a pattern closely resembling delayed-type hypersensitivity reactions [37]. Analysis of cytokines produced by infiltrating cells indicates, indeed, a Th1(T helper 1)-type functional differentiation of CD4 T lymphocytes [38]. Among several lymphocyte and macrophage cytokines produced in inflammatory lesions, interferon gamma seems to be crucial for the full development of the granulomatous reaction that typically characterizes GCA lesions [38]. In addition, identification of dendritic cells with a putative antigen-presenting cell function in temporal artery lesions further suggests that GCA lesions might develop as a consequence of a T-cell–mediated immune response directed toward antigens residing in the arterial wall [37].

By expanding CD4 lymphocyte clones obtained from temporal artery biopsy specimens, Weyand *et al.* [39] demonstrated that a minority of infiltrating lymphocytes obtained from different temporal artery segments share identical sequences at the third complementary determining region (CDR3) of their T-cell receptors. These sequences are absent in lymphocytes obtained from peripheral blood. T-lymphocyte clonal expansion provides a strong support to the hypothesis that temporal arteritis lesions may represent an immune response toward an antigen present in the arterial wall. Identical T-cell specificities have also been found in the right and left temporal arteries of the same patient [40•]. However, sequence homology at the CDR3 of the T-cell receptor

has not been demonstrated to be over-represented in T-cell clones derived from different patients [40•]. These observations suggest either a heterogeneity in the putative triggering agents or, more likely, a high variability in individual responses. At least some of the potentially disease-relevant T-cell clones from involved temporal arteries are able to proliferate when cocultured with irradiated peripheral blood mononuclear cells previously exposed to temporal artery extracts obtained from patients with GCA and from patients with PMR. However, proliferation did not occur when these T cells were cocultured with peripheral blood mononuclear cells that had been exposed to tissue from patients with unrelated diseases [40•]. Although this study includes only a few cases, it may indicate that temporal arteries from different patients might share some disease-relevant antigenic determinants. Wagner *et al.* [41•] suggested that antigen recognition may occur at the outer part of the artery. This hypothesis is based on the observation that proliferating, interleukin-2–producing T cells predominate in the adventitial layer [41•].

Mechanisms of vascular injury

Although only a minority of T lymphocytes become directly activated by antigen recognition and these appear to be predominantly located at the adventitial layer [39,41•], full-blown GCA lesions predominate in the intima-media junction and frequently expand throughout the entire thickness of the artery. Proliferating adventitial CD4 T lymphocytes actively produce interferon gamma, a potent activator of macrophages (which are the predominant cell population in GCA lesions). It has been shown in other granulomatous diseases that interferon gamma is a crucial cytokine in the development of granulomatous reaction and in macrophage transformation into giant cells [42,43].

Although other macrophage-derived cytokines such as interleukin-1, tumor necrosis factor-α, and T-cell–derived cytokines such as interleukin-2 can be found in temporal arteries from both GCA and patients with apparently isolated PMR, interferon gamma seems to be found only in patients with inflamed arteries [38]. To support the concept that interferon gamma is necessary for the full development of typical granulomatous GCA lesions, higher levels of interferon gamma mRNA can be demonstrated in patients with a prominent granulomatous reaction with giant cells and significant cranial vascular manifestations, compared with patients with only constitutional symptoms [44••].

Activated macrophages produce oxygen radicals, nitric oxide, and proteolytic enzymes that participate in disruption of vessel architecture and tissue destruction. Indeed, it has been demonstrated that macrophages present in temporal arteritis lesions produce inducible nitric oxide synthase and matrix metalloproteinases, particularly matrix metalloproteinase-2 and matrix metalloproteinase-9 [45,46,47••]. These enzymes have elastinolytic activity that might contribute to rupture of the elastic lamina, a typical finding in temporal arteritis lesions. Elevated concentrations of circulating matrix metalloproteinase-9 can also be found in sera from patients with GCA [45]. Its pathophysiologic significance is unknown.

Infiltrating macrophages in GCA seem to be functionally heterogeneous and appear to be topographically organized [47••]. Interleukin-1, TGF-β, and interleukin-6–producing macrophages are mainly located at the adventitial layer. Based on the known functional activities of these cytokines, they may contribute to the recruitment of inflammatory cells and to the nonspecific systemic acute phase response. Macrophages located at the tunica media produce matrix metalloproteinases, allowing the progression of inflammatory infiltrates and contributing to tissue destruction. Macrophages in the intimal layer produce inducible nitric oxide synthase. Through the generation of nitric oxide, these cells may further contribute to tissue damage [47••].

In addition, polymorphonuclear neutrophils have been shown to be a small proportion of the cellular infiltrate in GCA [48]. It has been recently shown that interferon gamma, a cytokine that appears to be crucial in the development of granulomatous inflammation in temporal arteritis, is also a potent activator of neutrophils [49]. Infiltrating neutrophils in temporal arteritis produce neutrophil elastase, which may also contribute to internal elastic lamina destruction and tissue damage [48].

Vascular response to inflammation

Rather than being passive spectators of leukocyte infiltration, vessel wall components, particularly endothelial cells, actively and dynamically react to the products released by infiltrating leukocytes. During the past few years it has become apparent that endothelial response to cytokines and growth factors amplifies the inflammatory response by three main mechanisms: adhesion molecule expression, cytokine and growth factor production, and neovascularization [50–53]. Angiogenesis is a prominent phenomenon in GCA [54] (Fig. 1) and other large and medium sized vessel vasculitides [50,55•]. Neovessels, rather than the endothelial cells of the large vessel lumen, strongly express adhesion molecules for leukocytes, providing new surfaces through which additional leukocytes invade the blood vessel. E selectin, intercellular adhesion molecule 1, vascular endothelial cell adhesion molecule 1, and platelet and endothelial cell adhesion molecule 1 expression can be detected in adventitial vasa vasorum and neovessels [54].

Vessel occlusion

Vascular response to inflammation may lead eventually to vessel occlusion. This occlusion may result from spasm, thrombosis, and, more frequently, from intimal hyperpla-

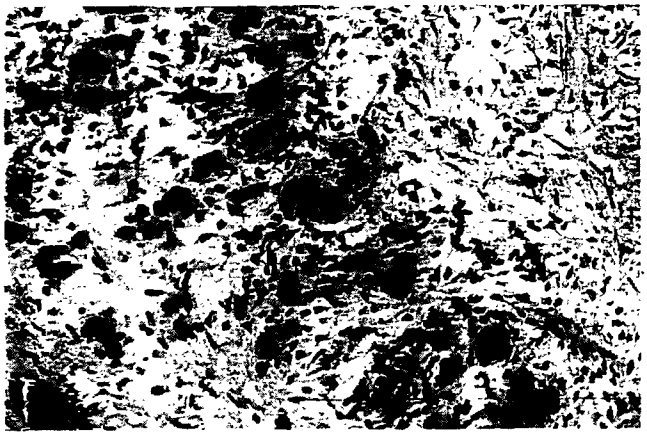

Fig. 1. Intense neovascularization within giant cell arteritis inflammatory infiltrates as assessed by *Ulex Europaeus* staining.

sia and fibrosis [53]. Several cytokines and growth factors produced in GCA lesions may contribute to vessel occlusion. Interleukin-1 and tumor necrosis factor-α have prothrombotic effects [51]. Interleukin-1 and TGF-β have fibrogenic activity [56]. In fact, a relationship between the presence of TGF-β–producing macrophages and intimal hyperplasia has been demonstrated in GCA [47••].

Prominent vascular occlusion may lead to ischemic complications. Typical GCA ischemic events include anterior ischemic optic neuropathy and central retinal artery thrombosis, which often result in visual loss. Less frequently, cerebrovascular accidents or scalp necrosis may also occur. In a recent study we found that irreversible cranial ischemic events tended to occur in patients with little evidence of systemic inflammatory reaction as defined by clinical and laboratory markers. Patients with strong clinical and laboratory inflammatory abnormalities were at very low risk of ischemic complications [57] (Fig. 2). The mechanisms through which the inflammatory response may relate to vessel oclusion are unknown. As mentioned previously, several cytokines produced in inflamed arteries have both systemic effects and potent biologic activities on endothelial and smooth muscle cells [52]. Consequently, cytokines and, perhaps, acute phase proteins might regulate vascular biologic responses, which may favor or prevent vessel occlusion [52, 58].

Relationship between polymyalgia rheumatica and giant cell arteritis

Polymyalgia rheumatica is a clinically defined syndrome consisting of aching and stiffness in neck, shoulder, and pelvic girdles. About 30% to 40% of patients with temporal arteritis report PMR symptoms, whereas between 0% to 80% of patients primarily diagnosed with PMR have a temporal artery biopsy showing arteritis [3••,59••]. Such variability among series is probably related to the application of different selection criteria. Besides their coincidence in a substantial proportion of individuals, GCA and PMR have a similar epidemiologic distribution and a similar immunogenetic background [3••,59••]. With a few exceptions [60], both are characterized by a strong nonspecific acute-phase response and, typically, respond dramatically to corticosteroid treatment, although the doses required to induce remission in patients with isolated PMR are usually lower than reported for GCA [61,62]. In addition, both conditions share a number of nonspecific immunologic abnormalities, such as decreased numbers of circulating CD8 lymphocytes [30,31], elevated levels of soluble interleukin-2 receptors [33,59••], and they show the same pattern of circulating monocyte activation with an increased production of interleukin-6 [63]. However, the nature of the relationship between both disorders is intriguing and not well understood.

The observation of clinically apparent joint swelling in some cases, abnormal findings on scintigraphic imaging, and histopathologic findings from synovial samples has led to the conviction that synovitis of the proximal joints is the main underlying cause of musculoskeletal symptoms in patients with PMR [61,64,65]. T_2-weighted magnetic resonance imaging has demonstrated that in addition to glenohumeral and hip synovitis, subdeltoid, subacromial, and ileopectineal bursitis and bicipital tenosynovitis are prominent findings in patients with active PMR [59••]. Recently, Meliconi *et al.* [66••] defined the immunopathologic characteristics of the PMR synovitis. The pattern of leukocyte infiltrates is similar to that observed in GCA, namely, predominance of macrophages, CD4 T lymphocytes, a small proportion of neutrophils, and absence of B cells and natural killer cells. Perivascular infiltrates have been observed in a few cases and granulomatous inflammation was noted. PMR synovi-

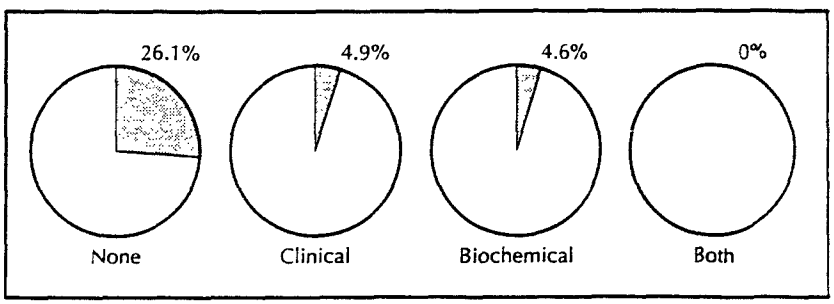

Fig. 2. Prevalence of irreversible cranial ischemic events in 200 patients with biopsy-proven giant cell arteritis according to the presence of clinical (fever and weight loss) or laboratory (erythrocyte sedimentation rate ≥ 85 mm and hemoglobin < 11 g/dL) inflammatory parameters [57].

tis differs from that of rheumatoid arthritis in the lack of B lymphocytes, γ/δ T lymphocytes, and in the absence of significant proliferation of the synovial lining cells. Neovascularization is a prominent finding in PMR synovium and there is a remarkable expression of adhesion molecules E selectin, intercellular adhesion molecule 1, and vascular endothelial cell adhesion molecule 1 by endothelial cells, which decreases on corticosteroid treatment [67••]. Therefore, neovascularization and adhesion molecule expression also contribute to the development of synovial inflammatory infiltrates in PMR. Weyand *et al.* [38] showed that even in the absence of overt inflammation at histopathologic examination, T-cell–derived cytokines could be detected in temporal artery specimens from patients with apparently isolated PMR. Only interferon gamma was absent from normally appearing temporal arteries of PMR patients. These findings led the authors to consider PMR as a "forme frustre" of temporal arteritis.

However, PMR has a well-defined histopathologic substrate that is absent in about 50% of patients with temporal arteritis. Additional observations suggest that isolated PMR may encompass a heterogeneous group of patients [60]. Several investigators have pointed out that at least a subset of patients with isolated PMR would be more closely related to late-onset seronegative rheumatoid arthritis [68,69]. From an immunogenetic point of view, PMR may also be heterogeneous. Although GCA occurs predominantly in individuals bearing the HLA DRB1*0401 allele, all DRB1 variants can be found among patients with PMR [28,29,70]. Moreover, in a series of patients with only PMR studied by Haworth *et al.* [70], there was an increased prevalence of the rheumatoid arthritis—associated sequence QKRAA, whereas the proposed GCA-related motif, DRYF, was present in a frequency similar to that observed in the general population. At this point, the nature of the relationship between GCA and PMR and between the latter and late-onset seronegative rheumatoid arthritis remains an unanswered question.

Response to treatment and outcome

Giant cell arteritis and PMR have been considered to be highly corticosteroid-responsive disorders. Response to corticosteroid therapy has even been considered one of some widely used criteria for clinical diagnosis of both PMR and GCA [61,70,71]. Indeed, corticosteroids induce a dramatic symptomatic relief of cranial, systemic, and PMR symptoms, and prevent in most but not all cases the occurrence of ischemic complications [72–74]. However, both diseases have a remarkable tendency to relapse and active histologic lesions have been demonstrated in patients with GCA who have been presumably in clinical remission for months and even years [3••,75] (Fig. 3). Evans *et al.* [6,22,76] have demonstrated that patients with GCA are at higher risk of developing aortic aneurysms, even in patients thought to be in remission. These facts suggest that some components of the disease are highly corticosteroid sensitive, whereas the major underlying pathogenic mechanisms may continue to be active despite the apparent clinical remission. Clinical symptoms, hematologic abnormalities, and acute-phase reactants such as erythrocyte sedimentation rate and C-reactive protein quickly return to normal [3••]. Within hours of treatment, circulating interleukin-6, typically elevated in GCA patients, return to normal levels in most patients [35]. In just a few days, interleukin-2 receptor expression disappears from tissue-infiltrating lymphocytes [37]. Downregulation of endothelial adhesion molecules may take longer (Cid *et al.*, Unpublished data) and clearing the white blood cell infiltrate may require months or even years [75]. In an animal model consisting of xenotransplantation of an involved temporal artery into a severe combined immunodeficiency mouse, Brack *et al.* [77••] demonstrated that corticosteroid therapy suppresses the production of proinflammatory, NFκB-dependent cytokines such as interleukin-2, interleukin-1β, and interleukin-6, whereas TGF-β production does not seem to be influenced and persists despite high-dose corticosteroid treatment.

Since its initial description, GCA has been considered a self-limiting disease. About 50% of patients are able to withdraw from corticosteroids after 1 to 2 years of treatment with no recurrences [3••,78], and, in some patients in remission, temporal artery biopsy specimens show progression to a healing stage. The reason why some patients develop a self-limiting inflammatory response to the putative triggering antigen—whereas in others it lasts for years or perhaps indefinitely—remains a mystery. Although corticosteroid treatment is effective in suppressing clinical

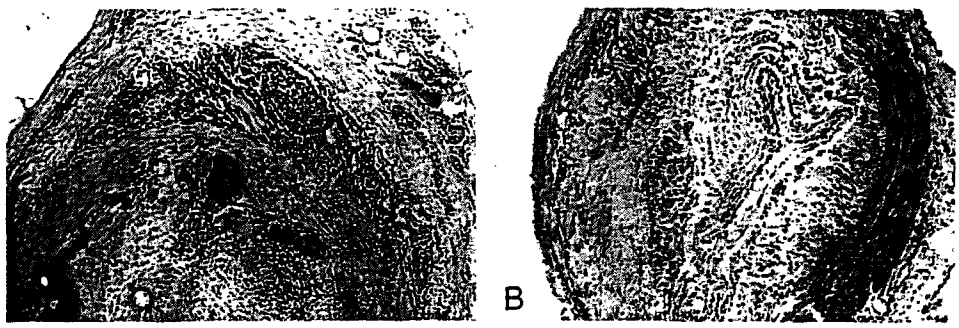

Fig. 3. A, Full-blown giant cell arteritis lesions in a patient treated with corticosteroids for 3 months. **B**, Progression to a healing stage in a patient in sustained clinical remission after corticosteroid withdrawal 6 years earlier. Persistent small inflammatory foci can still be observed.

manifestations, proof does not exist to demonstrate that treatment actually influences the course of the disease. The existence of a subset of polymyalgia patients requiring long-term therapy has also been identified [65,79].

Steroid tapering is usually guided by clinical assessment and determination of erythrocyte sedimentation rate, which closely correlates with disease activity in most patients [3••]. Preliminary reports indicate that other parameters such as circulating interleukin-6, interleukin-2 receptors, or soluble adhesion molecules may have some usefulness in assessing disease activity, but this needs to be confirmed in larger studies [32,35,80,81]. In a prospective study performed on 38 patients with PMR, Salvarani *et al.* [33] showed that persistent decreased circulating CD8 lymphocytes after 6 months of corticosteroid treatment was significantly associated with a longer duration of treatment, higher cumulative corticosteroid doses, and a higher relapse rate. High concentrations of von Willebrand factor–related antigen have been demonstrated in plasma from patients with GCA and PMR. High concentrations of von Willebrand factor–related antigen continue to be elevated despite clinical remission induced by corticosteroid therapy [82]. Elevated von Willebrand factor–related antigen concentrations in patients with temporal arteritis in apparent clinical remission may reflect persistent exposure of endothelial cells to a persistent inflammatory microenvironment rather than endothelial injury. We have recently shown that von Willebrand factor–related antigen levels eventually return to normal in patients in long-lasting remission [83]. Whether this or other parameters may help in differentiating corticosteroid-requiring clinical activity from true remission remains to be elucidated.

Takayasu's arteritis

Takayasu's arteritis is much less frequent than GCA in Western countries. The reported incidence in the United States is 2.6 cases per million people per year [84]. Although TA appears to occur much more frequently in Asian and Hispanic countries, the exact incidence rates in these settings are unknown. Clinical presentation may be insidious and diagnosis is often delayed [2,8••,11]. Response to corticosteroid therapy is far more uncertain. Heterogeneity in response to treatment, new therapeutic options including surgery, and percutaneous transluminal angioplasty have been addressed in recent prospective studies and in-depth reviews [2,8••,9,11].

Pathogenesis

Several observations indicate that immune mechanisms may play a role in the pathogenesis of TA. Indeed, nonspecific immunologic abnormalities such as hypergammaglobulinemia or autoantibodies are frequently found in patients with TA [2,11,85]. Occasional association with other immune-mediated diseases such as inflammatory bowel disease and sarcoidosis has also been described [2,11,86]. Histopathologic examination of vascular lesions shows a granulomatous inflammation and cytotoxic T lymphocytes. Contrary to GCA and PMR where they are rarely found [67••,87], γ/δ T lymphocytes recognizing heat-shock proteins seem to contribute to vessel damage in TA. The pathogenic mechanisms underlying vessel inflammation and stenoses in TA continue to progress slowly. The scarce availability of samples from involved tissues has largely restricted the application of modern immunohistochemical and molecular techniques, which have allowed more substantial progress in other vasculitides syndromes.

Genetic predisposition has been suspected in TA based on its ethnic and geographic distribution and occasional familial clustering, including the identification of the disease in monozygotic twins [2]. Several investigators have hypothesized that susceptibility to TA may be linked to HLA genes. Attempts to relate TA genetic predisposition to class II HLA genes by both standard microlymphocytotoxicity assays and molecular techniques have led to weak and variable associations that are not conclusive, particularly in studies of Western patients [2,11]. A more consistent association with class I antigens has been demonstrated [13]. Disease susceptibility appears to be associated with HLA-B5 in India and with one of its variants, B52, in Japan and Korea [88•]. Some of the previously reported weakly associated class II alleles, namely DRB1*1502 and DRB1*0901, are indeed in close linkage desequilibrium with B52 [89•]. A recent study shows that B39.2 is independently and significantly associated with TA in Japan [89•]. Interestingly, B52 and B39.2 display structural similarities near the peptide-binding site. However, the prevalence of these antigens in affected individuals is rather low. Only 59.4% of the Japanese patients studied by Kimura *et al.* [89•] carry either B52 or B39.2, whereas the prevalence of the putative disease-associated gene is much higher in other diseases. This observation points out that B52 and B39.2 may not be the true disease-susceptibility genes but are in close linkage desequilibrium with the real disease-associated gene.

New diagnostic criteria

During the past few years, it has become apparent that age at presentation and disease expression may vary in different geographic areas [13]. For instance, the strong female predominance observed in Japanese patients may not be so apparent in other settings. In Southeast Asia, the male-to-female ratio approaches 1:2 [14••]. In addition, although in most cases TA is diagnosed between the second and third decades of life, there is increasing recognition of the disease in people older than 40 years of age who lack additional criteria for temporal arteritis [90]. About 13% to 15% of patients are older than 40 in Japan and also in North America [2,91•]. Moreover, classic involvement of the aortic arch and its branches described in Japanese series may be less frequent in India and Thailand where involvement of the abdominal aorta and

renal arteries predominates [2,11,14••]. Consequently, dizziness, vertigo, aortic regurgitation, and upper limb pulselessness are the more common presenting symptoms in Japan, whereas in Southeast Asia reno-vascular hypertension leads to TA diagnosis in a substantial proportion of individuals [14••]. Ignorance of these deviations in the classic picture may result in important omissions and unnecessary delays in appropriate diagnosis and treatment.

According to Ishikawa's [92] initial criteria, age at presentation less than 40 years was an obligatory item for diagnosis. Its indiscriminate application may risk not making an appropriate diagnosis in a significant proportion of patients. In 1990 the American College of Rheumatology proposed a new set of classification criteria in which age less than 40 was no longer an obligatory requirement. However, patients with disease restricted to the aorta and renal arteries (type III and IV of the new angiographic classification), which account for 30% of patients diagnosed in Southeast Asia [13,14••], or those with disease limited to the pulmonary arteries [93] may not fullfil enough American College of Rheumatology criteria to allow TA diagnosis. In order to overcome these difficulties, Sharma *et al.* [91•] proposed some changes in Ishikawa's diagnostic criteria. Major modifications consist of removal of age as an obligatory requirement, including coronary and pulmonary involvement as additional criteria, and removal of terminal aortic involvement as an exclusion criterion [91•] (Table 1). Application of these criteria to an Indian population of 106 patients rendered a sensitivity of 92.5%, whereas the American College of Rheumatology and the original Ishikawa's criteria provided a sensitivity of 77.4% and 60.4%, respectively [91•]. However, the criteria of Sharma *et al.* [91•] easily allow the diagnosis of TA in temporal arteritis patients with large-vessel involvement. Further international collaborative efforts are necessary to improve existing diagnostic criteria and, perhaps, to allow slight modifications in particular ethnic groups.

Improving diagnosis and assessment of disease activity by new imaging techniques

Diagnosis of TA is usually confirmed by invasive angiography. Typical findings include stenoses and, often, aneurysms with a variable distribution along the aorta, its major branches, and pulmonary arteries [2,8••,11]. According to their arrangement, TA lesions can be classified into five angiographic patterns (reviewed by Numano [13] and Hata *et al.* [14••]). At present, invasive angiography is the best method to assess disease extent. Given the individual variability in disease distribution, total aortography and pulmonary angiography should be performed in every patient. As previously discussed, routine angiographic examination has allowed the recognition of peculiar distribution patterns in different geographic areas. Angiography is also essential in assessing disease progression and response to therapy because clinical and laboratory findings have not been found to be reliable to evaluate disease activity in many patients [2,8••,11].

However, periodic invasive angiography carries some inconveniences such as repeated radiation exposure and risk of uncommon but possible complications such as infection, hemorrhage, or aneurysm rupture. Moreover, angiography only evaluates the luminal appearance of the vessel and is unable to distinguish whether stenoses are caused by active inflammation or fibrotic scarring. These facts have prompted a search to identify safer and potentially more

Table 1

Proposed diagnostic criteria for Takayasu's arteritis

Ishikawa's [92] criteria*	Amencan College of Rheumatology [84]†	Sharma *et al.* [91•]‡ modified criteria
Obligatory criterion	Age < 40 years	Three major criteria
Age < 40 years	Claudication of extremities	Left midsubclavian artery lesion
Two major criteria	Decreased brachial artery pulse	Right midsubclavian artery lesion
Left midsubclavian stenoses	Blood pressure difference > 10 mm Hg	Characteristic signs and symptoms of at least 1 month duration
Right midsubclavian stenoses	Bruit over subclavian arteries or aorta	Ten minor criteria
Nine minor criteria	Arteriogram abnormality	Elevated ESR
Elevated ESR		Carotid artery tenderness
Carotid artery tenderness		Aortic regurgitation
Hypertension		Annuloaortic ectasia
Aortic regurgitation or annuloaortic ectasia		Pulmonary artery lesion
Pulmonary artery lesion		Left midcommon carotid lesion
Left midcommon carotid lesion		Distal brachiocephalic trunk lesion
Distal brachiocephalic trunk lesion		Descending thoracic aorta lesion
Descending thoracic aorta lesion		Abdominal aorta lesion
Abdominal aorta lesion		Coronary artery lesion

*In addition to the obligatory criterion, two major criteria, or one major and two or more minor criteria, or four minor criteria are required for Takayasu's arteritis diagnosis. †At least three criteria are required for classification purposes. ‡Presence of two major, or one major and two minor, or four minor criteria suggests a high probability of Takayasu's arteritis.
ESR—erythrocyte sedimentation rate.

informative imaging techniques such as computed tomography or magnetic resonance angiography, both of which are able to provide qualitative information about the vessel wall. Computed tomography, for instance, is able to detect calcifications, intraluminal or intra-aneurysmal clots, wall dissection, and wall thickening [94••]. Magnetic resonance angiography can provide additional information about flow and it has been proved to be useful in detecting subclavian steal syndrome [94••]. Compared with conventional invasive angiograms, magnetic resonance angiography appears to be equally sensitive in detecting lesions in the aorta and its brachiocephalic branch vessels [94••]. However, it is much less sensitive in detecting involvement of smaller branch vessels and may overestimate the degree of stenoses in renal and subclavian arteries [94••].

Several investigators have tried to detect abnormalities in the vessel wall that may correlate with either the existence of active inflammation or with the presence of fibrotic tissue. Early after the administration of bolus injection of contrast material, computed tomography angiography is able to detect heterogeneous mural high attenuation areas, which may reflect hypervascularization of the tunica media. In a delayed phase, high attenuation coefficient in the aortic wall might indicate active inflammation, whereas low attenuation rings in the intimal layer seen at any phase may represent intimal thickening [95]. Transcutaneous B-mode ultrasonography can also differentiate the intimal, medial, and adventitial layer, particularly in the aorta, subclavian, and carotid arteries [96,97]. Several case reports indicate that computed tomography scan and magnetic resonance angiography are able to detect a decrease in arterial thickness with corticosteroid treatment [98,99]. Preliminary findings suggest that T_2-weighted magnetic resonance imaging techniques, which are sensitive to tissue water content associated with edema, might also help in distinguishing thickness due to active inflammation from that resulting from scar [100]. Prospective large studies are necessary to correlate images provided by these new techniques with patient outcome.

Although the administration of weekly methotrexate has been a substantial achievement in the treatment of patients with TA, inducing remission in 81% of patients refractory to previous steroid therapy [101], 44% of patients experience subsequent relapses and 23% never achieve remission with any treatment and continue to develop additional lesions. Two major challenges in the care of patients with TA include developing more effective therapies and developing better measures of disease activity to guide treatment [8••].

Acknowledgments

We thank Mrs. Carme Carbonell for superb secretarial work. Supported by a grant from Fondo de Investigación Sanitaria (FIS 98/0443).

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COMUNICACIONS A CONGRESSOS

XLVI Reunión Anual de la Sociedad Española de Neurología.
Barcelona. Desembre de 1994

Neurología 1994;9:472

VASCULITIS MUSCULARES ASOCIADAS A LA INFECCION POR EL VIRUS DE LA INMUNODEFICIENCIA HUMANA (VIH). ESTUDIO DE 13 CASOS.

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Introducción: Es conocido que en el curso de la infección por el VIH se producen alteraciones inmunológicas tales como vasculitis. A nivel muscular se han descrito microvasculitis linfocitarias y arteritis necrotizantes tipo PAN.
Objetivo: Caracterizar el perfil clínico de los pacientes VIH + con vasculitis muscular.
Pacientes y método: 8 pacientes VIH+ diagnosticados de microvasculitis y 5 de arteritis tipo PAN mediante biopsia muscular. Se recogen sus características clínicas, analíticas e histológicas.
Resultados: 11 hombres y 2 mujeres. Edad media: 36 años. Nueve eran antiguos drogadictos, 2 homosexuales y 2 sin factores de riesgo. Los estadíos (CDC) eran: IVC2: 5 pacientes, IVC1: 4, III: 3 y II: 1. Tres pacientes referían mialgias y 9 pérdida de fuerza. Cinco tenían atrofia muscular y 6 polineuropatía periférica. La creatincinasa sérica fue normal. El HBsAg fue positivo en 1 caso. Un paciente presentó afección renal discreta. Valor medio de linfocitos Cd4: 214/mm3 y el de Beta2-microglobulina: 4,5 mg/ml. Cuatro pacientes habían recibido antirretrovirales. Seis pacientes con corticoterapia evolucionaron favorablemente y 7 no acudieron a controles.
Conclusiones: Las vasculitis que afectan al músculo en los pacientes VIH + se caracterizan por ser paucisintomáticas, aparentemente localizadas, presentarse en estadíos precoces de la infección y responder a la corticoterapia.

First Congress of the European Federation of Neurological Societies. Marseille, França. Setembre de 1995

European Journal of Neurology 1995;2:100

NY2

Leukocyte–endothelial cell adhesion receptors in muscle biopsies from patients with idiopathic inflammatory myopathies (IIM)

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Background. Interactions between leukocytes and endothelial cells through specific adhesion receptors play a crucial role in the development of inflammatory infiltrates in chronic inflammatory diseases.

Objective. To investigate the adhesion molecule expression in muscle biopsies from IIM.

Patients/Methods. Definite cases of dermatomyositis (DM) (18), polymyositis (PM) (6), inclusion-body myositis (IBM) (5) and normal controls (8). APAAP technique was used with monoclonal antibodies recognizing LFA-1 and VLA-4, their endothelial counter-receptors ICAM-1 and VCAM-1, and the endothelial cell markers CD31 and vWFAg.

Results. ICAM-1 expression was up-regulated and VCAM-1 induced in muscle capillaries of DM samples. In both DM and PM cases, endothelial cells from vessels surrounded by inflammatory infiltrates strongly expressed ICAM-1 and VCAM-1. Corresponding infiltrating leukocytes were LFA-1 and VLA-4 positive.

Conclusions. Leukocyte-endothelial cell interactions mediated by the receptor–ligand pairs LFA-1/ICAM-1 and VLA-4/VCAM-1 play a role in the development of muscle inflammatory infiltrates in IIM. ICAM-1 overexpression by capillary endothelial cells in DM reinforces the hypothesis of activation and/or injury of such cells in this disease.

Supported by FIS 95/0860.

59th National Scientific Meeting. American College of Rheumatology. San Francisco, California. Octubre de 1995

Arthritis and Rheumatism 1995;38:S156

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SOLUBLE INTERCELLULAR ADHESION MOLECULE-1, VASCULAR ADHESION MOLECULE-1, E-SELECTIN AND L-SELECTIN IN POLYARTERITIS NODOSA

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Soluble forms of cell adhesion molecules are thought to play an important role in regulating the inflammatory response. The purpose of this study was to evaluate whether changes in levels of soluble adhesion molecules reflect disease activity in patients with polyarteritis nodosa (PAN) histologically demonstrated by muscle and/or nerve biopsy.

Methods. A sandwich ELISA was used to measure soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule 1 (sVCAM-1), soluble E-selectin and soluble L-selectin in sera from 24 patients with PAN at the time of diagnosis, in sera from 11 of these patients serially during follow-up (mean 15 months), and in sera from 14 healthy controls.

Results. At the time of diagnosis of PAN, serum levels of sICAM-1 (491±194 ng/mL), sVCAM-1 (1057±594 ng/mL) and sE-selectin (61±26 ng/mL) were significantly higher in patients than in controls ($p<0.001$, $p<0.05$ and $p<0.01$ respectively). In contrast, sL-selectin levels (725±194 ng/mL) were significantly lower than in controls ($p<0.05$). Within the first 7 days after starting treatment, there was a significant increase of sICAM-1 levels ($p=0.0001$), which fell thereafter without reaching normal levels with clinical remission. sE-selectin and sL-selectin remained significantly elevated and decreased respectively during all follow-up. sVCAM-1 also fell without reaching normal values in inactive disease.

Conclusions. Increased levels of sICAM-1, sVCAM-1 and sE-selectin and decreased levels of sL-selectin in active PAN suggest immune and endothelial stimulation during disease activity. Failing of normalization of all them in inactive disease suggests that treatment may supress clinical and biological manifestations rather than target the underlying pathogenic mechanisms. BC-V is an award recipient from HCP Barcelona.

V[th] European Congress of Neuropathology. París, França. Abril de 1996

Neuropathology and Applied Neurobiology 1996;22(suppl 1):40

W17.2 Grau J. M., Coll-Vinent B. & Cid M. C. Muscle Research Unit Hospital Clinic Villarroel 170, 08036 Barcelona, Spain

Leukocyte-endothelial cell adhesion molecules in muscle and nerve vasculitis: immunohistochemical approach and detection of some soluble forms

As adhesion molecules are thought to play an important role in the regulation of the inflammatory response, the authors investigated whether changes in levels of some soluble(s) forms of such molecules (ICAM-1, VCAM-1, E-selectin and P-selectin) measured by sandwich ELISA, reflect disease activity in patients with polyarteritis nodosa (PAN). In addition, an IHQ evaluation in muscle and nerve biopsies from patients with vasculitis (PAN) and from HIV-related vasculitis (either PAN type or microvasculitis) was made using APAAP and PAP methods with the following monoclonal antibodies: *Ulex europeus*, granulocytic elastase, E-selectin, L-selectin, P-selectin, CD18, CD31, ICAM-1, ICAM-2, ICAM-3, VCAM-1 and VLA4. Increased serum levels of s-ICAM-1, s-VCAM and E-selectin with respect to the matched controls suggest an immune and endothelial stimulation during disease activity. An abnormal expression of VCAM-1 was detected at IHQ level in some middle sized vessels surrounded by VLA-4 positive mononuclear cells. The relationship between the levels of soluble forms and the detection by IHQ will be discussed.

XXII Congreso Nacional de la Sociedad Española de Reumatología. Saragossa. Maig de 1996

Revista Española de Reumatología 1996;23:209

FACTORES DE RIESGO ASOCIADOS A ACCIDENTES ISQUÉMICOS CRANEALES IRREVERSIBLES (AICI) EN LA ARTERITIS DE CÉLULAS GIGANTES (ACG).

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Propósito del estudio: Analizar los factores de riesgo asociados a AICI en pacientes con ACG.

Métodos utilizados: Revisión de las historias clínicas de 147 pacientes con diagnóstico de ACG con biopsia positiva.

Resultados: 26 pacientes (17.8%) presentaron AICI (23 déficit visual parcial o total, 2 accidente vascular cerebral y 1 ambos). Los pacientes con AICI presentaron menos frecuentemente fiebre (25.9% vs 59.3%, p=0.0024) y pérdida de peso (29.6% vs 65.2%, p=0.001), y más frecuentemente accidentes isquémicos craneales transitorios (AICT) tales como diplopia (18.5% vs 3.4%, p=0.0113) y amaurosis fugax (30.8% vs 5%, p=0.0006) respecto los pacientes sin AICI. Asimismo, los pacientes con AICI presentaron menor VSG (x=87 vs x=108, p=0.0101), menor anemia (niveles medios de hemoglobina x=12 vs x=11, p=0.0021) y menor grado de hipoalbuminemia (x=36 vs x=31, p=0.0107).

Los pacientes con AICT presentaron un mayor riesgo para padecer un AICI (RR: 4.55; CI 95%:2.47-8.36; p<0.001). Definimos un subgrupo con menor respuesta inflamatoria clínica (sin fiebre ni pérdida de peso) o biológica (VSG<90, albúmina >34, hemoglobina >11). Los pacientes con menor respuesta inflamatoria clínica (RR: 3.76; CI 95%: 1.91-7.41; p<0.001) y biológica (RR: 4.00, CI 95%: 2.07-7.72;p<0.001) presentaron un riesgo aumentado de padecer AICI. Ningún paciente del grupo con mayor respuesta inflamatoria clínica y biológica presentó AICI.

Conclusiones: Los pacientes con mayor respuesta inflamatoria y sin AICT tienen menor riesgo de padecer AICI y en ellos podría ensayarse un tratamiento menos agresivo.

60th National Scientific Meeting. American College of Rheumatology. Orlando, Florida. Novembre de 1996

Arthritis and Rheumatism 1996;39(suppl):S67

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POLYMORPHONUCLEAR LEUKOCYTES (PMN) AND E-SELECTIN EXPRESSION IN INFLAMMATORY LESIONS OF GIANT CELL ARTERITIS (GCA)

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Background: The presence of PMN in vascular inflammatory infiltrates has been considered one of the ACR classification criteria for polyarteritis nodosa. However it is not clear whether PMN can be consistently found in other large or medium- sized vessel vasculitides.
Objective: To investigate the presence of PMN in GCA lesions and its relationship to E-selectin expression, disease duration, and acute phase response.
Methods: Immunohistocnemical study with monoclonal antibodies recognizing granulocytic elastase and endothelial adhesion molecule E-selectin performed on frozen sections of temporal arteries from 32 patients with biopsy-proven GCA.
Results: PMN were present in all GCA samples. In 19 cases (61%) PMN accounted for more than 5% and in 10 (32%) cases for more than 10% of the infiltrating cells. The amount of PMN correlated inversely with disease duration prior to biopsy (P<0.05). PMN were more abundant in patients with a high inflammatory response (fever and ESR > 90mm) (p=0.0092). E-selectin expression was observed mainly in adventitial microvasculature and neovessels. E-selectin immunoreactivity was detected in 22 (69%) of cases and was more prevalent in samples with > 5% of PMN (p=0.000092).
Conclusions: PMN are usually present in GCA lesions. The higher amount of PMN found in early stages of the disease and their relationship to clinical and biological markers of inflammation suggest that their products may participate in the development of GCA lesions and in the generation of the acute phase response. Concurrent expression of E-selectin suggests that PMN/E-selectin interactions participate in PMN recruitment in the vessel wall. Supported by FIS 95/0860 and FIS 96/03-47

60th National Scientific Meeting. American College of Rheumatology. Orlando, Florida. Novembre de 1996

Arthritis and Rheumatism 1996;39 (suppl):S67

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A STRONG INFLAMMATORY RESPONSE PREVENTS VISUAL LOSS AND OTHER IRREVERSIBLE CRANIAL ISCHEMIC EVENTS IN GIANT-CELL ARTERITIS PATIENTS.

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Background: Irreversible cranial ischemic events (ICIE), particularly blindness, are the more frequent cause of permanent disability in giant-cell arteritis (GCA) patients.

Design and methods: Retrospective review of clinical findings in 147 (44 males, 103 females) biopsy-proven giant-cell arteritis (GCA) patients in order to identify risk factors for ICIE.

Results: 26 patients had ICIE (23 total or partial permanent visual loss, 2 cerebral infarction and 1 both). No differences were found in the prevalence of major clinical findings (headache, jaw claudication, polymyalgia rheumatica). However, patients with ICIE had less frequently fever (25.9 % vs 59.3 %) (p=0.0024) or weight loss (29.6 % vs 65.2 %) (p=0.001) and more frequently transient cranial ischemic events (TCIE) (diplopia 18.5% vs 3.4%, p=0.0113 or amaurosis fugax 30.8% vs 5%)(p=0.0006) than patients without ICIE. In addition patients with ICIE had lower ESR (x= 87 vs x= 108) (p=0.0101) and higher hemoglobin (x=12 vs x=11) (p=0.0021) or albumin (x=36 vs x=31) (p=0.0107) levels.

Patients with TCIE (RR:4.55; CI 95%: 2.47-8.36; p<0.001) had an increased risk to develop ICIE. We empirically defined a clinical (fever or weight loss) or "biological" (ESR >90, albumin <34, or hemoglobin <11) inflammatory status. Patients without clinical (RR: 3.76; CI 95%: 1.91-7.41; p<0.001) or biological (RR: 4.00, CI 95%: 2.07-7.72; p<0.001) inflammatory status presented an increased risk to develop ICIE. No patients with both clinical and biological inflammatory status ever developed ICIE.

Conclusion: The presence of a strong inflammatory response defines a subgroup of GCA patients at very low risk for developping ICIE. Our findings provide a rationale for testing less aggressive therapies in these individuals. Conversely, the absence of an inflammatory status and the presence of TCIE provide a higher risk for ICIE and would require a prompt therapeutic intervention. Supported by FIS 95/0860.

XLVIII Reunión Anual de la Sociedad Española de Neurología.
Barcelona. Desembre de 1996

Estudio inmunohistoquímico de moléculas de adhesión leucocito-endotelio en vasculitis tipo PAN en biopsias de músculo y nervio.
(Comunicació oral de Blanca Coll-Vinent)

61th National Scientific Meeting. American College of Rheumatology. Washington, DC. Novembre de 1997

Arthritis and Rheumatism 1997;40(suppl):S166

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DYNAMIC PATTERN OF ENDOTHELIAL CELL ADHESION MOLECULE EXPRESSION IN MUSCLE AND PERINEURAL VESSELS FROM PATIENTS WITH CLASSICAL POLYARTERITIS NODOSA (PAN). Blanca Coll-Vinent, Mireia Cebrian, Maria C Cid, Carme Font, Manuel Juan, Jordi Yagüe, Álvaro Urbano-Márquez, Josep M. Grau. Hospital Clinic, 08036 Barcelona, Spain.

Background. Polyarteritis nodosa (PAN) is a systemic disease characterized by inflammation of small and medium-sized vessels. Adhesion molecules are thought to have a crucial role in the development of inflammatory infiltrates.

Objective. To investigate endothelial cell adhesion molecule expression in vessels from patients with classical polyarteritis nodosa (PAN).

Methods. Immunohistochemical study with specific monoclonal antibodies (MAb) recognizing adhesion molecules performed on frozen sections of 21 muscle and 16 nerve samples from 30 patients with biopsy-proven PAN,and 12 histologically normal muscle and 2 nerve samples obtained from 12 controls. Adhesion molecules identified were ICAM-1, ICAM-2, ICAM-3, VCAM-1, PECAM-1, E-selectin, P-selectin, L-selectin, LFA-1, and VLA-4. Neutrophils were identified with a MAb recognizing neutrophilic elastase. Endothelial cells were identified with *Ulex Europeus* lectin.

Results. In *early* PAN lesions, VCAM-1 and E-selectin were induced in the luminal endothelium. PECAM-1, ICAM-1, ICAM-2 and P-selectin (constitutive adhesion molecules) expression was similar to controls. In *advanced* lesions, immunostaining for adhesion molecules diminished or disappeared in the luminal endothelium, whereas they were clearly expressed in new formed vessels. Endothelia from vessels in a *healing* stage tended to be negative. A high proportion of infiltrating leukocytes expressed LFA-1 and VLA-4, and only a minority expressed L-selectin. No relationship was observed between the expression pattern of adhesion molecules and clinical features, disease duration or previous treatment with corticosteroids.

Conclusions. Endothelial adhesion molecule expression in PAN is a dynamic process that varies along the histopathological stages of the vascular lesions. The preferent expression of constitutive and inducible adhesion molecules in neovessels suggests that angiogenesis contributes to the persistence of inflammatory infiltrates in PAN.

61th National Scientific Meeting. American College of Rheumathology. Washington, DC. Novembre de 1997

Arthritis and Rheumatism 1997;40(suppl):S68

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CELL ADHESION MOLECULES IN THE DEVELOPMENT OF INFLAMMATORY INFILTRATES IN GIANT CELL ARTERITIS (GCA)

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Background: Leukocyte/endothelial cell interactions are crucial in the development of inflammatory infiltrates in a variety of diseases.

Objective: To investigate cell adhesion molecule expression in inflammatory lesions of GCA.

Methods: Immunohistochemical study with specific monoclonal antibodies performed on frozen sections of temporal arteries from 25 patients with biopsy-proven GCA and 5 samples from normal controls. Cell adhesion molecules identified were: sialyl-Le^x , LFA-1, and VLA-4 on the leukocyte membrane and their endothelial ligands E-selectin, ICAM-1, and VCAM-1. Endothelial cells were identified with the *Ulex Europaeus* lectin.

Results: Neovascularization in the adventitia and within the inflammatory infiltrates (assessed by *Ulex Europaeus* staining), was remarkable in GCA samples and absent in controls. ICAM-1 and PECAM-1 were constitutively expressed in the luminal endothelium and in the adventitial micro-vasculature in both GCA involved and normal arteries. Both molecules were also expressed by neovessels in GCA samples. E-selectin and VCAM-1 expression was observed in adventitial microvasculature and in neovessels in 18 (72%) and in 23 (92%) of GCA specimens respectively. E selectin and VCAM-1 expression was more frequent in samples from patients with a high inflammatory response (fever and ESR > 90) ($p=0.0092$ and $p<0.02$ respectively). No E-selectin or VCAM-1 expression was observed in normal controls. Only very scattered leukocytes expressed sialyl-Le^x. LFA-1 and VLA-4 were expressed by the majority of infiltrating leukocytes.

Conclusion: Endothelial cell adhesion molecules expressed in GCA lesions may participate in the reclutment of inflammatory cells in the vessel wall. Their preferent expression in neovessels suggests that angiogenesis contributes to the perpetuation of inflammatory infiltrates in GCA. Supported by FIS 95/0860 and FIS 96/0347

Xv[th] Annual General Meeting. British Society for Rheumatology. Brighton, England. Abril de 1998

British Journal of Rheumatology 1998;37(suppl 1):24

DYNAMIC PATTERN OF ENDOTHELIAL CELL ADHESION MOLECULE EXPRESSION IN MUSCLE AND PERINEURAL VESSELS FROM PATIENTS WITH CLASSICAL POLYARTERITIS NODOSA (PAN). B Coll-Vinent, M Cebrian, MC Cid, C Font, M Juan, J Yague, A Urbano-Márquez, JM Grau. Hospital Clínic, 08036 Barcelona

Background: Polyarteritis nodosa (PAN) is a systemic disease characterized by inflammation of small and medium-sized vessels. Adhesion molecules are through to have a crucial role in the development of inflammatory infiltrates.
Objective: To investigate endothelial cell adhesion molecule expression in vessels from patients with classical polyarteritis nodosa (PAN).
Methods: Immunohistochemical study with specific monoclonal antibodies (MAb) recognizing adhesion molecules performed on frozen sections of 21 muscle and 16 nerve samples from 30 patients with biopsy-proven PAN and 12 histologically normal muscle and 2 nerve samples obtained from 12 controls. Adhesion molecules identified were ICAM-1, ICAM-2, ICAM-3, VCAM-1, PECAM-1, E-selectin, P-selectin, L-selectin, LFA-1, and VLA-4. Neutrophils were identified with Mab recognizing neutrophilic elastase. Endothelial cells were identified with *Ulex Europeus* lectin.
Results: In *early* PAN lesions, VCAM-1, and E-selectin were induced in the luminal endothelium. PECAM-1, ICAM-1 and P-selectin (constitutive adhesion molecules) expression was similar to controls. In *advanced* lesions, immunostaining for adhesion molecules diminished or disappeared in the luminal endothelium, whereas they were clearly expressed in new formed vessels. Endothelia from vessels in a *healing* stage tended to be negative. A high proportion of infiltrating leukocytes expressed LFA-1 and VLA-4, and only a minority expressed L-selection. No relationship was observed between the expression pattern of adhesion molecules and clinical features, disease duration or previous treatment with corticosteroids.
Conclusion: Endothelial adhesion molecule expression in PAN is a dynamic process that varies along the histopathological stages of the vascular lesions. The preferent expression of constitutive and inducible adhesion molecules in neovessels suggests that angiogenesis contributes to the persistence of inflammatory infiltrates in PAN.

XV[th] Annual General Meeting. British Society for Rheumatology. Brighton, England. Abril de 1998

British Journal of Rheumatology 1998;37(suppl):88

CELL ADHESION MOLECULES IN THE DEVELOPMENT OF INFLAMMATORY INFILTRATES IN GIANT CELL ARTERITIS (GCA). MC Cid, M Cebrian, C Font, B Coll-Vinent, E Sánchez, A López-Soto, A Urbano-Márquez, JM Grau. Hospital Clínic. 08036 Barcelona, SPAIN.

Background: Leukocyte/endothelial cell interactions are crucial in the development of inflammatory infiltrates in a variety of diseases.
Objective: To investigate cell adhesion molecule expression in inflammatory lesions of CGA.
Methods: Immunohistochemical arteries from 25 patients with biopsy-proven GCA and 5 samples from normal controls. Cell adhesion molecules identified were: sialy-Le^x, LFA-1, and VLA-4 on the leukocyte membrane and their endothelial ligands E-selectin, ICAM-1 and VCAM-1. Endothelial cells were identified with the *Ulex Europaeus* lectin.
Results: Neovascularization in the adventitia and within the inflammatory infiltrates (assessed by *Ulex Europaeus* staining), was remarkable in CGA samples and absent in controls. ICAM-1 and PECAM-1 were constitutively expressed in the luminal endothelium and in the adventitial micro-vasculature in both GCA involved and normal arteries. Both molecules were also expressed by neovessels in GCA samples. E-selectin and VCAM-1 expression was observed in adventitial microvasculature and in neovessels in 18 (72%) and in 23 (92%) of CGA specimens respectively. E-selectin and VCAM-1 expression was more frequent in samples from patients with a high inflammatory response (fever and ESR > 90) ($p=0.0092$ and $p>0.02$ respectively). No E-selectin or VCAM-1 expression was observed in normal controls. Only very scattered leukocytes expressed sialy-Le^x. LFA-1 and VLA-4 were expressed by the majority of infiltrating leukocytes.
Conclusion: Endothelial cell adhesion molecules expressed in CGA lesions may participate in the recruitment of inflammatory cells in the vessel wall. Their preferent expression in neovassels suggests that angiogenesis contributes to the perpetuation of infiltrates in CGA. Supported by FIS 95/0860 and FIS 96/0347.

62nd National Scientific Meeting. American College of Rheumatology. San Diego, California. Novembre de 1998

Arthritis and Rheumatism 1998;41(suppl):S117

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CIRCULATING SOLUBLE ADHESION MOLECULES IN PATIENTS WITH GIANT CELL ARTERITIS. CORRELATION BETWEEN SOLUBLE INTERCELLULAR ADHESION MOLECULE-1 (sICAM-1) LEVELS AND DISEASE ACTIVITY. Blanca Coll-Vinent, Carme Vilardell, Carme Font, Joaquim Orozco, Josep Hernández-Rodríguez, Alfons Lopez-Soto, Jordi Yagüe, Alvaro Urbano-Márquez, Josep M Grau, and Maria C Cid. Hospital Clinic, University of Barcelona, 08036 Barcelona, Spain.

Objective. To evaluate whether changes in levels of circulating adhesion molecules are related to disease activity in patients with giant cell arteritis (GCA).

Methods. A sandwich ELISA was used to measure soluble intercellular adhesion molecule-1 (sICAM-1), sICAM-3, vascular cell adhesion molecule-1 (sVCAM-1), E-selectin (sE-selectin) and L-selectin (sL-selectin) in sera and plasma from patients with GCA. A cross-sectional study was performed on 64 GCA patients at different activity stages: 36 active, 13 in recent remission (less than 2 years after starting treatment) and 15 in long-term remission (more than 2 years after starting treatment). 12 age and sex-matched healthy donors served as controls. Thirteen of GCA patients were evaluated longitudinally at the time of diagnosis and serially during follow-up.

Results. At the time of diagnosis, sICAM-1 levels were significantly higher in active GCA patients than in controls (350.91 ± 122.54 ng/mL versus 239.18 ± 52.21 ng/mL, $p<0.01$). In contrast, sICAM-3, sVCAM-1, sE-selectin and sL-selectin levels did not differ from those obtained in normal donors. With steroid administration, a decline in sICAM-1 levels was observed, reaching normal values when clinical remission was achieved (263.18 ± 92.7 ng/mL globally, 293.59 ± 108.39 ng/mL in the group of patients in recent remission, and 236.83 ± 70.02 ng /mL in those in long-term remission). In the thirteen patients followed longitudinally, sICAM-1 levels also normalized with clinical remission (225.87 ± 64.25 ng/mL in patients in recent remission, and 256.29 ± 75.15 ng/mL in those in long-term remission).

Conclusions. Circulating sICAM-1 levels clearly correlate with clinically apparent disease activity in GCA patients. Differences with results previously found in patients with other vasculitides may indicate that different pathogenic mechanisms contribute to vascular inflammation in different disorders.

Disclosure: work reported in this abstract was supported by:
Spanish Health Ministry (FIS 98/0443)

62nd National Scientific Meeting. American College of Rheumatology. San Diego, California. Novembre de 1998

Arthritis and Rheumatism 1998;41(suppl):S121

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CRANIAL ISCHEMIC EVENTS IN GIANT-CELL ARTERITIS PATIENTS (GCA) PRESENTING WITH APPARENTLY ISOLATED POLYMYALGIA RHEUMATICA (PMR).

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Background: Several studies indicate that GCA patients presenting with isolated PMR with no cranial symptoms are at very low risk of developing GCA-related ischemic events. However, this issue remains controversial since the occurrence of irreversible cranial ischemic complications in patients who have been suffering from isolated PMR for several months has been repeatedly reported

Objective. To assess the risk of developing cranial ischemic events in GCA patients presenting with apparently isolated PMR

Patients and Methods: Forty five GCA patients in whom disease manifested as an apparently isolated PMR for at least 2 months were selected among 220 individuals with biopsy-proven GCA. Clinical manifestations, as well as their chronological appearance before diagnosis were retrospectively recorded

Results: Thirty four patients presented with PMR for an average of 8 months (range 2 months-5 years) and later developed cranial symptoms for 5 weeks (range 1-48 weeks) which eventually led to diagnosis (group 1). Eleven patients, after presenting a self-limiting brief course of mild cranial symptoms lasting 2 weeks (range 1-8 weeks) developed PMR which was their chief complaint for 4 months (range 2-10 months) and the reason for admission (group 2)

Ten patients suffered permanent cranial ischemic complications at the moment of diagnosis (7 monocular blindness, 2 binocular blindness, 1 cerebral infarct), 1 transient diplopia and 2 amaurosis fugax. Thirteen out of 34 patients in group 1 (38.2%) suffered ischemic complications, either transient or permanent, and no patients in group 2 ever developed ischemic events ($p=0.0195$)

Conclusions: Isolated PMR may precede for long time the development of cranial GCA symptoms with all its potential complications. Among patients with GCA presenting with an apparently isolated PMR, the only patients who are at low risk of developing ischemic complications seem to be those in whom a self-limiting episode of cranial symptoms can be recorded.

Disclosure: work reported in this abstract was supported by

Spanish Health Ministry 98-0443 (FIS)

62nd National Scientific Meeting. American College of Rheumatology. San Diego. California. Novembre de 1998

Arthritis and Rheumatism 1998;41(suppl):S117

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INFLAMMATION-INDUCED ANGIOGENIC RESPONSE AND THE DEVELOPMENT OF CRANIAL ISCHEMIC COMPLICATIONS IN GIANT-CELL ARTERITIS PATIENTS

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Background: Neovascularization is frequently found in vasculitis lesions (Cid et al JCI 1993;91:977-85, Cid et al Arthritis Rheum 1994;37:1055-61). Angiogenesis may play a dual role in vasculitis. We have previously shown that newly-formed vessels strongly express adhesion molecules for leukocytes (Coll-Vinent et al Arthritis Rheum 1998;41:435-44) and, therefore, angiogenesis may amplify the inflammatory reaction. On the other hand, neovascularization may compensate for ischemia by generating new vessels.

Objective: To measure the intensity of neovascularization in temporal arteries from patients with giant-cell arteritis (GCA). To correlate the degree of neovascularization with the intensity of systemic inflammatory response and the development of cranial ischemic events.

Patients and methods: The study group consisted of 31 patients with biopsy-proven GCA. Five normal temporal arteries from patients with an alternate diagnosis were used as controls. Endothelial cells were immunostained with the lectin *Ulex Europaeus*. Neovascularization was quantitated as percentage of the artery circumference occupied by neovessels and multiplied by the number of layers in which they were distributed and a 0-6 score was established. Clinical data, particularly the occurrence of cranial ischemic events, and the presence of inflammatory parameters (fever, weight loss, ESR>85 and Hb<11 mg/dL) were recorded.

Results: In normal specimens, only scattered vasa vasorum at the adventitia were observed. In GCA lesions, neovascularization occurred at the adventitial layer and at the intima/media junction. Ischemic events occurred in 13 patients. Six had transient events (*amaurosis fugax* or diplopia) and 7 permanent visual loss. The neovascularization score was lower in patients with ischemic events (1.14 vs 2.15, p=0.03) and was higher in patients with 3 or more inflammatory parameters (2.55 vs 0.9, p=0.036).

Conclusion: Inflammation-induced angiogenic response is variable among GCA patients and correlates with the intensity of the systemic inflammatory response. Angiogenesis may compensate for ischemia at distal sites since a poor inflammatory response was associated with a higher frequency of cranial ischemic events.

Disclosure: work reported in this abstract was supported by:

Spanish Health Ministry FIS 98-0443