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BARCELONA

Autoimmunitat sinàptica com a causa d'encefalitis associada a teratoma, epilèpsia i recidives post-encefalitis herpètica a la infância
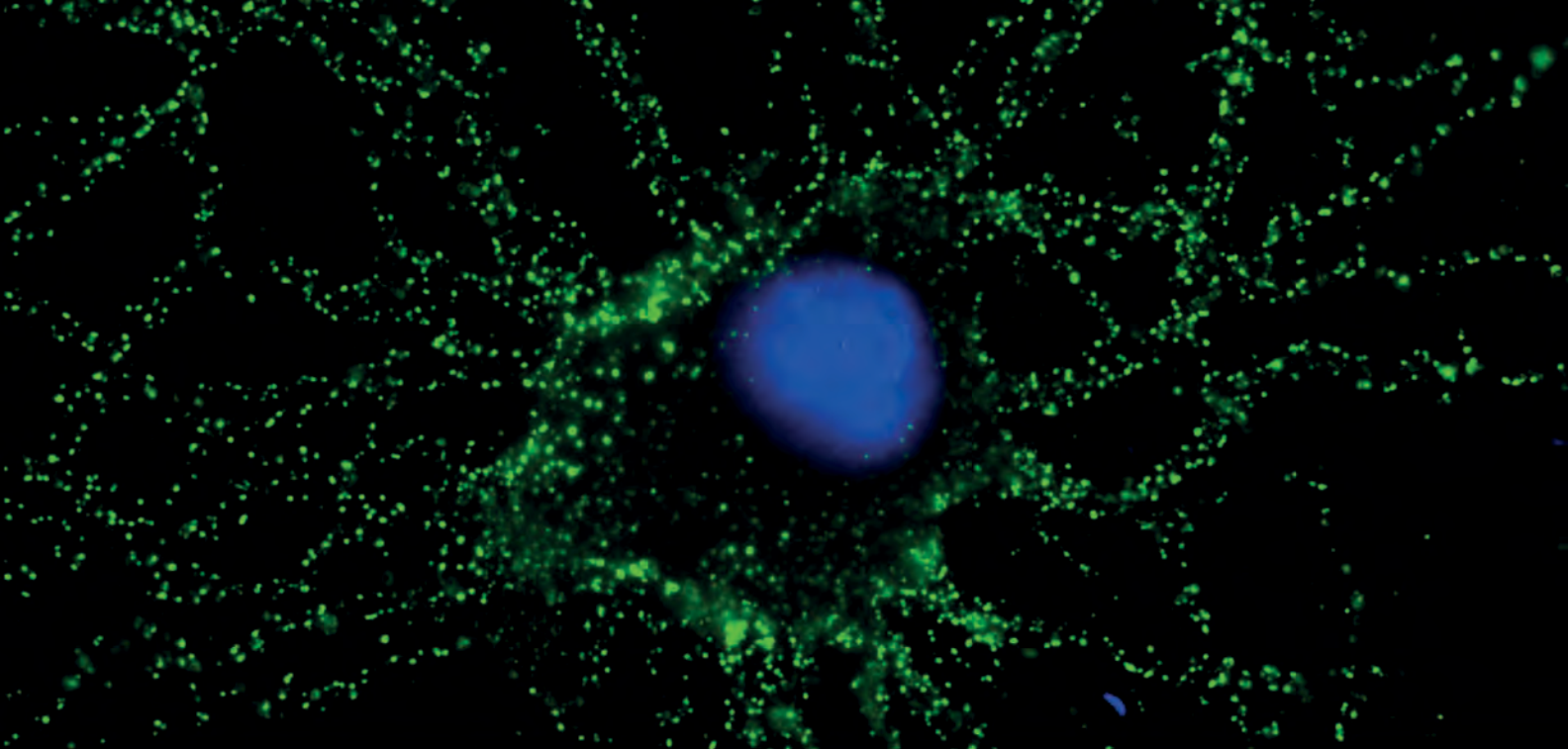
Thaís Armangué

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Thaís Armangué

Barcelona, Juny de 2015

Autoimmunitat sinàptica com a causa

d'encefalitis associada a teratoma, epilèpsia i

recidives post-encefalitis herpètica a la infància

Tesi doctoral realitzada per

Thaís Armangué Salvador

Per optar al grau de

Doctor per la Universitat de Barcelona

Programa de doctorat de Medicina
Línia de recerca
Neurociències clíniques i experimentals

Director de tesi

Josep Dalmau

Els estudis descrits han estat suportats en part per

Informe per la presentació en format de compendi de publicacions de la tesi doctoral titulada: "Autoimmunitat sinàptica com a causa d'encefalitis associada a teratoma, epilèpsia i recidives post-encefalitis herpètica a la infància"

Les publicacions presentades per l'obtenció del grau de doctor per la Universistat de Barcelona, Programa de Doctorat de Medicina, indicant factor d'impacte i participació de la doctoranda són les següents:

Publicacions:

I. **Títol: A novel treatment-responsive encephalitis with frequent opsoclonus and teratoma.**

   Autors: Armangue T, Titulaer MJ, Sabater L, Pardo-Moreno J, Gresa-Arribas N, Barbero-Bordallo N, Kelley GR, Kyung-Ha N, Takeda a, Nagao T, Takahashi Y, Lizcano A, Carr AS, Graus F, Dalmau J
   Revista: Ann Neurol 2014;75:435-41(highlighted)
   Factor d'impacte (percentil per especialitat): 11.193 (1er decil)
   Participació del doctorand: La doctoranda ha participat en el disseny conceptual i experimental de l'estudi, ha realitzat el treball tècnic en la seva totalitat, ha contactat amb els metges dels pacients i revisat la informació clínica de tots ells, ha escrit el manuscrit i ha desenvolupat les figures i taules. Aquest treball no es preveu que s'utilitzi en cap més tesi.

II. **Títol: Encephalitis with refractory seizures, status epilepticus, and antibodies to the GABA_A receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies.**

   Autors: Petit-Pedrol M*, **Armangue T***, Peng X*, Bataller L, Cellucci T, Davis R, McCracken L, Martinez-Hernandez E, Mason WP, Kruer MC, Ritacco DG, Grisold W, Meaney BF, Alcalá C, Sillevis-Smitt P, Titulaer MJ, Balice-Gordon R, Graus F, Dalmau J .*igual contribució
   Revista: Lancet Neurol 2014;13:276-86.
   Factor d'impacte (percentil per especialitat): 23.92 (1er decil)
   Participació del doctorand: Aquest treball té una co-autoria compartida per la Dra. Armangué (doctoranda) i per les biòlogues Mar Petit (predoctoral) i Xiaoyu Pen (postdoctoral). La doctoranda ha participat en el disseny conceptual i experimental de l'estudi, ha participat en el diagnòstic i seguiment de pacients de inclosos, ha contactat amb els

metges dels altres pacients i revisat la informació clínica de tots ells. La doctoranda ha participat en el treball tècnic per al desenvolupament del test diagnòstic per anticossos contra GABA$_A$R i ha escrit el manuscrit conjuntament amb Mar petit. Aquest treball es preveu que pugui ser utilitzat en la tesi doctoral en biomedicina de la biòloga Mar Petit.

III. **Títol: Pediatric Anti-N-methyl-D-Aspartate Receptor Encephalitis- Clinical Analysis and Novel Findings in a Series of 20 Patients.**

Autors: Armangue T, Titulaer MJ, Málaga I, Bataller L, Gabilondo I, Graus F, Dalmau J; Spanish Anti-N-methyl-D-Aspartate Receptor (NMDAR) Encephalitis Work Group.
Revista: J Pediatr 2013; 162:850-856.e2 (highlighted)
Factor d'impacte (percentil per especialitat): 4.358 (1er decil)
Participació del doctorand: La doctoranda ha participat en el disseny conceptual i experimental de l'estudi, ha realitzat el treball tècnic en la seva totalitat, ha participat en el diagnòstic i seguiment de pacients inclosos, contactat amb els metges dels altres pacients, i revisat la informació clínica de tots ells. La doctoranda ha escrit el manuscrit i ha desenvolupat les figures i taules. Aquest treball no es preveu que s'utilitzi en cap més tesi.

IV. **Títol: Herpes simplex virus encephalitis is a trigger for brain autoimmunity.**

Autors: Armangue T,* Leypoldt F*, Málaga I, Raspall-Chaure M, Marti I, Nichter C, Pugh J, Vicente-Rasoamalala M, Lafuente-Hidalgo M, Macaya A, Ke M, Titulaer MJ, Höftberger R, Sheriff H, Glaser C, Dalmau J. *igual contribució
Revista: Ann Neurol 2014;75:317-23.
Factor d'impacte (percentil per especialitat): 11.193 (1er decil)
Participació del doctorand: Aquest article té una co-autoria compartida amb el Dr. Leypoldt, MD, PhD. La doctoranda ha participat en el disseny conceptual i experimental de l'estudi, ha participat en el diagnòstic i seguiment de pacients inclosos, ha contactat metges dels altres pacients participants i revisat la informació clínica de tots ells, ha realitzat el treball tècnic, ha escrit el manuscrit i ha desenvolupat les figures i taules, juntament amb el Dr. Leypoldt. Aquest treball no es preveu que s'utilitzi en cap més tesi.

V. **Títol: Autoimmune relapses post-herpes simplex encephalitis in teenagers and adults.**

Autors: Armangue T, Moris G, Cantarín-Extremera V, Conde CE, Rostasy K, Erro ME, Portilla-Cuenca JC, Turón-Viñas E, Málaga I, Cabello-Muñoz B, Torres-Torres C, Llufriu S, González-Gutiérrez-Solana L, González G, Casado-Naranjo I, Rosenfeld M, Graus F, Dalmau J.

Revista: Neurology (acceptat, en premsa)

Factor d'impacte (percentil per especialitat): 8.303 (1er decil)

Participació del doctorand: La doctoranda és co-investigadora principal de l'estudi multicèntric prospectiu d'autoimmunitat en encefalitis herpètica. La doctoranda participat en el disseny conceptual i experimental de l'article, ha realitzat el treball tècnic en la seva totalitat, ha participat en el diagnòstic i seguiment de pacients inclosos, ha contactat metges dels altres pacients participants i revisat la informació clínica de tots ells, ha escrit el manuscrit i ha desenvolupat les figures i taules. Aquest treball no es preveu que s'utilitzi en cap més tesi.

Barcelona, 14 de juliol de 2015,

Director de la Tesi

Josep Dalmau

A Juan, als meus amics i a la meva família

A tu iaia

Abreviatures

ADEM	encefalomielitis aguda disseminada
AMPAR	receptor àcid propiònic α-amino-3-hidroxi-5-metil-4-isoxazol
Caspr2	proteïna associada a contactina-like 2
D2R	receptor dopamina 2
DPPX	proteïna semblant a dipeptidil-peptidasa 6
EHS	encefalitis pel virus herpes simple
GABA$_{A/B}$R	receptor àcid γ-amino-butíric (A / B)
GAD65	àcid glutàmic descarboxilasa 65
IgG	immunoglobulines G
LCR	líquid cefalorraquidi
LGI1	proteïna 1 inactivada del glioma rica en leucina
mGluR5	receptor metabotròpic del glutamat 5
NMDAR	receptor N-metil-D-aspartat
PCR	reacció amb cadena de la polimerasa
PERM	encefalomielitis progressiva amb rigidesa i mioclònies
RM	ressonància magnètica
SCLC	càncer pulmonar de cèl·lules petites
SNC	sistema nerviós central
TLR	toll-like receptor
TPO	peroxidasa tiroïdal
VGCC	canals de calci depenents de voltatge
VGKC	canals de potassi dependents de voltatge
VHS	virus herpes simple

Índex de continguts

Resum

Autoimmunitat sinàptica com a causa d'encefalitis associada a teratoma, epilèpsia i recidives post-encefalitis herpètica a la infància

Introducció: En els últims 7 anys, s'ha identificat una nova categoria d'encefalitis associada a anticossos contra superfície neuronal, que cursen amb psicosis, catatonia, crisis epileptiques, i moviments anormals. Aquests desordres són potencialment letals, però curables si es reconeixen i es tracten.

Hipòtesis: Un subgrup d'encefalitis pediàtriques prèviament considerades idiopàtiques o post-infeccioses estan causades per anticossos dirigits contra antígens sinàptics o de la membrana neuronal.

Objectius: Caracterització clínica e immunològica d'encefalitis pediàtriques d'etiologia no filiada, centrant-nos en 3 grups de pacients amb evidència preliminar d'un origen immunomediat: 1) encefalitis associada a la presència d'un teratoma a nivell sistèmic, 2) encefalitis amb crisis refractàries i status epilèptic, i 3) recidives neurològiques post-encefalitis herpètica (EHS).

Metodologia: Estudi clínic e immunològic dels pacients amb els desordres proposats. Per a la identificació dels antígens s'ha utilitzat una estratègia prèviament validada consistent en una selecció de pacients amb fenotips clínics similars associada a tècniques de cribratge immunològic desenvolupades i adaptades per la detecció d'anticossos dirigits contra antígens de superfície neuronal, incloent: immunohistoquímica de teixit nerviós, immunocitoquímica de cultius de neurones, immunoprecipitació, i caracterització d'antígens mitjançant espectrometria de masses. Els efectes dels anticossos dels pacients sobre els antígens diana s'han investigat en cultius de neurones disociades de hipocamp de rata.

Resultats: Per objectius específics: 1) Hem identificat un nou fenotip paraneoplàstic de la síndrome d'opsoclonus-mioclonus i d'encefalitis amb afectació de tronc i cerebel associades a la presència d'un teratoma sistèmic, sense anticossos NMDAR, que afecta predominantment a pacients joves, i que respon favorablement a immunoteràpia i a resecció tumoral. 2) Hem caracteritzat un nou anticòs dirigit contra el receptor sinàptic $GABA_A$ involucrat en encefalitis amb crisis refractàries i status epilepticus, que afecta de forma predominant a nens i adults joves. En el model in vitro aquests anticossos produeixen una disminució dels receptors $GABA_A$ a la sinapsis, i aquests efectes són reversibles en eliminar els anticossos del medi de cultiu. 3) Hem demostrat que l'EHS és un fort desencadenant d'autoimmunitat sinàptica i no restringida a NMDAR. Hem descrit per primer cop que els anticossos contra NMDAR i/o altres proteïnes sinàptiques poden ser causa de recidives autoimmunes post-EHS. En nens petits aquestes recidives es manifesten en forma de "coreoatetosis post-EHS", amb o sense crisis refractàries i status epilèptic acompanyants; i en adolescents i adults es manifesten predominantment amb símptomes psiquiàtrics.

Conclusions: Aquests estudis han resultat en la identificació de nous síndromes i respostes inmunològiques, en la caracerizació de les dianes antigèniques responsables, i en el desenvolupament d´un test diagnòstic per un grup de encefalitis infantils inmunomediades. Els estudis han canviat paradigmes de diagnòstic i tractament de les encefalitis infantils. Això es deu en gran part a que les investigacions han facilitat el diagnòstic i tractament precoç de desordres d'etiologia prèviament desconeguda, amb freqüent resposta a immunoteràpia.

I. Pròleg

Jane had been near the top of her class. She had "glandular fever" and did a little less well—but the light still danced in her green eyes. Then, when we walked the dog one evening, something seemed different. I had often read to her the old Scots ballad of Bonnie Kilmeny—the girl who was spirited off by fairies. A little of the sparkling precision of her conversation had gone. But if you have four kids you can't worry all the time.

I was away when it happened. She forgot to let a friend's dog out over the weekend and lost the memory of that weekend. Over a beer a paediatrician friend who had seen her because of amenorrhoea said he was worried—he didn't know why. A good neurologist could find nothing wrong. But Jane couldn't remember.

The psychiatrist was helpful, kind, and in the event one of the best doctors I have ever met. He was unwilling to commit himself to a name for this psychiatric syndrome. She was depressed; he thought depression needed treatment and wisely treated it with appropriate drugs. Two weeks later her feet started to sink into the carpet, and the floor to lift. Hallucinations. She was in hospital early next morning. We knew now that Super Jane would never be quite the same—nor would we. She was interviewed, talked to, and tested psychometrically, and (by now her blood cobalt and urinary rhubarb had been measured at least three times) as a standard precaution, her chest was X-rayed. There was a considerable mass in one lung.

The doctors were kind and talked of sarcoid. But it was clear to me that Jane had some sort of paraneoplastic syndrome. Jane herself was now aware of something wrong and yet somehow not too worried. In the next weeks Jane went from hallucinations into a parkinsonian state under the influence of the necessary drugs. A grey shaking shadow sitting in a corner of the room asking in a faraway terror-stricken voice, "What is happening to me? Why me?"

Mediastinoscopy clinched the diagnosis, and the sections were the saddest that ever crossed my microscope stage. Hodgkin's disease. In a psychotic. What right did we have to leave a psychotic in a twilight world for God knew how long? Jane, as she had been, would not have wanted it. There was considerable published evidence that the changes might, if related to the cancer, be due to an irreversible demyelinating lesion or a neurotropic virus. Could we allow staging of the Hodgkin's disease and treatment? The psychiatrist said we must not lose hope—and the medical machine ground on. Laparotomy. Splenectomy. Could an apparently psychotic frail teenager stand it? She did. Her psychotic condition got

worse—and all through this time, despite the anguish of dealing with someone who was mentally very sick, and in a ward with some even sicker people, her friends still came, week by week, to see her and sit and talk to her.

Two courses of chemotherapy (we felt) should do something if chemotherapy was going to work. Intravenous drugs. Bruised arms. Vomiting. Falling hair. We had been in to see her every day for three months and now went away for a weekend. When we came back she remembered what she had been doing the day before. Not just a straw in the wind but bending branches. It went on. More chemotherapy. The snow went and the instant prairie summer came. Day by day she was remembering more and more. Radiotherapy—more vomiting. The whole back of Jane's head bald. More radiotherapy. The oddest choice I have had to make; what do you give your teenage daughter the day she starts radiotherapy? Flowers of course. At last a weak, wan Jane—the back of her head bald—went on holiday. Soon Jane was remembering.

We learned that the standards of care in our university hospital are extremely high and are matched by the humanity of my colleagues. That the courage of a 16-year-old girl can meet extreme demands. That most of the concerns that beset the tranquillity of our daily lives are as chaff before the wind when it really blows; and it bloweth where it listeth. That our children, like our possessions and our lives, are lent not given. As Jane is lent, once more.

I record this story because it may help someone to a difficult diagnosis. In summary, recent memory loss may rarely be due to Hodgkin's disease, probably as a paraneoplastic event. It may be reversible and can be remembered as the Ophelia syndrome.

Extret de : Personal Paper, Alan Carr, The Lancet 1982[1]

La que trobeu aquí és l'emotiva carta que el radiòleg canadenc Alan Carr, va escriure a *The Lancet* l'any 1982. En ella aquest afligit pare explicava com en poques setmanes va veure com la super-Jane, la seva filla de 16 anys, que havia estat una de les alumnes més destacades de la seva classe desenvolupà dèficits de memòria i alteracions de conducta progressius que la portaren a ingressar en un hospital psiquiàtric. Gairebé per casualitat en una radiografia de tòrax de rutina es detectà una massa pulmonar. Tenia Hodgkin. Va rebre quimioteràpia. I la memòria, i amb ella la seva filla, retornaren.

Quan el Dr. Carr va escriure aquesta carta l'any 1982, no sabia la causa del que li havia passat a la seva filla però estava convençut de que la pèrdua de memòria de la Jane i l'alteració del seu comportament tenien una relació directa amb la seva malaltia de Hodgkin i el més important, havien sigut reversibles amb la quimioteràpia. El Dr. Carr anomenà aquest fenomen "Síndrome d'Ofèlia" en honor a la deessa grega que no podia recordar, i es decidí a publicar-lo perquè com ell mateix va explicar, potser podria ajudar a algú amb un diagnòstic difícil.

I això és precisament el més apassionant i bonic de la neurologia pediàtrica, l'especialitat per excel·lència de les malalties minoritàries. Sovint ens trobem amb casos difícils, excepcionals. Però el més important és observar-los, intentar-los entendre i sobretot, compartir-los. Aquí és on entra la meva altra gran passió, la recerca. Intentar anar un granet més enllà, intentar comprendre el per què del que has vist al costat del llit del pacient!

Gràcies a la recerca i als treballs previs del director d'aquesta tesi el Dr. Dalmau, que han sigut la base del treball que presentem en aquest manuscrit, ara podem explicar què li passava a la Jane. La síndrome d'Ofèlia o l'encefalitis paraneoplàstica associada al limfoma de Hodgkin, és una encefalitis autoimmune produïda per autoanticossos dirigits contra el receptor metabotròpic del glutamat 5 (mGluR5). Aquests anticossos, probablement

desencadenats per una disfunció dels mecanismes de tolerància immunològica generada pel seu limfoma, alteren la funció del receptor produint una disfunció de la transmissió sinàptica neuronal que causa els símptomes de la Jane i, el més important i tal i com havia observat el seu pare fa 30 anys sense entendre ben bé el perquè, aquesta alteració és reversible en eliminar aquests anticossos i el seu desencadenant, el tumor. El descobriment dels mecanismes responsables d'aquestes malalties i la possibilitat d'identificar-ne biomarcadors ha sigut l'inici indispensable per la correcta comprensió d'aquests desordres, establir-ne factors pronòstics, i segurament serà la base per explorar-ne possibles futures dianes terapèutiques.

En aquesta tesi el que pretendrem és precisament això, quelcom tan simple i alhora tan difícil com és donar "noms nous" a antigues malalties.

Thaís Armangué

Barcelona, 15 de maig de 2015
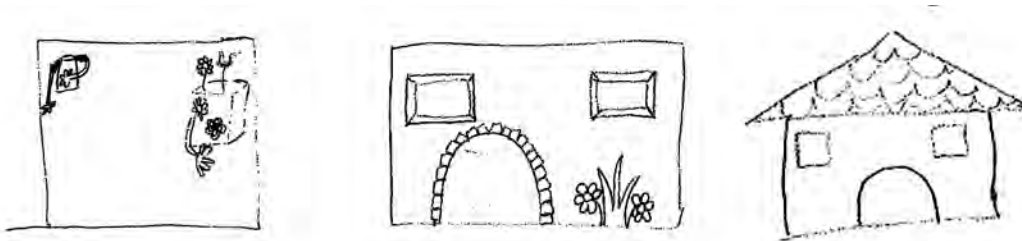
Representació d'una casa per part d'una pacient amb una recidiva autoimmune post-herpètica, abans (esquerra) i després de rebre tractament amb immunoteràpia (dreta). Armangue et al. Neurology (in press), i Article 5 de la Tesi.

II. Introducció

L'Encefalitis, encara un repte diagnòstic

Encefalitis es un terme referit a una patologia inflamatòria del cervell que causa alteració aguda de l'estat mental, crisis epilèptiques o dèficits neurològics focals, que freqüentment s'acompanya d'inflamació del líquid cefaloraquidi (LCR) i amb troballes a la ressonància magnètica cerebral (RM) variables que poden comprendre des de la normalitat fins a extenses lesions.[2] Aquesta, és una malaltia greu que resulta en 7.3 hospitalitzacions per 100.000 persones/any,[2] i que afecta a pacients de totes les edats i de totes les condicions immunològiques, doncs pot afectar a individus prèviament sans o a pacients severament immunodeprimits tot i que, les condicions basals del pacient seran determinants a l'hora d'establir-ne l'etiologia. Al debut de l'encefalitis el pronòstic dels pacients és incert ja que tot i ser una patologia severa amb una alta mortalitat durant el període agut (és causa de 1.400 morts/any als Estats Units) i amb un alt risc de seqüeles neurològiques i cognitives, depenent fonamentalment de la seva etiologia, la precocitat d'instauració d'un tractament etiològic quan és possible, i la qualitat de les mesures de suport durant la fase aguda, els pacients poden eventualment recuperar-se completament. Per tant, la identificació precoç de l'etiologia de l'encefalitis és fonamental per establir un potencial tractament etiològic i establir un pronòstic adequat i, es converteix en el principal repte del metge en el maneig d'aquests pacients. No obstant, les causes d'encefalitis són múltiples i la majoria de pacients són sotmesos a múltiples tests etiològics infecciosos sense arribar a identificar l'agent causal. Un estudi recent del *California Encephalitis Project*, un centre destinat a l'estudi de l'epidemiologia i de les etiologies dels pacients amb encefalitis, va descriure que el 63% de pacients romanen amb una etiologia desconeguda després d'una bateria de més de 16 agents infecciosos.[3] Múltiples estudis prospectius multicèntrics a nivell nacional amb pacients amb encefalitis realitzats en diferents països occidentals coincideixen en les mateixes xifres alarmants de pacients sense un diagnòstic etiològic després d'extensos i costosos estudis

microbiològics.[4,5] El recent descobriment de que varis tipus d'encefalitis són immunomediades i són causades per autoanticossos dirigits contra proteïnes sinàptiques o de la superfície neuronal és un concepte nou que ha donat un diagnòstic definitiu a molts d'aquests casos i que ha canviat el maneig diagnòstic i terapèutic dels pacients amb encefalitis de totes les edats.[6-10]

Antecedents històrics

Dels anticossos contra antígens intracel·lulars com a biomarcadors d'encefalitis límbica paraneoplàstica en pacients d'edat avançada als anticossos contra epítops extracel·lulars com a causa d'encefalitis autoimmune a l'edat pediàtrica

El terme de "*síndrome paraneoplàstica*" es refereix al conjunt de símptomes i/o signes que es produeixen com a conseqüència d'un càncer però que no es poden explicar per un efecte massa del tumor ni per la presència local de cèl·lules tumorals o metàstasis. Tot i que aquest terme no es va introduir fins a mitjans dels anys 1950,[11] i no va ser àmpliament usat a la literatura mèdica fins als anys 1970,[12] havien estat descrites síndromes neurològiques i no neurològiques associades a càncer com la *phlegmasia alba* (actualment coneguda com la *síndrome de Trousseau o* tromboembolisme venós associada a adenocarcinoma de pulmó o pàncrees), molts anys abans.[13] Les primeres descripcions clíniques de desordres neurològics associats a càncer remunten al segle XIX quan l'any 1887 Oppenheim i Siemerling van descriure 2 pacients amb carcinoma gàstric i neuropatia perifèrica.[14] Oppenheim també va ser el primer en formular la hipòtesis el 1888 que símptomes neurològics centrals podien ser manifestacions a distància d'un càncer quan descriví una dona amb hemiparèsia dreta i afàsia desenvolupades només uns dies abans de morir per un càncer estès sense trobar restes tumorals metastàtiques a nivell del sistema nerviós central (SNC) a l'autòpsia.[15] Tot i que en aquell moment Oppenheim suggerí

l'efecte d'una "substància tòxica" produïda pel càncer com a causant dels símptomes, és probable que aquesta pacient presentés micro-metàstasis no detectades en aquell moment doncs actualment sabem que l'hemiparèsia i l'afàsia rarament representen síndromes paraneoplàstiques.[16] La primera descripció clínica d'una síndrome neurològica que actualment podem reconèixer com a "veritablement paraneoplàstica" va ser l'any 1919 quan Brouwer va descriure una dona amb una síndrome cerebel·losa subaguda i un probable adenocarcinoma d'ovari (a qui ell anomenà sarcoma de pelvis).[17] Altre cop en aquest cas es va formular la hipòtesis d'una potencial "substància tòxica" produïda pel càncer com a causant dels símptomes al trobar-se a l'autòpsia una pèrdua de cèl·lules de Purkinje del cerebel. Posteriors hipòtesis de la relació entre càncer i símptomes neurològics inclogueren la competició entre les cèl·lules cancerígenes i les neurones del gangli dorsal per un "substrat essencial",[18] i un reflex resultant de l'estimulació del nervi vague per part del tumor com a causa de neuronopatia sensitiva associada a càncer,[19] corresponent actualment a una de les síndromes paraneoplàstiques més reconegudes, la qual es caracteritza per neuropatia i càncer pulmonar de cèl·lules petites (SCLC), associats a anticossos contra la proteïna intracel·lular Hu.[16]

El concepte de l'autoimmunitat com a possible causa de síndromes neurològiques no es va plantejar fins als anys 1960 quan diferents descripcions clíniques i patològiques associaren la presència d'un càncer amb quadres clínics neurològics diversos i troballes inflamatòries a les necròpsies, suggerint una possible resposta del sistema immune contra les cèl·lules del sistema nerviós.[20,21] En aquell moment però, no es descartava que els infiltrats inflamatoris fossin producte d'una infecció viral[22] no identificada o d'una reacció inflamatòria secundària a la destrucció tissular produïda pel càncer.[23,24] L'any 1968, *Corsellis i col·laboradors* van descriure per primer cop l'encefalitis límbica paraneoplàstica com a entitat clínicopatològica.[25] L'encefalitis límbica es

caracteritza per afectació inflamatòria d'àrees límbiques, típicament cursant amb alteració de la memòria, crisis epilèptiques del lòbul temporal i trastorns afectius. Per al seu diagnòstic és precís demostrar alteracions inflamatòries en aquestes àrees a nivell patològic (en biòpsia o necròpsia); o en el seu defecte afectació d'àrees mediotemporals a la RM cerebral, alentiment o anomalies epileptiformes localitzades en àrees temporals en l'electroencefalograma (EEG), i/o un LCR de característiques inflamatòries.[26]

Actualment sabem que l'encefalitis límbica afecta de forma gairebé exclusiva a pacients adults i s'associa en la majoria dels casos a la presència d'un càncer (principalment SCLC en majors de 40 anys o germinoma testicular en pacients més joves).[26] El seu diagnòstic continua sent un repte doncs símptomes similars (alteracions de memòria, crisis epilèptiques i alteracions del comportament), poden ser causats per altres complicacions del càncer, incloent metàstasis cerebrals, infeccions, dèficits nutricionals o metabòlics, episodis vasculars o complicacions del tractament. A més l'aparició dels símptomes neurològics sovint precedeix a la identificació del càncer subjacent complicant-ne encara més el diagnòstic.[26] Als anys 1980 i 90 el descobriment de que alguns d'aquests pacients associaven en sèrum i LCR anticossos dirigits contra epítops intracel·lulars neuronals com Hu, Yo, CV2/CRMP5 o Ma2 (Ta) ,[27-29] va ser de gran ajuda per al diagnòstic d'aquestes entitats. La identificació d'aquests anticossos intracel·lulars o onconeuronals en un pacient amb encefalitis límbica o amb una altra síndrome paraneoplàstica ben definida, com la degeneració cerebel·losa, l'encefalomielitis o una polineuropatia perifèrica sensitiva, permetia establir l'origen paraneoplàstic de la síndrome neurològica, i alertar de la presència d'un tumor ocult en els pacients en els quals la síndrome neurològica precedia el diagnòstic del càncer.[30,31] Tot i la seva importància a la pràctica clínica com a biomarcadors diagnòstics en les síndromes paraneoplàstiques, el fet que l'epítop d'aquests anticossos sigui intracel·lular, que els pacients presentin una escassa resposta a tractament immunosupressor, la falta de reproducció del

quadre neurològic en models animals en els quals s'injecten aquests anticossos i, la presència d'aquests anticossos tot i que en títols menors, en pacients amb neoplàsies sense símptomes neurològics associats, han fet qüestionar la seva patogenicitat. Per aquests motius, actualment es postula que aquests anticossos no són els responsables directes dels símptomes neurològics associats sinó que representen el component humoral d'una complexa resposta immunològica mitjançada per cèl·lules T citotòxiques contra antígens intraneuronals expressats per les cèl·lules tumorals.[32]

L'any 2001 es va descriure que alguns pacients amb encefalitis límbica amb associació tumoral (e.x. timoma) o sense, que eren seronegatius per als anticossos onconeuronals clàssics, tenien en sèrum i/o LCR anticossos dirigits contra les subunitats Kv1.1 i Kv1.2 dels canals de potassi dependents de voltatge (VGKC).[33] Tot i que més tard es va descobrir que aquesta assumpció era errònia, i que l'epítop al qual anaven dirigits aquests anticossos no era el VGKC sinó dues proteïnes sinàptiques associades (la proteïna 1 inactivada del glioma rica en leucina [LGI1] i la proteïna associada a contactina-like 2 [Caspr2],[34-38] és important destacar que per primer cop aquests autoanticossos no anaven dirigits contra proteïnes intracel·lulars sinó contra proteïnes sinàptiques o de la membrana cel·lular. A més, aquests pacients a diferència dels pacients amb anticossos onconeuronals clàssics, sí que responien favorablement a immunoteràpia i amb freqüència no es trobava un tumor subjacent.

No va ser fins l'any 2005 amb el treball publicat per *Ances i col.laboradors*[39] quan el concepte d'encefalitis amb resposta favorable a immunoteràpia associada a anticossos contra superfície neuronal deixà de restringir-se a pacients amb encefalitis límbica i a pacients amb anticossos contra "VGKC", marcant l'inici de la identificació molecular dels autoantígens diana i de la comprensió d'aquests desordres. En aquest treball, dirigit pel director d'aquesta tesi, es presentaren 6 pacients adults amb diferents tipus

d'encefalitis amb o sense associació tumoral (2 tenien teratomes, 2 timomes i, 2 no tenien càncer), que respongueren de forma notòria quan van ser tractats amb immunoteràpia i/o extirpació tumoral quan fou indicat. Aquesta diferència en la resposta a la immunoteràpia comparat amb les síndromes paraneoplàstiques clàssiques i la troballa en tots ells d'anticossos que reaccionaven contra la superfície neuronal (només un d'ells amb un patró en immunohistoquímica similar al prèviament descrit com "anti-VGKC"), va fer pensar als investigadors que es tractava d'una nova categoria d'encefalitis autoimmune.

Poc després, en el treball publicat per *Vitaliani i col.laboradors*[40] es descrivia minuciosament una nova síndrome neurològica comú (símptomes psiquiàtrics predominants, crisis epilèptiques, dèficits de memòria i disfunció autonòmica) en 4 dones joves amb un teratoma d'ovari, que associaven anticossos contra superfície neuronal diferents a "anti-VGKC". Gràcies a l'optimització en aquest treball de les tècniques d'immunohistoquímica en teixit cerebral de rata adaptades per detectar anticossos contra la superfície neuronal (Figura 1), i la identificació en poc temps de centenars de pacients d'arreu del món afectes d'aquesta nova síndrome, van permetre l'any 2007 a *Dalmau i col·laboradors*, la identificació de l'autoantigen diana associat mitjançant immunoprecipitació: la subunitat GluN1 (o NR1) del receptor N-metil-D-aspartat (NMDAR),[6] així com la caracterització clínica d'aquesta nova entitat, avui coneguda com encefalitis anti-NMDAR. Actualment se sap que aquesta encefalitis afecta a pacients de totes les edats, principalment nens i adults joves, i que l'associació tumoral, generalment un teratoma d'ovari, és sexe i edat depenent, sent la seva associació a l'edat pediàtrica molt inusual (veure: Focus en l'encefalitis autoimmune a l'edat pediàtrica i noves perspectives, pàgina 30).[41]

Figura 1: Demostració d'anticossos antineuronals amb tècniques d'immunofluorescència e immunohistoquímica
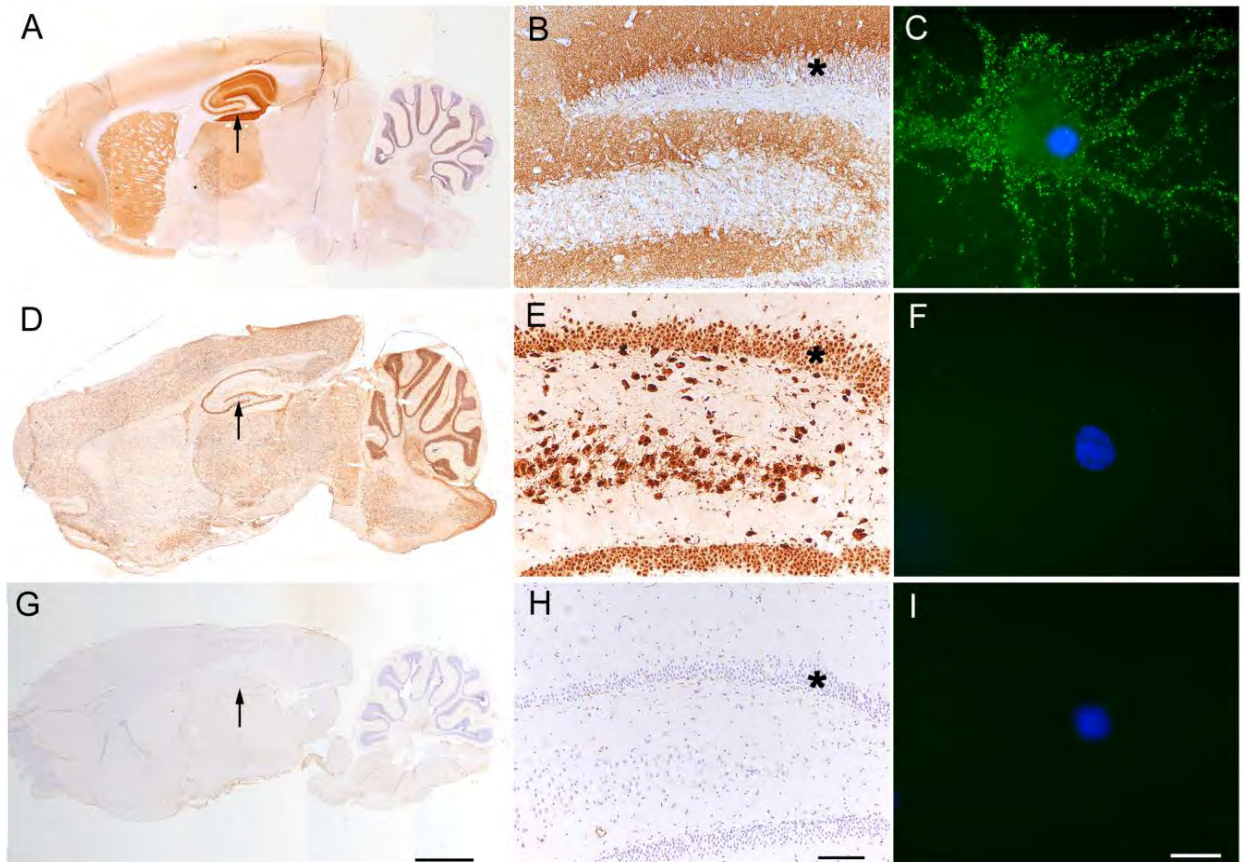
Immunohistoquímica en cervell de rata usant LCR de pacients amb anticossos anti-NMDAR (A), Hu (D), i LCR d'un pacient control (G). Les fletxes marquen àrees en les que es mostra major amplificació en B, E i H. S'observa que el patró de reactivitat dels anticossos anti-NMDAR és expressat a la superfície cel·lular i en regions sinàptiques, i és diferent del patró de reactivitat dels anticossos anti-Hu (en el quals la diana, HuD, és intracel·lular). En B, E i H l'asterisc indica el girus dentat. C, F i I mostren el LCR corresponent incubat amb cultius de neurones vives dissociades d'hipocamp de rata (vives, no permeabilitzades). Només els anticossos anti-NMDAR reaccionen contra antígens de superfície en les neurones vives (tinció verda en C); els anticossos anti-Hu no mostren reactivitat donat que els anticossos no penetren la cèl·lula (F), a més hi ha una falta de reactivitat en el LCR control (I). En C, F, i I el nucli de les neurones es marca amb DAPI. Escala en G = 2000 μm, escala en H = 100 μm, escala en I = 20 μm.

Figura adaptada de:
Armangue T, Petit M, Dalmau J. J Child Neurol 2012;27:1460-1469.

Mètodes similars permeteren durant als anys següents, la caracterització dels diferents antígens sinàptics i receptors de membrana diana dels anticossos associats a les altres síndromes neurològiques descrites per *Ances i col·laboradors* uns anys abans com: els anticossos dirigits contra els receptors de l'àcid propiònic α-amino-3-hidroxi-5-metil-4-isoxazol (AMPAR, Figura 2)[42] o de l'àcid γ-amino-butíric B (GABA$_B$R), [43] ambdós anticossos associats a encefalitis límbica paraneoplàstica considerada prèviament seronegativa, o els auto-antígens responsables dels quadres originàriament atribuïts als anticossos contra "VGKC", que en realitat anaven dirigits contra altres proteïnes sinàptiques associades incloent la proteïna LGI1 [44] i la proteïna Caspr2.[34,35] Aquestes tècniques també permeteren l'any 2011 la identificació del receptor metabotròpic del glutamat mGluR5 com autoantigen diana de l'encefalitis autoimmune associada al limfoma de Hodgkin o Síndrome d'Ofèlia (veure Pròleg),[45] i més recentment la identificació dels anticossos contra la proteïna semblant a dipeptidil-peptidasa 6 (DPPX), aquest cop sí una subunitat reguladora dels canals de potassi Kv4.2, associada a encefalitis i hiperexcitabilitat del SNC.[46]

Figura 2: Identificació del receptor AMPA com un autoantigen relacionat amb pèrdua de memòria i alteració de conducta
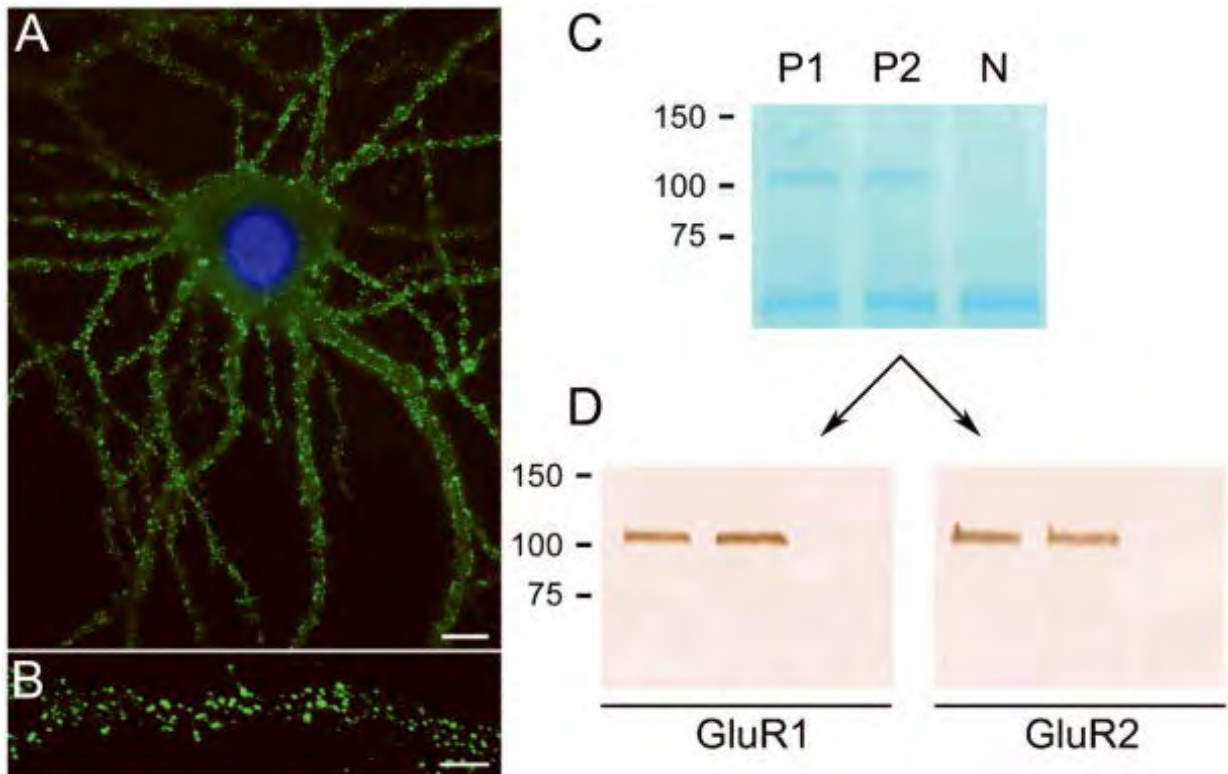
Cultiu de neurones d'hipocamp dissociades i incubades (vives, no permeabilitzades) amb el LCR del pacient. S'observa la intensa reactivitat dels anticossos del pacient amb antígens de superfície (A); escala = 10 μm. L'anàlisis amb microscòpia confocal suggereix que els antígens estan concentrats en complexes (clusters) a les dendrites (B); escala = 10 μm. La precipitació d'aquest antígens amb el LCR de 2 pacients es mostra en un gel en el que les proteïnes estan visualitzades amb EZBlue (C). Els anticossos dels pacients (P1 i P2) precipiten una banda de ~100 kDa; aquesta banda no s'observa en el precipitat amb el LCR control (N). L'anàlisis d'una banda de 100 kDa mitjançant espectrometria de masses demostrà seqüències derivades de les subunitats GluR1/GluR2 del receptor AMPA. La banda de ~50 kDa que s'observa en totes las mostres correspon a IgG. La transferència de la banda proteica a nitrocel·lulosa e immunoblot amb anticossos específics contra GluR1 y GluR2 confirmà que la banda de 100 kDa contenia les subunitats GluR1 i GluR2 del receptor AMPA (panells en D).

Figura adaptada de:
Meizan Lai M, Hughes EG, Peng X et al. Ann Neurol 2009; 65(4): 424–434.

Estat actual del tema: Classificació de les encefalitis autoimmunes segons a localització de l'autoantigen diana

Des de l'any 2005, la freqüència del descobriment de noves síndromes neurològiques associades a anticossos contra superfície neuronal i proteïnes sinàptiques ha estat de una o dos per any. En aquestes entitats, els anticossos van dirigits contra receptors i proteïnes sinàptiques involucrades en la transmissió sinàptica, plasticitat, o excitabilitat neuronal, i s'associen a síndromes que tot i ser severes, freqüentment responen a immunoteràpia.[10,47,48] Les síndromes resultants varien segons l'anticòs involucrat, amb fenotips que semblen aquells en els que la funció de l'antigen és modificada genèticament o farmacològicament (Taula 1).[10,47,48]

Taula 1: Encefalitis causades per anticossos contra antígens sinàptics

Antigen	Símptomes Neurològics	Edat, sexe, presència de tumor, resposta a immunoteràpia
NMDAR (subunitat GluN1)	Símptomes psiquiàtrics, alteració en el llenguatge, moviments anormals, crisis epilèptiques, disminució del nivell de consciència, inestabilitat autonòmica	Nens (40%) i adults joves (edat mediana 19 anys), 80% dones. La presència del tumor (majoritàriament un teratoma) varia segons l'edat, el sexe, la raça (9-55%); la majoria teratoma d'ovari 80%; bona recuperació amb immunoteràpia
GABA$_B$R (subunitat B1)	Encefalitis límbica clàssica. Crisis prominents (GABA$_B$R), símptomes psiquiàtrics aïllats (AMPAR), hiponatrèmia i breus crisis tònico-clòniques (LGI1)	Adults (edat mediana 62, 80 i 60 anys respectivament), Dones (50, 90, i 35% respectivament) Associació a càncer (pulmó, mama, timus) freqüent (60-70%) en GABA$_B$R i AMPAR i infreqüent (<10%) en LGI1 Bona resposta a immunoteràpia
AMPAR (subunitat Glu R1/2)		
LGI1		
CASPR2	Síndrome de Morvan, encefalitis límbica, hiperexcitabilitat del nervi perifèric	Adults (edat mediana 60 anys, predomini masculí) Experiència limitada, ~30% timoma
mGluR5	Síndrome d'Ofèlia: Canvis de personalitat, crisis epilèptiques, dèficit de memòria i regressió cognitiva	Adolescents i adults joves Associació freqüent a limfoma de Hodgkin
DPPX	Encefalitis difusa, símptomes prodròmics (diarrea), símptomes psiquiàtrics, tremolor, mioclònies, atàxia, nistagmus i hiperekplèxia	Adults Experiència limitada, no associació a càncer, resposta a immunoteràpia

Donat a l'ampli espectre de símptomes provocats per aquetes encefalitis, incloent alteracions de comportament, psicosis, catatonia, dèficits de memòria, crisis epilèptiques, moviments anormals i desregulació autonòmica, els pacients requereixen un maneig multidisciplinari, i el seu descobriment ha afectat a múltiples especialitats com la neurologia, la psiquiatria, la pediatria, la medicina interna e intensiva, la psicologia clínica o la medicina rehabilitadora.[49]

Al contrari que les síndromes paraneoplàstiques clàssiques com l'encefalitis límbica associada a anticossos intracel·lulars o onconeuronals, les encefalitis causades per autoimmunitat sinàptica afecten a pacients de totes les edats, inclús algunes d'elles com l'encefalitis anti-NMDAR afecten de forma predominant a l'edat pediàtrica, poden desenvolupar-se amb o sense la presència d'un tumor, i s'associen a anticossos que es dirigeixen contra epítops o dianes extracel·lulars de la membrana neuronal. A més a més, en els desordres en els quals els efectes dels anticossos s'han investigat, s'ha demostrat que l'aplicació dels anticossos (LCR o immunoglobulines G [IgG] del sèrum dels pacients) a cultius primaris de neurones hipocampals *in vitro,* produeix una alteració funcional i/o morfològica dels antígens corresponents, i aquests efectes són reversibles en eliminar els anticossos del mètode de cultiu.[41]

Més recentment, *Planagumà i col·laboradors* han desenvolupat un modelmurí en el qual la transmissió passiva de LCR de pacients amb encefalitis anti-NMDAR per infusió ventricular contínua (mitjançant bombes osmòtiques durant 14 dies), provoca alteracions de memòria i comportament d'aquests animals.[50] L'estudi necròptic dels hipocamps dels ratolins mostrà un dipòsit progressiu d'anticossos anti-NMDAR i una disminució progressiva exclusiva dels receptors NMDA de la sinapsis, sense afectar a altres proteïnes o receptors sinàptics com PSD95 o AMPAR. Tant els símptomes com els nivells dels receptors NMDA de la sinapsis es restabliren gradualment després de la cessió

de la bomba d'infusió, establint definitivament la patogenicitat d'aquests anticossos (Fig. 3).[50]

La reversibilitat dels efectes dels anticossos és recolzada a nivell clínic pel fet que la disfunció neurològica associada a les encefalitis autoimmunes causades per anticossos contra superfície neuronal amb freqüència respon a immunoteràpia, i en el cas de l'encefalitis anti-NMDAR fins un 70-85% dels pacients poden presentar una recuperació completa.[51]

Figura 3: Model murí d'encefalitis anti-NMDAR
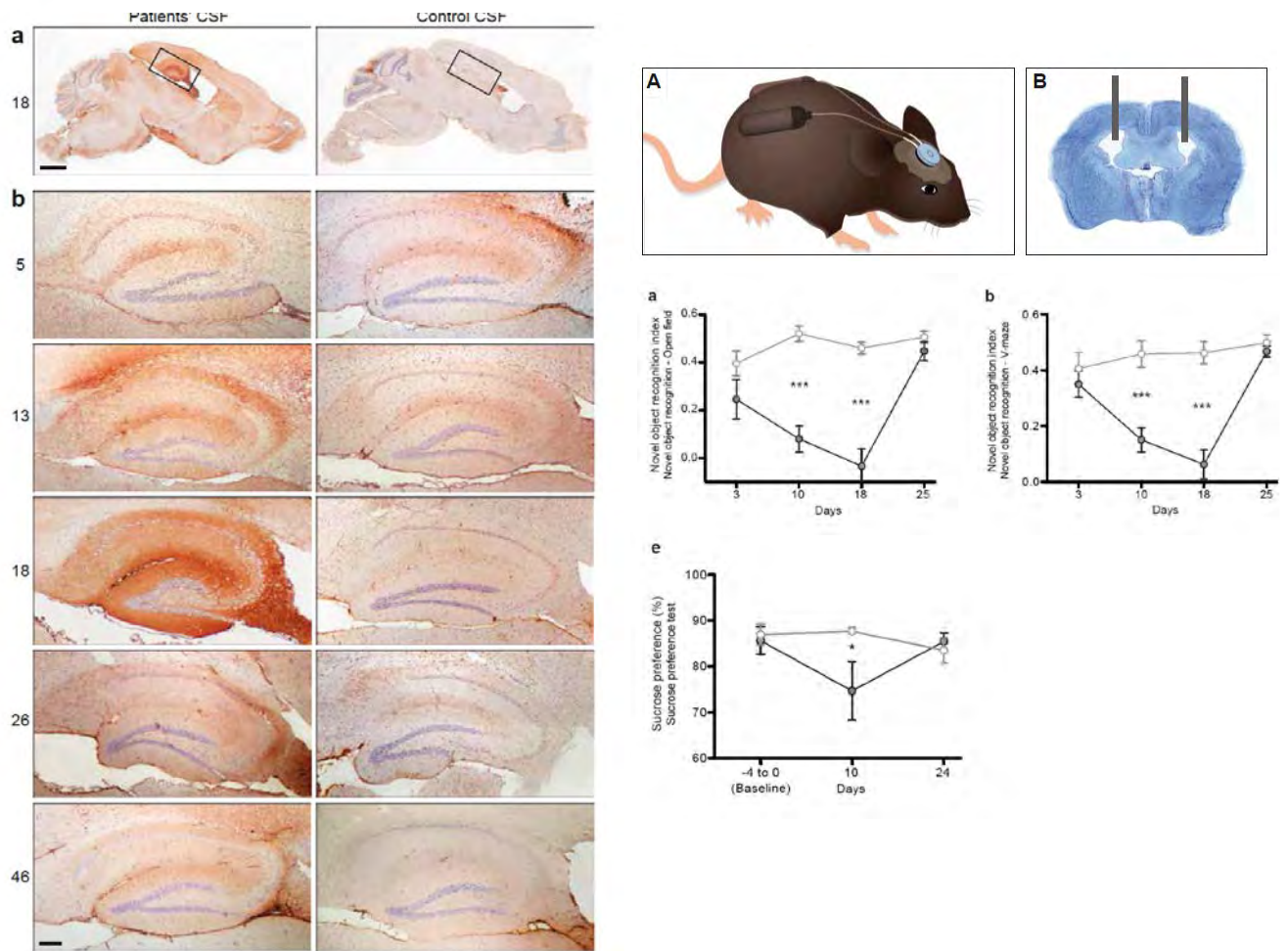
1. (Panells a i b esquerres): Animals infosos amb LCR de pacients amb anticossos NMDAR tenen un increment progressiu de dipòsit de IgG humana a l'hipocamp. (a i b) Immunotinció de IgG humana en seccions cerebrals sagitals (a) i hipocamp (b) d'animals representatius infosos amb LCR de pacients (esquerra) i LCR control (dreta), sacrificats als dies experimentals indicats. Escala: A=2mm; B=200microm. **2.** (Panells A i B superiors drets): Model experimental murí i localització dels catéters ventriculars per la infusió de LCR de pacients o LCR control mitjançant bombes osmòtiques durant 14 dies. **3.** (Panells mitjos e inferior drets): La infusió de LCR de pacients amb anticossos contra NMDAR causa dèficits de memòria, anhedònia, i comportament depressiu. (a i b) Índex de reconeixement d'un nou objecte (NOR) en el paradigma NOR de camp obert (a) o V-maze (b), en animals tractats amb LCR de pacients (cercles grisos) o LCR control (cercles blancs). Un índex elevat indica una millor memòria de reconeixement de l'objecte. (e) Preferència per l'aigua amb sucrosa en animals infosos amb LCR de pacients (gris) o LCR control (blanc). Un percentatge menor indica anhedònia.

Figura adaptada de:
Planaguma J, Leypoldt F, Mannara F, et al. Brain 2015;138:94-109.

Focus en l'encefalitis autoimmune a l'edat pediàtrica i noves perspectives

En la població pediàtrica, l'encefalitis causada per anticossos més freqüent, és aquella en la que els anticossos van dirigits contra el receptor NMDA (encefalitis anti-NMDAR) .[52] Estudis recents mostren que la seva freqüència en edats joves pot ser superior a la freqüència individual de les diferents encefalitis virals i, que és la segona causa d'encefalitis autoimmune després de l'encefalomielitis aguda disseminada (ADEM).[53] L'encefalitis anti-NMDAR en la qual fins el 40% dels pacients són pediàtrics, es presenta en adolescents i adults joves en forma d'una síndrome clínica molt característica. Inicialment, després d'una primera fase prodròmica amb febre o amb símptomes pseudogripals, els pacients debuten típicament amb trastorns psiquiàtrics i alteracions de la conducta, incloent pensaments paranoides, insomni, al·lucinacions visuals i auditives. En unes setmanes la síndrome progressa i els pacients presenten alteracions del moviment (característicament discinèsies de predomini orofacial) , crisis epilèptiques, disminució del nivell de consciència i disautonomia.[6] En nens petits, tot i que la síndrome és similar, els símptomes de presentació poden ser diferents.[52] L'estudi més extens que inclogué més de 500 pacients amb aquesta encefalitis, dels quals 180 eren menors de 18 anys, mostrà que la forma més comú de presentació en nens menors de 12 anys eren els trastorns de moviment, les crisis epilèptiques i el comportament anormal.[51] En nens petits les alteracions del comportament inclouen disminució d'interacció social, agitació, agressió, i canvis en l'humor i de personalitat. Els mecanismes que indueixen la producció d'aquests anticossos són desconeguts, tot i que en un subgrup de pacients, la presència d'un tumor (freqüentment teratoma d'ovari), que expressa l'antigen neuronal diana probablement contribueix a desencadenar la resposta immunològica.[41] En nens, la presència d'un tumor (principalment teratoma), és infreqüent. Mentre

que aproximadament el 56% de dones majors de 18 anys tenen teratomes uni- o bilaterals d'ovari, aquests tumors es troben en el 30% de les noies menors de 18 anys, i en un 9% de les nenes menors de 14.[52] En homes la presència d'un teratoma testicular és rara, i aquest tumor no s'ha descrit en nens de sexe masculí amb encefalitis anti-NMDAR.

Tot i que en la majoria dels pacients sense un teratoma la causa de l'encefalitis anti-NMDAR roman desconeguda, estudis preliminars descriuen una diferent estacionalitat en el debut de l'encefalitis anti-NMDAR en pacients joves amb o sense teratoma associat. En un estudi recent en el que s'inclogueren retrospectivament 29 pacients menors de 21 anys diagnosticats d'encefalitis anti-NMDAR en un sol centre, s'evidencià que 18/23 (78%) dels pacients sense un teratoma associat, havien debutat durant els mesos càlids (març-setembre), mentre que els 6 pacients amb un tumor associat (5 teratomes, 1 sarcoma d'Ewing) ho havien fet durant els mesos més freds (octubre-febrer), p<0,001).[54] Aquest biaix en l'estacionalitat al debut, no s'evidencià però en pacients amb la Síndrome d'opsoclonus-mioclonus amb o sense neuroblastoma associat diagnosticats en el mateix centre. Aquestes troballes obren interessants qüestions sobre l'etiopatogènia de l'encefalitis anti-NMDAR. Recentment s'ha descrit que variacions estacionals en malalties autoimmunes podrien estar produïdes per un perfil immunitari i una composició cel·lular de la sang variable al llarg de l'any, e invertida entre Europa i Oceania.[55] L'estudi de *Dopico* et al. mostra que durant els mesos més freds, el sistema immunològic tindria un perfil pro-inflamatori amb nivells incrementats del receptor de IL-6 soluble, proteïna C reactiva, i biomarcadors de risc per malalties cardiovasculars, psiquiàtriques i autoimmunes. El fet de que s'hagi descrit de que l'encefalitis anti-NMDAR no relacionada amb teratoma es produeixi més freqüentment en mesos càlids, xoca però amb aquestes noves troballes d'un perfil més auto-inflamatori durant els mesos freds, i ens podria portar a la hipòtesis preliminar de que altres factors com les infeccions podrien jugar un paper. Les infeccions són

àmpliament reconegudes per tenir diferències estacionals. Mentre que l'Enterovirus, el virus de l'Oest del Nil o el Lyme, predominen a la primavera o a l'inici de l'estiu, altres patògens com el virus influenza tenen un predomini durant els mesos d'hivern. Tot i això, encara que són freqüents els símptomes pseudogripals precedint l'encefalitis anti-NMDAR, i que els estudis microbiològics no es realitzen de forma estandarditzada, són una minoria els casos en els que es demostra un patogen infecciós en aquesta encefalitis. De totes maneres les troballes d'aquests estudis, van en contra de que l'encefalitis anti-NMDAR estigui relacionada amb les vacunes (generalment d'administració durant tot l'any), o amb patògens de predomini a l'hivern com el virus influenza.[54]

El recent descobriment d'aquestes entitats ha permès en els últims anys el diagnòstic i tractament de pacients fins ara catalogats amb termes descriptius com *encefalitis letàrgica* o *l'encefalitis de Hashimoto*, que no n'explicaven els mecanismes patogènics responsables. A mode d'exemple, dos estudis recents han mostrat que aproximadament el 50% dels pacients categoritzats prèviament com *encefalitis letàrgica hipercinètica* tenien en realitat encefalitis anti-NMDAR.[56] La identificació d'un biomarcador comú (anticossos contra NMDAR) també ha permès reconèixer com una mateixa entitat (l'encefalitis anti-NMDAR), és la única responsable d'algunes síndromes neurològiques pediàtriques prèviament descrites a la literatura mèdica com entitats diferenciades d'etiologia desconeguda com foren: la "Síndrome autista reversible en nens amb encefalopatia aguda"[57] (Delong, 1981), el "Coma amb moviments anormals i dèficits cognitius a llarg termini"[58] (Sebire 1992), o la "Síndrome encefalopàtica i corea immunomediada pediàtrica"[59] (Hartley 2002).

Tot i que tradicionalment el diagnòstic de les encefalitis autoimmunes en nens ha estat menys freqüent que en adults, el fet de que en aquestes edats hi hagi una alta incidència de patologies autoimmunes probablement provocada

per la immaduresa del sistemes de tolerància immunològica, i el fet de l'encefalitis per autoimmunitat sinàptica més freqüent, l'encefalitis anti-NMDAR, afecti de manera predominant a pacients pediàtrics i adults joves, recolzen la hipòtesis de que són patologies infradiagnosticades en aquestes edats. Per aquest motiu, creiem que és molt possible que l'augment del reconeixement en els últims 2 anys d'aquestes malalties en nens es tradueixi en la identificació de noves síndromes infantils causades per autoanticossos contra superfície neuronal tal com ha succeït en pacients d'edats més avançades.

En aquest projecte de tesi ens proposem caracteritzar noves síndromes pediàtriques causades per anticossos contra superfície neuronal e identificar nous autoantígens responsables d'encefalitis d'etiologia no aclarida amb un especial focus en aquests grups d'edat. Amb aquest objectiu ens centrarem en 3 grups de pacients pediàtrics en els quals tenim evidència preliminar de patologia neurològica immunomediada: 1) encefalitis associada a teratoma sistèmic, 2) encefalitis amb crisis refractàries i/o status epilèptic, i 3) pacients amb recidives neurològiques post-encefalitis herpètica. En detall:

Grup 1: Pacients amb encefalitis associada a teratoma sistèmic

El teratoma (del greg teras-, teratos "malson", "monstre", i –oma "tumor o tumoració"), és el tumor de cèl·lules germinals més freqüent i, les seves cèl·lules o teixits poden derivar dels tres tipus de capes germinals embrionàries (ectoderma, endoderma i mesoderma). Els teratomes es divideixen en quatre categories: madurs (quístics o sòlids, benignes, els quals són els més freqüents), immadurs (malignes), malignes per un component d'una altra neoplàsia somàtica maligne, i per últim monodermals o altament especialitzats, en els quals és freqüent la presència de teixit d'estroma ovàric, que conté teixit tiroïdal, o teixit de tipus carcinoide, constituint neoplàsies neuroendocrines ben diferenciades.

Els teratomes quístics madurs o quists dermoides representen més del 95% de tots els teratomes ovàrics, sent els tumors ovàrics més freqüents en dones entre la segona i la tercera dècada de la vida.[60] El quist dermoide conté teixit madur d'un origen ectodèrmic (ex., pell, fol·licles pilosos, glàndules sebàcies), mesodèrmic (ex., múscul, teixit urinari), i/o endodèrmic (ex., pulmó i gastrointestinal). Aquests tumors poden ser bilaterals en el 10-17% dels casos, i característicament tenen l'aparença d'una massa multiquística amb una prominència sòlida (protuberància de Rokitansky) localitzada entre el teratoma i l'ovari normal. Tot i que són gairebé sempre benignes, fins un 2% poden presentar una degeneració maligne, principalment en dones majors de 45 anys, en tumoracions de mida major de 10 cm de diàmetre i en tumors de ràpid creixement. Els teratomes immadurs o malignes són més comuns en les dues primeres dècades de la vida, i tot i que representen menys d'un 1% dels teratomes ovàrics són el tipus de tumor maligne més freqüent d'aquest òrgan (36%) seguits dels disgerminomes (33%).[61] Els teratomes immadurs típicament estan composats per teixits de les tres capes germinals disposades de manera erràtica. Histològicament és freqüent la diferenciació a teixit neural tot i que elements d'estroma immadurs també poden estar presents i, segons la proporció de teixit neural immadur es poden estratificar en graus des de l' I (ben diferenciats) fins al III (pobrament diferenciats).[62]

Per tant, a diferència d'altres tumors com els carcinomes, donat a que la majoria de teratomes són benignes i que les dones amb quists dermoides amb freqüència estan asimptomàtiques, clàssicament no s'havia considerat el teratoma com un possible causant de síndromes paraneoplàstiques. De forma excepcional, s'havia associat la presència d'aquests tumors amb quadres paraneoplàstics no neurològics com l'anèmia hemolítica, la poliartritis, i la dermatomiositis.[63-65] Algunes publicacions aïllades també havien suggerit una associació entre teratoma d'ovari o testicular i encefalitis paraneoplàstica.[6] Molts d'aquests pacients eren homes joves amb teratomes testiculars, i

anticossos Ma2, i una síndrome que es caracteritza per encefalitis límbica i disfunció de tronc cerebral i diencèfal.[66]

Aquest pensament ha canviat de forma radical des de la identificació l'any 2007 de l'encefalitis anti-NMDAR,[6] en la qual entre el 9 i el 55% dels pacients presenten un teratoma associat a nivell sistèmic,[51] augmentant la sospita de la presència d'un teratoma com a causa d'encefalitis autoimmune. Tot i així l'associació tumoral en aquesta encefalitis és edat i sexe depenent, sent molt infreqüent en edats inferiors als 12 anys.[67] Tot i que aquesta encefalitis s'ha associat a la presència de teratomes madurs i immadurs, el 29% dels teratomes associats a encefalitis anti-NMDAR són immadurs a diferència d'un percentatge inferior a l'1% descrit en sèries de pacients sense encefalitis.[68] Aquest fet i la demostració en teixit neural tumoral de l'expressió de receptors NMDA recolzen la presència del tumor com a desencadenant de l'encefalitis.[6] El percentatge de teratomes bilaterals en pacients amb encefalitis anti-NMDAR i teratoma es detecta amb una freqüència similar a la de les cohorts generals de pacients amb teratoma (14% versus 12%).[41,69]

La identificació del teratoma com a causant d'encefalitis autoimmune resulta molt rellevant doncs aquests pacients responen a l'extirpació tumoral i immunoteràpia.[51] Els pacients amb encefalitis anti-NMDAR associada a teratoma presenten un menor risc de recidives que els pacients en els que no se'ls detecta un tumor associat.[51] Actualment resulta imprescindible per tant, la cerca d'un teratoma ocult en un pacient amb aquesta encefalitis, tot i que el tipus i la freqüència del cribratge caldrà adaptar-los al risc d'associació tumoral del pacient, que varia segons l'edat i el sexe. Actualment es considera d'elecció la RM de la pelvis com a cribratge en dones i/o la ecografia abdominal i testicular en el cas dels homes.[67]

Des de 2007, al laboratori de Neuroimmunologia del Dr. Dalmau (director de la tesi), hem estudiat 249 pacients amb encefalitis associada a teratoma. Tot

i que la majoria d'aquests pacients tenen anticossos contra la subunitat GluN1 del receptor NMDAR, hem identificat 38 pacients amb teratoma i sospita d'encefalitis autoimmune però negatius per aquests anticossos. La pregunta que sorgeix d'aquesta troballa és si la negativitat dels anticossos en aquests pacients és deguda a una baixa sensibilitat de la tècnica o si estem davant d'un quadre paraneoplàstic diferent. En aquesta tesi pretendrem contestar aquesta pregunta (Veure Objectiu 1).

Grup 2: Pacients amb encefalitis amb crisis refractàries i status epilepticus

El recent descobriment d'una nova categoria d'encefalitis autoimmune causada per autoimmunitat sinàptica com a causa tractable d'encefalitis ha renovat l'interès per identificar causes autoimmunes d'epilèpsia. Aquest fet és degut a que la identificació d'aquesta nova categoria d'encefalitis autoimmune ha canviat els paradigmes diagnòstics i terapèutics de múltiples síndromes potencialment tractables que cursen amb crisis i *status epilepticus*, i que prèviament eren atribuïts a infeccions virals o a etiologies idiopàtiques. Les encefalitis autoimmunes que cursen amb crisis i/o *status epilepticus* es divideixen seguint la classificació de les encefalitis autoimmunes segons la localització de l'auto-antigen associat. També poden dividir-se entre encefalitis límbiques o encefalitis difuses, i/o en funció de si són paraneoplàstiques o no. En el cas de les encefalitis paraneoplàstiques associades a anticossos intracel·lulars o onconeuronals aquestes inclouen les associades a Hu, Ma2, CV2/CRMP5 i amfifisina. D'aquestes 4 respostes immunològiques, els anticossos anti-Hu són els que amb major freqüència es descriuen en pacients amb crisis, *epilèpsia partialis continua*,, i *status epilepticus*. Els tumors subjacents més freqüents són els càncers de pulmó, en especial SCLC (tots els anticossos), els tumors testiculars de cèl·lules germinals (Ma2), i els timomes (CRMP5).

Aquestes síndromes afecten de forma gairebé exclusiva a pacients adults i es caracteritzen per tenir una resposta limitada a la immunoteràpia.

Els anticossos intracel·lulars contra GAD65, tot i que típicament s'han associat a la síndrome de la persona rígida no paraneoplàstica i a disfunció cerebel·losa, recentment hi ha hagut un increment en el nombre de casos publicats associats a encefalitis límbica, i/o epilèpsia refractària.[70] En aquesta síndrome que pot afectar a pacients joves, la resposta a immunoteràpia és limitada a diferència dels síndromes per anticossos contra proteïnes de superfície o sinàptics.

Totes les encefalitis causades per anticossos contra antígens de superfície neuronal o sinàptics descrites cursen freqüentment amb crisis i/o *status epilepticus*. La seva identificació és de gran importància doncs aquests pacients responen a immunoteràpia. Aquestes encefalitis poden afectar a pacients amb o sense tumors i la freqüència de l'associació tumoral dependrà de l'autoanticòs associat i de les característiques epidemiològiques (edat, sexe i ètnia) del pacient. En el cas de l'encefalitis anti-NMDAR, més del 70% dels pacients desenvolupen crisis epilèptiques.[71] A més, en nens petits i en adults de sexe masculí les crisis o l'*status epilepticus* són sovint la forma de presentació d'aquesta encefalitis.[71,72] Tot i així, la majoria de pacients tractats amb immunoteràpia, i un cop superada la fase aguda de l'encefalitis, romanen lliures de crisis i sense necessitat de tractament antiepilèptic durant el seguiment.

En altres encefalitis associades a anticossos contra superfície neuronal (LGI1, Caspr2, GABA$_B$, AMPA) també es freqüent la presència de crisis epilèptiques, tot i que aquestes síndromes afecten de forma predominant o gairebé exclusiva a pacients adults. Els pacients amb encefalitis límbica associada a anticossos contra LGI1 típicament presenten dèficits de memòria i diferents tipus de crisis, incloent característicament crisis tòniques breus d'afectació facio-braquial.[36] El reconeixement d'aquestes crisis que es poden

presentar a l'inici de la malaltia, i la conseqüent instauració d'immunoteràpia precoç pot prevenir la progressió de l'encefalitis límbica i millorar el pronòstic cognitiu d'aquests pacients.[73,74] De manera interessant LGI1 és una proteïna secretada que forma part d'un complex transinàptic que interacciona amb el VGKC presinàptic a través de la proteïna ADAM23. Els ratolins genoanul.lats per LGI1 moren durant les dues primeres setmanes de vida. En humans, mutacions en LGI1 són causa d'epilèpsia del lòbul temporal autosòmica dominant.[75] Els anticossos contra Caspr2 a més d'associar-se a encefalitis límbica, també s'han associat a la síndrome de Morvan (encefalitis i neuromiotonia) i molt menys freqüentment a neuromiotonia aïllada.[37] Actualment el significat dels anticossos contra VGKC determinats per radioimmunoassaig que no són dirigits contra LGI1 o Caspr2 és incert donat a que s'han identificat en pacients amb desordres molt variats incloent pacients amb malalties no immunomediades.[71,76] Els anticossos contra el receptor GABA$_B$ s'associen a una encefalitis límbica que típicament es manifesta amb crisis refractàries prominents.[77] Aquests anticossos es troben en la majoria de pacients amb encefalitis límbica i SCLC sense anticossos anti-Hu, i a diferència dels pacients amb Hu, els pacients amb GABA$_B$ responen amb favorablement a immunoteràpia.[77] Els anticossos contra el receptor AMPA també s'associen a encefalitis límbica caracteritzada per crisis epilèptiques amb resposta favorable a immunoteràpia, però aquesta última cursa freqüentment amb símptomes psiquiàtrics prominents.[78] Els tumors associats a aquesta síndrome són el càncer de mama, de pulmó i de timus. A més aquests pacients amb freqüència presenten recidives clíniques i poden tenir altres auto-anticossos (peroxidasa tiroïdal [TPO], o canals de calci depenents de voltatge [VGCC] tipus N), suggerint una tendència a la autoimmunitat. Anticossos contra DPPX, una subunitat reguladora dels canals de potassi Kv4.2, s'han descrit recentment en pacients adults amb encefalitis caracteritzada per hiperexcitabilitat del SNC incloent crisis epilèptiques, mioclònies i agitació,[46] i en pacients joves (incloent un adolescent de 16 anys), amb encefalomielitis

progressiva amb rigidesa i mioclònies (PERM) .[79] En alguns pacients els símptomes neurològics són precedits per diarrea greu de llarga evolució que ha motivat prèviament extensos estudis microbiològics (incloent malaltia de Whipple) i de cribratge tumoral. La identificació de l'expressió de DPPX en el plexe mientèric de ratolins i la seva alta reactivitat amb els anticossos dels pacients, han fet postular un mecanisme immunològic dels símptomes gastrointestinals.[46] Els malalts amb aquesta encefalitis solen tenir bona resposta a immunoteràpia, tanmateix, poden fer-se dependents dels corticosteroides, amb recidives neurològiques al intentar reduir la dosi.

Com s'ha exposat en aquesta secció, tot i l'alta freqüència de trastorns autoimmunes a l'edat pediàtrica, només una (encefalitis anti-NMDAR) de les sis encefalitis per autoimmunitat sinàptica descrites aquí afecten de forma predominant a infants i adolescents. Aquest fet suggereix que possiblement altres auto-antígens encara pendents d'identificar poden ser responsables d'encefalitis autoimmune en nens. Des de gener de 2008 fins abril de 2014 hem identificat 350 pacients (un 40% nens), amb sospita d'encefalitis autoimmune que van cursar amb status epilèptic. Disposem de LCR i/o sèrum de tots els pacients, dels quals 140 tenen un patró en la immunohistoquímica suggestiu d'anticossos contra antígens desconeguts de la membrana neuronal. En aquesta tesi ens proposarem com a objectiu identificar nous auto-antígens associats a encefalitis i crisis epilèptiques que afectin a l'edat pediàtrica (Objectiu 2).

Grup 3: Pacients amb recidives neurològiques post-encefalitis herpètica

Generalitats i etiopatogènia de l'encefalitis herpètica:

L'encefalitis pel virus herpes simple (EHS) segueix sent una causa important de morbimortalitat a totes les edats a nivell mundial tot i la disponibilitat d'un tractament antiviral com és l'aciclovir. Aproximadament un terç dels pacients són nens o adolescents. Com a resultat d'aquesta encefalitis típicament es produeix necrosis d'àrees límbiques, i la síndrome clínica associada es manifesta amb febre i/o dèficits neurològics focals que inclouen alteració del nivell de consciència, afectació de parells cranials, hemiparèsia, disfàsia, afàsia, atàxia i/o crisis focals.[80-82] També s'han descrit varis tipus d'alteració del comportament com hipomania, síndrome de Kluver-Bucy i amnèsia associats a l'EHS.[83,84] Actualment, la prova de referència pel seu diagnòstic és la detecció del DNA viral mitjançant la reacció en cadena de la polimerasa (PCR) que mostra una alta sensibilitat (98%) i especificitat (94-100%). Excepte durant el període neonatal en el qual aquesta encefalitis pot estar causada pels serotips del virus herpes simple (VHS) 1 o 2, generalment adquirida per transmissió materna pel canal del part, en el resta de grups d'edat la majoria de casos són causats pel serotip VHS-1. Es creu que l'arribada del virus al SNC fora del període neonatal es pot produir mitjançant 3 vies: 1) invasió directe mitjançant el nervi trigemin u olfactori seguint una primoinfecció pel VHS-1 a la orofaringe (principal causa en menors de 18 anys), 2) invasió del SNC després d'una infecció recurrent del virus, representant una reactivació viral i la seva expansió, i 3) infecció del SNC sense observar-se una infecció primària o recurrent, representant una reactivació latent del virus in situ al SNC. Una altra potencial via d'inoculació és la virèmia.

La causa de per què la infecció pel VHS-1 només és causa d'encefalitis en un percentatge petit de pacients que pateixen una infecció per aquest virus és encara desconeguda. Tot i que en humans l'encefalitis pel VHS-1 de forma

general no és més comú en pacients immunodeprimits que en immunocompetents,[80] recentment s'han identificat algunes mutacions genètiques involucrades en vies de la resposta immunològica innata que podrien conferir una susceptibilitat a patir aquesta encefalitis, principalment en infants. Diferents mutacions que alteren diferents proteïnes (UNC93B, TLR3, TRAF3, TIR, TRIF) implicades en la via de la producció d'interferó mitjançant l'activació del receptor Toll-Like (TLR) 3 s'han vist involucrades. Els TLR són un tipus de receptors de reconeixement de patrons de patògens (PRR) que formen part de la resposta immunològica innata. Aquests receptors són capaços de reconèixer RNA de doble cadena de diversos patògens i activar factors de transcripció que estimulen la producció d'interferons i d'altres citocines. La variació regional dels diferents subtipus de TLR contribueix a explicar els diferents patrons d'afectació del SNC del VHS i d'altres virus amb neurotropisme. El subtipus TLR3 és el predominant en les cèl·lules del SNC i té una gran importància per evitar la disseminació del VHS dels ganglis al SNC mitjançant la producció d'interferó alfa. Resulta interessant el fet de que els pacients amb mutacions que afecten de forma exclusiva a la via de senyalització del TLR3 presenten un risc incrementat d'encefalitis o queratitis per VHS, però no per infeccions produïdes pel VHS en altres localitzacions com són les infeccions labials o mucoses recurrents, o per altres microorganismes al SNC. Al contrari, pacients amb immunodeficiències severes com els afectats per mutacions en IRAK-4 (receptor cinasa 4 associat a interleuquina (IL) 1) que produeixen una alteració en les vies dels TLR7, 8 i 9 i també del receptor de la IL-1, però no del TLR3, presenten un risc incrementat d'infeccions piògenes per bactèries gram positives encapsulades però no per EHS.

Tot i aquestes interessants troballes que representen sense dubte un gran pas per entendre l'etiopatogènia de l'EHS, aquesta última qüestió no està ni molt menys resolta. Tot i que mitjançant estudis funcionals *in vitro* s'han observat alteracions en la via de producció d'interferó alfa mitjançat l'activació

del TLR3 en un alt percentatge d'infants amb EHS, són una minoria els casos en els que es detecten mutacions en aquesta via (8-9%). A més, en els casos en que es detecten mutacions, aquestes no presenten una penetrància completa doncs també es troben en familiars asimptomàtics de pacients. Es desconeix si aquestes vies també estan alterades funcionalment en pacients adults amb EHS. D'altra banda, tot i que la introducció del tractament amb aciclovir a mitjans dels anys 1980 va suposar una davallada important de la mortalitat d'aquesta encefalitis, actualment més de dos terços dels pacients amb EHS encara queden amb greus seqüeles neurològiques. Per aquest motiu es recomana iniciar el tractament amb aciclovir endovenós el més aviat possible davant una sospita clínica en espera de la confirmació microbiològica. Un altre factor molt important a tenir en compte en la patogènesis de l'EHS és la hipòtesis d'un possible origen immunomediat del dany tissular en aquesta encefalitis.[85] Aquest fet explicaria l'observació que l'EHS no és més freqüent en immunodeprimits tot i l'alta freqüència d'infeccions mucocutànies pel VHS-1 en aquests hostes. En canvi l'EHS en immunodeprimits a vegades segueix un curs diferent, caracteritzat per una evolució lenta i únicament amb canvis mínims histopatològics observats en biòpsies cerebrals.[86]

Per tant, tot i que la patogènesis de la EHS és poc coneguda es creu que ambdós factors, els directament relacionats amb el virus, i d'altres indirectes immunomediats desencadenats per la infecció, juguen un paper en el dany al SNC.[80] Resulta poc clar si la quantitat de virèmia està relacionada amb l'extensió o la severitat de les lesions. En un estudi amb 8 pacients amb EHS, el nivell de virèmia determinat per PCR, no es correlacionà amb la severitat dels símptomes.[87]

D'altra banda diverses dades recolzen el paper del sistema immunològic en la EHS:

- L'EHS es caracteritza per lesions focals inflamatòries que es creuen secundàries a l'expressió d'una resposta mitjançada per cèl·lules T específica contra el virus.[88] Com a exemple, cèl·lules T citotòxiques que lisen específicament les cèl·lules infectades pel virus in vitro, predominen en els llocs d'infecció focals en models murins.[89]

- Altres models animals han demostrat la correlació entre la producció de citocines i òxid nítric amb dany cerebral.[90]

- L'administració exògena de IL-4 intranasal en ratolins infectats amb el VHS-1 incrementa la severitat de l'encefalitis posterior mitjançant l'increment de producció de IL-4 per part de les cèl·lules T CD4+ locals.[91]

- S'han observat correlacions entre la infiltració del SNC per cèl·lules immunes durant la infecció viral i desmielinització en models en ratolins.[85]

- S'han observat immunocomplexes IgM contra VHS-1 en les parets dels vasos en un pacient amb EHS.[92]

També resulta interessant la recent descripció de les *"interferonopaties"*, una nova categoria de malalties genètiques que inclou entre d'altres la *Síndrome d'Aicardi Goutières* o el Lupus eritematós sistèmic congènit, que associen una predisposició congènita a la autoimmunitat, i són causades per una hiperestimulació congènita dels factors de transcripció dependents d'interferó, una situació similar a la que hom postula que es produeix de forma adquirida davant una infecció pel VHS.

La demostració del paper de l'autoimmunitat en l'etiopatogènia de l'EHS podria tenir implicacions terapèutiques directes doncs ja s'ha demostrat en el model animal que la teràpia adjuvant amb corticoides juntament amb el tractament antiviral, és més efectiva que el tractament antiviral únicament en la reducció de les anomalies estructurals cerebrals observades en la RM.[93]

<u>Recidives post-encefalitis herpètica:</u>

Tot i que tradicionalment s'ha considerat que l'EHS és una malaltia monofàsica, en la literatura mèdica es descriu que fins el 14-24% dels pacients poden presentar recidives. [94] La patogènesis de les recidives és heterogènia i, s'ha postulat que mentre alguns casos representen recidives virals (nova detecció del virus mitjançant PCR positiva en LCR, noves lesions necròtiques a la RM, i resposta al tractament antiviral); d'altres puguin ser immunomediades (PCR viral negativa en LCR, falta de noves lesions necròtiques en RM, i falta de resposta a aciclovir).[94,95] Aquesta última síndrome d'etiologia desconeguda, però postulada de ser immunomediada, ha sigut més freqüentment descrita a l'edat pediàtrica on és coneguda com "*coreoatetosis post-EHS*", donada la presentació típica d'aquests pacients a les 3-4 setmanes posteriors al debut de l'encefalitis viral en forma greu alteració de moviment (status coreoatetòsic i discinèsies), juntament alteració greu del nivell de consciència.[95] Donada la falta de resposta a l'aciclovir en aquests pacients i la presumpció d'una etiopatogènia immunomediada s'havien publicat a la literatura diversos casos tractats amb immunoteràpia (corticoides i/o gammaglobulines endovenoses) amb resposta variable. Recentment l'estudi retrospectiu d'una cohort de pacients adults dels quals es disposava de mostres congelades obtingudes en diferents estadis d'encefalitis herpètica detectà anticossos tipus IgG contra NMDAR en un 11% dels pacients, postulant que aquests anticossos podrien ser causants de formes atípiques d'EHS.[96] En aquesta tesi pretendrem estudiar si una resposta immunològica per autoimmunitat sinàptica contra NMDAR i/o d'altres proteïnes sinàptiques pot estar involucrada en la "coreoatetosis post-herpètica" i/o en altres formes de recidiva neurològica post-encefalitis herpètica (Objectiu 3).

III. Hipòtesis i Objectius

Hipòtesis

El descobriment de que moltes encefalitis prèviament considerades idiopàtiques són causades per anticossos contra proteïnes sinàptiques o receptors de la superfície neuronal ha revolucionat en els últims 7 anys la neurologia, la pediatria, la psiquiatria i les neurociències. La malaltia amb més impacte en el camp de la pediatria, la encefalitis anti-NMDAR descrita l'any 2007, ha marcat una nova línia de treball i estratègies diagnòstiques i terapèutiques en encefalitis pediàtrica. La identificació d'aquestes síndromes es va realitzar mitjançant la observació de pacients amb síndromes que suggerien un procés immunomediat però en els quals no es detectaven anticossos coneguts. Això va suggerí la hipòtesi que aquests pacients tenien anticossos que no eren detectats amb les tècniques disponibles.

D'aquesta manera creiem que algunes encefalitis pediàtriques prèviament considerades idiopàtiques o post-infeccioses dels grups clínics seleccionats: 1) encefalitis associada a la presència d'un teratoma sistèmic, 2) encefalitis amb crisis refractàries i/o status epilèptic, i 3) recidives neurològiques post-encefalitis herpètica, estan causades per anticossos dirigits contra antígens sinàptics o de la membrana neuronal. Aquests anticossos podrien ser identificats amb les noves tècniques altament sensibles i específiques per la detecció d'anticossos/antígens de superfície neuronal, recentment desenvolupades pel Dr. Dalmau (director de la tesi), que han permès en els últims anys el descobriment de vàries síndromes associades a aquesta categoria d'anticossos (receptors AMPA, NMDA, GABAB, mGluR5 i proteïnes DPPX, LGI1, Caspr2).[49]

Objectius

Objectiu general:

Caracterització clínica e immunològica d'encefalitis pediàtriques d'etiologia no filiada, centrant-nos en tres grups de pacients amb evidència preliminar d'un origen immunomediat: grup 1) encefalitis associada a la presència d'un teratoma sistèmic, grup 2) encefalitis amb crisis refractàries i/o status epilèptic, i grup 3) recidives neurològiques post-encefalitis herpètica.

Objectius específics per grups de pacients:

- Grup 1: Encefalitis associada a la presència d'un teratoma sistèmic: Caracteritzar clínica e immunològicament els pacients identificats amb sospita d'encefalitis autoimmune associada a un teratoma sistèmic sense anticossos contra NMDAR per establir si la negativitat d'aquests anticossos es deu a una baixa sensibilitat en la tècnica o si estem davant d'un quadre paraneoplàstic diferent.

- Grup 2: Encefalitis amb crisis refractàries i/o status epilèptic: Identificar nous auto-antígens responsables d'encefalitis per autoimmunitat sinàptica associats a crisis i *status epilepticus* que afectin de manera predominant a l'edat pediàtrica.

- Grup 3: Recidives neurològiques post-encefalitis herpètica: Estudiar si una resposta immunològica per autoimmunitat sinàptica contra NMDAR i/o d'altres proteïnes sinàptiques està involucrada en la "*coreoatetosis post-herpètica*" i/o en altres formes de recidiva neurològica post-encefalitis herpètica.

IV. Articles

Objectiu 1:

Article I: A novel treatment-responsive encephalitis with frequent opsoclonus and teratoma.

Autors: Armangue T, Titulaer MJ, Sabater L, Pardo-Moreno J, Gresa-Arribas N, Barbero-Bordallo N, Kelley GR, Kyung-Ha N, Takeda a, Nagao T, Takahashi Y, Lizcano A, Carr AS, Graus F, Dalmau J

The Rapidly Expanding World of Rapidly Progressive Encephalopathy

Historically, patients with rapidly declining mental status and progressive neurological dysfunction were presumed to suffer the effects of an extrinsic agent, existential (ie, "demonic possession"[1]) or otherwise (ie, encephalitis of unknown origin[2]). In 2005, however, the description of 4 patients with psychiatric symptoms, seizures, memory deficits, alterations in consciousness, and autonomic dysfunction, occurring in association with ovarian teratoma,[3] focused attention on the potential contributions of the demons within to brain dysfunction. The subsequent isolation of a pathogenic antibody directed against N-methyl-D-aspartate receptors (NMDARs) within the serum and cerebrospinal fluid of these patients[4] heralded remarkable growth in the understanding and characterization of autoimmune brain diseases, and reports of robust responses to immunotherapy[5] transformed the approach to the patient with rapidly progressive encephalopathy.

In this issue of *Annals of Neurology*, Armangue et al report a novel and distinct phenotype of teratoma-associated encephalopathy in 38 young adults (median age = 28 years; range = 12–55 years).[6] Patients were identified from a cohort of 249 cases of suspected anti-NMDAR encephalitis on the basis of negative serum and cerebrospinal fluid tests for autoantibodies against known central nervous system antigens. The authors identified distinct clinical phenotypes through careful characterization of affected patients and comparison with the anti-NMDAR antibody-positive cohort. In doing so, they extend the path of Josep Dalmau's translational research, setting the stage for future studies investigating disease etiology.

Twenty-two of the 38 antibody-negative patients (58%) developed a brainstem–cerebellar syndrome within the first month of presentation, characterized by ataxia (86%), dysarthria (36%), decreased level of consciousness (32%), and diplopia or ophthalmoparesis (18%). The majority of patients were treated with immunotherapy and tumor resection, with an excellent response observed in 74% (14 of 22). Opsoclonus was the leading symptom in 10 female patients with the brainstem–cerebellar syndrome (45%, 10 of 22), with a median age of 27 years (range = 15–32). The population demographics of patients with opsoclonus in this study (females of childbearing age) distinguish this cohort from those reported previously in case series of adult onset opsoclonus–myoclonus (52% female; median age = 47 years; range = 27–78 years)[7] and those associated with carcinoma[8] or neuroblastoma.[9] These differences may reflect a selection bias, as only patients with a teratoma and suspected anti-NMDAR encephalitis were considered in this study. Alternatively, the younger age of onset and female sex predominance may be viewed as further evidence supporting an autoimmune origin of symptoms, acknowledging the importance of estrogen to the breakdown of self-tolerance and propagation of the immune response.[10]

The association between teratoma and rapidly progressive encephalopathy is increasingly recognized in young adults,[11] and should trigger the clinician to consider a diagnosis of anti-NMDAR encephalitis or teratoma-associated encephalopathy. A high response rate to immunotherapy, including CD-20–avid agents, is reported in patients with either syndrome (71% in anti-NMDAR encephalitis; 74% in patients with teratoma-associated encephalopathy), strongly implicating antibody-producing plasma cells in disease etiology. Yet despite the similarities in the patient population and a plausible common pathogenesis, it is the clinical differences that establish teratoma-associated encephalopathy as a unique entity. As outlined in the current report, patients with anti-NMDAR encephalitis more commonly presented with psychiatric and behavioral disturbances (including memory deficits), seizures, dyskinesias, impairments of consciousness, and autonomic instability (including central hypoventilation). Patients without antibodies, conversely, were significantly more likely to present with brainstem–cerebellar dysfunction, including opsoclonus, and lower cranial nerve involvement.[6] Further work remains to be completed characterizing the role of host- and tumor-specific factors in initiation and propagation of rapidly progressive encephalopathy.[12]

Josep Dalmau's exceptional work has done much to advance the care of children and adults with newly acquired progressive neurological deficits and encephalopathy. The present study builds upon this work, characterizing a new clinical entity, and offering an approach to diagnostic testing and treatment of affected patients. Yet, as the evolving story of anti-NMDAR encephalitis reminds us, early descriptions of a syndrome may change

with time and experience. Anti-NMDAR encephalitis was first reported in female patients (10:1 female:male ratio) of childbearing age (median age = 23 years; range = 5–76 years).[13] More recent numbers, however, confirm a contracting female:male ratio (approaching 8:1), with patients affected at the extremes of age.[14,15] It remains important, therefore, to consider the diagnosis of teratoma-associated encephalopathy in a broad population, until the epidemiology is more clearly defined by multisite population studies. Additionally, although a paraneoplastic association was presumed in early reports of anti-NMDAR encephalitis,[4] tumor was reported in a minority of cases in more recent case series (38%, 220 of 577).[5] Thus, although the search for specific antibodies and hidden malignancies, such as teratomas, is an essential part of the diagnostic evaluation of patients with rapidly progressive encephalopathy,[6] neither tumor nor antibody testing should be relied upon to define the clinical syndrome.

Limitations notwithstanding, this brief communication represents a critical step toward understanding rapidly progressive encephalopathy. In 2005, clinical characterization of 4 female patients with ovarian teratomas[3] directly preceded the discovery of the pathogenic antibody against NMDARs.[4] Less than 9 years later, the accrual of hundreds of patients with anti-NMDAR encephalitis by the same group[5] and others[14,16] speaks to the exponential rate at which this disease is being diagnosed, and to the importance of clinical characterization as the rate-limiting step in this process. Recognizing this, the report from Armangue et al does more than define a population of patients for further study. By emphasizing classification based upon clinical presentation, this report offers an invitation to clinicians and researchers to participate in the research process— no laboratory required.

Global collaboration is critical when approaching patients with rare diseases, and will be essential to future efforts to characterize the pathogenesis of autoimmune brain disease and optimize patient outcomes. Following precedent in anti-NMDAR encephalitis, hopes are high that the seminal description of teratoma-associated encephalopathy with brainstem–cerebellar dysfunction will herald further advances in the understanding of rapidly progressive encephalopathy, signaling a move from clinical characterization and localization to the development and implementation of clinical strategies for the treatment of autoimmune brain disease in all its forms.

Potential Conflicts of Interest

Gregory S. Day, MD, MSc

Division of Neurology
University of Toronto
Toronto, Ontario, Canada

Susanne M. Benseler, MD, PhD

Division of Rheumatology, Department of Pediatrics
Alberta Children's Hospital
University of Calgary
Calgary, Alberta, Canada

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A Novel Treatment-Responsive Encephalitis with Frequent Opsoclonus and Teratoma

Thaís Armangue MD,[1,2] Maarten
J. Titulaer MD, PhD,[1,3†]
Lidia Sabater PhD,[1] Javier
Pardo-Moreno MD,[4]
Nuria Gresa-Arribas PhD,[1]
Natalia Barbero-Bordallo MD,[4]
Gordon R. Kelley MD,[5]
Noh Kyung-Ha MD,[6]
Akitoshi Takeda MD, PhD,[7]
Takahiro Nagao MD,[8]
Yukitoshi Takahashi MD,[9]
Angélica Lizcano MD,[10] Aisling
S. Carr MRCP, PhD,[11]
Francesc Graus MD,[1] and
Josep Dalmau MD, PhD[1,3,12]

Among 249 patients with teratoma-associated encephalitis, 211 had N-methyl-D-aspartate receptor antibodies and 38 were negative for these antibodies. Whereas antibody-positive patients rarely developed prominent brainstem–cerebellar symptoms, 22 (58%) antibody-negative patients developed a brainstem–cerebellar syndrome, which in 45% occurred with opsoclonus. The median age of these patients was 28.5 years (range = 12–41), 91% were women, and 74% had full recovery after therapy and tumor resection. These findings uncover a novel phenotype of paraneoplastic opsoclonus that until recently was likely considered idiopathic or postinfectious. The triad of young age (teenager to young adult), systemic teratoma, and high response to treatment characterize this novel brainstem–cerebellar syndrome.

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The discovery of anti–N-methyl-D-aspartate receptor (NMDAR) encephalitis in 2007[1] has brought attention to a relationship between systemic teratomas and autoimmune encephalitis. Since 2007, we have studied 249 patients with teratoma-associated encephalitis; most of these patients had antibodies against the NR1 subunit of the NMDAR, but 38 were NMDAR antibody negative. When these 38 patients were compared with those with NMDAR antibodies, a novel brainstem–cerebellar syndrome that frequently associates with opsoclonus emerged. The current study describes the clinical differences between NMDAR antibody-positive and antibody-negative patients with systemic teratoma, and focuses on the novel brainstem–cerebellar syndrome and the subgroup of patients with opsoclonus.

Patients and Methods

From January 2007 until September 2012, serum and CSF of 249 patients with teratoma-associated encephalitis were studied at the Department of Neurology, Hospital of the University of Pennsylvania and at the Neurology Service, Hospital Clinic, August Pi i Sunyer Biomedical Research Institute, University of Barcelona. The presence of a systemic teratoma was confirmed pathologically in 234 patients and radiologically in 15. Information was obtained by the authors or provided by referring physicians at symptom onset and at regular intervals during the course of the disease using a comprehensive questionnaire that includes all symptoms shown in the Figure.[2] Sera and cerebrospinal fluid (CSF) were examined for antibodies to NMDA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, γ-aminobutyric acid (B), and mGluR5 receptors, LGI1, Caspr2, onconeuronal proteins (Hu, CRMP5, Ma1–2, amphiphysin), and GAD65, using reported techniques including brain immunohistochemistry, immunoblot, and cell-based assays.[3–5] Patients without NMDAR antibodies were further studied for antibodies to dipeptidyl-

FIGURE 1: Comparison of symptoms of patients with teratoma-associated encephalitis and N-methyl-D-aspartate receptor (NMDAR) antibodies with those without NMDAR antibodies. (A) Patients without NMDAR antibodies (indicated in dark gray) less frequently developed symptoms considered characteristic of anti-NMDAR encephalitis (behavioral abnormalities, memory deficits, seizures, dyskinesias, speech disorder, and central hypoventilation, all $p < 0.0005$, and impaired level of consciousness and autonomic dysfunction, $p < 0.05$). (B) In addition, patients without NMDAR antibodies more frequently developed brainstem–cerebellar symptoms and opsoclonus, which are rare in anti-NMDAR encephalitis (all $p < 0.0005$). From 1 patient without NMDAR antibodies and 4 with antibodies, detailed clinical information was not available, and these patients were excluded from analysis; in 3 additional patients without NMDAR antibodies, information for memory deficits and speech disorder was not available.

peptidase-like protein-6 (DPPX), α1-glycine receptor, D2 subunit of the dopamine receptor, and unknown cell-surface antigens using reported techniques.[3–5]

Outcome was assessed with the modified Rankin scale (mRS),[6] grading it as full recovery (mRS = 0), substantial improvement (mRS = 1–2), partial improvement (mRS > 2 after having had at least 1 point of improvement), and no improvement. Three patients without NMDAR antibodies have been previously reported.[7–9] Studies were approved by the internal review boards of the University of Pennsylvania and University of Barcelona.

Statistical Analysis

Comparative analyses between patients with and without NMDAR antibodies were performed with SPSS version 20 (IBM, Armonk, NY), using the Fisher exact test for contingency tables and Mann–Whitney U tests for continuous variables.

Results

Two hundred eleven patients were found to have NMDAR antibodies, and 38 were negative for these antibodies. Compared with antibody-positive patients, the 38 patients without NMDAR antibodies showed no differences with respect to gender and age of symptom onset (NMDAR antibody-negative patients: 92% female, median age = 28 years [interquartile range (IQR) = 20–32, range = 12–55] vs antibody-positive patients: 99% female, median age = 25 years [IQR = 19–30, range = 7–65], $p = 0.05$ and $p = 0.11$, respectively). However, significant differences were identified with respect to symptom presentation and repertoire of symptoms during the first month of the disease (see Fig 1). Whereas 18 (47%) patients without NMDAR antibodies initially presented with brainstem–cerebellar dysfunction, this presentation did not occur in any of the patients with NMDAR antibodies ($p < 0.0005$). In contrast, whereas 144 of 211 (68%) patients with NMDAR antibodies presented with psychosis and behavioral abnormalities, this presentation occurred only in 4 of 38 (11%) patients without these antibodies ($p < 0.0005$).

The Figure shows that during the first month of the disease, 76% of the patients with NMDAR antibodies developed dyskinesias, often involving the face and mouth, whereas only 1 (3%) patient without these antibodies developed dyskinesias, without affecting the face and mouth ($p < 0.0005$); similar differences were seen for most symptoms typical of anti-NMDAR encephalitis. In contrast, 22 of 38 (58%) patients without NMDAR

TABLE 1. Clinical Features in Patients with Brainstem–Cerebellar Syndrome and Systemic Teratoma without N-Methyl-D-Aspartate Receptor Antibodies

Opsoclonus	Patient No.	Age, yr/Sex	Main Symptoms	Neurologic Symptoms before Tumor Diagnosis	Brain MRI	CSF	Treatment	Response to Treatment	Immunological Studies with Cultures of Neurons
With	1	20/F	Opsoclonus–myoclonus, limb ataxia, dysarthria, meningeal signs, drowsiness, tonic seizures, autonomic instability (ileus, urinary retention)	Yes	Meningeal enhancement	182 WBC/µl (87% L), 99mg/dl protein; repeat study: 326 WBC/µl, 159mg/dl protein	Tumor removal, steroids	Complete, related to immunotherapy	Negative
	2	15/F	Opsoclonus–myoclonus, ataxia, drowsiness, vomiting, blurred vision	Yes	Normal	37 WBC/µl, 64mg/dl protein	Tumor removal, steroids, IVIg	Complete, related to tumor removal	Negative
	3	26/F	Opsoclonus–myoclonus, ataxia, dysarthria, aphasia, 3 days after tumor removal	No	Normal	72 WBC/µl (69% L), 49mg/dl protein, OB positive	Steroids, IVIg, plasma exchange (3×), rituximab (3 cycles); 1 cycle of bleomycin, etoposide, and carboplatin; 3 cycles of etoposide and cisplatin	Partial response to steroids, IVIg, plasma exchange; complete recovery after chemotherapy and rituximab	Negative
	4	31/F	Opsoclonus–myoclonus, ataxia, tinnitus	Yes	Normal	Mild pleocytosis with increased lymphocytes and protein concentration	Tumor removal (bilateral), steroids, IVIg, plasma exchange, chlorambucil	Complete, related to immunotherapy and tumor removal; relapsed 7 years later with mild ataxia and memory deficits	Reactivity of serum with cell surface of neurons
	5	22/F	Opsoclonus–myoclonus, ataxia, abnormal behavior, impaired consciousness; severe bradycardia requiring sinus pacemaker	Yes	Normal	10 WBC/µl, 57mg/dl protein, OB positive	Tumor removal, steroids	Partial, related to immunotherapy; complete after tumor removal	Negative
	6	24/F	Opsoclonus without myoclonus, truncal ataxia, vertigo, abdominal pain, generalized weakness, hyporeflexia	Yes	Normal[a]	<5 WBC/µl, <45mg/dl protein	Tumor removal, steroids, IVIg	Complete, related to immunotherapy	Negative
	7	30/F	Opsoclonus without myoclonus, dizziness, meningeal signs, seizures, abnormal behavior (not psychotic), weakness, hyporeflexia, central hypoventilation	Yes	1st normal; repeat study: brainstem edema and meningeal enhancement	134 WBC/µl (88% L), 88 mg/dl protein; repeat study: 414 WBC/µl (97% L), 110mg/dl protein	Steroids, IVIg, plasma exchange	Complete, related to immunotherapy; remained with motor weakness 3 months after disease onset	Negative
	8	29/F	Opsoclonus–myoclonus, sense of unsteadiness and body "shakiness" (26th week of pregnancy)	No	Not done	<5 WBC/µl, <45mg/dl protein, OB positive	Tumor removal, steroids	Complete, related to immunotherapy	Negative

TABLE 1. (Continued)

	Patient No.	Age, yr/Sex	Main Symptoms	Neurologic Symptoms before Tumor Diagnosis	Brain MRI	CSF	Treatment	Response to Treatment	Immunological Studies with Cultures of Neurons
Opsoclonus	9	28/F	Opsoclonus–myoclonus, dysarthria, ataxia, behavioral disinhibition, hypersexuality, hyperphagia, cognitive decline	No	Normal	12 WBC/μl, <45mg/dl protein	Tumor removal, steroids, IVIg, plasma exchange, azathioprine	Partial, related to immunotherapy; improved dysarthria and opsoclonus; ataxia still improving at last follow-up (15 months)	Reactivity of serum with cell surface of neurons
	10	32/F	Opsoclonus–myoclonus, dysarthria, diplopia, ataxia	Yes	Normal	30 WBC/μl, <45mg/dl protein	Tumor removal, steroids, IVIg, rituximab (4 doses)	Partial, related to immunotherapy and tumor removal; mild ataxia and dysarthria at last follow-up (13 months)	Negative
Without	11	19/F	Right hand tremor, ataxia, bilateral dysdiadochokinesia, dysmetria	Yes	Normal	124 WBC/μl, <45mg/dl protein	Tumor removal, steroids	Complete, related to immunotherapy and tumor removal	Negative
	12	32/F	Subacute tremor, unsteady gait	Yes	Normal	<5 WBC/μl, <45mg/dl protein	Tumor removal	Complete, related to tumor removal	Negative
	13	31/F	Subacute onset of vomiting, nystagmus, ataxic gait, dysarthria, myoclonus; all symptoms resolved after removal of ovarian teratoma, but the patient developed abnormal behavior, memory deficit, labile affect, and optic neuritis; recurrence of symptoms and oculomotor paresis 4 months later	Yes	1st normal; at clinical relapse 7 months later: abnormality at the level of oculomotor nuclei	16 WBC/μl, <45mg/dl protein, OB negative	Tumor removal, steroids, IVIg	Partial with tumor removal, complete with immunotherapy; relapse 8 months later (4 months after recovery)	Negative
	14	23/M	Cerebellar ataxia	Yes	NA	NA	NA	NA	Negative
	15	33/F	Severe cerebellar ataxia	Yes	NA	NA	Tumor removal	NA	Negative
	16	15/F	Left side ataxia, dysarthria, paresthesias, dysdiadochokinesia with left hand (onset 1.5 months after tumor removal)	No	Normal brain MRI and PET scan	<5 WBC/μl, <45mg/dl protein	Not treated	Symptoms stable with no improvement 4 months after presentation	Reactivity of CSF with cell surface of neurons

TABLE 1. (Continued)

Opsoclonus	Patient No.	Age, yr/Sex	Main Symptoms	Neurologic Symptoms before Tumor Diagnosis	Brain MRI	CSF	Treatment	Response to Treatment	Immunological Studies with Cultures of Neurons
	17	33/F	6-week episode of severe cerebellar ataxia that resolved without any specific treatment; relapse 2 years later: ataxia and memory problems; ovarian teratoma found	Yes	Normal	<5 WBC/μl, <45mg/dl protein	Not treated	Complete without treatment, but relapsed 2 years later	Negative
	18	36/F	Subacute ataxia, memory problems, confabulation, bilateral intention tremor, bilateral gaze directed nystagmus	Yes	Diffuse bilateral atrophy, enlarged ventricles (history of alcohol abuse)	<5 WBC/μl, <45mg/dl protein, OB negative	NA	NA	Negative
	19	28/F	Suspected viral meningoencephalitis (drowsiness, fever, headache), followed by seizures, brainstem symptoms, ataxia, cognitive and behavioral abnormalities	Yes	1st normal; 1 year later: diffuse brain atrophy (predominant in cerebellum)	66 WBC/μl, 126mg/dl protein	Tumor removal	No response; dependent for activities of daily living (dressing, feeding, ambulation) due to cognitive deficits and tetraparesis	Negative
	20	12/M	Left side ataxia, bilateral tremor, weakness, short-term memory loss; status epilepticus after testis teratoma removal	Yes	FLAIR hyperintensities in limbic region; right cortical atrophy	13 WBC/μl, <45mg/dl protein, OB positive	Tumor removal, steroids, IVIg	Partial, ataxia and coordination problems 4 months after onset	Negative
	21	41/F	Subacute diplopia, ophthalmoplegia, impaired consciousness; left ovarian teratoma discovered at workup of encephalitis (history of a right ovarian teratoma removed 10 years earlier)	Yes	Normal	25 WBC/μl, 85mg/dl protein	IVIg, plasma exchange	Complete, related to immunotherapy	Reactivity of serum with cell surface of neurons
	22	31/F	Myoclonus of lips, diplopia, confusion, catatonia, orthostatic hypotension	Yes	Increased FLAIR signal in medial temporal lobes	106 WBC/μl (98% L), 63.2mg/dl protein	Tumor removal, steroids	Complete, related to tumor removal and immunotherapy	Negative

[a]Normal brain MRI, but decreased degree of tracer accumulation in the brainstem and bilateral cerebral hemispheres on single photon emission computed tomography.
CSF = cerebrospinal fluid; F = female; FLAIR = fluid-attenuated inversion recovery; IVIg = intravenous immunoglobulin; L = lymphocyte; M = male; MRI = magnetic resonance imaging; NA = not available; OB = oligoclonal bands; PET = positron emission tomography; WBC = white blood cell count.

antibodies developed brainstem–cerebellar symptoms during the first month of the disease, 10 (45%) of them with opsoclonus, whereas these symptoms rarely occurred in patients with NMDAR antibodies. The identification of a predominant brainstem–cerebellar syndrome led us to focus on this disorder and the subgroup of patients with opsoclonus, both described below (the other 16 patients are shown in the Supplementary Table).

Brainstem–Cerebellar Syndrome

The median age of the 22 patients with brainstem–cerebellar symptoms was 28.5 years (IQR = 22–32, range = 12–41). Twenty (91%) were female, all with ovarian teratoma; 2 male patients had testicular teratoma. Main symptoms included ataxia in 86%, opsoclonus–myoclonus in 45% (described below), dysarthria in 36%, decreased level of consciousness in 32%, diplopia or ophthalmoparesis in 18%, and seizures in 18%. Other symptoms are listed in the Table 1.

Neurological symptoms developed before tumor diagnosis in 18 patients (82%; median = 1 month, IQR = 0.9–2 months, range = 3 days to 24 months) and after tumor diagnosis in 4 (10 days and 1.5, 2, and 3.5 months, respectively). Two of these 4 patients had the tumor removed 3 days and 1.5 months before developing encephalitis, respectively. All patients had mature teratomas, except 1 who had an immature ovarian teratoma. Serum of 3 patients (2 with opsoclonus) and CSF of another patient showed weak immunolabeling of cultures of rat neurons (data not shown); no antibodies were identified in the other patients.

Treatment and follow-up information was available for 19 (86%) patients, including all patients with opsoclonus (described below). Fifteen (79%) received immunotherapy, 13 of them with tumor resection; 2 had tumor resection without immunotherapy, and 2 were not treated (1 had tumor removal before developing encephalitis). With a median follow-up of 15 months (range = 3–84), 14 patients (74%) had full recovery, 3 (16%) had partial improvement, and 2 had no improvement (1 of them was not treated). Two patients with complete recovery and 1 with partial recovery relapsed 2 years, 7 years, and 8 months after disease onset, respectively.

Opsoclonus–Myoclonus Syndrome

Ten women (median age = 27 years, IQR = 22–30, range = 15–32) with brainstem–cerebellar syndrome developed opsoclonus; accompanying symptoms are listed in the Table 1. Four had prodromal fever or viral-like symptoms, and another one was 26 weeks pregnant. Symptoms developed before the tumor diagnosis in 7

(median = 1 month, IQR = 0.1–1.5 months, range = 3 days to 2 months) and after tumor diagnosis in 3 (10 days, 2 months, and 3.5 months, respectively). One of these 3 patients had undergone tumor resection 3 days before developing opsoclonus; the other 2 patients had not had tumor treatment.

At symptom onset, 7 patients had CSF lymphocytic pleocytosis (median = 37 white blood cells/μl, range = 10–182), 6 had increased protein concentration (median = 64/dl, range 49–100), and 3 of 3 had oligoclonal bands. Brain magnetic resonance imaging and electroencephalographic studies were abnormal in 2 of 9 and 3 of 5 patients (see Table 1).

All patients were treated with methylprednisolone: 3 alone, 3 combined with intravenous immunoglobulin (IVIg), and 4 with IVIg and plasma exchange. Two patients received rituximab after failing initial immunotherapy, and 1 received azathioprine (see Table 1). Nine patients had resection of the teratoma; pathological studies showed mature teratoma in 8, including 1 with bilateral teratomas, and immature teratoma in 1. Chemotherapy was used in 2 patients (see Table 1). Valproic acid, clonazepam, levetiracetam, or phenobarbital did not control the opsoclonus–myoclonus (data not shown).

The median time of follow-up was 19.5 months (IQR = 6–39, range = 3–84). Eight patients had full recovery, and 2 had mild residual dysarthria and ataxia at 13- and 15-month follow-up, respectively. Six of the 8 patients with full recovery became asymptomatic within the first 3 months of treatment, and the other 2 patients within 6 and 12 months, respectively.

Discussion

This study shows that patients with systemic teratoma can develop several forms of encephalitis without NMDAR antibodies, among which a syndrome that associates with brainstem–cerebellar symptoms stands out. Almost 50% of patients with this syndrome developed opsoclonus in association with the triad of young age (teenager to young adult), presence of an ovarian teratoma, and high response to treatment. The subacute presentation of symptoms, frequent CSF pleocytosis, and response to immunotherapy coupled with the detection of antibodies to neuronal cell-surface antigens in some patients suggest an immune-mediated pathogenesis.

All patients with opsoclonus were young women (aged 15–32 years), considered too young for carcinoma-associated opsoclonus, which usually occurs in patients >50 years old,[10] and too old for neuroblastoma-associated opsoclonus, which usually affects children <5 years old.[11] It is likely that this type of opsoclonus has been previously considered idiopathic or postinfectious and

that the presence of a teratoma was missed or not felt to be related.

Compared with patients with anti-NMDAR encephalitis, those without these antibodies were less likely to initially present with psychosis and behavioral change. Although there was overlap of some symptoms, such as limbic dysfunction and psychiatric manifestations, the frequency of other symptoms, such as dyskinesias, rarely occurred in patients without NMDAR antibodies. In contrast, patients with anti-NMDAR encephalitis did not initially present with brainstem–cerebellar dysfunction or opsoclonus. Of note, ataxia can be a presentation of anti-NMDAR encephalitis in children[2,12]; this is not reflected here, because young children usually do not have teratomas.

This study has several practical implications. Any teenager or young adult, especially if female, who develops subacute brainstem–cerebellar symptoms or opsoclonus–myoclonus suspected to be immune-mediated (because of the rapid onset of symptoms and/or CSF pleocytosis) should be investigated for a teratoma in the ovary (or testes for male patients). Detection of a teratoma should prompt its removal along with the use of immunotherapy (most patients described here received steroids, IVIg, and/or plasma exchange). A limitation of this study is that it is retrospective; future studies will establish the frequency of these disorders and may identify patients with higher levels of cell-surface antibodies that could lead to the characterization of the antigens.

Acknowledgment

Potential Conflicts of Interest

J.D.: grants/grants pending, Euroimmun, NIH; patents, royalties, Athena Diagnostics Euroimmun; editorial board, *Up-To-Date*.

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Supplementary Table 1: Clinical and immunological features of patients with teratoma associated encephalitis (without brainstem-cerebellar syndrome) without anti-NMDAR antibodies

	Age, sex	Main symptoms	Neurologic symptoms before tumor diagnosis	Brain MRI	CSF	Treatment	Response to treatment	Immunological studies with cultures of neurons
23	24, F	Actue onset of decreased level of consciousness, status epilepticus, suspected limbic encephalitis. Remained with seizures and memory deficits	Yes	1st, normal. 12 days later: T2/FLAIR increased signal in temporal lobes	Mild pleocytosis with increased lymphocytes, and protein concentration	Tumor removal, steroids	Partial, related to immunotherapy and tumor removal At last follow up (3 years after symptom onset) has mild memory deficits, and infrequent seizures (2 seizures per year)	Negative
24	49, F	Intractable seizures for 2 months, memory deficits, suspected limbic encephalitis. In addition, leg weakness, inability to walk	Yes	FLAIR increased signal in right occipital, temporal, parietal cortex, with areas of restricted diffusion	Mild pleocytosis with increased lymphocytes, protein: <45mg/dL	NA	NA	Negative
25	29, F	Left extremity weakness, followed 1 month later by altered mental status, poor attention, impulsive behavior, agitation, short-term memory deficits. Left ovarian teratoma discovered at workup of encephalitis (history of a right ovarian teratoma removed 9 years earlier)	Yes	T2/FLAIR increased signal in insula, putamen and right thalamus (non-enhancing, no diffusion restriction)	13 WBC/ μL protein: <45mg/dL OB negative	Tumor removal, steroids, IVIG, plasma exchange, rituximab	No improvement 5 months after symptom onset.	CSF reactivity with the cell surface of neurons. Cell-based assays demonstrated AMPA receptor antibodies
26	39, M	Confusion, memory loss, narcolepsy, ptosis, and eye movement disorder (history of orchiectomy for immature teratoma 14 months earlier).	No	Intense contrast enhancement in diencephalon and medial temporal lobes	3 WBC/ μL protein: 57 mg/dL	Plasma exchange, chemotherapy	No response	Negative Ma2 antibodies in serum (westernblot)
27	29, F	Isolated psychiatric symptoms with diffuse slowing of cerebral electrical activity in EEG	Yes	Normal	24 WBC/ μL protein: <45mg/dL	Not treated	Complete, spontaneous improvement	Negative
28	17, F	Isolated psychiatric symptoms; personality change, hallucinations. Six month later: visual changes associated with increased intracranial pressure without papilledema	Yes	Normal	NA	Tumor removal, IVIG	No response	Negative
29	16, F	Isolated psychiatric symptoms; depression, weight loss, non-epileptic spells, poor school performance (developed a few days after tumor removal)	No	Normal	<5 WBC/ μL protein: <45mg/dL	Tumor removal (before neurological symptoms)	Symptoms remained 7 months after onset	Negative
30	56, F	Isolated psychiatric symptoms; confusion aggressive behavior	Yes	Normal	<5 WBC/ μL protein: <45mg/dL	Tumor removal	Substantial improvement, related to tumor removal	Negative
31	19, F	17 weeks pregnant: developed abdominal pain, and nystagmus, followed by agitation, inappropriate behavior, tachycardia. Later developed mild sensory neuropathy	Yes	1st, normal; repeat MRI: FLAIR abnormalities in bilateral medial thalamic region	NA	Tumor removal, steroids, plasma exchange	Partial, related to immunotherapy and tumor removal	Negative

32	20, F	Delirium, decreased level of consciousness, meningeal signs, nystagmus, hypoventilation, limb tremor	Yes	Mild meningeal enhancement	Mild pleocytosis with increased lymphocytes	Steroids	No response	Negative
33	26, F	Decreased level of consciousness, disorientation, memory deficit	Yes	Normal	21 WBC/ µL protein: <45mg/dL Repeat study: 4 WBC/ µL protein: <45mg/dL	Tumor removal	Complete, related to tumor removal	Negative
34	39, F	Clonic seizures, abnormal behavior, confusion	No	Normal	1000 WBC/ µL (99% L)	Tumor removal (before neurological symptoms)	Symptoms remained 5 months after onset	Negative
35	47, F	Meningoencephalitis. Recurrence of right ovarian teratoma previously removed discovered at workup of encephalitis	Yes	Parietal cortical and meningeal enhancement	Mild pleocytosis with increased lymphocytes	NA	NA	Negative
36	19, F	Decreased level of consciousness, seizures, myoclonus, hypoventilation	Yes	Abnormal findings in DWI in temporal lobes	28 WBC/ µL protein: <45mg/dL OB negative	Steroids	No response	Negative
37	25, F	Three weeks after delivery: subacute onset of drowsiness, unresponsiveness and altered speech	Yes	Normal	111 WBC/ µL protein: <45mg/dL	NA	NA	Reactivity of serum with cell surface of neurons
38	16, F	Dizziness and vomiting, abdominal pain, autonomic instability (urinary retention and syncope). Several months later: limb dyskinesias	Yes	Normal	8 WBC/ µL protein: <45mg/dL OB negative	NA	NA	Negative

CSF = cerebrospinal fluid, F= female, M= male, FLAIR = fluid attenuated inversion recovery, DWI= diffusion-weighted imaging, NA = not available, WBC = white blood cells count, OB= oligoclonal bands, IVIG= intravenous immunoglobulin.

Objectiu 2:

Article II: Encephalitis with refractory seizures, status epilepticus, and antibodies to the GABA$_A$ receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies.

Autors: Petit-Pedrol M*, **Armangue T***, Peng X*, Bataller L, Cellucci T, Davis R, McCracken L, Martinez-Hernandez E, Mason WP, Kruer MC, Ritacco DG, Grisold W, Meaney BF, Alcalá C, Sillevis-Smitt P, Titulaer MJ, Balice-Gordon R, Graus F, Dalmau J. *igual contribució

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A new encephalitis with GABA$_A$ receptor antibodies

In the past decade, several autoimmune neurological syndromes have been identified. Many of these syndromes, especially those responsive to immunotherapy, involve antibodies to cell-surface antigens, such as the NMDA receptor, LGI1, and the GABA$_B$ receptor.[1,2] Detailed investigations have led to the recognition of distinct features that aid in early diagnosis. For example, faciobrachial dystonic seizures—very brief (<3 seconds) contractions of the arm and usually the ipsilateral face—are highly suggestive of the non-paraneoplastic, autoimmune syndrome associated with anti-voltage-gated potassium channel complex antibodies, specifically the anti-LGI1 variant.[3] This is a treatable disorder with an excellent prognosis when recognised early. The faciobrachial dystonic seizures usually precede the rest of the syndrome, which consists of hyponatraemia and limbic encephalitis. Treatment with immunotherapy, especially corticosteroids, in the early phase can not only lead to improvement but can prevent the limbic encephalitis.[4] Similarly, the clinical features of anti-NMDA receptor encephalitis are quite distinct, with psychiatric presentation, followed by rapid deterioration, including central apnoea requiring ventilatory support, and prominent dyskinesias, often in young women, and often in those with ovarian teratomas.[5] Extreme delta brushes on EEG might also be suggestive of this syndrome.[6] Identification of the antibodies to cell-surface antigens in these syndromes not only suggests good response to immunotherapy but also raises suspicion of specific yet-to-be-detected underlying malignancies—eg, small-cell lung cancer is present in half of patients with limbic encephalitis with GABA$_B$ (metabotropic) receptor antibodies.[7]

In *The Lancet Neurology*, Mar Petit-Pedrol and colleagues elegantly describe yet another treatable autoimmune neurological syndrome, based primarily on findings in six patients.[8] In this disorder, the culprit antigen is the GABA$_A$ receptor, the primary ligand-gated, ionotropic, fast-acting inhibitory receptor in the brain. The six patients were two young children, one teenager, and three adults aged 28–63 years. The syndrome is one of status epilepticus refractory to antiepileptic drugs with cortical and subcortical MRI abnormalities. The teenager and the three adults had weeks to months of prodromal mood and cognitive changes prior to status epilepticus. Analysis of CSF samples showed mild pleocytosis in four of six patients with white blood cell counts of up to 154 cells per μL. MRI scans showed abnormal signal in the temporal lobes, often with extensive extratemporal abnormalities. Two patients died during the acute illness, two recovered fully, and two recovered partially. Three of the four who improved received immunotherapy.

How did the investigators identify this syndrome? They first found the antibody in two index patients. They then re-examined serum and CSF samples from 140 patients with encephalitis, severe seizures, and antibodies to rat neuropil, but to a previously unknown target. Controls were 75 healthy individuals and 416 patients with other neurological diseases. High titres (>1:160) of antibodies to the GABA$_A$ receptor were seen in an additional four of 140 patients with encephalitis and status epilepticus, but not in control individuals. The antibodies targeted the α1 or β3 subunits of the GABA$_A$ receptor. Using CSF samples from one of the patients on a rat cell culture assay, the investigators show that the patient's antibodies cause the specific loss of synaptic GABA$_A$ receptors (as is seen in refractory status epilepticus of other causes[9]), but not dendritic GABA$_A$ receptors or other synaptic proteins. This finding makes sense, because α1 subunits are concentrated in synapses but not in dendrites.[10] An

interesting observation is that several patients also had other autoimmune disorders or antibodies, primarily to intracellular targets such as GAD65 or TPO, as has been shown in other immune encephalitides. Three of these six patients would have qualified for the diagnosis of steroid-responsive encephalopathy associated with autoimmune thyroiditis (Hashimoto's encephalopathy). The investigators postulate that at least some cases of that syndrome and other syndromes associated with antibodies to intracellular targets (including GAD65) are actually due to antibodies to cell-surface antigens such as the $GABA_A$ and $GABA_B$ receptors.[8]

The main limitation of this study is the small number of patients. Additionally, neuroimaging, CSF, or EEG data are incomplete from some patients. The study's findings do not help establish which antiepileptic drugs might work (are GABAergic drugs effective?), if any, or which immunotherapies are best. Whether these antibodies might also have a role in milder syndromes, or perhaps in cryptogenic epilepsy, warrants further study. For now, the important point is that the investigators have uncovered yet another neurological syndrome, which is severe but treatable, with specific auto-antibodies and a plausible pathophysiological mechanism. More details about this syndrome, and additional antibodies and syndromes, are sure to emerge in the coming years.

Lawrence J Hirsch
Department of Neurology, Yale University, New Haven, CT 06831, USA
lawrence.hirsch@yale.edu

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Corrections

Olanow CW, Kieburtz K, Odin P, et al, for the LCIG Horizon Study Group. Continuous intrajejunal infusion of levodopa-carbidopa intestinal gel for patients with advanced Parkinson's disease: a randomised, controlled, double-blind, double-dummy study. Lancet Neurol 2014; **13:** 141–49—In part A of figure 2 of this Article, the p value for "on-time without troublesome dyskinesia" should have been 0·0059. In the fifth paragraph of the Discussion section, the citation to table 3 should have been to table 2. These corrections have been made to the online version as of Feb 17, 2014.

Encephalitis with refractory seizures, status epilepticus, and antibodies to the GABA$_A$ receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies

Mar Petit-Pedrol*, Thaís Armangue*, Xiaoyu Peng*, Luis Bataller, Tania Cellucci, Rebecca Davis, Lindsey McCracken, Eugenia Martinez-Hernandez, Warren P Mason, Michael C Kruer, David G Ritacco, Wolfgang Grisold, Brandon F Meaney, Carmen Alcalá, Peter Sillevis-Smitt, Maarten J Titulaer, Rita Balice-Gordon, Francesc Graus, Josep Dalmau

Summary

Background Increasing evidence suggests that seizures and status epilepticus can be immune-mediated. We aimed to describe the clinical features of a new epileptic disorder, and to establish the target antigen and the effects of patients' antibodies on neuronal cultures.

Methods In this observational study, we selected serum and CSF samples for antigen characterisation from 140 patients with encephalitis, seizures or status epilepticus, and antibodies to unknown neuropil antigens. The samples were obtained from worldwide referrals of patients with disorders suspected to be autoimmune between April 28, 2006, and April 25, 2013. We used samples from 75 healthy individuals and 416 patients with a range of neurological diseases as controls. We assessed the samples using immunoprecipitation, mass spectrometry, cell-based assay, and analysis of antibody effects in cultured rat hippocampal neurons with confocal microscopy.

Findings Neuronal cell-membrane immunoprecipitation with serum of two index patients revealed GABA$_A$ receptor sequences. Cell-based assay with HEK293 expressing α1/β3 subunits of the GABA$_A$ receptor showed high titre serum antibodies (>1:160) and CSF antibodies in six patients. All six patients (age 3–63 years, median 22 years; five male patients) developed refractory status epilepticus or epilepsia partialis continua along with extensive cortical-subcortical MRI abnormalities; four patients needed pharmacologically induced coma. 12 of 416 control patients with other diseases, but none of the healthy controls, had low-titre GABA$_A$ receptor antibodies detectable in only serum samples, five of them also had GAD-65 antibodies. These 12 patients (age 2–74 years, median 26·5 years; seven male patients) developed a broader spectrum of symptoms probably indicative of coexisting autoimmune disorders: six had encephalitis with seizures (one with status epilepticus needing pharmacologically induced coma; one with epilepsia partialis continua), four had stiff-person syndrome (one with seizures and limbic involvement), and two had opsoclonus-myoclonus. Overall, 12 of 15 patients for whom treatment and outcome were assessable had full (three patients) or partial (nine patients) response to immunotherapy or symptomatic treatment, and three died. Patients' antibodies caused a selective reduction of GABA$_A$ receptor clusters at synapses, but not along dendrites, without altering NMDA receptors and gephyrin (a protein that anchors the GABA$_A$ receptor).

Interpretation High titres of serum and CSF GABA$_A$ receptor antibodies are associated with a severe form of encephalitis with seizures, refractory status epilepticus, or both. The antibodies cause a selective reduction of synaptic GABA$_A$ receptors. The disorder often occurs with GABAergic and other coexisting autoimmune disorders and is potentially treatable.

Introduction

Seizures and status epilepticus can result from immunological responses to excitatory or inhibitory synaptic receptors or associated cell-surface proteins.[1–3] These include the N-methyl-D-aspartate receptor (NMDAR),[4] the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR),[5] the gamma-aminobutyric acid-B receptor (GABA$_B$R),[6] leucine-rich glioma inactivated protein 1 (LGI1),[7] contactin-associated protein-like 2 (Caspr2),[8,9] dipeptidyl-peptidase-like protein-6 (DPPX),[10] and the metabotropic glutamate receptor 5 (mGluR5).[11]

The seizures that accompany any of these disorders are often refractory to antiepileptic treatment unless the immune mechanism is identified and treated.[6,12,13] In some patients, generalised seizures or status epilepticus can be the first manifestation of the disease, with patients needing heavy sedation or induced pharmacological coma.[6,14–16] These treatments might conceal other symptoms such as dyskinesias or psychiatric alterations, delaying the recognition of the syndrome. Hitherto, the main epilepsy-related inhibitory receptor known to be a target of autoimmunity was the GABA$_B$R.[9,16,17] Most

patients with GABA_BR antibodies develop early seizures or status epilepticus as a component of limbic encephalitis. About 50% of these patients have an underlying small-cell lung cancer, and the neurological symptoms usually respond to immunotherapy and treatment of the cancer.[9,16,17] Although the GABA_BR belongs to the category of metabotropic G protein-coupled receptors, the GABA_A receptor (GABA_AR) is a ligand-gated ion channel that modulates most of the fast inhibitory synaptic transmission in the brain and has not been previously recognised as a target of autoimmunity.

The identification of the above-mentioned disorders, all potentially treatable with immunotherapy,[1–11] has enhanced awareness of autoimmune mechanisms in patients with encephalitis associated with refractory seizures or status epilepticus, leading to an increased recognition of cases in which the antigens are unknown. Some patients might have several autoantibodies, suggesting that they have a propensity to autoimmunity, but also leading investigators to attribute the disorder to intracellular antigens that are not accessible to circulating antibodies, such as thyroid peroxidase or glutamic acid decarboxylase 65 (GAD65),[5,6] and therefore of questionable pathogenic significance. In such patients, other more relevant, yet unknown cell-surface antigens can be overlooked, as occurred in previously reported patients who were eventually shown to have AMPAR or GABA_BR antibodies.[5,6] We aimed to establish the identity of a novel synaptic antigen in a subset of patients with encephalitis and refractory seizures or status epilepticus. We report the clinical features of this new syndrome, the identity of the antigen, and the effects of patients' antibodies on neuronal cultures.

Methods
Study design and participants
Between Aug 20, 2012, and Dec 10, 2012, we identified two patients with encephalitis, refractory seizures, and serum and CSF antibodies with a similar pattern of reactivity against the neuropil of rat brain (appendix). The severity of the symptoms and unknown identity of the antigen prompted us to immunoprecipitate the antigen and to retrospectively review clinical and immunological information from patients with similar symptoms. We assessed serum and CSF samples, collected worldwide between April 28, 2006, and April 25, 2013, from 1134 patients with encephalitis and seizures that were suspected to be autoimmune. The samples had been sent to two referral centres (Department of Neurology, Hospital of the University of Pennsylvania, PA, USA, and Center of Neuroimmunology, Institut d'Investigacions Biomediques August Pi i Sunyer [IDIBAPS], Hospital Clinic, University of Barcelona, Barcelona, Spain) for confirmation of the presence of cell-surface antibodies or investigation for novel antibodies after standard laboratory studies were negative. Serum and CSF samples were kept frozen at –80° C. For all patients, we obtained clinical information using a questionnaire completed by the treating physicians when the samples were sent to our centres. The treating physician also did subsequent clinical follow-up via email or phone.

Of these 1134 patients, 356 (44%) had antibodies that reacted with known cell-surface or synaptic antigens such as NMDAR, AMPAR, and LGI1, and 140 (including the two index patients) had the triad of encephalitis, seizures, and antibodies against unknown rat brain neuropil antigens. In all instances, the assessment of antibodies to brain neuropil antigens was done independently by two investigators (FG and JD), with results kept in a database. We then re-examined serum and CSF samples from these 140 patients with immunohistochemistry of rat brain, cultured live neurons, and a cell-based assay to establish whether they had similar antibodies to the two index patients. We also examined serum samples from 75 otherwise healthy individuals (blood donors) and serum or CSF samples from 416 patients with a range of neurological disorders (worldwide referrals). These 416 patients with diverse disorders were re-examined for neuropil antibodies and antibodies to α1/β3 subunits of the GABA_AR. They included 41 seronegative patients with encephalitis and seizures or status epilepticus, 59 with opsoclonus-myoclonus, 20 with non-inflammatory degenerative ataxia, nine with herpes-simplex-virus encephalitis, 30 with multiple sclerosis, 101 with antibodies against GAD65 (16 limbic encephalitis, 33 epilepsy, 13 ataxia, 39 stiff-person syndrome), 90 with stiff-person syndrome without GAD65 antibodies, 30 with NMDAR antibodies, 19 with GABA_BR antibodies, and 17 with LGI1 antibodies. Only serum samples were available from 238 patients, and only CSF samples were available from 35 patients, with both types of samples available from 143 patients.

Partial clinical information about two patients with coexisting GABA_BR antibodies (patients 4 and 6) has been reported elsewhere.[9,18] Our final protocol was approved by the institutional review boards of the University of Pennsylvania and the Hospital Clinic, and written informed consent was obtained from all patients or representatives.

Laboratory procedures
All laboratory techniques are described in the appendix and elsewhere.[5,19–22] Briefly, we did immunohistochemistry on rodent brain, immunocytochemistry of rodent neuronal cultures, immunoprecipitation, mass spectrometry, immunoabsorption and immune-competition studies, immunocytochemistry on live or fixed HEK293 cells (cell-based assays), quantitative analysis of neuronal GABA_AR immunoreactivity of patients' antibodies, and analysis of the effects of these antibodies on GABA_AR using confocal microscopy.

Role of the funding source
The study sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of

Vienna, Austria (Prof W Grisold MD); Division of Neurology, Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada (B F Meaney MD); Department of Neurology, Erasmus Medical Center, Rotterdam, Netherlands (Prof P Sillevis-Smitt MD, M J Titulaer MD); Hospital Clinic, University of Barcelona, Barcelona, Spain (M Petit-Pedrol, T Armangue, Prof F Graus, Prof J Dalmau); and Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain (Prof J Dalmau)

Correspondence to: Prof Josep Dalmau, IDIBAPS-Hospital Clínic, Universitat de Barcelona, Department of Neurology, c/ Villarroel 170, Barcelona, 08036, Spain jdalmau@clinic.ub.es

the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

On immunohistochemistry with rat brain, the serum and CSF samples of the two index patients produced a similar and intense pattern of neuropil reactivity (figure 1, appendix). This neuropil reactivity resembled that reported for $GABA_BR$ antibodies (figure 1, appendix),[6] but specific testing for these antibodies with a cell-based assay was negative in both index patients (data not shown). Findings from a subsequent assessment with cultured live rodent hippocampal neurons showed that the novel antigen was on the cell surface (figure 1). Immunoprecipitation of neuronal proteins reacting with antibodies from the two index patients, followed by electrophoretic protein separation and EZBlue gel staining, did not produce any specific band compared

***Figure 1*: Reactivity with brain tissue and neuron cultures of the CSF of patients with $GABA_AR$ or $GABA_BR$ antibodies**
The CSF of patient 2 showed extensive and diffuse immunostaining of the neuropil of cortical and subcortical regions (A; see the appendix for higher magnifications of selected brain regions). This pattern of brain and cerebellar staining is similar to that produced by the CSF of a patient with $GABA_BR$ antibodies (C). However, patient 2 was negative for $GABA_BR$ antibodies in a specific cell-based assay (data not shown). These findings suggested the presence of antibodies against a novel neuronal cell-surface antigen, which was confirmed in cultures of live rat hippocampal neurons (B). The CSF of the patient with $GABA_BR$ antibodies also reacted with the neuronal cell surface, as expected (D). E and F show a similar study using CSF of a control patient without neuronal cell-surface antibodies. In B, D, and F the nucleus of the neurons was counterstained with DAPI. In A, C, and E the tissue was counterstained with haematoxylin.

with serum samples from individuals in the healthy control group (data not shown). Mass spectrometry of all separated proteins showed that serum samples from the two index patients but not from otherwise healthy control individuals had precipitated protein fragments containing sequences of the β3 subunit of the $GABA_AR$ (sequences shown in appendix).

Because the β3 subunit of the $GABA_AR$ forms complexes with the α1 subunit, we tested the reactivity of patients' antibodies with HEK293 cells transfected with the human α1 or β3 subunits or both. This cell-based assay identified $GABA_AR$ antibodies in six patients, including four of the 140 patients with encephalitis, seizures or status epilepticus, and antibodies to unknown neuropil antigens, and two of the 19 patients with $GABA_BR$ antibodies. All six patients' serum and CSF samples reacted with cells coexpressing α1 and β3 subunits, but when the subunits were individually assessed, four patients' samples reacted with both the α1 and β3 subunit, one patient's sample reacted with only the α1 subunit, and another patient's sample reacted with only the coexpression of α1 and β3 subunits. For this reason, we subsequently used α1 and β3 heteromers to assess antibody titres. To optimise the cell-based assay, we compared the sensitivity of the assay with live or fixed and permeabilised α1/β3 receptor-expressing HEK293 cells (live cell-based assay vs fixed cell-based assay). These studies showed that all patients' CSF antibodies were detectable with either live or fixed cell-based assay, but serum antibodies were mostly visible with live cell-based assay (figure 2).

Immunocompetition assays with serum antibodies of the six patients showed that all recognised the same epitopes of the $GABA_AR$ (appendix). Immunoabsorption of a representative serum sample with HEK293 cells expressing the α1/β3 subunits resulted in abrogation of reactivity in rat brain and culture neurons, further confirming the reactivity with the $GABA_AR$ (figure 3).

Using live cell-based assay in cells coexpressing α1/β3, we identified two clinical-immunological groups of patients: the first comprised the six patients with $GABA_AR$ antibodies identified from the cohort of 140 patients with encephalitis and unknown neuropil antigens and from the group of 19 patients with $GABA_BR$ antibodies; all six patients had a high titre (>1:160) of serum (when available) and CSF $GABA_AR$ antibodies (patients 1–6; table. The second group consisted of 12 patients from the disease control groups, but not from the group of healthy individuals. In these 12 patients, the serum antibody titre was always 1:160 or lower; CSF samples were available from three individuals (patients 7, 12, and 18) and all three were negative. Moreover, whereas in patients in the first group the antibodies were detectable with three techniques (immunohistochemistry with rat brain, cultured neurons, and live or fixed cell-based assay), in patients of the second group the antibodies were
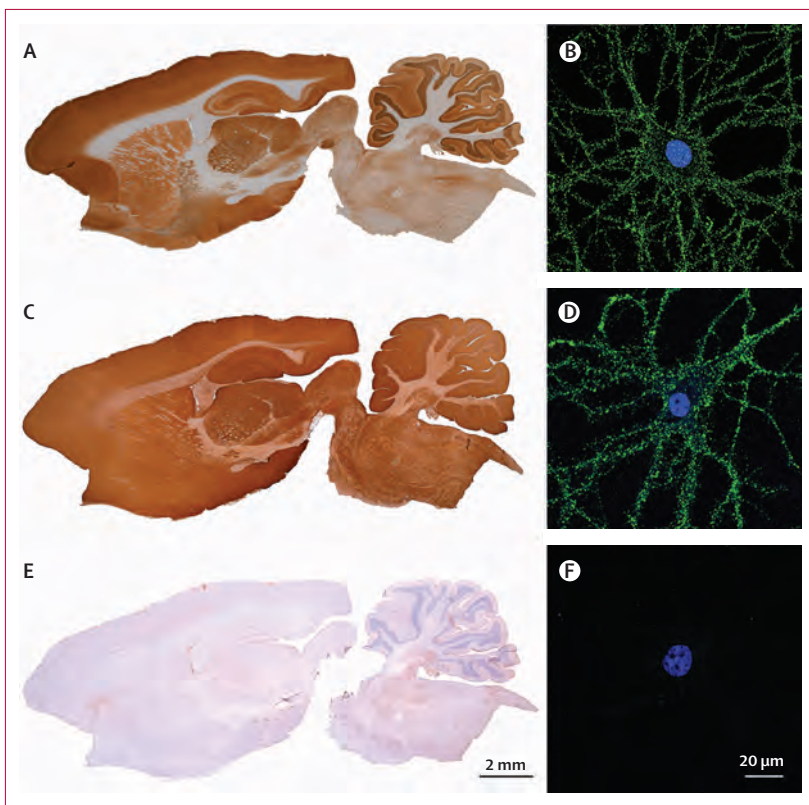
detectable only with live cell-based assay (all individuals) and cultured live neurons (patients 7–13).

Five of the six patients with high concentrations of GABA$_A$R antibodies in serum and CSF samples were male; three were children and three were adults (age range 3–63 years). All developed a rapidly progressive encephalopathy that eventually resulted in refractory seizures, five of the six had status epilepticus and one of them (patient 2) also had epilepsia partialis continua (table). In all six patients, epileptic symptoms were preceded or associated with a change in behaviour or level of cognition. A 3-year-old child (patient 4), who also had GABA$_B$R antibodies, developed seizures along with confusion, opsoclonus, ataxia, and chorea. Another patient (patient 5) developed a progressive hemiparesis before seizures. One patient had normal CSF white-cell count and protein concentration and the other five had at least one abnormality, including pleocytosis in four of six patients, increased protein concentration in four of six patients, and oligoclonal bands in two of patients (patients 2 and 6 in the table). All six patients had abnormal brain MRI, often showing extensive abnormalities on FLAIR and T2 imaging, with multifocal or diffuse cortical involvement without contrast enhancement (figures 4 and 5); one patient had involvement of the basal ganglia. The EEG showed seizures in all patients, two of whom had periodic generalised discharges (appendix). In addition to GABA$_A$R antibodies, three patients had thyroid peroxidase antibodies, one had GAD65 antibodies, and two had GABA$_B$R antibodies. Other findings suggestive of a propensity to autoimmunity or immune dysregulation included a past history of Hodgkin's lymphoma in one patient, and idiopathic thrombo-cytopenic purpura in another.

Treatment and follow-up were assessable in all six patients: one child received levetiracetam without immunotherapy and had substantial recovery, although 3 years after symptom onset he still requires antiepileptic treatment to avoid seizure recurrence. The other five received immunotherapy and multiple antiepileptic drugs, and four patients needed a pharmacologically induced coma. Three of these patients had total or partial recovery and two died as a result of sepsis during status epilepticus. One death was a child (patient 4) with concomitant GABA$_B$R antibodies indicated above (clinical and autopsy findings have been reported in detail elsewhere;[18] the GABA$_A$R antibodies were identified in archived serum and CSF samples). The oldest of the six patients (age 63 years) also had GABA$_B$R antibodies; the GABA$_A$R antibodies were identified in samples that had been archived for 7 years. This patient fully recovered from the encephalopathy associated with antibodies against both GABARs, but 7 years later developed diplopia and hemiataxia with GAD65 antibodies (without antibodies to GABARs) from which he fully recovered.

12 patients (age 2–74 years, median 26·5 years; seven male patients) had low serum concentrations of GABA$_A$R
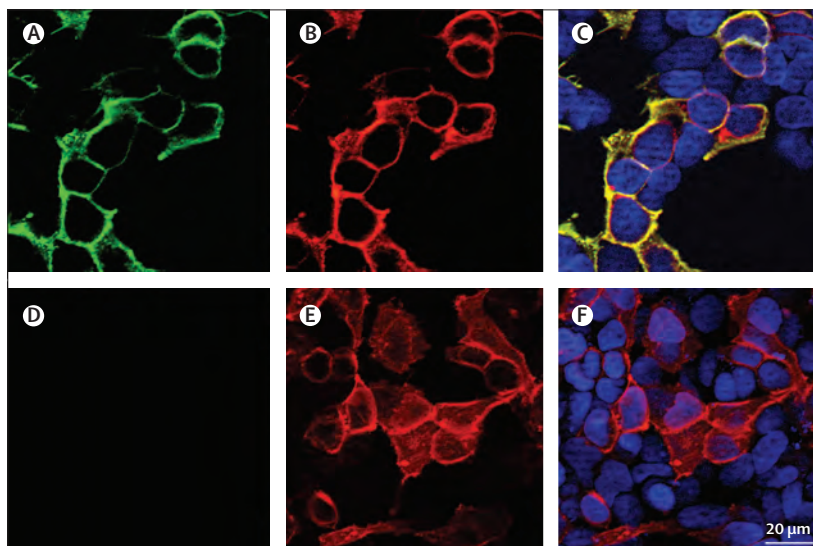
Figure 2: Reactivity of a patient's serum with live HEK293 cells expressing GABA$_A$R
Reactivity of live HEK293 cells expressing human a1/b3 subunits of the GABA$_A$R with a patient's serum (A) and a monoclonal antibody against the a1 subunit (B). Merged reactivities (C). A similar assay with serum from a control individual is shown in (D–F). The nuclei of the cells are shown with DAPI in C and F. Note the specific reactivity of patient's antibodies with cells expressing GABA$_A$R and the co-localisation with the reactivity of the commercial antibody.
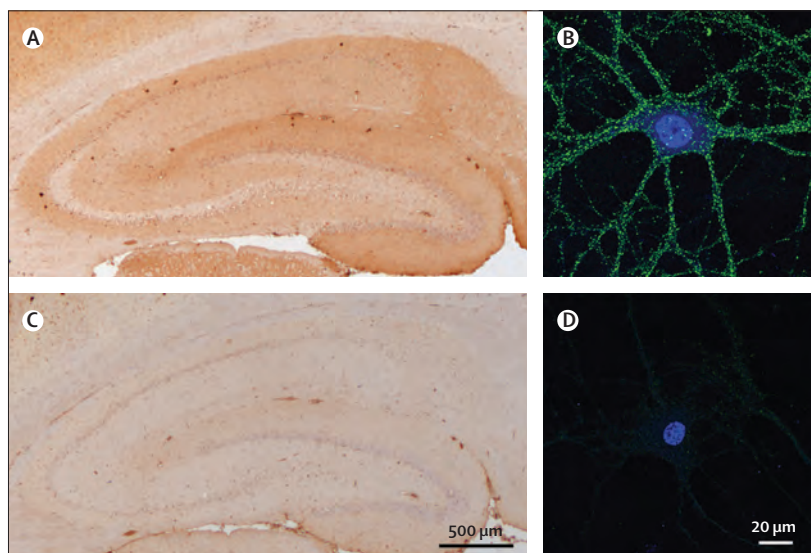
Figure 3: Abrogation of serum antibody reactivity with brain and cultures of neurons after GABA$_A$R immunoabsorption
Panels A and B show the reactivity of a patient's serum after immunoabsorption with non-transfected HEK293 cells. C and D show that this reactivity is abolished after the serum has been immunoabsorbed with HEK293 cells expressing the a1/β3 subunits of the GABA$_A$R.

antibodies (table). Briefly, all six patients with encephalitis had seizures; one of them (patient 7; a 2-year-old boy) with refractory status epilepticus that needed a pharmacologically induced coma, and another patient (patient 8; a 41-year-old man) with epilepsia partialis continua. In the other six patients, four had stiff-person syndrome (one of them associated with seizures), and two had opsoclonus-myoclonus.

Six of the 12 patients had other neuronal antibodies in addition to GABA$_A$R antibodies: five had GAD65 and one had NMDAR antibodies. Additional findings suggestive of a propensity to autoimmunity or immuno-logical dysfunction included: Hashimoto's thyroiditis with thyroid peroxidase antibodies in one patient, and type 1 diabetes mellitus in two patients. None of the patients with stiff-person syndrome had amphiphysin or glycine receptor (GlyR) antibodies. Treatment and follow-up were assessable in nine patients. Immunotherapy was used in seven patients: one had full recovery, five had partial recovery, and one died. The two patients who did not receive immunotherapy had stiff-

person syndrome that was controlled symptomatically with clonazepam or baclofen.

We did the following studies with CSF of a representative patient (index patient 1) with high titre serum and CSF antibodies reacting with only GABA$_A$R. The reactivity was abrogated by pre-absorption with HEK293 cells expressing GABA$_A$R (figure 3), and by immunocompetition assays with antibodies from the other five patients with high antibody titres, indicating that all patients' antibodies targeted the same GABA$_A$R epitopes (appendix). To examine the extent of recognition of GABA$_A$R by patients' CSF antibodies, we quantified GABA$_A$R immunelabelling by confocal microscopy

	Sex, age in years	Presentation and main symptoms	CSF	MRI	EEG	History of autoimmunity or cancer	Treatment	Outcome	Sample: subunit target (α1/β3 titres)
Patients with high titres of serum GABA$_A$R antibodies and with antibodies detectable in CSF									
1 (index patient 1)	F, 16	Memory, cognitive, and affective problems for several months. Developed headache and 9 days later tonic-clonic seizures progressing to status epilepticus	23 WBC/µL; protein 60 mg/dL	Multifocal increased T2/FLAIR signal with cortical-subcortical involvement	Generalised slowing, bilateral temporal seizures. Generalised periodic discharges	Hodgkin's lymphoma 10 months before onset of encephalitis	Anticonvulsants: LEV, TPM, MDZ, barbiturate coma. Immunosuppressants: MTP, IVIG, PEX, RTX, CPH	Progressive neurological recovery after 12 weeks in hospital. At 15-month follow-up she had returned to school with mild cognitive deficit that is improving	Serum: α1, β3 (>1/1280) CSF: α1 (>1/320)
2 (index patient 2)	M, 51	Behavioural change, depression, psychosis, and mutism for several weeks. Developed partial clonic seizures and epilepsia partialis progressing in 48 h to status epilepticus	Normal WBC and protein concentration; OCB-positive	Multifocal increased T2/FLAIR signal with extensive cortical-subcortical involvement	Right temporal ictal activity, secondary generalisation. Generalised periodic discharges	Idiopathic thrombocytopenic purpura; TPO and thyroglobulin antibodies	Anticonvulsants: LEV, DZP, LCM, PHT, MDZ, PPF, barbiturate coma. Immunosuppressants: MTP, IVIG, PEX, CPH, RTX.	After 10 weeks, status epilepticus persisted and the patient died of sepsis	Serum: α1, β3 (1/1280) CSF: α1 (1/320)
3	M, 28	Subacute presentation of behavioural and cognitive deficits followed 5 days later by complex partial seizures and status epilepticus	Normal WBC and protein concentration	Bilateral mesiotemporal high T2/FLAIR signal	Ictal activity	TPO antibodies	Anticonvulsants: PPF, MDZ, LEV, PHT, TPM, CLB, barbiturate coma. Immunosuppressants: MTP with PDN taper.	After 8 weeks in the intensive care unit he gradually returned to baseline function. At last follow-up (18 months) he was seizure free and back to work	Serum: α1, β3 (1/640) CSF: α1, β3 (1/160)
4	M, 3	Acute development of confusion, lethargy, dystonic tongue movements, chorea of limbs and trunk, opsoclonus, ataxia, evolving in 24 h to complex partial seizures and status epilepticus	154 WBC/µL; protein 59 mg/dL	Multifocal high T2/FLAIR signal in brainstem and cerebellum with involvement of basal ganglia and hippocampi	Generalised slowing and bioccipital ictal activity	GABA$_A$R antibodies in serum and CSF	Anticonvulsants: multiple, barbiturate coma. Decompressive posterior craniectomy due to cerebral oedema. Immunosuppressants: MTP, IVIG	After 4 weeks, status epilepticus persisted and the patient died of sepsis	Serum: NA CSF: α1, β3 (1/320)
5	M, 4	Progressive right hemiparesis; 2 months later, partial seizures progressing to status epilepticus	Increased WBC and protein concentration	Abnormal FLAIR changes suggesting encephalitis	Generalised slowing and ictal activity	No	Anticonvulsants: LEV. Immunosuppressants: No	Substantial recovery but, 2·5 years after symptom onset, still requires antiepileptics to prevent seizures.	Serum: α1 (1/320) CSF: α1/β3 (1/40)
6	M, 63	Subacute memory problems, gustatory and olfactory hallucinations, facial cramps, psychomotor agitation, tinnitus	75 WBC/µL; increased protein concentration; OCB-positive	Right temporal cortex high T2/FLAIR signal	Frontotemporal ictal activity	GABA$_A$R, GAD65, TPO and thyroglobulin antibodies	Anticonvulsants: VPA, LEV, barbiturate. Immunosuppressants: PDN	Full recovery. 7 years later: diplopia and hemiataxia that spontaneously resolved (positive GAD65 but negative GABA$_A$R and GABA$_B$R antibodies)	Serum: NA CSF: α1/β3 (1/20)

(Table continues on next page)

	Sex, age in years	Presentation and main symptoms	CSF	MRI	EEG	History of autoimmunity or cancer	Treatment	Outcome	Sample: subunit target (α1/β3 titres)
(Continued from previous page)									
Patients with low titres of serum GABAₐR antibodies and without antibodies detectable in CSF									
7	M, 2	Subacute onset partial seizures; 4 months later, choreathetoid movements and status epilepticus.	Normal WBC and protein concentration	Cortical atrophy	Generalised slowing and right parietal ictal activity	No	Anticonvulsants: CBZ, VPA, MDZ, LEV, ketogenic diet, barbiturate coma. Immunosuppressants: MTP with PDN taper	Partial response, cognitive and motor skills improved. At last follow-up (2 years), partial seizures persist	Serum: β3 (1/160) CSF: negative
8	M, 41	Subacute onset generalised seizures with fever, epilepsia partialis continua, aphasia. 2 years later, status epilepticus	Normal WBC and protein concentration	Multifocal cortical-subcortical high T2/FLAIR signal in both hemispheres	Bifrontal ictal activity	GAD65 antibodies	Anticonvulsants: VPA, OXC, LEV. Immunosuppressants: MTP with PDN taper	Partial response at initial presentation. Status epilepticus responded to anticonvulsants	Serum: α1 (1/160) CSF: NA
9	F, 15	Reduced verbal output and seizures	8 WBC/μL; normal protein concentration	Bilateral fronto-temporal increased T2/FLAIR signal, leptomeningeal enhancement	Multifocal ictal activity	GAD65 antibodies	NA	NA	Serum: α1, β3 (1/160) CSF: NA
10	F, 32	Multifocal refractory seizures	Normal WBC and protein concentration	Normal	Bilateral temporal ictal activity and multifocal interictal epileptiform discharges	Type 1 diabetes mellitus, Hashimoto's thyroiditis. GAD65, TPO and thyroglobulin antibodies	Anticonvulsants: OXC, CBZ, LCM, LEV, ZNS, TPM, CLB, PHT, LTG. Immunosuppressants: IVIG, PDN, ciclosporin	After 7 years she still has uncontrolled seizures	Serum: α1/β3 (1/40) CSF: NA
11	F, 74	Subacute onset of lethargy and alternating changes in level of consciousness. Suspected temporal lobe seizures	Normal WBC and protein concentration	Normal	NA	Previous history of ovarian cancer	NA	NA	Serum: α1/β3 (1/40) CSF: NA
12	F, 16	Behavioural changes, insomnia, orofacial dyskinesia, decreased level of consciousness, brief seizure, dysautonomia	17 WBC/μL; normal protein concentration	Left temporal cortical-subcortical high T2/FLAIR signal	Generalised slowing	NMDAR antibodies in serum and CSF samples	Anticonvulsants: VPA Immunosuppressants: MTP, IVIG, PEX, RTX	Full recovery, gradual improvement over many months	Serum: α1/β3 (1/20) CSF: negative
13	M, 19	Stiff-person syndrome since age 14 years	Normal WBC; protein 85 mg/dL	Not done	Not done	Type 1 diabetes mellitus. GAD65 antibodies	Clonazepam, baclofen. Immunosuppressants: No	Marked improvement. At last follow-up (16 years) he is independent for all daily life activities	Serum: α1/β3 (1/40) CSF: NA
14	M, 12	Stiff-person syndrome since age 5 years; brief episodes of seizures	NA	Hippocampal high T2/FLAIR signal	Right temporal seizures, bifrontal sharp waves	GAD65 antibodies	Anticonvulsants: LEV. Immunosuppressants: IVIG, RTX	Partial improvement of stiff-person symptoms, free of seizures	Serum: α1/β3 (1/20) CSF: NA
15	M, 21	Stiff-person syndrome since age 16 years	Normal WBC and protein concentration	Normal	Normal	Antinuclear antibodies; anti-endomysial immunoglobulin A	Anticonvulsants: CBZ, OXC. Immunosuppressants: IVIG	Partial improvement	Serum: α1/β3 (1/20) CSF: NA
16	M, 46	Stiff-limb syndrome	Not done	Not done	Not done	No	Baclofen Immunosuppressants: No	Substantial improvement	Serum: α1/β3 (1/20) CSF: NA
17	F, 34	Opsoclonus-myoclonus syndrome	NA	Normal	Not done	No	NA	NA	Serum: α1/β3 (1/40) CSF: NA
18	M, 65	Opsoclonus-myoclonus syndrome	Normal WBC and protein concentration	Normal	Not done	Antinuclear antibodies	Immunosuppressants: MTP	No response, died few months after onset	Serum: α1/β3 (1/20) CSF: negative

CBZ=carbamazepine. CLB=clobazam. CPH=cyclophosphamide. DZP=diazepam. F=female. GABAR=gamma-aminobutyric acid receptor. GAD65=glutamic acid decarboxylase 65. IVIG=intravenous immunoglobulin. LCM=lacosamide. LEV=levetiracetam. LTG=lamotrigine. M=male. MDZ=midazolam. MTP=intravenous methylprednisolone. NA=not available. NMDAR=N-methyl-D-aspartate receptor. OCB=oligoclonal bands. OXC=oxcarbazepine. PDN=oral prednisone. PEX=plasma exchange. PHT=phenytoin. PPF=propofol. RTX=rituximab. TPM=topiramate. TPO=thyroid peroxidase. VPA=valproate. WBC=white blood cell count. ZNS=zonisamide.

Table: Clinical characteristics of patients with GABAₐR antibodies in serum and CSF samples
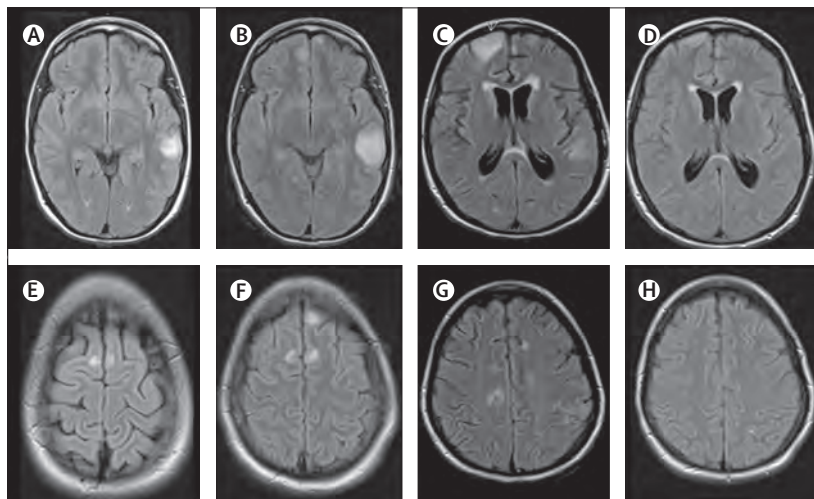
Figure 4: MRI findings in index patient 1
On day 3 of admission, the MRI of this 16-year-old girl showed multiple cortical-subcortical abnormalities with increased FLAIR and T2 signal involving the left temporal lobe and frontal parasagittal regions (A, E). On day 10, a repeat MRI showed an increase of the size of the temporal lesion and a new cortical lesion in the left frontal lobe (B, F). Repeat MRIs on days 22 and 48 did not show substantial changes (data not shown). Another MRI done 4 months after disease onset showed many new multifocal abnormalities and diffuse atrophy and increase of the size of the ventricles (C, G). A repeat MRI 2 months later, 6 months after symptom onset, showed substantial improvement and resolution of the abnormalities as well as improvement of the ventricular dilatation (D, H).

(figure 6). These results showed that 89% of patient's antibodies labelled GABA_AR-containing clusters (figure 6). To examine the effects of the antibodies on inhibitory synapses containing GABA_AR, neurons were treated with patient's CSF antibodies or a control CSF for 48 h. These studies showed that the density of GABA_AR clusters along dendrites was not affected (figure 6), but the clusters of GABA_AR at synapses, measured as cluster density co-labelled by the presynaptic marker vGAT (vesicular GABA transporter), were substantially reduced (figure 6). This finding suggests that the antibodies in the patient's CSF, but not control CSF, removed GABA_AR from synapses. The effect was specific for GABA_AR because the cluster density of other synaptic markers such as gephyrin (figure 6) and the GluN1 subunit of the NMDAR (data not shown) were not affected.

Discussion

We report the identification of high titre serum and CSF antibodies against the GABA_AR in a subset of patients with encephalitis and refractory seizures or status epilepticus, who often needed pharmacologically induced coma. This finding is important because the disorder is potentially treatable. However, because of the rapid development of seizures and frequent presence of coexisting autoimmune disorders, recognition of the disorder might be difficult. Findings from the four following sets of experiments establish GABA_AR as a relevant autoantigen: direct immunoprecipitation of the receptor by patients' antibodies, specific immunostaining of HEK293 cells expressing α1/β3 subunits of the receptor, competition of patients' antibodies for the same

GABA_AR epitopes, and demonstration that patients' antibodies selectively remove GABA_AR from synapses without affecting NMDAR or gephyrin (a scaffold protein that anchors the receptor at post-synaptic sites).

Most fast inhibitory neurotransmission in the adult brain is mediated by ligand-gated GABA_AR.[23] These receptors are regulated by many positive (barbiturates, benzodiazepines) and negative (picrotoxin, bicuculline) allosteric modulators, providing several models of GABA_AR-antagonist induced seizures.[24,25] The GABA_ARs are pentamers, the five subunits of which originate from eight gene families that encode different isoforms ((α1–6, β1–3, γ1–3, δ, ε, □, π, and □1–3). The subunit composition of the receptor governs the intrinsic properties of the channel, such as affinity for GABA, receptor conductance, kinetics, and modulation.[26] These 19 subunits combine in different ways to form functional receptors, but at synaptic sites most receptors contain two α subunits (α1–3 isoforms), two β subunits, and a γ subunit arranged in the order γ-β-α-β-α. By contrast with receptors at synaptic sites, those at perisynaptic or extrasynaptic sites are mainly composed of α4 or α6 subunits combined with β and δ subunits.[27] The antibodies of our patients reacted with the α1, β3, or both subunits (we did not assess other subunits), and when the reactivity with each subunit was individually assessed, the α1 subunit was always recognised by patients' CSF. Therefore, that the main effects of patients' antibodies occurred at synaptic sites, where the α1 receptors are enriched, is not surprising. Indeed, using cultures of rat hippocampal neurons, patients' antibodies caused a decrease in the density of GABA_AR at synaptic sites. The total density of GABA_ARs, including synaptic and extrasynaptic receptors, was not affected, suggesting a relocation of receptors from synaptic to extrasynaptic sites. This finding contrasts with the effects of antibodies identified in other autoimmune encephalitis, such as anti-NMDAR or anti-AMPAR, in which the decrease of the corresponding receptors occurs at both synaptic and extrasynaptic sites.[5,22,28]

At least four mutations in the α1 subunit of the GABA_AR are associated with generalised epilepsy.[27] Findings from in-vitro studies have shown that each of these mutations results in a substantial loss of α1-subunit function or level of expression.[27] Additionally, mutations of the β3 subunit have been reported in children with absence epilepsy.[29] In line with these findings, in our study, all patients with high titres of serum and CSF α1/β3 receptor antibodies developed seizures, status epilepticus, or epilepsia partialis continua. Most of these patients had an abnormal EEG with multifocal seizures and, in two cases, generalised periodic discharges. These findings were associated with extensive cortical and subcortical brain MRI abnormalities in all six patients with high serum antibody titres (all with CSF antibodies) and in three (25%) of 12 patients with low serum titres. We do not know if the MRI findings were caused by the immune

response or resulted from the lengthy seizures. However, the multifocal and extensive brain MRI abnormalities were different from those seen in other autoimmune encephalitis, in which the MRI is often normal (NMDAR)[30] or shows predominant involvement of the hippocampus (AMPAR, GABA$_B$R, LGI1).[5,7,16] The comparison with other autoimmune encephalitis shows other differences: 39% of patients with GABA$_A$R antibodies are younger than 18 years, whereas most patients with other encephalitis (except anti-NMDAR) are adults.[31] Patients with GABA$_A$R antibodies do not seem to frequently have an underlying tumour (similar to LGI1 autoimmunity), whereas about 30–60% of patients with other antibodies (Caspr2, GABA$_B$R, or AMPAR) have a tumour[32] and, for patients with NMDAR antibodies, the frequency of tumours varies with age, sex, and ethnicity.[30] Since the end of this study, we have identified a patient with a malignant thymoma and encephalitis, seizures, multifocal cortical FLAIR MRI abnormalities, and LGI1 and GABA$_A$R antibodies, suggesting that patients with thymoma and seizures should be tested for GABA$_A$R antibodies (data not shown).

In the group with low serum titres of antibodies and absent CSF antibodies, all patients with encephalitis developed seizures; the youngest patient, a 2-year-old child, also required pharmacologically induced coma for status epilepticus. The frequent presence of other relevant autoimmunities could explain the broader spectrum of symptoms in this group. Indeed, two of the four patients with stiff-person syndrome had coexisting GAD65 antibodies, and another patient with GABA$_A$R antibodies only detected in serum had high titres of NMDAR antibodies in serum and CSF samples that were responsible for most of the clinical features (anti-NMDAR encephalitis).

Findings from this and previous studies suggest that patients with encephalitis or seizures attributed to GAD65 antibodies should be examined for other relevant antibodies against cell-surface antigens, such as GABA$_A$Rs and other synaptic receptors (panel).[6,33–35] Additionally, the increasing recognition of autoimmune encephalitis with neuronal cell-surface antibodies and concurrent thyroid peroxidase antibodies (as in four of 18 patients in this study) suggests that Hashimoto's encephalitis should be a diagnosis of exclusion—that is, the detection of thyroid peroxidase antibodies and symptom response to steroids are not sufficient criteria to establish the diagnosis of Hashimoto's encephalitis.[9,35,36]

Evidence suggests that status epilepticus can lead to chronic epilepsy. The development of epilepsy is usually preceded by a silent period during which there is increasing hyperexcitability and a progressive decrease of synaptic GABA$_A$R.[26] This effect has been attributed in part to a disruption of the GABA$_A$R-anchoring protein, gephyrin.[26,37] Additionally, lengthy seizures reduce GABA$_A$R inhibition, which might lead to the
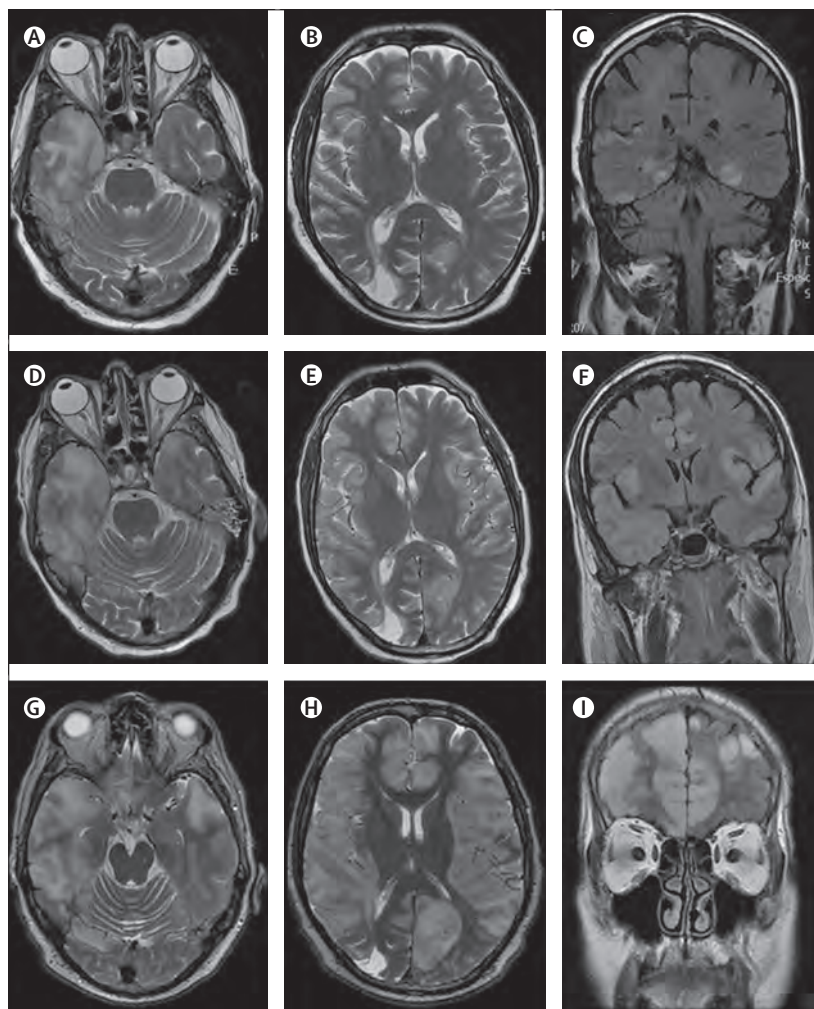
Figure 5: MRI findings in index patient 2
On day 2 of admission, the MRI of this patient showed multiple areas of FLAIR and T2 signal abnormality predominantly involving cortical regions (A–C), without oedema, mass effect, or contrast enhancement (data not shown), but with blurring of the grey-white matter junction. On day 14, repeated MRI showed interval increase of the cortical-subcortical involvement, with oedema in the right temporal lobe (D–F). Subsequent MRIs showed a pronounced worsening of these abnormalities now extensively involving cortical and subcortical regions (G–I).

development of status epilepticus.[38] These findings and the antibody-mediated decrease of synaptic GABA$_A$R seen in neuronal cultures exposed to patients' antibodies suggest a model whereby the GABA$_A$Rs are removed from synapses leading to status epilepticus, which in turn causes a further decrease of receptors along with reduced GABA$_A$R inhibition, resulting in a pathogenic reinforcement. This would explain the severity and refractory nature of the seizures associated with high concentrations of GABA$_A$R antibodies, and why this disorder seems to be more difficult to treat than the syndromes associated with NMDAR, GABA$_B$R, AMPAR, or LGI1 antibodies, emphasising the importance of prompt diagnosis and treatment. Despite the difficulties in treatment, 12 of 15 patients had partial or complete response to immunotherapy
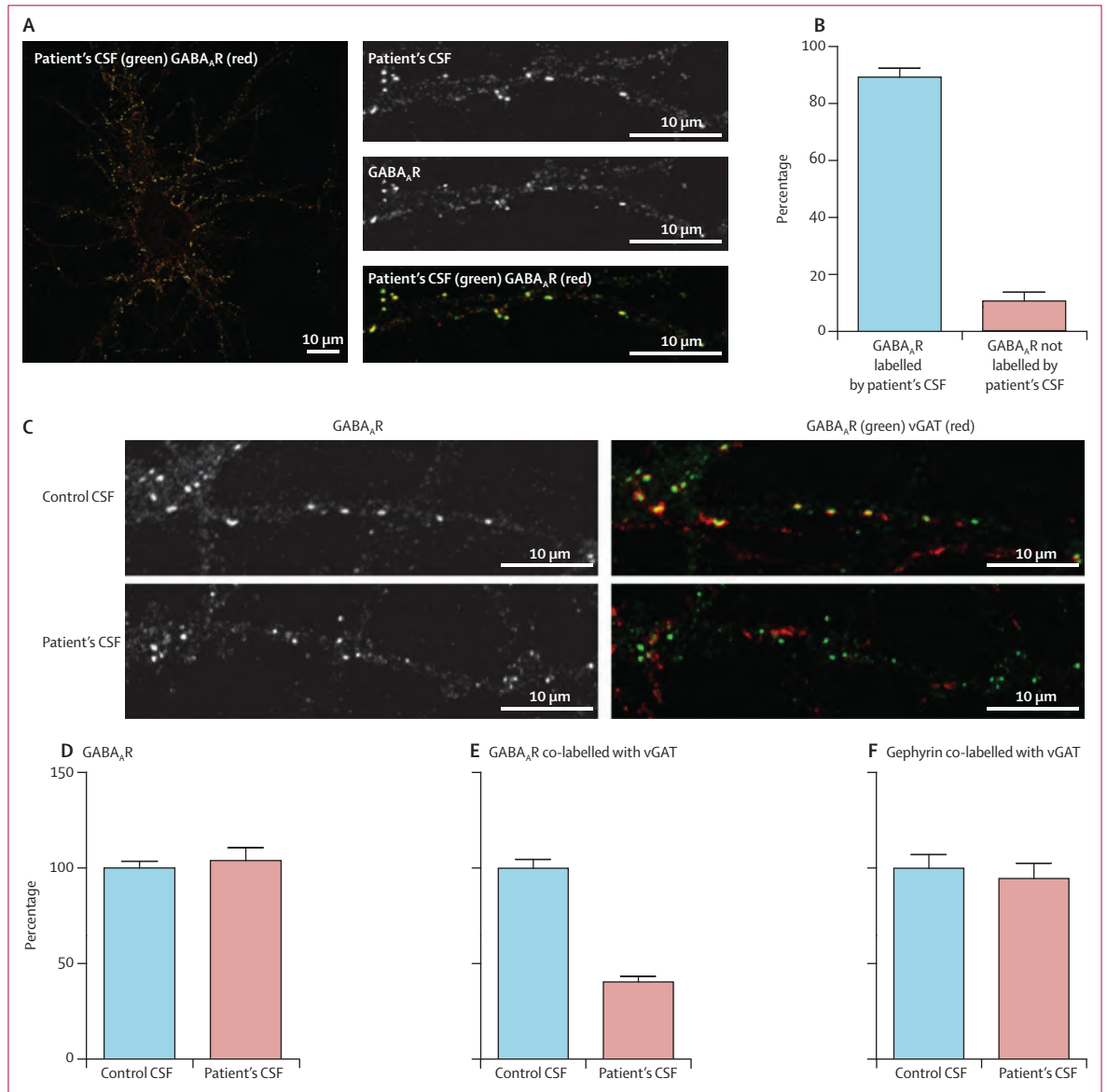
Figure 6: **Effect of patient's antibodies on the density of GABA$_A$R clusters in cultures of hippocampal neurons**
Live 14-day-in-vitro cultures of dissociated rat hippocampal neurons were stained with patient's CSF containing GABA$_A$R antibodies (green), then fixed and stained with commercial GABA$_A$R antibodies (red; A). Quantification of colocalisation between patient's CSF antibodies and the commercial GABA$_A$R antibody shows that 89% (SE 3%) of receptors labelled by patient's antibodies were colabelled with the commercial antibody against GABA$_A$R (B). In a similar assay, neurons were incubated with patient's CSF for 48 h and subsequently stained for postsynaptic GABA$_A$R (green) and presynaptic vesicular GABA transporter (vGAT) (red; C). The synaptic GABA$_A$Rs (shown as yellow clusters in control conditions) were greatly reduced after treatment with patient's CSF (C). The number of GABA$_A$R clusters along dendrites of neurons treated with patient's CSF is not different from neurons treated with control CSF (Mann-Whitney test p=0·6; D). The number of GABA$_A$Rs localised in synapses, however, decreased significantly in neurons treated with a patient's CSF compared with neurons treated with control CSF (40% [3%] compared with control as 100%; p<0·0001; E). Patients' CSF did not affect the clusters of post-synaptic gephyrin colabelled with presynaptic vGAT when compared with the effects of control CSF (p=0·5; F).

(nine patients), symptomatic therapy (three patients), and extended intensive care support (all patients with encephalitis).

Our study has several limitations, including the retrospective assessment of most patients (except the two index patients), and the absence of CSF samples from nine of the 12 patients with low serum titres of GABA$_A$R

antibodies. Therefore, the clinical implications of low serum antibody titres should be interpreted with caution, especially because in some patients (eg, the patients with NMDAR antibodies) other coexisting immunological mechanisms could have contributed to the patients' symptoms. Future studies should establish, in a prospective manner, the incidence of serum and CSF

Panel: Research in context

Systematic review

We searched Medline and Embase up to Nov 1, 2013, for articles published in English with the search terms "gamma-aminobutyric acid-A receptor", "GABA$_A$", "GABA$_B$", "GABA", "receptors", "antibodies" and "encephalitis" [MeSH terms]. We restricted searches to studies in human beings. We also reviewed the reference lists of the papers identified by this search. We identified 36 papers, of which none was related to encephalitis associated with GABA$_A$R antibodies; of the papers identified, three were series of patients with GABA$_B$R antibodies, 18 were reviews of autoimmune encephalitis, 12 were original research articles, two were editorials, and one was a letter.

Interpretation

Our findings suggest that the GABA$_A$ receptor is a novel target antigen of autoimmune encephalitis, and provides an unambiguous test for the detection of patients' antibodies in serum and CSF samples. Although we found high titres of serum and CSF GABA$_A$R antibodies in patients with seizures, refractory status epilepticus, or epilepsia partialis continua, low titre serum antibodies were associated with a wider spectrum of symptoms, probably due to the high prevlalence of coexisting autoimmunities. We also show that patients' GABA$_A$R antibodies cause a selective decrease of the clusters of GABA$_A$R at synapses, but not along dendrites, without altering other post-synaptic proteins such as the NMDAR or gephyrin. These findings are important for three reasons: they define a novel form of autoimmune epileptic disorder, usually non-paraneoplastic, that affects children and adults and is severe but potentially treatable; that the severity of the seizures (often needing pharmacologically induced coma) and frequent presence of other less relevant antibodies against intracellular antigens (eg, thyroid peroxidase, GAD65) can mislead diagnosis (eg, Hashimoto's encephalitis, anti-GAD65 encephalitis or seizures); and that patients' antibodies have a direct effect on the GABA$_A$R receptors, which provides a useful reagent (purified patients' antibodies) to understand how selective disruption of GABA$_A$R leads to neuronal hyperexcitability, seizures, or status epilepticus.

GABA$_A$R antibodies in patients with seizures or status epilepticus, opsoclonus-myoclonus, and stiff-person syndrome with or without GAD65 autoimmunity, and whether the presence of antibodies in CSF always associates with seizures or status epilepticus.

Findings from this study have several clinical implications. The presence of GABA$_A$R antibodies should be tested in patients with severe seizures or status epilepticus in the context of encephalitis of unclear cause with MRI and CSF abnormalities suggestive of an inflammatory process, patients with opsoclonus-myoclonus or stiff-person syndrome, and any patients with GAD65 or thyroid peroxidase antibodies and other clinical features suggesting a propensity to autoimmunity.

In addition to the clinical implications, the identification of a disorder in which patients' antibodies specifically eliminate GABA$_A$Rs from synapses provides a useful reagent (purified patients' antibodies) to understand how selective disruption of these receptors leads to neuronal hyperexcitability, seizures, or status epilepticus.

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Supplementary webappendix

Supplemental information

Index case 1
This 16 year-old girl presented to the hospital with a four-day history of severe fatigue
and headache, accompanied by vertigo, nausea, and scintillating scotomas. She
complained of several months of memory difficulties, cognitive dysfunction, anxiety,
depressed mood and fatigue. Her past medical history was significant for Hodgkin's
lymphoma which was in remission since completing chemotherapy and radiation 10
months earlier. On the fifth day of admission, she had a generalized tonic-clonic seizure
and rapidly progressed to having frequent seizures. Complete blood cell count, C-
reactive protein and erythrocyte sedimentation rate were normal. Testing for anti-
thyroid peroxidase, anti-thyroglobulin, anti-nuclear antibodies, anti-neutrophil
cytoplasmic antibodies, and paraneoplastic antibodies (Hu, Ri, Yo, CRMP5,
amphiphysin) were negative. Brain MRI on day 3 demonstrated multiple foci of
increased T2/FLAIR signal in both hemispheres (Figure 4A, E). CSF analysis showed
normal opening pressure, 23 white blood cells (WBC)/ mm3 (69% lymphocytes),
protein concentration 60 mg/dL,normal glucose concentration and negative cytology.
Gram stain, routine cultures and PCR testing for Herpes simplex virus, Enterovirus and
Mycoplasma pneumoniae were negative. Serology for Cytomegalovirus, Epstein-Barr
virus, Arbovirus, *Bartonella henselae*, and Lyme disease were negative.
Treatment with high-dose methylprednisolone was initiated on day 7. Very high doses
of phenobarbital were required to suppress electrographic seizures. A subsequent course
of plasma exchange on alternating days for one week failed to improve the seizure
pattern. On day 10, repeat brain MRI showed increase of the size of the FLAIR/T2
abnormalities, mainly in the left temporal lobe, and multifocal new cortical and
subcortical lesions in both cerebral hemispheres (Figure 4B, F). Brain biopsy on day 14
demonstrated intense diffuse reactive astrocytic gliosis throughout the cortex associated
with microglial activation and a population of reactive T lymphocytes. Several days
later, antibodies against unknown neuronal cell-surface antigens were identified in her
CSF. She received high-dose corticosteroids, intravenous immunoglobulin, rituximab
and cyclophosphamide. Phenobarbital coma was continued for four months; during this
time breakthrough clinical and electrographic seizures occurred if the phenobarbital
level was allowed to decrease. EEG recordings demonstrated generalized periodic
discharges late in the first month of admission (Supplemental Figure 3 A).

After three months, the EEG showed more focal left-sided ictal activity. The
phenobarbital dose was weaned and she began a slow neurological recovery with
gradual resolution of the encephalopathic EEG pattern. Four months after admission a
repeat lumbar puncture showed resolution of the leukocytosis; however, repeat MRI
showed numerous new multifocal lesions throughout the brain with diffuse atrophy and
moderate ex-vacuo ventricular dilatation (Figure 4 C, G). Six months after her initial
presentation, she began to show more rapid neurological recovery. Repeat MRI
demonstrated no new lesions, improvement or resolution of all previous lesions, and
reduction of the previous seen diffuse atrophy (Figure 4 D, H). She was transferred to
an inpatient rehabilitation facility seven months after presentation and over the
subsequent three months made significant gains to the point that she was able to
communicate, eat, dress and groom herself. She could walk short distances with
minimal assistance. Ten months after symptom onset, she was discharged home able to
carry out most activities of daily living independently. At the last follow-up, 15 months
after symptom onset, she walks with no assistance and is able to perform all daily

activities independently. She has returned to school with a modified course load due to mild cognitive deficits that continue to improve.

<u>Index case 2</u>
A 51 year-old man was admitted to the hospital for rapidly progressive symptoms of change of behavior and new-onset psychosis. Prior to admission the patient was seen several times in the emergency department of another hospital where he was diagnosed with new onset depression and treated with sertraline and alprazolam. In addition, he had complained of generalized pruritis and developed worsening high blood pressure. On several occasions the family heard the patient saying he was going to kill other people and himself. A few days prior to admission, he refused to get out of bed, and became apathetic with almost total reduction of verbal output. His past medical history was relevant for high blood pressure, diabetes mellitus, hypercholesterolemia, stroke (from which he had fully recovered), and thrombotic thrombocytopenic purpura treated a few years earlier with splenectomy and steroids.

At admission, the clinical picture resembled akinetic mutism, with brief periods in which the patient spontaneously uttered a few incoherent sentences. The day of admission, he was noted to have clonic seizures involving the left side of the face and left arm that resolved with intravenous diazepam and levetiracetam. Over the next 24 hours he developed acute respiratory failure due to pneumonia, requiring intubation and admission to intensive care unit. Two days later he developed status epilepticus characterized by clonic movements of the left side of the face and left arm, associated with continuous saccadic eye movements to the left that were refractory to all treatments, including levetiracetam, lacosamide, and phenytoin. The patient was maintained in a pharmacological coma, sequentially using midazolam, propofol, and thiopental. The seizures persisted until the patients' death 10 weeks after presentation.

The initial EEG showed seizures in the right temporal lobe with a tendency to generalization that in subsequent recordings progressed to a pattern of generalized periodic discharges (Supplemental Figure 3 B). The MRI showed multiple increased FLAIR/T2 signal abnormalities, extensively involving cortex without mass effect or contrast enhancement, blurring the grey-white matter junction (Figure 5). The initial CSF study was normal, but a repeat CSF analysis several days later showed IgG and IgM oligoclonal bands without matching serum bands. The following tests were negative: 1) Blood studies for syphilis, hepatitis virus B and C, *Brucella melitensis*, *Borrelia burgdorferi*, *Toxoplasma gondii*, *Streptococcus pneumoniae*, and *Legionella pneumophila*; 2) CSF studies for bacterial and fungal infections, Herpes simplex virus 1 and 2; Human herpesvirus 6, Cytomegalovirus, Varicella zoster virus, JC virus and Enterovirus, 3) panel for paraneoplastic antibodies, and connective tissue disorders (antibodies to GAD65, Hu, Ri, Yo, CRMP5, amphiphysin, DNAdc, Sm, Rib-P, PCNA, U1-RNP, SS-A/Ro, SS-B/La, Scl-70, CENP-B, RNA Pol III, Jo-1, Mi-2, PM-Scl, and ANCA), complement levels, 4) serum protein electrophoresis, 5) tumor markers: CEA, AFP, Ca 19.9, PSA, and B-2-microglobulina. The patient was found to have low levels of thyroid peroxidase antibodies (156 IU/ml) and thyroglobulin antibodies (158 IU/ml).

After excluding an infectious etiology, the patient was started on corticosteroids and IVIG without significant effect. One week later, he received 5 plasma exchange treatments without clinical effect and no change in the MRI (Figure 5 D-F). By this time laboratory studies revealed serum and CSF antibodies against unknown neuronal cell-surface antigens, and he was started on cyclophosphamide (1 g per m^2/ month) and

rituximab (1 g every 2 weeks). Despite these treatments the patient showed no clinical or radiological improvement and continued with electrographic status epilepticus. Repeat MRIs showed new FLAIR/T2 abnormalities diffusely involving cortex (Figure 5 G-I), and the patient died two months after admission.

Supplemental methods

Immunohistochemistry of rat brain
Adult female Wistar rats were sacrificed without perfusion, and the brain was removed and fixed by immersion in 4% paraformaldehyde for 1 hour at 4°C, cryoprotected in 40% sucrose for 48 hours, embedded in freezing compound media, and snap frozen in isopentane chilled with liquid nitrogen. Seven-micrometer-thick tissue sections were then sequentially incubated with 0.3% H_2O_2 for 15 minutes, 5% goat serum for 1 hour, and patient or control serum (1:200), or CSF (1:5) at 4°C overnight. After using a secondary biotinylated antibody goat anti-human IgG (diluted 1:2000, Vector, BA-3000), the reactivity was developed with the avidin-biotin-peroxidase method, as reported.[1]

Immunocytochemistry on neuronal cultures
Rat hippocampal neuronal cultures were prepared as reported.[2] Live neurons grown on coverslips were incubated for 1 hour at 37°C with patient or control serum (final dilution 1:200) or CSF (1:10). After removing the media and extensive washing with phosphate-buffered saline (PBS), neurons were fixed with 4% paraformaldehyde, permeabilized with 0.3% Triton X-100, and immunolabeled with Alexa Fluor 488 goat anti-human IgG (diluted 1:1000, Invitrogen, A11013). Results were photographed under a fluorescence microscope using Zeiss Axiovision software (Zeiss, Thornwood, NY).

Immunocytochemistry on HEK293 cells
Fixed cells:
HEK293 cells were transfected with plasmids containing the human α1 subunit of the GABAaR (accession number: NM_000806.3; Origene catalog number: SC119668) or the human β3 subunit of the receptor (accession number: NM_000814.3; Origene catalog number: SC125324); cells transfected with a plasmid without insert was used as control. Cells were grown for 24 hours after transfection before assessment. Transfected cells were fixed in 4% paraformaldehyde, permeabilized with 0.3% Triton X-100 and then incubated with patients' serum (1:20 and higher serial dilutions) or CSF (1:5 and higher serial dilutions) along with a commercial mouse antibody against the α1 subunit of the GABAaR (dilution 1:5000, Millipore, MAB339) or the β3 subunit (dilution 1:5000, Abcam, AB4046) for 2 hours at room temperature, and the corresponding fluorescent secondary antibodies (Alexa Fluor 488 goat anti-human IgG, diluted 1:1000, A11013; and Alexa Fluor 594 goat anti-mouse IgG, diluted 1:1000, A11032, both from Invitrogen). Results were photographed under a fluorescence microscope using Zeiss Axiovision software.
Live cells:
Live HEK cells were incubated with serum (1:20 and higher serial dilutions) or CSF (1:5 and higher serial dilutions) of the patient together with the same commercial antibody against GABAaR indicated above for 1 hour at 37ºC, washed, and fixed with 4% paraformaldehyde for 5 minutes. After washing cells were then incubated with the corresponding Alexa Fluor secondary antibodies indicated above.

Immunoprecipitation and immunoblot

Live neurons obtained as above, were grown in 100 mm plates (density 1.5 x 10^6 neurons/plate), and incubated at 37°C with filtered patient serum (diluted 1:200) for 1 hour. Neurons were then washed with PBS, lysed with buffer (NaCl 150mM, EDTA 1mM, tris (hydroxymethyl) aminomethane [Tris]-HCl 100mM, deoxycholate acid 0.5%, 1% Triton X-100, pH 7.5) containing protease inhibitors (P8340; Sigma Labs), and centrifuged at 16.1 x 10^3 *g* for 20 minutes at 4°C. The supernatant was retained and incubated with protein A/G agarose beads (20423; Pierce, Rockford, IL) overnight at 4°C, centrifuged, and the pellet containing the beads with patients' antibodies bound to the target cell-surface antigen was then washed with lysis buffer, aliquoted, and kept at -80°C. An aliquot of this pellet was resuspended in Laemmli buffer, boiled for 5 minutes, separated in a 4 to 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the proteins visualized with EZBlue gel staining (G1041; Sigma Labs). Due to the lack of differences between the EZBlue-visible bands between patient's and control samples, all precipitated proteins run along the gel were analyzed using mass spectrometry.

Mass spectrometry

Mass spectrometry was performed at the Proteomics Facility at the Abramson Cancer Center of the University of Pennsylvania. Protein bands were trypsin digested and analyzed with a nano liquid chromatography (nano LC)/nanospray/linear ion trap (LTQ) mass spectrometer (Thermo Electron Corporation, San Jose, CA) as reported.[3] Briefly, 3 ml trypsin digested sample was injected with autosampler from Eksigent (Dublin, CA). The digested samples were separated on a 10 cm C18 column, using nano LC from Eksigent with 200 ml/minute flow rate, 45 minute gradient. Online nanospray was used to spray the separated peptides into LTQ, and Xcalibur software (Thermo Scientific, Waltham, MA) was utilized to acquire the raw data. The raw data files were searched using Mascot (Matrix Science, Boston, MA) against the NCBI and Swissprot databases (Swiss Institute of Bioinformatics (Basel, Switzerland).

Immunoabsorption and immunocompetition studies

In order to determine whether the brain reactivity of patient's antibodies was specifically due to GABAaR binding, six 60 mm plates of HEK 293 cells expressing GABAaR were sequentially incubated with patient's serum (1:200), each plate for 1 hour at 37°C. After incubation with the six plates, the immunoabsorbed serum was incubated with sections of rat hippocampus, as above. Patient's serum absorbed with non-transfected HEK 293 cells served as control.

To determine whether patients' antibodies were directed against similar antigens and epitopes of GABAaR, immunocompetition studies were performed. IgG was isolated from a patient whose serum contained high levels of IgG antibodies against GABAaR using protein A and G sepharose beads, and subsequently eluted and labeled with biotin (Vector, SP1200), as reported.[4] Then, sections of rat brain were incubated with other patients' or control sera (diluted 1:5) overnight at 4°C, washed in PBS, and subsequently incubated with the indicated human biotinylated IgG containing GABAaR antibodies (diluted 1:40) for 1 hour at room temperature, and the reactivity was developed using the avidin-biotin-peroxidase method. Two sera were considered to compete for the same GABAaR epitopes, when pre-incubation of the tissue with one serum abrogated the reactivity of the other patient's IgG.

Quantitative analysis of neuronal GABAaR immunolabeling by patient's antibodies

To determine the degree of immunolabeling of GABAaRs by patient's antibodies, 14-day *in vitro* (div) rat hippocampal neurons were incubated with a representative patient's CSF (diluted 1:20) for 30 minutes, then washed, fixed, and incubated with a commercial mouse monoclonal antibody (Millipore 05-474; 1:500) against a sequence contained in the β2/3 subunit (which is a component of most GABAaR[5]) followed by appropriate fluorescent-conjugated secondary antibodies, Alexa Fluor 488 goat anti-human IgG (1:200, Invitrogen, A11013) and Alexa Fluor 594 donkey anti-mouse IgG (1:200, A21203, both from Invitrogen). Images were obtained with a laser-scanning confocal microscope (Leica TCS SP5). Laser light levels and detector gain and offset were adjusted in every experiment so that no pixel values were saturated in any treatment conditions. Images were thresholded, and the number of individual clusters along neuronal dendrites was determined using interactive software (ImageJ).

Analysis of the structural effects of patient's antibodies on GABAaR clusters
To determine the effects of patient's antibodies on the number and localization of GABAaR clusters, 14 *div* rat hippocampal neurons were treated with patient's or control CSF (1:20 dilution in Neuro-Basal supplemented with B27 medium; GIBCO, Carlsbad, CA) for 2 days. Every day, 20 of the 300 μl medium in each culture well were removed and replaced with 20 μl fresh patient or control CSF. On 16 *div*, neurons were fixed in freshly made paraformaldehyde (4% paraformaldehyde, 4% sucrose in phosphate-buffered saline) for 5 minutes, permeabilized in 0.25% Triton X-100 for 10 minutes, and blocked in 5% normal goat serum for 1 hour. Neurons were then incubated with the indicated monoclonal antibody against the GABAaR β2/3 (1:500), or a mouse monoclonal antibody against Gephyrin (1:200, Synaptic Systems, 147011), or a guinea pig polyclonal antibody against vesicular-GABA transporter (VGAT, 1:1000; Synaptic Systems,131004) or a rabbit antibody against GluN1 (anti-NMDAR1, 1:100; Millipore AB9864R) for 2 hours, followed by the appropriate fluorescent-conjugated secondary antibodies (Alexa Fluor 488 goat anti mouse IgG, 1:200, A-11001; Alexa Fluor 594 goat anti-guinea pig IgG, A-11076, 1:200; Cy5 donkey anti-rabbit IgG, 1:200, Jackson ImmunoResearch 711-175-152). Images were obtained and analyzed as above.

Supplemental Table 1: Sequences isolated by immunoprecipitation with patient's serum

Sequence	β3 subunit of the GABAaR, peptide identification probability	Sequest XCorr	Sequest deltaCn
(R)LHPDGTVLYGLR(I) (+3H)	95%	2.97	0.21
R)NVVFATGAYPR(L) (+2H)	95%	3.21	0.55
(R)VADqLWVPDTYFLnDKK(S) (+3H)	95%	2.98	0.42

Mass spectral data was analyzed using the search engine Sequest. Peptide confidence was determined by the cross-correlation scoring which represent sensitivity, comparing the experimental fragmentation spectrum of the peptides against the theoretical predicted fragmentation spectrum; and by the DeltaCn, which represents specificity for the peptide identification. Xcorr > 2 (+2 H), 2.5 (+3 H) and deltaCn > 0.2) indicate a good spectrum.

Legends to Supplemental figures

Supplemental Figure 1: Comparison of reactivity of CSF of a patient with GABAaR antibodies with that of a patient with GABAbR antibodies using rat brain immunohistochemistry (high magnification of figure 1)
Panel A shows the reactivity of the CSF (dilution 1:4) of patient #2 with hippocampus; the asterisk indicates the area shown at higher magnification in panel B. Panel C shows the reactivity with cerebellum. Panels D-F correspond to the same brain regions immunostained with CSF of a patient with GABAbR antibodies, and panels G-I with CSF of a control subject (without GABAaR or GABAbR antibodies). Note that the pattern of reactivity of GABAaR antibodies is very similar to that of the GABAbR antibodies which makes difficult to distinguish one from the other by plain immunohistochemistry. Scale bar for G = 100 μm, Scale bar for H and I = 200 μm

Supplemental Figure 2: Immunocompetition studies demonstrating that patients' antibodies compete for the same epitopes of the GABAaR
Reactivity with rat brain of biotinylated IgG from a patient with GABAaR antibodies in which the tissue has been pre-incubated with serum from a control individual (A and B), the serum from the same patient whose IgG has been biotinylated (C, D), and the serum of another patient with GABAaR antibodies. Note the dramatic decrease of reactivity (competition for the same GABAaR epitopes) in panels E and F compared with A and B. Panels C and D (competition with same patient's serum serves to demonstrate the background reactivity). Scale bar for A, C, E = 1 mm; Scale bar for B, DD and E = 200 μm

Supplemental Figure 3: Generalized periodic discharges in patients with encephalitis and antibodies to GABAaR

The recording in A corresponds to the EEG of patient #1 obtained one month after admission; note the presence of generalized epileptiform discharges. Settings: gain (sensitivity): 5 µV/mm; low frequency filter: 1 Hz, high frequency filter: 70 Hz.
The recording in B corresponds to patient #2; this patient initially showed epileptiform activity in the right temporal lobe with tendency to generalization in posterior recordings, as shown in B. Settings: gain (sensitivity): 15 µV/mm; low frequency filter: 0.5 Hz, high frequency filter: 35 Hz.

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Objectiu 3:

Article III: Pediatric Anti-N-methyl-D-Aspartate Receptor Encephalitis-Clinical

Analysis and Novel Findings in a Series of 20 Patients.

Autors: Armangue T, Titulaer MJ, Málaga I, Bataller L, Gabilondo I, Graus F, Dalmau J; Spanish Anti-N-methyl-D-Aspartate Receptor (NMDAR) Encephalitis Work Group.

III

branding in online advergaming, in school buses, on speed-bumps, and in social media.[7] The increased exposure to branding through these means might result in greater behavioral and biological response to food logos in future generations. Further, minorities often are targeted for increased marketing of unhealthy foods, especially African-American and Hispanic youth. Targeted advertising has been related to greater consumption of high-calorie foods (eg, fast foods) by African-American and Hispanic children.[8] This trend might also be related to greater neural response to food logos in children from racial/ethnic groups at increased risk of obesity, which might highlight the need for policy to restrict the targeted food marketing to minority children.

In summary, the research conducted by Bruce et al[4] helps lay essential groundwork for an important area of future research—the impact of aggressive marketing by the food industry on the brain. As literature in this area grows, it may play a key role not only in the development of more effective intervention but also in encouraging environmentally focused policy initiatives to turn back the tide of childhood obesity. ∎

Ashley N. Gearhardt, PhD
Institute for Human Adjustment
University of Michigan
Ann Arbor, Michigan

Kelly D. Brownell, PhD
Rudd Center for Food Policy & Obesity
Yale University
New Haven, Connecticut

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Anti-N-methyl-D-aspartate Receptor Encephalitis: What's in a Name?

In 2009, Dalmau et al[1] described the first cohort of children with anti–N-methyl-D-aspartate receptor (NMDAR) encephalitis. Their article provided a name for a previously unknown, severe pediatric encephalitis, manifested as behavioral and psychiatric symptoms in combination with dyskinesias, dystonia, seizures, progressive somnolence, and occasionally autonomic instability, notably hypoventilation and variable fever. The authors detailed the differences in presentation from adults. They also pointed out that most children with anti-NMDAR encephalitis do not commonly harbor neoplasms, unlike adults with the disease. The etiology was unclear. They reported some early success treating these children with immune-modulating therapy. Today, anti-NMDAR encephalitis is diagnosed more often than any single viral encephalitis in the US.[2]

See related article, p 850

Ultimately, however, as more children are being diagnosed with the disorder, the questions remain: What is pediatric anti-NMDAR encephalitis? Is it a disease? A syndrome? What is/are the cause(s)? Has anti-NMDAR encephalitis always existed, or is it new? Certainly, we know from the work by Dalmau et al,[3] and reproduced by others,[4] that the putative antibody binds to an extracellular epitope region of the N-terminal domain of the NR1 subunit of the NMDAR, causing a pronounced and specific decrease of NMDAR protein at synapses. We know that pharmacologic antagonists of NMDARs produce a similar profile of symptoms (eg, psychosis, agitation, memory disturbance, unresponsiveness). We have hints that the disease may be immune-mediated, given the high ratio of B cells to T cells, the presence of plasma cell infiltrates on pathologic specimens, and the dramatic response to immune

EEG	Electroencephalography
HSE	Herpes simplex encephalitis
NMDAR	N-methyl-D-aspartate receptor

suppression[1,5]; however, the triggering mechanism eludes us. Is the initial insult responsible for triggering antibody production virally mediated, paraneoplastic, or the result of an autoimmune disorder? Is this the same or a different disease than that seen in adults? Adults with anti-NMDAR encephalitis frequently harbor occult neoplasms, yet very few children have tumors. Moreover, the incidence of childhood tumors has remained largely unchanged for decades.[6]

In this issue of The Journal, Armangue et al[7] take some first steps toward elucidating the etiology of pediatric anti-NMDAR encephalitis. The authors identified and followed 20 children with the disorder with similar presentations, clinical courses, responses to treatment, and long-term outcomes as previously reported cases. Interestingly, they report that 60% of their patients presented with neurologic symptoms and just 40% exhibited psychiatric manifestations, and assert that neurologic presentations were more frequent in patients aged <12 years. Is this a clue? Maybe. However, all patients ultimately developed a combination of neurologic and psychiatric impairment. Furthermore, young children often have delays in presentation and diagnosis, and given their relative inability to articulate and our slowness in recognizing psychiatric disturbances, the distinction could be arbitrary.

In addition to the clinical contrast from adults, Armangue et al report two novel findings for pediatric anti-NMDAR encephalitis. First, they identify an extreme delta brush pattern on electroencephalography (EEG) in one patient. This pattern was described previously in 30% of an adult cohort with NMDAR encephalitis,[8] but had not yet been reported in a child. This EEG pattern is unique to this disease and correlates with disease severity. Thus, if identified early, this finding can hasten accurate diagnosis and appropriate treatment.

The authors' second novel finding is the most provocative: the presence of NMDAR antibodies in a patient recovering from herpes simplex encephalitis (HSE). They describe a 2-year-old girl with choreoathetosis and orofacial dyskinesias, symptoms seen in both anti-NMDAR encephalitis and, rarely, in convalescent stages of HSE.[9-11] This girl had been diagnosed earlier with HSE and treated appropriately. At 4 weeks after the diagnosis, she began exhibiting abnormal movements, and was found to have anti-NMDAR antibodies and a negative herpes polymerase chain reaction test. In previous reports of choreoathetosis seen after HSE, investigators presumed that the patients had relapsed HSE or an immuno-inflammatory response to disease and treated them with antivirals or steroids, with varying results. A recent study identified NMDAR antibodies in 30% of patients with HSE.[12] That study, in combination with the patient described in the present report, provides the first suggestion of a possible viral trigger for the production of NMDAR antibodies.

Further work must elucidate the cause of NMDAR antibody production in children with encephalitis and the constellation of signs and symptoms reported currently in The Journal. Such understanding will provide guidance in how to evaluate these patients and to what extent, and possibly how best to treat them. Until then, the diagnosis of anti-NMDAR encephalitis should be considered in those children with the aforementioned symptoms and signs. In pediatric patients, consideration should be given to infection, such as HSE, and seldom tumor, namely ovarian teratoma. There is no standard workup with EEG or magnetic resonance imaging. Lumbar puncture, performed once or multiple times, should cast a wide net for bacterial, viral, postinfectious, autoimmune, and neoplastic causes. In females, consideration should be given to the possibility of ovarian teratoma, with screening with ultrasound or magnetic resonance imaging performed during the first year. At this time, there is no evidence to support endless screening for cancer in children, and certainly no role for prophylactic oophorectomy or exploratory laparotomy. Earlier treatment with steroids or intravenous immunoglobulin, or possibly plasmapheresis, is indicated, and if unsuccessful, followed days or weeks later by rituximab or even cyclophosphamide, although there is no standard protocol. Outcomes can be very good, but relapses can occur. Clearly our approach must be empiric, thoughtful, and cautious, until we can identify just what lies behind the term anti-NMDAR encephalitis. ∎

Cynthia J. Campen, MD
Department of Neurology

Paul Graham Fisher, MD
Departments of Neurology, Pediatrics
Neurosurgery, and Human Biology
Stanford University
Palo Alto, California

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Reply

To the Editor:

We thank Tang et al for their interest in our article. We agree that obese adolescents who have cardiovascular risk factors (dyslipidemia and insulin resistance), as did our subjects, could have coronary atherosclerosis. However, the presence of coronary atherosclerosis does not necessarily mean there is an obstructive coronary lesion and ischemic myocardial disease, particularly in adolescents. In fact, data from both the Bogalusa Heart study[1] and Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study[2] demonstrated that atherosclerotic coronary changes involved less than 15% of the coronary luminal surface and, therefore, were not associated with coronary artery stenosis or ischemic heart disease. Thus, it is unlikely our subjects had "silent myocardial ischemia" that was responsible for myocardial dysfunction. Moreover, we would like to underscore that our study demonstrated an association between increased intrahepatic triglyceride (IHTG) content and early myocardial dysfunction in obese adolescents, but was not designed to determine the mechanism responsible for this dysfunction. Therefore, even if our subjects did have myocardial ischemia, this would not change our conclusion that nonalcoholic fatty liver disease (NAFLD) is an early marker of cardiac ventricular dysfunction in asymptomatic obese adolescents.

The data from our study also demonstrated that IHTG content was associated with insulin resistance in obese adolescents, as we have previously found in obese adults.[3] As noted by Tang et al, intra-abdominal adipose tissue mass correlates with IHTG content and increased intra-abdominal adipose tissue is also associated with insulin resistance. Therefore, as noted in our paper,[4] we conducted a multiple stepwise regression analysis with age, Tanner stage, percent body fat, intra-abdominal adipose tissue volume, and IHTG content as putative predictive variables, and found IHTG content was the only significant independent determinant of insulin sensitivity, assessed as either the insulin sensitivity index (b = −0.770) or the homeostasis model assessment of insulin resistance (b = 0.738). These results demonstrate that an increase in IHTG content was associated with a decrease in insulin action, independent of intra-abdominal adipose tissue volume.

We appreciate the opportunity to clarify these issues, which reinforces the notion that the presence of NAFLD in obese adolescents helps identify those who have subclinical cardiometabolic abnormalities that likely increase their risk for developing type 2 diabetes and heart failure.

Gautam Singh, MD
Faidon Magkos, PhD
Samuel Klein, MD
Center for Human Nutrition
Washington University School of Medicine
St Louis, Missouri
http://dx.doi.org/10.1016/j.jpeds.2013.06.059

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Anti-N-methyl-D-aspartate receptor encephalitis and parvovirus B19: A possible link?

To the Editor:

The article by Armangue et al[1] prompted us to report a patient who we treated in 2008.[2] This previously healthy 17-month-old boy had developed a severe encephalopathy with prominent abnormal movements, preceded by cognitive disturbances that were difficult to assess given his age. A discrepancy between the presentation of severe encephalopathy and normal magnetic resonance imaging findings caught our attention.

Similar cases had been reported in the United Kingdom,[3,4] one of which was associated with parvovirus B19 (PVB19) infection.[4] Our patient also had serologic evidence of acute PVB19 infection. Oligoclonal bands and PVB19 DNA were detected in cerebrospinal fluid by polymerase chain reaction. Cytomegalovirus DNA was detected as well, but without serologic evidence of acute infection. The patient had an initial good outcome, but long-term follow-up revealed moderate cognitive and severe behavioral disturbances, possibly as a result of the encephalopathy, a form that was called choreaencephalopathy.

Anti-N-methyl-D-aspartate receptor encephalitis is an immune-mediated condition that is frequently associated with neoplasms in adults but is rare in children, in whom infection could be a trigger. Armangue et al report an association with herpes simplex virus in one case. Symptomatic and especially asymptomatic PVB19 infections are very common in children; some cases of anti-N-methyl-D-aspartate receptor encephalitis could be triggered by this virus.

Eugenio Grillo, MD
Ronaldo Jose M. da Silva, MD
Department of Neurology
Hospital Infantil Joana de Gusmão
Florianopolis, Brazil
http://dx.doi.org/10.1016/j.jpeds.2013.06.079

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Pediatric Anti-*N*-methyl-D-Aspartate Receptor Encephalitis—Clinical Analysis and Novel Findings in a Series of 20 Patients

Thaís Armangue, MD[2,4], Maarten J. Titulaer, MD, PhD[2], Ignacio Málaga, MD, PhD[5], Luis Bataller, MD, PhD[6], Iñigo Gabilondo, MD[2], Francesc Graus, MD, PhD[2], and Josep Dalmau, MD, PhD[1,2,3], on behalf of the Spanish Anti-*N*-methyl-D-Aspartate Receptor (NMDAR) Encephalitis Work Group*

Objective To report the clinical features of 20 pediatric patients with anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis.

Study design Review of clinical data, long-term follow-up, and immunologic studies performed in a single center in Spain in the last 4 years.

Results The median age of the patients was 13 years (range, 8 months-18 years), 70% were female. In 12 patients (60%), the initial symptoms were neurologic, usually dyskinesias or seizures, and in the other 40% psychiatric. One month into the disease, all patients had involuntary movements and alterations of behavior and speech. All patients received steroids, intravenous immunoglobulin or plasma exchange, and 7 rituximab or cyclophosphamide. With a median follow up of 17.5 months, 85% had substantial recovery, 10% moderate or severe deficits, and 1 died. Three patients had previous episodes compatible with anti-NMDAR encephalitis, 2 of them with additional relapses after the diagnosis of the disorder. Ovarian teratoma was identified in 2 patients, 1 at onset of encephalitis and the other 1 year later. Two novel observations (1 patient each) include, the identification of an electroencephalographic pattern ("extreme delta brush") considered characteristic of this disorder, and the development of anti-NMDAR encephalitis as post herpes simplex encephalitis choreoathetosis.

Conclusions The initial symptoms of pediatric anti-NMDAR encephalitis vary from those of the adults (more neurologic and less psychiatric in children), the development of a mono-symptomatic illness is extremely rare (except in relapses), and most patients respond to treatment. Our study suggests a link between post herpes simplex encephalitis choreoathetosis and anti-NMDAR encephalitis. *(J Pediatr 2013;162:850-6).*

Since its initial description in 2007,[1] anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis has been recognized as the most frequent autoimmune encephalitis in children after acute demyelinating encephalomyelitis.[2] In a center focused on the etiology and epidemiology of encephalitis (California Encephalitis Project) the frequency of anti-NMDAR encephalitis surpassed that of any viral encephalitis.[3] Patients develop serum and cerebrospinal fluid (CSF) antibodies to a restricted epitope region of the NR1 subunit of the NMDAR.[4] In cultures of hippocampal neurons, patients' immunoglobin G (IgG) or CSF produce a substantial decrease of the levels of NMDAR and NMDAR-mediated currents that is reversible upon removal of patients' antibodies. A similar effect was obtained when antibodies were injected in vivo into the hippocampus of rats.[5,6] In pediatrics, increased awareness of this disorder is largely due to single case reports or small series,[7,8] with the largest experience being an American series of 32 patients, 8 from a single institution.[9] This and subsequent studies suggested that the younger the patient, the less likely a tumor would be found, and that the disease onset

CSF	Cerebrospinal fluid
EEG	Electroencephalography
FLAIR	Fluid-attenuated inversion recovery
HSE	Herpes simplex encephalitis
HSV	Herpes simplex virus
IgG	Immunoglobin G
IVIG	Intravenous immunoglobulin
MRI	Magnetic resonance imaging
NMDAR	*N*-methyl-D-aspartate receptor
PCPC	Pediatric Cerebral Performance Category
PCR	Polymerase chain reaction

in children may be different from that of adults.[9,10] However, in those studies, the retrospective diagnosis of some patients and conservative treatment approach because of the novelty of the disease limited the information on symptom onset, treatment, and outcome. To address these issues, we report our experience with 20 pediatric patients with anti-NMDAR encephalitis focusing on disease presentation, spectrum of symptoms, treatment, and relapses. Moreover, the identification of a patient who developed the disorder 1 month after onset of herpes simplex encephalitis (HSE) provides an explanation for a rare and poorly understood complication of HSE[11-13] that often presents with choreoathetosis and occurs without viral reactivation.

Methods

From January 2008 until February 2012, we identified 61 patients (median age 22 years; range 8 months-76 years) with anti-NMDAR encephalitis whose serum and CSF were referred for antibody testing to Hospital Clinic, University of Barcelona. All patients were suspected of having autoimmune encephalitis after being extensively studied by their physicians. Twenty patients (33%) were younger than 19 years and are the focus of this study. Analysis of serum and CSF for NMDAR antibodies was performed using 2 different tests, immunohistochemistry with rodent brain tissue and a highly specific cell-based assay, following reported criteria.[14] None of the patients had antibodies to other cell surface or synaptic proteins using cell-base assays for the following proteins: AMPA receptor, GABA(B) receptor, mGluR1, mGluR5, LGI1, Caspr2, glycine receptor, and dopamine receptor. All patients were seen by the authors. Disease severity and residual deficits were determined with the Pediatric Cerebral Performance Category (PCPC) scale (Table I; available at www.jpeds.com).[15] There was not standardized protocol for ancillary tests; all patients underwent electroencephalography (EEG), magnetic resonance imaging (MRI), CSF analysis, and extensive bacterial and viral studies, including in all instances herpes simplex virus (HSV) among others. Treatment decisions were based on the physician's discretion. Three patients have been previously reported as part of a series of patients with relapses of encephalitis.[16]

The study was approved by the Ethics Committee of the Hospital Clinic. Samples are deposited in a collection of biological samples registered in the Biobank of Institut d'Investigació Biomèdica August Pi i Sunyer, Barcelona.

Results

The median age of the patients was 13 years (8 months-18 years); 14 were Caucasian, 5 Hispanic, and 1 Asian. NMDAR antibodies were identified in the CSF of all patients and serum of 9; the serum of 2 patients was negative and was not available from the other 9. Seventy percent of the patients were female. The ratio female/male varied according to age, so that 33% of patients younger than 12 years and all above this age were female.

Initial Symptom

Eleven patients (55%) developed prodromal symptoms a few days before the onset of the disease, including fever (n = 7), headache (6), and vomiting (4). A 2-year-old girl developed anti-NMDAR encephalitis 1 week after completing treatment
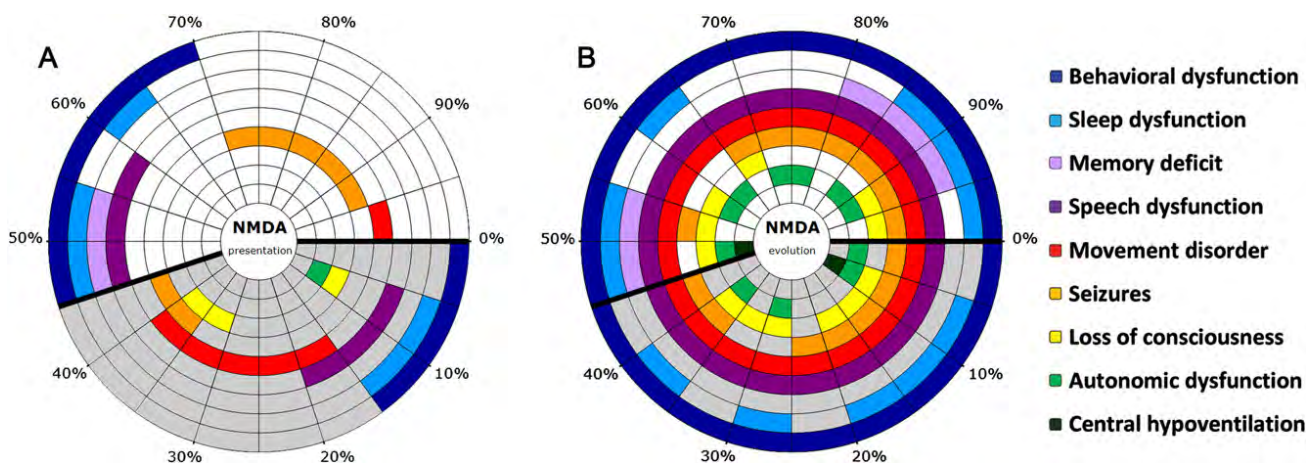
Figure 1. Symptoms at presentation and during the first month of the disease. **A,** The initial symptoms of each patient are shown in a panel; every radial segment represents 1 patient. The percentages assist to determine the percentage of patients with a specific symptom or combination of symptoms, each symptom coded with a different color. Patients >12 years-old are shown in the section with white background, and those ≤12 in the section with grey background. Behavioral dysfunction included agitation and aggression (4 patients), psychosis, delusional thoughts, and hallucinations (3), nonspecific behavioral disturbance (3), anxiety (2), stereotyped behavior and obsessions (1), and negativism and autolytic thoughts (1). **B,** The symptoms during the first month of the disease; patients are represented in the same order as in **A**.

Table II. Clinical features, diagnostic tests, treatment, and outcome

Patient's age	<12 y	12-18 y	All patients
Number of patients	9	11	20
Female	3 (33%)	11 (100%)	14 (70%)
Median age (range), y	3 (0,7-11)	15 (13-18)	13 (0,7-18)
Associated tumor	0	2 (18%), both ovarian teratoma	2 (10%)
Prodomal symptoms	6 (67%)	5 (45%)	11 (55%)
Symptom presentation*			
Psychiatric features	3 (33%)	5 (45%)	8 (40%)
Neurological	6 (67%)	6 (55%)	12 (60%)
Abnormal EEG†	8 (89%)	10 (90%)	18 (90%)
Abnormal MRI‡	5 (55%)	4 (36%)	9 (45%)
CSF pleocytosis	4 (44%)	10 (91%)	14 (70%)
Hospitalization, median (range) days	56 (13-90)	56 (17-336)	56 (13-336)
PCPC maximum, median (range)	4 (4-5)	4 (4-6)	4 (4-6)
Treatment			
Steroids	9 (100%)	11 (100%)	20 (100%)
IVIG	6 (67%)	9 (82%)	15 (75%)
Plasma exchange	1 (11%)	0	1 (5%)
Rituximab alone	2 (22%)	3 (27%)	5 (25%)
Rituximab combined with cyclophosphamide	1 (11%)	1 (9%)	2 (10%)
Other§	2 (22%)	1 (9%)	3 (15%)
Follow up, median (range), mo	18 (9-149)	15 (4-103)	17,5 (4-149)
Delay of treatment from disease onset			
>1 mo	3 (33%)	4 (36%)	7 (35%)
>2 mo	0	2 (18%)	2 (10%)
First sign of improvement since disease onset, median (range), days	20 (7-180)	46 (25-276)	40 (7-276)
First sign of improvement since immunotherapy, median (range), days	7 (2-176)	15 (2-90)	11,5 (2-176)
Outcome (PCPC score)			
1: Full recovery	5	7	12 (60%)
2: Mild disability	3	2	5 (25%)
3: Moderate disability	0	0	0
4: Severe disability	1	1	2 (10%)
5: Coma	0	0	0
6: Death	0	1	1 (5%)

For patients with relapses, the information used in the Table corresponds to the first episode, except treatment that includes all episodes.
*See Figure 1, A for detailed information about symptom presentation.
†Abnormal EEG findings included 7 patients with generalized slowing, 6 with generalized slowing and focal epileptic activity, 3 with asymmetrical or focal slowing, 1 with generalized epileptiform activity, and 1 with "extreme delta brush"[17] (Figure 2).
‡Brain MRI abnormalities included 6 patients with T2/FLAIR abnormalities in the temporal lobes (1 with contrast enhancement, and 2 with abnormal diffusion weighted images), 1 patient with minimal changes in arterial spin labeled perfusion in insular regions, 1 patient with cystic lesions in the left temporal lobe with perilesional gliosis, and 1 patient with transient brain atrophy attributed to steroids.
§One patient received tacrolimus before the diagnosis of anti-NMDAR encephalitis for suspected Rasmussen encephalitis. Two other patients received oral mycophenolate mofetil after completing second-line immunotherapies.

with acyclovir for HSE (Appendix 2 and Videos 1-3; available at www.jpeds.com).

In 12 patients (60%), the first symptom was neurologic, usually abnormal movements or seizures, and in the other 8 patients (40%), the first symptom was psychiatric or cognitive dysfunction. Neurologic presentations were more frequent in patients younger than age 12 years (67% vs 55%) (Figure 1, A). Three patients were admitted to psychiatric units.

Symptoms and Diagnostic Studies During the First 4 Weeks of Disease
Figure 1, B shows the predominant symptoms that occurred within the first month of the disease grouped in 9 categories. The median number of symptoms was 5.5 (range 4-8), and all patients developed psychiatric dysfunction, impaired speech, and abnormal movements, usually orofacial dyskinesias and choreoathetosis.

The EEG was abnormal in 18 patients (90%) and the brain MRI in 9 (45%). A list of these findings is shown in Table II. One of the patients had an EEG pattern named "extreme

delta brush" (Figure 2; available at www.jpeds.com).[17] Extreme delta brush consists of a nearly continuous combination of delta activity with superimposed fast activity, usually in the beta range, in patients who are not under effects of sedation or anesthetics. This pattern resembles the delta brushes described in premature infants but extreme delta brush is more symmetric and synchronous involving predominantly frontal regions.[17] We have not been able to review all EEG studies performed in other patients.

In 1 patient, the MRI obtained when the patient developed HSE showed increased T2/fluid-attenuated inversion recovery (FLAIR) signal in opercular regions, medial temporal lobes, and posterior aspect of the basal ganglia (Figure 3, A-D). In contrast to these rapid and irreversible viral-induced abnormalities, the subsequent development of anti-NMDAR encephalitis did not result in additional changes to the MRI (Figure 3, E-H).

The initial CSF study showed lymphocytic pleocytosis in 14 (70%) patients (median 30 cells/µL; range 5-140). Only 1 patient had elevated CSF protein concentration. CSF
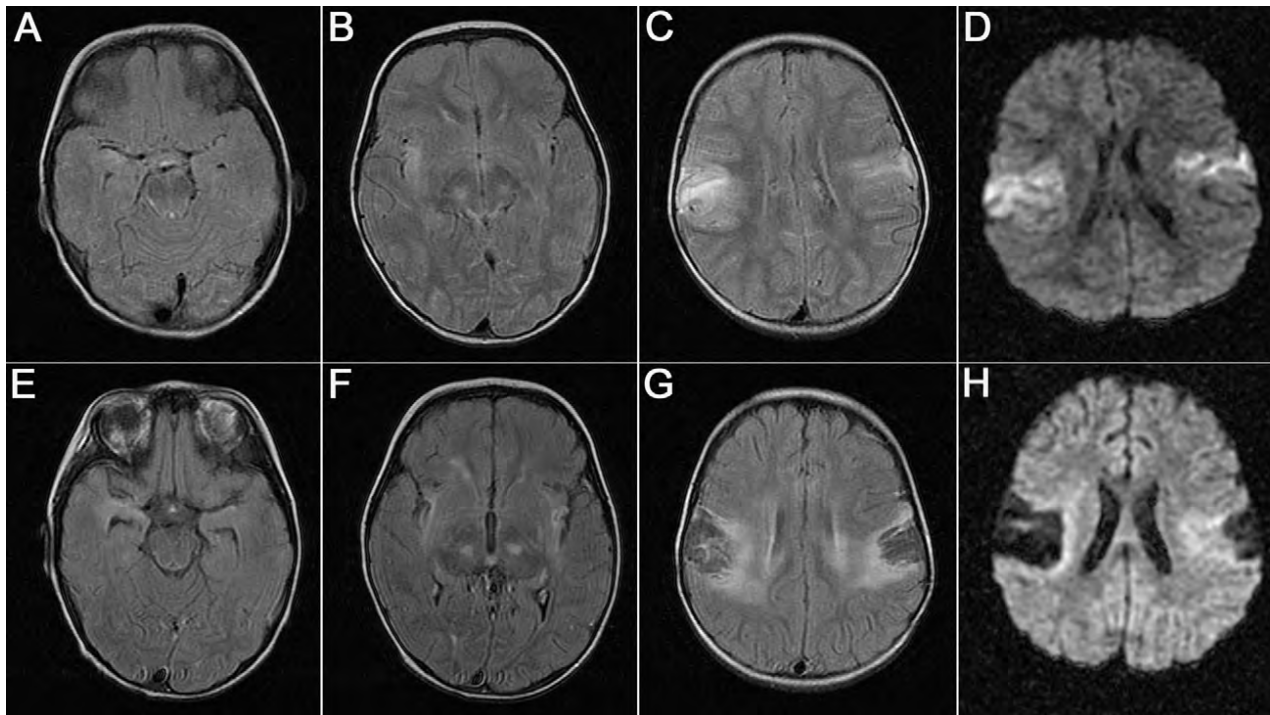
Figure 3. MRI findings in a patient who developed HSE followed by anti-NMDAR encephalitis. **A-D,** The MRI findings during the first week of HSE. **A-C,** Increased T2-FLAIR signal was demonstrated in the right medial temporal lobe, right insula, posterior basal ganglia, and bilateral opercular regions, and **D,** increased signal in diffusion weighted images (DWI). **E-H,** The MRI obtained during admission for anti-NMDAR encephalitis, 1 month after HSE onset, showed no additional changes other than the interval evolution of areas of encephalomalacia in opercular regions and hippocampal atrophy.

specific oligoclonal bands were identified in 3 of 6 (50%) patients. One patient underwent a brain biopsy that was normal.

In all patients, the CSF polymerase chain reaction (PCR) for HSV-1 was negative. Four patients had specific viral or bacteriologic findings, including a throat swab positive for *Haemophilus influenzae*, a CSF PCR positive for *Human herpesvirus 6*, serum immunoglobin M and IgG antibodies to mumps virus, and a nasopharyngeal aspirate positive for *Enterovirus*.

All patients had tumor screening with MRI of the abdomen and pelvis, or abdominal or testicular ultrasound. An ovarian mass suggesting a teratoma was identified in 2 patients, leading to unilateral oophorectomy; in 1 of the patients (a 17-year-old), pathologic studies demonstrated a mature teratoma, and in the other (a 13-year-old), a benign follicular cyst.

Treatment

During the first episode of encephalitis, 19 (95%) patients received first-line immunotherapies (1 patient was only treated at third relapse). All patients received at least a short course of high-dose steroids (median 1, range 1-3 courses), followed in 13 patients by oral steroid tapering for a median of 12 weeks (range 3-47). In addition, 14 patients received intravenous immunoglobulin (IVIG) (median 2 cycles, range 1-12) and 1 patient had plasmapheresis. In 1 patient, steroids were

stopped because of worsening symptoms of psychosis; no side effects of first line therapies occurred in the other patients.

At last follow-up, all patients had received immunotherapy: 20 had first-line therapies (steroids, IVIG and/or plasma exchange), and 7 (35%) had second-line therapies (rituximab alone or combined with cyclophosphamide) (Table II). The reasons for using second-line therapies included unsatisfactory response to first-line drugs in 6 patients and multiple relapses in 1. The median number of treatments with rituximab was 4 weekly doses (range 4-6) and the median number of cycles of cyclophosphamide was 5.5 monthly doses (range 4-7). Although 18 patients (90%) were treated with antiepileptic drugs, none of them developed chronic or recurrent seizures, and at the last follow-up only 1 continued with antiepileptic medication. Abnormal movements were symptomatically treated with a variety of medications (tetrabenazine, piracetam), none of them clearly effective.

Disease Severity and Outcome

At the peak of the disease the median degree of disability was 4 in the PCPC scale (all patients had ≥4, and 1 died [PCPC = 6]). Nine patients were admitted to pediatric intensive care units, 2 of them requiring mechanical ventilation. The median time of hospitalization was 56 days (13-336).

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Table III. Clinical features of episodes of relapses

	Patient 1	Patient 2	Patient 3
Total number of episodes of encephalitis	2	3	6
Time between episodes (mo)	31	108 and 36	13, 49, 16, 5, and 12
Number of episodes before the diagnosis of anti-NMDAR encephalitis	1	1	2
Age (y)	13	8	18 and 19
First symptom	Leg dystonia	Dyskinesias	Behavior/speech disorder
Course	Full syndrome	Full syndrome	Full syndrome/full syndrome
PCPC maximum	4	4	4/4
Immunotherapy	Steroids, IVIG	Steroids	No treatment/no treatment
Outcome	Full recovery (PCPC 1) in 8 mo	Full recovery (PCPC 1) In 6 mo	Partial recovery; slow improvement in both episodes (PCPC 2)
Episode that led to the diagnosis of anti-NMDAR encephalitis			
Age (y)	15	17	23
First symptom	Speech disorder	Behavior	Behavior and speech disorder
Course	Only speech Dysfunction	Full syndrome	Behavior and speech symptoms
PCPC maximum	3	4	3
Anti-NMDAR antibodies	CSF 1:80, serum NA	CSF 1:20, serum NA	CSF 1:160, serum 1:1600
Immunotherapy	Steroids	Steroids	Steroids, monthly IVIG for long period of time
Response	Full recovery (PCPC 1) in 1 mo	Full recovery (PCPC 1) in 6 mo	Recovered to previous baseline, (PCPC 2), in less than 1 mo
Number of episodes after the diagnosis of anti-NMDAR encephalitis	0	1	3
Age (y)	-	20	24; 25; 26
First symptom	-	Aphasia	All 3 episodes: behavioral and speech deficits (more severe psychiatric symptoms in last episode)
Course	-	Only aphasia	
PCPC maximum	-	3	3/3/4
Anti-NMDAR antibodies	-	CSF 1:80, serum 1:400	Between relapses (CSF NA, serum 1:1600) Last episode (CSF 1:80, serum 1:3200), after second-line drugs (CSF 1:20, serum 1:1600)
Immunotherapy	-	Steroids	Steroids, IVIG. Last episode also: rituximab, cyclophosphamide and myophenolate mofetil
Response	-	Still improving (1 mo of follow-up)	Recovery to previous baseline (PCPC, 2), episodes #4 and 5 in 1 mo, episode #6 in 3 mo
Follow-up since first episode/since last relapse (mo)	62/31	149/1	103/8

NA, not available.

After a median follow up of 17.5 months (4-149), 17 (85%) patients had substantial improvement (PCPC of 1 or 2: 60% complete recovery and 25% minimal residual deficits), 2 (10%) moderate or severe disability (PCPC of 3 or 4), and 1 died. The 2 patients with moderate or severe disabilities (follow-up of 4 and 9 months) are still improving at the time of writing this manuscript.

The median time from symptom onset until the first sign of improvement was 40 days (7-276), and from the start of immunotherapy until the first sign of improvement 11.5 days (2-176). Full recovery was achieved in 12 patients; this occurred between 8 and 12 months after symptom onset in 8 patients, and 3 and 5 months in 4 patients. For the 17 patients with substantial improvement, the last symptoms to improve in 16 patients (94%) were related to executive functions; in 1 patient the last symptom to improve was gait ataxia.

The patient who died was a 15-year-old girl who presented with psychiatric symptoms (auditory hallucinations, memory disturbance, and anxiety) and subsequently developed abnormal movements, episodes of bradycardia, hypertension, and coma requiring mechanical ventilation and vasoactive drugs. She developed multiple organ failure and intestinal perforation; during surgery, multiple areas of ischemia were noted in the liver and intestine. No tumor was identified; she was treated with intravenous steroids and IVIG without effect, and died 8 weeks after onset of encephalitis.

Before the diagnosis of anti-NMDAR encephalitis, 3 patients (15%) had at least 1 episode of encephalitis that had been classified as "encephalitis lethargica" (Table III). In retrospect, these episodes were likely prior episodes of anti-NMDAR encephalitis; in all instances, symptoms included dyskinesias, language dysfunction, and psychiatric manifestations. Since the diagnosis of anti-NMDAR encephalitis, 1 of these 3 patients has developed a new relapse, and another patient 3 relapses. In the latter patient, the 2 episodes preceding the diagnosis of anti-NMDAR encephalitis were not treated with immunotherapy, episode number 3 (when the diagnosis of anti-NMDAR encephalitis was made) and episode numbers 4 and 5 were treated with first-line immunotherapy, and episode number 6 with rituximab and cyclophosphamide followed by mycophenolate mofetil. At last follow-up, 8 months after rituximab, the patient had

substantial neurologic improvement without further relapses (**Table III**).

A 14-year-old girl, whose neurologic symptoms of anti-NMDAR encephalitis resolved in 8 months, developed acute abdominal pain without neurologic symptoms 4 months later (1 year from the diagnosis of encephalitis). A large ovarian mass was identified and removed, with pathologic studies demonstrating an immature teratoma. Review of the tumor screening performed 1 year earlier with computed tomography, MRI, and ultrasound of abdomen and pelvis confirmed that no tumor was visible at the time she developed encephalitis.

Discussion

Our study suggests that the presentation of anti-NMDAR encephalitis in children can be different from that reported in adults. Although 60% of children presented with seizures, abnormal movements, and focal neurologic deficits, previous experience with large series of predominantly young adults demonstrated that 70% presented with psychosis and other psychiatric symptoms.[14] In the current study, patients older than age 18 years were excluded, but a similar trend was noted comparing the symptom presentation of patients older and younger than age 12 years: those older than age 12 presented more often with psychiatric symptoms (45% vs 33%). Subsequently, during the first month of the disease, all patients developed abnormal movements, psychiatric symptoms, and language dysfunction. Similar features were recently described by Titulaer et al in a series of 568 patients of all ages in which 95.6% developed at least 3 of the groups of symptoms shown in **Figure 1**, and only 0.7% had a monosymptomatic illness.[10] In patients who develop the full syndrome, there is usually a gradual progression of symptoms from those mentioned above towards decreased level of consciousness, autonomic instability, and hypoventilation. Overall, these findings suggest caution in accepting the diagnosis of anti-NMDAR encephalitis in monosymptomatic cases or when symptoms do not fit the expected syndrome. A prudent approach to these cases is reassessment of CSF and serum for antibodies. However, clinical relapses can be monosymptomatic and less severe than the initial presentation,[16] as occurred in 1 of our patients who, over a period of 3 years, developed 4 episodes of isolated behavioral and language dysfunction.

The frequency of CSF alterations in this study appears lower than in prior series (70% vs 91%) in adults.[14] This may be due to an earlier recognition of the disease resulting in prompt diagnosis and reduction of repeat spinal taps that may have shown alterations. The MRI and EEG findings are, in most respects, similar to those reported in adults including, in 1 of the patients, a unique EEG pattern ("extreme delta brush") recently described in 30% of adults with anti-NMDAR encephalitis.[17]

Eighty-five percent of the patients had remarkable clinical improvement or full recovery. The delay between treatment and first sign of improvement was 11.5 days, but in most cases the recovery was achieved 8-12 months after symptom onset. It has been postulated that the prolonged duration of the disease and slow response to immunotherapy are due, in part, to the production of antibodies within the central nervous system as well as systemically. This is supported by the almost constant detection of intrathecal synthesis of antibodies[14,18,19] (identified in all 9 patients studied here), and the demonstration of parenchymal and meningeal infiltrates of plasma cells, which are long-lived and difficult to eliminate.[20] By abrogating systemic B-cells, rituximab would prevent the entry of these cells in the central nervous system and subsequent development into antibody-producing plasma cells.[21] Cyclophosphamide, which can penetrate the blood-brain barrier, affects T and B cells, and increases anti-inflammatory cytokines contributing to immunosuppression.[22] In the current study, all 7 patients who received rituximab (2 with cyclophosphamide) responded to treatment without further relapses, including 1 patient who had had 5 previous episodes. None of the patients had significant side effects of the treatment.

As noted in previous series and case reports,[7,9] most children with anti-NMDAR encephalitis do not have an underlying tumor. However, some patients (usually older than age 12 years) do have a teratoma and, as occurs in young adults, the tumor may be detectable after the patient has recovered from encephalitis, similar to 1 of our patients.[23] The frequency and duration of tumor surveillance in children is a question that needs to be answered in future studies.

A 2-year-old girl developed anti-NMDAR encephalitis 4 weeks after HSE. Her symptoms were remarkably similar to the choreoathetosis and orofacial dyskinesias that occur in some patients during the first month of onset of HSE. This complication has an unclear etiology.[11-13] In these cases, viral reactivation seems unlikely because CSF and brain viral studies are negative, the MRI studies do not show new necrotic-hemorrhagic lesions, and symptoms are refractory to acyclovir. The abnormal movements may last several months or years and are refractory to anti-epileptics and dopamine receptor antagonists.[11] These observations have suggested a postinfectious immune-mediated etiology.[11-13] Taken together, the biphasic course of symptoms of our patient, along with the CSF findings (negative PCR HSV, positive NMDAR antibodies), no additional changes in the brain MRI, and lack of response to acyclovir but improvement after rituximab and cyclophosphamide, suggest that some patients with post-HSE choreoathetosis may in fact have anti-NMDAR encephalitis. This link between both disorders is supported by a recent study showing IgG NMDAR antibodies in serum or CSF of 11% of patients with past history of HSE.[24]

Our study was not prospective and did not have a uniform systematic treatment approach (eg, same criteria and timing to change from first line immunotherapy to second line immunotherapy, and duration of treatments). Future studies should address these issues in the context of a clinical trial. In addition, studies with larger number of patients will provide predictor factors of the response to treatment and relapses.

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Findings from this study suggest that in children the first symptom of anti-NMDAR encephalitis may be different from that of the adults (more neurologic in children, more psychiatric in adults); the development of a monosymptomatic illness is extremely rare (except in relapses), and although the disease is potentially lethal, most patients respond to immunotherapy. Moreover, second line immunotherapy, mostly including rituximab, is often effective and well tolerated. A notable feature not previously reported in children is the EEG pattern named extreme delta brush. Further studies are needed to determine the frequency of NMDAR antibodies in patients with post-HSE choreoathetosis and whether this disorder is, in fact, anti-NMDAR encephalitis. ■

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Appendix 1

Members of the Spanish anti-NMDAR Encephalitis Work Group include: Verónica Cantarín-Extremera (Hospital Universitario Niño Jesús, Madrid), Maite Carreras-Rodríguez (Hospital Princesa, Madrid), Mireia Del Toro (Hospital Universitari Materno-Infantil Vall d'Hebron, Barcelona), Jordi Espinosa-Garcia (Hospital General de Granollers, Barcelona); Alfonso Falcón (Hospital San Pedro de Alcántara, Cáceres), Jaime Gállego Pérez-Larraya (Clínica Universitaria de Pamplona), Verónica Garcia-Sanchez (Hospital Sant Joan de Déu, Barcelona), Enriqueta Gómez (Hospital Universitari Politècnic La Fe, Valencia), Verónica González-Álvarez (Hospital Sant Joan de Déu, Barcelona), Juan Carlos Gómez-Esteban (Hospital Universitario de Cruces, Bilbao), Rocío Jadraque (Hospital Universisstario de Alicante), África Jiménez-Vidal (Hospital Clínico Universitario "Lozano Blesa", Zaragoza), Francisco López-Pisón (Hospital Universitario Miguel Servet, Zaragoza), Alfons Macaya (Hospital Universitari Materno-Infantil Vall d'Hebron, Barcelona), Emilio Monteagudo (Hospital Universitari Politècnic La Fe, Valencia). Guillermo Navarro-Mascarell (Hospital Universitario Virgen Macarena, Sevilla), Ernest Palomeras-Soler (Hospital de Mataró, Barcelona), Anna Pellisé-Guinjoan (Hospital Universitari Joan XXIII, Tarragona), Rogelio Simón De Las Heras (Hospital Universitario 12 de Octubre, Madrid), Eulàlia Turón-Viñas (Hospital Universitari de la Santa Creu i Sant Pau, Barcelona), and Alvaro Vela (Hospital Moncloa, Madrid).

Appendix 2

Development of anti-NMDAR Encephalitis after HSE—Clinical Case

A 2-year-old previously healthy Caucasian girl presented with partial seizures and fever. CSF studies showed 180 white blood cells/μL (69% lymphocytes), normal protein and glucose concentrations, and the PCR for HSV-1 was positive. Brain MRI showed increased T2/FLAIR signal in opercular regions, medial temporal lobes, and posterior aspect of the basal ganglia (Figure 3, A-D). She was treated with intravenous acyclovir during 3 weeks, resulting in substantial recovery but with residual bilateral facial weakness and anarthria secondary to bilateral opercular encephalomalacia (Foix-Chavany-Marie syndrome). One week after being discharged home (1 month after HSE onset) she developed diarrhea and low grade fever followed by behavioral abnormalities, including agitation, inability to sleep, and periods of hyperexcitability alternating with somnolence. The patient was admitted to the Pediatric Intensive Care Unit for extreme agitation, and treatment with midazolam was initiated. While waiting for the CSF results, treatment with acyclovir was started for suspicion of HSV reactivation. CSF analysis showed a normal white blood cell count and protein concentration, and the PCR for HSV was negative. Over the next days she developed choreathetosis in the limbs, orofacial dyskinesias, generalized tonic-clonic seizures, and decreased level of consciousness. Severe tachychardia and transient hypoventilation were noted during the episodes of agitation, but mechanical ventilation was not required. A repeat brain MRI showed areas of encephalomalacia in opercular regions, where the MRI obtained 1 month earlier had demonstrated the most prominent FLAIR/T2 signal abnormalities; other previously noted abnormalities were unchanged or improved, without new findings identified (Figure 3, E-H). The EEG demonstrated diffuse but asymmetric slow activity (more evident in the right hemisphere), without evidence of epileptic activity. The absence of new MRI findings or areas of necrosis, negative HSV testing in CSF studies, and the clinical picture of choreoathetosis and orofacial dyskinesias that did not respond to acyclovir led to a diagnosis of post-HSV "choreoathetosis," which is a disorder suspected to be immune-mediated. Therefore, treatment with methyl-prednisolone (30 mg/k/d for 3 days) and IVIG (0,4 g/k/d for 5 days, 2 courses) was started, followed by a 4-week taper of steroids. Additional symptomatic medication included midazolam for agitation and valproate for seizures. However, her symptoms did not improve and she continued with prominent choreaoathetosis (Video 1), catatonic features, and episodes of tachycardia. Four months after onset of this new episode of encephalitis, testing for antibodies to cell surface or synaptic proteins demonstrated high levels of NMDAR antibodies in serum and CSF. The patient was then started on 5 monthly courses of IVIG along with second line immunotherapy, including rituximab (375 mg/m^2 weekly, 4 doses), and cyclophosphamide (monthly IV pulses, first dose: 500 mg/m^2, second and subsequent doses: 750 mg/m^2). Screening for an underlying ovarian teratoma with abdominal and pelvic ultrasound was negative. Five weeks after second line drugs were started (5 months since the onset of anti-NMDAR encephalitis), the first signs of improvement were noted. The patient's level of consciousness as well as the involuntary movements and abnormal behavior started to improve (Video 2). At the last follow-up, 9 months after onset of anti-NMDAR encephalitis, she is still improving and is now completing the fourth cycle of cyclophosphamide. Because of the residual opercular syndrome (sequelae of HSE), she cannot speak or swallow and her feedings are via a percuatenous endoscopic gastrostomy tube. Her gait is mildly dystonic/ataxic but she is able to walk with mild support. Her cognition is intact, she follows commands appropriately, and communicates by signs (Video 3).

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Table I. PCPC[15]

Score	Category	Description
1	Normal	Normal; at age-appropriate level; school-age child attending regular school classroom
2	Mild disability	Conscious, alert, and able to interact at age-appropriate level; school-age child attending regular school classroom, but grade perhaps not appropriate for age; possibility of mild neurologic deficit
3	Moderate disability	Conscious; sufficient cerebral function for age-appropriate independent activities of daily life; school-age child attending special education classroom and/or learning deficit present
4	Severe disability	Conscious; dependent on others for daily support because of impaired brain function
5	Coma or vegetative state	Any degree of coma without the presence of all brain death criteria; unaware, even if awake in appearance, without interaction with environment; cerebral unresponsiveness and no evidence of cortex function (not aroused by verbal stimuli); possibility of some reflexive response, spontaneous eye-opening, and sleep-wake cycles
6	Brain death	Apnea, areflexia, and/or electroencephalographic silence
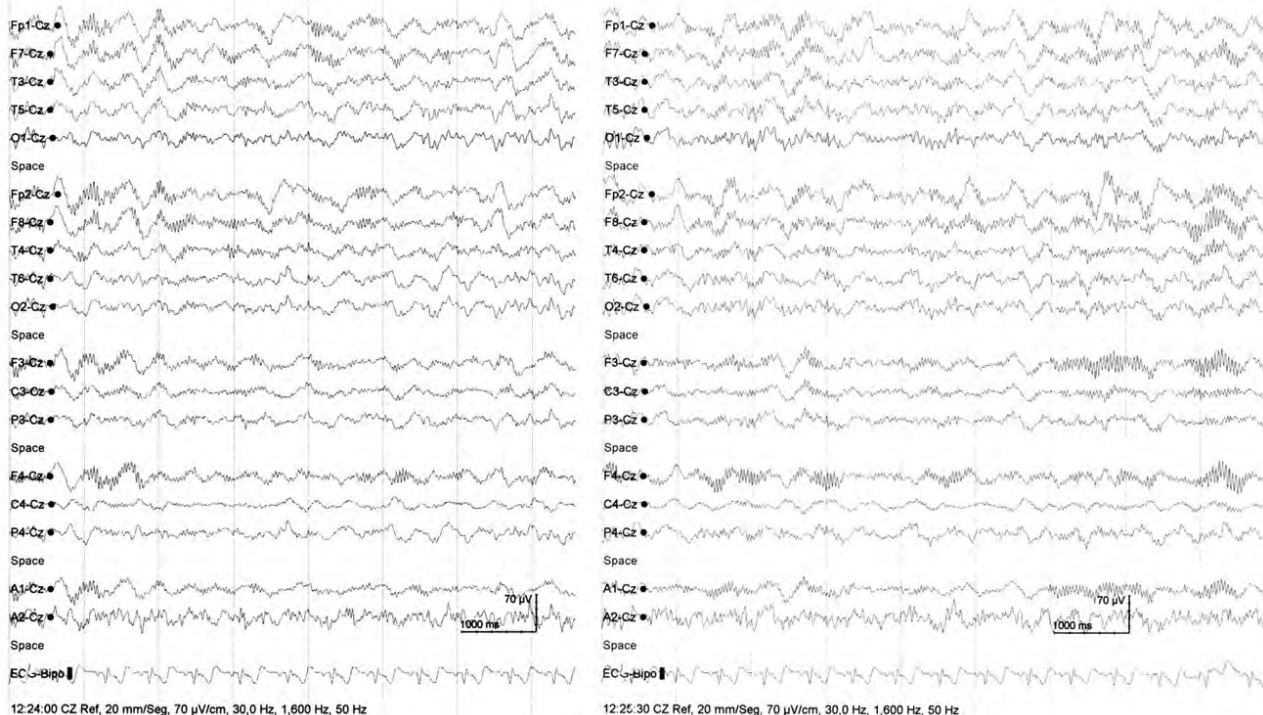
Figure 2. Extreme delta brush. A 14-year-old previously healthy girl presented with secondarily generalized seizures. Brain MRI was normal and initial EEG showed slow activity in right temporal lobes. She was diagnosed with temporal lobe epilepsy and treatment with levetiracetam was initiated. Two weeks later, she was readmitted to the hospital for severe obsessive-compulsive behavior and visual hallucinations. Levetiracetam was discontinued and oral carbamazepine was started. EEG during the first 48 hours of admission showed a pattern consistent with "extreme delta brush,"[17] including continuous combination of delta frequency transients with superimposed fast activity in the beta range, symmetrically involving all head regions, with frontal preference. Screening for an underlying tumor was negative and she was treated with IVIG and steroids. At the last follow-up, 10 months after symptom onset, she was fully recovered.

Article IV: Herpes simplex virus encephalitis is a trigger for brain autoimmunity.

Autors: Armangue T,* Leypoldt F*, Málaga I, Raspall-Chaure M, Marti I, Nichter C, Pugh J, Vicente-Rasoamalala M, Lafuente-Hidalgo M, Macaya A, Ke M, Titulaer MJ, Höftberger R, Sheriff H, Glaser C, Dalmau J. *igual contribució

IV

Herpes Simplex Virus Encephalitis Is a Trigger of Brain Autoimmunity

Thaís Armangue, MD,[1,2]
Frank Leypoldt, MD, PhD,[1,3]
Ignacio Málaga, MD, PhD,[4]
Miquel Raspall-Chaure, MD,[2]
Itxaso Marti, MD,[5] Charles Nichter, MD,[6]
John Pugh, MD,[6]
Monica Vicente-Rasoamalala, MD,[7]
Miguel Lafuente-Hidalgo, MD,[5]
Alfons Macaya, MD, PhD,[2]
Michael Ke, MD, PhD,[8]
Maarten J. Titulaer, MD, PhD,[9]
Romana Höftberger, MD,[1,10]
Heather Sheriff,[11]
Carol Glaser, MD, PhD,[11]
and Josep Dalmau, MD, PhD[1,12,13]

In 5 prospectively diagnosed patients with relapsing post–herpes simplex encephalitis (HSE), N-methyl-D-aspartate receptor (NMDAR) antibodies were identified. Antibody synthesis started 1 to 4 weeks after HSE, preceding the neurological relapse. Three of 5 patients improved postimmunotherapy, 1 spontaneously, and 1 has started to improve. Two additional patients with NMDAR antibodies, 9 with unknown neuronal surface antibodies, and 1 with NMDAR and unknown antibodies, were identified during retrospective assessment of 34 HSE patients; the frequency of autoantibodies increased over time (serum, $p = 0.004$; cerebrospinal fluid, $p = 0.04$). The 3 retrospectively identified NMDAR antibody–positive patients also had evidence of relapsing post-HSE. Overall, these findings indicate that HSE triggers NMDAR antibodies and potentially other brain autoimmunity.

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R elapsing post–herpes simplex virus (HSV) encephalitis (post-HSE) is a potentially lethal complication that occurs in 13 to 24% of patients as a result of viral reactivation or suspected immunological mechanisms.[1–3] In the latter, patients frequently develop choreoathetosis, symptoms do not respond to acyclovir, and the HSV polymerase chain reaction in cerebrospinal fluid (CSF) is negative.[1–4] These findings and the observation that some patients (11%) with HSE develop N-methyl-D-aspartate receptor (NMDAR) immunoglobulin G (IgG) antibodies[5] led us to postulate that these antibodies could be involved in neurological relapses,[4] a hypothesis supported by a few case reports.[4,6–8] In the current study, we provide a robust link between NMDAR antibodies and relapsing post-HSE by demonstrating novel NMDAR antibody synthesis during the weeks that lapse between HSE and the development of new symptoms. We also show that HSE is a robust trigger of cell-surface/synaptic autoimmunity not limited to NMDAR. These findings are important because they assist in establishing the correct diagnoses and direct appropriate treatment approaches.

Patients and Methods

From June 2012 until May 2013, serum and CSF of 5 patients seen by the authors with relapsing post-HSE were studied at the Hospital Clinic and August Pi i Sunyer Biomedical Research Institute (IDIBAPS), University of Barcelona. In addition, 34 patients with definite or probable HSE were included to determine the frequency of neuronal antibodies after HSE (Supplementary Table 1). From these 34 patients, archived serum and/or CSF obtained 1 to 88 days after HSE were available for study. Information was retrospectively provided by investigators of the California Encephalitis Project (Supplementary Methods, Identification of Patients). All patients were examined for antibodies to cell-surface/synaptic antigens (see Supplementary Methods, List of antibodies tested in all

patients).[9–12] The appearance of antibodies over time was assessed using the Mann–Whitney U test (SPSS version 20; IBM, Armonk, NY). Studies were approved by the internal review board of Hospital Clinic-IDIBAPS. Partial data on 2 patients (Cases #2 and #5) were previously reported[4,6]; in this report, we demonstrate NMDAR antibody seroconversion after HSE of Case #2.

Results

Prospective Cases

The 5 patients prospectively identified included 4 children (median age = 7 months, range = 2–28; 2 female) and a 24-year-old man (Table 1). Detailed information and videos are provided in the Supplementary Material; the timing of initial antibody synthesis and symptoms is outlined in Figure 1. Overall, relapsing or new neurological symptoms started 7 to 41 days (median = 24) after onset of HSE. In 4 patients, the symptoms occurred after having improved from HSE, and in case #1 the symptoms (choreiclike movements) developed in contiguity 7 days after hospital admission for HSE. In the 4 children, choreoathetosis was the most prominent finding (Supplementary Video); other symptoms included irritability, sleep disorder, and unresponsiveness. The adult patient developed abnormal behavior and personality change. CSF showed pleocytosis in 4 of 5 patients, with a white blood cell count (WBC) similar to that of the viral phase (HSE: median = 49 WBC/μl, range = 6–120; relapse: median = 69 WBC/μl, range = 10–153). Brain magnetic resonance imaging (MRI) did not show new T2/fluid-attenuated inversion recovery (FLAIR) signal abnormalities in 3 patients; Patient #5 had a mild increase of size of a frontotemporal T2/FLAIR abnormality without new necrotic lesions, and 1 patient did not have follow-up MRI.

All patients were treated for relapsing symptoms with a second 15- to 21-day course of intravenous acyclovir, and 4 received immunotherapy (see Table). Immunotherapy was not used in Patient #1, who improved spontaneously (see Supplementary Video). Within 4 weeks of relapsing symptoms, only Patient #5 improved with steroids; the other 3 did not respond to steroids and intravenous (IV) Ig and were subsequently treated with rituximab (375mg/kg, weekly, 4 weeks) and cyclophosphamide (monthly IV pulses, first dose = 500mg/m^2, second and subsequent doses = 750mg/m^2). At the last follow-up, 2 patients had returned to the prerelapse level (Case #2, 24-month follow-up; Case #3, 7-month follow-up), and the patient with the shortest follow-up (Case #4, 4-month follow-up) had started to improve.

Serum and CSF samples of all 5 patients were available from the time of symptom relapse, and in 4 from the time of HSE. None of the patients had NMDAR antibodies during HSE, but all had high antibody titers, both in CSF and in serum, 3 to 5 weeks later by the time of relapsing symptoms (see Fig 1). All 5 patients had IgG and IgM NMDAR antibodies, and 2 had mild IgA antibody reactivity (Supplementary Table 2). All patients' serum/CSF reacted with the GluN1 subunit of the NMDAR, but not with the subunit mutated at amino acid 368, as reported in typical anti-NMDAR encephalitis (see Supplementary Material).[13] Antibodies to the other indicated antigens were not identified.

Retrospective Cases

Among the 34 patients with HSE whose archived serum/CSF samples were retrospectively studied, 17 had the samples obtained during the first week of the infection, 10 afterward, and 7 during the first week and afterward (see Supplementary Table 1). Twelve of 34 patients had antibodies against neuronal cell-surface antigens, 2 of them against NMDAR, 9 against unknown antigens, and 1 against both. In the 2 patients with only NMDAR antibodies (Cases #6 and #7 in Supplementary Table 3, and vignette, Case #7), the serum/CSF samples had been obtained after the first week of HSE (74 and 61 days). In case #8, CSF from the first week of HSE was available and showed extensive neuropil/cell-surface staining without NMDAR antibodies, suggesting an early presence of antibodies to uncharacterized antigens (Fig 2); however, NMDAR antibodies were identified on day 42, indicating seroconversion after the first week. For all 3 patients, the antibody isotype was IgG (2 also had IgM) against the GluN1 subunit of the receptor (see Fig 2, Supplementary Table 2). In these 3 patients with NMDAR antibodies, the reason for serum/CSF analysis was "new onset or worsening neurological symptoms." The antibodies of the other 9 patients (4 detected during the first week of HSE and 5 afterward) were directed against cell-surface antigens of unknown identity (1 representative case is shown in Fig 2).

Cell-surface autoantibodies were identified in 1 of 17 sera and 5 of 22 CSF samples during the first week of HSE and in 5 of 13 sera and 7 of 12 CSF samples afterward (see Fig 2D). The frequency of autoantibodies increased over time in both serum and CSF (serum, $p = 0.004$; CSF, $p = 0.04$; Supplementary Fig), suggesting that HSE triggers brain autoimmunity.

Discussion

This study shows that relapsing post-HSE is often anti-NMDAR encephalitis, that this immune response underlies different complications (eg, choreoathetosis in children, abnormal behavior in adults), which may occur in

TABLE 1. Clinical Features of Patients Prospectively Identified with Neurological Relapse after HSE

Patient No.	Age, Sex	HSV-1 Encephalitis						Relapse								Outcome (follow-up after HSE onset)
		Symptoms	CSF	MRI T2 Lesions	HSV PCR	Treatment	NMDAR Antibodies	Time to Relapse, Days	Symptoms	CSF	MRI: New T2 Lesions	HSV PCR	NMDAR Antibodies	Intrathecal Synthesis NMDAR Antibodies[a]	Treatment	
1	2 months, M	Fever, focal seizures	WBC 77, prot. 78	Extensive bilateral occipital and right temporal	+	Acyc	–	7	Choreoathetosis, irritability, sleep disorder	WBC 120, prot. 249	n/a	–	+	75.41	Acyc	Day 180: improved; deficits in visual tracking
2[b]	28 months, F	Fever, irritability, focal seizures, dysphagia, dysarthria	WBC 18, prot. 25	Extensive bilateral temporal	+	Acyc	–	23	Fever, diarrhea, agitation, insomnia, choreoathetosis	WBC <5, prot. <45	–	–	+	18.85	Acyc, IVMP, IVIg, Ritux, CycP	Two years; improved; normal exam, residual bio-percular syndrome
3	6 months, F	Fever, diarrhea, focal seizures; residual right hemiparesis	WBC 10, prot. 38	Extensive bilateral temporal	+	Acyc	–	30	Fever, diarrhea, irritability, insomnia, choreoathetosis, unresponsiveness	WBC 6, prot. 45	–	–	+	4.71	Acyc, IVMP, IVIg, Ritux, CycP	Day 210: partial improvement; no chorea, residual dysphagia and hemiparesis
4	8 months, M	Fever, irritability, focal seizures	WBC 85, prot. 36	Extensive bilateral fronto temporal	+	Acyc	n/a	24	Irritability, unresponsiveness, seizures, choreoathetosis	WBC 74, prot. 82, OCB	–	–	+	18.85	Acyc, IVMP, IVIg, Ritux, CycP	Day 120: slight improvement
5[b]	24 years, M	Confusion, delusions, coma; residual memory impairment	WBC 153, prot. <45, OCB neg	Extensive bilateral temporal, insular	+	Acyc	–	41	Progressive mania, irritability, disorientation, memory dysfunction	WBC 24, prot. 86	+ (adjacent to prior lesions)	–	+	34.46	Acyc, IVMP	Day 119: improved; residual memory impairment

[a]Calculated according to Reiber and Peter[20]; normal value ≤ 2.4.
[b]Partial data previously published.[4,6]

Acyc = acyclovir; CSF = cerebrospinal fluid; CycP = monthly intravenous cyclophosphamide; F = female; HSE = herpes simplex virus encephalitis; HSV = herpes simplex virus; IVIg = intravenous immunoglobulins; IVMP = intravenous methylprednisolone; M = male; MRI = magnetic resonance imaging; n/a = not available/not done; neg = negative; NMDAR = N-methyl-D-aspartate receptor; OCB = oligoclonal bands; PCR = polymerase chain reaction; prot. = CSF total protein in mg/dl; Ritux = rituximab; WBC = white blood cells/μl in CSF.
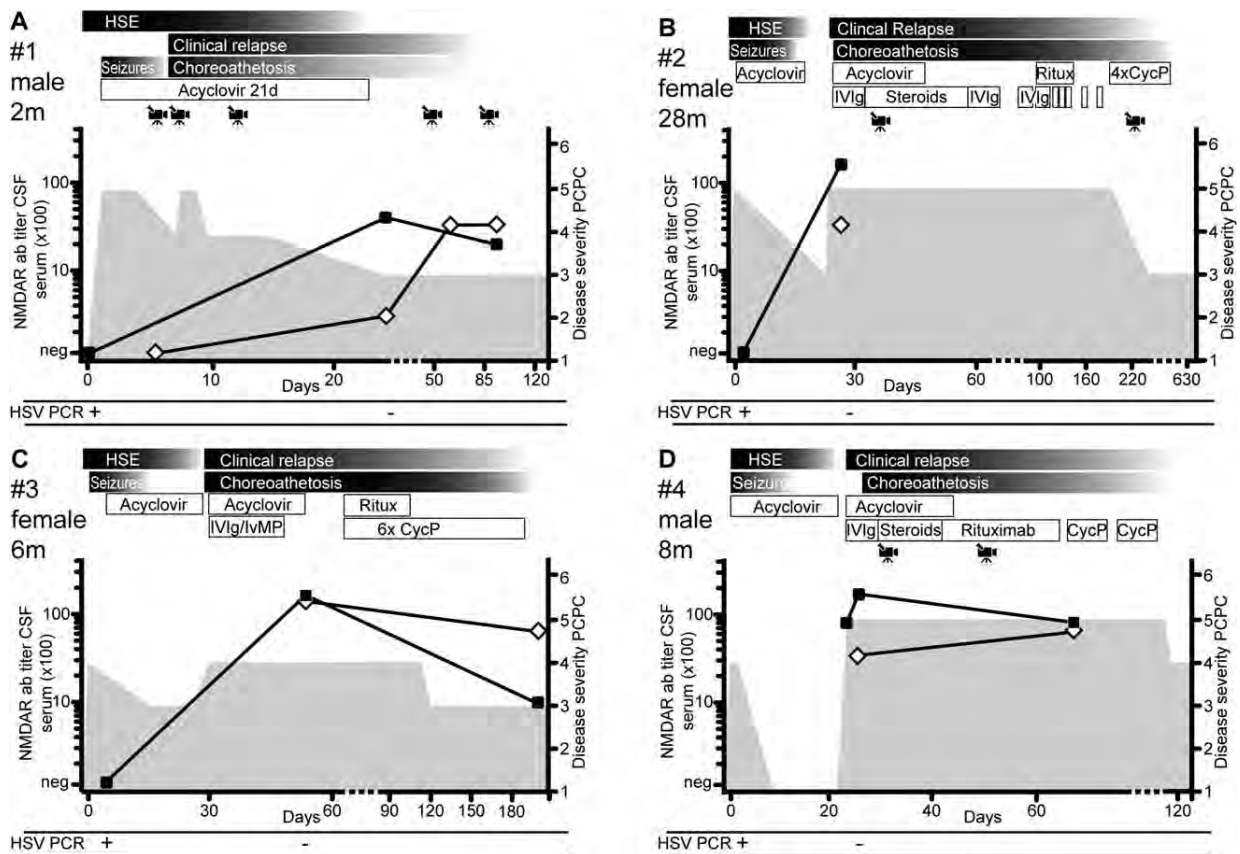
FIGURE 1: Clinical course, treatment, and cerebrospinal fluid (CSF)/serum N-methyl-D-aspartate receptor (NMDAR) antibody (ab) titers in 4 patients with neurological relapses after herpes simplex virus (HSV) encephalitis (HSE). Scaling of x-axes is different in all patients, reflecting length of follow-up. Broken x-axis represents discontinuous axis and change of tick interval. Right y-axis and gray curve represent quantitative measure of disease severity. PCPC = Pediatric Cerebral Performance Category[19] (1 = normal, 2 = mild disability, 3 = moderate disability, 4 = severe disability, 5 = coma or vegetative state, 6 = dead). Left y-axis: filled boxes represent NMDAR ab titer in CSF, open diamonds represent NMDAR ab titer in serum (multiplied × 100 to fit the same axis as CSF). Camera symbol represents available video segments in the Supplementary Video. CycP = cyclophosphamide; IVIg = intravenous immunoglobulins; IvMP = intravenous methylprednisolone; PCR = polymerase chain reaction; Ritux = rituximab; Steroids = IvMP followed by oral taper of steroids.

contiguity or a few weeks after HSE, and that HSE is a trigger of cell-surface/synaptic autoimmunity.

The involvement of immune mechanisms in HSE has been previously suggested by the observation that immunocompetent patients have a more severe disease course than immunocompromised patients,[14] the beneficial effect of combining steroids with acyclovir,[15,16] and the recent identification of NMDAR antibodies in some patients with HSE.[5] We report here 8 patients with relapsing post-HSE related to NMDAR antibodies, 5 of them with serum/CSF samples available from both the episode of HSE and the episode of neurological relapse. In these 5 patients (4 prospectively studied), the synthesis of NMDAR antibodies started shortly after HSE, preceding the neurological relapse, providing a robust link between these entities. Altogether, 4 of 8 patients were children, and all developed choreoathetosis. In contrast, behavior,

personality, and memory deficits were the main symptoms in adults, with only 1 (Case #7) presenting abnormal movements. These differences regarding symptom presentation according to age are in line with those reported in non–HSE-related anti-NMDAR encephalitis.[4,17]

Patient #1 (2 months old), the youngest patient we know with NMDAR antibody-associated encephalitis, developed choreiclike movements 7 days after diagnosis of HSE. The awareness of 1 of the authors of choreoathetosis post-HSE led to antibody testing on day 1 of admission (without detection of NMDAR antibodies), and on day 24, which demonstrated NMDAR antibodies in serum and CSF. Subsequently, the patient improved spontaneously, with a decrease of antibodies in CSF. The seroconversion demonstrated in this patient and in the other 4 prospectively identified cases, together with the detection of antibodies to cell-surface antigens in more than half of the patients tested after
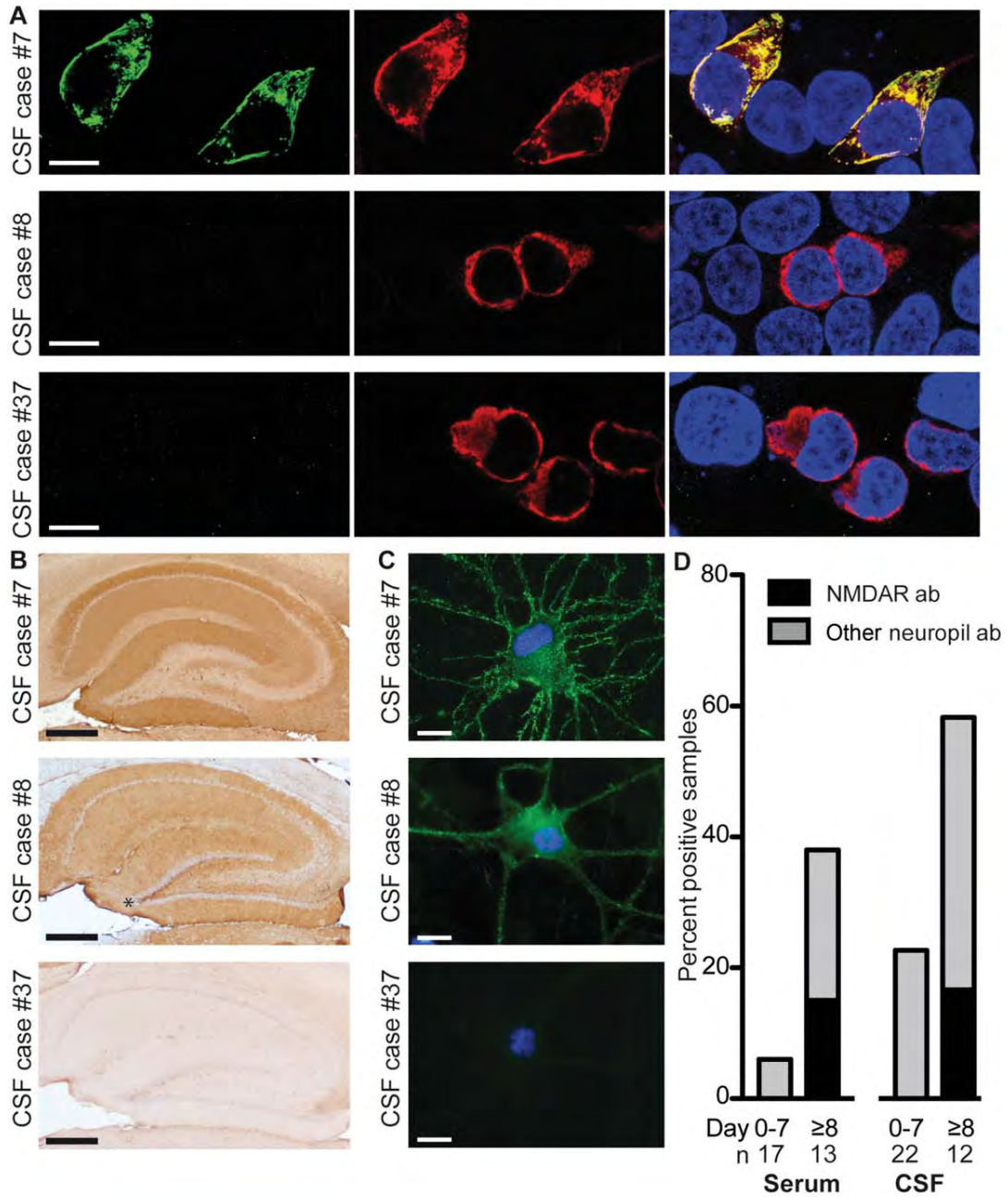
FIGURE 2: Determination and frequency of antibodies to N-methyl-D-aspartate receptor (NMDAR) and uncharacterized cell-surface antigens in a retrospective cohort of patients with herpes simplex virus encephalitis (HSE). (A) HEK cells expressing GluN1/GluN2 subunits of the NMDAR incubated with cerebrospinal fluid (CSF) of the indicated patients (left column, green fluorescence), and a monoclonal antibody to GluN1 (middle column, red); the merged immunostaining is shown in the right column. Nuclei of neurons are demonstrated with DAPI. Scale bars = 10μm. Note that only Patient #7 had antibodies to NMDAR (a similar staining was obtained with cells transfected only with GluN1, not shown). (B, C) Reactivity of the CSF of the same patients with sagittal sections of rat brain (B), and live rat hippocampal neurons (C). The CSF of Patient #7 shows a typical pattern of NMDAR reactivity with the neuropil of hippocampus as well as with the cell-surface of neurons; the CSF of Patient #8 shows reactivity with a neuropil antigen expressed on the cell-surface of neurons (the identity of the antigen is unknown), and the CSF of Patient #37 was negative in both tests. Scale bars in B = 500μm; scale bars in C = 10μm. (D) Percentage of patients' serum and CSF samples harboring immunoglobulin G antibodies (ab) to NMDAR (black) or to other neuronal cell-surface antigens (gray) during and after the first week of HSE; the identity of other neuronal antigens was unknown. Frequency increased over time (serum, $p = 0.004$; CSF, $p = 0.04$; Mann–Whitney U test).

1 week of HSE, suggest that this infection often triggers brain autoimmunity, which may fade spontaneously (eg, Case #1) or lead to progressive neurological symptoms.

Whether a mechanism of molecular mimicry or the release of antigens by viral neuronal lysis and inflammation leads to synaptic autoimmunity is unknown. We favor the second mechanism for 3 reasons. First, the broad immune response of some patients, mostly against unknown autoantigens, suggests release of multiple potential autoantigens. Second is the description of similar neurological complications in other viral encephalitis, such as Japanese B encephalitis, which may associate with a bimodal clinical course, including choreoathetosis and behavioral changes (to our knowledge, cell-surface autoimmunity has not been examined in these cases).[18] Third, the identification of other viruses such as Epstein–Barr or cytomegalovirus in the CSF of some patients with anti-NMDAR encephalitis further supports a nonspecific viral-induced immunological mechanism (unpublished observations).

Findings from this study have several practical implications: (1) patients with prolonged, worsening or relapsing symptoms after HSE should be tested for NMDAR and other antibodies to cell-surface/synaptic antigens; (2) identification of these immune responses is important, because NMDAR-related symptoms are potentially responsive to immunotherapy; and (3) immunotherapy appears to be safe in patients with relapsing post-HSE. Future studies should determine prospectively the frequency of brain autoimmunity in patients with HSE, identify those who progress to develop post-HSE symptoms, characterize the unknown antigens, and further assess the effects of immunotherapy.

Authorship

T.A. and F.L. contributed equally. T.A. and F.L. contributed to study design, collecting and analyzing data, and writing the manuscript. I.Mál., M.R.-C., I.Mar., C.N., J.P., M.V.-R., M.L.-H., A.M., and M.K. contributed to data acquisition and interpretation, and writing the manuscript. M.J.T. and R.H. contributed to data interpretation and writing the manuscript. H.S. contributed to data acquisition and interpretation, and writing the manuscript. C.G. contributed to study design, data acquisition and interpretation, and writing the manuscript. J.D. contributed to study design, collecting and analyzing data, and writing the manuscript.

Potential Conflicts of Interest

M.J.T.: fellowships, Erasmus MC, Dutch Cancer Society. J.D.: patents, use of NMDAR as an autoantibody test; grants/grants pending, EuroImmun research support.

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Supplementary material

<u>Clinical vignettes</u>

Case #1

A 2-month-old previously healthy boy presented with seizures and fever. CSF studies showed 77 white blood cells (WBC)/microliter (87% lymphocytes), elevated protein concentration (78mg/dL) and normal glucose concentration; PCR for herpes simplex virus -1 (HSV-1) was positive. EEG showed periodic lateralized discharges predominant in posterior regions, and the brain MRI showed bilateral T2/FLAIR increased signal in the occipital and temporal lobes (right >left). Intravenous acyclovir ($1500mg/m^2$ daily) was started, and he was admitted to Pediatric Intensive Care Unit for convulsive status epilepticus. Seizures were treated with midazolam, levetiracetam, phenobarbital and phenytoin, achieving good seizure control by day 6 (Video segment 1). Seven days after onset of HSE the patient developed generalized choreoathetosis with prominent orofacial dyskinesias (Video segment 2) without EEG correlate in serial video-EEG recordings, insomnia and periods of irritability alternating with somnolence. The choreoathetosis was severe and persisted for 24 hours, followed by a gradual improvement over the ensuing 60 days. On day 24 after onset of HSE, CSF studies showed 120 WBC/µl (96% lymphocytes), 1568 red blood cells/µl, protein of 249 mg/dL, and normal glucose concentration; HSV PCR was negative, and intravenous acyclovir treatment was stopped. At that point, NMDAR antibodies were detected in CSF (1:40, intrathecal synthesis 75.41) and serum (1:200), but not in CSF and serum obtained at onset of HSE (day 0 and day 5 respectively). Because of continuous neurological improvement, no immunotherapy was initiated. The patient was discharged from hospital on levetiracetam, and oral acyclovir ($300 \ mg/m^2$ daily). Abnormal movements and insomnia continued to improve (Video segment 3 and 4), and

completely resolved two months after HSE onset (Video segment 5). The CSF was normal, HSV-PCR negative, CSF titers decreased (1:20, intrathecal synthesis 2.36), serum NMDAR titers increased (1:3200). At the last follow up (four month after onset of HSE), the patient now 6 month, he is awake, attentive, and has residual deficits in visual tracking that have been attributed to viral-related occipital lesions (PCPC 3).

Case #2

See details on early disease course in Armangue et al. J Pediatr 2013.[1] At the last follow-up two years after onset of HSE, the patient is alert and able to interact at age-appropriate level, although she has residual dysphagia and dysphonia due to bilateral opercular necrotic lesions caused by the initial viral infection (PCPC 2). Retrospective study of archived serum obtained at the time of onset of HSE showed no NMDAR antibodies, at day 23 NMDAR antibodies were positive (serum 1/3200, CSF 1/160) demonstrating NMDAR seroconversion after HSE.

Case #3

A previously healthy 6-month-old girl presented with fever, diarrhea and focal seizures. CSF studies showed 10 WBC/μl (76% mononuclear), normal protein and glucose concentration, and positive HSV-1 PCR. Brain MRI showed bilateral increase in T2/FLAIR signal in the temporal lobes (right>left). She was treated with intravenous acyclovir for three weeks (1500mg/m2 daily). Phenobarbital was added because of recurrent seizures 15 days after symptom onset, and she continued to improve. CSF 21 days after symptom onset showed negative PCR for HSV-1 and acyclovir was stopped. One week later (one month after HSE onset), irritability, insomnia, oral dyskinesias and choreoathetosis commenced. Treatment with acyclovir ($1500mg/m^2$ daily) was

reinitiated, and the patient was admitted to the Pediatric Intensive Care Unit for a decreased gag reflex, hypoventilation, and episodes of bradycardia. Repeat brain MRI showed encephalomalacia in previously affected areas but no new T2/FLAIR abnormalities. CSF was normal and HSV PCR negative. NMDAR antibodies were found in the CSF but not in the CSF sample obtained at onset of HSE. Treatment with IVIg (0,4g/k/day for 5 days) and methylprednisolone (30mg/k/day for three days) was started. Additional medication included clobazam, valproate and tetrabenazine. No improvement occurred during the next month, she continued with prominent choreoathetosis, unresponsiveness, dysphagia and dysphonia leading to placement of a nasogastric tube and posterior gastrostomy tube. Fifty-two days after HSE, repeat serum/CSF antibody titers showed persistently elevated titers (serum 1/12800, CSF 1/80, intrathecal synthesis 4.71). Screening for an underlying ovarian teratoma was negative. The patient developed a transient syndrome of inappropriate antidiuretic hormone secretion (SIADH). Fifty days after neurological relapse (80 days after onset of HSE), second line immunotherapy was started (rituximab 375 mg/m^2 weekly, 4 doses; cyclophosphamide monthly intravenous pulses, first dose: 500 mg/m^2, second-sixth dose 750 mg/m^2). Two months later (4 months after the onset of neurological relapse), improvement was noted. At the last follow-up 7 months after HSE, the antibody titers have decreased (serum 1/6400, CSF 1/10, intrathecal synthesis 0.59) and all symptoms related to the relapse post-HSE have resolved, but she has residual deficits related to HSE (hemiparesis, difficulty sitting and dysphagia, PCPC 3).

Case #4

A previously healthy 8-month-old boy presented with fever and focal seizures. CSF studies showed 85 WBC/µl (95% lymphocytes), normal protein and glucose

concentration, and the PCR for HSV-1 was positive. Brain MRI showed bilateral increase of T2/FLAIR signal in frontal and temporal lobes. He was treated with intravenous acyclovir ($1500mg/m^2$ daily) for three weeks, resulting in substantial recovery, and was discharged on day 21 without neurological symptoms. Twenty-three days after onset of HSE, he developed extreme irritability and insomnia, and treatment with acyclovir ($1500mg/m^2$ daily) was restarted. Two focal seizures were successfully treated with phenytoin, but he developed prominent generalized choreoathetosis. CSF analysis showed 74 WBC (95% lymphocytes), protein concentration of 85 mg/dL, normal glucose concentration and negative HSV-1 PCR. NMDAR antibodies were detected in CSF (1:160, intrathecal synthesis 18.85) and serum (1:3200). Repeat brain MRI showed residual encephalomalacia from the viral infection but no new T2/FLAIR lesions. Screening for an underlying testicular teratoma was negative. Treatment with IVIg (0,4gr/kg/day for 5 days) and methylprednisolone (30mg/kg/day for three days) was started, followed by oral taper of methylprednisolone over 6 weeks. Symptoms did not improve (Video segment 1), and 40 days after onset of HSE, rituximab was started (375 mg/m^2 weekly, 4 doses). Repeat NMDAR antibody studies showed mild decrease of CSF titers (1:80, intrathecal synthesis 4.71), and mild increase of serum titers (1:6400), but without significant change of the neurological status. At this time symptoms included, prominent choreoathetosis, unresponsiveness, dysphagia and dysarthria (Video segment 2). On day 70 after HSE, monthly intravenous pulses of cyclophosphamide (1st dose: 500 mg/m^2, second dose: 750 mg/m^2) were started. He developed pneumonia by *Pneumocystis carinii* requiring one week of mechanical ventilation. Immunosuppression was temporarily discontinued. At the last follow-up 120 days after HSE, the patient has started to improve (more alert, less irritable, PCP4), but still has choreoathetosis and dysphagia.

Case #7

A 41 year-old man presented with confusion and fever. An MRI showed extensive T2/FLAIR abnormalities bilaterally involving temporal, opercular, gyrus rectus and insular regions, with increased signal on diffusion weighted images (DWI), and contrast enhancement. A spinal tap was not performed for concern of increased intracranial pressure. He was treated with intravenous acyclovir for a total of 14 days. Repeat brain MRI nine days after symptom onset showed improvement, he returned to baseline and was discharged without residual symptoms 18 days after onset. Forty-nine days after onset of HSE, he developed confusion, social inappropriate behavior, perseveration, complains of generalized weakness, and double vision. CSF showed 25 WBC/ml (lymphocytic predominance), protein of 65 mg/dL, and normal glucose. Repeat MRI did not show new T2/FLAIR lesions; EEG showed right hemispheric slowing. CSF HSV-PCR performed 61 days after onset of HSE was negative; he had elevated HSV-specific IgG antibody index (4.86) indicating intrathecal antibody synthesis; HSV-1 IgM was negative. NMDAR antibodies were identified in both, CSF (1:640) and serum (1:3200), intrathecal synthesis 40.32.

He was treated with intravenous acyclovir (600mg/kg iv Q 8 hours) for 21 days. A modest improvement of mental status was noted; however, he had persistent rhythmic movements with the right arm, left face twitching and grimacing, and abnormal tongue movements without EEG correlate. He was treated with phenytoin, levetiracetam, lorazepam, valproate, lacosamide, and clonazepam without clear effect on the abnormal movements. He developed hypoventilation, fever, tachycardia, confusion, agitation, sexual disinhibition, aggressive behavior and sleep dysfunction. Over the next five months, neurological symptoms slowly improved without immunotherapy. He was

discharged 9 months after onset of HSE; at the last follow-up three months later (one year after HSE) he was fully recovered (mRS 0).

Supplementary video legend:

Case #1: Segment 1 (day 5 of HSE): 2-months old boy sleeping on video EEG register without abnormal movements. Segment 2 (day 7 of HSE): Two days later, prominent choreoathetosis with orofacial dyskinesias and tongue movements are noted for first time. Segment 3 (day 12 of HSE): Initial improvement of abnormal movements and level of consciousness is observed. Segment 4, (day 48 of HSE): Mild choreoathetosis is still present but the patient is awake and responsive. Segment 5 (day 88 of HSE): Choreoathetosis has resolved; psychomotor development is appropriate for age except for deficits in visual tracking that have been attributed to viral-related occipital lesions

Case #2: Segment 1 (day 38 after onset HSE): 28-months old girl shows prominent generalized choreoathetosis and unresponsiveness, which developed 21 days after onset of HSE. Segment 2 (1 year after HSE): Choreoathetosis has notably improved, residual dysphagia and dysarthria remain but otherwise development is appropriate for age. The patient was treated with steroids, IVIg, rituximab and intravenous cyclophosphamide.

Case #4: Segment 1 and 2 (38 and 55 days after HSE): 8-months old boy shows prominent choreoathetosis, orofacial and tongue dyskinesias and unresponsiveness 38 and 55 days after HSE. He is undergoing treatment with immunotherapy and at 90 days after HSE is still severely impaired.

Supplementary methods

Identification of patients

All five prospectively studied patients were diagnosed after June 2012 and followed by at least one of the authors. The identification of these patients was due in part to the increased awareness raised by the diagnosis of a patient with relapsing symptoms post-HSE who had NMDAR antibodies,[1] and a report suggesting that some patients with HSE develop NMDAR antibodies.[2] Since those publications, the number of consultations has steadily increased; we report here the initial 5 patients from whom extensive clinical and immunological information is available, including follow-up with video clips. In addition, we examined available serum and CSF samples of 34 patients with HSE provided by the California Encephalitis Project. Samples were obtained 1-88 days after HSE over a 6-year period as previously described.[3]

Criteria of definitive or probable HSE

Criteria of definite HSE included development of a typical clinical picture, presence of temporal lobe lesions on MRI, CSF pleocytosis, and positive CSF HSV-PCR. Probable HSE was defined by the same clinical criteria and negative HSV-PCR, but high intrathecal synthesis of HSV antibodies; or typical clinical features and positive HSV-PCR in CSF, but no temporal lobe abnormalities in the MRI.

Clinical scales

Pediatric patients were scored using the Pediatric Cerebral Performance Category scale[4] (PCPC, 1=normal, 2=mild disability, 3=moderate disability, 4=severe disability, 5= coma or vegetative state, 6= death). Adult patients were scored using the modified Rankin Scale.[5]

List of antibodies tested in all patients:

Cell surface/synaptic antigens: NMDA receptor, AMPA receptor, GABA(B)-receptor, mGluR5, dopamine-2 receptor, LGI1, Caspr2, and DPPX proteins.

Intracellular antigens: Hu, CRMP5, Ma1-2, amphiphysin, GAD65.

Tissue based antibody screening

Serum and CSF samples were tested for NMDAR-antibodies following previously reported methods unless indicated otherwise.[6] Briefly, non-perfused rat brains were removed, split sagittally, and fixed in 4% paraformaldehyde (PFA) for 1h, cryoprotected with 40% sucrose for 24h, and snap frozen in chilled isopentane. Seven micron-thick sections were then incubated with 0.3% hydrogen peroxide for 20 minutes, with 10% goat serum in PBS for 1h, and then labeled with patient's or control sample (serial dilutions starting for serum: 1:200; and CSF: 1:2) at 4°C overnight. The next day, sections were incubated with the appropriate secondary antibody for 1h at room temperature and visualized with an avidin-biotin-peroxidase method. To determine IgG subtypes, biotinylated secondary goat anti-human IgG or IgA antibodies at dilution 1:2000 were used (Vector Labs, Burlingame, CA, USA); for IgM antibodies, goat anti-human IgM at dilution 1:1000 was used (Southern Biotechnology, Birmingham, AL, USA).

Cell-based assays

The CBA for NMDAR was performed as described previously.[6] In brief, HEK293 cells were transfected with the NR1 and NR2B subunits of the NMDAR. Twenty-four hours after transfection, cells were fixed in 4% PFA for 10 minutes, permeabilized with 0.3% Triton X-100 (Sigma-Aldrich, St Louis, MO, USA) and incubated with 1% bovine-

serum-albumin (BSA) for 1.5 hours. HEK cells were then incubated with patient's or control samples (serum: 1:40; CSF: 1:2) at 4°C overnight. The next day, cells were labeled with a mouse monoclonal NR1 antibody (1:20 000; Millipore, Billerica, MA, USA) for 1h at room temperature, followed by the corresponding Alexa Fluor 488 and 594 secondary antibodies against human IgG (γ), IgM (μ) and mouse IgGs (1:1000; Molecular Probes, Invitrogen, Eugene, OR, USA) or human IgA (α) (1:1000; Jackson ImmunoResearch, Pennsylvania, USA). CBAs for AMPA receptor,[7] GABA(B) receptor,[8] mGluR5,[9] dopamine-2 receptor,[10] LGI1,[11] Caspr2[12] and DPPX[13] were performed as previously described.

Epitope analysis was done with NR1 mutants as previously described.[14] In brief, G369I and G369S are single point mutations where glycine 369 was replaced by an isoleucine or serine, respectively. The "top lobe construct" carries a deletion of residues 26-140 and 275-349 in the top lobe of the amino-terminal domain (ATD). The ATD-TM4 construct keeps the ATD region and the transmembrane domain 4 (TM4), missing the in-between regions (S1, S2, and TM1-3) corresponding to residues 401-792. HEK293 cells were transfected with either wild-type NR1/N2B, the indicated NR1 mutants or NR2A, NR2C subunits and treated as described above. For transfection with ATD-TM4 construct, the commercial antibody used was a rabbit polyclonal antibody against amino acid 918-938 (dilution 1:2000; G8913, Sigma).

Embryonic rat hippocampal neurons were cultured and the reactivity of patients' antibodies with live neurons assessed as previously described.[6] In brief, 21 days in vitro rat hippocampal neurons were incubated with patients' CSF (1:5) or serum (1:200) at 37°C for one hour, washed in cold PBS, fixed and subsequently incubated with Alexa

Fluor 488 secondary antibody against human IgG (1:1000; Molecular Probes, Invitrogen, Eugene, OR, USA). Microscopy was done as previously described.[6]

Supplementary results

Epitope analysis of NMDAR antibodies in post-HSE

All samples from patients with post-HSE and NMDAR antibodies showed reactivity with NR1 subunit of the NMDAR, but not with NR2A, NR2B or NR2C. The reactivity with NR1 was abolished in all cases by the G369I mutation. Deletion of amino acids 26-140 and 275-349 of the NR1 subunit corresponding to the "top-lobe" of the ATD abolished reactivity in 6/8 and reduced reactivity in 2/8 post-HSE patients. These findings, indicating the presence of a major epitope in G369, and the variable effect of the top-lobe deletion are similar to those reported in classical anti-NMDAR encephalitis.[13] In summary, the epitope repertoire in patients with NMDAR antibodies post-HSE is similar to that found in anti-NMDAR encephalitis.

Supplementary Tables

Supplementary Table 1: Characteristics of a retrospective cohort of 34 patients with

HSE

Case #	AGE	GEN-DER	DEFINITE/PROBABLE HSE*	HSV TYPE	NEUROLOGICAL SYMPTOMS	MRI FLAIR/T2 CHANGES OF TEMPORAL LOBE	ONSET OF HSE TO CSF (days)	ANTIBODIES DETECTED[¶] IN CSF	ONSET OF HSE TO SERUM (days)	ANTIBODIES DETECTED[¶] IN SERUM
6	45	F	DEF	1	H,S,W	+	74	NMDAR (IgG, IgM)	n/a	n/a
7	41	M	PROB	1	L	+	61	NMDAR (IgG, IgM)	61	NMDAR (IgG, IgM)
8	82	M	DEF	1	H,L,Fo,S,W,LOC	+	4	Neuronal surface	42	NMDAR and Additional neuronal surface
9	74	M	DEF	1	L,I,S,W,MoD	+	1	-	1	-
10	85	F	DEF	1	n/a	+	1	Neuronal surface (IgG, IgM)	1	-
11	13	M	DEF	1	L,A	+	1	-	2	-
12	60	F	DEF	1	n/a	+	1	-	1	-
13	63	F	DEF	1	Fo,S,C	+	2	-	2	-
14	85	M	DEF	1	L,S	+	2	-	14	-
15	86	F	DEF	1	L,I,LOC	n/a, CT normal	2	-	2	-
16	53	F	DEF	1	H,S	+	3	-	n/a	n/a
17	58	F	PROB	1	H,S	+	3	-	3	-
18	0	M	DEF	1	Fo,S	+/multi-focal	3	-	3	-
19	66	M	PROB	1	H,L,A,Fo,C,W	+	4	Neuronal surface	13	-
20	3	M	DEF	1	L,S	n/a	4	-	4	-
21	25	M	DEF	1	H,S	+	4	-	4	-
22	0	F	DEF	1	L,Fo,S	+	4	-	4	-
23	46	F	PROB	1	Fo	+	4	-	5	-
24	17	M	DEF	1	H,L	+	5	-	5	-
25	14	F	DEF	1	H,L,I,A	+	5	-	n/a	n/a
26	87	M	DEF	1	L,I,W,Aph	+	5	Neuronal surface	6	Neuronal surface
27	1	F	DEF	1	L,Fo,S,LOC	+/multi-focal	5	-	18	-
28	55	M	PROB	1	H,L,I,A,S	+	6	Neuronal surface	24	-
29	56	F	PROB	1	L,W,My	n/a, CT normal	7	-	7	-
30	1	F	DEF	1	L,I	+	10	Neuronal surface (gG, IgM)	26	Neuronal surface

31	0	M	DEF	1	L,I,S,W	+	12	Neuronal surface (IgG, IgM)	n/a	n/a
32	32	M	PROB	1	A,Fo	+	13	-	3	-
33	54	M	PROB	1	L,I,Fo,S,C,W	+	17	-	31	-
34	85	F	PROB	1	L,I,Fo,S	+	18	Neuronal surface	20	Neuronal surface
35	7	M	DEF	1	L,Fo,S,W	+	20	Neuronal surface	16	-
36	11	F	DEF	1	L,Fo,W,LOC	+	20	-	19	-
37	1	M	DEF	1	L,I,A,S,LOC	-	22	-	3	-
38	66	M	DEF	1	L	+	22	Neuronal surface	23	Neuronal surface
39	20	F	PROB	1	L,Fo,S,W	+	88	-	88	-

* DEF: definite HSE, PROB: probable HSE; ¶only IgG unless indicated otherwise; A: ataxia, F: female, Fo: focal neurologic signs, H: headache, I: irritability/confusion, L: lethargy, LOC: reduced level of consciousness, M: male, Men: meningitis, MoD: movement disorder, My: myoclonus, n/a: not available, Phot: photophobia, S: seizures, W: weakness.

Supplementary Table 2: IgG NMDAR antibody titers, intrathecal synthesis of IgG NMDAR antibodies, and presence or absence of IgM and IgA antibodies

CASE #	ONSET OF HSE TO SAMPLE (DAYS)	SAMPLE TYPE	IgG* NMDAR ANTIBODY TITERS	INTRATHECAL IgG NMDAR ANTIBODY SYNTHESIS§	IgM** NMDAR ANTIBODIES	IgA** NMDAR ANTIBODIES
1	24	Serum	1:200	75.41	negative	negative
	24	CSF	1:40		positive	negative
2	23	Serum	1:3200	18.85	positive	negative
	23	CSF	1:160		positive	negative
3	52	Serum	1:12800	4.71	positive	negative
	52	CSF	1:80		positive	negative
4	23	Serum	1:3200	18.85	positive	negative
	23	CSF	1:160		positive	positive
5	45	Serum	1:800	34.46	positive	negative
	45	CSF	1:160		positive	positive
6	n/a	Serum	n/a	n/a	n/a	n/a
	74	CSF	1:40		positive	negative
7	61	Serum	1:3200	40.32	positive	negative
	61	CSF	1:640		positive	negative
8	42	Serum	1:320	n/a	negative	negative
	4	CSF	negative		negative	negative

CSF: cerebrospinal fluid, NMDAR: N-methyl-D-aspartate receptor, n/a: not available. [§]calculated according to[15] normal value ≤2.4. *IgG NMDAR antibody titers were calculated using serial dilutions of CSF or serum on immunohistochemistry with rat brain sections as reported,[6] except in case #8 that due to concomitant reactivity against other unknown neuronal surface antigens NMDAR antibody titers were calculated with cell based-assays. **IgM and IgA NMDAR antibodies were determined using cell-based assay (serum 1:40, CSF 1:2) and with immunohistochemistry on rat brain sections (serum 1:200, CSF 1:2).

Supplementary Table 3: Clinical characteristics of retrospectively identified HSE cases with NMDAR antibodies

		HSV-1 ENCEPHALITIS						RELAPSE							
#	AGE SEX	SYMPTOMS	CSF	MRI LESIONS	HSV-PCR	TREAT-MENT	NMDAR ANTI-BODIES	TIME TO RELAPSE (DAYS)	SYMPTOMS	CSF	MRI: NEW T2 LESIONS	HSV-PCR	NMDAR ANTI-BODIES	TREAT-MENT	OUTCOME (Time after HSE onset)
6	45 y, f	Fever, headache, confusion, psychosis, seizures.	WBC 327 prot. 91	bilateral temporal	+	Acyc	n/a	38	Memory dysfunction, personality changes	n/a	n/a	n/a	+	n/a	residual complex partial seizures
7	41 y, m	Mental status change, lethargy, confusion	WBC 25 prot. 65	bilateral temporal	-§	Acyc	n/a	49	Confusion, choreic-like movements/seizures, autonomic and respiratory dysfunction	WBC 25 prot. 65	-	-	+	Acyc	9 months hospitalization 12 months: complete recovery
8	82 y, m	Fever, headache, seizures, aphasia, confusion, coma, Bell's palsy, hemiparesis	WBC 98 prot. 137	bilateral temporal	+	Acyc	-	120	Cognitive decline	missing	missing	n/a	+	n/a	n/a

§ high intrathecal HSV IgG. Acyc: acyclovir, CSF: cerebrospinal fluid, HSV: Herpes simplex virus, HSE: Herpes virus simplex encephalitis, f: female, m: male, NMDAR: N-methyl-d-aspartate receptor, n/a: not available/not done, PCR: polymerase chain reaction, prot.: CSF total protein in mg/dL, WBC: white blood cells/µl, y: years

Supplementary Figure 1: Antibody positivity related to the time of onset of HSE

Time from onset of HSE (in days) comparing patients with and without neuronal surface antibodies in serum (A) and CSF (B). The vertical lines depict the median. Note that antibodies are mainly detected in samples obtained after 1 week from onset of HSE (black symbols). Grey symbols represent samples obtained during the first week of disease.

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Article V: Autoimmune relapses post-herpes simplex encephalitis in teenagers and adults.

Autors: Armangue T, Moris G, Cantarín-Extremera V, Conde CE, Rostasy K, Erro ME, Portilla-Cuenca JC, Turón-Viñas E, Málaga I, Cabello-Muñoz B, Torres-Torres C, Llufriu S, González-Gutiérrez-Solana L, González G, Casado-Naranjo I, Rosenfeld M, Graus F, Dalmau J.

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MS TITLE: Autoimmune Post-herpes Simplex Encephalitis of

Adults and Teenagers

Thaís Armangue, Germán Morís, Verónica Cantarín-Extremera, Carlos Enrique Conde,

Kevin Rostasy, Maria Elena Erro, Juan Carlos Portilla-Cuenca, Eulàlia Turón-Viñas,

Ignacio Málaga, Beatriz Muñoz-Cabello, Maria Carmen Torres-Torres, Sara Llufriu,

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Autoimmune Post-herpes Simplex Encephalitis of

Adults and Teenagers

Thaís Armangue, MD[1]; Germán Moris, MD[2]; Verónica Cantarín-Extremera, MD[3]; Carlos Enrique Conde, MD[4]; Kevin Rostasy, MD, PhD;[5] Maria Elena Erro;[6] Juan Carlos Portilla-Cuenca, MD,[7] Eulàlia Turón-Viñas, MD;[8] Ignacio Málaga, MD, PhD;[9] Beatriz Cabello-Muñoz, MD;[10] Carmen Torres-Torres, MD;[11] Sara Llufriu, MD, PhD;[1] Luis González-Gutiérrez-Solana, MD, PhD,[3] Guillermo González, MD;[4] Ignacio Casado-Naranjo, MD,[6] Myrna Rosenfeld, MD, PhD;[1] Francesc Graus, MD, PhD;[1] Josep Dalmau, MD, PhD[1,12,13]

on behalf of the Spanish prospective multicentric study of Autoimmunity in Herpes Simplex Encephalitis

Affiliations: [1]Neuroimmunology Program, August Pi Sunyer Biomedical Research Institute (IDIBAPS); Hospital Clínic, University of Barcelona, Barcelona (Spain); [2]Department of Neurology, Hospital Universitario Central de Asturias, Oviedo (Spain); [3]Department of Pediatric Neurology, Hospital Universitario Niño Jesús, Madrid (Spain); [4]Department of Neurology, Hospital Universitario Hernando Moncaleano Perdomo, Neiva (Colombia); [5]Department of Pediatric Neurology, Children's Hospital Datteln, Witten/Herdecke University, Witten (Germany); [6]Department of Neurology, Complejo Hospitalario de Navarra, Pamplona (Spain); [7]Department of Neurology, Hospital San Pedro de Alcántara, Cáceres (Spain); [8]Pediatric Neurology Unit, Hospital de la Santa Creu i Sant Pau, Barcelona (Spain); [9]Pediatric Neurology Unit, Pediatrics Department, Hospital Universitario Central de Asturias, Oviedo (Spain); [10]Pediatric Neurology Unit, Hospital Universitario Virgen del Rocio, Sevilla (Spain); [11]Department of Pediatric Neurology, Hospital General La Mancha Centro, Alcázar de San Juan (Spain); [12]Department of Neurology, University of Pennsylvania, Philadelphia (USA); [13]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona (Spain)

Corresponding author: Josep Dalmau, MD, PhD, Service of Neurology, IDIBAPS-Hospital Clínic, University of Barcelona, Casanova, 143; Floor 3,Department A, Barcelona 08036 (Spain), [Josep.dalmau@uphs.upenn.edu] Phone: +34 932 271 738 Fax: +34 932271726

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Abstract

Objective: To report 14 patients with immune-mediated relapsing symptoms post-herpes simplex encephalitis (HSE) and to compare the clinical and immunological, features of the teenager and adult group with those of young children.

Methods: Prospective observational study of patients diagnosed between June 2013 and February 2015. Immunological techniques have been previously reported.

Results: Among the teenager and adult group (8 patients, median age 40 years, range 13-69; 5 male), 3 had an acute symptom presentation suggesting a viral relapse, and 5 a presentation contiguous with HSE suggesting a recrudescence of previous deficits. Seven patients developed severe psychiatric/behavioral symptoms disrupting all social interactions, and one refractory status epilepticus. Blepharospasm occurred in one patient. Five patients had CSF antibodies against NMDAR and three against unknown neuronal cell-surface proteins. In 5/6 patients the brain MRI showed new areas of contrast-enhancement that decreased after immunotherapy and clinical improvement. Immunotherapy was useful in 7/7 patients, sometimes with impressive recoveries, returning to their baseline HSE-residual deficits. Compared with the 6 younger children (median age 13 months, range 6-20, all with NMDAR-antibodies), the teenagers and adults were less likely to develop choreoathetosis (0/8 vs 6/6, p<0.01) and decreased level of consciousness (2/8 vs 6/6, p<0.01) and had longer delays in diagnosis and treatment (interval relapse-antibody testing 85 days, range 17-296 vs 4 days, range 0-33, p=0.037).

Conclusion: In teenagers and adults the immune-mediated relapsing syndrome post-HSE is different from that known in young children as "choreoathetosis post-HSE" and is under-recognized. Prompt diagnosis is important because immunotherapy can be highly effective.

Introduction

Herpes simplex-virus encephalitis (HSE) is a frequent cause of severe, potentially fatal encephalitis among children and adults worldwide. The disease usually follows a monophasic course but 12-27% of the patients develop relapsing neurological symptoms a few weeks after the CSF viral studies become negative and the treatment with acyclovir has been discontinued.[1-3] Most of these patients are children who develop an encephalopathy with abnormal movements named "choreoathetosis post-HSE" or "relapsing symptoms post-HSE".[4] The hypothesis that the disorder is immune mediated has received strong support by the recent discovery that many of these patients develop IgG antibodies against the GluN1 subunit of the N-methyl-d-aspartate receptor (NMDAR)[5-11] and sometimes to other known[7] or unknown synaptic proteins.[6] This clinical complication is less well known in adults and teenagers, suggesting a lower frequency in these age groups or a different and less recognizable syndrome. Over the last 21 months we have prospectively identified 14 new patients with relapsing symptoms post-HSE, 8 of them adults or teenagers. In the current study we show that the clinical picture of these patients is indeed different from that of young children with choreoathetosis leading to delays in the diagnosis and treatment. Prompt recognition of this disorder is important because immunotherapy is effective in reducing the burden of the immune-mediated deficits and improving the quality of life of patients and families.

Patients and Methods

From June 2013 until February 2015, serum and CSF of 14 patients with non-viral relapsing symptoms post-HSE were prospectively studied at the Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), Hospital Clinic, University of Barcelona. Five of the patients (#3, 5, 8-10) were examined as part of a 2-year multicenter prospective Spanish study in which all patients with HSE are clinically and immunologically

followed after being diagnosed with HSE. The other 9 patients were diagnosed either before this multicenter HSE study was initiated (January 1st 2014, #1, 4, 12) and/or at centers that do not participate in the study (2 from Spain, 4 from other countries). In the prospective multicenter study, physicians are blinded of the immunological findings unless patients develop relapsing symptoms. Of 20 HSE patients (6 children and 14 adults) enrolled to date, the indicated 5 cases (25%) have developed relapsing neurological symptoms post-HSE.

CSF and/or serum of all patients were extensively examined for antibodies to cell-surface/synaptic proteins, including NMDA, mGluR5, AMPA, $GABA_B$, $GABA_A$, D2 receptors and LGI1, Caspr2, and DPPX proteins using previously reported techniques that included tissue immunohistochemistry, live cultured neurons, and cell-based-assays.[12-16] All patients underwent repeat CSF PCR for HSV, and MRI of the brain (9 with contrast and 5 without). Clinical information was obtained by the authors or from referring physicians. No data of any patient has been reported previously.

Standard protocol approvals, registrations, and patient consents:

Written informed consent for participating in the study was obtained from all patients or guardians of patients. Studies were approved by the internal review board of Hospital Clinic-IDIBAPS and the ethical standards committee on human experimentation of IDIBAPS.

Statistical analysis:

Comparative analyses between the group of teenagers and adults and the group of children were performed with STATA version 13.1 (StataCorp, Texas, USA), using Fisher-Exact, Chi2 Square Test, or Mann Whitney U test when appropriate. The

Wilcoxon rank-sum test was used for comparatives studies between the CSF obtained during the stage of viral encephalitis and that obtained during the autoimmune relapse.

Results

Eight of the 14 patients with non-viral relapsing neurological symptoms post-HSE were adults or teenagers (median age 40 years, range 13-69; 5 male) and the other six were young children (median age 13 months, range 6-20 months; 3 male). Repeat CSF PCR for HSV was negative in all patients. The six young children developed a classical syndrome of "choreathetosis post-HSE" in association with IgG antibodies against the GluN1 subunit of the NMDAR and one of them also had antibodies against the GABA$_A$R (Supplemental Figure 1). In many respects these children were clinically similar to previously reported cases and are not the focus of this study (see information in Table 1e, #9-14). In contrast, the eight patients of the teenager and adult group did not develop "choreoathetosis" and are the main focus of this report (Table 1, #1-8, and Clinical Vignettes in Supplemental material).

In these eight patients the onset of relapsing symptoms started 12-51 days (median 39, interquartile range [IQR] 26-43 days) after onset of HSE. In three patients (#2-4) symptoms presented acutely mimicking a viral relapse (biphasic course) and all three were re-started on acyclovir along with antipsychotic drugs or benzodiazepines while waiting for the results of repeat CSF PCR studies. In the other five patients (#1, 5-8) the symptoms developed while recovering in rehabilitation centers or at home, or in contiguity with those of HSE, without a clear biphasic stage, and none of them was initially considered to have relapsing symptoms. In these patients the symptoms were initially attributed to a recrudescence of residual viral-related deficits and managed with

antipsychotics and antidepressants. The severity and persistence of neuropsychiatric symptoms eventually led to reconsider the possibility of an independent complication. Three of these patients (case # 3, 5 and 8) were diagnosed with immune-mediated symptoms when they returned for a routine outpatient visit as part of the prospective study.

Seven patients presented with acute or subacute change of behavior, agitation, aggression, suicidal ideation, confusion, or delusional thoughts, and one patient with refractory seizures and status epilepticus requiring mechanical ventilation and barbiturate coma (case #6). An example of the severe alteration of mental functions and imaginary graphic representation is shown in the drawings of patient #8 (Figure 1). In three patients (cases #1, 3 and 4) the neuropsychiatric manifestations were heralded by intense headache accompanied in one case by drug-resistant high blood pressure (Table 1, and Case Vignettes in Supplemental Data). In another patient (case #7) the change of behavior was followed a few days later by fever, decreased level of consciousness, and severe blepharospasm. Except for this case, no abnormal movements were identified in the other patients.

The median time between onset of relapsing symptoms and CSF routine and viral studies was 30 days (IQR 14-81, range 0-136), and for antibody testing 85 days (IQR 37-126, range 17-296). The PCR for HSV was negative in all 8 patients. Five patients had pleocytosis (median 10 WBC, IQR 7-10, range 5-27) and four had increased protein concentration (median 100 mg/dL, IQR 79-110). The level of pleocytosis was substantially lower than that previously found during the viral encephalitis (median 80 WBC, IQR 30-245, range 2-460, p<0,01). Patients #1-5 had IgG antibodies against the GluN1 subunit of the NMDAR (all 5 in CSF, 2 also in serum; Figure 2 and Supplementary Figure 2); patients #6-8 had antibodies against unknown neuronal

antigens (all three in CSF, 1 also in serum). Archived CSF and serum obtained at the time of the HSE were available in 4 patients (case # 1, 3, 5 and 7) and all were negative for NMDAR or other autoantibodies (Supplementary Figure 2).

All eight patients underwent brain MRI; six had prior MRI studies obtained by the time of HSE, and the other two had CT scans (#6 and 7). In the 6 patients with repeat MRI, this showed mild to moderate interval progression of T2/FLAIR abnormalities compared with that obtained during HSE (Figure 3, A-D). Gadolinium was used in six of the eight patients at symptom relapse, showing in five contrast enhancement (4 intense, 1 mild) in the same areas with T2/FLAIR abnormalities (Figure 3, F, G, L). In 4 cases the findings could be compared with those of the MRI obtained during HSE, which showed absence or mild enhancement. Several additional follow-up MRIs were obtained in three patients; two of them showed dramatic reduction or absence of contrast enhancement after clinical improvement (#3 and 8, Figure 3 H), and the third patient, who had not received immunotherapy and continued with severe deficits showed persistent contrast enhancement one year after onset of relapsing symptoms (#4, Figure 3 L).

Before immunotherapy, 6 patients (#1, 2, 4, 6-8) received acyclovir, antipsychotics, and/or antidepressants, and all continued to deteriorate: 5 developed drug-resistant psychiatric symptoms (one of them, case #7, progressing to coma), and 1 developed refractory status epilepticus needing barbiturate coma. Only one patient (#3) improved without immunotherapy (described below).

The median time between onset of relapsing symptoms and immunotherapy was 79 days (IQR 22-148, range 17-352 days). This included steroids in 4 patients, steroids and IVIg in 1, and steroids, IVIG, and plasma exchange in the other 2 patients. In all 7 cases

a substantial improvement was noted after the immunotherapy was started. At last follow up (median 12 months, IQR 4.5-15, range 2-20 months), 2 patients had full or near complete recovery (case # 2 and 6), and the other 5 had substantial improvement of the neuropsychiatric abnormalities or seizures returning to their baseline residual deficits of HSE (Table 1 and Supplemental Case vignettes). The patient with refractory status epilepticus improved transiently after the first plasma exchange but because of recurrent electrographic seizures he was started on IVIg and rituximab. This treatment resulted in complete seizure control and improved level of consciousness, but he was left with HSE-related residual aphasia and critical illness neuropathy.

The patient that was not treated with immunotherapy (#3, Table 1 and Case Vignettes in Supplemental Data) was tested for antibodies 3 months after the neurological relapse as part of the prospective multicenter study of patients with HSE. This patient on day 40 post-HSE developed agitation, delusional thoughts, and insomnia that improved with alprazolam. By the time he was found to have NMDAR antibodies in CSF (serum negative), his symptoms had substantially improved and the contrast enhancement in the MRI had decreased.

When the clinical features of these 8 teenagers and adults were compared with those of the 6 young children with autoimmune relapses (Supplementary Table e-2), the young children were more likely to have choreoathetosis (6/6 vs 0/8, p<0.01) and decreased level of consciousness (6/6 vs 2/8, p<0.01). In addition, 3/6 young children developed refractory seizures and status epilepticus (2 preceding choreoathetosis) while only 1/8 in the teenager and adult group had seizures. MRI with contrast was obtained in three young children; one showed contrast enhancement and the other two, who had the study performed after immunotherapy, did not show enhancement.

The interval from onset of HSE to relapsing symptoms was similar in the teenager and adult group (median 39 days, range 12-51) and in the young children group (median 27 days, range 17-40, p=0.25), but the first group had a longer delay in the recognition of the relapsing symptoms than the young children group (interval onset of symptom relapse-antibody testing 85 days, range 17-296 vs 4 days, range 0-55 in children, p=0.037). Moreover, all young children were promptly treated with immunotherapy while one of the adult patients did not receive immunotherapy and the other 7 were treated with substantial delay (interval from relapsing symptoms to immunotherapy in the teenager and adult group 79 days, range 17-352 vs 4 days, range 0-12 in the young children group, p=0,043).

Discussion

The recent identification of antibodies to NMDAR and other synaptic proteins has provided a proof of principle to the long-held theory that relapsing symptoms post-HSE (or choreoathetosis post-HSE) can be immune mediated, and has increased awareness for this complication in children and adults.[5,6,9] In the current study, we report several novel findings in the age group of adults and teenagers demonstrating that 1) the main clinical manifestations are different from those of young children, 2) the symptom presentation may occur as a relapse of encephalitis (biphasic course), or in contiguity with HSE suggesting progression or recrudescence of residual deficits after the viral infection, 3) an immune-mediated pathogenesis is often not suspected, or is considered late in the course of the disease likely explaining substantial delays in immunotherapy, 4) the brain MRI frequently shows contrast enhancement during the autoimmune relapse, 5) in addition to NMDAR, patients may develop antibodies to $GABA_AR$ or other, yet unknown, neuronal cell-surface antigens, and 6) prompt diagnosis and

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immunotherapy improve symptoms and favorably affect the quality of life of patients and families despite persistence of HSE-related deficits.

The current data confirm that in young children the most characteristic manifestation of the disorder is choreoathetosis,[4,6,17] which in some patients may be accompanied or preceded by refractory seizures or status epilepticus and the most common autoantibody is against the NMDAR. In contrast, none of the teenagers or adults developed choreoathetosis; in these patients the NMDAR antibodies also predominated in the antibody repertoire, but some patients had antibodies against unknown neuronal cell-surface proteins. The novel finding of $GABA_AR$ antibodies in one of the young children with choreoathetosis and status epilepticus and a previous report demonstrating dopamine receptor antibodies[7] support the concept that the viral encephalitis triggers an immune response against a wide number of antigens. Further support is provided by the reactivity of the CSF or serum of some patients with live neuronal cultures even when studies with cell-based assays expressing all known surface antigens are negative.[6] Given that we have always found that CSF IgG antibodies to GluN1 associate with anti-NMDAR encephalitis[18,19] and these antibodies are pathogenic in models of cultured neurons[20] and mice,[21] we postulate they contribute to patients' symptoms. The pathogenic role of the other antibodies is unclear.

Preliminary data of our ongoing prospective study in which all patients with HSE are clinically and immunologically followed after the viral infection, show that 5/20 patients (25%) developed immune-mediated neurological symptoms, suggesting that this complication might be under-recognized. In teenagers and adults the problem of syndrome under-recognition is worse than in younger children given that they had substantial longer delays in antibody testing (unless they were part of the prospective study) and initiation of immunotherapy. The two main reasons for these delays included

the type of syndrome which in teenagers and adults was less stereotyped (e.g., absence of choreoathetosis), and the initial symptom presentation which in some patients was not suggestive of a clinical relapse. Indeed, the symptom presentation in most patients of the teenager and adult group was initially attributed to a progression or recrudescence of residual deficits and therefore not suspected to be autoimmune nor viral-induced; the clinical interval change noted in the scheduled visits suggested the autoimmune process. These findings have led to modify the protocol to include early follow-up visits (e.g., 1 month after hospital discharge).

Compared with the brain MRIs obtained during HSE (which showed mild or absent contrast enhancement), the MRIs obtained during symptom relapse had intense contrast enhancement that decreased or disappeared after the use of immunotherapy and clinical improvement. This observation has not been previously reported and deserves further study with larger number of patients in order to assess if contrast-enhancing MRI is a potential biomarker of the autoimmune response.

An important finding of this study is the symptom response to immunotherapy. In addition to the remarkable improvement of the patient shown in Figure 1, the clinical response in other patients was similarly impressive despite their residual deficits caused by the viral encephalitis. Before the autoimmune relapse all patients were collaborative or able to communicate and carry some activities of daily living according to the expected limitations caused by the areas of viral-induced necrosis (usually affecting short-term memory and language). However, this clinical picture contrasted with that observed during the autoimmune relapse, when most of the patients were agitated, aggressive, not collaborative, some of them with suicidal thoughts, or with seizures or decreased level of consciousness progressing to coma. In all but one case who improved with symptomatic treatment, immunotherapy (usually first line, such as steroids, IVIg or

plasma exchange) restored the clinical picture to the baseline deficits, allowing continuation of rehabilitation or discharge home.

The current findings suggest that patients with HSE should be carefully followed for any symptom relapse, worsening of deficits, or development of behavioral-psychiatric alterations with or without choreoathetosis or abnormal movements. Any of these symptoms, should raise concern for a viral relapse or an immune-mediated complication. Determination of CSF and serum neuronal cell-surface antibodies (mainly NMDAR) is a relatively new and important aid in the diagnosis of immune-mediated relapses post-HSE, and should be considered in all patients. If NMDAR antibodies are negative and testing for other antibodies is not available, contact a research lab for further studies. Meanwhile if the CSF PCR for HSV is negative, it seems reasonable to start these patients with empiric immunotherapy (e.g., first line steroids, IVIG, or plasma exchange) and depending on the symptom response and antibody results consider more intense therapies such as rituximab. The ongoing prospective multicenter study will clarify whether neuronal cell surface antibodies may occur without relapsing symptoms post-HSE, or if there is a titer threshold required for symptom development. The significance of MRI contrast enhancement in the areas previously affected by HSE, and the identity of additional target autoantigens should be goals of future studies.

Legends to Figures and Tables

Table 1: Clinical features of adults and teenagers with autoimmune relapsing symptoms post-herpes simplex encephalitis:

Abreviations: ACYC: Acyclovir, ADC rest: apparent diffusion coefficient restriction, CSF: cerebrospinal fluid, CT: cranial tomography, CYC: cyclophosphamide, d: days, enhanc: enhancement, F: female, FB: frontobasal, FT: frontotemporal, M: male, HSV: herpes simplex virus, IV: intravenous, Ig: immunoglobulin, mo: months, MP: methylprednisolone, neg: negative, NMDAR Ab: N-methyl-D-aspartate receptor antibodies, NSAb: neuronal surface antibodies, n/a: no available, OCB: oligoclonal bands, PEX: plasma exchange, pos: positive, prot.: CSF total protein in mg/dL, RTX: rituximab, T: temporal, WBC: white blood cell count /µl in CSF, WM: white mater, y: years.

Figure 1: Drawings of patient #8 at presentation of relapsing symptoms post-HSE and after immunotherapy

Drawing s of patient #8 by the time of relapsing symptoms (tree, family and house, A, D, and G), three weeks after immunotherapy (B, E, H) and at a 6 month follow-up (C, F, I). At presentation of relapsing symptoms the patient had severe anterograde amnesia, confusion, disorganized thoughts, and disorientation to place, time and person. After immunotherapy, her symptoms resolved except for amnesia and temporal orientation.

Figure 2: <u>Demonstration of brain autoantibodies in a patient with autoimmune relapse post-herpes simplex encephalitis</u>

Consecutive sections of rat brain immunostained with CSF of a subject without NMDAR antibodies (A, negative control), a patient with classical anti-NMDAR encephalitis (B, positive control), and the CSF of patient #9 by the time of HSE (C) and on day 19 when relapsing neurological symptoms due to autoimmune encephalitis occurred (D). The CSF of patient 9 shows a pattern of antibody reactivity typical of NMDAR but superimposed with diffuse background staining (compare B with D) likely representing disruption of the blood-brain-barrier and/or additional antibodies against other autoantigens (targets unknown). A similar background staining was noted in the CSF of the other patients; this dirty background usually clears up during CSF follow-up studies and eventually disappears (e.g., the reactivity becomes clear and indistinguishable from that seen in B; data not shown). In B and D the presence of NMDAR antibodies was confirmed with cell-based assay (not shown). Bar = 500μm

Figure 3: <u>MRI findings in patients with relapsing symptoms post-herpes simplex encephalitis</u>

Axial FLAIR-sequences of patients #1 (A, B) and #2 (C, D) during HSE (A, C) and during relapsing symptoms due to autoimmune encephalitis (B, D). In both cases there is an interval change due to areas of encephalomalacia, brain atrophy, and white matter changes. Panels E-H correspond to T1-sequences with contrast from patient #3 obtained during HSE (E), a few weeks later during relapsing symptoms due to autoimmune encephalitis (F,G), and after symptom improvement (H). Note that the areas of contrast enhancement during autoimmune encephalitis resolved after symptom improvement.

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Panels I-L correspond to patient #4 during HSE (I, T2; J, T1 with contrast) and one year later (K, T2; L, T1 with contrast). In this patient the relapsing symptoms post-HSE were not recognized as autoimmune encephalitis for 1 year; during this year he did not receive immunotherapy and had persistent symptoms and contrast enhancement in the MRI.

Legend to Supplementary Material:

Supplementary Table e-1: Clinical features of children with autoimmune relapsing symptoms post-herpes simplex encephalitis:

Abbreviations: ACYC: Acyclovir, ADC rest: apparent diffusion coefficient restriction, CSF: cerebrospinal fluid, CT: cranial tomography, CYC: cyclophosphamide, d: days, enhanc: enhancement, F: female, FT: frontotemporal, M: male, HSV: herpes simplex virus, IV: intravenous, Ig: immunoglobulin, mo: months, MP: methylprednisolone, neg: negative, NMDAR Ab: N-methyl-D-aspartate receptor antibodies, NSAb: neuronal surface antibodies, n/a: no available, OCB: oligoclonal bands, PEX: plasma exchange, pos: positive, PO: parietooccipital lobes, prot.: CSF total protein in mg/dL, RTX: rituximab, WBC: white blood cell count /µl in CSF, WM: white mater, y: years.

Supplementary Table e-2: Patients with post-HSE autoimmune encephalitis according to age and antibody findings

Legend: * p< 0,01; ** p=0,037; *** p=0,043. Abbreviations: CSF: cerebrospinal fluid, GABAAR: gamma amino butyric acid A receptor, HSE: herpes simplex virus encephalitis, IVIg: intravenous immunoglobulins, mo: months, mRS: modified Rankin Score, NMDAR: N-methyl-D-aspartate receptor

Supplemental Figure 1: Concurrent NMDAR and GABA$_A$R antibodies in the CSF of a patient with choreoathetosis post-HSE

HEK293 cells expressing human NR1/2 subunits of the NMDAR immunolabeled with patient's CSF (A) and a monoclonal antibody against the NR1 subunit (B). The merged reactivities are shown in (C). The same patient´s CSF also reacted with HEK cells expressing human α1/β3 subunits of the GABA$_A$R (D); panel E shows the reactivity with a monoclonal antibody against the α1 subunit of the GABA$_A$R, and the merged reactivities are shown in F. A similar assay (α1/β3 of GABA$_A$R) with serum from a normal subject is negative (G). Bar = 50µm

Supplemental Figure 2: Development of antibodies in patients with relapsing symptoms due to autoimmune encephalitis post-HSE

The graph shows the antibody titers in CSF (solid lines) and serum (dotted lines) of 5 patients who had antibody determination during HSE and afterwards (#1, dark green; #3 blue; #5, light green; #9, black; #10, red). Note that the antibodies became detectable in CSF before than in serum, and in one of the patients the serum studies have been negative. All 5 patients (4 of them part of the indicated prospective HSE protocol) are currently being followed clinically and immunologically.

Table 1: Clinical features of adults and teenagers with autoimmune relapsing symptoms post-herpes simplex encephalitis

| # sex, age | HSE | | | | AUTOIMMUNE RELAPSE | | | | |
	Symptoms	Brain MRI	CSF and immunological studies	Treatment and response	Symptoms	Brain MRI	CSF and immunologic al studies	Treatment	Outcome
#1 M, 13 y	Fever, seizures, aphasia, cardio-respiratory arrest	**D2:** Left FT, right FB necrotic lesions with ADC restr.	**D5:** HSV pos. **D23:** HSV neg, WBC 27, prot. 51, neg NSAb	IV Acyc (21 d) Motor and cognitive residual deficits	**D42:** Aggressive behavior, headache, high blood pressure	**D186:** No new necrosis, ↑ WM changes; Contrast: n/a	**D178:** HSV neg, WBC 7, prot. ≤45, NMDAR Ab (1:160) (also in serum 1:800)	**D45:** Risperidone , high blood pressure drugs **D190:** IV MP	Partial improvement of behavioral deficits **FU: 15 mo**, motor and cognitive deficits
#2 M, 15 y	Headache, focal seizures, encephalo pathy	**D3:** Bilateral FT necrosis, + ADC restr. No enhanc	**D2:** HVS pos, WBC 2, prot. 50	IV Acyc (21 d) Complete recovery	**D51:** Agitation, cognitive deficits, aggressive behavior	**D 69:** No new necrosis, ↑ WM changes; enhanc +	**D62:** HSV neg, WBC 0, prot ≤45, pos OCB, NMDAR Ab (1:80) **D102:** NMDAR Ab (1:20)	**D60:** Acyc, lorazepam **D102:** IV MP, oral MP, IVIg	Rapid resolution of behavior abnormalities **FU: 12 mo**, complete recovery
#3 M, 45 y	Headache, fever, confusion, aphasia	**D10:** Left T necrosis, + ADC restr. Mild enhanc **Day 23:** no changes	**D10:** HSV pos, WBC 110, prot. 74, **D25:** HSV neg, WBC 56, prot.78	IV Acyc (21 d) Residual global aphasia	**D44:** Headache, confusion, agitation. Delusional thoughts and insomnia	**D51:** No new necrosis, ↑ WM changes; enhanc: ++ **D141:** enhanc: +	**D44:** HSV neg, WBC 5, prot.93, **D145:** HSV neg, WBC 10, Prot. 43, pos OCB, NMDAR Ab (1:40)	**D44:** Acyc (5 d), alprazolam	Relapsing symptoms that faded spontaneously **FU: 6 mo**, residual aphasia
#4 M, 50 y	Fever, aphasia and memory deficits	**D2:** Bilateral T necrosis; + ADC restr. No enhanc	**D2:** HVS pos, WBC 239, prot. 66	IV Acyc (14 d) Good recovery	**D40:** Headache, aggression, suicidal ideation, tremor, sleep disorder	**D86:** No new necrosis, ↑ WM changes; enhanc: + **D360:** enhanc: +++	**D86:** HSV neg, WBC 27, prot. 107 **D336:** WBC 15, prot.88, NMDAR Ab (1:2) **D585:** HSV neg, WBC 7, prot. 80	**D86:** Acyc (14 d), Risperidone **D392:** MP, IVIg **D586:** starting RTX, CYC	Improvement of behavioral symptoms **FU: 20 mo**, moderate behavioral deficits.
#5 F, 34 y	Fever, focal seizures, aphasia, memory deficits	**D2:** Left T necrosis; + ADC restr. Enhanc n/a	**D2:** HVS pos, WBC 460, prot. 51, neg NSAb	IV Acyc (14 d) Persistent aphasia and memory deficits	**D38:** Insomnia, anxiety, restlessness, irritability, delusions	**D65:** No new necrosis, ↑ WM changes; No enhanc	**D60:** HSV neg, WBC 10, prot. 65, NMDAR Ab (1:80) (also in serum 1:400)	**D60:** IV MP	Improvement of behavior **FU: 2 mo**, mild aphasia and memory deficits
#6 F, 69 y	Speech problems, confusion, fever, partial seizures	**D2:** Normal brain CT	**D2:** HSV pos, WBC 32, prot. ≤45	IV Acyc (21 d) Improvemen t of seizures after 6 days of treatment	**D12:** Confusion, new onset non-convulsive status epilepticus	**D8:** Left T cortical and WM changes, no ADC restr. Enhanc: ++	**D29:** HSV neg, WBC 0, prot. ≤45, pos NSAb (also in serum)	**D12:** Acyc, barbiturate coma. **D29:** MP, IVIg, PEX **D60:** RTX	Transient response to PEX; seizure control post-RTX **FU: 3 mo**, mild aphasia.

#7 M, 29 y	Fever, respiratory failure, seizures, abnormal behavior	**D3:** right FT hypointensity in CT	**D2:** HSV pos, WBC 49, prot. 60	IV Acyc (13 d); motor and cognitive residual deficits	**D21:** Abnormal behavior. **D60**: fever, ↓ consciousness, blepharospasm	**D70**: No new necrosis. Bilateral FT WM changes. Enhanc: n/a	**D58:** HSV neg, WBC 2, Prot. 112 **D90:** HSV neg, WBC 12, prot. 63, pos NSAb	**D15:** Acyc (21 d), haloperidol, risperidone **D100**: IV MP	Improvement. after MP and local botox **FU: 12 mo,** minor deficits (back to work)
#8 F, 56 y	Fever, diarrhea, somnolence, catatonia	**D7:** bilateral T necrosis; no ADC restr; No enhanc	**D7:** HSV pos, WBC 250, prot. 62, **D21:** HSV neg, WBC 90 prot. 61,	IV Acyc (15 d) Residual anterograde amnesia	**D30:** Emotional lability, suicidal ideation, confusion	**D150:** No new necrosis; ↑WM changes; Enhanc: ++ **D314:** No enhanc	**D146:** HSV neg, WBC 10, prot. 45, pos OCB, pos NSAb (neg serum)	**D30:** Quetiapine, citalopram, paroxetine **D160:** IV MP	Improvement psychiatric symptoms; **FU: 15 mo,** anterograde amnesia

Acyc: Acyclovir, ADC rest: apparent diffusion coefficient restriction, CSF: cerebrospinal fluid, CT: cranial tomography, CYC: cyclophosphamide, D: days, enhanc: contrast enhancement, F: female, FB: frontobasal, FT: frontotemporal, T: temporal, M: male, HSV: herpes simplex virus, IV: intravenous, Ig: immunoglobulin, mo: months, MP: methylprednisolone, neg: negative, NMDAR Ab: N-methyl-D-aspartate receptor antibodies, NSAb: neuronal surface antibodies (unknown identity), n/a: no available, OCB: oligoclonal bands, PEX: plasma exchange, pos: positive, prot.: CSF total protein in mg/dL, RTX: rituximab, WBC: white blood cell count /µl in CSF, WM: white mater, y: years.

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FIGURE 1

Drawings of patient #8 at presentation of relapsing symptoms post-HSE and after immunotherapy

Drawing s of patient #8 by the time of relapsing symptoms (tree, family and house, A, D, and G), three weeks after immunotherapy (B, E, H) and at a 6 month follow-up (C, F, I). At presentation of relapsing symptoms the patient had severe anterograde amnesia, confusion, disorganized thoughts, and disorientation to place, time and person. After immunotherapy, her symptoms resolved except for amnesia and temporal orientation.

FIGURE 2

Demonstration of brain autoantibodies in a patient with autoimmune relapse post-herpes simplex encephalitis

Consecutive sections of rat brain immunostained with CSF of a subject without NMDAR antibodies (A, negative control), a patient with classical anti-NMDAR encephalitis (B, positive control), and the CSF of patient #9 by the time of HSE (C) and on day 19 when relapsing neurological symptoms due to autoimmune encephalitis occurred (D). The CSF of patient 9 shows a pattern of antibody reactivity typical of NMDAR but superimposed with diffuse background staining (compare B with D) likely representing disruption of the blood-brain-barrier and/or additional antibodies against other autoantigens (targets unknown). A similar background staining was noted in the CSF of the other patients; this dirty background usually clears up during CSF follow-up studies and eventually disappears (e.g., the reactivity becomes clear and indistinguishable from that seen in B; data not shown). In B and D the presence of NMDAR antibodies was confirmed with cell-based assay (not shown). Bar = 500μm

FIGURE 3

MRI findings in patients with relapsing symptoms post-herpes simplex Encephalitis

Axial FLAIR-sequences of patients #1 (A, B) and #2 (C, D) during HSE (A, C) and during relapsing symptoms due to autoimmune encephalitis (B, D). In both cases there is an interval change due to areas of encephalomalacia, brain atrophy, and white matter changes. Panels E-H correspond to T1-sequences with contrast from patient #3 obtained during HSE (E), a few weeks later during relapsing symptoms due to autoimmune encephalitis (F,G), and after symptom improvement (H). Note that the areas of contrast enhancement during autoimmune encephalitis resolved after symptom improvement. Panels I-L correspond to patient #4 during HSE (I, T2; J, T1 with contrast) and one year later (K, T2; L, T1 with contrast). In this patient the relapsing symptoms post-HSE were not recognized as autoimmune encephalitis for 1 year; during this year he did not receive immunotherapy and had persistent symptoms and contrast enhancement in the MRI.

Supplementary Table 1e: Clinical features of young children with autoimmune relapsing symptoms post-herpes simplex encephalitis

		HSE			AUTOIMMUNE RELAPSE				
Case, sex, age	**Symptoms**	**Brain MRI**	**CSF and immunological studies**	**Treatment and response**	**Symptoms**	**Control MRI**	**CSF and immunological studies**	**Treatment**	**Response to immunotherapy, residual deficits at last follow up**
9# M, 11 mo	Fever, status epilepticus	Day 3: Bilateral PO necrotic lesions with ADC restr, no contrast enhanc	Day 1: HSV pos, WBC 26, prot. ≤45, neg NSAb	IV ACYC (21 d) complete recovery	Day 19: Alternating periods of lethargy and irritability, loss of contact with environment, refractory seizures, status epilepticus. 60 days after onset of HSE: generalized choreoathetosis	Day 22: No new necrotic lesions, encephalomalacia Expansion of WM abnormalities Contrast enhanc +++ Day 110: encephalomalacia, no enhanc	Day 19: HSV neg, WBC 54, prot. 64, pos NMDAR Ab (neg serum) Day 85: pos NMDAR Ab (also in serum)	Day 20: ACYC, IV MP, IVIg, RTX, CYC, ketogenic diet Day 70: PEX	Partial improvement of choreoathetosis and level of consciousness after PEX. Seizures controlled with ketogenic diet. FU: 9 mo, developmental delay with decrease of communicative skills and refractory epilepsy
10# M, 15 mo	Fever, status epilepticus	Day 5: Right PO necrotic lesions with ADC restr, meningeal constrast enhanc +	Day 1: HSV pos, WBC 7, prot. ≤45. Day 4: HSV pos, WBC 55 prot. ≤45	IV ACYC (21 d) complete recovery	Day 27: Confusion, irritability, refractory seizures and status epilepticus. 15 days after onset of relapse: generalized choreoathetosis	Day 27: Brain CT, progression to encephalomalacia of previous lesions Contrast enhanc: n/a	Day 27: HSV neg, WBC 47, prot. 115, GABA$_A$R Ab Day 44: HSV neg, pos NMDAR and GABA$_A$R Ab (both also in serum)	Day 27: ACYC, IV MP, induced coma Day 44: PEX, RTX	Improvement of level of consciousness and seizures after PEX and RTX. FU: 4 mo, seizure free, minor choreoathetosis Rapidly improving motor and social skills
11# F, 20 mo	Fever, upper respiratory infection, complex seizures	Brain CT: right temporal hypointensity. MRI not obtained	Day 18: HSV pos, WBC 12, prot. 76	IV ACYC (21 d), residual left hemiparesis	Day 40: Irritability and hemiballistic movements Day 47: Prominent lingual dyskinesia, encephalopathy and refractory seizures	Day 72: No new necrotic lesions, encephalomalacia Contrast enhanc: n/a	Day 52: HSV neg, pos NMDAR Ab Day 140 and 360: pos NMDAR Ab (also in serum)	Day 52: ACYC, PEX	Transient improvement after PEX
12# M, 6 mo	Fever, irritability, decreased level consciousness, complex seizures	D9: left temporal and PO necrotic lesions without contrast enhanc	Spinal tap not performed (coagulopathy) Diagnosed by blood serology	IV ACYC (13 d) complete recovery	Day 27: Decreased level of consciousness, irritability, hypotonia, general choreoathetosis, orolingual dyskinesias	Day 40: No new necrotic lesions, No contrast enhanc	Day 31: HSV neg, WBC 29, prot. ≤45 pos NMDAR Ab Day 120: pos NMDAR Ab (also	Day 31: ACYC, IV MP, IVIg Day 120: starting RTX	Almost full recovery of abnormal movements FU: 4 mo, developmental delay

		MRI (initial)	CSF (initial)	Treatment	Clinical (later)	MRI (later)	CSF (later)	Treatment	Outcome/FU
							in serum)		
			(1st sample: IgM-, IgG low+; 2nd IgM+, IgG++)						
13# F, 8 mo	Fever, decreased level of consciousness, complex seizures	**Day 4:** Bilateral frontal and PO necrotic lesions with ADC restr and contrast enhanc.	**Day 3:** HSV pos, WBC 10, prot. 69	IV ACYC (21 days), started to improve	**Day 17:** choreoathetosis, decreased level of consciousness	**Day 30:** No new lesions; enlargement of previous necrotic lesions Contrast enhanc: n/a	**Day 24:** HSV neg, WBC 8, prot. 56 **Day 72:** pos NMDAR Ab (also in serum)	**Day 24** ACYC, IVIg repeated courses of IVIg at 72, 100 and 130 days	Substantial improvement of choreoathetosis after IVIg **FU: 5 mo,** developmental delay; requires 3 antiepileptics
14# M, 15 mo	Fever, decreased level of consciousness, complex seizures	**Day 4:** Left FT necrotic lesions with ADC restr No contrast enhanc.	**Day 1:** HSV pos, WBC 20, prot. ≤45	IV ACYC (21 days), clear improvement	**Day 34:** insomnia, low grade fever and irritability, 2 days later choreoathetosis, continuous dyskinesias and decreased level of consciousness	**Day 40:** No new necrotic lesions, Encephalomalacia Expansion of WM abnormalities. No contrast enhanc	**Day 38:** HSV neg, WBC 25, prot. ≤45 NMDAR Ab (also in serum)	**Day 38:** ACYC, IV MP, IVIg, RTX	**FU: day 40,** Just starting immunotherapy

Table e-1 Legend: Abbreviations: ACYC: Acyclovir, ADC rest: apparent diffusion coefficient restriction, CSF: cerebrospinal fluid, CT: cranial tomography, CYC: cyclophosphamide, d: days, enhanc: enhancement, F: female, FT: frontotemporal, M: male, HSV: herpes simplex virus, IV: intravenous, Ig: immunoglobulin, mo: months, MP: methylprednisolone, neg: negative, NMDAR Ab: N-methyl-D-aspartate receptor antibodies, NSAb: neuronal surface antibodies, n/a: no available, OCB: oligoclonal bands, PEX: plasma exchange, pos: positive, PO: parietooccipital lobes, prot.: CSF total protein in mg/dL, RTX: rituximab, WBC: white blood cell count /µl in CSF, WM: white mater, y: years

Supplementary Table e-2: Patients with post-HSE autoimmune encephalitis according to age and antibody findings

	Adult-teenagers =8	Young Children =6
Male (%)	5 (63%)	3 (50%)
Age, median (range)	40 years (13-69)	13 months (6-20)
Residual deficits post viral infection **Mild (mRS ≤2)** **Severe (mRS >2)**	4 (50%) 4 (50%)	5 (83%) 1 (17%)
Median time from HSE to relapsing symptoms (range)	39 days (12-51)	27 days (17-40)
Symptoms at relapse - Seizures - Choreoathetosis - Irritability - Abnormal behavior - Autonomic instability - Decreased level of consciousness	1 (13%) 0* 6 (75%) 7 (88%) 1 (13%) 2 (25%)*	3 (50%) 6 (100%)* 6 (100%) Not assessable Not reported 6 (100%)*
Ancillary tests at relapse - Pleocytosis - Increased CSF proteins - Contrast enhancing brain MRI	5 (63%) 4 (50%) 5/6 (83%) (n/a in 2)	5/5 (100%) (n/a in 1) 3/5 (60%) (n/a in 1) 1/3 (33%) (n/a in 3)
Median time from relapse to antibody testing (range)	87 days (17-296)**	4 days (0-55)**
Median time from relapse to immunotherapy (range)	79 days (17-352)*. one not treated	4 days (0-12)*
Antibody Findings	5 NMDAR (62.5%); 3 unknown antigens	6 NMDAR (100%) (one concurrent GABA$_A$R)
Immunotherapy - Steroids - IVIg - Plasma exchange - Rituximab alone - Rituximab combined with cyclophosphamide	7 (88%) 3 (38%) 1 (13%) 1 (13%) 1 (13%)	4 (67%) 4 (67%) 3 (50%) 3 (50%) 1 (17%)
Follow up >1mo, **median (range)**	8 patients 12 months (2-20)	5 patients 5 months (4-12)
Improvement of relapsing symptoms (return to base line deficits post-HSE)	Complete 7 (87%) Partial 1 (13%) No improvement 0	Complete 0 Partial 3 (60%) No improvement 2 (40%)

<u>Table e-2 Legend</u>: * p< 0,01; ** p=0,037; *** p=0,043. Abbreviations: CSF: cerebrospinal fluid,

GABAAR: gamma amino butyric acid A receptor, HSE: herpes simplex virus encephalitis, IVIg:

intravenous immunoglobulins, mo: months, mRS: modified Rankin Score, NMDAR: N-methyl-D-

aspartate receptor

SUPPLEMENTARY FIGURE 1

Concurrent NMDAR and GABA$_A$R antibodies in the CSF of a patient with choreoathetosis post-HSE

HEK293 cells expressing human NR1/2 subunits of the NMDAR immunolabeled with patient's CSF (A) and a monoclonal antibody against the NR1 subunit (B). The merged reactivities are shown in (C). The same patient´s CSF also reacted with HEK cells expressing human α1/β3 subunits of the GABA$_A$R (D); panel E shows the reactivity with a monoclonal antibody against the α1 subunit of the GABA$_A$R, and the merged reactivities are shown in F. A similar assay (α1/β3 of GABA$_A$R) with serum from a normal subject is negative (G). Bar = 50µm

Development of antibodies in patients with relapsing symptoms due to autoimmune encephalitis post-HSE

The graph shows the antibody titers in CSF (solid lines) and serum (dotted lines) of 5 patients who had antibody determination during HSE and afterwards (#1, dark green; #3 blue; #5, light green; #9, black; #10, red). Note that the antibodies became detectable in CSF before than in serum, and in one of the patients the serum studies have been negative. All 5 patients (4 of them part of the indicated prospective HSE protocol) are currently being followed clinically and immunologically.

Case vignettes

Patient 1

A previous healthy 13 year old male without past medical history of interest, was admitted for fever, headache and emesis. Over the next 2 days he developed aphasia, refractory seizures, and decreased level of consciousness. A brain MRI revealed extensive left frontotemporal and mild right frontobasal T2 and fluid-attenuated inversion recovery (FLAIR) abnormalities, with mass effect and restricted apparent diffusion coefficient (ADC) (Figure 3, panel A). Intravenous (IV) acyclovir was initiated for suspected herpes simplex encephalitis (HSE) although the lumbar puncture was not performed due to increased intracranial pressure. Cerebrospinal fluid (CSF) obtained three days later during placement of an external ventricular shunt confirmed the viral infection (PCR positive for Herpes simplex virus 1, [HSV1]). During the patient's stage at the ICU, he developed severe hypotension, becoming pulseless and requiring cardiopulmonary resuscitation. Acyclovir was discontinued after 21 days at which time the CSF showed 27 white blood cells (WBC)/µl, protein concentration 51 mg/dL and the PCR was negative for HSV1. Studies for N-methyl-D-aspartate receptor (NMDAR) and other antibodies to cell surface antigens were negative. During the next 2 weeks he had progressive slow improvement but on day 42 post-HSE he developed a drastic change in behavior including prominent sexual disinhibition, cursing and insulting people, and aggressive behavior, biting the pillow and objects. These behavioral changes were felt to be sequelae of the HSE and he was started on risperidone. He also developed refractory hypertension, requiring four antihypertensive drugs (labetalol, amlodipine, enalapril and hydralazine). The patient was discharged home two months after onset of HSE but he was re-admitted 4 months later (6 months after HSE onset), due to persistent and severe behavioral problems. An MRI without

contrast showed encephalomalacia in the previous areas of viral involvement without new necrotic regions but with expansion of the surrounding white matter changes (Figure 3, panels A and B). The CSF showed mild pleocytosis (7 WBC/µL) with normal protein concentration, negative PCR to HSV1, and high titers of NMDAR antibodies (CSF titer 1:160; serum titer 1:800). He was treated with 5 daily doses of high dose IV methylprednisolone with rapid recovery of the behavioral abnormalities. Fifteen months after onset of HSE he has stable motor and cognitive deficits considered residual from the viral infection.

Patient 2

A previously healthy 15 year-old male presented with headache, focal seizures, and encephalopathy. MRI showed bilateral right greater than left frontotemporal T2-FLAIR hyperintensities with ADC restriction. The CSF had 2 WBC/µL, mildly elevated proteins (50 mg/dL) and the PCR was positive for HSV1; NMDAR antibodies were not tested. He completed a 21 day course of IV acyclovir and had a complete recovery. Fifty-two days after HSE onset he developed agitation, memory and cognitive deficits, and inappropriate behavior including aggressiveness. A relapse of the HSE was suspected but the CSF showed 0 WBC/µL, normal protein concentration and the PCR was negative for HSV1. The MRI showed encephalomalacia of the previous right frontotemporal viral involvement, no new necrotic lesions, but mild worsening of T2/FLAIR white matter changes bilaterally affecting the frontal lobes (Figure 3, panel C and D), with mild contrast enhancement when compared to the prior MRI taken during the HSE. Re-evaluation of the CSF showed high titers of NMDAR antibodies (1:80) that were not detected in serum. He was treated with one course of intravenous immunoglobulins (IVIg) and IV methylprednisolone followed by oral

methylprednisolone that was tapered and discontinued over 2 weeks. A repeat CSF study showed decrease in NMDAR antibody titers (1:20). The patient had good clinical response and at last follow up, 12 months after HSE onset, he is at baseline with no neuropsychiatric symptoms or behavioral problems.

Patient 3

A previously healthy 45 year old man developed sudden onset headache, fever, confusion, and speech problems. A brain CT showed a left temporal hypointense lesion with mass effect suspected to be a glioma but the MRI demonstrated left temporal T2/FLAIR hyperintensities with ADC restriction suggestive of HSE encephalitis (Figure 3, panel E). The CSF showed 110 WBC/µL, increased protein concentration (74 mg/dL), and PCR positive for HSV1; NMDAR and other antibodies to cell surface antigens were negative. He was started on IV acyclovir and repeat CSF two weeks later showed 56 WBC/µL, protein of 78 mg/dL and the PCR was no longer positive for HSV1 (NMDAR antibodies not tested in this CSF sample). After completing 21 days of acyclovir the MRI showed stability of the previously noted brain lesions and the patient was discharged with residual global aphasia. Ten days after discharge (44 days after HSE onset) he was admitted to a local community hospital for acute onset headache, confusion, agitation, severe insomnia, and delusional thoughts. MRI showed slight progression of the T2/FLAIR hyperintensities in the left temporal lobe with new intense contrast enhancement and involvement of the right temporal lobe (Figure 3, panels F and G) that was not present in the MRI performed 2 weeks earlier. Acyclovir was restarted for presumed HSE relapse but it was discontinued 5 days later when the CSF PCR came back negative for HSV; this revealed 5 WBC/µL, and protein concentration of 93 mg/dL (NMDAR antibodies were not tested). He improved with no further

treatment and was discharged with his baseline aphasia. Three months after this episode (and as part of a prospective study of patients with HSE) he was re-evaluated in our center. The MRI showed marked improvement of the previous contrast-enhancing temporal lobe abnormalities (Figure 3, panel H), and the CSF showed 10 WBC/μL with normal protein concentration, unmatched oligoclonal bands, negative PCR for HSV-1, and positive NMDAR antibodies (1:40; serum negative), suggesting that the episode he developed after the HSE was NMDAR-antibody related. At the last follow up, 6 months after HSE onset, he had residual baseline aphasia due to HSE but no neuropsychiatric symptoms or behavioral problems.

Patient 4

A previous healthy 50 year old male was admitted for subacute onset of fever, speech difficulties and memory deficits. Brain MRI showed right greater than left temporal and hippocampal T2/FLAIR hyperintensities with ADC restriction without contrast enhancement (Figure 3, panel I and K). The CSF showed 239 WBC/μL, increased protein (66 mg/dL), and PCR positive for HSV1 (NMDAR antibodies were not tested). He received IV acyclovir for 14 days with good recovery. One month after discharge (40 days after HSE onset), he developed severe headache and abnormal behaviors. He was depressed with suicidal ideation and episodes of aggression. He had an intention tremor with no other abnormal movements. These symptoms were initially attributed to the previous HSE and risperidone was started. He was admitted 2 months later with refractory headaches and behavioral symptoms. The MRI showed mild progression of the previous T2/FLAIR white matter bilateral temporal lobe hyperintensities with new intense contrast enhancement (data not shown). The CSF showed 27 WBC/μL, increased proteins 107 mg/dL, and the PCR was negative for HSV1. NMDAR

antibodies were not tested. Although a viral relapse was not confirmed by PCR, he empirically received IV acyclovir for two weeks without improvement, and his symptoms were attributed to residual deficits of HSE. Due to persistent symptoms he was re-evaluated 12 month after HSE onset. The MRI showed more intense contrast enhancement compared to the prior study (Figure 3, panels J and L). The CSF showed mild pleocytosis (15 WBC/μL), high protein concentration (88 mg/dL), negative HSV1 PCR and low titers of NMDAR antibodies (1:2) (serum negative). He was treated with IV corticosteroids and IVIg with substantial but not complete recovery of the headache and psychiatric symptoms, and he is currently receiving rituximab and cyclophosphamide.

Patient 5

A previous healthy 34 year old woman was admitted for subacute onset of fever, speech difficulties, memory deficits, and focal seizures. An MRI without contrast showed left temporal and hippocampal T2/FLAIR hyperintensities with ADC restriction. The CSF showed 460 WBC/μL, increased protein (51 mg/dL), PCR positive for HSV1, and absence of NMDAR and other autoantibodies. She was discharged after completing 14 days of intravenous acyclovir with residual aphasia and memory deficits. Twenty-three days after discharge (38 days after HSE onset) she developed progressive behavioral abnormalities, including anxiety, restlessness, delusional thoughts, irritability and insomnia. In a routine follow up visit sixty days after HSE onset, her change of behavior led to consider an autoimmune relapse and she was re-admitted. The CSF PCR was negative for HSV, but showed 10 WBC/μL, protein concentration of 65 mg/dL, and NMDAR antibodies (CSF titer 1:40; serum titer 1:200). She was started on IV methylprednisolone with rapid improvement of the behavioral abnormalities, returning

to her pre-relapse level. A brain MRI performed 5 days after methylprednisolone was initiated showed mild expansion of the T2/FLAIR white matter changes in the left temporal lobe compared with the MRI obtained during the HSE.

Patient 6

A 69 year old female was recently admitted for speech difficulties and an acute confusional syndrome associated with fever. A brain CT was normal but the CSF showed 32 WBC/μL, normal protein level, and the PCR was positive for HSV1. NMDAR and other antibodies to cell surface antigens were not tested. IV acyclovir was started and over the next 6 days she developed simple partial seizures that were initially well controlled with levetiracetam and valproate. Brain MRI performed 8 days after HSE onset showed left temporal and hippocampal T2/FLAIR hyperintensities without ADC restriction but with intense contrast enhancement. Over the next days the patient had a progressive increase in frequency of seizures that evolved to a non-convulsive status epilepticus. The patient was readmitted to the ICU for refractory status epilepticus receiving treatment with continuous infusion of midazolam and propofol, followed by barbiturate coma. After completing 21 days of acyclovir the CSF showed absence of WBC, normal protein concentration, and the PCR was negative for HSV1. Antibodies to unknown neuronal cell surface antigens were identified in CSF and serum. She was then started on IV methylprednisolone, IVIG, and plasma exchange resulting in a transient improvement of the EEG pattern. Four weeks after first line immunotherapies she was started on rituximab leading to complete seizure control. Currently, 3 months after HSE onset, the patient has moderate aphasia and proximal weakness due to critical illness neuropathy.

Patient 7

A previously healthy 29 year old male was admitted for fever, seizures, and abnormal behavior. After admission he developed respiratory failure requiring mechanical ventilation. A brain CT showed a right frontotemporal hypointensity. The CSF demonstrated 49 WBC/μL, protein concentration 60 mg/dL and positive PCR for HSV1. NMDAR and other neuronal cell surface autoantibodies were not tested. He was treated with 2 weeks of IV acyclovir with improvement but one week later developed abnormal behavior which was thought to be secondary to the HSE. He was discharged one month after admission requiring haloperidol and risperidone and was then re-admitted 1 month later due to the development of decreased level of consciousness and fever. At admission he was noted to have blepharospasm without other abnormal movements. An MRI without contrast showed extensive bilateral right greater than left frontotemporal T2/FLAIR hyperintensities. There was no prior MRI for comparison. The CSF showed 2 WBC/μL and increased proteins (112 mg/dL). He was re-treated with IV acyclovir for 21 days for a suspected viral relapse although the CSF PCR for HSV1 was negative. He did not improve and progressed to a comatose state that required mechanical ventilation. Re-evaluation of the CSF showed 12 WBC/μL, increased proteins (63 mg/dL) and antibodies to unknown neuronal cell surface antigens; the serum was not studied. He was treated with IV methylprednisolone for 5 days and his level of consciousness improved as did the abnormal behavior. Persistent blepharospasm was treated with botulin toxin. At the last follow up 12 months after the initial admission he is almost fully recovered and has returned to work.

Patient 8

A healthy 56 year old female developed low grade fever and diarrhea followed the next day by apathy and somnolence. She was seen by a psychiatrist and diagnosed with reactive depression due to the recent death of her father. She was started on citalopram but several days later she became catatonic. An MRI of the brain showed bilateral T2/FLAIR abnormalities without contrast enhancement in the temporal lobes, right greater left. The CSF showed 250 WBC/μL, increased proteins (62 mg/dL), positive PCR for HSV1 and absence of antibodies to cell surface antigens. The patient received 15 days of IV acyclovir and was discharged to a rehabilitation center with severe anterograde amnesia. Repeat CSF obtained one day before the acyclovir was discontinued showed 90 WBC/μL, protein concentration 61 mg/dL, negative HSV1 PCR, and absence of antibodies to neuronal cell surface antigens. Over the next 2 weeks she developed emotional lability, continuous crying, and suicidal ideation. She was treated with quetiapine, citalopram and paroxetine with partial improvement, but continued having episodes of severe agitation, and confrontational and oppositional behavior. Five months after onset of HSE the patient was seen in a follow up visit of a prospective study of patients with HSE in our center. She had stable, severe anterograde amnesia, and was disoriented to place, time and person (Minimental State Examination 19/30) and continuously confused (Figure 1, panels A, D and G). MRI showed slight progression of T2/FLAIR hyperintensity in the temporal lobes with new contrast enhancement. Repeat CSF studies showed 10 WBC/μL, normal protein concentration, negative HSV1 PCR, unmatched oligoclonal bands, and antibodies to unknown neuronal cell surface antigens that were not present in serum. Although her symptoms were stable, based on these laboratory findings she was treated with 5 days of IV methylprednisolone. Within the next three weeks her severe behavioral problems resolved and became oriented to person and place (Minimental State Examination

22/30) (Figure 1, panels B, E and H). Her anterograde amnesia and orientation to time remained unchanged, but overall she was able to return home and participate in conversations with her family and friends. She received 2 additional monthly cycles of methylprednisolone. At the last follow 15 months after HSE, she was clinically stable (Figure 1, panels C, F, I) and the MRI showed resolution of the contrast enhancement.

V. Resum i discussió

L'encefalitis continua sent a dia d'avui una causa important de morbimortalitat a nivell mundial que afecta a totes les edats. Tot i així, estudis multicèntrics realitzats a nivell mundial coincideixen en el fet de que més de la meitat de casos romanen sense un diagnòstic etiològic definitiu després d'extenses bateries microbiològiques.[3-5] El recent descobriment d'una nova categoria d'encefalitis causada per anticossos dirigits contra proteïnes de la superfície neuronal ha donat un diagnòstic definitiu a molts d'aquests casos, i ha identificat la causa de diversos desordres prèviament considerats idiopàtics o atribuïts a infeccions víriques.[41,7] A diferència de les síndromes paraneoplàstiques clàssiques associades a anticossos intracel·lulars que afecten de forma casi exclusiva a pacients adults, les encefalitis per autoimmunitat sinàptica afecten a pacients de totes les edats, e inclús algunes d'elles com l'encefalitis anti-NMDAR, de forma predominant a nens i adolescents.[8,9,49] Aquestes entitats poden desenvolupar-se amb o sense la presència d'un tumor, i s'associen a anticossos que es dirigeixen contra epítops o dianes extracel·lulars de la membrana neuronal amb fenotips clínics que varien en funció de l'anticòs associat, semblant aquells fenotips en el que l'antigen diana és alterat farmacològicament o genèticament.[8,9,49] Mentre que els anticossos intracel·lulars probablement representen el component humoral d'una complexa resposta immunològica mediada per cèl·lules T citotòxiques, els anticossos dirigits contra proteïnes de superfície neuronal han demostrat en els desordres en els que s'ha investigat, que produeixen una alteració funcional i/o morfològica dels antígens corresponents quan són aplicats en models *in vitro* o *in vivo*, i que aquestes alteracions són reversibles després d'eliminar els anticossos.[41,50] La distinció entre aquestes dues categories d'encefalitis segons si l'anticòs associat va dirigit contra un epítop intra o extracel·lular resulta de gran importància a la pràctica clínica, doncs els pacients que associen anticossos contra epítops extracel·lulars amb freqüència responen a immunoteràpia i/o resecció tumoral quan està indicat.[8,9,49]

El descobriment d'aquesta nova categoria de síndromes associades a anticossos contra antígens de superfície neuronal ha canviat l'enfocament diagnòstic i terapèutic de problemes tan diversos com la catatonia, les alteracions agudes de la memòria, l'epilèpsia, o els trastorns del moviment. Per exemple, alguns processos prèviament atribuïts a infeccions víriques o manifestacions atípiques d'esquizofrènia són actualment identificats com processos autoimmunes tractables.[53] La transferibilitat d'aquestes investigacions a la indústria i a la pràctica clínica han produït test diagnòstics que s'utilitzen a tot el món i a patents per l'ús d'aquesta tecnologia. En la població pediàtrica, el descobriment per part del director de la tesi de l'encefalitis anti-NMDAR, una causa freqüent d'encefalitis autoimmune en nens i adolescents, ha canviat el maneig diagnòstic i terapèutic de l'encefalitis en l'edat pediàtrica.[51,52] Cal destacar però, que en els últims anys la descripció dels anticossos contra proteïnes sinàptiques a excepció dels dirigits contra NMDAR, s'ha focalitzat principalment en pacients d'edat avançada. D'aquesta manera, mentre que en població adulta s'han descrit nombrosos anticossos contra superfície neuronal responsables d'encefalitis autoimmune, a l'edat pediàtrica l'única encefalitis per autoimmunitat sinàptica ben caracteritzada fins el moment havia sigut l'encefalitis anti-NMDAR.

En aquesta tesi, amb l'objectiu principal de caracteritzar clínica e immunològicament encefalitis pediàtriques d'etiologia no filiada, ens hem centrat en tres grups de desordres amb evidència preliminar d'una etiologia immunomediada i que afecten de forma predominant a nens i adolescents: grup 1) encefalitis associada a la presència d'un teratoma sistèmic, grup 2) encefalitis amb crisis refractàries i/o status epilèptic, i grup 3) recidives neurològiques post-encefalitis herpètica. Els resultats obtinguts en els estudis que es presenten aquí, han donat lloc a la publicació de 5 articles originals en revistes internacionals d'alt impacte, responent als 3 objectius específics plantejats. Cal destacar que de les quatre publicacions que ja han estat difoses (la cinquena

està en premsa), en tres se n'ha publicat una editorial dedicada en el mateix número de la revista (veure articles), i que tots ells han estat ben rebuts en el camp de la pediatria, la neurologia i les neurociències. Un exemple de la repercussió que han tingut els estudis publicats en aquesta tesi han estat els 4 articles de revisió sobre encefalitis autoimmune que la doctoranda i el director de tesi han publicat durant el període de la realització d'aquesta tesi, prèvia invitació, en revistes de prestigi; incloent: *The Journal of Child Neurology 2012* (on la revisió que vàrem fer d'encefalitis autoimmune va ser l'article més llegit mensualment d'aquesta revista durant més de dos anys des de la seva publicació), *Current Opinion in Neurology 2014, Annals of the New York Academy of Sciences 2014*, i un nou capítol dedicat a encefalitis autoimmunes en *"Nelson Textbook of Pediatrics"* 20th edition, el llibre de referència de pediatria a nivell mundial (Veure CV i publicacions no defensades en Annexa).

Respecte el compliment dels objectius específics de la tesi, en el primer d'ells, ens plantejàvem caracteritzar clínica e immunològicament els pacients identificats amb sospita d'encefalitis autoimmune associada a un teratoma sistèmic sense anticossos contra NMDAR, per establir si la negativitat d'aquests anticossos es devia a una baixa sensibilitat en la tècnica de detecció o si estàvem davant d'un quadre paraneoplàstic diferent. En aquest treball (**Article 1, Armangue et al. Ann Neurol 2013**), sobre una cohort de 249 pacients amb encefalitis associada a teratoma, 211 tenien encefalitis anti-NMDAR, i 38 eren negatius per aquests anticossos. Un acurat estudi clínic dels pacients permeté identificar que 22 dels 38 pacients sense anticossos anti-NMDAR presentaven una síndrome clínica amb afectació predominant de cerebel i tronc, amb freqüent opsoclonus. El fet de que aquests símptomes es presentin de forma molt excepcional en pacients amb encefalitis anti-NMDAR, recolza fermament que ens trobem davant la descripció d'un nou quadre paraneoplàstic. A més a més, comparat amb els pacients amb anticossos contra NMDAR, els pacients sense aquests anticossos presentaren amb menys freqüència alteració de

comportament, disminució del nivell de consciència i crisis epilèptiques, i rarament presentaren discinèsies. Tot i que en la majoria d'aquests pacients no detectàrem anticossos en sèrum ni en LCR, la identificació d'aquesta nova síndrome neurològica és clínicament molt rellevant doncs amb freqüència aquests pacients responen a immunoteràpia (corticoides, gammaglobulines i/o recanvi plasmàtic), i/o a resecció tumoral. Les deu pacients de l'estudi que presentaren opsoclonus foren dones joves (rang 15-32 anys), considerant-se massa joves per la síndrome d'opsoclonus-mioclonus associada a carcinoma, que amb freqüència afecta a pacients majors de 50 anys, i massa grans per la síndrome d'opsoclonus-mioclonus associada a neuroblastoma, característica de nens menors de 5 anys. El reconeixement d'aquesta nova entitat destapa per tant un nou fenotip paraneoplàstic d'opsoclonus-mioclonus i d'encefalitis amb afectació de tronc i cerebel associada a la presència d'un teratoma sistèmic, que afecta predominantment a pacients joves. Aquests pacients presenten una resposta favorable a immunoteràpia i a resecció tumoral, i probablement havien sigut prèviament infradiagnosticats, i catalogats de patir un quadre "idiopàtic" o "post-infecciós". Per tant és important destacar que la cerca de la presència d'un tumor com el teratoma és essencial en pacients amb un quadre d'encefalopatia ràpidament progressiva com el descrit, i no s'hauria de restringir en pacients amb anticossos contra NMDAR.

En el següent objectiu de la tesi ens proposàvem identificar nous auto-antígens responsables d'encefalitis per autoimmunitat sinàptica associats a crisis i *status epilepticus* que afectessin de forma predominant a l'edat pediàtrica. En el segon article d'aquesta tesi (**Article 2, Petit-Pedrol M*, Armangue T*, Peng X* et al. Lancet Neurol 2014, *igual contribució**), hem caracteritzat un nou anticòs dirigit contra el receptor sinàptic GABA$_A$ que es troba involucrat en encefalitis amb crisis refractàries i *status epilepticus*, i que afecta de forma predominant a nens i adults joves. En aquest treball, identificàrem inicialment dos pacients índex de 15 i 56 anys d'edat

respectivament, que compartien característiques clíniques similars altament suggestives de patir una síndrome immunomediada: encefalitis amb crisis refractàries i *status epilepticus*, signes inflamatoris al LCR i alteracions de senyal córtico-subcorticals a la RM cerebral. A més a més, l'estudi immunològic de les mostres de sèrum i LCR d'ambdós pacients mostrà un mateix patró de reactivitat en les tècniques immunohistoquímica adaptades per la detecció d'anticossos contra auto-antígens sinàptics, suggerint que el quadre en ambdós casos era produït pel mateix auto-anticòs. La selecció d'aquests dos pacients amb un fenotip clínic e immunològic similar permeté com s'exposa en el treball, la identificació de l'autoantigen responsable del quadre: les subunitats alfa1/beta3 del receptor ionotròpic $GABA_A$. En aquest treball a més a més demostràrem els efectes d'aquests anticossos sobre la sinapsis en un model experimental *in vitro* en cultius de neurones hipocampals, en el qual l'aplicació dels anticossos dels pacients (LCR) produïa una disminució dels receptors $GABA_A$ a la sinapsis, i la reversibilitat dels efectes en remoure els anticossos dels pacients del medi de cultiu. Un cop identificat aquest nou anticòs, i desenvolupar un test diagnòstic amb cèl·lules HEK transfectades per expressar el receptor $GABA_A$, re-examinàrem les mostres de 150 pacients amb encefalitis i crisis epilèptiques identificant 4 pacients més afectes d'aquesta nova síndrome autoimmune, associant tots ells alts títols d'aquests anticossos en LCR i crisis epilèptiques refractàries. En aquest treball no identificàrem anticossos contra $GABA_AR$ en cap dels 75 controls sans examinats, però sí títols més baixos d'aquests anticossos ($<1/160$) en sèrum de pacients amb altres síndromes neurològiques immunomediades com la síndrome de la persona rígida i la síndrome d'opsoclonus-mioclonus. El significat d'aquesta última troballa encara és incert, ja que aquests pacients no tenien anticossos associats en LCR, i mereix més estudis posteriors. Resulta interessant l'observació de que alguns dels pacients amb encefalitis per anticossos contra $GABA_AR$ presentaven altres malalties autoimmunes o altres auto-anticossos concomitants com els

anticossos contra GAD$_{65}$ o els dirigits contra TPO. D'aquesta experiència, del fet de que els anticossos contra TPO es poden trobar fins en un 10% d'adults asimptomàtics, i de que no s'ha demostrat la seva patogenicitat, podem concloure que és important descartar la presència d'altres anticossos més rellevants com els dirigits contra proteïnes sinàptiques, en pacients amb encefalitis en els quals detectem anticossos TPO, abans de classificar-los amb el terme descriptiu d'encefalitis associada a malaltia tiroïdal o Encefalitis de Hashimoto.

En el tercer i últim objectiu d'aquesta tesi ens proposàrem estudiar si una resposta immunològica per autoimmunitat sinàptica contra NMDAR i/o contra altres proteïnes sinàptiques estava involucrada en la "*coreoatetosis post-herpètica*" i/o en altres formes de recidiva neurològica post-encefalitis herpètica. En el tercer article d'aquesta tesi (**Article 3, Armangue et al. J Pediatr 2013**) identificàrem i seguírem una cohort de 20 nens amb encefalitis anti-NMDAR de diferents centres de l'estat espanyol. En aquesta cohort de pacients identificàrem una nena de 2 anys que havia desenvolupat coreoatetosis, discinèsies orofacials i disminució severa del nivell de consciència (símptomes característics de l'encefalitis anti-NMDAR), poques setmanes després d'haver estat correctament diagnosticada i tractada per una EHS. Amb un diagnòstic inicial de "*coreoatetosis post-herpètica*", entitat d'etiologia prèviament desconeguda descrita en fases convalescents de l'EHS, va ser tractada empíricament amb corticoides i gammaglobulines sense evidenciar-se una resposta favorable. Setmanes més tard, la similitud del quadre clínic amb l'encefalitis anti-NMDAR i la confirmació d'alts títols d'anticossos contra NMDAR en el LCR de la pacient, recolzaren la hipòtesis d'una etiologia immunomediada del quadre, i permeteren ser més agressius en el tractament immunosupressor administrant rituximab i ciclofosfamida (abans no administrats per l'antecedent de la infecció viral). Després d'aquests tractaments, la pacient va presentar una recuperació progressiva dels símptomes de la recidiva (veure Vídeos Article 3),

tot i que els símptomes residuals de l'EHS prèvia (síndrome biopercular) persistiren. Aquest estudi, juntament amb la descripció gairebé simultània de que un 11% de pacients d'una cohort retrospectiva d'adults amb EHS tenien anticossos IgG contra NMDAR, van aportar per primer cop evidència de que un procés viral podia desencadenar la producció d'anticossos contra NMDAR, i de que aquests anticossos podien ser causa de recidives post-herpètiques incloent la forma clàssicament anomenada *"coreoatetosis post-herpètica"*.

Aquesta hipòtesis va ser ràpidament confirmada amb la identificació en els següents mesos de més de 22 nous casos (7 per altres autors[97-100] i 15 pel nostre grup, veure articles 4 i 5) de recidives post-herpètiques produïdes per anticossos anti-NMDAR. A més, en el quart article d'aquesta tesi (**Article 4, Armangue T*, Leypoldt F* et al. Ann Neurol 2014, *igual contribució**), mitjançant la identificació prospectiva de 5 pacients, incloent un lactant de tan sols 2 mesos d'edat, el pacient més jove descrit amb encefalitis anti-NMDAR, evidenciàrem la inexistència dels anticossos NMDAR en les mostres obtingudes durant la fase infecciosa de l'encefalitis, demostrant que la infecció viral és el desencadenant dels anticossos NMDAR, i per tant de la encefalitis associada, en aquest grup de pacients.

En el mateix treball identificàrem anticossos contra superfície neuronal (la majoria contra auto-antígens desconeguts) en el 58% de pacients d'una cohort retrospectiva de 34 pacients (adults i nens) amb EHS, dels quals es disposava d'una mostra de LCR obtinguda després de la primera setmana del debut de la infecció viral. El fet de que trobéssim que l'EHS havia desencadenat una resposta per autoimmunitat sinàptica en més de la meitat de pacients de la sèrie, incloent casos de totes les edats, però en canvi les recidives autoimmunes post-EHS només havien estat descrites fins aquell moment de forma excepcional, i gairebé exclusivament en nens menors de 3 anys, ens portà a plantejar la hipòtesis de que l'autoimmunitat desencadenada per l'EHS era un

fenomen infradiagnosticat. Amb l'objectiu d'estudiar la freqüència real d'aquest fenomen, així com la patogenicitat i la identitat dels anticossos contra superfície neuronal desencadenats per l'EHS, vàrem iniciar al gener de 2014 un estudi observacional multicèntric prospectiu amb més de 40 centres participants repartits entre les diferents comunitats autònomes. En aquest estudi, pacients amb EHS de totes les edats són avaluats immunològicament de forma cega a la informació clínica (amb l'excepció de que presentin símptomes suggestius de recidiva), des de l'inici de l'encefalitis viral i durant els 12 mesos posteriors. Tot i que aquest estudi encara està en curs, resultats preliminars després de la inclusió dels primers 20 pacients, ens han permès no només confirmar la hipòtesis de que les recidives autoimmunes post-EHS són freqüents (un 25% de la nostra sèrie prospectiva), sinó que hem identificat una nova forma clínica de recidiva autoimmune post-herpètica que afecta a adolescents i adults, i que amb freqüència és infradiagnosticada.

Aquests resultats han estat publicats recentment en el cinquè i últim article d'aquesta tesi (**Article 5, Armangue et al. Neurology 2015, en premsa**), en el qual es descriuen 14 nous pacients (6 nens petits i 8 adolescents-adults) amb recidives autoimmunes post-EHS identificats pel nostre grup en els últims 21 mesos, cinc d'ells procedint de l'estudi multicèntric prospectiu. La comparació entre les recidives post-herpètiques autoimmunes en nens petits (menors de tres anys) i en el grup d'adolescents i adults, ens permeté observar que les característiques clíniques de les recidives depenen de l'edat del pacient. Mentre que en el grup de 6 nens petits (3 d'ells de sexe masculí, mitjana d'edat de 13 mesos, rang 6-20 mesos, tots amb anticossos NMDAR, i un amb anticossos contra $GABA_AR$ concomitants), tots presentaren alteracions de moviment de tipus coreoatetòsic i/o discinètic típics de la *"coreoatetosis post-herpètica"*; 7 dels 8 adolescents i adults (5/8 de sexe masculí, mitjana d'edat de 40 anys, rang 13-69 anys, 5 amb anticossos NMDAR i 3 amb anticossos contra antígens desconeguts), es presentaren amb símptomes psiquiàtrics i alteracions

del comportament sense alteracions del moviment. Mentre que en el grup dels nens petits, la forma abrupta i dramàtica de debut portà a una ràpida identificació i tractament del quadre, la forma de presentació subaguda i purament o gairebé exclusivament psiquiàtrica en els adolescents i adults, condicionà que els símptomes fossin atribuïts inicialment a símptomes residuals de l'encefalitis viral i tractats inicialment amb fàrmacs simptomàtics. En els pacients adults i adolescents participants a l'estudi prospectiu, els símptomes de recidiva no foren identificats com a tals fins que van acudir a visites de seguiment establertes pel protocol de l'estudi. Aquest fet, ens portà a una modificació del protocol inicial, avançant la primera visita de seguiment al mes de l'alta hospitalària (aproximadament 50 dies post debut de l'encefalitis viral), i no als tres mesos com s'havia establert inicialment. Tot i l'important retràs diagnòstic en adults i adolescents (mitjana des de l'inici dels símptomes de recidiva fins el diagnòstic d'encefalitis autoimmune de 85 dies, rang 17-296 dies, respecte una mitjana en els nens petits de 4 dies, rang 0-55 dies, p=0.037), aquests pacients respongueren molt favorablement a immunoteràpia. Mentre que abans de la recidiva autoimmune els pacients eren capaços de col·laborar, de comunicar-se i de portar a terme algunes activitats de la vida diària d'acord amb les limitacions esperades causades per la necrosis induïda per la infecció viral (principalment afectant la memòria anterògrada i el llenguatge), durant la recidiva autoimmune els pacients es van tornar agressius, no col·laboradors, alguns d'ells amb idees suïcides, i fins i tot en algun cas progressant a disminució de consciència, crisis i coma. El tractament amb immunoteràpia (generalment amb corticoides endovenosos), va restablir la seva activitat basal prèvia a la recidiva (veure Figura 1, article 5).

Una altra troballa rellevant d'aquest estudi fou la demostració d'una captació intensa de contrast endovenós en les RM cerebrals obtingudes durant la recidiva autoimmune comparat amb una captació de contrast absent o mínima en les RM cerebrals obtingudes durant la fase viral. A més, la captació

de contrast presentà una evolució paral·lela a la resposta clínica dels pacients, persistint en pacients no tractats i simptomàtics, i disminuint o desapareixent coincidint amb el tractament i/o la milloria clínica dels pacients. Aquesta observació no havia estat prèviament publicada i mereix futurs estudis per veure si la RM cerebral amb contrast endovenós pot ser un potencial biomarcador per detectar i/o seguir recidives post-herpètiques autoimmunes.

En els grup dels nens petits, es confirmà en tots els casos estudiats que la *"coreoatetosis post-herpètica"* corresponia en realitat a encefalitis anti-NMDAR. La resposta a immunoteràpia en aquests casos va ser variable i no tan favorable a l'esperada comparat amb el primer cas descrit de *"coreoatetosis post-HSE"* associat a anticossos NMDAR (article 3) o amb els pacients amb l'encefalitis anti-NMDAR no relacionada amb l'EHS. A més de les diferències en la resposta a la immunoteràpia, es detectaren altres diferències entre l'encefalitis anti-NMDAR relacionada o no amb l'EHS. A mode d'exemple, tot i que la variació dels símptomes edat depenent observada en les recidives autoimmunes post-EHS van en la línia de les diferències en la forma de presentació depenents de l'edat descrites en l'encefalitis anti-NMDAR clàssica o no relacionada amb EHS (moviments anormals en nens petits, i símptomes psiquiàtrics en adolescents i adults), en aquesta última, la majoria dels pacients amb independència de l'edat acaben desenvolupant alteracions de moviment i/o crisis epilèptiques durant el curs de la malaltia. Per contra, cap dels pacients adolescents i adults amb encefalitis anti-NMDAR post-EHS desenvolupà crisis ni moviments anormals durant l'evolució. Les causes de les diferències observades entre l'encefalitis anti-NMDAR post-EHS respecte a l'encefalitis anti-NMDAR clàssica o no relacionada amb el virus són encara desconegudes. L'estudi del repertori dels epítops dels pacients amb anticossos anti-NMDAR post-EHS fou similar al repertori descrit en pacients amb encefalitis anti-NMDAR clàssica (veure Apèndix de l'article 3). Descartat aquest factor, hom podria postular que un diferent ambient immunològic en ambdues situacions, amb un major rol de la

immunitat cel.lular (cèl.lules T citotòxiques) en els casos secundaris a EHS, o la destrucció tissular i necrosis prèvia induïda per la infecció viral podrien ser factors implicats en explicar aquestes diferències.

Una altra troballa important en aquest treball fou que 3 dels 6 nens petits amb recidives autoimmunes post-herpètiques van desenvolupar crisis i *status epilepticus* durant la recidiva autoimmune (en dos d'ells precedint la coreoatetosis característica). En un d'aquests pacients, un lactant de 11 mesos d'edat (pacient 9), la recidiva es presentà en forma de espasmes epilèptics i hipsarrítmia (*Málaga I, Armangue T i Dalmau J, observació personal*), seguit d'un estatus coreic. La identificació d'anticossos NMDAR en aquest pacient podria representar la primera evidència de que un subgrup de casos d'epilèpsia de tipus espasmes infantils o Síndrome de Westpodrien ser secundaris o desencadenats per anticossos contra el receptor NMDAR. Aquesta hipòtesis ha estat recentment recolzada per la identificació de mutacions en GRIN2A i GRIN2B (gens codificants per subunitats del receptor NMDA) com a causants de Síndrome de West,[101,102] i pel model animal d'espasmes epilèptics causats per l'administració d'agonistes NMDA.[103] Mutacions *de novo* en GRIN1 (subunitat del receptor NMDA sobre la que van dirigits els anticossos dels pacients amb encefalitis anti-NMDAR), s'han descrit recentment en pacients amb encefalopatia epilèptica precoç amb debut abans del 3 mesos d'edat, dèficit cognitiu, i moviments hipercinètics, amb alteracions focals i difuses a l'EEG, però sense mostrar patrons de salva-supressió o hipsarrítmia.[104] També resulta interessant la identificació concomitant a anticossos contra NMDAR, d'anticossos contra GABA$_A$R (auto-antigen descrit recentment en encefalitis i crisis refractàries, veure Article 2), en un altre dels pacients de la sèrie de 15 mesos d'edat (pacient 10), que debutà en forma de *status epilepticus* refractari i coreoatetosis als 23 dies de l'EHS, i respongué favorablement a immunoteràpia agressiva (corticoides, gammaglobulines, recanvi plasmàtic i rituximab). Aquesta troballa, juntament amb la descripció prèvia de pacients amb recidives

autoimmunes post-EHS associades a anticossos contra D2R,[98] confirmen que l'EHS és un desencadenant d'autoimmunitat sinàptica no restringit a anticossos contra NMDAR.

Els resultats d'aquesta tesi suporten fermament la hipòtesis prèviament postulada de que els mecanismes autoimmunes tenen un paper important en la patogènesis de l'EHS. Donat a que els anticossos IgG dirigits contra GluN1 quan es troben en LCR sempre s'han associat a encefalitis anti-NMDAR,[51,105] i que aquests anticossos són patogènics en models de cultius de neurones[68] i en ratolins,[50] postulem que els anticossos contribueixen a produir els símptomes dels pacients. El paper patogènic dels altres auto-anticossos encara no és clar. Per tant, la determinació d'anticossos contra superfície neuronal (principalment NMDAR) en sèrum i LCR hauria de ser considerada en tots els pacients amb recidives post-EHS. En cas de positivitat, si la PCR per EHS en LCR és negativa, sembla raonable començar immunoteràpia empírica (per exemple: corticosteroides, gammaglobulines, i/o recanvi plasmàtic), i depenent de la resposta es podria plantejar tractaments més agressius com el rituximab. L'estudi multicèntric prospectiu en curs aclarirà si els anticossos contra superfície neuronal poden desenvolupar-se sense símptomes de recidiva post-EHS, o si existeix un títol llindar d'aquests anticossos per desenvolupar els símptomes. Futurs estudis esclariran si l'autoimmunitat desencadenada per l'EHS està relacionada amb les recents descripcions de defectes congènits relacionats amb l'estimulació de l'interferó, tant per defecte (per mutacions en la via dels TLR3 en pacients amb EHS), com per excés (en pacients amb *"interferonopaties"* i activació constant dels factors de transcripció derivats de l'interferó, i autoimmunitat associada).

VI. Conclusions

Conclusions generals:

- La selecció de pacients amb fenotips clínics similars i l'aplicació de tècniques altament sensibles i específiques per la identificació d'anticossos contra superfície neuronal ens ha permès caracteritzar el perfil clínic e immunològic de varis processos neurològics autoimmunes pediàtrics d'etiologia prèviament desconeguda, que amb freqüència responen a immunoteràpia.

Conclusions específiques Subgrup 1:

- És important el reconeixement d'un nou fenotip paraneoplàstic d'opsoclonus-mioclonus i d'encefalitis amb afectació de tronc i cerebel associada a la presència d'un teratoma sistèmic, sense anticossos NMDAR, que afecta predominantment a pacients joves, i que respon favorablement a immunoteràpia i a resecció tumoral.

- La cerca d'un tumor com el teratoma és essencial en pacients amb quadres d'encefalopatia ràpidament progressives com el descrit, i no s'hauria de restringir en pacients amb anticossos anti-neuronals.

Conclusions específiques Subgrup 2:

- Hem caracteritzat un nou anticòs dirigit contra el receptor sinàptic $GABA_A$ que es troba involucrat en encefalitis amb crisis refractàries i *status epilepticus*, que afecta de forma predominant a nens i adults joves, i es caracteritza per LCR de característiques inflamatòries, alteracions córtico-subcorticals en la RM cerebral, associació a altres factors de propensió a l'autoimmunitat (ex. anticossos contra TPO, GAD65 concomitants), i amb potencial resposta a immunoteràpia.

- Els anticossos contra GABA$_A$R són patogènics en el model *in vitro* basat en cultius de neurones hipocampals murines, produint una disminució dels receptors GABA$_A$ a la sinapsis, i aquests efectes són reversibles en eliminar els anticossos del medi de cultiu.

Conclusions específiques Subgrup 3:

- L'EHS és un fort desencadenant d'autoimmunitat sinàptica no restringida a NMDAR.

- Anticossos contra NMDAR i/o altres proteïnes sinàptiques poden ser causa de recidives autoimmunes post-EHS. En nens petits aquestes recidives es manifesten en forma de "coreoatetosis post-EHS" amb o sense crisis refractàries i status epilèptic acompanyant; i en adolescents i adults es manifesten predominantment en forma d'alteracions de comportament i psiquiàtriques.

- La identificació de recidives autoimmunes post-EHS, fenomen prèviament infradiagnosticat, és molt important doncs aquests pacients poden respondre a immunoteràpia.

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VIII.   Annexes

Traducción tesis en castellano

Resumen

Autoinmunidad sináptica como causa de encefalitis asociada a teratoma, epilepsia y recidivas post-encefalitis herpética en la infancia

Introducción: En los últimos 7 años, se ha identificado una nueva categoría de encefalitis asociada a anticuerpos contra superficie neuronal, que cursan con psicosis, catatonia, crisis epilépticas, y movimientos anormales. Estos desórdenes son potencialmente letales, pero curables si se reconocen y se tratan.

Hipótesis: Un subgrupo de encefalitis pediátricas previamente consideradas idiopáticas o post-infecciosas están causadas por anticuerpos dirigidos contra antígenos sinápticos o de la membrana neuronal.

Objetivos: Caracterización clínica e inmunológica de encefalitis pediátricas de etiología no filiadas, centrándonos en 3 grupos de pacientes con evidencia preliminar de un origen inmunomediado: 1) encefalitis asociada a la presencia de un teratoma a nivel sistémico; 2) encefalitis con crisis refractarias y status epiléptico; y 3) recidivas neurológicas post-encefalitis herpética (EHS).

Metodología: Estudio clínico e inmunológico de pacientes con los desórdenes propuestos. Para la identificación de los antígenos se ha utilizado una estrategia previamente validada consistente en una selección clínica de pacientes con fenotipos similares asociado a técnicas de despistaje inmunológico desarrolladas y adaptadas para la detección de anticuerpos dirigidos contra antígenos de superficie neuronal, incluyendo: inmunohistoquímica en cultivos de neuronas, inmunoprecipitación, y caracterización de antígenos mediante espectrometría de masas. Los efectos de los anticuerpos de los pacientes sobre los antígenos diana se han investigado en cultivos de neuronas disociadas de hipocampo de rata.

Resultados: Por objetivos específicos: 1) Hemos identificado un nuevo fenotipo paraneoplásico del síndrome de opsoclonus-mioclonus y de encefalitis con afectación de tronco y cerebelo asociada a la presencia de un teratoma sistémico, sin anticuerpos anti-NMDAR, que afecta de forma predominante a pacientes jóvenes, y que responde favorablemente a inmunoterapia y a resección tumoral. 2) Hemos caracterizado un nuevo anticuerpo dirigido contra el receptor sináptico GABA$_A$ involucrado en encefalitis con crisis refractarias y *status epilepticus*, que afecta de forma predominante a niños y adultos jóvenes. En el modelo in vitro estos anticuerpos producen una disminución de los receptores GABA$_A$ en la sinapsis, y estos efectos son reversibles en eliminar los anticuerpos del medio de cultivo. 3) Hemos demostrado que la EHS es un fuerte desencadenante de autoinmunidad sináptica no restringida a NMDAR. Hemos descrito por primera vez que anticuerpos contra NMDAR o otras proteínas sinápticas pueden ser causa de recidivas autoinmunes post-EHS. En niños pequeños estas recidivas se manifiestan en forma "coreoatetosis post-EHS" con o sin crisis refractarias y status epiléptico acompañante, y en adolescentes y adultos se manifiestan predominantemente con síntomas psiquiátricos.

Conclusiones: Estos estudios han resultado en la identificación de nuevos síndromes y respuestas inmunológicas, en la caracterización de las dianas antigénicas, y en el desarrollo de un test diagnóstico para un grupo de encefalitis infantiles inmunomediadas. Los estudios han cambiado los paradigmas de diagnóstico y tratamiento de las encefalitis infantiles. Esto se debe en gran parte a que las investigaciones han facilitado el diagnóstico y tratamiento precoz de desórdenes de etiología previamente desconocida con frecuente respuesta favorable a la inmunoterapia.

Prólogo

La que encontraréis aquí es la emotiva carta que el radiólogo canadiense Alan Carr, escribió a The Lancet el año 1982. En ella, este afligido padre explicaba como en pocas semanas vio como super-Jane, su hija de 16 años, que había sido una de las alumnas más destacadas de su clase desarrollaba déficits de memoria y alteraciones de conducta progresivos que la llevaron a ingresar en un hospital psiquiátrico. Casi por casualidad en una radiografía de tórax de rutina se detectó una masa pulmonar. Tenia Hodgkin. Recibió quimioterapia. I la memoria, y con ella su hija, volvieron.

Cuando el Dr. Carr escribió esta carta el año 1982, no sabía la causa del que le había sucedido a su hija pero estaba convencido que la pérdida de memoria de Jane y la alteración de su comportamiento tenían una relación directa con su enfermedad de Hodgkin y el más importante, habían sido reversibles con la quimioterapia. El Dr. Carr nombró a este fenómeno "Síndrome de Ofelia" en honor a la diosa griega que no podía recordar, y se decidió a publicarlo porque como el mismo explicó, a lo mejor podría ayudar a alguien con un diagnóstico difícil.

Y esto es precisamente lo más apasionante y bonito de la neurología pediátrica, la especialidad por excelencia de las enfermedades minoritarias. Frecuentemente nos encontramos con casos difíciles, excepcionales. Pero lo más importante es observarlos, intentarlos entender y sobretodo compartirlos. Es aquí donde entra mi otra gran pasión, la investigación. Intentar ir un poquito más allá, intentar comprender el por qué de lo que has visto al lado de la cama del paciente!

Gracias a la investigación y a los trabajos previos del director de esta tesis, el Dr. Dalmau, que han sido la base del trabajo que presentamos en este

manuscrito, ahora podemos explicar qué le pasaba a Jane. El síndrome de Ofelia o la encefalitis asociada al linfoma de Hodgkin, es una encefalitis autoinmune producida por anticuerpos dirigidos contra el receptor metabotrópico del glutamato 5 (mGluR5. Estos anticuerpos, probablemente desencadenados por una disfunción de los mecanismos de tolerancia inmunológica generada por su linfoma, alteran la función del receptor produciendo una disfunción de la transmisión sináptica neuronal que causa los síntomas de Jane, y lo más importante, y tal y como había observado su padre hace 30 años sin entender bien por qué, esta alteración es reversible en eliminar los anticuerpos y su desencadenante, el tumor. El descubrimiento de los mecanismos responsables de estas enfermedades y la posibilidad de identificar biomarcadores ha sido el inicio indispensable para una correcta comprensión de estos desórdenes, establecer factores pronósticos, y seguramente será la base para explorar posibles futuras dianas terapéuticas.

En esta tesis lo que pretendemos es precisamente esto, algo tan simple y a la vez tan difícil como es dar "nuevos nombres" a antiguas enfermedades.

Thaís Armangué

Barcelona, 15 de mayo de 2015
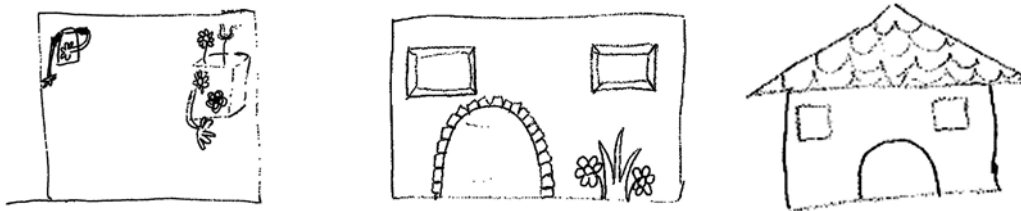
Representació d'una casa per part d'una pacient amb una recidiva autoimmune post-herpètica, abans (esquerra) i després de rebre tractament amb immunoteràpia (dreta). Armangue et al. Neurology (in press), i Article 5 de la Tesi.

Introducción

Encefalitis, aún un reto diagnóstico

Encefalitis es un término referido a una patología inflamatoria del cerebro que causa alteración aguda del estado mental, crisis epilépticas o déficits neurológicos focales, que frecuentemente se acompaña de inflamación del líquido cefalorraquídeo (LCR) y con hallazgos en la resonancia magnética cerebral (RM) variables que pueden ir desde la normalidad hasta extensas lesiones.[2] Esta, es una enfermedad grave que resulta en 7.3 hospitalizaciones por 100.000 personas/año,[2] y que afecta a pacientes de todas las edades y de todas las condiciones inmunológicas, dado que afecta a pacientes de todas las edades y condiciones inmunológicas, dado que puede afectar individuos previamente sanos o a pacientes severamente inmunodeprimidos, aunque, las condiciones basales del paciente serán determinantes a la hora de establecer la etiología. Al debut de la encefalitis el pronóstico de los pacientes es incierto dado que aunque es una patología severa con a alta mortalidad durante el período agudo (es causa de 1.400 muertes/año en Estados Unidos) y con un alto riesgo de secuelas neurológicas y cognitivas, dependiente principalmente de su etiología, la precocidad de instauración de un tratamiento etiológico cuando es posible, y la calidad de las medidas de soporte durante la fase aguda, los pacientes pueden eventualmente recuperarse completamente. Por lo tanto, la identificación precoz de la etiología de la encefalitis es fundamental para establecer un potencial tratamiento etiológico y establecer un pronóstico adecuado y, se convierte en el principal reto del médico en el manejo de estos pacientes. No obstante, las causas de encefalitis son múltiples y la mayoría de pacientes son sometidos a múltiples test etiológicos infecciosos sin llegar a identificar el agente causal. Un estudio reciente del *California Encephalitis Project*, un centro destinado al estudio de la epidemiología y las etiologías de pacientes con encefalitis, describió que el 63% de pacientes permanecen con

una etiología desconocida después de una batería de más de 16 agentes infecciosos.[3] Múltiples estudios prospectivos multicéntricos a nivel nacional con pacientes con encefalitis en diferentes países occidentales coinciden en las mismas cifras alarmantes de pacientes sin un diagnóstico etiológico después de extensos y costosos estudios microbiológicos.[4,5] El reciente descubrimiento de que varios tipos de encefalitis son inmunomediadas y son causadas por anticuerpos dirigidos contra proteínas sinápticas o de la superficie neuronal es un concepto nuevo que ha dado un diagnóstico definitivo a muchos de estos casos y que ha cambiado el manejo diagnóstico y terapéutico de pacientes con encefalitis de todas las edades.[6-10]

Antecedentes históricos

De los anticuerpos contra antígenos intracelulares como biomarcadores de encefalitis límbica paraneoplásica en pacientes de edad avanzada a los anticuerpos contra epítopos extracelulares como causa de encefalitis autoinmune en la edad pediátrica

El término de "*síndrome paraneoplásico*" se refiere al conjunto de síntomas y/o signos que se producen como consecuencia de un cáncer pero que no se pueden explicar por un efecto masa del tumor ni por la presencia local de células tumorales o metástasis. Aunque este término no se introdujo hasta medianos de los años 1950,[11] y no se usó ampliamente en la literatura médica hasta los años 1970,[12] habían estado descritos síndromes neurológicos y no neurológicos asociados a cáncer como la *phlegmasia alba* (actualmente conocida como el *síndrome de Trousseau o* tromboembolismo venoso asociado a adenocarcinoma de pulmón o páncreas), muchos años antes.[13] Las primeras descripciones clínicas de desórdenes neurológicos asociados a cáncer remontan al siglo XIX cuando el año 1887 Oppenheim y Siemerling describieron 2 pacientes con carcinoma gástrico y neuropatía periférica.[14] Oppenheim también

fue el primero en formular la hipótesis en 1888 que síntomas neurológicos centrales podían ser manifestaciones a distancia de un cáncer cuando describió una mujer con hemiparesia derecha y afasia desarrolladas solo unos días antes de morir por un cáncer extendido sin hallar restos tumorales metastásicas a nivel del sistema nervioso central (SNC) en la autopsia.[15] Aunque en ese momento Oppenheim sugirió el efecto de una "sustancia tóxica" producida por el cáncer como causa de los síntomas, es probable que esta paciente presentara micro-metástasis no detectadas en ese momento, puesto que actualmente sabemos que la hemiparesia y la afasia raramente representan síndromes paraneoplásicos.[16] La primera descripción clínica de un síndrome neurológico que actualmente podemos reconocer como "realmente paraneoplásico" fue en el año 1919 cuando Brouwer describió una mujer con un síndrome cerebeloso subagudo y un probable adenocarcinoma de ovario (que él llamó sarcoma pélvico).[17] Nuevamente en este caso se formuló la hipótesis de una potencial "sustancia tóxica" producida por el cáncer como causante de los síntomas al encontrarse en la autopsia una pérdida de células de Purkinje del cerebelo. Posteriores hipótesis de la relación entre cáncer y síntomas neurológicos incluyeron la competición entre células cancerígenas y las neuronas del ganglio dorsal por un "sustrato esencial",[18] y un reflejo resultante de la estimulación del nervio vago por parte del tumor como causa de neuropatía sensitiva asociada a cáncer,[19] correspondiendo actualmente a uno de los síndromes paraneoplásicos más reconocidos, el cuál se caracteriza por neuropatía y cáncer pulmonar de células pequeñas (SCLC), asociados a anticuerpos contra la proteína intracelular Hu.[16]

El concepto de autoinmunidad como posible causa de síndromes paraneoplásicos no se planteó hasta los años 1960 cuando diferentes descripciones clínicas y patológicas asociaron la presencia de un cáncer con cuadros clínicos neurológicos diversos y hallazgos inflamatorios en las necropsias, sugiriendo una posible respuesta del sistema inmune contra las

células del sistema nervioso.[20,21] En ese momento pero, no se descartaba que los infiltrados inflamatorios fueran producto de una infección viral[22] no identificada o de una reacción inflamatoria secundaria a la destrucción tisular producida por el cáncer.[23,24] En el año 1968, *Corsellis y colaboradores* describieron por primera vez la encefalitis límbica paraenoplásica como entidad clínicopatológica.[25] La encefalitis límbica se caracteriza por afectación inflamatoria de áreas límbicas, típicamente cursando con alteración de la memoria, crisis epilépticas del lóbulo temporal y trastornos afectivos. Para su diagnóstico es preciso demostrar alteraciones inflamatorias en estas áreas a nivel patológico (en biopsia o necropsia); o en su defecto afectación de áreas mediotemporales en la RM cerebral, enlentecimiento o anomalías epileptiformes localizadas en áreas temporales del electroencefalograma (EEG), y/o un LCR de características inflamatorias.[26]

Actualmente sabemos que la encefalitis límbica afecta de forma casi exclusiva a pacientes adultos y se asocia en la mayoría de los casos a la presencia de un cáncer (principalmente un SCLC en mayores de 40 años o un germinoma testicular en pacientes más jóvenes).[26] Su diagnóstico continua siendo un reto dado que síntomas similares (alteraciones de memoria, crisis epilépticas y alteraciones del comportamiento), pueden ser causados por otras complicaciones del cáncer, incluyendo metástasis cerebrales, infecciones, déficits nutricionales o metabólicos, episodios vasculares o complicaciones del tratamiento. Además, la aparición de los síntomas neurológicos con frecuencia precede a la identificación del cáncer subyacente complicando aún más el diagnóstico.[26] En los años 1980 y 90 el descubrimiento de que algunos de estos pacientes asociaban en suero y LCR anticuerpos dirigidos contra epítopos intracelulares como Hu, Yo, CV2/CRMP5 o Ma2 (Ta),[27-29] fue de gran ayuda para el diagnóstico de estas entidades. La identificación de anticuerpos intracelulares o onconeuronales en un paciente con encefalitis límbica o con otro síndrome paraneoplásico bien definido, como la degeneración cerebelosa, la

encefalomielitis o una polineuropatía periférica sensitiva, permitía establecer el origen paraneoplásico del síndrome neurológico, y alertar de la presencia de un tumor oculto en los pacientes en los cuales el síndrome neurológico precedía al diagnóstico del cáncer.[30,31] Aunque su importancia en la práctica clínica como biomarcadores diagnósticos de los síndromes paraneoplásicos, el hecho que el epítopo de estos anticuerpos sea intracelular, que los pacientes presenten una escasa respuesta a tratamiento inmunosupresor, la falta de reproducción del cuadro neurológico en modelos animales en los cuales se inyectan estos anticuerpos y, la presencia de estos anticuerpos, aunque en títulos menores, en pacientes con neoplasias sin síntomas neurológicos asociados, han hecho cuestionar su patogenicidad. Por estos motivos, actualmente se postula que estos anticuerpos no son los responsables directos de los síntomas neurológicos asociados sino que representan el componente humoral de una compleja respuesta inmunológica mediada por células T citotóxicas contra antígenos intracelulares expresados por las células tumorales.[32]

En el año 2001 se describió que algunos pacientes con encefalitis límbica con o sin asociación tumoral (ej. timoma), que eran seronegativos para los anticuerpos onconeuronales clásicos, tenían en suero y/o LCR anticuerpos dirigidos contra las subunidades Kv1.1 i Kv1.2 de los canales de potasio dependientes de voltaje (VGKC).[33] Aunque más tarde se descubrió que esta asunción era errónea, y que el epítopo al que iban dirigidos estos anticuerpos no era el VGKC sino dos proteínas sinápticas asociadas (la proteína 1 inactivada del glioma rica en leucina [LGI1] y la proteína asociada a contactina-like 2 [Caspr2],[34-38] es importante destacar que por primera vez estos autoanticuerpos no iban dirigidos contra proteínas intracelulares sino contra proteínas sinápticas o de la membrana celular. Además, estos pacientes, a diferencia de los pacientes con anticuerpos onconeuronales clásicos, sí que respondían favorablemente a inmunoterapia y con frecuencia no se encontraba un tumor subyacente.

No fue hasta el año 2005 con el trabajo publicado para *Ances y colaboradores*[39] cuando el concepto de encefalitis con repuesta favorable a inmunoterapia asociada a anticuerpos contra superficie neuronal dejó de restringirse a pacientes con encefalitis límbica y a pacientes con anticuerpos contra "VGKC", marcando el inicio de la identificación molecular de los autoantígenos diana y de la comprensión de estos desórdenes. En este trabajo, dirigido por el director de esta tesis, se presentaron 6 pacientes adultos con diferentes tipos de encefalitis con o sin asociación tumoral (2 tenían teratomas, 2 timomas, y 2 no tenían cáncer), que respondieron de favorablemente cuando fueron tratados con inmunoterapia y/o resección tumoral cuando estuvo indicado. Esta diferencia en la respuesta a inmunoterapia comparado con los síndromes paraneoplásicos clásicos y el hallazgo en todos ellos de anticuerpos que reaccionaban contra la superficie neuronal (solo uno de ellos con un patrón en la inmunohistoquímica similar al previamente descrito como "anti-VGKC"), hizo pensar a los investigadores que se trataba de una categoría de encefalitis autoinmune.

Poco después, en el trabajo publicado por *Vitaliani y colaboradores*[40] se describía minuciosamente un nuevo síndrome común (síntomas psiquiátricos predominantes, crisis epilépticas, déficits de memoria, y disfunción autonómica) en 4 mujeres jóvenes afectas de un teratoma de ovario, que asociaban anticuerpos contra superficie neuronal diferentes a "anti-VGKC". Gracias a la optimización en este trabajo de las técnicas de inmunohistoquímica en tejido cerebral de rata adaptadas para detectar anticuerpos contra la superficie neuronal (Figura 1), y a la identificación en poco tiempo de centenares de pacientes de todo el mundo afectos de este nuevo síndrome, permitieron en el año 2007 a *Dalmau y colaboradores*, la identificación del autoantígeno diana asociado mediante inmunoprecipitación: la subunidad GluN1 (o NR1) del receptor N-metil-D-aspartato (NMDAR),[6] así como la caracterización clínica de esta nueva entidad, hoy conocida como encefalitis anti-NMDAR. Actualmente se

sabe que esta encefalitis afecta a pacientes de todas las edades, principalmente niños y adultos jóvenes, y que la asociación tumoral, generalmente un teratoma de ovario, es sexo y edad dependiente, siendo su asociación en la edad pediátrica muy inusual (ver: Foco en la encefalitis autoinmune en la edad pediátrica y nuevas perspectivas, página 237).[41]

Figura 1: Demostración de anticuerpos antineuronales con técnicas de inmunofluorescencia e inmunohistoquímica
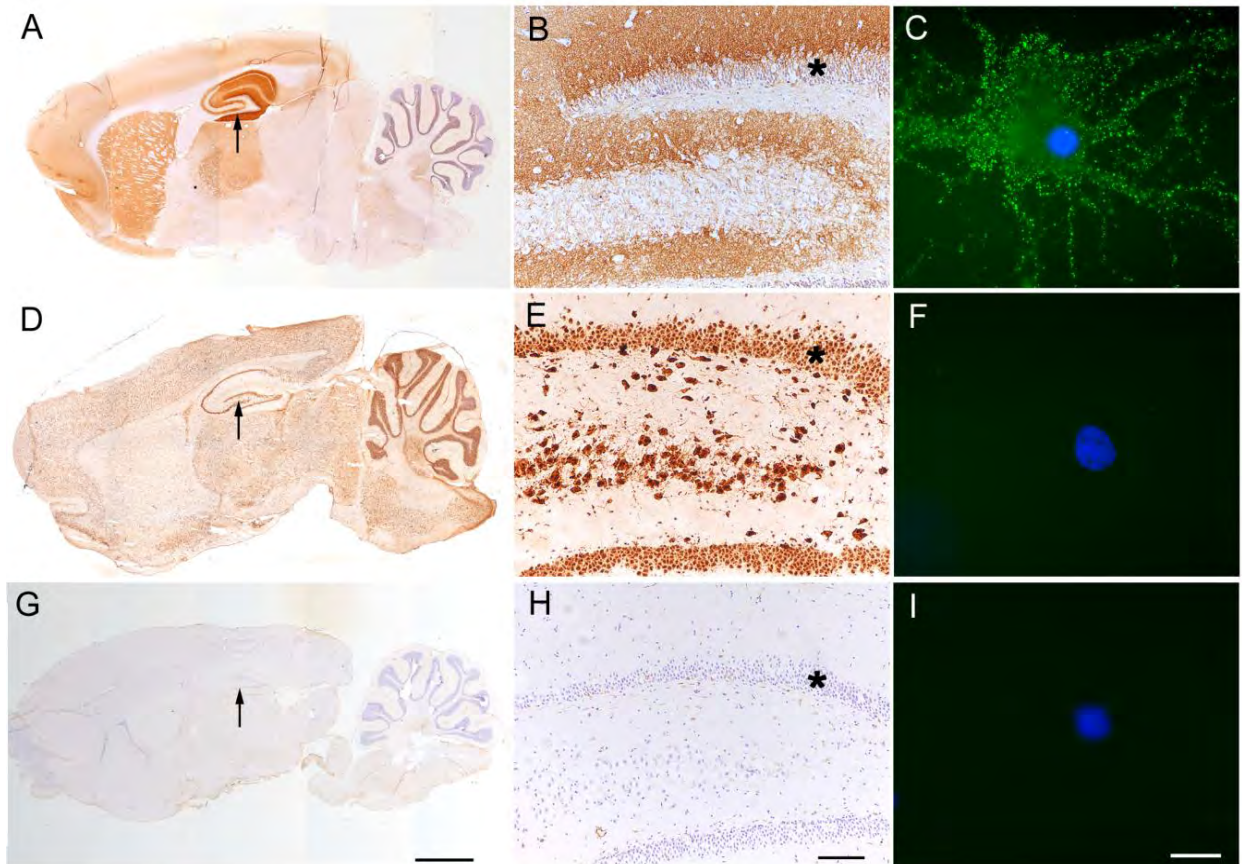

Inmunohistoquímica en cerebro de rata usando LCR de pacientes con anticuerpos anti-NMDAR (A), Hu (D), y LCR de un paciente control (G). Las flechas marcan áreas en las que se muestra una mayor amplificación en B, E y H. Se observa que el patrón de reactividad de los anticuerpos anti-NMDAR es expresado en la superficie celular y en regiones sinápticas, y es diferente del patrón de reactividad de los anticuerpos anti-Hu (en los cuales su diana, HuD, es intracelular). En B, E y H el asterisco indica el giro dentado. C, F y I muestran el correspondiente LCR incubado en cultivos de neuronas disociadas de hipocampo de rata (vivas, no permeabilizadas). Solamente los anticuerpos anti-NMDAR reaccionan contra antígenos de superficie en las nueronas vivas (tinción verde en C); los anticuerpos anti-Hu no muestran reactividad dado que los anticuerpos no penetran en la célula (F), además hay una falta de reactividad en el LCR control (I). En C, F, y I el núcleo de las neuronas se marca con DAPI. Escala en G = 2000 µm, escala en H = 100 µm, escala en I = 20 µm.

Figura adaptada de:
Armangue T, Petit M, Dalmau J. J Child Neurol 2012;27:1460-1469.

Métodos similares permitieron durante los años siguientes, la caracterización de los diferentes antígenos sinápticos y receptores de membrana diana de los anticuerpos asociados a los ostros síndromes neurológicos descritos por *Ances y colaboradores* unos años antes como: los anticuerpos dirigidos contra los receptores del ácido propiónico α-amino-3-hidroxi-5-metil-4-isoxazol (AMPAR, Figura 2)[42] o del ácido γ-amino-butírico B (GABA$_B$R),[43] ambos anticuerpos asociados a encefalitis límbica paranoplásica considerada previamente seronegativa, o los autoantígenos responsables de los cuadros originariamente atribuidos a los anticuerpos contra "VGKC", que en realidad iban dirigidos contra otras proteínas sinápticas asociadas incluyendo la proteína LGI1[44] y la proteína Caspr2.[34,35] Estas técnicas también permitieron en el año 2011 la identificación del receptor metabotrópico del glutamato mGluR5 como autoantígeno diana de la encefalitis autoinmune asociada al linfoma de Hodgkin o Síndrome de Ofelia (ver Prólogo),[45] y más recientemente, la identificación de los anticuerpos contra la proteína parecida a dipeptidil-peptidasa 6 (DPPX), esta vez sí, una subunidad reguladora de los canales de potasio Kv4.2, asociada a encefalitis y hiperexcitabilidad del SNC.[46]

Figura 2: Identificación del receptor AMPA como un autoantígeno relacionado con pérdida de memoria y alteración de conducta
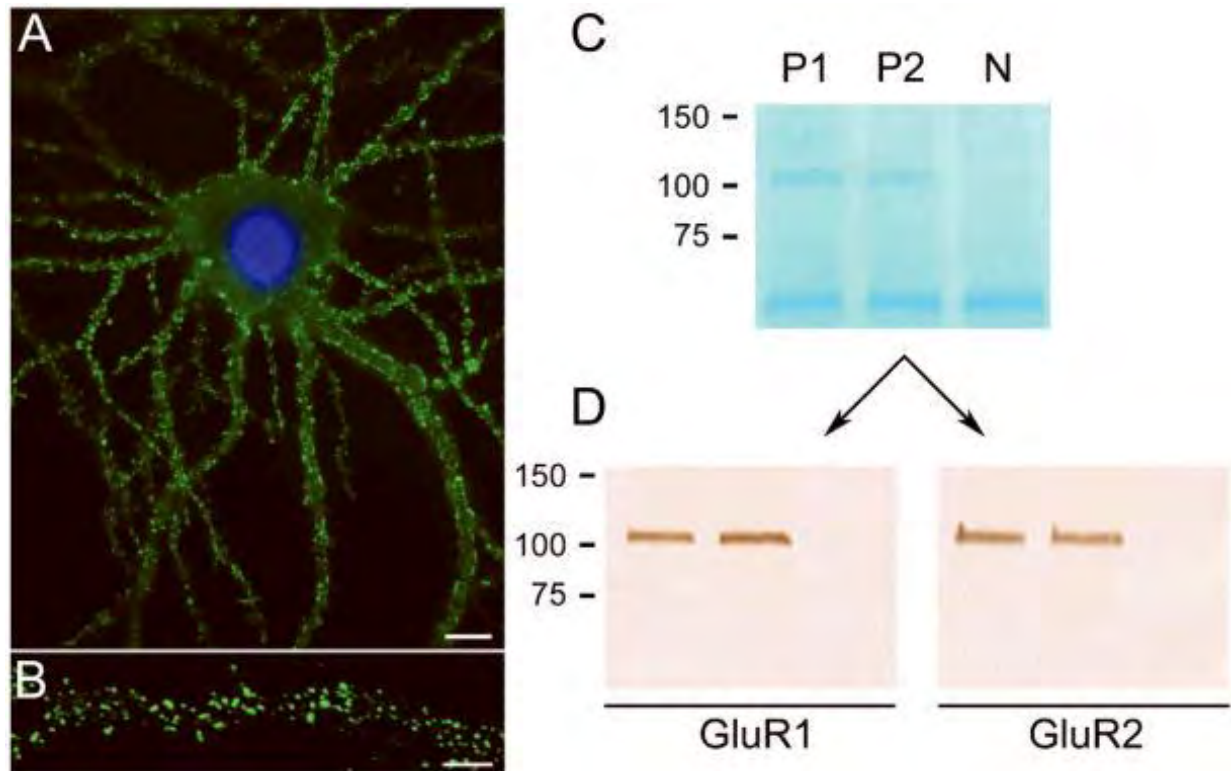
Cultivo de neuronas de hipocampo disociadas e incubadas (vivas, no permeabilizadas) con el LCR del paciente. Se observa la intensa reactividad de los anticuerpos del paciente con antígenos de superficie (A); escala = 10 μm. El análisis con microscopia confocal sugiere que los antígenos están concentrados en complejos (*clusters*) en las dendritas (B); escala = 10 μm. La precipitación de estos antígenos con el LCR de 2 pacientes se muestra en un gel en el cual las proteínas se visualizan con EZBlue (C). Los anticuerpos de los pacientes (P1 y P2) precipitan una banda de ~100 kDa; esta banda no se observa en el precipitado con el LCR control (N). El análisis de una banda de 100 kDa mediante espectrometría de masas demostró secuencias derivadas de las subunidades GluR1/GluR2 del receptor AMPA. La banda de ~50 kDa que se observa en todas las muestras corresponde a IgG. La transferencia de la banda proteica a nitrocelulosa e immunoblot con anticuerpos específicos contra GluR1 y GluR2 confirmó que la banda de 100 kDa contenía las dos subunidades GluR1 y GluR2 del receptor AMPA (paneles en D).

Figura adaptada de:
Meizan Lai M, Hughes EG, Peng X et al. Ann Neurol 2009; 65(4): 424–434.

Estado actual del tema: Clasificación de las encefalitis autoinmunes según la localización del autoantígeno diana

Desde el año 2005, la frecuencia del descubrimiento de nuevos síndromes neurológicos asociados a anticuerpos contra superficie neuronal y proteínas sinápticas ha sido de una o dos por año. En estas entidades, los anticuerpos van dirigidos contra receptores y proteínas sinápticas involucradas en la transmisión sináptica, plasticidad, o excitabilidad neuronal, y se asocian a síndromes que aunque severos, frecuentemente responden a inmunoterapia.[10,47,48] Los síndromes resultantes varían en función del anticuerpo involucrado, con fenotipos que parecen aquellos en los que la función del antígeno es modificada genéticamente o farmacológicamente (Tabla 1).[10,47,48]

Tabla 1: Encefalitis causadas por anticuerpos contra antígenos sinápticos

Antígeno	Síntomas Neurológicos	Edad, sexo, presencia de tumor, respuesta a inmunoterapia
NMDAR (subunidad GluN1)	Síntomas psiquiátricos, alteración del lenguaje, movimientos anormales, crisis epilépticas, disminución del nivel de conciencia, inestabilidad autonómica	Niños (40%) y adultos jóvenes (edad mediana 19 años), 80% mujeres. La presencia del tumor (mayoritariamente un teratoma) varía según la edad, el sexo, la raza (9-55%); la mayoría teratoma de ovario 80%; buena recuperación con inmunoterapia
GABA$_B$R (subunidad B1)	Encefalitis límbica clásica. Crisis prominentes (GABA$_B$R), síntomas psiquiátricos aislados (AMPAR), hiponatremia y breves crisis tónico-clónicas (LGI1)	Adultos (edad mediana 62, 80 y 60 años respectivamente), Mujeres (50, 90, y 35% respectivamente) Asociación a cáncer (pulmón, mama, timo) frecuente (60-70%) en GABA$_B$R y AMPAR infrecuente (<10%) en LGI1 Buena respuesta a inmunoterapia
AMPAR (subunidad Glu R1/2)		
LGI1		
CASPR2	Síndrome de Morvan, encefalitis límbica, hiperexcitabilidad del nervio periférico	Adultos (edad mediana 60 años, predominio masculino) Experiencia limitada, ~30% timoma
mGluR5	Síndrome de Ofelia: Cambios de personalidad, crisis epilépticas, déficit de memoria, y regresión cognitiva	Adolescentes y adultos jóvenes Asociación frecuente a linfoma de Hodgkin
DPPX	Encefalitis difusa, síntomas prodrómicos (diarrea), síntomas psiquiátricos, temblor, mioclonías, ataxia, nistagmus e hiperekplexia	Adultos Experiencia limitada, no asociación a cáncer, respuesta a inmunoterapia

Dado al amplio espectro de síntomas provocados por estas encefalitis, incluyendo alteraciones del comportamiento, psicosis, catatonia, déficits de memoria, crisis epilépticas, movimientos anormales y desregulación autonómica, los pacientes requieren un manejo multidisciplinar, y su descubrimiento ha afectado a múltiples especialidades como la neurología, la psiquiatría, la pediatría, la medicina interna e intensivista, la psicología clínica o la medicina rehabilitadora.[49]

Al contrario que los síndromes paraneoplásicos clásicos como la encefalitis límbica asociada a anticuerpos intracelulares o onconeuronales, las encefalitis causadas por autoinmunidad sináptica afectan a pacientes de todas las edades, incluso algunas de ellas como la encefalitis anti-NMDAR afectan de forma predominante a niños, pueden desarrollarse con o sin la presencia de un tumor, y se asocian a anticuerpos que se dirigen contra epítopos o dianas extracelulares de la membrana neuronal. Además , en los desórdenes en los que los efectos de los anticuerpos se han investigado, se ha demostrado que la aplicación de los anticuerpos (LCR o inmunoglobulinas G [IgG] del suero de los pacientes) en cultivos primarios de neuronas hipocampales *in vitro*, produce una alteración funcional y/o morfológica de los antígenos correspondientes, y estos efectos son reversibles en eliminar los anticuerpos del medio de cultivo.[41]

Más recientemente, *Planagumà y colaboradores* han desarrollado un modelo murino en el cual, la transmisión pasiva de LCR de pacientes con encefalitis anti-NMDAR por infusión ventricular continua (mediante bombas osmóticas durante 14 días), provoca alteraciones de memoria y comportamiento de estos animales.[50] El estudio necrópsico los hipocampos de los ratones mostró un depósito progresivo de anticuerpos anti-NMDAR y una disminución progresiva exclusiva de los receptores NMDA de la sinapsis, sin afectar a otras proteínas o receptores sinápticos como PSD95 o AMPAR. Tanto los síntomas como los niveles de los receptores NMDA de la sinapsis se

restablecieron gradualmente tras la cesión de la bomba de infusión, demostrando definitivamente la patogenicidad de estos anticuerpos (Fig.3).[50]

La reversibilidad de los efectos de los anticuerpos es apoyada a nivel clínico por el hecho que la disfunción neurológica asociada a las encefalitis autoinmunes causadas por anticuerpos contra superficie neuronal con frecuencia responde a inmunoterapia, y en el caso de la encefalitis anti-NMDAR hasta un 70-85 % de los pacientes pueden presentar una recuperación completa.[51]

Figura 4: Modelo murino de encefalitis anti-NMDAR
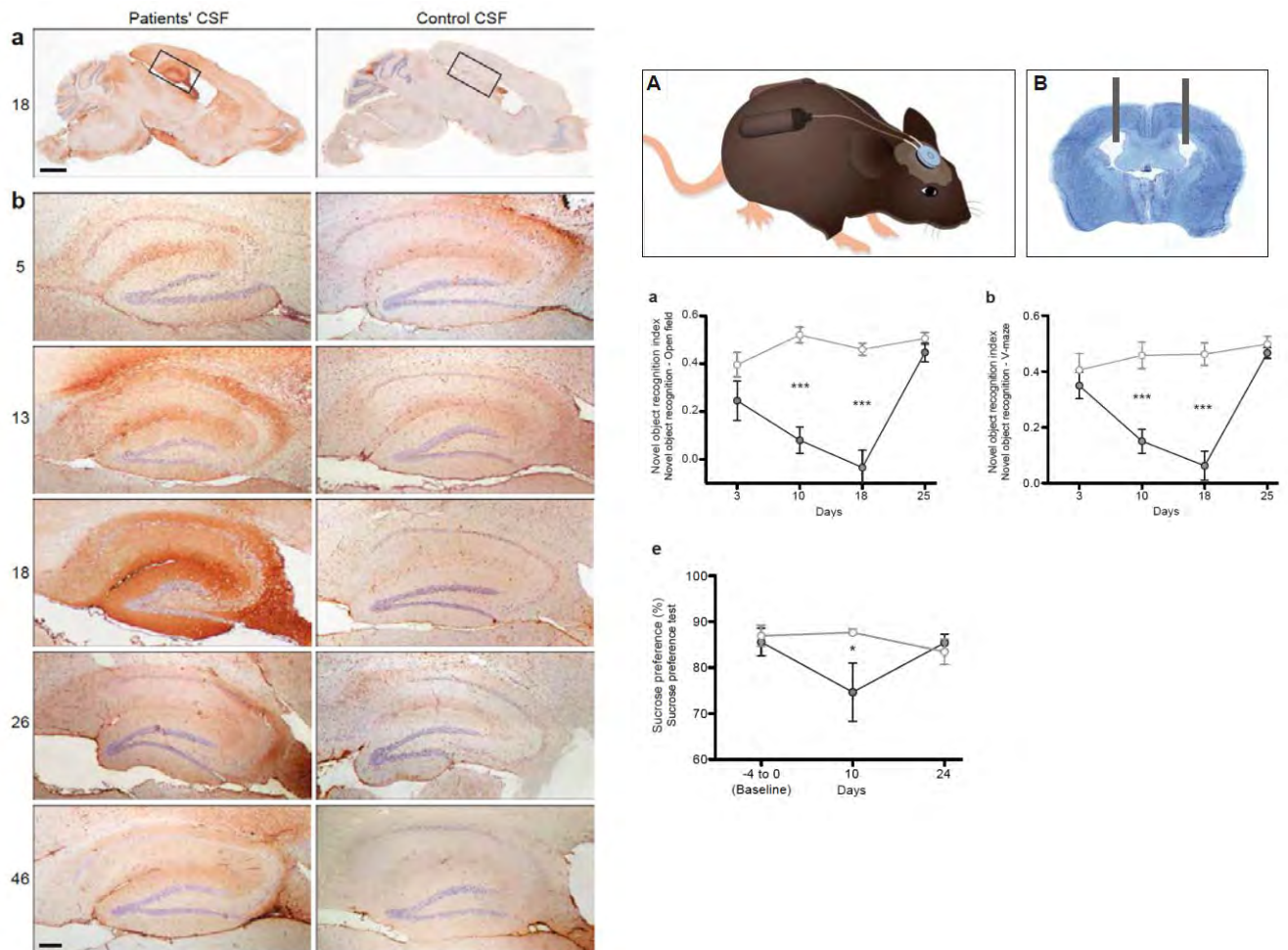
1.(Paneles a y b izquierdos) Animales infundidos con LCR de pacientes con anticuerpos NMDAR tienen un incremento progresivo de depósito de IgG humana en el hipocampo. (a y b) Inmunotinción de IgG humana en secciones cerebrales sagitales (a) y hipocampo (b) de animales representativos infundidos con LCR de pacientes (izquierda) y LCR control (derecha), sacrificados en los días experimentales indicados. Escala: A=2mm; B=200microm. **2.** (Paneles A Y B superiores derechos) Modelo experimental murino y localización de los catéteres ventriculares para la infusión de LCR de pacientes o LCR control mediante bombas osmóticas durante 14 días. **3.** (Paneles medios e inferior derechos) La infusión de LCR de pacientes con anticuerpos contra NMDAR causa déficits de memoria, anhedonia, y comportamiento depresivo. (a y b) Índice de reconocimiento de un nuevo objeto (NOR) en el el paradigma NOR de campo obierto (a) o V-maze (b), en animales tratados con LCR de pacientes (círculos grises) o LCR control (círculos blancos). Un índice elevado indica una mejor memoria de reconocimiento del objeto. (e) Preferencia para el agua con sucrosa en animales infundidos con LCR de pacientes (gris) o LCR control (blanco). Un porcentaje menor indica anhedonia.

Figura adaptada de:
Planaguma J, Leypoldt F, Mannara F, et al. Brain 2015;138:94-109.

Foco en la encefalitis autoinmune en la edad pediátrica y nuevas perspectivas

En la población pediátrica, la encefalitis causada por anticuerpos más frecuente, es aquella en la que los anticuerpos van dirigidos contra el receptor NMDA (encefalitis anti-NMDAR).[52] Estudios recientes muestran que en edades jóvenes, su frecuencia puede ser superior a la frecuencia individual de las diferentes encefalitis virales y, que es la segunda causa de encefalitis autoinmune después de la encefalomielitis aguda diseminada (ADEM). [53] La encefalitis anti-NMDAR en la que hasta el 40% de los pacientes son pediátricos, se presenta en adolescentes y adultos jóvenes en forma de un síndrome clínico muy característico. Inicialmente, después de una primera fase prodrómica con fiebre o con síntomas pseudogripales, los pacientes debutan típicamente con trastornos psiquiátricos y alteraciones de la conducta, incluyendo pensamientos paranoides, insomnio, alucinaciones visuales y auditivas. En unas semanas el síndrome progresa y los pacientes presentan alteraciones del movimiento (característicamente disquinesias de predominio orofacial), crisis epilépticas, disminución del nivel de conciencia y disautonomía.[6]

En niños pequeños, aunque el síndrome es similar, los síntomas de presentación pueden ser diferentes.[52] El estudio más extenso, que incluyó más de 500 pacientes con esta encefalitis, de los cuales 180 eran menores de 18 años, mostró que la forma más común de presentación en niños menores de 12 años eran los trastornos de movimiento, las crisis epilépticas y el comportamiento anormal.[51] En niños pequeños las alteraciones del comportamiento incluyen disminución de interacción social, agitación, agresión, y cambios en el humor y de personalidad. Los mecanismos que inducen la producción de estos anticuerpos son desconocidos, aunque en un subgrupo de pacientes, la presencia de un tumor (frecuentemente teratoma de ovario), que expresa el

antígeno neuronal diana, probablemente contribuye a desencadenar la respuesta inmunológica.[41] En niños, la presencia de un tumor (principalmente teratoma), es infrecuente. Mientras que aproximadamente el 56% de mujeres mayores de 18 años tienen teratomas uni- o bilaterales de ovario, estos tumores se encuentran en el 30% de las chicas menores de 18 años, y en un 9% de las niñas menores de 14.[52] En hombres la presencia de un teratoma testicular es rara, y este tumor no se ha descrito en niños de sexo masculino con encefalitis anti-NMDAR.

Aunque en la mayoría de los pacientes sin un teratoma la causa de la encefalitis anti-NMDAR es desconocida, estudios preliminares describen una diferente estacionalidad en el debut de la encefalitis anti-NMDAR en pacientes jóvenes con o sin teratoma asociado. En un estudio reciente en el que se incluyeron retrospectivamente 29 pacientes menores de 21 años diagnosticados de encefalitis anti-NMDAR en un solo centro, se evidenció que 18/23 (78%) de los pacientes sin un teratoma asociado, habían debutado durante los meses cálidos (de marzo a septiembre), mientras que los 6 pacientes con un tumor asociado (5 teratomas, un sarcoma de Ewing) lo habían hecho durante los meses más fríos (de octubre a febrero, p <0,001).[54] Este sesgo en la estacionalidad en el debut, no se evidenció en pacientes con el Síndrome de opsoclonus-mioclonus con o sin neuroblastoma asociado diagnosticados en el mismo centro. Estos hallazgos abren interesantes cuestiones sobre la etiopatogenia de la encefalitis anti-NMDAR. Recientemente se ha descrito que variaciones estacionales en enfermedades autoinmunes podrían estar producidas por un perfil inmunitario y una composición celular de la sangre variable a lo largo del año, e invertida entre Europa y Oceania.[55] El estudio de *Dopico et al.* muestra que durante los meses más fríos, el sistema inmunológico tendría un perfil pro-inflamatorio con niveles incrementados del receptor de IL-6 soluble, proteína C reactiva, y biomarcadores de riesgo para enfermedades

cardiovasculares, psiquiátricas y autoinmunes. El hecho de que se haya descrito de que la encefalitis anti-NMDAR no relacionada con teratoma se produzca más frecuentemente en meses cálidos, es contrario no obstante con estos nuevos hallazgos de un perfil más auto-inflamatorio durante los meses fríos, y nos podría llevar a la hipótesis preliminar de que otros factores como las infecciones podrían jugar un papel. Las infecciones son ampliamente reconocidas por tener diferencias estacionales. Mientras que el Enterovirus, el virus del Oeste del Nilo o el Lyme, predominan en la primavera o al inicio del verano, otros patógenos como el virus influenza tienen un predominio durante los meses de invierno. Sin embargo, aunque son frecuentes los síntomas pseudogripales precediendo la encefalitis anti-NMDAR, y que los estudios microbiológicos no se realizan de forma estandarizada, son una minoría los casos en los que se demuestra un patógeno infeccioso en esta encefalitis. No obstante, los hallazgos de estos estudios van en contra de que la encefalitis anti-NMDAR esté relacionada con las vacunas (generalmente de administración durante todo el año), o con patógenos de predominio en invierno como el virus influenza.[54]

El reciente descubrimiento de estas entidades ha permitido en los últimos años el diagnóstico y tratamiento de pacientes hasta ahora catalogados con términos descriptivos como *encefalitis letárgica o la encefalitis de Hashimoto*, que no explicaban los mecanismos patogénicos responsables. A modo de ejemplo, dos estudios recientes han mostrado que aproximadamente el 50% de los pacientes categorizados previamente como *encefalitis letárgica hipercinética* tenían en realidad encefalitis anti-NMDAR.[56] La identificación de un biomarcador común (anticuerpos contra NMDAR) también ha permitido reconocer como una misma entidad (la encefalitis anti-NMDAR), es la única responsable de algunos síndromes neurológicos pediátricos previamente descritos en la literatura médica como entidades diferenciadas de etiología desconocida como fueron: el "Síndrome autista reversible en niños con encefalopatía aguda"[57] (Delong , 1981), el "Coma con movimientos anormales y

déficits cognitivos a largo plazo"[58] (Sebire 1992), o el "Síndrome encefalopático y corea immunomediada pediátrica"[59] (Hartley 2002).

Aunque tradicionalmente el diagnóstico de las encefalitis autoinmunes en niños ha sido menos frecuente que en adultos, el hecho de que en estas edades haya una alta incidencia de patologías autoinmunes probablemente provocada por una inmadurez de los sistemas de tolerancia inmunológica, y el hecho de la encefalitis por autoinmunidad sináptica más frecuente, la encefalitis anti-NMDAR, afecte de manera predominante a pacientes pediátricos y adultos jóvenes, apoyan la hipótesis de que son patologías infradiagnosticadas en estas edades. Por este motivo, creemos que es muy posible que el aumento del reconocimiento en los últimos 2 años de estas enfermedades en niños se traduzca en la identificación de nuevos síndromes infantiles causados por autoanticuerpos contra superficie neuronal tal y como ha sucedido en pacientes de edades más avanzadas.

En este proyecto de tesis nos proponemos caracterizar nuevos síndromes pediátricos causados por anticuerpos contra superficie neuronal e identificar nuevos autoantígenos responsables de encefalitis de etiología no aclarada con un foco especial en estos grupos de edad. Con este objetivo nos centraremos en 3 grupos de pacientes pediátricos en los que tenemos evidencia preliminar de patología neurológica immunomediada: 1) encefalitis asociada a teratoma sistémico, 2) encefalitis con crisis refractarias y/o status epiléptico, y 3) pacientes con recidivas neurológicas post-encefalitis herpética. En detalle:

Grupo 1: Pacientes con encefalitis asociada a teratoma sistémico

El teratoma (del griego teras-, teratos "pesadilla", "monstruo", y -oma "tumor o tumoración"), es el tumor de células germinales más frecuente y, sus células o tejidos pueden derivar de los tres tipos de capas germinales embrionarias (ectodermo, endodermo y mesodermo). Los teratomas se dividen en cuatro categorías: maduros (quísticos o sólidos, benignos, los más

frecuentes), inmaduros (malignos), malignos por un componente de otra neoplasia somática maligna, y por último monodermales o altamente especializados, en los que es frecuente la presencia de tejido de estroma ovárico, que contiene tejido tiroideo o tejido de tipo carcinoide, constituyendo neoplasias neuroendocrinas bien diferenciadas.

Los teratomas quísticos maduros o quistes dermoides representan más del 95% de todos los teratomas ováricos, siendo los tumores ováricos más frecuentes en mujeres entre la segunda y la tercera década de la vida.[60] El quiste dermoide contiene tejido maduro de un origen ectodérmico (ej., piel, folículos pilosos, glándulas sebáceas), mesodérmico (ej., músculo, tejido urinario), y/o endodérmico (ej., pulmón y gastrointestinal). Estos tumores pueden ser bilaterales en el 10-17% de los casos, y característicamente tienen la apariencia de una masa multiquística con una prominencia sólida (protuberancia de Rokitansky) localizada entre el teratoma y el ovario normal. Aunque son casi siempre benignos, hasta un 2% pueden presentar una degeneración maligna, principalmente en mujeres mayores de 45 años, en tumoraciones de tamaño mayor a 10 cm de diámetro y en tumores de rápido crecimiento. Los teratomas inmaduros o malignos son más comunes en las dos primeras décadas de la vida, y aunque representan menos de un 1% de los teratomas ováricos son el tipo de tumor maligno más frecuente de este órgano (36%) seguidos de los disgerminomas (33%).[61]

Los teratomas inmaduros típicamente están compuestos por tejidos de las tres capas germinales dispuestas de manera errática. Histológicamente es frecuente la diferenciación en tejido neural aunque elementos de estroma inmaduros también pueden estar presentes y, según la proporción de tejido neural inmaduro se pueden estratificar en grados desde el I (bien diferenciados) hasta el III (pobremente diferenciados). [62]

De este modo, a diferencia de otros tumores como los carcinomas, dado a que la mayoría de teratomas son benignos y que las mujeres con quistes dermoides con frecuencia están asintomáticas, clásicamente no se había considerado el teratoma como un posible causante de síndromes paraneoplásicos. De forma excepcional, se había asociado la presencia de estos tumores con cuadros paraneoplásicos no neurológicos como la anemia hemolítica, la poliartritis, y la dermatomiositis.[63-65] Algunas publicaciones aisladas también habían sugerido una asociación entre teratoma de ovario o testicular y encefalitis paraneoplásica.[6] Muchos de estos pacientes eran hombres jóvenes con teratomas testiculares, y anticuerpos Ma2, y un síndrome que se caracteriza por encefalitis límbica y disfunción de tronco cerebral y del diencéfalo.[66]

Este pensamiento ha cambiado de forma radical desde la identificación en 2007 de la encefalitis anti-NMDAR,[6] en la que entre el 9 y el 55% de los pacientes presentan un teratoma asociado a nivel sistémico,[51] aumentando la sospecha de la presencia de un teratoma como causa de encefalitis autoinmune. Aun así, la asociación tumoral en esta encefalitis es edad y sexo dependiente, siendo muy infrecuente en edades inferiores a los 12 años.[67] Aunque esta encefalitis se ha asociado a la presencia de teratomas maduros e inmaduros, el 29% de los teratomas asociados a encefalitis anti-NMDAR son inmaduros a diferencia de un porcentaje inferior al 1% descrito en series de pacientes sin encefalitis.[68] Este hecho y la demostración en tejido neural tumoral de la expresión de receptores NMDA apoyan la presencia del tumor como desencadenante de la encefalitis.[6] El porcentaje de teratomas bilaterales en pacientes con encefalitis anti-NMDAR y teratoma se detecta con una frecuencia similar a la de las cohortes generales de pacientes con teratoma (14% versus 12%).[41,69]

La identificación del teratoma como causante de encefalitis autoinmune resulta muy relevante pues estos pacientes responden a la extirpación tumoral y a la inmunoterapia.[51] Los pacientes con encefalitis anti-NMDAR asociada a teratoma presentan un menor riesgo de recidivas que los pacientes en los que no se detecta un tumor associado.[51] Actualmente resulta imprescindible por tanto, la búsqueda de un teratoma oculto en un paciente con esta encefalitis, aunque el tipo y frecuencia del cribado habrá que adaptarlos al riesgo de asociación tumoral del paciente, que varía según la edad y el sexo. Actualmente se considera de elección la RM de la pelvis como cribado en mujeres y/o la ecografía abdominal y testicular en el caso de los homes.[67]

Desde 2007, en el laboratorio de Neuroinmunología del Dr. Dalmau (director de la tesis), hemos estudiado 249 pacientes con encefalitis asociada a teratoma. Aunque la mayoría de estos pacientes tienen anticuerpos contra la subunidad GluN1 del receptor NMDAR, hemos identificado 38 pacientes con teratoma y sospecha de encefalitis autoinmune pero negativos para estos anticuerpos. La pregunta que surge de este hallazgo es si la negatividad de los anticuerpos en estos pacientes se debe a una baja sensibilidad de la técnica o si estamos ante un cuadro paraneoplásico diferente. En esta tesis pretendemos contestar esta pregunta (Ver Objetivo 1).

Grupo 2: Pacientes con encefalitis con crisis refractarias y *status epilepticus*

El reciente descubrimiento de una nueva categoría de encefalitis autoinmune causada por autoinmunidad sináptica como causa tratable de encefalitis ha renovado el interés por identificar causas autoinmunes de epilepsia. Este hecho se debe a que la identificación de esta nueva categoría de encefalitis autoinmune ha cambiado los paradigmas diagnósticos y terapéuticos de múltiples síndromes potencialmente tratables que cursan con crisis y *status epilepticus*, y que previamente eran atribuidos a infecciones virales o etiologías idiopáticas. Las encefalitis autoinmunes que cursan con crisis y/o *status*

epilepticus se dividen siguiendo la clasificación de las encefalitis autoinmunes según la localización de la auto-antígeno asociado. También pueden dividirse entre encefalitis límbicas o encefalitis difusas, y/o en función de si son paraneoplásicas o no. En el caso de las encefalitis paraneoplásicas asociadas a anticuerpos intracelulares u onconeuronales, estas incluyen las asociadas a Hu, Ma2, CV2/CRMP5 y anfifisina. De estas cuatro respuestas inmunológicas, los anticuerpos anti-Hu son los que con mayor frecuencia se describen en pacientes con crisis, *epilepsia partialis continua*, y *status epilepticus*. Los tumores subyacentes más frecuentes son los cánceres de pulmón, en especial SCLC (todos los anticuerpos), los tumores testiculares de células germinales (Ma2), y los timomas (CRMP5). Estos síndromes afectan de forma casi exclusiva a pacientes adultos y se caracterizan por tener una respuesta limitada a la inmunoterapia.

Los anticuerpos intracelulares contra GAD65, aunque típicamente se han asociado al síndrome de la persona rígida no paraneoplásico y a disfunción cerebelosa, recientemente ha habido un incremento en el número de casos publicados asociados a encefalitis límbica, y/o epilepsia refractaria.[70] En este síndrome que puede afectar a pacientes jóvenes, la respuesta a inmunoterapia es limitada a diferencia de los síndromes producidos por anticuerpos contra proteínas de superficie o sinápticos.

Todas las encefalitis causadas por anticuerpos contra antígenos de superficie neuronal o sinápticos descritos cursan frecuentemente con crisis y/o *status epilepticus*. Su identificación es de gran importancia pues estos pacientes responden a inmunoterapia. Estas encefalitis pueden afectar a pacientes con o sin tumores y la frecuencia de la asociación tumoral dependerá del autoanticuerpo asociado y de las características epidemiológicas (edad, sexo y etnia) del paciente. En el caso de la encefalitis anti-NMDAR, más del 70% de los pacientes desarrollan crisis epilépticas.[71] Además, en niños pequeños y en

adultos de sexo masculino las crisis o el *status epilepticus* son a menudo la forma de presentación de esta encefalitis.[71,72] Sin embargo, la mayoría de pacientes tratados con inmunoterapia, y una vez superada la fase aguda de la encefalitis, permanecen libres de crisis y sin necesidad de tratamiento antiepiléptico durante el seguimiento.

En otras encefalitis asociadas a anticuerpos contra superficie neuronal (LGI1, Caspr2, GABAB, AMPA) también es frecuente la presencia de crisis epilépticas, aunque estos síndromes afectan de forma predominante o casi exclusiva a pacientes adultos. Los pacientes con encefalitis límbica asociada a anticuerpos contra LGI1 típicamente presentan déficits de memoria y diferentes tipos de crisis, incluyendo característicamente crisis tónicas breves de afectación facio-braquial.[36] El reconocimiento de estas crisis que se pueden presentar al inicio de la enfermedad, y la consecuente instauración de inmunoterapia precoz puede prevenir la progresión de la encefalitis límbica y mejorar el pronóstico cognitivo de estos pacientes.[73,74] De manera interesante LGI1 es una proteína secretada que forma parte de un complejo transináptico que interacciona con el VGKC presináptico mediante la proteína ADAM23. Los ratones genoanulados para LGI1 mueren durante las dos primeras semanas de vida. En humanos, mutaciones en LGI1 son causa de epilepsia del lóbulo temporal autosómica dominante.[75] Los anticuerpos contra Caspr2 además de asociarse a encefalitis límbica, también se han asociado al síndrome de Morvan (encefalitis y neuromiotonía) y mucho menos frecuentemente a neuromiotonía aislada.[37] Actualmente el significado de los anticuerpos contra VGKC determinados por radioinmunoensayo que no van dirigidos contra LGI1 o Caspr2 es incierto, dado a que se han identificado en pacientes con desórdenes muy variados incluyendo pacientes con enfermedades no inmunomediadas.[71,76] Los anticuerpos contra el receptor $GABA_B$ se asocian a una encefalitis límbica que típicamente se manifiesta con crisis refractarias prominentes.[77] Estos anticuerpos se encuentran en la mayoría de pacientes con encefalitis límbica y

SCLC sin anticuerpos anti-Hu y, a diferencia de los pacientes con Hu, los pacientes con GABA$_B$ responden favorablemente a inmunoterapia.[77] Los anticuerpos contra el receptor AMPA también se asocian a encefalitis límbica caracterizada por crisis epilépticas con respuesta favorable a inmunoterapia pero esta última, se acompaña frecuentemente de síntomas psiquiátricos prominentes.[78] Los tumores asociados a este síndrome son el cáncer de mama, de pulmón y de timo. Además estos pacientes con frecuencia presentan recidivas clínicas y pueden tener otras auto-anticuerpos (peroxidasa tiroidea [TPO], o canales de calcio dependientes de voltaje [VGCC] tipo N), sugiriendo una tendencia a la autoinmunidad. Anticuerpos contra DPPX, una subunidad reguladora de los canales de potasio Kv4.2, se han descrito recientemente en pacientes adultos con encefalitis caracterizada por hiperexcitabilidad del SNC incluyendo crisis epilépticas, mioclonías y agitación,[46] y en pacientes jóvenes (incluyendo un adolescente de 16 años), con encefalomielitis progresiva con rigidez y mioclonías (PERM).[79] En algunos pacientes los síntomas neurológicos son precedidos por diarrea grave de larga evolución que ha motivado previamente extensos estudios microbiológicos (incluyendo despistaje para la enfermedad de Whipple) y de cribado tumoral. La identificación de la expresión de DPPX en el plexo mientérico de ratones y su alta reactividad con los anticuerpos de los pacientes, han hecho postular un mecanismo inmunológico de los síntomas gastrointestinales.[46] Los enfermos con esta encefalitis suelen tener buena respuesta a inmunoterapia, sin embargo, pueden hacerse dependientes de los corticosteroides, con recidivas neurológicas al intentar reducir la dosis.

Como se ha expuesto en esta sección, a pesar de la alta frecuencia de trastornos autoinmunes en la edad pediátrica, sólo una (encefalitis anti-NMDAR) de las seis encefalitis por autoinmunidad sináptica descritas aquí afectan de forma predominante a niños y adolescentes. Este hecho sugiere que posiblemente otros auto-antígenos aún pendientes de identificar pueden ser

responsables de encefalitis autoinmunes en niños. Desde enero de 2008 hasta abril de 2014 hemos identificado 350 pacientes (un 40% niños), con sospecha de encefalitis autoinmune que cursaron con status epiléptico. Disponemos de LCR y/o suero de todos los pacientes, de los cuales 140 tienen un patrón en la inmunohistoquímica sugestivo de anticuerpos contra antígenos desconocidos de la membrana neuronal. En esta tesis nos proponemos como objetivo identificar nuevos auto-antígenos asociados a encefalitis y crisis epilépticas que afecten a la edad pediátrica (Objetivo 2).

Grupo 3: Pacientes con recidivas neurológicas post-encefalitis herpética

<u>Generalidades y etiopatogenia de la encefalitis herpética:</u>

La encefalitis por el virus herpes simple (EHS) sigue siendo una causa importante de morbimortalidad en todas las edades a nivel mundial a pesar de la disponibilidad de un tratamiento antiviral como es el aciclovir. Aproximadamente un tercio de los pacientes son niños o adolescentes. Como resultado de esta encefalitis típicamente se produce necrosis de áreas límbicas, y el síndrome clínico asociado se manifiesta con fiebre y/o déficits neurológicos focales que incluyen alteración del nivel de conciencia, afectación de pares craneales, hemiparesia, disfasia, afasia, ataxia y/o crisis focales.[80-82] También se han descrito varios tipos de alteración del comportamiento como hipomanía, síndrome de Kluver-Bucy y amnesia asociados la EHS.[83,84] Actualmente, la prueba de referencia para su diagnóstico es la detección del ADN viral mediante la reacción en cadena de la polimerasa (PCR) que muestra una alta sensibilidad (98%) y especificidad (94-100%). Excepto durante el período neonatal en el que esta encefalitis puede estar causada por los serotipos del virus herpes simple (VHS) 1 o 2, generalmente adquirida por transmisión materna por el canal del parto, en el resto de grupos de edad la mayoría de casos son causados por serotipo VHS-1. Se cree que la llegada del virus al SNC fuera del periodo

neonatal se puede producir mediante 3 vías: 1) invasión directa mediante el nervio trigémino u olfatorio siguiendo una primoinfección por el VHS-1 en la orofaringe (principal causa en menores de 18 años), 2) invasión del SNC tras una infección recurrente del virus, representando una reactivación viral y su expansión, y 3) infección del SNC sin observarse una infección primaria o recurrente, representando una reactivación latente del virus in situ en el SNC. Otra potencial vía de inoculación es la viremia.

La causa de por qué la infección por el VHS-1 sólo es causa de encefalitis en un porcentaje pequeño de pacientes que sufren una infección por este virus es aún desconocida. Aunque en humanos la encefalitis por el VHS-1 de forma general no es más común en pacientes inmunodeprimidos que en inmunocompetentes,[80] recientemente se han identificado algunas mutaciones genéticas involucradas en vías de la respuesta inmunológica innata que podrían conferir una susceptibilidad a padecer esta encefalitis, principalmente en niños. Diferentes mutaciones que alteran diferentes proteínas (UNC93B, TLR3, TRAF3, TIR, TRIF) implicadas en la vía de la producción de interferón mediante la activación del receptor *Toll-Like* (TLR) 3 se han visto involucradas. Los TLR son un tipo de receptores de reconocimiento de patrones de patógenos (PRR) que forman parte de la respuesta inmunológica innata. Estos receptores son capaces de reconocer RNA de doble cadena de varios patógenos y activar factores de transcripción que estimulan la producción de interferones y otras citosinas. La variación regional de los diferentes subtipos de TLR contribuye a explicar los diferentes patrones de afectación del SNC del VHS y de otros virus con neurotropismo. El subtipo TLR3 es el predominante en las células del SNC y tiene una gran importancia para evitar la diseminación del VHS de los ganglios al SNC mediante la producción de interferón alfa. Resulta interesante el hecho de que los pacientes con mutaciones que afectan de forma exclusiva en la vía de señalización del TLR3 presentan un riesgo incrementado de encefalitis o

queratitis por el VHS, pero no por infecciones producidas por el VHS en otras localizaciones como son las infecciones labiales o mucosas recurrentes, o por otros microorganismos en el SNC. Por el contrario, pacientes con inmunodeficiencias severas como los afectados por mutaciones en IRAK-4 (receptor quinasa 4 asociado a interleucina (IL) 1) que producen una alteración en las vías de los TLR7, 8 y 9 y también del receptor de la IL-1 , pero no del TLR3, presentan un riesgo incrementado de infecciones piógenas por bacterias gram positivas encapsuladas pero no por EHS.

A pesar de estos interesantes hallazgos, que representan sin duda un gran paso para entender la etiopatogenia del EHS, esta última cuestión no está ni mucho menos resuelta. Aunque mediante estudios funcionales *in vitro* han observado alteraciones en la vía de producción de interferón alfa mediante la activación del TLR3 en un alto porcentaje de niños con EHS, son una minoría los casos en los que se detectan mutaciones en esta vía (8-9%). Además, en los casos en que se detectan mutaciones, estas no presentan una penetrancia completa pues también se encuentran en familiares asintomáticos de pacientes. Se desconoce si estas vías también están alteradas funcionalmente en pacientes adultos con EHS. Por otra parte, aunque la introducción del tratamiento con aciclovir a mediados de los años 1980 supuso un descenso importante de la mortalidad de esta encefalitis, actualmente más de dos tercios de los pacientes con EHS aún quedan con graves secuelas neurológicas. Por este motivo se recomienda iniciar el tratamiento con aciclovir endovenoso lo antes posible ante una sospecha clínica en espera de la confirmación microbiológica. Otro factor muy importante a tener en cuenta en la patogénesis de la EHS es la hipótesis de un posible origen inmunomediado del daño tisular en esta encefalitis.[85] Este hecho explicaría la observación de que la EHS no es más frecuente en inmunodeprimidos pesar de la alta frecuencia de infecciones mucocutáneas por el VHS-1 en estos huéspedes. En cambio la EHS en inmunodeprimidos a veces sigue un curso diferente, caracterizado por una

evolución lenta y únicamente con cambios mínimos histopatológicos observados en biopsias cerebrales.[86]

De este modo, aunque la patogénesis de la EHS es poco conocida se cree que ambos factores, los directamente relacionados con el virus, y otros indirectos inmunomediados desencadenados por la infección, juegan un papel en el daño al SNC.[80] Resulta poco claro si la cantidad de viremia está relacionada con la extensión o la severidad de las lesiones. En un estudio con 8 pacientes con EHS, el nivel de viremia determinado por PCR, no se correlacionó con la severidad de los síntomas.[87]

Por otra parte varios datos apoyan el papel del sistema inmunológico en la EHS:

-La EHS se caracteriza por lesiones focales inflamatorias que se consideran secundarias a la expresión de una respuesta mediada por células T específica contra el virus.[88] Como ejemplo, células T citotóxicas que lisan específicamente las células infectadas por el virus *in vitro*, predominan en los sitios focales de infección focales en modelos murinos.[89]

- Otros modelos animales han demostrado la correlación entre la producción de citosinas y óxido nítrico con daño cerebral.[90]

- La administración exógena de IL-4 intranasal en ratones infectados con el VHS-1 incrementa la severidad de la encefalitis posterior mediante el incremento de producción de IL-4 por parte de las células T CD4 + locales.[91]

- Se han observado correlaciones entre la infiltración del SNC por células inmunes durante la infección viral y desmielinización en modelos en ratones.[85]

- Se han observado inmunocomplejos IgM contra VHS-1 en las paredes de los vasos en un paciente con EHS.[92]

También resulta interesante la reciente descripción de las *"interferonopatías"*, una nueva categoría de enfermedades genéticas que incluye entre otros el síndrome de *Aicardi Goutières* o el Lupus eritematoso

sistémico congénito, que asocian una predisposición congénita a la autoinmunidad, y que son causadas por una hiperestimulación congénita de los factores de transcripción dependientes de interferón, una situación similar a la que se postula que se produce de forma adquirida frente a una infección por el VHS.

La demostración del papel de la autoinmunidad en la etiopatogenia de la EHS podría tener implicaciones terapéuticas directas pues ya se ha demostrado en el modelo animal que la terapia adyuvante con corticoides junto con el tratamiento antiviral, es más efectiva que el tratamiento antiviral únicamente en la reducción de las anomalías estructurales cerebrales observadas en la RM.[93]

Recidivas post-encefalitis herpética:

Aunque tradicionalmente se ha considerado que la EHS es una enfermedad monofásica, en la literatura médica se describe que hasta el 14-24% de los pacientes pueden presentar recidivas.[94] La patogénesis de las recidivas es heterogénea y, se ha postulado que mientras algunos casos representan recidivas virales (nueva detección del virus mediante PCR positiva en LCR, nuevas lesiones necróticas en la RM, y respuesta al tratamiento antiviral); otros puedan ser inmunomediados (PCR viral negativa en LCR, falta de nuevas lesiones necróticas en RM, y falta de respuesta a aciclovir).[94,95] Este último síndrome de etiología desconocida pero postulada ser inmunomediada, ha sido más frecuentemente descrita en la edad pediátrica, donde es conocida como *"coreoatetosis post-EHS"*, dada la presentación típica de estos pacientes a las 3-4 semanas posteriores al debut de la encefalitis viral en forma de grave alteración de movimiento (status coreoatetósico y disquinesias), junto con alteración grave del nivel de conciencia.[95] Dada la falta de respuesta al aciclovir

en estos pacientes y la presunción de una etiopatogenia inmunomediada habían sido publicados en la literatura varios casos tratados con inmunoterapia (corticoides y/o gammaglobulinas endovenosas) con respuesta variable. Recientemente en el estudio retrospectivo de una cohorte de pacientes adultos de los que se disponía de muestras congeladas obtenidas en diferentes estadios de encefalitis herpética se detectaron anticuerpos tipo IgG contra NMDAR en un 11% de los pacientes, postulando que estos anticuerpos podrían ser causantes de formas atípicas de EHS.[96] En esta tesis pretendemos estudiar si una respuesta inmunológica por autoinmunidad sináptica contra NMDAR y/u otras proteínas sinápticas puede estar involucrada en la *coreoatetosis post-herpética*" y/o en otras formas de recidiva neurológica post-encefalitis herpética (Objetivo 3).

Hipótesis

El descubrimiento de que muchas encefalitis previamente consideradas idiopáticas son causadas por anticuerpos contra proteínas sinápticas o receptores de la superficie neuronal ha revolucionado en los últimos 7 años la neurología, la pediatría, la psiquiatría y las neurociencias. La enfermedad con mayor impacto en el campo de la pediatría, la encefalitis anti-NMDAR descrita en 2007, ha marcado una nueva línea de trabajo y estrategias diagnósticas y terapéuticas en encefalitis pediátrica. La identificación de estos síndromes se realizó mediante la observación de pacientes con síndromes que sugerían un proceso inmunomediado pero en los que no se detectaban anticuerpos conocidos. Esto sugirió la hipótesis de que estos pacientes tenían anticuerpos que no eran detectados con las técnicas disponibles.

De esta manera creemos que algunas encefalitis pediátricas previamente consideradas idiopáticas o post-infecciosas de los grupos clínicos seleccionados: 1) encefalitis asociada a la presencia de un teratoma sistémico, 2) encefalitis con crisis refractarias y/o status epiléptico, y 3) recidivas neurológicas post-encefalitis herpética, están causadas por anticuerpos dirigidos contra antígenos sinápticos o de la membrana neuronal. Estos anticuerpos podrían ser identificados con las nuevas técnicas altamente sensibles y específicas para la detección de anticuerpos /antígenos de superficie neuronal, recientemente desarrolladas por el Dr. Dalmau (director de la tesis), que han permitido en los últimos años el descubrimiento de varios síndromes asociados a esta categoría de anticuerpos (receptores AMPA, NMDA, GABAB, mGluR5 y proteínas DPPX, LGI1, Caspr2).[49]

Objetivos

Objetivo general:

Caracterización clínica e inmunológica de encefalitis pediátricas de etiología no filiada, centrándonos en tres grupos de pacientes con evidencia preliminar de un origen inmunomediado: grupo 1) encefalitis asociada a la presencia de un teratoma sistémico, grupo 2) encefalitis con crisis refractarias y/o status epiléptico, y grupo 3) recidivas neurológicas post-encefalitis herpética.

Objetivos específicos para grupos de pacientes:

- Grupo 1: Encefalitis asociada a la presencia de un teratoma sistémico:
Caracterizar clínica e inmunológicamente los pacientes identificados con sospecha de encefalitis autoinmune asociada a un teratoma sistémico sin anticuerpos contra NMDAR para establecer si la negatividad de estos anticuerpos se debe a una baja sensibilidad en la técnica o si estamos ante un cuadro paraneoplásico diferente.

- Grupo 2: Encefalitis con crisis refractarias y/o status epiléptico:
Identificar nuevos auto-antígenos responsables de encefalitis por autoinmunidad sináptica asociados a crisis y *status epilepticus* que afecten de manera predominante a la edad pediátrica.

- Grupo 3: Recidivas neurológicas post-encefalitis herpética:
Estudiar si una respuesta inmunológica por autoinmunidad sináptica contra NMDAR y/o de otras proteínas sinápticas está involucrada en la *"coreoatetosis post-herpética"* y/o en otras formas de recidiva neurológica post-encefalitis herpética.

Resumen y discusión

La encefalitis sigue siendo a día de hoy una causa importante de morbimortalidad a nivel mundial que afecta a todas las edades. Aun así, estudios multicéntricos realizados a nivel mundial coinciden en el hecho de que más de la mitad de casos permanecen sin un diagnóstico etiológico definitivo después de extensas baterías microbiològicas.[3-5] El reciente descubrimiento de una nueva categoría de encefalitis causada por anticuerpos dirigidos contra proteínas de la superficie neuronal ha dado un diagnóstico definitivo a muchos de estos casos, y ha identificado la causa de varios desórdenes previamente considerados idiopáticos o atribuidos a infecciones víricas.[41,7] A diferencia de los síndromes paraneoplásicos clásicos asociados a anticuerpos intracelulares que afectan de forma casi exclusiva a pacientes adultos, las encefalitis por autoinmunidad sináptica afectan a pacientes de todas las edades, e incluso algunos de ellos como la encefalitis anti-NMDAR, de forma predominante a niños y adolescentes.[8,9,49] Estas entidades pueden desarrollarse con o sin la presencia de un tumor, y se asocian a anticuerpos que se dirigen contra epítopos o dianas extracelulares de la membrana neuronal con fenotipos clínicos que varían en función del anticuerpo asociado, semejando aquellos fenotipos en los cuales antígeno diana es alterado farmacológicamente o genéticamente.[8,9,49] Mientras que los anticuerpos intracelulares probablemente representan el componente humoral de una compleja respuesta inmunológica mediada por células T citotóxicas, los anticuerpos dirigidos contra proteínas de superficie neuronal han demostrado en los desórdenes en los que se ha investigado, que producen una alteración funcional y/o morfológica de los antígenos correspondientes cuando son aplicados en modelos *in vitro* o *in vivo*, y que estas alteraciones son reversibles al eliminar los anticuerpos.[41,50] La distinción entre estas dos categorías de encefalitis según si el anticuerpo asociado va dirigido contra un epítopo intra o extracelular resulta de gran

importancia en la práctica clínica, pues los pacientes que asocian anticuerpos contra epítopos extracelulares con frecuencia responden a inmunoterapia y/o resección tumoral cuando está indicada.[8,9,49]

El descubrimiento de esta nueva categoría de síndromes asociados a anticuerpos contra antígenos de superficie neuronal ha cambiado el enfoque diagnóstico y terapéutico de problemas tan diversos como la catatonia, las alteraciones agudas de la memoria, la epilepsia, o los trastornos del movimiento. Por ejemplo, algunos procesos previamente atribuidos a infecciones víricas o manifestaciones atípicas de esquizofrenia son actualmente identificados como procesos autoinmunes tratables.[53] La transferibilidad de estas investigaciones a la industria y a la práctica clínica han producido test diagnósticos que se utilizan en todo el mundo y a patentes por el uso de esta tecnología. En la población pediátrica, el descubrimiento por parte del director de la tesis de la encefalitis anti-NMDAR, una causa frecuente de encefalitis autoinmune en niños y adolescentes, ha cambiado el manejo diagnóstico y terapéutico de la encefalitis en la edad pediátrica.[51,52] Cabe destacar sin embargo que en los últimos años la descripción de los anticuerpos contra proteínas sinápticas, a excepción de los dirigidos contra NMDAR, se ha focalizado principalmente en pacientes de edad avanzada. Así, mientras que en población adulta se han descrito numerosos anticuerpos contra superficie neuronal responsables de encefalitis autoinmune, en la edad pediátrica la única encefalitis por autoinmunidad sináptica bien caracterizada hasta el momento había sido la encefalitis anti-NMDAR.

En esta tesis, con el objetivo principal de caracterizar clínica e inmunológicamente encefalitis pediátricas de etiología no filiada, nos hemos centrado en tres grupos de desórdenes con evidencia preliminar de una etiología inmunomediada y que afectan de forma predominante a niños y adolescentes: grupo 1) encefalitis asociada a la presencia de un teratoma sistémico, grupo 2) encefalitis con crisis refractarias y/o status epiléptico, y

grupo 3) recidivas neurológicas post-encefalitis herpética. Los resultados obtenidos en los estudios que se presentan aquí, han dado lugar a la publicación de 5 artículos originales en revistas internacionales de alto impacto, respondiendo a los 3 objetivos específicos planteados. Cabe destacar que de las cuatro publicaciones que ya han sido difundidas (la quinta está en prensa), en tres se ha publicado una editorial dedicada en el mismo número de la revista (ver artículos), y que todos ellos han sido muy bien recibidos en el campo de la pediatría, la neurología y las neurociencias. Un ejemplo de la repercusión que han tenido los estudios publicados en esta tesis han sido los 4 artículos de revisión sobre encefalitis autoinmune que la doctoranda y el director de tesis han publicado durante el periodo de la realización de esta tesis, previa invitación, en revistas de prestigio; incluyendo: *The Journal of Child Neurology* 2012 (donde la revisión que hicimos de encefalitis autoinmune fue el artículo más leído mensualmente en esta revista durante más de dos años desde su publicación), *Current Opinion in Neurology* 2014, *Annals of the New York Academy of Sciences* 2014, y un nuevo capítulo dedicado a encefalitis autoinmunes en "*Nelson Textbook of Pediatrics*" 20th edition, el libro de referencia de pediatría a nivel mundial (Ver CV y publicaciones no defendidas en Anexo).

En referencia al cumplimiento de los objetivos específicos de la tesis, en el primero de ellos, nos planteábamos caracterizar clínica e inmunológicamente los pacientes identificados con sospecha de encefalitis autoinmune asociada a un teratoma sistémico sin anticuerpos contra NMDAR, para establecer si la negatividad de estos anticuerpos se debía a una baja sensibilidad en la técnica de detección o si estábamos ante un cuadro paraneoplásico diferente. En este trabajo (**Artículo 1, Armangue et al. Ann Neurol 2013**), sobre una cohorte de 249 pacientes con encefalitis asociada a teratoma, 211 tenían encefalitis anti-NMDAR, y 38 eran negativos para estos anticuerpos. Un cuidadoso estudio

clínico de los pacientes permitió identificar que 22 de los 38 pacientes sin anticuerpos anti-NMDAR presentaban un síndrome clínico con afectación predominante de cerebelo y tronco, con frecuente opsoclonus. El hecho de que estos síntomas se presenten de forma muy excepcional en pacientes con encefalitis anti-NMDAR, apoya firmemente que nos encontramos ante la descripción de un nuevo cuadro paraneoplásico. Además, comparado con los pacientes con anticuerpos contra NMDAR, los pacientes sin estos anticuerpos presentaron con menos frecuencia alteración de comportamiento, disminución del nivel de conciencia y crisis epilépticas, y raramente presentaron disquinesias. Aunque en la mayoría de estos pacientes no detectamos anticuerpos en suero ni en LCR, la identificación de este nuevo síndrome neurológico es clínicamente muy relevante pues con frecuencia estos pacientes responden a inmunoterapia (corticoides, gammaglobulinas y/o recambio plasmático), y/o a resección tumoral. Las diez pacientes del estudio que presentaron opsoclonus fueron mujeres jóvenes (rango 15-32 años), considerándose demasiado jóvenes para el síndrome de opsoclonus-mioclonus asociado a carcinoma, que con frecuencia afecta a pacientes mayores de 50 años, y demasiado mayores para el síndrome de opsoclonus-mioclonus asociado a neuroblastoma, característico de niños menores de 5 años. El reconocimiento de esta nueva entidad descubre un nuevo fenotipo paraneoplásico de opsoclonus-mioclonus y de encefalitis con afectación de tronco y cerebelo asociado a la presencia de un teratoma sistémico, que afecta predominantemente a pacientes jóvenes. Estos pacientes presentan una respuesta favorable a inmunoterapia y a resección tumoral, y probablemente habían sido previamente infradiagnosticados y catalogados de sufrir un cuadro "idiopático" o "post-infeccioso". Por lo tanto es importante destacar que la búsqueda de la presencia de un tumor como el teratoma es esencial en pacientes con un cuadro de encefalopatía rápidamente progresiva como el descrito, y no se debería restringir en pacientes con anticuerpos contra NMDAR.

En el siguiente objetivo de la tesis nos proponíamos identificar nuevos auto-antígenos responsables de encefalitis por autoinmunidad sináptica asociados a crisis y *status epilepticus* que afectaran de forma predominante a la edad pediátrica. En el segundo artículo de esta tesis (**Artículo 2, Petit-Pedrol M *, Armangue T *, Peng X * et al. Lancet Neurol 2014, *igual contribución**), hemos caracterizado un nuevo anticuerpo dirigido contra el receptor sináptico GABA$_A$ que se encuentra involucrado en encefalitis con crisis refractarias y *status epilepticus*, y que afecta de forma predominante a niños y adultos jóvenes. En este trabajo, identificamos inicialmente dos pacientes índice de 15 y 56 años de edad respectivamente, que compartían características clínicas similares altamente sugestivas de padecer un síndrome inmunomediado: encefalitis con crisis refractarias y *status epilepticus*, signos inflamatorios en el LCR y alteraciones de señal cortico- subcorticales en la RM cerebral. Además, el estudio inmunológico de las muestras de suero y LCR de ambos pacientes mostró un mismo patrón de reactividad en las técnicas inmunohistoquímica adaptadas para la detección de anticuerpos contra auto-antígenos sinápticos, sugiriendo que el cuadro en ambos casos era producido por el mismo auto-anticuerpo. La selección de estos dos pacientes con un fenotipo clínico e inmunológico similar permitió como se expone en el trabajo, la identificación del auto-antígeno responsable del cuadro: las subunidades alfa1/beta3 del receptor inotrópico GABA$_A$. En este trabajo además demostramos los efectos de estos anticuerpos sobre la sinapsis en un modelo experimental *in vitro* en cultivos de neuronas hipocampales, en el que la aplicación de los anticuerpos de los pacientes (LCR) producía una disminución de los receptores GABA$_A$ en la sinapsis, y la reversibilidad de los efectos en remover los anticuerpos de los pacientes del medio de cultivo. Una vez identificado este nuevo anticuerpo, y desarrollar un test diagnóstico con células HEK transfectadas para expresar el receptor GABA$_A$, re-examinamos las muestras de 150 pacientes con encefalitis y crisis epilépticas identificando 4 pacientes más afectos de este nuevo síndrome

autoinmune, asociando todos ellos altos títulos de estos anticuerpos en LCR y crisis epilépticas refractarias. En este trabajo no identificamos anticuerpos contra GABA en ninguno de los 75 controles sanos examinados, pero sí títulos más bajos de estos anticuerpos (<1/160) en suero de pacientes con otros síndromes neurológicos inmunomediados como el síndrome de la persona rígida y el síndrome de opsoclonus-mioclonus. El significado de este último hallazgo aún es incierto, debido a que estos pacientes no tenían anticuerpos asociados en LCR, y merece más estudios posteriores. Resulta interesante la observación de que algunos de los pacientes con encefalitis por anticuerpos contra GABA$_A$ R presentaban otras enfermedades autoinmunes u otras auto-anticuerpos concomitantes como los anticuerpos contra GAD65 o los dirigidos contra TPO. De esta experiencia, del hecho de que los anticuerpos contra TPO se pueden encontrar hasta en un 10% de adultos asintomáticos, y de que no se ha demostrado su patogenicidad, podemos concluir que es importante descartar la presencia de otros anticuerpos más relevantes como los dirigidos contra proteínas sinápticas, en pacientes con encefalitis en los que detectamos anticuerpos TPO, antes de clasificarlos con el término descriptivo de encefalitis asociada a enfermedad tiroidea o Encefalitis de Hashimoto.

En el tercer y último objetivo de esta tesis nos propusimos estudiar si una respuesta inmunológica por autoinmunidad sináptica contra NMDAR y/o contra otras proteínas sinápticas estaba involucrada en la "*coreoatetosis post-herpética*" y/o en otras formas de recidiva neurológica post-encefalitis herpética. En el tercer artículo de esta tesis (**Artículo 3, Armangue et al. J Pediatr 2013**) identificamos y seguimos una cohorte de 20 niños con encefalitis anti-NMDAR de diferentes centros de España. En esta cohorte de pacientes identificamos una niña de 2 años que había desarrollado coreoatetosis, disquinesias orofaciales y disminución severa del nivel de conciencia (síntomas característicos de la encefalitis anti-NMDAR), pocas semanas después de haber

sido correctamente diagnosticada y tratada por una EHS. Con un diagnóstico inicial de "*coreoatetosis post-herpética*", entidad de etiología previamente desconocida descrita en fases convalecientes de la EHS, fue tratada empíricamente con corticoides y gammaglobulinas sin evidenciarse una respuesta favorable. Semanas más tarde, la similitud del cuadro clínico con la encefalitis anti-NMDAR y la confirmación de altos títulos de anticuerpos contra NMDAR en el LCR de la paciente, apoyaron la hipótesis de una etiología inmunomediada del cuadro, y permitieron ser más agresivos con el tratamiento inmunosupresor administrando rituximab y ciclofosfamida (antes no administrados por el antecedente de la infección viral). Después de estos tratamientos, la paciente presentó una recuperación progresiva de los síntomas de la recidiva (ver Videos Artículo 3), a pesar de que los síntomas residuales de la EHS previa (síndrome biopercular) persistieron. Este estudio, junto con la descripción casi simultánea de que un 11% de pacientes de una cohorte retrospectiva de adultos con EHS tenían anticuerpos IgG contra NMDAR, aportaron por primera vez evidencia de que un proceso viral podía desencadenar la producción de anticuerpos contra NMDAR, y de que estos anticuerpos podían ser causa de recidivas post-herpéticas incluyendo la forma clásicamente llamada "*coreoatetosis post-herpética*".

Esta hipótesis fue rápidamente confirmada con la identificación en los siguientes meses de más de 22 nuevos casos (7 por otros autores[97-100] y 15 por nuestro grupo, ver artículos 4 y 5) de recidivas post-herpéticas producidas por anticuerpos anti-NMDAR. Además, en el cuarto artículo de esta tesis (**Artículo 4, Armangue T *, Leypoldt F * et al. Ann Neurol 2014, * igual contribución**), mediante la identificación prospectiva de 5 pacientes, incluyendo un lactante de tan sólo 2 meses de edad, el paciente más joven descrito con encefalitis anti-NMDAR, evidenciamos la inexistencia de los anticuerpos NMDAR en las muestras obtenidas durante la fase infecciosa de la encefalitis, demostrando

que la infección viral es el desencadenante de los anticuerpos NMDAR, y por tanto de la encefalitis asociada, en este grupo de pacientes. En el mismo trabajo identificamos anticuerpos contra superficie neuronal (la mayoría contra auto-antígenos desconocidos) en el 58% de pacientes de una cohorte retrospectiva de 34 pacientes (adultos y niños) con EHS, de los que se disponía de una muestra de LCR obtenida después de la primera semana del debut de la infección viral. El hecho de que encontráramos que la EHS había desencadenado una respuesta por autoinmunidad sináptica en más de la mitad de pacientes de la serie, incluyendo casos de todas las edades, pero en cambio las recidivas autoinmunes post-EHS sólo habían sido descritas hasta el momento de forma excepcional, y casi exclusivamente en niños menores de 3 años, nos llevó a plantear la hipótesis de que la autoinmunidad desencadenada por la EHS era un fenómeno infradiagnosticado. Con el objetivo de estudiar la frecuencia real de este fenómeno, así como la patogenicidad y la identidad de los anticuerpos contra superficie neuronal desencadenados por la EHS, iniciamos en enero de 2014 un estudio observacional multicéntrico prospectivo con más de 40 centros participantes repartidos entre las diferentes comunidades autónomas. En este estudio, pacientes con EHS de todas las edades son evaluados inmunológicamente de forma ciega a la información clínica (con la excepción de que presenten síntomas sugestivos de recidiva), desde el inicio de la encefalitis viral y durante los 12 meses posteriores. Aunque este estudio todavía está en curso, resultados preliminares tras la inclusión de los primeros 20 pacientes, nos han permitido no sólo confirmar la hipótesis de que las recidivas autoinmunes post-EHS son frecuentes (un 25% de nuestra serie prospectiva), sino que hemos identificado una nueva forma clínica de recidiva autoinmune post-herpética que afecta a adolescentes y adultos, y que con frecuencia es infradiagnosticada.

Estos resultados han sido publicados recientemente en el quinto y último artículo de esta tesis (**Artículo 5, Armangue et al. Neurology 2015, en prensa**),

en el que se describen 14 nuevos pacientes (6 niños pequeños y 8 adolescentes-adultos) con recidivas autoinmunes post-EHS identificados por nuestro grupo en los últimos 21 meses, cinco de ellos procediendo del estudio multicéntrico prospectivo. La comparación entre las recidivas post-herpéticas autoinmunes en niños pequeños (menores de tres años) y en el grupo de adolescentes y adultos, nos permitió observar que las características clínicas de las recidivas dependen de la edad del paciente. Mientras que en el grupo de 6 niños pequeños (3 de ellos de sexo masculino, edad mediana de 13 meses, rango 6-20 meses, todos con anticuerpos NMDAR, y uno con anticuerpos contra GABA$_A$R concomitantes), todos presentaron alteraciones de movimiento de tipo coreoatetósico y/o discinéticos típicos de la "*coreoatetosis post-herpética*"; 7 de los 8 adolescentes y adultos (5/8 de sexo masculino, edad media de 40 años, rango 13-69 años, 5 con anticuerpos NMDAR y 3 con anticuerpos contra antígenos desconocidos), se presentaron con síntomas psiquiátricos y alteraciones del comportamiento sin alteraciones del movimiento. Mientras que en el grupo de los niños pequeños, la forma abrupta y dramática de debut llevó a una rápida identificación y tratamiento del cuadro, la forma de presentación subaguda y puramente o casi exclusivamente psiquiátrica en los adolescentes y adultos, condicionó que los síntomas fueran atribuidos inicialmente síntomas residuales de la encefalitis viral y tratados inicialmente con fármacos sintomáticos. En los pacientes adultos y adolescentes participantes en el estudio prospectivo, los síntomas de recidiva no fueron identificados como tales hasta que acudieron a visitas de seguimiento establecidas por el protocolo del estudio. Este hecho, nos llevó a una modificación del protocolo inicial, avanzando la primera visita de seguimiento al mes del alta hospitalaria (aproximadamente 50 días post debut de la encefalitis viral), y no a los tres meses como se había establecido inicialmente. A pesar del importante retraso diagnóstico en adultos y adolescentes (mediana desde el inicio de los síntomas de recidiva hasta el diagnóstico de encefalitis autoinmune de 85 días, rango 17-296 días, respecto una mediana en los niños pequeños de 4

días, rango 0-55 días, p=0,037), estos pacientes respondieron muy favorablemente a inmunoterapia. Mientras que antes de la recidiva autoinmune los pacientes eran capaces de colaborar, de comunicarse y de llevar a cabo algunas actividades de la vida diaria de acuerdo con las limitaciones esperadas causadas por la necrosis inducida por la infección viral (principalmente afectando la memoria anterógrada y el lenguaje), durante la recidiva autoinmune los pacientes se volvieron agresivos, no colaboradores, algunos de ellos con ideas suicidas, e incluso en algún caso progresando a disminución de conciencia, crisis y coma. El tratamiento con inmunoterapia (generalmente con corticoides endovenosos), restableció su actividad basal previa a la recidiva (ver Figura 1, artículo 5).

Otro hallazgo relevante de este estudio fue la demostración de una captación intensa de contraste endovenoso en las RM cerebrales obtenidas durante la recidiva autoinmune comparado con una captación de contraste ausente o mínima en las RM cerebrales obtenidas durante la fase viral. Además, la captación de contraste presentó una evolución paralela a la respuesta clínica de los pacientes, persistiendo en pacientes no tratados y sintomáticos, y disminuyendo o desapareciendo coincidiendo con el tratamiento y/o la mejoría clínica de los pacientes. Esta observación no había sido previamente publicada y merece futuros estudios para ver si la RM cerebral con contraste endovenoso puede ser un potencial biomarcador para detectar y/o seguir recidivas post-herpéticas autoinmunes.

En el grupo de los niños pequeños, se confirmó en todos los casos estudiados que la "*coreoatetosis post-herpética*" correspondía en realidad a encefalitis anti-NMDAR. La respuesta a inmunoterapia en estos casos fue variable y no tan favorable a la esperada comparado con el primer caso descrito de "*coreoatetosis post-HSE*" asociado a anticuerpos NMDAR (Artículo 3) o con los pacientes con la encefalitis anti-NMDAR no relacionada con el EHS. Además de las diferencias en la respuesta a la inmunoterapia, se detectaron otras

diferencias entre la encefalitis anti-NMDAR relacionada o no con la EHS. A modo de ejemplo, aunque la variación de los síntomas edad dependiente observada en las recidivas autoinmunes post-EHS va en la línea de las diferencias en la forma de presentación dependientes de la edad descritas en la encefalitis anti-NMDAR clásica o no relacionada con EHS (movimientos anormales en niños pequeños, y síntomas psiquiátricos en adolescentes y adultos), en esta última, la mayoría de los pacientes con independencia de la edad acaban desarrollando alteraciones de movimiento y/o crisis epilépticas durante el curso de la enfermedad. Por el contrario, ninguno de los pacientes adolescentes y adultos con encefalitis anti-NMDAR post-EHS desarrolló crisis o movimientos anormales durante la evolución. Las causas de las diferencias observadas entre la encefalitis anti-NMDAR post-EHS respecto a la encefalitis anti-NMDAR clásica o no relacionada con el virus son aún desconocidas. El estudio del repertorio de los epítopos de los pacientes con anticuerpos anti-NMDAR post-EHS fue similar al repertorio descrito en pacientes con encefalitis anti-NMDAR clásica (ver Apéndice del artículo 3). Descartado este factor, se podría postular que un diferente ambiente inmunológico en ambas situaciones, con un mayor papel de la inmunidad celular (células T citotóxicas) en los casos secundarios a EHS, o la destrucción tisular y necrosis previa inducida por la infección viral podrían ser factores implicados en explicar estas diferencias.

Otro hallazgo importante en este trabajo fue que 3 de los 6 niños pequeños con recidivas autoinmunes post-herpéticas desarrollaron crisis y status epilepticus durante la recidiva autoinmune (en dos de ellos precediendo la coreoatetosis característica). En uno de estos pacientes, un lactante de 11 meses de edad (paciente 9), la recidiva se presentó en forma de espasmos epilépticos y hipsarritmia (*Málaga I, Armangue T y Dalmau J, observación personal*), seguido de un status coreico. La identificación de anticuerpos NMDAR en este paciente podría representar la primera evidencia de que un subgrupo de casos de epilepsia de tipo espasmos infantiles o Síndrome de West podrían ser

secundarios o desencadenados por anticuerpos contra el receptor NMDAR. Esta hipótesis ha sido recientemente apoyada por la identificación de mutaciones en GRIN2A y GRIN2B (genes codificantes para subunidades del receptor NMDA) como causantes de Síndrome de West,[101,102] y por el modelo animal de espasmos epilépticos causados por la administración de agonistas NMDA.[103] Mutaciones de novo en GRIN1 (subunidad del receptor NMDA sobre la que van dirigidos los anticuerpos de los pacientes con encefalitis anti-NMDAR), se han descrito recientemente en pacientes con encefalopatía epiléptica precoz con debut antes de los 3 meses de edad, déficit cognitivo, y movimientos hipercinéticos, con alteraciones focales y difusas en el EEG, pero sin mostrar patrones de salva-supresión o hipsarrítmia.[104] También resulta interesante la identificación concomitante a anticuerpos contra NMDAR, de anticuerpos contra GABA$_A$R (auto-antígeno descrito recientemente en encefalitis y crisis refractarias, ver Artículo 2), en otro de los pacientes de la serie de 15 meses de edad (paciente 10), que debutó en forma *de status epilepticus* refractario y coreoatetosis los 23 días del EHS, y respondió favorablemente a inmunoterapia agresiva (corticoides, gammaglobulinas, recambio plasmático y rituximab). Este hallazgo, junto con la descripción previa de pacientes con recidivas autoinmunes post-EHS asociadas a anticuerpos contra D2R,[98] confirman que el EHS es un desencadenante de autoinmunidad sináptica no restringido a anticuerpos contra NMDAR.

Los resultados de esta tesis respaldan firmemente la hipótesis previamente postulada de que los mecanismos autoinmunes tienen un papel importante en la patogénesis de la EHS. Dado que los anticuerpos IgG dirigidos contra GluN1 cuando se encuentran en LCR siempre se han asociado a encefalitis anti-NMDAR,[51,105] y que estos anticuerpos son patogénicos en modelos de cultivos neuronales[68] y en ratones,[50] postulamos que los anticuerpos contribuyen a producir los síntomas de los pacientes. El papel patogénico de los otros auto-

anticuerpos todavía no está claro. De esta manera, la determinación de anticuerpos contra superficie neuronal (principalmente NMDAR) en suero y LCR debería ser considerada en todos los pacientes con recidivas post-EHS. En caso de positividad, si la PCR para EHS en LCR es negativa, parece razonable empezar inmunoterapia empírica (por ejemplo: corticosteroides, gammaglobulinas, y/o recambio plasmático), y dependiendo de la respuesta se podría plantear tratamientos más agresivos como el rituximab. El estudio multicéntrico prospectivo en curso aclarará si los anticuerpos contra superficie neuronal pueden desarrollarse sin síntomas de recidiva post-EHS, o si existe un título umbral de estos anticuerpos para desarrollar los síntomas. Futuros estudios esclarecerán si la autoinmunidad desencadenada por la EHS está relacionada con las recientes descripciones de defectos congénitos relacionados con la estimulación del interferón, tanto por defecto (por mutaciones en la vía de los TLR3 en pacientes con EHS), como por exceso (en pacientes con "*interferonopatías*" y activación constante de los factores de transcripción derivados del interferón, y autoinmunidad asociada).

Conclusiones

Conclusiones generales:

- La selección de pacientes con fenotipos clínicos similares y la aplicación de técnicas altamente sensibles y específicas para la identificación de anticuerpos contra superficie neuronal nos han permitido caracterizar el perfil clínico e inmunológico de varios procesos neurológicos autoinmunes pediátricos de etiología previamente desconocida que, con frecuencia responden a inmunoterapia.

Conclusiones específicas Subgrupo 1:

- Es importante el reconocimiento de un nuevo fenotipo paraneoplásico de opsoclonus-mioclonus y de encefalitis con afectación de tronco y cerebelo asociada a la presencia de un teratoma sistémico, sin anticuerpos NMDAR, que afecta predominantemente a pacientes jóvenes, y que responde favorablemente a inmunoterapia y resección tumoral.

- La búsqueda de un tumor como el teratoma es esencial en pacientes con cuadros de encefalopatía rápidamente progresivas como el descrito, y no se debería restringir a pacientes con anticuerpos anti-neuronales.

Conclusiones específicas Subgrupo 2:

- Hemos caracterizado un nuevo anticuerpo dirigido contra el receptor sináptico GABAAR que se encuentra involucrado en encefalitis con crisis refractarias y status epilepticus, que afecta de forma predominante a niños y adultos jóvenes, y se caracteriza por LCR de características inflamatorias, alteraciones cortico-subcorticales en la RM cerebral,

asociación a otros factores de propensión a la autoinmunidad (ej. anticuerpos contra TPO, GAD65 concomitantes), y con potencial respuesta a inmunoterapia.

- Los anticuerpos contra GABAAR son patogénicos en el modelo in vitro basado en cultivos de neuronas hipocampales murinas, produciendo una disminución de los receptores GABAA en la sinapsis, y estos efectos son reversibles en eliminar los anticuerpos del medio de cultivo.

Conclusiones específicas Subgrupo 3:

- La EHS es una fuerte desencadenante de autoinmunidad sináptica no restringida a NMDAR.

- Anticuerpos contra NMDAR y/o otras proteínas sinápticas pueden ser causa de recidivas autoinmunes post-EHS. En niños pequeños estas recidivas se manifiestan en forma de "coreoatetosis post-EHS" con o sin crisis refractarias y status epiléptico acompañante; y en adolescentes y adultos se manifiestan predominantemente en forma de alteraciones de comportamiento y psiquiátricas.

- La identificación de recidivas autoinmunes post-EHS, fenómeno previamente infradiagnosticado, es muy importante pues estos pacientes pueden responder a inmunoterapia.

English translation of summary and conclusions

Synaptic autoimmunity as a cause of encephalitis associated to teratoma, epilepsy and relapses post-herpetic encephalitis in children

Summary

Background: In the last 7 years, a new category of encephalitis associated with antibodies against neuronal surface epitopes has been identified, in which patients develop psychosis, catatonia, seizures, and abnormal movement. These disorders are potentially lethal but curable if recognized and treated.

Hypothesis: A subgroup of pediatric encephalitis previously considered idiopathic or post-infectious are caused by antibodies against neuronal surface receptors and proteins.

Objectives: Clinical and immunological characterization of pediatric encephalitis of unknown origin, focusing on three groups of patients with evidence of an immune-mediated origin: 1) encephalitis associated with the presence of systemic teratoma; 2) encephalitis with refractory seizures and status epilepticus; and 3) neurological relapses post-herpes simplex encephalitis (HSE).

Methods: Clinical and immunological study of patients and serum/CSF. To identify antibodies and target antigens we used a previous validated strategy consisting in selecting patients with similar clinical phenotypes and immunological screening with techniques developed for the identification of antibodies against neuronal surface antigens, including: immunohistochemistry in cultures of live neurons, immunoprecipitation, and antigen characterization by mass spectrometry. The effects of the antibodies on the target antigens have been investigated in cultures of dissociated rat hippocampal neurons.

Results: By specific objectives: 1) We have identified a new paraneoplastic phenotype of opsoclonus-myoclonus syndrome and brainstem and cerebellar encephalitis associated with the presence of a systemic teratoma, without anti-NMDAR antibodies that predominantly affects young patients, and that responds favorably to immunotherapy and tumor resection. 2) We have characterized a novel antibody directed against the synaptic GABA$_A$ receptor involved in encephalitis with refractory seizures and status epilepticus in children and young adults. In an in vitro model we showed that these antibodies produce a reduction of GABA$_A$ receptors in the synapse, and that these effects were reversible upon removing antibodies from the culture medium. 3) We have shown that HSE is a strong trigger for synaptic autoimmunity that is not restricted to NMDAR antibodies. We have described for first time that antibodies to NMDAR and/or other synaptic proteins can cause autoimmune relapses post-HSE. In young children these relapses present with "post-HSE choreoathetosis" with or without associated refractory seizures and status epilepticus, and in teenagers and adults presents with predominant psychiatric symptoms.

Conclusions: These studies have resulted in the identification of new syndromes and immunological responses, in the characterization of antigenic targets, and in the development of a diagnostic test for a group of children with immune-mediated encephalitis. These studies have changed diagnostic and treatment paradigms of childhood encephalitis by facilitating early diagnosis and treatment of patients affected with disorders of previously unknown etiology, and that frequently respond to immunotherapy.

Conclusions

General conclusions:

- Selection of patients with similar clinical phenotypes and immunological screening using techniques with high sensitivity and specificity for the identification of antibodies against neuronal surface antigens led us to characterize the clinical and immunological profile of several pediatric autoimmune neurological syndromes of previous unknown etiology that frequently respond to immunotherapy.

Specific Conclusions from Subgroup 1:

- The recognition of a new paraneoplastic phenotype of Opsoclonus-myoclonus syndrome and encephalitis with brainstem and cerebellar involvement associated with teratoma without NMDAR antibodies that predominantly affects young women and responds to immunotherapy and tumor resection.

- Tumor screening is important in patients with rapidly progressive encephalopathic syndromes such as that described above, and should not be restricted to patients with anti-neuronal antibodies.

Specific Conclusions from Subgroup 2:

- We have characterized a new antibody directed against the synaptic $GABA_A$ receptor that is associated with a syndrome of refractory epileptic seizures and status epilepticus which predominantly affects children and is characterized by inflammatory CSF, cortico-subcortical abnormalities on brain MRI, association to other autoimmune features (e.g. concomitant

TPO and/or GAD65 antibodies), and is potentially responsive to immunotherapy.

- Patient $GABA_A$ receptor antibodies are pathogenic. This has been demonstrated in vitro using cultures of live murine hippocampal neurons in which the antibodies produce a selective decrease in synaptic $GABA_A$ receptors; these effects are reversible when the antibodies are removed from the culture medium.

Specific Conclusions from Subgroup 3:

- HSE is a trigger for brain synaptic autoimmunity not restricted to NMDAR.

- Antibodies against NMDAR and/or other synaptic proteins can cause autoimmune relapses post-EHS. In young children these relapses are characterized by "choreoathetosis post-EHS" with or without refractory seizures and status epilepticus and in teenagers and adults by abnormal behavior and psychiatric symptoms.

- Autoimmune relapses post-EHS are frequent and under diagnosed. Recognition of these relapses is important as patients can respond to immunotherapy.

Currículum vitae resumit

La Dra. Armangué es llicencià en Medicina amb premi extraordinari de llicenciatura per la Universitat Autònoma de Barcelona al 2006. Va completar la seva residència en Pediatria i àrees específiques (2007-2011) a l'Hospital Universitari Vall d'Hebron (Barcelona) i va rebre la beca de la Societat Espanyola de Neurologia Pediàtrica (SENEP) per realitzar l'especialització en Neurologia pediàtrica en el mateix hospital (2011-2013, mentor: Dr. Macaya), mitjançant el programa Màster de la Universitat Autònoma de Barcelona (UAB). L'any 2012 va rebre el Diploma d'Estudis Avançats pel treball "Cerca de reactivitat enfront antígens de superfície neuronal en casos pediàtrics amb sospita de malaltia autoimmune"(UAB), i el 2015 la diplomatura en Metodologia de la Investigació: Disseny i estadística en ciències de la salud, per la mateixa Universitat. La Dra. Armangué es va incorporar al Programa de Neuroimmunologia de l'Hospital Clínic-IDIBAPS (Universitat de Barcelona) dirigit pel Dr. Josep Dalmau al 2011 com a fellow clínico-investigadora. Al 2012, rebé la beca predoctoral PFIS de l'Institut Carles III, per prosseguir els seus estudis de doctorat, i a l'octubre de 2014, l'ajuda per a contractes en investigació Rio Hortega de la mateixa entitat, sent la candidata millor valorada a nivell estatal en ambdues convocatòries. Actualment està participant en la creació i coordinació d'una Unitat Clínica i de Recerca en Neuroimmunologia Pediàtrica conjunta entre l'Hospital Clínic-IDIBAPS i l'Hospital Sant Joan de Déu, i ha estat becada per realitzar una estància post-doctoral de 6 mesos a l'Hospital Children's National de Washington DC (USA), al programa "Research Fellowship in Heritable Disorders of the White Matter" dirigit per la Dra. Vanderver.

El seu projecte d'investigació implica tant estudis de ciències bàsiques com clíniques, per identificar nous antígens associats a encefalitis autoimmune pediàtrica i trastorns desmielinitzants, per caracteritzar aquestes malalties i el

seu pronòstic a la població pediàtrica. A mode d'exemple, la Dra. Armangué ha participat en el disseny i el desenvolupament de dos estudis multicèntrics que involucren més de 45 centres pediàtrics a Espanya: "Estudi prospectiu multicèntric de pacients amb encefalitis per herpes simple i la seva relació amb encefalitis autoimmune"(disseny de l'estudi i co-investigadora principal); i "Diagnòstic i biomarcadors pronòstics en l'esclerosis múltiple pediàtrica i trastorns relacionats" (co-disseny de l'estudi i investigadora, financiat per Marató de TV3 2014). La recerca de la Dra. Armangué ha rebut nombrosos premis i reconeixements incloent la Beca d'Assistència Sanitària, el premi de Mutual Mèdica, i més recentment la beca DODOT de l'Associació Espanyola de Pediatria per a un projecte d'Investigació, i la *Travel award* de la American Neurological Association. Des de l'any 2010, és membre de la Societat Catalana de Pediatria i del consell de redacció de la revista "Pediatria Catalana", i desde 2013 de la Societat española de Neurologia pediátrica, i del Grup Internacional d'Estudi de l'Esclerosis múltiple pediátrica (IPMSSG). La Dra. Armangué ha participat com a ponent invitada en més de 20 conferències en cursos i congressos nacionals e internacionals, el seus treballs han estat presentats en més de 40 abstracts incloent la reunió anual de la Societat Espanyola de Neurologia, la Societat Espanyola de Neurologia Pediàtrica, l'Associació Americana de Neurologia, i ha publicat com a primera autora en *The Journal Child Neurology* 2012, *The Journal of Pediatrics* 2012, *Annals of Neurology* 2012 y 2013, *Current Opinion in Neurology* 2014, *The Lancet Neurology* 2014, *Neurology 2015,* y *Nelson Textbook of Pediatrics, 20th edition*, entre altres publicacions internacionals.

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Índex Alfabètic

Agraïments

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Lab de Neuroimmunologia sense dubte el millor ambient de treball i un gran equip professional i personal: Myrna always there to help! L'Albert amb tanta feina però trobant sempre el moment per nosaltres, l'Eugènia la supermurciana a qui tinc de referent!, l'Helena companya de batalla de la superdatabase i de crêpes, és un vincle forever!, la Maria sense tu tot seria un caos i ho saps!, l'Esther increïble la dedicació de treball i humana, merci!!, la Mar companya de tesi des del principi, ja et falta poquet!, la Lídia companya de penes i alegries de l'opsoclonus project;), en Jesús l'artista del lab, merci per l'ajuda en el disseny i pel teu bon humor!, la Núria que ha anat a pel Nobel, la Merche la xispa del lab i junt amb l'Eva les supremamis!, en Francesco l'altre artista de la pista i del vermut!, la Sheila superincorporació, i l'Anna que ja va sortir del niu. I què dir dels adoptats over the world: En Frank (our f..hero!), en Kevin (Mog and Mountain man, jo vull ser com tu!), en Maarten (trying to follow your steps, dank!), Romana (I love your chocolat and smiles ;), Jenny (exciting projects to come!), Rebecca (perfect flatmate!), Laurent (merci!), Mariana (benvenuta!), Mateus (muito obrigada!), i en Makoto (歡迎).

Però la tesi no només és el resultat del treball al lab, he tingut la sort de poder formar-me en molts bons hospitals i per tant enriquir-me com a professional i també com a persona gràcies a molts companys i companyes, i sobretot a grans amics i amigues. De Sant Pau i per sempre: en Marc, l'Anna, i l'Aina. I també és clar en Xavier, en Narcís i la Rita. L'erasmus a la *Università degli Studi di Padova* amb la Paula. Pediatria a Valle, a tots els R's, co-R's, al *grup gafe* i als adjunts. Guàrdies amb l'Esther, la Mònica i l'Àlex. Cafès amb crema de llet a la UCI amb en Juan. A l'equip 08035! Les súper Núries, la muntanyenca i la panameña! Per gran aventura al Darien i a *Isla Iguana*! La Raquelilla, i a la súper R gran Marteta. A l'Equipo Senegal: No t'oblidem Laura! Neuropediatria a Valle (uns cracks com a professionals i persones): en Miquel, la Susana, la Francina i la Mireia un equipazo, merci per tot el suport! Mastereando amb la Marina, la Laura, l'Ana, la Marta, la Geles, l'Eduardo, l'Isaam, la Sandra i l'Amparo! Al Manel per creure en mi des del començament, gràcies! A l'Eli i a sus "chicos": l'Àngel i el Nacho. Equipo electros: la Mònica, l'Elena i en Joan. Neuro a Valle sense pedia: La Mar a la muntanya, i en Manu i en Xavier companys de trinxeres. De Neuroimmuno al Clínic: l'hospital de dia de luxe amb la Maria, la Núria, la Sara, la Yolanda, la Montse i l'Anna, i l'equip via visual amb el Pablo, la Irati, la Elena i supercoordinats per l'Eva. Neuro sense immuno: la Elen i l'Oriol a les cavernes, en Joan a la zona dels somnis i la Bea compartint la passió pels peques. Les meves psiquiatres preferides: l'Àstrid, la Gisela i l'Imma.

De més lluny, i sempre compartint generosament la il.lusió per col.laborar: en Nacho (*pionero e imán para la autoinmunidad ;)*), en Sergio, la Itxaso i en Miguel del Nord, la Verónica de los Madriles, en Miguel i en Luís de terres valencianes, en Javier de la franja, i l'Íñigo, l'Eduardo i en Joaquín del Sud.

Als (ja ex ;)) companys de guàrdies de la Diagonal. I als nous companys i a la meva nova etapa que m'espera a Sant Joan, segur que plena d'experiències!
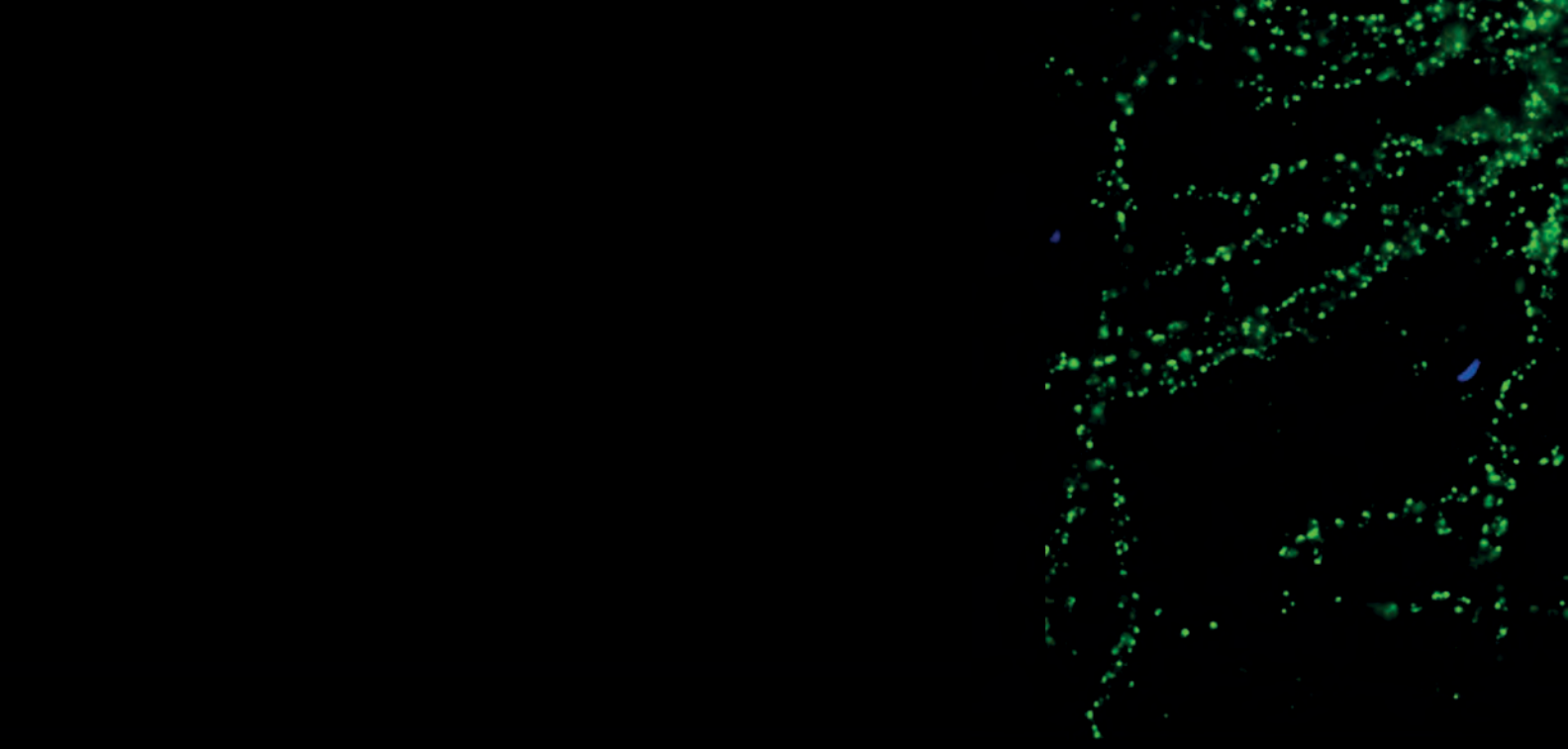
I finalment una tesi no es fa sense el caliu de la teva família i amics, que cel.lebren els dies bons però més important també t'aguanten els dies dolents!

Als meus pares, germana i gran família pel seu amor incondicional. A la Roser superpadrina i la Gemma superfillola i la seva marona (de ciències tb ;)). A totes "les ties i oncles" i cosins i cosines, i tb als seus peques. A les meves iaies, tan fortes i divertides. L'una tossuda i viatgera (a qui dec haver sortit?), i l'altre carinyosa i una gran persona! Això del Messi ha funcionat iaia, sé que n'estaries orgullosa. Juguem al Rumi o a la Canasta?

Als meus grans amics i amigues, el meu gran pilar. Gràcies pels viatges, els sopars, les converses i les rialles: De petits als pumes i panteres, i a la Jordina, l'Anna, i l'Aina que no ens veiem gaire però sé que sempre hi sou! La gran colla de Bagà (dita pessigolla) que em va adoptar com una englantinaire i patumarie més i que encara ens en queden mil per viure!: l'Aida, l'Ariadné, la Mercè, en Gerard, l'Albert, en Pau, la Núria, en Toni, en Xavi, la Maria, l'Anna, l'Ali, en Galde, i tots els peques. A la colla dels grans. Als brasilenys and company! Del Sud, a la gran família, amics i companys d'en Juan, una mica del Sud però també ja del Momo's i del Kibuka, i dels verdi,.. la Maite. A les nenes! Can Palom, casa meva per sempre!, us estimo Júlia i Gemma.

A ti Juan

Moltes gràcies.

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