trophil autoantibodies (p-ANCA) in unaffected relatives of patients with ulcerative colitis (UC). Suggestions against familial aggregation [resumen]. Gut 1994; 35 (Supl 4): A31.
24. Castellino F, Rosina F, Bansi DS, Bauducci M, Touscoz GA, Giorda L et al. Antineutrophil cytoplasmic antibodies in inflammatory bowel disease: do they recognize different subsets of a heterogeneous disease? Eur J Gastroenterol Hepatol 1995; 7: 859-864.
25. Lee JCW, Lennard-Jones JE, Cambridge G. Antineutrophil antibodies in familial inflammatory bowel disease. Gastroenterology 1995; 108: 428-433.
26. Kossa K, Coulthart A, Ives CT, Pusey CD, Hodgson HJF. Antigen specificity of circulating antineutrophil cytoplasmic antibodies in inflammatory bowel disease. Eur J Gastroenterol Hepatol 1995; 7: 783-789.
27. Hertervig E, Wieslander J, Johansson C, Wiik A, Nilsson A. Antineutrophil cytoplasmic antibodies in chronic inflammatory bowel disease. Scand J Gastroenterol 1995; 30: 693-698.
28. Esteve M, Mallolas J, Klaassen J, Abad-Lacruz A, González-Huix F, Cabré E et al. Antineutrophil cytoplasmic antibodies in sera from colectomised ulcerative colitis patients and its relation to the presence of pouchitis. Gut 1995; 38: 894-898
29. García Herola A, Bustamante M, Hoyos M, Sánchez-Cuenca JM, Nos P, Carmona E et al. Prevalencia de anticuerpos frente al citoplasma de los neutrófilos (ANCA) en la enfermedad inflamatoria intestinal [resumen]. Rev Esp Enf Digest 1996; 88 (Supl 1): 6.
30. Vasiliauskas EA, Plevy SE, Landers CJ, Binder SW, Ferguson DM, Yang H et al. Perinuclear antineutrophil cytoplasmic antibodies in patients with Crohn's disease define a clinical subgroup. Gastroenterology 1996; 110: 1.810-1.819.
31. Ellerbroek PM, Oudkerk Pool M, Ridwan BU, Dolman KM, Von Blomberg BME, Von dem Borne AEGKR et al. Neutrophil cytoplasmic antibodies (p-ANCA) in ulcerative colitis. J Clin Pathol 1994; 47: 257-262.
32. Stoffel MP, Csernok E, Herzberg C, Johnston T, Carroll F, Gross W. Antineutrophil cytoplasmic antibodies (ANCA) directed against bactericidal/permeability increasing protein (BPI): a new seromarker for inflammatory bowel disease and associated disorders. Clin Exp Immunol 1996; 104: 54-59.
33. Shanahan F. Neutrophil autoantibodies in inflammatory bowel disease: are they important? Gastroenterology 1994; 107: 586-589.
34. Dignass A, Goebell H. Genetics of inflammatory bowel disease. Curr Opin Gastroenterol 1995; 11: 292-297.
35. Lennard-Jones JE. Classification of inflammatory bowel disease. Scand J Gastroenterol 1989; 24 (Supl 170): 2-6.
36. Truelove SC, Witts LJ. Cortisone in ulcerative colitis. Final report of a therapeutic trial. Br Med J 1955; 2: 1.041-1.048.
37. Harvey RF, Bradshaw JM. A simple index of Crohn's disease activity. Lancet 1980; 1: 514-515.
38. Wiik A. Delineation of a standard procedure for indirect immunofluorescence detection of ANCA. APMIS 1989; 97 (Supl 6): 12-13.
39. Shanahan F. Pathogenesis of ulcerative colitis. Lancet 1994; 342: 407-411.
40. Jacquot S, Boumsell L, Bensussan A, Modigliani R. Anomalies du système immunitaire dans les maladies inflammatoires chroniques de l'intestin. Gastroenterol Clin Biol 1994; 18: 945-953.
41. Papo M, Quer JC, Pastor RM, García-Pardo G, Prats E, Mirapeix E et al. Antineutrophil cytoplasmic antibodies in relatives of patients with inflammatory bowel disease. Am J Gastroenterol 1996; 91: 1.512-1.515.
42. Rump JA, Wörner I, Roth M, Schölmerich J, Hänsch M, Peter HH. p-ANCA of undefined specificity in ulcerative colitis: correlation to disease activity and therapy. Adv Exp Med Biol 1993; 336: 507-513.
43. Seibold F, Slametschka D, Gregor M, Weber P. Neutrophil autoantibodies: a genetic marker in primary sclerosing cholangitis and ulcerative colitis. Gastroenterology 1994; 107: 532-536.
44. Lindgren S, Florén CH, Lindhagen T, Starck M, Stewenius J, Nässberger L. Low prevalence of antineutrophil cytoplasmic antibodies in ulcerative colitis patients with long-term remission. Eur J Gastroenterol Hepatol 1995; 7: 563-568.
45. Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Association of antineutrophil cytoplasmic antibodies with resistance to treatment of left-sided ulcerative colitis: results of a pilot study. Mayo Clin Proc 1996; 71: 431-436.

4.2. ESTUDIO 2

ANTICUERPOS ANTICITOPLASMA DE NEUTROFILO EN FAMILIARES DE PACIENTES CON ENFERMEDAD INFLAMATORIA INTESTINAL.

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Antineutrophil Cytoplasmic Antibodies in Relatives of Patients with Inflammatory Bowel Disease

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Background: **The occurrence of antineutrophil cytoplasmic antibodies (ANCA) has been reported more frequently than expected in healthy first-degree relatives of patients with ulcerative colitis, suggesting that these antibodies may represent a subclinical marker of genetic disease susceptibility.** ***Aim:*** **To determine the prevalence of ANCA in unaffected first-degree relatives of inflammatory bowel disease patients in a Spanish population.** ***Methods:*** **Three hundred and seventy sera obtained from 80 patients with inflammatory bowel disease (55 ulcerative colitis, 25 Crohn's disease), 217 unaffected first-degree relatives (157 from ulcerative colitis and 60 from Crohn's disease patients), 62 healthy controls, and 11 celiac disease patients were tested using an indirect immunofluorescence assay.** ***Results:*** **Antibodies were detected in 64% of patients with ulcerative colitis but in only 12.5% of patients with Crohn's disease. ANCA were seldom present in their unaffected first-degree relatives (4.6%), control subjects (1.6%), and celiac disease patients (0%).** ***Conclusions:*** **In the Spanish population studied, antineutrophil cytoplasmic antibodies occur more commonly in ulcerative colitis than in Crohn's disease, as reported in other Caucasian populations. Moreover, their presence is not increased in their first-degree relatives. These findings indicate that ANCA are not a subclinical marker of genetic susceptibility to inflammatory bowel disease in this population.**

INTRODUCTION

The etiopathogenesis of the chronic inflammatory bowel diseases (IBD), ulcerative colitis (UC) and Crohn's disease (CD), remains poorly understood. The most comprehensive theory proposed is that the disease occurs in a genetically susceptible subject, as a result of the interaction between environmental agents and host inmunological responses (1). Evidence for genetic predisposition is strongly supported by epidemiological and genetic studies showing ethnic differences in disease frequency, familial aggregation, increased monozygotic twin concordance, and different HLA associations for UC and CD (2).

Antineutrophil cytoplasmic antibodies (ANCA) directed against the cytoplasmic components of neutrophil granules were first described as an important marker in patients with Wegener's granulomatosis and other forms of vasculitis (3–5). Their clinical relevance has been well established in the diagnosis and management of the vasculitic syndromes (6). More recently, the occurrence of a subset of ANCA has been recognized in the sera of patients with IBD. The reported frequency of ANCA in patients with UC varies from 23 to 88%. In CD, ANCA have been detected much less frequently, with a prevalence of 0–25% and usually in low titers (7–15). In contrast to the vasculitides, however, the potential clinical significance of ANCA in IBD is still unclear. Currently available evidence indicates that they are not directly involved in its pathogenesis. On the other hand, their high degree of specificity for ulcerative colitis in comparison with other colitides, lack of correlation with disease extent and severity, and persistence after colectomy, suggests that they are not simply an epiphenomenon related to active colonic inflammation. Rather, they may reflect an underlying immunoregulatory disturbance in a subgroup of UC patients.

For the last few years, a higher frequency of ANCA has been reported in unaffected family members of patients with UC than in control subjects (16, 17). These findings raised the question of whether such antibodies may represent a subclinical marker of genetic susceptibility to UC. This increased prevalence, however, has not been confirmed by other studies (18–21). The discrepancy between the various reports may reflect population and ethnic differences in the patients studied. In the present study we have evaluated the frequency of ANCA in first-degree family members of patients with IBD in a well-defined Spanish population.

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MATERIALS AND METHODS

Study population

Serum samples obtained from patients with IBD, their first-degree relatives, and controls were coded and stored at −70°C until assayed.

All patients, family members, and controls analyzed in the present report are Caucasians, and were born and live in the same geographic area (province of Tarragona, Catalonia).

IBD patients

Eighty patients with IBD attending the Gastroenterology Section and the Department of Internal Medicine of two participating medical centers were included in the study. All patients were well known to the investigators, their diagnosis having been established based on conventional clinicopathological criteria according to those described by Lennard-Jones (22). There were 55 patients with UC (25 men, 30 women; 40 yr mean age) and 25 patients with CD (8 men, 17 women; 31 yr mean age). The main clinical features of the patients studied are shown in Table 1.

TABLE 1

Clinical Characteristics of Patients with Inflammatory Bowel Disease at the Time of Serum Sampling

Features	Ulcerative Colitis	Crohn's Disease
Number	55	25
Gender (Male/Female)	25/30	8/17
Mean age, yr (range)	40 (18–76)	31 (17–61)
Disease location		
Proctitis	6 (10.9%)	—
Proctosigmoiditis	16 (29.1%)	—
Left-sided colitis	14 (25.4%)	—
Extensive colitis	4 (7.3%)	—
Total colitis	10 (18%)	—
Small bowel	—	6 (24%)
Ileocolic	—	6 (24%)
Colonic	—	10 (40%)
Postresection	5[a]	3[b]
Disease activity		
Activity	19 (38%)	8 (36%)
Remission	31 (62%)	14 (64%)
Medication		
Steroids	10	7
Azathioprine	3	4

[a] 3 ileostomy, 2 ileoanal anastomosis.
[b] 3 ileocolic anastomosis.

First-degree relatives of patients with IBD

One hundred fifty-seven first-degree relatives of patients with UC ranging in age from 13 to 78 yr (mean 39.5) were tested for ANCA. There were 51 parents (30 women and 21 men), 19 brothers, 39 sisters, and 48 children (27 daughters and 21 sons). The group of first-degree relatives of patients with CD consisted of 60 subjects ranging in age from 15 to 73 (mean 42). There were 10 fathers, 19 mothers, 10 brothers, 13 sisters, 7 sons, and 1 daughter.

At the time of the blood collection, relatives were interviewed and a detailed medical history was obtained to yield information concerning any significant gastrointestinal symptom or extraintestinal manifestation that may relate to as-yet-undiagnosed IBD. Five relatives were found to have IBD and were not included in the study population.

Control subjects

As controls, we examined sera from 40 healthy blood donors (25 men, 15 women, 35 yr mean age) without any history of gastrointestinal disease, nor family members affected with IBD. We also investigated 22 healthy spouses of patients with IBD (20 UC, 2 CD; 14 men, 8 women; 45 yr mean age) as environmental controls, and 11 patients with celiac disease.

Indirect immunofluorescence assay

A standard indirect immunofluorescence (IIF) method was used for detection of ANCA (23). Sera were diluted 1:20 in phosphate-buffered saline (PBS), and approximately 35 μL of the diluted sera was placed onto test wells on glass slides containing ethanol-fixed neutrophils as antigen substate (INOVA Diagnostics, San Diego, CA). All incubations were performed in humidified boxes at room temperature for 30 min. The slides were then washed with three changes of PBS for 5 min each. After a 30-min incubation period with anti-human IgG conjugated to fluorescein with Evans blue counterstain (INOVA Diagnostics), they were washed again as above, mounted under a coverslip with PBS-glycerin, and finally examined with a fluorescent microscope. Positive and negative controls were included for all assays. The slides were analyzed in a blinded fashion, and perinuclear or cytoplasmic immunofluorescence staining pattern was regarded as positive. All positive results and 20 negative results randomly selected were retested in another laboratory (Laboratory of Nephrology, Hospital Clinic i Provincial of Barcelona) following the same IIF test procedure by another investigator. Only sera with positive test results in both laboratories were regarded as positive in the final results. Sera with different results between the two laboratories were excluded from the final analysis.

Sera that produced a nuclear or equivocal perinuclear ANCA staining pattern in neutrophils were tested for the presence of antinuclear antibodies (ANA) using commercially prepared slides with fixed HEp2 cells as substrate (INOVA Diagnostics).

Statistical analysis

Statistical comparisons of prevalence of ANCA in the study groups were performed by the chi-square test. To measure the correlation of tests performed in the two different laboratories, Cohen's kappa coefficient was used.

TABLE 2
Prevalence of ANCA in Patients with UC, CD, First-Degree Relatives, Healthy Controls, and Celiac Disease

Diagnosis	n	ANCA Positive (n) (%)
Ulcerative colitis	53	34 (64)*
Crohn's disease	24	3 (12.5)
First-degree relatives of UC patients	155	6 (3.9)**
First-degree relatives of CD patients	60	4 (6.7)**
Healthy controls	40	1 (2.5)
Spouses of IBD patients	22	0
Celiac disease	11	0

* $p < 0.0001$ *vs* all other groups.
** p NS *vs* healthy controls.

RESULTS

Interlaboratory correlation of the immunofluorescence test

Results of the sera (n = 72) tested in the two laboratories were as follows. Forty-seven sera tested ANCA positive and 20 tested ANCA negative in both laboratories. Only five sera tested different results. Thus, a high degree of correlation was found between the two laboratories (kappa score = 0.84; $p < 0.001$).

Prevalence of ANCA in IBD patients

Thirty-four of 53 (64%) UC patients showed the presence of circulating ANCA. Perinuclear staining around neutrophil nuclei was the predominant IIF pattern observed (76.5%), similar to the proportion described in previous reports. In addition, a homogeneous cytoplasmic staining was given by eight sera. There was no correlation between ANCA positivity and duration, course, extent, and activity of the disease (data not shown). In CD, ANCA were detected in 3 of 24 patients (12.5%). Two patients had the disease confined to the colon and the other to the ileum.

Prevalence of ANCA in first-degree relatives

Of the unaffected first-degree relatives of patients with UC studied, only 6 sera of 155 (3.9%) tested ANCA positive. Four of the 60 (6.7%) relatives of patients with CD were ANCA positive. All of these 10 positive ANCA relatives belonged to different families. Among them, one had seropositive rheumatoid arthritis and another ankylosing spondylitis, conditions known to exhibit ANCA. Table 2 summarizes the results.

Control subjects

Only one of the 40 healthy controls tested ANCA positive. ANCA were not detected in any of the 22 spouses of patients with IBD, nor in the 11 patients with celiac disease.

Antinuclear antibodies

Three patients with UC and one patient with CD had an equivocal ANCA or diffuse nuclear immunofluorescence staining of all leukocyte nuclei in ethanol-fixed human neutrophils. In all cases they were found to be positive for ANA when tested on HEp2 cells. A total of 9 sera among 11 of family members assessed were positive for ANA.

DISCUSSION

In recent years it has been firmly established that ANCA are often present in patients with UC but not in other IBDs, including CD (7–15). The results from the present study are within the limits of most previous reports. Thirty-four of 53 UC patients (64%) were ANCA positive compared with only 3 of 24 patients with CD (12.5%). Additionally, we did not detect an increased prevalence of ANCA in the unaffected first-degree relatives of IBD patients compared with healthy controls.

A familial predisposition to IBD is now widely recognized; the risk of developing the disease in family members is increased approximately 10-fold (24). At the present time, however, no subclinical markers are available to identify those at risk of developing the disease; studies of lymphocytotoxic antibodies, antibodies to colonic epithelial cells, colonic mucins, mucosal immunoglobulins, intestinal permeability, and complement system have not shown any consistent pattern (2). The role of ANCA as a potential subclinical marker of genetic predisposition to UC was first suggested by the finding of an increased prevalence of ANCA in the unaffected family members of patients with UC from two North American patient populations. The authors reported the presence of ANCA in 14 of 93 (15%) first-degree relatives to 38 UC patients in Los Angeles and in 9 of 43 (21%) first-degree relatives to 22 UC patients in Calgary, Canada (16). This observation was strengthened by a German study in which ANCA were detected in 30% of 142 first-degree relatives of patients with UC (17). In contrast to the American and German reports, however, four other studies from France, Italy, and England failed to detect a higher frequency of ANCA in the sera of relatives of IBD patients (18–21). In addition, Yang *et al.* have recently reported that ANCA were not significantly increased in healthy monozygot twin siblings to twins with UC, thus arguing against the hypothesis of ANCA as a subclinical marker of genetic susceptibility to UC (25).

It is uncertain why our results are in agreement with the latter studies but differ from those of the North American and German ones. It has been suggested that the different methods used for the detection of ANCA may explain in part these substantial differences. The whole cell ELISA method used by Shanahan *et al.* (16) has provided in most studies a higher sensitivity but lower specificity than the IIF test. Thus, the ELISA test might detect antineutrophil cytoplasmic antibodies other than those responsible for ANCA in UC. On the other hand, although subjective in interpretation, IIF results are more reproducible and correlate well in independent laboratories as shown by Oudkerk Pool *et al.* (12, 13) and our own study. Of note, the American investigators used IIF to verify every positive ELISA result, and

Seibold *et al.* (17) applied the same IIF method performed in the other European studies. Thus, these differences are unlikely to be entirely caused by technical variations. So far, the nature of the antigen(s) to which ANCA react in UC remain unknown. It seems likely that their identification will allow the development of more specific assays to overcome these methodological problems.

Another explanation for the divergent results among unaffected relatives may be related to variability in different populations and ethnic groups. In fact, the reported frequency of positivity of ANCA in UC patients varies widely in several different study populations. Positivity is more than 70% in North America (7, 8), 70% in Germany (10), but only 50% in France (9), 50% in England (11), and 40% in Australia (15). Here again, differences could be caused by the use of different detection methods as alluded to earlier. Nevertheless, studies performing simultaneous analysis of samples from distinct populations in different laboratories have confirmed significant regional differences, thus suggesting genetic differences between these populations (13, 17). Similarly, such variability may occur not only in probands, but also in relatives in different geographic areas.

In summary, we have not found an increased prevalence of ANCA among the unaffected first-degree relatives of patients with IBD compared with healthy controls. Therefore, our results indicate that, at least in our population, ANCA do not represent a subclinical marker of genetic susceptibility to IBD.

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REFERENCES

1. Shanahan F. Pathogenesis of ulcerative colitis. Lancet 1994;342:407–11.
2. Yang H, Rotter JI. The genetics of inflammatory bowel disease. In: Targan S, Shanahan F, eds. Inflammatory bowel disease: From bench to bedside. Baltimore: Williams & Wilkins, 1993: 32–64.
3. Davis DJ, Moran JE, Nial JF, et al. Segmental necrotizing glomerulonephritis with antineutrophil antibodies: Possible arbovirus aetiology. Br Med J 1982;285:606.
4. Falk RJ, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. N Engl J Med 1988;318:1651–7.
5. Van der Woude FJ, Rasmussen N, et al. Autoantibodies against neutrophils and monocytes: Tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet 1985;1:425–9.
6. Kallenberg CGM, Brouwer E, Weening JJ, et al. Antineutrophil cytoplasmic antibodies: Current diagnostic and pathophysiological potential. Kidney Int 1994;46:1–15.
7. Saxon A, Shanahan F, Landers C, et al. A distinct subset of antineutrophil cytoplasmic autoantibodies is associated with inflammatory bowel disease. J Allergy Clin Immunol 1990;86:202–10.
8. Duerr RH, Targan SR, Landers CJ, et al. Anti-neutrophil cytoplasmic antibodies in ulcerative colitis: Comparison with other colitides/diarrheal illnesses. Gastroenterology 1991;100:1590–6.
9. Colombel J-F, Reumaux D, Duthilleul P, et al. Antineutrophil cytoplasmic autoantibodies in inflammatory bowel diseases. Gastroenterol Clin Biol 1992;16:656–60.
10. Seibold F, Weber P, Klein R, et al. Clinical significance of antibodies against neutrophils in patients with inflammatory bowel disease and primary sclerosing cholangitis. Gut 1992;33:657–62.
11. Cambridge G, Rampton DS, Stevens TRJ, et al. Anti-neutrophil antibodies in inflammatory bowel disease: Prevalence and diagnostic role. Gut 1992;33:668–74.
12. Oudkerk Pool M, Ellerbroek PM, Ridwan BU, et al. Serum antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease are mainly associated with ulcerative colitis: A correlation study between perinuclear antineutrophil cytoplasmic autoantibodies and clinical parameters, medical and surgical treatment. Gut 1993;34:46–50.
13. Oudkerk Pool M, Roca M, Reumaux D, et al. The value of pANCA as a serological marker for ulcerative colitis in different European regions. Eur J Gastroenterol Hepatol 1994;6:399–403.
14. Vecchi M, Bianchi MB, Sinico RA, et al. Antibodies to neutrophil cytoplasm in Italian patients with ulcerative colitis: Sensitivity, specificity and recognition of putative antigens. Digestion 1994;55:34–9.
15. Romas E, Paspaliaris B, d'Apice AJF, et al. Autoantibodies to neutrophil cytoplasmic (ANCA) and endothelial cell surface antigens (AECA) in chronic inflammatory bowel disease. Aust NZ J Med 1992;22:652–9.
16. Shanahan F, Duerr RH, Rotter JI, et al. Neutrophil autoantibodies in ulcerative colitis: Familial aggregation and genetic heterogeneity. Gastroenterology 1992;103:456–461.
17. Seibold F, Slametschka D, Gregor M, Weber P. Neutrophil autoantibodies: A genetic marker in primary sclerosing cholangitis and ulcerative colitis. Gastroenterology 1994;107:532–6.
18. Reumaux D, Delecourt L, Colombel JF, et al. Antineutrophil cytoplasmic autoantibodies in relatives of patients with ulcerative colitis. Gastroenterology 1992;103:1706.
19. Monteleone G, Doldo P, Marasco R, et al. Perinuclear neutrophil autoantibodies (p-ANCA) in unaffected relatives of patients with ulcerative colitis (UC): Suggestions against familial aggregation. Gut 1994;35(suppl 4):A31 (abstract).
20. Lo SK, Fleming K, Chapman R. No increased frequency of p-ANCA in the families of patients with ulcerative colitis and primary sclerosing cholangitis. J Hepatol 1994;21:556 (abstract).
21. Lee JCW, Lennard-Jones JE, Cambridge G. Antineutrophil antibodies in familial inflammatory bowel disease. Gastroenterology 1995;108:428–33.
22. Lennard-Jones JE. Classification of inflammatory bowel disease. Scand J Gastroenterol 1989;24(suppl 7):2–6.
23. Wiik A. Delineation of a standard procedure for indirect immunofluorescence detection of ANCA. APMIS 1989;97(suppl 6):12–3.
24. Orholm M, Munkholm P, Langholz E, et al. Familial occurrence of inflammatory bowel disease. N Engl J Med 1991;324:84–8.
25. Yang P, Järnerot G, Danielsson D, et al. P-ANCA in monozygotic twins with inflammatory bowel disease. Gut 1995;36:887–90.

4.3. ESTUDIO 3

HETEROGENEIDAD GENETICA EN LA COLITIS ULCEROSA DETERMINADA POR UN POLIMORFISMO GENETICO DEL ANTAGONISTA DEL RECEPTOR DE LA INTERLEUCINA 1 Y ANTICUERPOS ANTICITOPLASMA DE NEUTROFILO.

Genetic heterogeneity within ulcerative colitis determined by an interleukin-1 receptor antagonist gene polymorphism and antineutrophil cytoplasmic antibodies

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Background **Although there is strong evidence implicating genetic predisposition in the pathogenesis of the chronic inflammatory bowel diseases, the number and identity of susceptibility genes remain uncertain. Cytokine genes are tentative candidate loci, but data regarding association studies in different populations are conflicting.**

Aims **To determine potential associations of interleukin-1 receptor antagonist (IL-1ra), tumour necrosis factor α (TNFα), and tumour necrosis factor β (TNFβ) gene polymorphisms with ulcerative colitis or subsets of ulcerative colitis in a Spanish population.**

Methods **Genotyping for IL-1ra, TNFα and TNFβ gene polymorphisms was performed by the polymerase chain reaction in 95 patients with ulcerative colitis and 74 healthy controls. A variable number of tandem repeats (VNTR) in the IL-1ra gene, and a single base pair polymorphism in the TNFα gene promoter region (−308) and in the first intron of the TNFβ gene were analysed. Anti-neutrophil cytoplasmic antibodies (ANCA) were detected using an indirect immunofluorescence assay.**

Results **There were no significant differences between ulcerative colitis patients and controls in either polymorphism analysed, nor between ulcerative colitis subgroups as a function of the clinical disease pattern. However, when stratified by their ANCA status, perinuclear ANCA (p-ANCA) ulcerative colitis showed an increased frequency of the genotype 1,2 of the IL-1ra gene compared with ANCA-negative ulcerative colitis (52% versus 28%; $P = 0.02$, $P_{corr} = 0.1$). Furthermore, p-ANCA ulcerative colitis had a statistically significant increase of this genotype compared with cytoplasmic ANCA (c-ANCA)/ ANCA-negative ulcerative colitis (52% versus 26.5%; $P = 0.01$, $P_{corr} = 0.05$).**

Conclusions **In the Spanish population studied, the polymorphisms analysed in the IL-1ra, TNFα and TNFβ genes are unlikely to be important in the overall susceptibility to ulcerative colitis. However, the combination of a subclinical (p-ANCA) and a genetic (IL-1ra gene) marker identified a distinct ulcerative colitis subgroup (p-ANCA; IL-1ra genotype 1,2). These findings provide further evidence of genetic heterogeneity within ulcerative colitis, and support the concept that ANCA may represent a subclinical marker of genetic heterogeneity.**
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Keywords: anti-neutrophil cytoplasmic antibodies, cytokine gene polymorphisms, inflammatory bowel disease, ulcerative colitis

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Introduction

The aetiopathogenesis of the chronic inflammatory bowel diseases (IBDs) ulcerative colitis and Crohn's disease remains unknown. The most comprehensive theory proposed is that the diseases occur in a genetically susceptible subject, as a result of the interaction between environmental triggers and host immunological responses [1]. Although numerous lines of evidence strongly support the contribution of genetic factors in the pathogenesis of ulcerative colitis and Crohn's disease, the pattern of inheritance is still unclear [2]. Currently available evidence indicates that they are not inherited in any simple Mendelian or polygenic models. Moreover, recent studies using the genome-wide screening technique have shown the involvement of multiple genes in different chromosomes [3–5]. In addition, epidemiological and genetic studies support the hypothesis that the IBDs may be genetic heterogeneous disorders of polygenic inheritance [6,7].

For the last few years, the identification of susceptibility genes in IBD has become a major focus of research.

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Considering the central role of the immune system in mediating the tissue damage in IBD, most studies have investigated the potential contribution of the HLA class II genes. Several reports from North America [8,9], Europe [10] and Japan [11] have shown that these genes are determinants of disease susceptibility and behaviour in ulcerative colitis. However, data regarding HLA class II associations with ulcerative colitis in different populations have been conflicting.

Other non-HLA genes also involved in the regulation of the immune response have been proposed recently as natural candidates for genetic IBD studies. Although their functional significance is still under evaluation, the description of several polymorphisms within the cytokine genes has allowed the investigation of associations with ulcerative colitis and Crohn's disease [12].

Interleukin-1 receptor antagonist (IL-1ra) is a natural receptor antagonist that modulates the pro-inflammatory effects of interleukin 1. Both experimental and clinical studies have pointed out the importance of IL-1ra in IBD [13]. Of note, an intestinal mucosal imbalance of IL-1 and IL-1ra has been described in patients with ulcerative colitis, which may contribute to the pathogenesis of chronic gut inflammation. The gene encoding the IL-1ra lies on chromosome 2 and contains a variable number of tandem repeats (VNTR) of an 86 base pair sequence in intron 2 [14]. Mansfield *et al.* [15] reported an association between ulcerative colitis and allele 2 of the polymorphic IL-1ra gene. This was the first observed association of ulcerative colitis with a gene outside the major histocompatibility complex (MHC). Although this finding was strengthened by two North American studies [16,17], data from subsequent European reports did not support it [18–23].

Tumour necrosis factor α (TNFα) and tumour necrosis factor β (TNFβ) are potent pro-inflammatory and immunomodulatory cytokines, implicated in the initiation and regulation of the immune response [12]. The genes for these cytokines are tandemly arranged in the class III region of the MHC, on the short arm of chromosome 6. A number of polymorphisms that might be responsible for differences in the secretion of these cytokines have been described in the TNF locus. Of particular interest, a polymorphism at position −308 in the promoter region of the TNFα gene may be important for the transcriptional control of TNFα [24–26], and has been implicated in genetic susceptibility to some autoimmune diseases [27]. Within the first intron of the TNFβ gene, an *Nco*I restriction fragment length polymorphism (RFLP) influencing TNFβ production has been described [28]. In addition, an association between this polymorphism and TNFα production has also been found [29]. Genetic variation within this polymorphism might be involved in the pathogenesis of certain autoimmune diseases [27].

During recent years, the occurrence of anti-neutrophil cytoplasmic antibodies (ANCA) has been recognized in patients with IBD, particularly ulcerative colitis [30]. The majority of ulcerative colitis-associated ANCA show an immunofluorescence perinuclear pattern (p-ANCA), but cytoplasmic (c-ANCA) and mixed patterns have also been described. The significance of ANCA in IBD, however, remains unclear. Their value as disease markers is supported by the high degree of specificity for IBD in comparison with other colitides, lack of correlation with disease extent and activity, and persistence after colectomy. Furthermore, it has been suggested that p-ANCA may allow stratification of clinically distinct subsets of IBD patients: they have been found to be particularly associated with patients with pouchitis [31], treatment-resistant left-sided ulcerative colitis [32], aggressive disease [33] and Crohn's disease patients with an 'ulcerative colitis-like' clinical phenotype [34]. On the other hand, ANCA may represent a genetic marker in IBD, although this issue is a matter of debate. This concept was raised by the finding of an increased prevalence of these antibodies in healthy relatives of ulcerative colitis patients, suggesting their role as a subclinical marker of genetic disease susceptibility [35,36]. In addition, the reported associations between ANCA and different genetic markers in ulcerative colitis (HLA class II and intercellular adhesion molecule 1 genes) point to their role as a potential marker of genetic heterogeneity [37,38].

In the present study, we report on the distribution of three cytokine gene polymorphisms (IL-1ra, TNFα and TNFβ) in a well-defined Spanish population of patients with ulcerative colitis. We have also investigated potential associations between these polymorphisms and subgroups of patients defined by their clinical disease pattern and ANCA status.

Materials and methods

Study subjects

Ninety-five non-operated patients with ulcerative colitis attending the Gastroenterology Section and the Department of Gastroenterology of two participating medical centres (Hospital Joan XXIII of Tarragona and Hospital Vall d'Hebron of Barcelona) were included in the study. The patients studied did not have any other conditions known to exhibit ANCA, nor anti-nuclear antibody positive serum samples. All patients were well known to the investigators, their diagnosis having been established based on conventional clinico-pathological criteria. Disease extent was determined by endoscopy and/or radiology, and classified as extensive (proximal to the splenic flexure) or distal. Patients were divided according to the clinical course into relapsing–remitting

and chronic–relapsing requiring immunosuppressive therapy. The main clinical features of the patients are shown in Table 1.

Seventy-four unrelated healthy blood donors without any history of gastrointestinal disease nor family members affected with IBD served as controls. All patients and controls analysed in the present report are non-Jewish Caucasians, and were born and live in the same geographical area (Catalonia).

Isolation of DNA

A 10 ml sample of venous blood was collected in an EDTA tube. Within 1 h of drawing, the buffy coat was separated from the blood by centrifugation at 800–900 *g* for 10 min. Genomic DNA was isolated from the buffy coat using QiaAMP spin columns (Qiagen, Chatsworth, California, USA).

Cytokine gene polymorphisms analysis

Genotyping for the cytokine gene polymorphisms was performed using the polymerase chain reaction (PCR).

IL-1ra VNTR gene polymorphism

A 100 ng aliquot of the extracted DNA was used as a template for amplifying the second intron of the IL-1ra gene, which contains a variable number of 86 bp sequence that gives rise to this polymorphism [15].

The primers used in the PCR were: 5′-CTCAGC-AACACTCCTAT-3′ and 5′-TCCTGGTCTGCAGG-TAA- 3′. The reaction was carried out in a final volume of 50 µl containing 2 mmol/l $MgCl_2$, 0.2 mmol/l of each dNTP (Boehringer Mannheim, Mannheim, Germany), 0.2 µmol/l of each primer and 2.5 units of Taq polymerase (Gibco Life Technologies). DNA was amplified for 30 cycles, with an initial denaturation of 30 s at 94°C and a final extension of 10 min at 70°C. Each cycle consisted of 30 s denaturation at 94°C, 1 min annealing at 58°C and 1 min extension at 70°C. PCR products were electrophoresed on a 2% agarose gel. VNTR was detected by ethidium bromide staining.

VNTR IL-1ra analysis revealed a five-allele polymorphism which produced five bands of different sizes. A 410 bp band corresponded to the class 1 allele (four repeats), a 240 bp band to the class 2 allele (two repeats), a 500 bp band to the class 3 allele (five repeats), a 325 bp band to the class 4 allele (three repeats), and a 595 bp band to the class 5 allele (six repeats).

Table 1 Characteristics of patients with ulcerative colitis

Total number	95
Gender (male/female)	45/50
Age (years)	
Mean	41
Range	16–79
Extent of ulcerative colitis	
Extensive	39 (41%)
Distal	56 (59%)
Clinical course	
Relapsing–remitting	76 (80%)
Chronic–relapsing	19 (20%)

TNFα gene polymorphism

A transition polymorphism G → A in the −308 position of the gene was detected [15]. A 100 ng aliquot of the extracted DNA was used as a template. The primers used were: 5′-AGGCAATAGGTTTTGAGGGCCAT-3′ and 5′-TCCTCCCTGCTCCGATTCCG-3′. The reaction was carried out in a final volume of 50 µl containing 3 mmol/l of $MgCl_2$, 0.5 mmol/l of each dNTP, 0.2 µmol/l of each primer and 2.5 units of Taq DNA polymerase. DNA was amplified for 35 cycles, each consisting of 1 min denaturation at 94°C, 1 min annealing at 60°C and 1 min extension at 72°C. Before the first cycle, one cycle of 3 min denaturation at 94°C, 1 min annealing at 60°C and 1 min extension at 72°C was performed. After all the cycles, a final cycle of 1 min denaturation at 94°C, 1 min annealing at 60°C and 5 min extension at 72°C was included. PCR products were digested with a 10-fold excess of *Nco*I restriction enzyme at 37°C for 2–4 h and electrophoresed on a 2.5% agarose gel. *Nco*I RFLPs were detected by ethidium bromide staining.

RFLP-TNFα gene analysis revealed a two-allele polymorphism that produced three bands of different sizes: a 107 bp fragment corresponding to the TNF2 allele (restriction site absent) and a set of 87 bp and 20 bp bands corresponding to the TNF1 allele (restriction site present).

TNFβ gene polymorphism

A 100 ng aliquot of the extracted DNA was used as a template for amplifying the first intron of the TNFβ gene, which contains a mutation that correlates 100% with a mutation in the second exon of this gene that produces a transversion of A → C [28]. The primers used in the PCR were: 5′-CCGTGCTTCGTGC-TTTGGACTA-3′ and 5′-AGAGCTGGTGGGGACA-TGTCTG-3′. The reaction was carried out in a final volume of 50 µl containing 1.75 mmol/l $MgCl_2$, 0.34 mmol/l of each dNTP, 0.38 µmol/l of each primer and 2.8 units of Taq polymerase. DNA was amplified for 30 cycles with an initial denaturation of 2 min at 94°C and a final extension of 7 min at 68°C. The cycles were divided into two programmes, one consisting of 10 cycles of 10 s denaturation at 94°C, 30 s annealing at 65°C and 2 min extension at 68°C, and the other consisting of 20 cycles with the same temperatures and times except for the extension time which increased by 20 s per cycle. PCR products were digested with a 10-fold excess of *Nco*I restriction enzyme at 37°C

overnight and electrophoresed on a 0.8% agarose gel. *Nco*I RFLPs were detected by ethidium bromide staining.

RFLP-TNFβ gene analysis revealed a two-allele polymorphism that produced three bands of different sizes: a 740 bp band corresponding to the TNFB1 allele (absence of restriction site) and a set of 555 bp and 185 bp bands corresponding to the TNFB2 allele (restriction site present).

ANCA indirect immunofluorescence assay

A standard indirect immunofluorescence (IIF) method was used for detection of ANCA as previously described [39]. In brief, sera were diluted 1:20 in phosphate-buffered saline (PBS), and approximately 35 µl of the diluted sera was placed onto test wells on glass slides containing ethanol-fixed neutrophils as antigen substrate (INOVA Diagnostics, San Diego, California, USA). All incubations were performed in humidified boxes at room temperature for 30 min. The slides were then washed with three changes of PBS for 5 min each. After a 30 min incubation period with anti-human immunoglobulin (IgG) conjugated to fluorescein with Evans blue counterstain (INOVA Diagnostics), they were washed again as above, mounted under a cover slip with PBS–glycerin, and finally examined with a fluorescent microscope. Positive and negative controls were included for all assays. The slides were analysed in a blinded fashion by two independent investigators, and a perinuclear or cytoplasmic immunofluorescence staining pattern was regarded as positive. Sera with an equivocal ANCA staining pattern were excluded from the final analysis.

Statistical analysis

Statistical comparisons of genotypes, allele frequencies and carriage rates between the study groups were performed using either a χ^2 test or Fisher's Exact Test when appropriate. For subgroup analysis as a function of clinical disease pattern (extent and clinical course) and ANCA status, correction was made using a Bonferroni correction for multiple analysis. Thus probability values were multiplied by a factor of 5 to obtain corrected probability values. A probability value of 0.05, after correction if necessary, was considered to be the threshold for statistical significance.

Results

Comparisons between ulcerative colitis patients and controls

The distribution of the IL-1ra, TNFα and TNFβ genotypes and allelic frequencies in patients with ulcerative colitis and healthy controls is shown in Tables 2 and 3. There were no significant differences in the IL-1ra VNTR genotypes and allelic frequencies between the two groups (Table 2). In patients with ulcerative colitis, the frequency of allele 2 (30.5%) was similar to that in the controls (31%). Moreover, no significant difference was observed between ulcerative colitis patients and controls in the allele 2 carriage rate (50.5% versus 53%). The polymorphisms studied in the TNFα and TNFβ genes did not either reveal any associations with ulcerative colitis when compared with the control group (Table 3).

Comparisons within subgroups of ulcerative colitis patients as a function of the clinical disease pattern and ANCA status

On subgroup analysis based on the clinical disease pattern, no associations between the IL-1ra VNTR genotypes and allelic frequencies, and groups defined by extent and clinical course were found. In patients with extensive colitis, the frequency of allele 2 (29%) was similar to that in the patients with distal colitis (31%). Nineteen of 39 patients (49%) with extensive colitis and 29 of 56 patients (52%) with distal disease were carriers of at least one copy of allele 2.

When we divided patients according to their ANCA status, we observed that p-ANCA ulcerative colitis patients had an increased frequency of the genotype 1,2 compared with ANCA-negative patients (52% versus 28%; $P = 0.02$, $P_{corr} = 0.1$) (Table 4). Similarly, p-ANCA ulcerative colitis patients had a higher frequency of the genotype 1,2 than c-ANCA ulcerative colitis patients (52% versus 22%), although the difference was not statistically significant probably due to the small numbers analysed. For further analysis, we combined c-ANCA and ANCA-negative patients, since both groups showed a similar genotype distribution, and compared them with p-ANCA ulcerative colitis patients. The result indicated that p-ANCA ulcerative colitis patients had a statistically significant increase of the genotype 1,2 compared with c-ANCA/ANCA-nega-

Table 2 **IL-1ra genotypes and allele frequencies in patients and controls**

	Genotype						Allelic frequencies (%)			
	1,1	1,2	1,3	1,5	2,2	3,3	1	2	3	5
Healthy controls ($n = 73$)	31	33	2	1	6	0	67	31	1	1
Ulcerative colitis ($n = 95$)	44	38	2	0	10	1	67.5	30.5	2	0

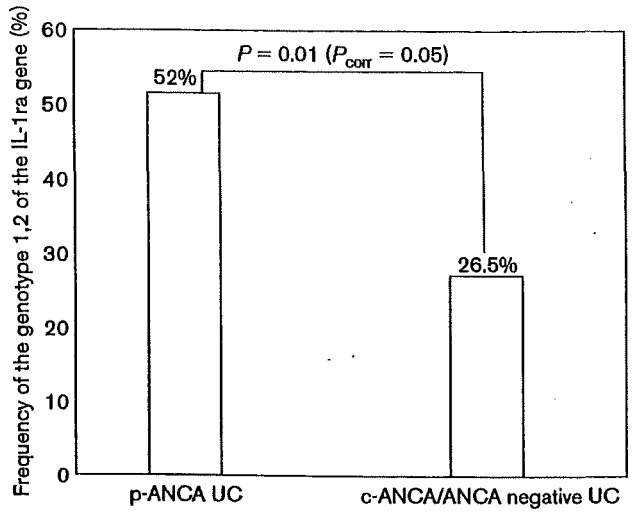
Table 3 TNFα and TNFβ genotypes and allele frequencies in patients and controls

	Genotype			Allelic frequencies (%)	
	1,1	2,2	1,2	1	2
TNFα					
Healthy controls ($n = 74$)	52	4	18	82.5	17.5
Ulcerative colitis ($n = 93$)	71	3	19	86.5	13.5
TNFβ					
Healthy controls ($n = 71$)	7	41	23	26	74
Ulcerative colitis ($n = 92$)	7	46	39	29	71

tive ulcerative colitis (52% versus 26.5%; $P = 0.01$, $P_{corr} = 0.05$) (Fig. 1). Finally, p-ANCA/IL-1ra genotype 1,2 ulcerative colitis was not associated with any clinical disease pattern when compared with the remaining ulcerative colitis patients (data not shown).

The genotype and allelic frequencies of the TNFα and TNFβ genes did not reveal any significant differences between subgroups defined by their clinical disease pattern, nor their ANCA status (data not shown).

Fig. 1



Frequency of the genotype 1,2 of the IL-1ra gene in p-ANCA ulcerative colitis and c-ANCA/ANCA-negative ulcerative colitis. p-ANCA ulcerative colitis patients showed an increased frequency of the genotype 1,2 ($P = 0.01$, $P_{corr} = 0.05$) compared with c-ANCA/ANCA-negative ulcerative colitis patients.

ANCA

Four patients had an equivocal ANCA staining pattern and were excluded from further analysis. Fifty-five of 91 (60.5%) ulcerative colitis patients showed the presence of circulating ANCA. Perinuclear staining around neutrophil nuclei was the predominant IIF pattern observed (50.5%), whereas a cytoplasmic staining was seen for nine samples (10%). There was no correlation between ANCA positivity and course, extent, and activity of the disease (data not shown).

Discussion

Considering the key role of cytokines in the control of the mucosal inflammatory response in IBD, genes involved in the regulation of their activity have recently been proposed as tentative candidate loci for genetic IBD studies. Mansfield *et al.* [15] first reported an association between the polymorphic gene for IL-1ra and ulcerative colitis in a British population. The rare allele 2 was over-represented in patients with ulcerative colitis (35%) compared with controls (24%). Furthermore, since this association was greatest in patients with total colitis, the authors suggested that allele 2 of the IL-1ra gene might represent a genetically specified severity factor in ulcerative colitis. This overall association was supported by two North American studies [16,17] which showed a significant increase in the allele 2 carriage rate in ulcerative colitis patients compared with controls. In contrast, however, other European studies from Germany [18,22], England [20,21], The Netherlands [19] and France [23] failed to detect a significant association of this allele with ulcerative colitis. The results of the present study are in agreement with the latter reports. Allele 2 frequencies and carriage rates were similar in patients with ulcerative colitis and healthy controls (30.5% versus 31% and 50.5% versus 53%, respectively). In addition, no differences were detected between patients with extensive and distal colitis (49% versus 52%). Our present results are also consistent with those of another study in a Spanish population, which showed no association between allele 2 and ulcerative colitis [40].

Table 4 IL-1ra genotypes in p-ANCA ulcerative colitis, c-ANCA ulcerative colitis and ANCA-negative ulcerative colitis patients

	Genotype 1,2	Genotypes 1,1 1,3 2,2 and 3,3
ANCA-negative ulcerative colitis ($n = 36$)	10 (28%)	26 (72%)
p-ANCA ulcerative colitis ($n = 46$)	24 (52%)*	22 (48%)
c-ANCA ulcerative colitis ($n = 9$)	2 (22%)	7 (78%)

*p-ANCA ulcerative colitis versus ANCA-negative ulcerative colitis: $P = 0.02$, $P_{corr} = 0.1$.

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The reasons for the discrepancies between these studies are unclear. Methodological reasons are unlikely to be responsible, since all of them used the same molecular genotyping procedures. There are several possible explanations for the divergent results. First, ethnic differences, which account for many of the inconsistencies reported in HLA association studies in ulcerative colitis, may in part explain them. Thus, the North American studies involved a mixed Jewish/non-Jewish population from Pittsburgh and a Hispanic one from Los Angeles, whereas the European studies included predominantly non-Jewish white European subjects. Moreover, Tountas *et al.* [41] have recently reported that the association with allele 2 of the IL-1ra gene was only relevant in a Jewish subgroup from a Caucasian population in Los Angeles, but not in the whole study cohort. Second, the different results could be due to disease heterogeneity. From a clinical point of view, ulcerative colitis is a heterogeneous disease, which may be a reflection of underlying genetic heterogeneous background. For instance, there is evidence that genes in the HLA region may influence disease behaviour in ulcerative colitis: the HLA-DRB1*1502 allele has been found to be associated with disease intractability [11] and corticosteroid treatment [42], whereas the DR3-DQ2 haplotype has been found to predict extensive disease [10]. Similarly, the allele 2 of IL-1ra might only be associated with a particular subgroup of ulcerative colitis patients. In fact, three of the above studies showed a significant association of allele 2 exclusively with extensive disease [19,21] or disease intractability [23], but not with the whole group of ulcerative colitis patients. Finally, it is possible that the reported weak association in some studies reflects the fact that the IL-1ra gene does not contribute in itself to genetic susceptibility to ulcerative colitis, but rather is a marker of other closely linked genes on chromosome 2 of primary importance, or alternatively, the synergistic association of specific alleles of the IL-1ra gene with other nearby genetic markers predisposes to ulcerative colitis. Supporting this last hypothesis, different associations of allelic variants of the IL-1β and IL-1ra genes in both ulcerative colitis and Crohn's disease patients compared to healthy controls have recently been reported [23,43].

Genetic variation within the TNF locus may be involved in the pathogenesis, or clinical manifestations, of some autoimmune and infectious diseases [27]. In IBD, there are relatively few studies of TNFα and TNFβ gene polymorphism associations with either ulcerative colitis or Crohn's disease. Plevy *et al.* [44] have recently reported an association of the TNF microsatellite haplotype a2b1c2d4e1 with Crohn's disease, which is the strongest genetic risk factor described so far in Crohn's disease. Bi-allelic single-base polymorphisms of either TNFα [15,20,21,45,46] or TNFβ genes [45–47] have shown weak [45] or no association [15,20,21,46,47] with ulcerative colitis. In agreement with these previous studies, we found no differences between ulcerative colitis patients and controls in the TNFα and TNFβ polymorphisms analysed. Taken as a whole, these data indicate that bi-allelic single-base polymorphisms of the TNF genes are not important determinants of overall disease susceptibility to ulcerative colitis.

A number of observations support the concept of genetic heterogeneity within IBD [2]. According to these, IBD is regarded not as a single disease, but rather as several aetiologically and genetically distinct diseases presenting a similar clinical picture. Furthermore, there is an increasing body of evidence suggesting genetic heterogeneity within each disease. The most relevant clues have come from genetic marker studies, which have demonstrated different HLA associations for ulcerative colitis and Crohn's disease, and distinct associations between subsets of ulcerative colitis as discussed earlier. Likewise, there are hints that subclinical markers, such as ANCA, may contribute to establish genetic heterogeneity within ulcerative colitis. This was first suggested by Shanahan *et al.* [35], who described a familial distribution of these antibodies: the relatives of ANCA-positive ulcerative colitis patients had an increased prevalence of the presence of ANCA compared with those of ANCA-negative patients. Additional evidence was provided by the same investigators from Los Angeles, who reported distinct associations between ANCA and various genetic markers. Thus, both ANCA-positive and ANCA-negative ulcerative colitis were associated with the HLA-DR2 and the HLA-DR4 alleles respectively in a mixed Jewish/non-Jewish population from Los Angeles [37]. In another study, ANCA-negative ulcerative colitis exhibited an increased frequency of allele R241 in codon 241 of the intercellular adhesion molecule 1 gene [38]. However, the role of ANCA as a subclinical marker of genetic heterogeneity in ulcerative colitis has been questioned, because further studies did not establish any relationship between ANCA and HLA status, particularly with the HLA-DR2 allele [9,19]. The discrepancies between the studies may reflect methodological differences and disease heterogeneity. Ethnic differences may also be relevant, as shown by Satsangi *et al.* [48] who recently reported that in North European patients with ulcerative colitis there is an association between ANCA and the HLA-DR3 DQ2 TNF2 haplotype, but not with HLA-DR2.

The results of the present study provide further support for the notion of ANCA as a subclinical marker of genetic heterogeneity within ulcerative colitis. We have shown an association between an inherited polymorphism of the IL-1ra gene and an immunologically

defined subgroup of ulcerative colitis patients: p-ANCA ulcerative colitis was positively associated with the genotype 1,2 (52%) compared with c-ANCA/ANCA-negative ulcerative colitis (26.5%). In contrast to our results, no association between p-ANCA and the IL-1ra gene polymorphism was found in reports from North America [16], Germany [18] and The Netherlands [19]. In a British study [21], the proportion of carriers of allele 2 was increased in the ANCA-positive group (38.2%) compared with the ANCA-negative group (11.8%). Here again, as for the HLA association studies, the conflicting results may reflect methodological differences, disease heterogeneity and/or variability between different study populations and ethnic groups.

The association of ANCA with ulcerative colitis is now well established. The majority of ulcerative colitis-associated ANCA exhibit a perinuclear immunofluorescence pattern, but cytoplasmic and mixed patterns have also been described [49–53]. There is no consensus, however, on which patterns should be considered associated with ulcerative colitis. While most authors only consider the perinuclear type, others also include the less frequent cytoplasmic or mixed patterns. The findings of our study provide evidence supporting this last view. When we first compared ANCA-positive ulcerative colitis with ANCA-negative ulcerative colitis patients, we did not find any association between ANCA and the cytokine gene polymorphisms analysed. It was only after the stratification of ulcerative colitis by their different ANCA immunofluorescence patterns, p-ANCA and c-ANCA, that we detected an association between p-ANCA and a specific IL-1ra genotype. c-ANCA and ANCA-negative ulcerative colitis patients showed a similar 1,2 genotype distribution, which was opposite to that of p-ANCA ulcerative colitis patients. However, these data should be interpreted with caution, first due to the small number analysed, and second because attempts to differentiate clinical disease patterns between p-ANCA ulcerative colitis patients and c-ANCA ulcerative colitis patients have so far been unsuccessful. Nevertheless, considering that the nature of the antigen(s) to which ANCA react in ulcerative colitis remains unknown, although it is likely that p-ANCA and c-ANCA are directed against different antigens, it does not seem reasonable to exclude any type of ANCA pattern when an unequivocal immunofluorescence staining is found.

In summary, this study shows that cytokine gene polymorphisms of the TNFα, TNFβ and IL-1ra are not important factors in determining genetic susceptibility to ulcerative colitis in the Spanish population studied. On the other hand, our findings are in accordance with the concept of genetic heterogeneity within ulcerative colitis, and provide further evidence that ANCA may represent a serological marker of genetic heterogeneity in this disease.

References

1 Shanahan F. Pathogenesis of ulcerative colitis. *Lancet* 1993; **342**:407–411.
2 Yang H, Rotter JI. The genetics of inflammatory bowel disease. In: *Inflammatory Bowel Disease: From Bench to Bedside.* Targan S, Shanahan F (editors). Baltimore: Williams & Wilkins; 1993. pp. 32–64.
3 Hugot JP, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugerie L, *et al.* Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 1996; **379**:821–823.
4 Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, *et al.* Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nature Genet* 1996; **14**:199–202.
5 Ohmen JD, Yang HY, Yamamoto KK, Zhao HY, Ma Y, Bentley LG, *et al.* Susceptibility locus for inflammatory bowel disease on chromosome 16 has a role in Crohn's disease, but not in ulcerative colitis. *Hum Mol Genet* 1996; **5**:1679–1683.
6 Satsangi J, Jewell DP, Rosenberg WMC, Bell JI. Genetics of inflammatory bowel disease. *Gut* 1994; **35**:696–700.
7 Satsangi J, Jewell DP, Bell JI. The genetics of inflammatory bowel disease. *Gut* 1997; **40**:572–574.
8 Toyoda H, Wang SJ, Yang HY, Redford A, Magalong D, Tyan D, *et al.* Distinct associations of HLA class II genes with inflammatory bowel disease. *Gastroenterology* 1993; **104**:741–748.
9 Duerr RH, Neigut DA. Molecularly defined HLA-DR2 alleles in ulcerative colitis and an antineutrophil cytoplasmic antibody-positive subgroup. *Gastroenterology* 1995; **108**:423–427.
10 Satsangi J, Welsh KI, Bunce M, Julier C, Farrant JM, Bell JI, *et al.* Contribution of genes of the major histocompatibility complex to susceptibility and disease phenotype in inflammatory bowel disease. *Lancet* 1996; **347**:1212–1217.
11 Masuda H, Nakamura Y, Tanaka T, Hayakawa S. Distinct relationship between HLA-DR genes and intractability of ulcerative colitis. *Am J Gastroenterol* 1994; **89**:1957–1962.
12 Koutroubakis I, Crusius JBA, Peña AS. Immunogenetics of cytokines. *Scand J Gastroenterol* 1995; **30**:1139–1146.
13 Cominelli F, Pizarro TT. Interleukin-1 and interleukin-1 receptor antagonist in inflammatory bowel disease. *Aliment Pharmacol Ther* 1996; **10(suppl 2)**:49–53.
14 Tarlow JK, Blakemore AIF, Lennard A, Solari R, Hughes HN, Steinkasserer A, *et al.* Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. *Hum Genet* 1993; **91**:403–404.
15 Mansfield JC, Holden H, Tarlow JK, Di Giovine FS, McDowell TL, Wilson AG, *et al.* Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology* 1994; **106**:637–642.
16 Duerr RH, Tran T. Association between ulcerative colitis and a polymorphism in intron 2 of the interleukin-1 receptor antagonist gene [Abstract]. *Gastroenterology* 1995; **108**:A812.
17 Tountas NA, Kam L, di Giovine FS, Casini-Raggi V, Cominelli F. Genetic association between allele 2 of IL-1 receptor antagonist (IL-1ra) and ulcerative colitis in a Los Angeles based Hispanic population [Abstract]. *Gastroenterology* 1995; **108**:A930.
18 Andus T, Caesar I, Vogl D, Schölmerich J, Gross V. Association of HLA-DR15, p-ANCA and IL-1 receptor antagonist allele 2 with ulcerative colitis [Abstract]. *Gastroenterology* 1995; **108**:A770.
19 Bioque G, Bouma G, Crusius JBA, Koutroubakis I, Kostense PJ, Meuwissen SGM, *et al.* Evidence for genetic heterogeneity in IBD: the interleukin-1 receptor antagonist in the predisposition to suffer from ulcerative colitis. *Eur J Gastroenterol Hepatol* 1996; **8**:105–110.
20 Louis E, Satsangi J, Roussomoustakaki M, Parkes M, Fanning G, Welsh K, *et al.* Cytokine gene polymorphisms in inflammatory bowel disease. *Gut* 1996; **39**:705–710.
21 Roussomoustakaki M, Satsangi J, Welsh K, Louis E, Fanning G, Targan S, *et al.* Genetic markers may predict disease behavior in patients with ulcerative colitis. *Gastroenterology* 1997; **112**:1845–1853.
22 Hacker UT, Gomolka M, Keller E, Eigler A, Folwaczny C, Fricke H, *et al.* Lack of association between an interleukin-1 receptor antagonist gene polymorphism and ulcerative colitis. *Gut* 1997; **40**:623–627.
23 Heresbach D, Alizadeh M, Dabadie A, Le Berre N, Colombel JF, Yaouanq J, *et al.* Significance of interleukin-1β and interleukin-1 receptor antagonist

genetic polymorphism in inflammatory bowel diseases. *Am J Gastroenterol* 1997; **92**:1164–1169.
24 Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997; **94**:3195–3199.
25 Kroeger KM, Carville KS, Abraham LJ. The −308 tumor necrosis factor-α promoter polymorphism effects transcription. *Mol Immunol* 1997; **34**:391–399.
26 Bouma G, Crusius JBA, Oudkerk Pool M, Kolkman JJ, von Blomberg BME, Kostense PJ, *et al.* Secretion of tumor necrosis factor α and lymphotoxin α in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. *Scand J Immunol* 1996; **43**:456–463.
27 Wilson AG, di Giovine FS, Duff GW. Genetics of tumor necrosis factor-α in autoimmune, infectious, and neoplastic diseases. *J Inflam* 1995; **45**:1–12.
28 Messer G, Spengler U, Jung MC, Honold G, Blömer K, Pape GR, *et al.* Polymorphic structure of the tumor necrosis factor (TNF) locus: an *Nco*I polymorphism in the first intron of the human TNF-β gene correlates with a variant amino acid in position 26 and a reduced level of TNF-β production. *J Exp Med* 1991; **173**:209–219.
29 Pociot F, Briant L, Jongeneel CV, Mölvig J, Worsaae H, Abbal M, *et al.* Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-α and TNF-β by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. *Eur J Immunol* 1993; **23**:224–231.
30 Duerr RH, Targan SR, Landers CJ, Sutherland LR, Shanahan F. Anti-neutrophil cytoplasmic antibodies in ulcerative colitis: comparison with other colitides/diarrheal illnesses. *Gastroenterology* 1991; **100**:1590–1596.
31 Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Antineutrophil cytoplasmic antibody correlates with chronic pouchitis after ileal pouch–anal anastomosis. *Am J Gastroenterol* 1995; **90**:740–747.
32 Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Association of antineutrophil cytoplasmic antibodies with resistance to treatment of left-sided ulcerative colitis: results of a pilot study. *Mayo Clin Proc* 1996; **71**:431–436.
33 Vecchi M, Bianchi MB, Sinico RA, Radice A, Meucci G, Torgano G, *et al.* Antibodies to neutrophil cytoplasm in Italian patients with ulcerative colitis: sensitivity, specificity and recognition of putative antigens. *Digestion* 1994; **55**:34–39.
34 Vasiliauskas EA, Plevy SE, Landers CJ, Binder SW, Ferguson DM, Yang H, *et al.* Perinuclear antineutrophil cytoplasmic antibodies in patients with Crohn's disease define a clinical subgroup. *Gastroenterology* 1996; **110**:1810–1819.
35 Shanahan F, Duerr RH, Rotter JI, Yang H, Sutherland LR, McElree C, *et al.* Neutrophil autoantibodies in ulcerative colitis: familial aggregation and genetic heterogeneity. *Gastroenterology* 1992; **103**:456–461.
36 Seibold F, Slametschka D, Gregor M, Weber P. Neutrophil autoantibodies: a genetic marker in primary sclerosing cholangitis and ulcerative colitis. *Gastroenterology* 1994; **107**:532–536.
37 Yang H, Rotter JI, Toyoda H, Landers C, Tyan D, McElree CK, *et al.* Ulcerative colitis: a genetically heterogeneous disorder defined by genetic (HLA class II) and subclinical (antineutrophil cytoplasmic antibodies) markers. *J Clin Invest* 1993; **92**:1080–1084.
38 Yang H, Vora DK, Targan SR, Toyoda H, Beaudet AL, Rotter JI. Intercellular adhesion molecule 1 gene associations with immunologic subsets of inflammatory bowel disease. *Gastroenterology* 1995; **109**:440–448.
39 Papo M, Quer JC, Pastor RM, García-Pardo G, Prats E, Mirapeix E, *et al.* Antineutrophil cytoplasmic antibodies in relatives of patients with inflammatory bowel disease. *Am J Gastroenterol* 1996; **91**:1512–1515.
40 García-Paredes J, Bioque G, Crusius JBA, García-González A, López-Nava G, Díaz-Rubio M, *et al.* The interleukin-1 receptor antagonist gene polymorphism in Spanish IBD patients [Abstract]. *Gastroenterology* 1996; **110**:A914.
41 Tountas NA, Yang H, Coulter DL, Rotter JI, Cominelli F. Increased carriage of allele 2 of IL-1 receptor antagonist (IL-1ra) in Jewish populations: the strongest known genetic association in ulcerative colitis [Abstract]. *Gastroenterology* 1996; **110**:A1029.
42 Futami S, Aoyama N, Honsako Y, Tamura T, Morimoto S, Nakashima T, *et al.* HLA-DRB1*1502 allele, subtype of DR15 is associated with susceptibility to ulcerative colitis and its progression. *Dig Dis Sci* 1995; **40**:814–818.
43 Bioque G, Crusius JBA, Koutroubakis I, Bouma G, Kostense PJ, Meuwissen SGM, *et al.* Allelic polymorphism in IL-1β and IL-1 receptor antagonist (IL-1Ra) genes in inflammatory bowel disease. *Clin Exp Immunol* 1995; **102**:379–383.
44 Plevy SE, Targan SR, Yang H, Fernandez D, Rotter JI, Toyoda H. Tumor necrosis factor microsatellites define a Crohn's disease-associated haplotype on chromosome 6. *Gastroenterology* 1996; **110**:1053–1060.
45 Bouma G, Xia B, Crusius JBA, Bioque G, Koutroubakis I, Von Blomberg BME, *et al.* Distribution of four polymorphisms in the tumor necrosis factor (TNF) genes in patients with inflammatory bowel disease (IBD). *Clin Exp Immunol* 1996; **103**:391–396.
46 Heresbach D, Ababou A, Bourienne A, Alizadeh M, Quelvennec E, Pagenault M, *et al.* Étude du polymorphisme des microsatellites et des gènes du tumor necrosis factor (TNF) au cours des maladies inflammatoires chroniques de l'intestin. *Gastroenterol Clin Biol* 1997; **21**:555–561.
47 Sugimura K, Asakura H, Mizuki N, Inoue M, Hibi T, Yagita A, *et al.* Analysis of genes within the HLA region affecting susceptibility to ulcerative colitis. *Hum Immunol* 1993; **36**:112–118.
48 Satsangi J, Landers C, Welsh K, Koss K, Targan S, Jewell DP. ANCA and HLA genes in North European patients with ulcerative colitis [Abstract]. *Gastroenterology* 1996; **110**:A1009.
49 Cambridge G, Rampton DS, Stevens TRJ, McCarthy DA, Kamm M, Leaker B. Anti-neutrophil antibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut* 1992; **33**:668–674.
50 Patel RT, Stokes R, Birch D, Ibbotson J, Keighley MRB. Influence of total colectomy on serum antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Br J Surg* 1994; **81**:724–726.
51 Kossa K, Coulthart A, Ives CT, Pusey CD, Hodgson JF. Antigen specificity of circulating anti-neutrophil cytoplasmic antibodies in inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 1995; **7**:783–789.
52 Castellino F, Rosina F, Bansi DS, Bauducci M, Touscoz GA, Giorda L, *et al.* Anti-neutrophil cytoplasmic antibodies in inflammatory bowel disease: do they recognize different subsets of a heterogeneous disease? *Eur J Gastroenterol Hepatol* 1995; **7**:859–864.
53 Hertervig E, Wieslander J, Johansson C, Wiik A, Nilsson A. Anti-neutrophil cytoplasmic antibodies in chronic inflammatory bowel disease. *Scand J Gastroenterol* 1995; **30**:693–698.

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5. DISCUSION

ESTUDIO 1.

Los resultados de este primer trabajo han demostrado la presencia de ANCA en 49 de 75 pacientes (65%) con CU, en 5 de 41 pacientes con EC (12%) y únicamente en un sujeto del grupo control (2,5%). Estos hallazgos coinciden con los de la mayoría de las series de la literatura, en las que estos anticuerpos tienen una alta prevalencia en la CU (50-80%)[49,50,200-204,285-288,290,291,293-295,297,299-303,306-310,312,313,316], se detectan con mucho menor frecuencia en la EC (5-25%)[49,50,202-204,207,285-291,293,298,299,301-306,308,310,311,316,318], y no se asocian a otras patologías del tracto gastrointestinal. Por el contrario, contrastan con los de otros trabajos que han comunicado una prevalencia sensiblemente inferior en la CU (30-40%)[202,207,208,289,304,305], y una mayor frecuencia a la reportada habitualmente en la EC (40-55.5%)[292,295,297,300,307,312-314].

Las causas de tan notables diferencias sobre la prevalencia de los ANCA en la EII no han sido del todo esclarecidas. Se han propuesto varias explicaciones posibles y no excluyentes. En primer lugar, la utilización de distintas técnicas para su detección puede ser parcialmente responsable. Como ya se ha señalado en la introducción, existen discrepancias en cuanto a la sensibilidad y especificidad de la IFI y el ELISA[290,358]. Sin embargo, el hecho de que también se perciban marcadas diferencias entre laboratorios que emplean únicamente la IFI, técnica con la que se consiguen resultados altamente reproducibles[290,296], sugiere que éstas no se deben únicamente a diferencias metodológicas. En segundo lugar, la divergencia de resultados puede atribuirse a diferencias reales de prevalencia en las distintas poblaciones estudiadas, entre las que cabe considerar diferencias

raciales y/o étnicas. Así, la frecuencia de ANCA reportada en la CU varía en distintas áreas geográficas, siendo del 72-85% en Norteamérica[49,204,285], 58-83% en Alemania[203,291,293], 45-79% en Holanda[290,292,296] y 41-76% en Inglaterra[286,297,305,366]. La prevalencia reportada en nuestro país oscila entre un 41% y 73%[296,302,303,318,352]. Estas cifras, exceptuando el estudio de García Herola y cols.[318], son superiores a las descritas en los otros países europeos del área mediterránea: 39,8-48% en Italia[298,304,365], 46-52% en Francia[288,296,364], y 30% en Grecia[289]. Estudios en los que se han analizado simultáneamente sueros de distintas poblaciones en laboratorios independientes han confirmado diferencias regionales significativas[296,363]. Finalmente, no se puede excluir que las diferencias de prevalencia puedan deberse a sesgos de tamaño y de selección de los pacientes estudiados. No obstante, esta última posibilidad es menos probable, teniendo en cuenta que no existe una relación demostrada entre el status ANCA y las principales variables demográficas y clínicas de la enfermedad.

Lógicamente, el valor de los ANCA como marcadores serológicos útiles para el diagnóstico de la CU, está en función de la prevalencia encontrada en los pacientes con CU y con EC, que como se ha discutido, varía notablemente entre las distintas series, muy posiblemente como consecuencia de diferencias metodológicas y/o poblacionales. En nuestro estudio, la sensibilidad y especificidad de los ANCA para la CU fue de un 65% y de un 88% respectivamente. El valor predictivo positivo fue del 91%, y el valor predictivo negativo del 58%. De esta forma, nuestros resultados coinciden con los de la mayoría de trabajos publicados que han reportado una sensibilidad y especificidad de los ANCA para el diagnóstico de la CU de entre un 50-80% y un 80-95%

respectivamente[49,204,285,287,288,290,296,298,302,303,309,311,318]. Por contra, los autores que detectan una baja prevalencia de los anticuerpos en la CU y/o una mayor frecuencia a la reportada habitualmente en la EC, señalan obviamente, que si bien los ANCA pueden ser de utilidad para diferenciar la CU de ciertas colítides, su valor discriminatorio frente a la EC es limitado[207,295,304,306,312,313].

A pesar de que nuestro estudio, al igual que otros, sugiere que la determinación de los ANCA puede contribuir al diagnóstico CU, ciertamente su utilidad en la práctica clínica tiene una serie de limitaciones que merecen ser comentadas. En primer lugar, es importante señalar que las técnicas utilizadas en la actualidad para la detección de los ANCA en la EII tienen el inconveniente de no ser antígeno-específicas. En este sentido, los estudios más recientes que ubican el antígeno de los ANCA asociados a la EII en el núcleo celular[326-330], han creado nuevas expectativas para conseguir finalmente su identificación, lo que debería permitir en último término el desarrollo de técnicas más específicas para soslayar los problemas metodológicos. En segundo lugar, la hipotética utilidad de los marcadores serológicos para el diagnóstico de la CU y la EC es discutible. En efecto, a diferencia de otras patologías (conectivopatías u otras enfermedades autoinmunes organoespecíficas por citar algunos ejemplos) en las que diversos autoanticuerpos tienen una considerable utilidad clínica, el valor potencial de los marcadores serológicos en la EII parece más limitado, teniendo en cuenta que el diagnóstico tanto de la CU como de la EC no plantea por lo general grandes dificultades cuando se combinan criterios convencionales clínicos, radiológicos, endoscópicos y anatomopatológicos. Por ello, la mayor utilidad de los marcadores serológicos en la EII, y en concreto la de los ANCA, consistiría en posibilitar el

diagnóstico diferencial en aquellas situaciones en las que realmente existen dificultades diagnósticas. Primero, deberían facilitar el diagnóstico diferencial de la EII con otras colítides, principalmente las infecciosas enteroinvasivas y algunas colitis isquémicas de curso evolutivo crónico. Como ya se ha señalado en otros apartados de este texto, los ANCA parecen cumplir este objetivo, ya que no se detectan en otras colopatías agudas y/o crónicas. Unicamente se ha sugerido su asociación con las colitis microscópicas[285], si bien este hallazgo no ha sido posteriormente ratificado[512]. Segundo, deberían posibilitar el diagnóstico diferencial entre la CU y la EC con afectación colónica, cuando las técnicas diagnósticas habituales no son del todo concluyentes. En este sentido, la alta prevalencia de los anticuerpos en los pacientes con EC que tienen un fenotipo *UC-like* reportada por algunos autores[314,359], obviamente limitaría su utilidad. Esta observación, sin embargo, no ha sido confirmada por otros investigadores[360,361]. Finalmente, la mayor utilidad de los ANCA consistiría en poder establecer el diagnóstico definitivo de CU o EC en los pacientes que tienen una de colitis indeterminada. No obstante, esta cuestión es difícil de evaluar, y hasta la fecha no se ha publicado ningún estudio al respecto.

En resumen, si bien los ANCA pueden contribuir al diagnóstico de la CU, posibilitando el diagnóstico diferencial con otras colítides, su valor clínico para diferenciar las formas de CU difícilmente distinguibles de la EC no se ha establecido. Es posible que la combinación de la detección de los ANCA con otros marcadores serológicos, como por ejemplo los anticuerpos anti-*Saccharomyces cerevisiae* (ASCA) que tienen una alta especificidad para la EC, pueda aumentar el rendimiento diagnóstico de los marcadores serológicos en la EII, tal como se ha sugerido recientemente en diversos trabajos[341,513,514].

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En nuestro estudio, al igual que en la mayoría de los trabajos publicados, no encontramos relación entre la seropositividad de los ANCA en la CU, y el tiempo de evolución, el curso clínico, la extensión, la actividad de la enfermedad, las manifestaciones extraintestinales, o el tratamiento farmacológico recibido. Tampoco existió relación entre el título o patrón de inmunofluorescencia y los parámetros clínicos valorados. Sin embargo, algunos autores sí han detectado relación con alguna de las variables analizadas. Tural[303] y Rump y cols.[293] encuentran una correlación entre la positividad de los ANCA y la actividad de la enfermedad. Rump y cols.[293] describen además su negativización en pacientes que entran en remisión tras tratamiento esteroidal. De forma similar, otros autores hallan relación entre el título de los ANCA y la actividad clínica de la CU[203,287,293,295]. Por otra parte, se ha sugerido que el status ANCA se relacionaría con la evolución de la enfermedad, de tal forma que su presencia se asociaría con un curso clínico más agresivo: se ha reportado una mayor prevalencia entre los pacientes que tienen más exacerbaciones anuales[298,515], y una muy baja prevalencia en enfermos que presentan una remisión clínica prolongada[516]. Finalmente, Sandborn y cols.[517] han encontrado una frecuencia incrementada en pacientes con colitis izquierda resistente al tratamiento médico, sugiriendo una posible asociación entre los ANCA y una resistencia relativa al tratamiento farmacológico. No obstante, como previamente se ha hecho referencia, la tendencia más constante observada en la bibliografía es la ausencia de relación entre el status ANCA y las características clínicas de la enfermedad. Lógicamente, la ausencia de relación entre la positividad y/o el título de los ANCA con la actividad de la CU, inhabilitan una hipotética utilidad de estos anticuerpos

como marcadores serológicos para la monitorización de la respuesta terapéutica y evolución clínica.

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ESTUDIO 2.

En este segundo trabajo se evaluó el posible papel de los ANCA como marcadores subclínicos de susceptibilidad genética a la EII en nuestro medio. Para ello se establecieron tres grupos de estudio. En el primero, formado por pacientes con EII, se detectó la presencia de ANCA en 34 de 53 pacientes (64%) con CU, y en 3 de 24 de pacientes (12,5%) con EC. El segundo grupo incluyó 215 familiares de primer grado de los pacientes con EII. Se encontró la presencia de los anticuerpos en 6 de 155 familiares (3,9%) de pacientes con CU, y en 4 de 60 familiares (6,7%) de pacientes afectados de EC. De esta forma, globalmente se detectó la presencia de ANCA en 10 de los 215 familiares estudiados de los pacientes con EII, lo que representa un 4.6%. Finalmente, en el grupo control, los ANCA se detectaron únicamente en uno de 40 sujetos sanos donantes de sangre (2,5%), en ninguno (0%) de 22 cónyuges de pacientes con EII (grupo control ambiental), y en ninguno (0%) de los 11 pacientes con enfermedad celíaca que también fueron evaluados. De esta forma, la presencia de ANCA fue estadísticamente más frecuente en los pacientes con CU respecto a todos los demás grupos de estudio. La prevalencia de los anticuerpos no se encontró incrementada en los familiares sanos de los pacientes con respecto al grupo control.

A pesar de los considerables esfuerzos realizados durante la última década con el objetivo de identificar los locus genéticos que expliquen el carácter hereditario de la CU y la EC, la naturaleza de los genes implicados sigue siendo desconocida.

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Consecuentemente, a diferencia de lo que sucede actualmente con numerosas enfermedades hereditarias monogénicas en las que el riesgo de recurrencia para los miembros de una familia específica puede determinarse con relativa facilidad, en la EII no se disponen de marcadores genéticos que permitan identificar a los sujetos susceptibles de desarrollar la enfermedad. Por ello, el estudio de diversos marcadores subclínicos en los familiares sanos de los pacientes, parámetros que permiten detectar a los sujetos con un genotipo anormal en ausencia de expresión fenotípica de una enfermedad, y que se han mostrado útiles en otros trastornos genéticos multifactoriales, ha sido objeto de una considerable atención[375]. Se han propuesto diversos candidatos que incluyen el sistema del complemento[518], las glicoproteínas colónicas[519], la flora anaerobia intestinal[520], las subclases de inmunoglobulinas IgG[521,522], el estudio de la permeabilidad intestinal[523-529], y varios anticuerpos como los anti-linfocitotóxicos[530], anticuerpos dirigidos contra antígenos epiteliales intestinales (ECAC)[531], contra el páncreas[532,533], anti-tropomiosina[534], anti-*globet cells*[535], anti-*Saccharomyces cerevisiae*[536], e incluso los ANA[537]. Sin embargo, para ninguno de estos potenciales marcadores subclínicos se han obtenido resultados consistentes.

El valor de los ANCA como marcadores subclínicos de susceptibilidad genética a la CU fue inicialmente sugerido por el grupo de Shanahan y cols.[362] que encontraron una frecuencia significativamente más alta de los anticuerpos en los familiares sanos de los pacientes que en sujetos controles. Estos autores reportaron la presencia de los anticuerpos en 14 de 93 (15,7%) de los familiares sanos de pacientes con CU en una población de Los Angeles, y en 9 de 43 (20,9%) de los de una población canadiense de

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Calgary. Estos hallazgos fueron confirmados dos años más tarde por Seibold y cols.[363] en Alemania: detectaron la presencia de ANCA en el 30% de los familiares sanos de pacientes con CU, y en el 25% de los de pacientes con CEP. No obstante, contrariamente a estos dos trabajos, cuatro estudios realizados en Francia[364], Italia[365] e Inglaterra[366,367], no evidenciaron una prevalencia incrementada de ANCA en los familiares sanos de pacientes con CU (0-6,6%). Nuestro estudio, al igual que estos últimos trabajos, tampoco ha mostrado una mayor frecuencia de los anticuerpos en los familiares de los enfermos con CU, ni en los de los de pacientes con EC, con respecto al grupo control. Más recientemente, otros autores han comunicado, ya sea en forma de artículos originales o de comunicaciones, resultados igualmente negativos[532,538-540]. Por otra parte, Yang y cols.[541] han reportado que la frecuencia de ANCA no está incrementada en hermanos gemelos monocigotos sanos de hermanos con CU, resultados que están en contra del hipotético papel de estos anticuerpos como marcadores genéticos en la EII.

En resumen, los resultados de los estudios del grupo de Shanahan y del de Seibold sugiriendo el papel de los ANCA como marcadores de susceptibilidad genética a la CU, y por extensión a la CEP, no se han reproducido en otros estudios europeos. Las diferencias entre estos trabajos, al igual que las encontradas con respecto a la prevalencia de los ANCA en los propios pacientes, también se han atribuido a diferencias metodológicas y/o poblacionales. Considerando esta última explicación, es posible que los ANCA representen marcadores genéticos en la CU únicamente en determinadas poblaciones. En cualquier caso, en la mayoría de las poblaciones estudiadas, incluida la nuestra, los ANCA no representan marcadores de susceptibilidad genética a la EII.

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ESTUDIO 3.

Durante los últimos años, los genes que codifican para diversas citocinas con importante actividad inflamatoria y/o inmunoreguladora, moléculas que participan de forma activa en la patogenia de la EII, se han considerado como importantes candidatos potenciales a la susceptibilidad genética de la CU y la EC. Los importantes avances realizados en el campo de la biología molecular, han llevado por una parte, a la clonación y secuenciación de estos locus genéticos, y por otra, la descripción de polimorfismos de los mismos, permitiendo de esta forma el estudio de posibles asociaciones con las enfermedades inflamatorias idiopáticas crónicas del intestino. Los trabajos publicados hasta la fecha han evaluado polimorfismos de los genes que codifican para el TNFα, el TNFβ, la IL-1β, el IL-1ra, la IL-2, y la IL-10[284,421,422].

Con respecto al gen del IL-1ra, desde que en el año 1994 Mansfield y cols.[460] reportaran una asociación entre el alelo 2 del polimorfismo VNTR en el intrón 2 del IL-1RN y la CU en una población de Sheffield, otros investigadores han comunicado resultados discordantes. Entre los estudios positivos, dos grupos norteamericanos[461,462] evidenciaron una mayor frecuencia de portadores del alelo 2 en la CU, confirmando los hallazgos de los investigadores ingleses. Recientemente, el grupo de Mansfield ha estudiado una serie considerablemente más amplia de pacientes con CU, ratificando los datos de su primer estudio[542]. Sin embargo, los resultados obtenidos al evaluar este polimorfismo en otras poblaciones europeas, de Alemania[463,464], Inglaterra[465,466], Holanda[373], Francia[467] y España[468], no han encontrado esta asociación. En nuestro estudio,

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al igual que en estos últimos trabajos, tampoco encontramos una asociación entre el alelo 2 del IL-1RN y la CU. En concreto, la frecuencia del alelo 2 en la CU (30,5%) fue similar a la detectada en el grupo control (31%), y la frecuencia de los portadores de este alelo en la CU (50,5%) fue también equiparable a la observada en los controles (53%).

Las discrepancias entre estos trabajos difícilmente se pueden atribuir a diferencias metodológicas, ya que las técnicas de biología molecular utilizadas en todos ellos son muy similares. Diferencias poblacionales, en concreto étnicas, así como la heterogeneidad clínica y genética propias de la EII, parecen ser más relevantes[469]. Con respecto a las posibles diferencias étnicas, en los dos trabajos realizados en Estados Unidos se evaluaron una población mixta de judíos/no judíos de Pittsburgh y una población hispánica de los Angeles, mientras que en los estudios europeos se incluyeron mayoritariamente sujetos blancos no judíos del norte de Europa. Más aún, Tountas y cols.[543] han reportado que la asociación entre el alelo 2 y la CU era relevante únicamente en el subgrupo de pacientes judíos de la población caucásica que estudiaron. En cuanto a la explicación que considera la heterogeneidad clínica y genética de la CU, ya en el estudio inicial del grupo de Mansfield[460], la asociación entre el alelo 2 y la CU era mucho más manifiesta en los pacientes con una afectación extensa del colon (pancolitis) que en los que tenían una colitis distal. De igual forma, en dos de los estudios europeos en los que no se objetivó una asociación del alelo 2 con la CU, sí se observó una asociación con la forma de afectación extensa de la enfermedad[373,466], y en otro trabajo, con la intratabilidad de la misma[467]. Así, los autores de estos trabajos han sugerido que el alelo 2 podría representar un marcador de "gravedad" en la CU. Su asociación con las formas más "graves" de CU,

sin embargo, no se ha objetivado en los demás estudios, incluido el nuestro. Finalmente, otra posible explicación a las discrepancias entre los estudios, propone que las asociaciones positivas comunicadas por algunos autores entre el alelo 2 y la CU, se deben a que el IL1RN, no siendo directamente responsable de la susceptibilidad a la enfermedad, está en desequilibrio de ligamiento con algún otro gen de importancia primaria en el cromosoma 2, o alternativamente, la acción sinérgica del ILRN con otros genes cercanos es la que determina la susceptibilidad genética a la CU. Sosteniendo esta última hipótesis, Bioque y cols.[544], y Heresbach y cols.[467], han descrito una asociación de distintas variantes alélicas de polimorfismos genéticos del IL1B y IL1RN en la CU, sugiriendo que puede ser la combinación de genotipos IL1B/IL1RN la que define las bases biológicas de la predisposición a la enfermedad.

Considerando que el TNF juega un importante papel en la patogenia de la EII, y que la variación genética de ciertos polimorfismos del TNFA y del TNFB se ha asociado a diversas enfermedades autoinmunes e infecciosas, estos dos locus se han considerado candidatos potenciales en la EII[495]. Sin embargo, los resultados de los diversos estudios de la literatura sobre polimorfismos RFLP del TNFA y del TNFB en la EII, realizados básicamente en poblaciones del norte de Europa, han mostrado una muy discreta o nula asociación con la CU y la EC[460,465,466,506-508,510,511]. En concordancia con estos estudios, en el nuestro no hemos detectado ningún tipo de asociación alélica ni genotípica de los dos polimorfismos analizados con la CU. A partir del análisis combinado de todos estos estudios, se infiere que los polimorfismos bialélicos del locus del TNF no son determinantes mayores de susceptibilidad global a la CU, ni a la EC. Es importante

señalar, no obstante, que esta asunción no descarta que los genes que codifican para estas citocinas puedan jugar un papel en la genética de la EII. En efecto, Plevy y cols.[546] han reportado una asociación del haplotipo a2b1c2d4e1 de microsatélites del TNF con la EC, que representa el mayor riesgo genético descrito hasta la fecha en esta enfermedad. Recientemente, Heresbach y cols.[507] no han podido reproducir los mismos resultados en un estudio realizado en Francia.

Uno de los avances más importantes realizados durante los últimos años en el estudio de la genética de la EII ha sido el reconocimiento de que la CU y la EC son enfermedades genéticamente heterogéneas. Como ya se ha señalado en otros apartados de este texto, la heterogeneidad genética de las dos enfermedades se sustenta en diversas evidencias aportadas por estudios experimentales, clínico-epidemiológicos, y genéticos. Así mismo, también se ha destacado que los marcadores subclínicos, y en concreto los ANCA, pueden contribuir a definir la heterogeneidad genética de la CU y la EC. Shanahan y cols.[362] fueron los primeros autores que lo sugirieron, al encontrar una mayor frecuencia de los anticuerpos en los familiares de pacientes con CU que eran ANCA+ que en los familiares de los pacientes ANCA– (21.4% vs 7%). Posteriormente, varios estudios han mostrado que se pueden definir subgrupos homogéneos de pacientes con CU, a partir de la asociación de marcadores genéticos (genes HLA, ICAM-1, y motilina)[368,369,370,374,547,548] y el status ANCA, y sugerir en consecuencia que la CU-ANCA+ y la CU-ANCA– son grupos genéticamente distintos. Sin embargo, otros trabajos no han conseguido establecer asociaciones de estas características, cuestionando

así el papel de los ANCA como marcadores de heterogeneidad genética[371-373]. Nuevamente aquí, las discrepancias entre estos trabajos se han atribuido a diferencias metodológicas y/o poblacionales.

Los resultados de nuestro estudio apoyan el papel de los ANCA como marcadores subclínicos de heterogeneidad genética en la CU, y consecuentemente proporcionan evidencia adicional al concepto de heterogeneidad genética de la enfermedad. En efecto, se encontró una asociación estadísticamente significativa entre el polimorfismo VNTR del IL-1RN y un subgrupo de pacientes con CU definidos por el status ANCA: los pacientes pANCA+ se asociaron con el genotipo 1,2 cuando se compararon con los pacientes ANCA–/c-ANCA (52% vs 26,5%; $p=0,01$; $p_c=0,05$). Estos hallazgos contrastan con los de otros autores que no han objetivado asociaciones entre la CU-pANCA y variantes genotípicas del polimorfismo del IL-1RN[373,461,463]. Unicamente Roussomoustakaki y cols.[466] han encontrado que la proporción de portadores del alelo 2 está aumentada en la CU-ANCA+ comparada con la CU-ANCA –.

La ventaja principal de contemplar a la CU y la EC como enfermedades genéticamente heterogéneas, consiste en la posibilidad de estratificar a los pacientes por la naturaleza de su alteración genética subyacente, por ejemplo como se ha descrito previamente, mediante el estudio de asociaciones con marcadores genéticos y subclínicos, para conseguir mejorar los conocimientos sobre los mecanismos fisiopatogénicos implicados en cada forma etiológica. Además, la identificación de grupos genéticamente homogéneos debería permitir el empleo de tratamientos más racionales y ensayar nuevas estrategias terapéuticas acorde con las alteraciones fisiopatológicas inherentes a cada uno

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de ellos. En este sentido, los estudios comentados más arriba, a pesar de que consiguen definir subgrupos homogéneos de CU a partir de marcadores genéticos y los ANCA, no encuentran que los subgrupos "genéticos" tengan características fenotípicas específicas, lo que sin duda debería ser el principal objetivo de este tipo de trabajos. Por citar un ejemplo, recientemente Plevy y cols.[549] han reportado que el estudio combinado de microsatélites del gen del TNFα y del status ANCA, identifica a un subgrupo de pacientes con EC caracterizados por una pobre respuesta al tratamiento con anticuerpos monoclonales anti-TNFα. Lógicamente, los resultados de este trabajo deberían ser ratificados para cobrar relevancia, y únicamente se cita aquí el mismo, para ilustrar lo que puede llegar a aportar este campo de la investigación en el estudio, y sobre todo, en el manejo de la EII. Por todo lo expuesto, se comprende el interés que existe en lograr identificar marcadores genéticos y subclínicos, como los ANCA, que nos permitan alcanzar en el futuro estos objetivos.

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6. CONCLUSIONES

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ESTUDIO 1.

1.- La presencia de ANCA se asocia a la CU y en mucha menor proporción a la EC en la población catalana estudiada. Su determinación mediante IFI puede ser de utilidad para el diagnóstico diferencial entre las dos enfermedades en nuestro medio.

2.- La seropositividad de los ANCA no se asocia con las diferentes características demográficas ni clínicas de la CU.

ESTUDIO 2.

1.- La prevalencia de los ANCA no se encuentra incrementada en los familiares sanos de primer grado de los pacientes con EII en la población catalana estudiada. Estos anticuerpos no representan marcadores subclínicos de susceptibilidad genética a la EII en esta población.

ESTUDIO 3.

1.- Los polimorfismos de los genes del TNFα, TNFβ, e IL-1ra analizados no tienen un papel determinante en la susceptibilidad genética global a la CU en la población catalana estudiada.

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2.- La combinación de un marcador genético (IL-1RN) y subclínico (ANCA) identifica a un subgrupo de pacientes con CU (genotipo 1,2 del gen IL-1ra; ANCAp). Este resultado:

2.1. Proporciona evidencia adicional al concepto de heterogeneidad genética en la CU.

2.2. Apoya el papel de los ANCA como marcadores subclínicos de heterogeneidad genética en la CU.

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7. BIBLIOGRAFIA

1. Wiik A. Current classification and definitions of autoantibodies to neutrophil granulocytes. APMIS 1990; Suppl 19: 24-25.

2. Davies DJ, Moran JE, Niall JF, Ryan GB. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology ? Br Med J 1982;285: 606.

3. Hall JB, Wadham BM, Wood CJ, Ashton V, Adam WR. Vasculitis and glomerulonephritis: a subgroup with an antineutrophil cytoplasmic antibody. Aust N Z J Med 1984;14: 277-278.

4. Van der Woude FJ, Rasmussen N, Lobatto S, Wiik A, Permin H, Van Es LA, Van der Giessen M, Van der Hem GK, The TH. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet 1985;1: 425-429.

5. Gross WL, Lüdemann G, Kiefer G, Lehmann H. Anticytoplasmic antibodies in Wegener´s granulomatosis. Lancet 1986;1: 806.

6. Lüdemann G, Gross WL. Autoantibodies against cytoplasmic structures of neutrophil granulocytes in Wegener´s granulomatosis. Clin Exp Immunol 1987;69: 350-357.

7. Specks U, Wheatley CL, McDonald TJ, Rohrbach MS, DeRemee RA. Anticytoplasmic autoantibodies in the diagnosis and follow-up of Wegener´s granulomatosis. Mayo Clin Proc 1989;64: 28-36.

8. Cohen Tervaert JW, van der Woude FJ, Fauci AS, Ambrus JL, Velosa J, Keane WF, Meijer S, van der Giessen M, The TH, van der Hem GK, Kallenberg CGM. Association between active Wegener´s granulomatosis and anticytoplasmic antibodies. Arch Intern Med 1989;149: 2461-2465.

9. Nölle B, Specks U, Lüdemann J, Rohrbach MS, DeRemee RA, Gross WL. Anticytoplasmic autoantibodies: their immunodiagnostic value in Wegener granulomatosis. Ann Inter Med 1989;111: 28-40.

10. Savage COS, Winearls CG, Jones S, Marshall PD, Lockwood CM. Prospective study of radioimmunoassay for antibodies against neutrophil cytoplasm in diagnosis of systemic vasculitis. Lancet 1987;1: 1389-1393.

11. Falk RJ, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. N Eng J Med 1988;318: 1651-1657.

12. Walters MDS, Savage COS, Dillon MJ, Lockwood CM, Barratt TM. Antineutrophil cytoplasm antibodies in crescentic glomerulonephritis. Arch Dis Child 1988;63: 814-817.

13. Parlevliet KJ, Henzen-Logmans SC, Oe PL, Bronsveld W, Balm AJM, Donker AJM. Antibodies to components of neutrophil cytoplasm: a new diagnostic tool in patients with Wegener´s granulomatosis and systemic vasculitis. Q J Med 1988;66: 55-63.

14. Jennette JC, Wilkman AS, Falk RJ. Antineutrophil cytoplasmic autoantibody-associated glomerulonephritis and vasculitis. Am J Pathol 1989;135: 921-930.

15. Andrassy K, Koderisch J, Rufer M, Erb A, Waldherr R, Ritz E. Detection and clinical implication of anti-neutrophil cytoplasm antibodies in Wegener´s granulomatosis and rapidly progressive glomerulonephritis. Clin Nephrol 1989;32: 159-167.

16. Nassberger L, Sjoholm AG, Bygren P, Thysell H, Hojer-Madsen M, Rasmussen N. Circulating anti-neutrophil cytoplasm antibodies in patients with rapidly progressive glomerulonephritis and extracapillary proliferation. J Intern Med 1989;225: 191-196.

17. Venning MC, Quinn A, Broomhead V, Bird AG. Antibodies directed against neutrophils (C-ANCA and P-ANCA) are of distinct diagnostic value in systemic vasculitis. Q J Med 1990;77: 1287-1296.

18. Mustonen J, Soppi E, Pasternack A, Hällström O. Clinical significance of autoantibodies against neutrophil cytoplasmic components in patients with renal disease. Am J Nephrol 1990;10: 482-488.

19. Cohen Tervaert JW, Goldschmeding R, Elema JD, van der Giessen M, Huitema MG, van der Hem GK, The TH, von dem Borne AEGKr, Kallenberg CGM. Autoantibodies against myeloid lysosomal enzymes in crescentic glomerulonephritis. Kidney Int 1990;37: 799-806.

20. Falk RJ, Hogan S, Carey TS, Jennette JC, and the Glomerular Disease Collaborative Network. Clinical course of anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and systemic vasculitis. Ann Inter med 1990;113: 656-663.

21. Cohen Tervaert JW, Goldschmeding R, Elema JD, Limburg PC, van der Giessen M, Huitema MG, Koolen MI, Hené RJ, The TH, van der Hem GK, von dem Borne AEGKr, Kallenberg CGM. Association of autoantibodies to myeloperoxidase with different forms of vasculitis. Arthritis Rheum 1990;33: 1264-1272.

22. MacIsaac AI, Moran JE, Davies DJ, Murphy BF, Georgiou T, Niall JF. Antineutrophil cytoplasm antibody (ANCA) associated vasculitides. Clin Nephrol 1990;34: 5-8.

23. Saxena R, Bygren P, Rasmussen N, Wieslander J. Circulating autoantibodies in patients with extracapillary glomerulonephritis. Nephrol Dial Transplant 1991;6: 389-397.

24. Niles JL, Pan GL, Collins AB, Shannon T, Skates S, Fienberg R, Arnaout MA, McCluskey RT. Antigen-specific radioimmunoassays for anti-neutrophil cytoplasmic antibodies in the diagnosis of rapidly progressive glomerulonephritis. J Am Soc Nephrol 1991;2:27-36.

25. Cohen Tervaert JW, Limburg PC, Elema JD, Huitema MG, Horst G, The TH, Kallenberg CGM. Detection of autoantibodies against myeloid lysosomal enzymes: a useful adjunct to classification of patients with biopsy-proven necrotizing arteritis. Am J Med 1991;91: 59-66.

26. Bosch X, Mirapeix E, Font J, Ingelmo M, Revert L. Anti-myeloperoxidase antibodies in crescentic glomerulonephritis. Nephron 1991;59:504-505.

27. Bosch X, Mirapeix E, Font J, Cervera R, Ingelmo M, Khamashta MA, Revert LL, Hughes GRV, Urbano-Márquez A. Anti-myeloperoxidase autoantibodies in patients with necrotizing glomerular and alveolar capillaritis. Am J Kidney Dis 1992;20: 231-239.

28. Velosa JA, Homburger HA, Holley KE. Prospective study of anti-neutrophil cytoplasmic autoantibody tests in the diagnosis of idiopathic necrotizing-crescentic glomerulonephritis and renal vasculitis. Mayo Clin Proc 1993;68:561-565.

29. Bindi P, Mougenot B, Mentre F, Noel LH, Peraldi MN, Vanhille P, Lesavre P, Mignon F, Ronco PM. Necrotizing crescentic glomerulonephritis without significant immune deposits: a clinical and serological study. Q J Med 1993;86: 55-68.

30. Bosch X, Mirapeix E, Font J, López-Soto A, Rodríguez R, Vivancos J, Revert L, Ingelmo M, Urbano-Márquez A. Anticuerpos anticitoplasma de neutrófilo: utilidad diagnóstica en vasculitis y glomerulonefritis. Med Clin (Barc) 1994;102: 412-417.

31. Davenport A, Lock RJ, Wallington TB, Feest TG. Clinical significance of anti-neutrophil cytoplasm antibodies detected by a standardized indirect immunofluorescence assay. Q J Med 1994;87: 291-299.

32. Davenport A, Lock RJ, Wallington TB. Clinical relevance of testing for antineutrophil cytoplasm antibodies (ANCA) with a standard indirect immunofluorescence ANCA test in patients with upper or lower respiratory tract symptoms. Thorax 1994;49: 213-217.

33. Rao JK, Allen NB, Feussner JR, Weinberger M. A prospective study of antineutrophil cytoplasmic antibody (c-ANCA) and clinical criteria in diagnosing Wegener´s granulomatosis. Lancet 1995;346: 926-931.

34. Rao JK, Weinberger M, Oddone EZ, Allen NB, Landsman P, Feussner JR. The role of antineutrophil cytoplasmic antibody (c-ANCA) testing in the diagnosis of Wegener granulomatosis. Ann Intern Med 1995;123: 925-932.

35. Blockmans D, Stevens E, Marien G, Bobbaers H. Clinical spectrum associated with positive ANCA titres in 94 consecutive patients: is there a relation with PR-3 negative c-ANCA and hypergammaglobulinemia ? Ann Rheum Dis 1998;57: 141-145.

36. Hagen EC, Daha MR, Hermans J, Andrassy K, Csernok E, Gaskin G, Lesavre P, Lüdemann J, Rasmussen N, Sinico RA, Wiik A, van der Woude FJ. Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. Kidney Int 1998;53: 743-753.

37. Jennette C, Wilkman AS, Falk RJ. Diagnostic predictive value of ANCA serology. Kidney Int 1998;53: 796-798.

38. Sneller MC, Fauci AS. Pathogenesis of vasculitis syndromes. Med Clin North Am 1997;81: 221-242.

39. Cid Xutglà MC. Mecanismos patogénicos de las vasculitis sistémicas. Nuevos conceptos. Med Clin (Barc) 1998;110: 587-596.

40. Jennette JC, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, Hagen EC, Hoffman GS, Hunder GG, Kallenberg CGM, McCluskey RT, Sinico RA, Rees AJ, van Es LA, Waldherr R, Wiik A. Nomenclature of systemic vasculitides. Arthritis Rheum 1994;37: 187-192.

41. Jennette J, Falk RJ. Small-vessel vasculitis. N Engl J Med 1997;337: 1512-1523.

42. Falk RJ, Jennette JC. ANCA small-vessel vasculitis. J Am Soc Nephrol 1997;8: 314-322.

43. Savage COS, Harper L, Adu D. Primary systemic vasculitis. Lancet 1997,349: 553-558.

ISBN:978-84-691-2705-6/DL: T.398-2008

44. Jayne DRW, Rasmussen N, for the European Community Systemic Vasculitis Clinical Trials Study Group (ECSYSVASTRIAL). Treatment of antineutrophil cytoplasmic autoantibody-associated systemic vasculitis: initiatives of the European community systemic vasculitis clinical trials study group. Mayo Clin Proc 1997;72: 737-747.

45. Kallenberg CGM, Brouwer E, Weening JJ, Cohen Tervaert JW. Anti-neutophil cytoplasmic antibodies: current diagnostic and pathophysiological potential. Kidney Int 1994;46: 1-15.

46. Gross W, Csernok E, Helmchen U. Antineutrophil cytoplasmic autoantibodies, autoantigens, and systemic vasculitis. APMIS 1995;103: 81-97.

47. Schnabel A, Hauschild S, Gross WL. Anti-neutrophil cytoplasmic antibodies in generalized autoimmune diseases. Int Arch Allergy Immunol 1996;109: 201-206.

48. Hoffman GS, Specks U. Antineutrophil cytoplasmic antibodies. Arthritis Rheum 1998;41: 1521-1537.

49. Saxon A, Shanahan F, Landers C, Ganz T, Targan S. A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. J Allergy Clin Immunol 1990;86: 202-210.

50. Rump JA, Schölmerich J, Gross V, Roth M, Helfesrieder R, Rautmann A, Lüdemann J, Gross WL, Peter HH. A new type of perinuclear anti-neutrophil cytoplasmic antibody (p-ANCA) in active ulcerative colitis but not in Crohn´s disease. Immunobiol 1990;181: 406-413.

51. Wieslander J. How are antineutrophil cytoplasmic antibodies detected ? Am J Kidney Dis 1991;18: 154-158.

52. Wiik A, Rasmussen N, Wieslander J. Methods to detect autoantibodies to neutrophilic granulocytes. In: van Venrooij WJ and Maini RN eds. Manual of biological markers of disease. Kluwer Academic Publishers, Dordrecht 1993: A9, 1-14.

53. Wiik A. Delineation of a standard procedure for indirect immunofluorescence detection of ANCA. APMIS 1989;97 (supl 6): 12-13.

54. Hagen EC, Andrassy K, Csernok E, Daha MR, Gaskin G, Gross WL, Lesavre Ph, Lüdemann J, Pusey CD, Rasmussen N, Savage CO, Sinico RA, Wiik A, Van der Woude FJ. The value of indirect immunofluorescence and solid phase thecniques for ANCA detection. A report on the first phase of an international cooperative study on the standardization of ANCA assays. J Immunol Methods 1993;159: 1-16.

55. Charles LA, Falk RJ, Jennette JC. Reactivity of anti-neutrophil cytoplasmic autoantibodies with HL-60-cells. Clin Immunol Immunopathol 1989;53: 243-253.

56. Rasmussen N, Borregaard N, Wieslander J. Alfa-ELISA determination of ANCA and characterization of the ANCA related antigen. Acta Pathol Microbiol Immunol 1989;S697: 40.

57. Charles LA, Jennette JC, Falk RJ. The role of HL-60 cells in the detection of anti-neutrophil cytoplasmic autoantibodies. J Rheumatol 1991;18: 492-494.

58. Wheeler FB, Saluta G, Wise CM, Semble EL, Pisko EJ. Detection of anti-neutrophil cytoplasmic autoantibodies using the promyelocytic HL-60 cell-line. Clin Exp Rheumatol 1991;9: 569-580.

59. Gallagher R, Collins S, Trujillo J. Characterization of the continous differentiating myeloid cell line (HL-60) from a patient with acute promyelocitic leukemia. Blood 1979;54: 713-733.

60. Leglise MC, Dent GA, Ayscue LH, Ross DW. Leukemic cell maturation: phenotypic variability and oncogene expression in HL60 cells: a review. Blood Cells 1988;13: 319-337.

61. Specks U, Wiegert EM, Homburger HA. Human mast cells expressing recombinant proteinase 3 (PR3) as substrate for clinical testing for anti-neutrophil cytoplasmic antibodies (ANCA). Clin Exp Immunol 1997;109: 286-295.

62. Wiik A, van der Woude FJ. The new ACPA/ANCA nomenclature: Neth J Med 1990;36: 107-108.

63. Wiik A, Stummann L, Kjeldsen L, Borregaard N, Ullman S, Jacobsen S, Halberg P. The diversity of perinuclear antineutrophil cytoplasmic antibodies (pANCA) antigens. Clin Exp Immunol 1995;101 (suppl 1): 15-17.

64. Zhao MH, Lockwood CM. ANCA defines the clinical disease manifestations of vasculitis. Sarcoidosis Vasc Diffuse Lung Dis 1996;13: 221-226.

65. Bang la Cour B, Wiik A, Hoier-Madsen M, Baslund B. Clinical correlates and substrate specificities of antibodies exhibiting neutrophil nuclear reactivity - A methodological study. J Immunol Methods 1995;187: 287-295.

66. Spickett GP, Broomhead V. Formalin fixation and patterns of antineutrophil cytoplasmic antibodies. J Clin Pathol 1995;48: 89-90.

67. Yang P. Comparative evaluation of unfixed and fixed human neutrophils for determination of antineutrophil cytoplasmic antibodies by indirect immunofluorescence. J Clin Pathol 1997;50: 677-680.

68. Chevailler A, Noël LH, Renier G, Gardembas-Pain M, Subra JF, Nusbaum P, Hurez D, Lesavre P. Determination of anti-neutrophil cytoplasm antibodies (ANCA) specificity by immunofluorescence on chronic myelocytic leukemia cells. J Immunol Methods 1992;147: 101-109.

69. Lock RJ. Detection of autoantibodies to neutrophil cytoplasmic antigens. J Clin Pathol 1994;47: 4-8.

70. Lesavre P, Noël LH, Gayno S, Nusbaum P, Reumaux D, Erlinger S, Grünfeld JP, Halbwachs-Mecarelli L. Atypical autoantigen targets of perinuclear antineutrophil cytoplasm antibodies (P-ANCA): specificity and clinical associations. J Autoimm 1993;6: 185-195.

71. Lüdemann J, Utecht B, Gross WL. Detection and quantitation of anti-neutrophil cytoplasm antibodies in Wegener´s granulomatosis by ELISA using affinity purified antigen. J Immunol Methods 1988;114:167-174.

72. Rasmussen N, Sjolin C, Isaksson B, Bygren P, Wieslander J. An ELISA for the detection of anti-neutrophil cytoplasm antibodies (ANCA). J Immunol Methods 1990;127: 139-145.

73. Wang G, Csernok E, de Groot K, Gross WL. Comparison of eight commercial kits for cuantitation of antineutrophil cytoplasmic antibodies (ANCA). J Immunol Methods 1997;208: 203-211.

74. Hagen EC, Andrassy K, Csernok E, Daha MR, Gaskin G, Gross WL, Hansen B, Heigl Z, Hermans J, Jayne D, Kallenberg CGM, Lesavre Ph, Lockwood CM, Lüdemann J, Mascart-Lemone F, Mirapeix E, Pusey CD, Rasmussen N, Sinico RA, Tzioufas A, Wieslander J, Wiik A, Van der Woude FJ. Development and standardization of solid phase assays for the detection of anti-neutrophil cytoplasmic antibodies (ANCA). A report on the second phase of an international cooperative study on the standardization of ANCA assays. J Immunol Methods 1996;196: 1-15.

75. Baslund B, Segelmark M, Wiik A, Szpirt W, Petersen J, Wieslander J. Screening for anti-neutrophil cytoplasmic antibodies (ANCA): is indirect immunofluorescence the method of choice ? Clin Exp Immunol 1995;99: 486-492.

76. Westman KWA, Selga D, Bygren P, Segelmark M, Baslund B, Wiik A, Wieslander J. Clinical evaluation of a capture ELISA for detection of proteinase-3 antineutrophil cytoplasmic antibody (PR3-ANCA). Kidney Int 1998;53: 1230-1236.

77. Borregaard N, Kjeldsen L, Lollike K, Sengelov H. Granules and secretory vesicles of the human neutrophil. Clin Exp Inmunol 1995;101 (suppl 1): 6-9.

78. Lockwood CM, Bakes D, Jones S, Whitaker KB, Moss DW, Savage COS. Association of alkaline phosphatase with an autoantigen recognised by circulating anti-neutrophil antibodies in systemic vasculitis. Lancet 1987;1: 716-719.

79. Rasmussen N, Borregaard N, Wiik A. Anti-neutrophil-cytoplasm antibodies in Wegener´s granulomatosis are not directed against alkaline phosphatase. Lancet 1987;1: 1488.

80. Gross WL, Lüdemann J, Schröder JM. Anti-neutrophil-cytoplasm antibodies in Wegener´s granulomatosis are not directed against alkaline phosphatase. Lancet 1987;1: 1488-1489.

81. Goldschmeding R, Tetteroo PAT, Von dem Borne AEGK, Kallenberg CGM. Anti-neutrophil-cytoplasm antibodies in Wegener´s granulomatosis are not directed against alkaline phosphatase. Lancet 1987;1: 1489.

82. Goldschmeding R, van der Schoot CE, Ten Bokkel Huinink D, Hack CE, van den Ende ME, Kallenberg CGM, vom dem Borne AEGKr. Wegener´s granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. J Clin Invest 1989;84: 1577-1587.

83. Niles JL, McCluskey RT, Ahmad MF, Arnaout MA. Wegener´s granulomatosis autoantigen is a novel serine proteinase. Blood 1989;74: 1888-1893.

84. Lüdemann J, Utecht B, Gross WL. Anti-neutrophil cytoplasm antibodies in Wegener´s granulomatosis recognize an elastinolytic enzyme. J Exp Med 1990;171: 357-362.

85. Goldschmeding R, Cohen-Tervaert JW, van der Schoot CE, van der Veen C, Kallenberg CGM, von dem Borne AEGKr. ANCA, anti-myeloperoxidase, and anti-elastase: three members of a novel class of autoantibodies against myeloid lysosomal enzimes. APMIS 1989;97(S6): 48-49.

86. Jennette JC, Hoidal JR, Falk RJ. Specificity of anti-neutrophil cytoplasmic autoantibodies for proteinase 3. Blood 1990;75: 2263-2264.

87. Jenne DE, Tschopp J, Lüdemann J, Utecht B, Gross WL. Wegener´s autoantigen decoded. Nature 1990;346: 520.

88. Kao RC, Wehner NG, Skubitz KM, Gray BH, Hoidal JR. Proteinase 3: a distinct human polymorphonuclear leukocyte proteinase that produces enphysema in hamsters. J Clin Invest 1988;82: 1963-1973.

89. Nässberger L, Jonsson H, Sjöholm AG, Sturfelt G, Heubner A. Circulating antielastase in systemic lupus erythematosus. Lancet 1989;1: 509.

90. Flesch BK, Lampe M, Rautman A, Gross WL. Anti-elastase, cathepsin G, and lactoferrin antibodies in sera with c-ANCA or atypical fluorescence staining pattern. Am J Kidney Dis 1991;18: 201.

91. Thomson RA, Lee SS. Anti-neutrophil cytoplasmic antibodies. Lancet 1989;1: 670-671.

92. Schmitt WII, Csernok E, Flesch BK, Hauschild S, Gross WL. Autoantibodies directed against lysozyme: a new target antigen for anti-neutrophil cytoplasmic antibodies (ANCA). Adv Exp Med Biol 1993;336: 267-272.

93. Nässberger L, Ljungh A, Schumacher G, Kollberg B. β-glucuronidase antibodies in ulcerative colitis. Lancet 1992;340: 734-735.

94. Moodie FDL, Leaker G, Cambridge NF, Totty NF, Segal AW. Alpha-enolase: a novel cytosolic autoantigen in ANCA positive vasculitis. Kidney Int 1993;43: 675-681.

95. Zhao MH, Jones SJ, Lockwood CM. Bactericidal/permeability-increasing protein (BPI) is an important antigen for anti-neutrophil cytoplasmic autoantibodies (ANCA) in vasculitis. Clin Exp Immunol 1995;99: 49-56.

96. Zhao MH, Lockwood CM. Azurocidin is a novel antigen for anti-neutrophil cytoplasmic autoantibodies (ANCA) in systemic vasculitis. Clin Exp Immunol 1996;103: 397-402.

97. Yang JJ, Tuttle R, Falk RJ, Jennette JC. Frequency of anti-bactericidal/permeability-increasing protein (BPI) and anti-azurocidin in patients with renal disease. Clin Exp Immunol 1996;105: 125-131.

98. Gallin MY, Jacobi AB, Büttner DW, Schönberger Ö, Marti T, Erttmann KD. Human autoantibody to defensin: disease association with hyperreactive onchocerciasis (sowda). J Exp Med 1995;182: 41-47.

99. Kain R, Matsui K, Exner M, Binder S, Schaffner G, Sommer EM, Kerjaschki D. A novel class of autoantigens of anti-neutrophil cytoplasmic antibodies in necrotizing and crescentic glomerulonephritis: the lysosomal membrane glycoprotein h-lamp-2 in neutrophil granulocytes and a related membrane protein in glomerular endothelial cells. J Exp Med 1995;181: 585-597.

100. Sobajima J, Ozaki S, Osakada F, Uesugi H, Shirakawa H, Yoshida M, Nakao K. Novel autoantigens of perinuclear anti-neutrophil cytoplasmic antibodies (P-ANCA) in ulcerative colitis: non-histone chromosomal proteins, HMG1 and HMG2. Clin Exp Immunol 1997;107: 135-140.

101. Sobajima J, Ozaki S, Uesugi H, Osakada F, Shirakawa H, Yoshida M, Nakao K. Prevalence and characterization of perinuclear anti-neutrophil cytoplasmic antibodies (P-ANCA) directed against HMG1 and HMG2 in ulcerative colitis (UC). Clin Exp Immunol 1998;111: 402-407.

102. Uesugi H, Ozaki S, Sobajima J, Osakada F, Shirakawa H, Yoshida M, Nakao K. Prevalence and characterization of novel pANCA, antibodies to the high mobility group non-histone chromosomal proteins HMG1 and HMG2, in systemic rheumatic diseases. J Rheumatol 1998;25:703-709.

103. Zeek PM. Periarteritis nodosa: a critical review. Am J Clin Pathol 1952;22: 777-790.

104. Fauci AS, Haynes BF, Katz P. The spectrum of vasculitis: clinical, pathologic, immunologic and therapeutic considerations. Ann Intern Med 1978;89:660-676.

105. Alarcón-Segovia D. Classification of the necrotizing vasculitides in man. Clin Rheum Dis 1980;6: 223-231.

106. Lie JT. The classification and diagnosis of vasculitis in large and medium-sized blood vessels. Pathol Annu 1987;22: 125-162.

107. Hunder GG, Arend WP, Bloch DA, Calabrese LH, Fauci AS, Fries JF, Leavitt RY, Lie JT, Lightfoot RW Jr, Masi AT, McShane DJ, Michel BA, Mills JA, Stevens MB, Wallace SL, Zvaifler NJ. The American College of Rheumatology 1990 criteria for the classification of vasculitis: introduction. Arthritis Rheum 1990;33: 1065-1067.

108. Wathen CW, Harrison DJ. Circulating anti-neutrophil antibodies in systemic vasculitis. Lancet 1987;1: 1037.

109. Cohen Tervaert JW, Goldschmeding R, Elema JD, von dem Borne AEGKr, Kallenberg CGM. Antimyeloperoxidase antibodies in the Churg-Strauss syndrome.Thorax 1991;46: 70-71.

110. Guillevin L, Visser H, Noel LH, Pourrat J, Vernier I, Gayraud M, Oksman F, Lesavre P. Antineutrophil cytoplasmic antibodies in systemic polyarteritis nodosa with and without hepatitis B virus infection and Churg-Strauss syndrome-62 patients. J Rheumatol 1993;20: 1345-1349.

111. Guillevin L, Lhote F, Amouroux J, Gherardi R, Callard P, Casassus P. Antineutrophil cytoplasmic antibodies, abnormal angiograms and pathological findings in polyarteritis nodosa and Churg-Strauss syndrome: indications for the classification of vasculitides of the polyarteritis nodosa group. Br J Rheumatol 1996;35: 958-964.

112. Schmitt WH, Csernok E, Kobayashi S, Klinkenborg A, Reinhold-Keller E, Gross WL. Churg-Strauss syndrome. Serum markers of lymphocyte activation and endothelial damage. Arthritis Rheum 1998;41: 445-452.

113. Egner W, Chapel HM. Titration of antibodies against neutrophil cytoplasmic antigens is useful in monitoring disease activity in systemic vasculitides. Clin Exp Immunol 1990;82: 244-249.

114. Cohen Tervaert JW, Huitema MG, Hené RJ, Sluiter WJ, The TH, van der Hem GK, Kallenberg CGM. Prevention of relapses in Wegener´s granulomatosis by treatment based on antineutrophil cytoplasmic antibody titre. Lancet 1990;336: 709-711.

115. Gaskin G, Savage CO, Ryan JJ, Jones S, Rees AJ, Lockwood CM, Pusey CD. Anti-neutrophil cytoplasmic antibodies and disease activity during long-term follow-up of 70 patients with systemic vasculitis. Nephrol Dial Transplant 1991;6: 689-694.

116. Chan TM, Frampton G, Jayne DRW, Perry GJ, Lockwood CM, Cameron JS. Clinical significance of anti-endothelial cell antibodies in systemic vasculitis: a longitudinal study comparing anti-endothelial cell antibodies and anti-neutrophil cytoplasm antibodies. Am J Kid Dis 1993;22: 387-392.

117. Stegeman CA, Cohen Tervaert JW, Sluiter WJ, Manson WL, de Jong PE, Kallenberg CGM. Association of chronic nasal carriage of staphylococcus aureus and higher relapse rates in Wegener granulomatosis. Ann Intern Med 1994;120: 12-17.

118. Markey BA, Warren JS. Use of anti-neutrophil cytoplasmic antibody assay to distinguish between vasculitic disease activity and complications of cytotoxic therapy. Am J Clin Pathol 1994;102: 589-594.

119. Jayne DRW, Gaskin G, Pusey CD, Lockwood CM. ANCA and predicting relapse in systemic vasculitis. Q J Med 1995;88: 127-133.

120. De´Oliviera J, Gaskin G, Dash A, Rees AJ, Pusey CD. Relationship between disease activity and anti-neutrophil cytoplasmic antibody concentration in long-term management of systemic vasculitis. Am J Kidney Dis 1995,25: 380-389.

121. Kerr GS, Fleisher TA, Hallahan CW, Leavitt RY, Fauci AS, Hoffman GS. Limited prognostic value of changes in antineutrophil cytoplasmic antibody titer in patients with Wegener´s granulomatosis. Arthritis Rheum 1993;36: 365-371.

122. Geffriaud-Ricouard C, Noël LH, Chauveau D, Houhou S, Grünfeld JP, Lesavre P. Clinical spectrum associated with ANCA of defined antigen specificities in 98 selected patients. Clin Nephrol 1993;39: 125-136.

123. Davenport A, Lock RJ, Wallington T. Clinical significance of serial measurement of autoantibodies to neutrophil cytoplasm using a standard indirect immunofluorescence test. Am J Nephrol 1995;15: 201-207.

124. Cohen P, Guillevin L, Baril L, Lothe F, Noël LH, Lesavre P. Persistence of antineutrophil cytoplasmic antibodies (ANCA) in asymptomatic patients with systemic polyarteritis nodosa or Churg-Strauss syndrome: follow-up of 53 patients. Clin Exp Rheumatol 1995;13: 193-198.

125. Cohen Tervaert JW, Stegeman CA, Kallenberg CG. Serial ANCA testing is useful in monitoring disease activity of patients with ANCA-associated vasculitides. Sarcoidosis Vasc Diffuse Lung Dis 1996;13: 241-245.

126. Lesavre P, Kyndt X, Vanhille P, Reumaux D, Guillevin L, Noël LH. Serial ANCA testing has limited value during the follow-up of disease activity in patients with ANCA-associated vasculitis. Sarcoidosis Vasc Diffuse Lung Dis 1996;13: 246-248.

127. Nässberger L, Johansson AC, Björck S, Sjöholm AG. Antibodies to neutrophil granulocyte myeloperoxidase and elastase: autoimmune responses in glomerulonephritis due to hydralazine treatment. J Int Med 1991;229: 261-265.

128. Cambridge G, Wallace H, Bernstein RM, Leaker B. Autoantibodies to myeloperoxidase in idiopathic and drug-induced systemic lupus erythematosus and vasculitis. Br J Rheumatol 1994;33:109-114.

129. Short AK, Lockwood CM. Antigen specificity in hydralazine associated ANCA positive systemic vasculitis. Q J Med 1995;88: 775-783.

130. Dolman KM, Gans ROB, Vervaat ThJ, Zevenbergen G, Maingay D, Nikkels RE, Donker AbJM, von dem Borne AEGKr, Goldschmeding R. Vasculitis and antineutrophil cytoplasmic autoantibodies associated with propylthiouracil therapy. Lancet 1993;342: 651-652.

131. D´Cruz D, Chesser AMS, Lightowler C, Comer M, Hurst MJ, Baker LRI, Raine AEG. Antineutrophil cytoplasmic antibody-positive crescentic glomerulonephritis associated with anti-thyroid drug treatment. Br J Reumatol 1995;34:1090-1091.

132. Tanemoto M, Miyakawa H, Hanai J, Yago M, Kitaoka M, Uchida S. Myeloperoxidase-antineutrophil cytoplasmic antibody-positive crescentic glomerulonephritis complicating the course of Graves´ disease: report of three adult cases. Am J Kidney Dis 1995;26: 774-780.

133. Kitahara T, Hiromura K, Maezawa A, Ono K, Narahara N, Yano S, Naruse T, Takenouchi K, Yasumoto Y. Case of propylthiouracil-induced vasculitis associated with anti-neutrophil cytoplasmic antibody (ANCA); review of literature. Clin Nephrol 1997;47: 336-340.

134. Harper L, Cockwell P, Savage COS. Case of propylthiouracil-induced ANCA associated small vessel vasculitis. Nephrol Dial Transplant 1998;13: 455-458.

135. Jones BF, Major GAC. Crescentic glomerulonephritis in a patient taking penicillamine associated with antineutrophil cytoplasmic antibody. Clin Nephrol 1992;38:293.

136. Hillis GS, Khan IH, Simpson JG, Rees AJ. Scleroderma, D-Penicillamine treatment, and progressive renal failure associated with positive antimyeloperoxidase antineutrophil cytoplasmic antibodies. Am J Kidney Dis 1997;30: 279-281.

137. Karpinski J, Jothy S, Radoux V, Levy M, Baran D. D-Penicillamine-induced crescentic glomerulonephritis and antimyeloperoxidase antibodies in a patient with scleroderma. Am J Nephrol 1997;17: 528-532.

138. Merkel PA. Drugs associated with vasculitis. Curr Opin Rheumatol 1998;10:45-50.

139. Van den Wall-Bake AWL, Lobatto S, Jonges L, Daha MR, van Es LA. IgA antibodies directed against cytoplasmic antigens of polymorphonuclear leukocytes in patients with Henoch-Schönlein purpura. Adv Exp Med Biol 1987;216: 1593-1598.

140. Ronda N, Esnault VLM, Layward L, Sepe V, Allen A, Feehally J, Lockwood CM. Antineutrophil cytoplasm antibodies (ANCA) of IgA isotype in adult Henoch-Schönlein purpura. Clin Exp Immunol 1994;95: 49-55.

141. O´Donoghue DJ, Nusbaum P, Noel LH, Halbwachs-Mecarelli L, Lesavre P. Antineutrophil cytoplasmic antibodies in IgA nephropathy and Henoch-Schönlein purpura. Nephrol Dial Transplant 1992;7: 534-538.

142. Robson WL, Leung AK, Woodman RC. The absence of anti-neutrophil cytoplasmic antibodies in patients with Henoch-Schönlein purpura. Pediatr Nephrol 1994;8: 295-298.

143. Kuester S, Andrassy K, Waldherr R, Ritz E. Contrasting clinical course of Henoch-Schönlein purpura in younger and elderly patients. Contr Nephrol 1993;105: 93-97.

144. Saulsbury FT, Kirkpatrick PR, Bolton WK. IgA antineutrophil cytoplasmic antibody in Henoch-Schönlein purpura. Am J Nephrol 1991;11: 295-300.

145. Sinico RA, Tadros M, Radice A, Pozzi C, Quarenghi M, Comotti C, Gregorini G, Castiglione A, Arrigo G, D´Amico G. Lack of IgA antineutrophil cytoplasmic antibodies in Henoch-Schönlein purpura and IgA nephopathy. Clin Immunol Immunopathol 1994;73: 19-26.

146. Coppo R, Cirina P, Amore A, Sinico RA, Radice A, Rollino C, for the Italian Group of Renal Immunopathology Collaborative Study on Henoch-Schönlein purpura in adults and in children. Nephrol Dial Transplant 1997;12: 2269-2276.

147. Lamprecht P, Schmitt WH, Gross WL. Mixed cryoglobulinaemia, glomerulonephritis, and ANCA: essential cryoglobulinaemic vasculitis or ANCA-associated vasculitis ? Nephrol Dial Transplant 1998;13: 213-221.

148. Khan IH, Catto GRD, MacLeod AM. Antineutrophil cytoplasmic autoantibody associated vasculitis and renal failure in Behçet's disease. Nephrol Dial Transplant 1994;9: 332.

149. Baleva M, Kolarov Z, Nikolov K. Antineutrophil cytoplasmic autoantibody in two patients with Behçet's disease. Nephrol Dial Transplant 1994;9: 876.

150. Burrows NP, Zhao MH, Norris PG, Lockwood CM. ANCA associated Behcet´s disease. J R Soc Med 1996;89: 47P-48P.

151. Ben Hmida M, Hachicha J, Kaddour N, Makñi H, Aydel FZ, Chakroun N, Bahloul Z, Ayadi H, Noël LH, Jarraya A. ANCA in Behçet's disease. Nephrol Dial Transplant 1997;12: 2465-2466.

152. Zeek PM, Smith CC, Weeter JC. Studies on periarteritis nodosa. The differentiation between the vascular lesions of periarteritis nodosa and of hypersensitivity. Am J Pathol 1948;24: 889-917.

153. Godman G, Churg J. Wegener´s granulomatosis. Pathology and review of the literature. Arch Pathol Lab Med 1954;58: 533-553.

154. Guillevin L, Lhote F, Cohen P, Sauvaget F, Jarrousse B, Lortholary O, Noël LH, Trépo C. Polyarteritis nodosa related to hepatitis B virus. Medicine 1995;74:238-253.

155. Savage CO, Tizard J, Jayne D, Lockwood CM, Dillon MJ. Antineutrophil cytoplasm antibodies in Kawasaki disease. Arch Dis Child 1989;64: 360-363.

156. Rider LG, Wener MH, French J, Sherry DD, Mendelman PM. Autoantibody production in Kawasaki syndrome. Clin Exp Rheumatol 1993;11: 445-449.

157. Guzman J, Fung M, Petty RE. Diagnostic value of anti-neutrophil cytoplasmic and anti-endothelial cell antibodies in early Kawasaki disease. J Pediatr 1994;124: 917-920.

158. Nash MC, Shah V, Reader JA, Dillon MJ. Anti-neutrophil cytoplasmic antibodies and anti-endothelial cell antibodies are not increased in Kawasaki disease. Br J Rheumatol 1995;34: 882-887.

159. Falcini F, Trapani S, Turchini S, Farsi A, Ermini M, Keser G, Khamashta MA, Hughes GR. Immunological findings in Kawasaki disease: an evaluation in a cohort of Italian children. Clin Exp Rheumatol 1997;15: 685-689.

160. Mc Hugh NJ, James IE, Plant GT. Anticardiolipin and antineutrophil antibodies in giant cell arteritis. J Rheumatol 1990;17: 916-922.

161. Bosch X, Font J, Mirapeix E, Cid M, Revert L, Ingelmo M. Antineutrophil cytoplasmic antibodies in giant cell arteritis. J Rheumatol 1991;18: 787-788.

162. Baranger TAR, Audrain MAP, Castagne A, Barrier JH, Esnault VLM. Absence of antineutrophil cytoplasmic antibodies in giant cell arteritis. J Rheumatol 1994;21: 871-873.

163. Vera O, Pérez-Hernández T, Mejía R, Ariza R, Frati A. Anticuerpos anticitoplasmáticos del neutrófilo (ANCA) en arteritis de Takayasu. Rev Mex Reumatol 1994;9: 71.

164. Dagenais P, Dalpé G, Fernandez MF, Boire G, Keystone EC, Gross WL. ANCAs in patients with Takayasu´s arteritis. Clin Exp Immunol 1993;93 (suppl 1): 534.

165. Eichhorn J, Sima D, Thiele B, Lindschau C, Turowski A, Schmidt H, Schneider W, Haller H, Luft FC. Anti-endothelial cell antibodies in Takayasu arteritis. Circulation 1996;94: 2396-2401.

166. García-Torres R, Noël LH, Reyes PA, Vera OL, Amigo MC, Silveira LH, Pineda C. Absence of ANCA in Mexican patients with Takayasu´s arteritis. Scand J Rheumatol 1997;26: 55-57.

167. Calabresi P, Edwards EA, Schilling RF. Fluorescent antiglobulin studies in leukopenic and related disorders. J Clin Invest 1959;38: 2091-2100.

168. Calabresi P, Thayer WR, Jr, Spiro HM. Demonstration of circulating antinuclear globulins in ulcerative colitis,. J Clin Invest 1961;40: 2126-2133.

169. Faber V, Elling P, Norup G, Mansa B, Nissen NI. An antinuclear factor specific for leucocytes. Lancet 1964;2: 344-345.

170. Barnett EV, North F Jr, Condemi JJ, Jacox RF, Vaughan JH. Antinuclear factors in systemic lupus erithematosus and rheumatoid arthritis. Ann Intern Med 1965;63: 100-108.

171. Faber V, Elling P. Leucocyte-specific anti-nuclear factors in patients with Felty´s syndrome, rheumatoid arthritis, systemic lupus erythematosus and other diseases. Acta Med Scand 1966;179:257-267.

172. Svec KH. Immunological and clinical observations of granulocyte-specific antinuclear antibodies. Arthritis Rheum 1969;12: 165-172.

173. Wiik A, Jensen E, Friis J. Granulocyte-specific antinuclear factors in synovial fluids and sera from patients with reumathoid arthritis. Ann Rheum Dis 1974;33: 515-522.

174. Wiik A. Granulocyte-specific antinuclear antibodies. Allergy 1980;35: 263-289.

175. Nielsen H, Wiik A, Elmegreen J. Granulocyte specific anti-nuclear antibodies in ulcerative colitis. Aid in differential diagnosis of inflammatory bowel disease. Acta Pathol Microbiol Immunol Scand (C) 1983;91: 23-26.

176. Snook JA, Chapman RW, Fleming K, Jewell DP. Anti-neutrophil nuclear antibody in ulcerative colitis, Crohn´s disease, and primary sclerosing cholangitis. Clin Exp Immunol 1989;76: 30-33.

177. Nässberger L, Sjöholm AG, Sturfelt G. Absence of circulating antineutrophil cytoplasm antibodies (ANCA) in severe vasculitis associated with rheumatoid arthritis. Scand J Rheumatol 1990;19: 189-192.

178. Savige JA, Gallicchio MC, Stockman A, Cunningham TJ, Rowley MJ, Georgiou T, Davies D. Anti-neutrophil cytoplasm antibodies in rheumatoid arthritis. Clin Exp Immunol 1991;86: 92-98.

179. Juby A, Johnston C, Davis P, Russell AS. Antinuclear and antineutrophil cytoplasmic antibodies (ANCA) in the sera of patients with Felty´s syndrome. Br J Rheumatol 1992;31: 185-188.

180. Coremans IEM, Hagen EC, Daha MR, van der Woude FJ, van der Voort EAM, Kleijburg-van der Keur C, Breedveld FC. Antilactoferrin antibodies in patients with rheumatoid arthritis are associated with vasculitis. Arthritis Rheum 1992;35: 1466-1475.

181. Mulder AHL, Horst G, van Leeuwen MA, Limburg PC, Kallenberg CGM. Antineutrophil cytoplasmic antibodies in rheumatoid arthritis. Arthritis Rheum 1993;36: 1054-1060.

182. Helsloot J, Virgo S, McGuigan L, Sturgess A. Antineutrophil cytoplasmic antibodies in inflammatory arthritis-potential for misdiagnosis ? Br J Rheumatol 1995;34: 820-824.

183. Bosch X, Llena J, Collado A, Font J, Mirapeix E, Ingelmo M, Muñoz-Gómez J, Urbano-Márquez A. Ocurrence of antineutrophil cytoplasmic and antineutrophil (peri)nuclear antibodies in rheumatoid arthritis. J Rheumatol 1995;22:2038-2045.

184. De Bandt M, Meyer O, Haim T, Kahn MF. Antineutrophil cytoplasmic antibodies in rheumatoid arthritis patients. Br J Rheumatol 1996;35: 38-43.

185. Afeltra A, Sebastiani GD, Galeazzi M, Caccavo D, Ferri GM, Marcolongo R, Bonomo L. Antineutrophil cytoplasmic antibodies in synovial fluid and in serum of patients with rheumatoid arthritis and other types of synovitis. J Rheumatol 1996;23: 10-15.

186. Braun MG, Csernok E, Schmitt WH, Gross WL. Incidence, target antigens, and clinical implications of antineutrophil cytoplasmic antibodies in rheumatoid arthritis. J Rheumatol 1996;23: 826-830.

187. Brimnes J, Halberg P, Jacobsen S, Wiik A, Heegaard NHH. Specificities of anti-neutrophil autoantibodies in patients with rheumatoid arthritis (RA). Clin Exp Immunol 1997,110: 250-256.

188. Mustila A, Korpela M, Mustonen J, Helin H, Huhtala H, Soppi E, Pasternack A, Miettinen A. Perinuclear antineutrophil cytoplasmic antibody in rheumatoid arthritis. Arthritis Rheum 1997;40: 710-717.

189. Merkel PA, Polisson RP, Chang YC, Skates SJ, Niles JL. Prevalence of antineutrophil cytoplasmic antibodies in a large inception cohort of patients with connective tissue disease. Ann Intern Med 1997;126:866-873.

190. Lawton JWM, Lee SS. Anti-neutrophil cytoplasmic antibody (ANCA) in systemic lupus erythematosus. Am J Kidney Dis 1991;18: 200-201.

191. Lee SS, Lawton JWM, Chan CE, Li CS, Kwan TH, Chau KF. Antilactoferrin antibody in systemic lupus erythematosus. Br J Rheumatol 1992;31: 669-673.

192. Pauzner R, Urowitz M, Gladman D, Gough J. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. J Rheumatol 1994;1670-1673.

193. Schnabel A, Csernok E, Isenberg DA, Mrowka C, Gross WL. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. Arthritis Rheum 1995;38: 633-637.

194. Spronk PE, Bootsma H, Horst G, Huitema MG, Limburg PC, Cohen Tervaert JW, Kallenberg CG. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. Br J Rheumatol 1996;35: 625-631.

195. Nishiya K, Chikazawa H, Nishimura S, Hisakawa N, Hashimoto K. Anti-neutrophil cytoplasmic antibody in patients with systemic lupus erythematosus is unrelated to clinical features. Clin Rheumatol 1997;16: 70-75.

196. Nässberger L, Sjöholm AG, Jonsson H, Sturfelt G, Akesson A. Autoantibodies against neutrophil cytoplasm components in systemic lupus erythematosus and in hydralazine-induced lupus. Clin Exp Immunol 1990;81: 380-383.

197. Mulder L, van Rossum M, Horst G, Limburg P, de Graeff-Meeder ER, Kuis W, Kallenberg C. Antineutrophil cytoplasmic antibodies in juvenile chronic arthritis. J Rheumatol 1997;24: 568-575.

198. Locht H, Peen E, Skogh T. Antineutrophil cytoplasmic antibodies in reactive arthritis. J Rheumatol 1995;22: 2304-2306.

199. Akimoto S, Ishikawa O, Tamura T, Miyachi Y. Antineutrophil cytoplasmic antibodies in patients with systemic sclerosis. Br J Dermatol 1996;134: 407-410.

200. Duerr RH, Targan SR, Landers CJ, LaRusso NF, Lindsay KL, Wiesner RH, Shanahan F. Neutrophil cytoplasmic antibodies: a link between primary sclerosing cholangitis and ulcerative colitis. Gastroenterology 1991;100: 1385-1391.

201. Klein R, Eisenburg J, Weber P, Seibold P, Berg PA. Significance and specificity of antibodies to neutrophils detected by western blotting for the serological diagnosis of primary sclerosing cholangitis. Hepatology 1991;14: 1147-1152.

202. Lo SK, Fleming KA, Chapman RW. Prevalence of anti-neutrophil antibody in primary sclerosing cholangitis and ulcerative colitis using an alkaline phosphatase technique. Gut 1992;33; 1370-1375.

203. Seibold F, Weber P, Klein R, Berg PA, Wiedmann KH. Clinical significance of antibodies against neutrophils in patients with inflammatory bowel disease and primary sclerosing cholangitis. Gut 1992;33: 657-662.

204. Hardarson S, LaBrecque DR, Mitros FA, Neil GA, Goeken JA. Antineutrophil cytoplasmic antibody in inflammatory bowel and hepatobiliary diseases. Am J Clin Pathol 1993;99: 277-281.

205. Mulder AHL, Horst G, Haagsma EB, Limburg PC, Kleibeuker JH, Kallenberg CGM. Prevalence and characterization of neutrophil cytoplasmic antibodies in autoimmune liver diseases. Hepatology 1993;17: 411-417.

206. Lo SK, Chapman RW, Cheeseman P, Charlton CP, Walker-Smith JA, Mieli-Vergani G, Fleming KA. Antineutrophil antibody: a test for autoimmune primary sclerosing cholangitis in childhood ? Gut 1993;34: 199-202.

207. Claise C, Johanet C, Bouhnik Y, Kapel N, Homberg JC, Poupon R. Antineutrophil cytoplasmic autoantibodies in autoimmune liver and inflammatory bowel diseases. Liver 1996;16: 28-34.

208. Bansi DS, Fleming KA, Chapman RW. Importance of antineutrophil cytoplasmic antibodies in primary sclerosing cholangitis and ulcerative colitis: prevalence, titre, and IgG subclass. Gut 1996;38: 384-389.

209. Peen E, Almer S, Bodemar G, Rydén BO, Sjölin C, Tejle K, Skogh T. Anti-lactoferrin antibodies and other types of ANCA in ulcerative colitis, primary sclerosing cholangitis, an Crohn's disease. Gut 1993;34: 56-62.

210. Orth T, Kellner R, Diekmann O, Faust J, Meyer Zum Büschenfelde KH, Mayet WJ. Identification and characterization of autoantibodies against catalase and α-enolase in patients with primary sclerosing cholangitis. Clin Exp Immunol 1998;112; 507-515.

211. Lo SK, Fleming KA, Chapman RW. A 2 year follow-up study of anti-neutrophil antibody in primary sclerosing cholangitis: relationship to clinical activity, liver biochemistry and ursodeoxycholic acid treatment. J Hepatol 1994;21: 974-978.

212. Pokorny CS, Norton ID, McCaughan GW, Selby WS. Anti-neutrophil cytoplasmic antibody: a prognostic indicator in primary sclerosing cholangitis. J Gastroenterol Hepatol 1994;9: 40-44.

213. Warny M, Brenard R, Cornu C, Tomasi JP, Geubel AP. Anti-neutrophil antibodies in chronic hepatitis and the effect of α-interferon therapy. J Hepatol 1993;17: 294-300.

214. Targan SR, Landers C, Vidrich A, Czaja AJ. High-titer antineutrophil cytoplasmic antibodies in type-1 autoimmune hepatitis. Gastroenterology 1995;108: 1159-1166.

215. Bansi DS, Fleming KA, Chapman RW. Antineutrophil cytoplasmic antibodies in autoimmune hepatitis. Gastroenterology 1995;109: 2049-2050.

216. Zauli D, Ghetti S, Grassi A, Descovich C, Cassani F, Ballardini G, Muratori L, Bianchi FB. Anti-neutrophil cytoplasmic antibodies in type 1 and 2 autoimmune hepatitis. Hepatology 1997;25: 1105-1107.

217. Kellner R, Orth T, Mayet WJ. Characterization of target antigens from anti-neutrophil cytoplasmic antibodies in autoimmune hepatitis type-1. Electrophoresis 1997;18: 507-510.

218. Orth T, Gerken G, Kellner R, Meyer Zum Büschenfelde KH, Mayet WJ. Actin is a target antigen of anti-neutrophil cytoplasmic antibodies (ANCA) in autoimmune hepatitis type-1. J Hepatol 1997;26: 37-47.

219. Malnick SD, Lurie Y, Fogel N, Cohen P, Geltner D, Bass DD. Chronic HCV hepatitis is associated with a high incidence of anti-neutrophil cytoplasmic antibodies and anti-cardiolipin antibodies. Hepatology 1995;22: 352A.

220. Efthimiou J, Spickett G, Lane D, Thompson A. Antineutrophil cytoplasmic antibodies, cystic fibrosis, and infection. Lancet 1991;337: 1037-1038.

221. Gallicchio MC, Savige JA. Detection of anti-myeloperoxidase and anti-elastase antibodies in vasculitides and infections. Clin Exp Immunol 1991;84: 232-237.

222. Davenport A. "False positive" perinuclear and cytoplasmic anti-neutrophil cytoplasmic antibody results leading to misdiagnosis of Wegener´s granulomatosis and/or microscopic polyarteritis. Clin Nephrol 1992;37;124-130.

223. Koderisch J, Andrassi K, Rasmussen N, Hartmann M, Tilgen W. "False-positive" anti-neutrophil cytoplasmic antibodies in HIV infection. Lancet 1990;335: 1227-1228.

224. Klaassen RJL, Goldschmeding R, Dolman KM, Vlekke ABJ, Weigel HM, Schattenkerk JKME, Mulder JW, Westedt ML, von dem Borne AEGKr. Anti-neutrophil cytoplasmic autoantibodies in patients with symptomatic HIV infection. Clin Exp Immunol 1992;87: 24-30.

225. Savige JA, Chang L, Crowe SM. Anti-neutrophil cytoplasm antibodies in HIV infection. Adv Exp Med Biol 1993;336: 349-352.

226. Savige JA, Chang L, Horn S, Crowe SM. Anti-nuclear, anti-neutrophil cytoplasmic and anti-glomerular basement membrane antibodies in HIV-infected individuals. Autoimmunity 1994;18: 205-211.

227. Pudifin DJ, Duursma J, Gathiram V, Jackson TFHG. Invasive amoebiasis is associated with the development of anti-neutrophil cytoplasmic antibody. Clin Exp Immunol 1994;97: 48-51.

228. Adebajo AO, Charles P, Maini RN, Hazleman BL. Autoantibodies in malaria, tuberculosis and hepatitis B in a West African population. Clin Exp Immunol 1993;92: 73-76.

229. Wenisch C, Wenisch H, Bankl HC, Exner M, Graninger W, Looareesuwan S, Rumpold H. Detection of anti-neutrophil cytoplasmic antibodies after acute plasmodium falciparum malaria. Clin Diagn Lab Immunol 1996;31: 132-134.

230. Yahya TM, Benedict S, Shalabi A, Bayoumi R. Anti-neutrophil cytoplasmic antibody (ANCA) in malaria is directed against cathepsin G. Clin Exp Immunol 1997;110: 41-44.

231. Medina F, Camargo A, Moreno J, Zonana-Nacach A, Aceves-Avila J, Fraga A. Anti-neutrophil cytoplasmic autoantibodies in leprosy. Br J Rheumatol 1998;37: 270-273.

232. Galperin C, Shoenfeld Y, Gilburd B, Esterre P, Meroni PL, Del Papa N, Halpern GM, Andriantsimahavandy A, Gershwin ME. Anti-neutrophil cytoplasmic antibodies in patients with chromomycosis. Clin Exp Rheumatol 1996;14: 479-483.

233. Milesi-Lecat AM, Aumaitre O, Deusebis T, Kaufman P, Tridon A, Cambon M, Marcheix JC. Semi-invasive diffuse pulmonary aspergillosis with antineutrophil cytoplasmic antibodies. 2 cases. Ann Med Interne (Paris) 1994;145: 140-146.

234. Stappaerts I, Bogers J, Ebo D, Vanden Broecke E, Stevens WJ, Van Marck E, Vermeire P. c-ANCA positivity in a Belgian patient with pulmonary paracoccidioidomycosis. Eur Resp J 1997;10: 2419-2422.

235. Byrd R, Hourany J, Cooper C, Roy TM. False-positive antineutrophil cytoplasmic antibodies in a patient with cavitary pulmonary sporotrichosis. Am J Med 1998;104: 101-103.

236. Schmitt WH, Csernok E, Gross WL. ANCA and infection. Lancet 1991:337: 1416-1417.

237. Mege JL, Escallier JC, Capo C, Bongrand P, Velut JG, Quiles N, Soubeyrand J, Durand JM. Anti-neutrophil cytoplasmic antibodies (ANCA) and infection. Adv Exp Med Biol 1993;336: 353-356.

238. Murphy EA, McVie A, Galbraith I, Capell HA. Antineutrophil cytoplasmic antibody titres in patients with recent infection. Br J Rheumatol 1993;32: 941-942.

239. Finnegan MJ, Hinchcliffe J, Russell-Jones D, Neill S, Sheffield E, Jayne D, Wise A, Hodson ME. Vasculitis complicating cystic fibrosis. Q J Med 1989;267: 609-621.

240. Zhao MH, Jayne DRW, Ardiles LG, Culley F, Hodson ME, Lockwood CM. Autoantibodies against bactericidal/permeability-increasing protein in patients with cystic fibrosis. Q J Med 1996;89: 259-265.

241. Sedivá A, Bartunková J, Kolarová I, Hrusák O, Vávrová V, Macek jr M, Lockwood CM, Dunn AC. Antineutrophil cytoplasmic autoantibodies (ANCA) in children with cystic fibrosis. J Autoimmunity 1998;11: 185-190.

242. Jayne DRW, Marshall PD, Jones SJ, Lockwood CM. Autoantibodies to GMB and neutrophil cytoplasm in rapidly progressive glomerulonephritis. Kidney Int 1990;37: 965-970.

243. Bosch X, Mirapeix E, Font J, Borrellas X, Rodriguez R, Lopes-Soto A, Ingelmo M, Revert L. Prognostic implication of anti-neutrophil cytoplasmic autoantibodies with myeloperoxidase specificity in anti-glomerular basement membrane disease. Clin Nephrol 1991;36: 107-113.

244. Niles JL, Böttinger EP, Saurina GR, Kelly KJ. Pan GL, Collins AB, McCluskey RT. The syndrome of lung hemorrhage and nephritis is usually an ANCA-associated condition. Arch Intern Med 1996;156: 440-445.

245. Westman KWA, Bygren PG, Eilert I, Wiik A, Wieslander J. Rapid screening assay for anti-GBM antibody and ANCAs; an important tool for the differential diagnosis of pulmonary renal syndromes. Nephrol Dial Transplant 1997;12: 1863-1868.

246. Hellmark T, Niles JL, Collins AB, McCluskey RT, Brunmark C. Comparison of anti-GBM antibodies in sera with or without ANCA. J Am Soc Nephrol 1997;8: 376-385.

247. Kemmett D, Harrison DJ, Hunter JA. Antibodies to neutrophil cytoplasmic antigens: serologic marker for Sweet's syndrome. J Am Acad Dermatol 1991;24: 967-969.

248. Allmaras E, Nowack R, Andrassy K, Waldherr R, van der Woude F, Ritz E. Rapidly progressive IgA nephropathy with anti-myeloperoxidase antibodies benefits from immunosuppression. Clin Nephrol 1997;48: 269-273.

249. Ramirez SB, Rosen S, Niles J, Somers MJG. IgG antineutrophil cytoplasmic antibodies in IgA nephropathy: a clinical variant ? Am J Kidney Dis 1998;31: 341-344.

250. Mezzano S, Valderrama G, Olavarria F, Ardiles L, Arriagada A, Castillo A, Caorsi I. Antineutrophil-cytoplasmic-autoantibodies in poststreptococcal nephritis. Adv Exp Med Biol 1993;336: 449-453.

251. Ardiles LG, Valderrama G, Moya P, Mezzano SA. Incidence and studies on antigenic specificities of antineutrophil-cytoplasmic autoantibodies (ANCA) in poststreptococcal glomerulonephritis. Clin Nephrol 1997;47: 1-5.

252. Savige JA, Chang L, Smith CL, Duggan JC. Anti-neutrophil cytoplasmic antibodies (ANCA) in myelodysplasia and other haematologic disorders. Aust N Z Med 1994;24: 282-287.

253. Hull DR, McMillan SA, Rea IM, Boyd N, McMullin MF. Antineutrophil cytoplasmic antibodies in myelodysplasia. Ulster med J 1996;65: 55-57.

254. Pietravalle P, Monteleone G, Morano S, Cristina G, De Rossi MG, Sagratella E, Biancone L, Parrello T, Oliva A, Pallone F, Di Mario U. Antineutrophil cytoplasmic antibodies are present in long standing type 1 diabetics but not correlate with selective proteinuria. J Autoimmunity 1996;9: 113-117.

255. Hagen EC, van de Vijver-Reenalda H, De Keizer RJW, Kijlstra A, Van Es LA, Daha MR, van der Woude FJ. Uveitis and anti-neutrophil cytoplasmic antibodies. Clin Exp Immunol 1994;95: 56-59.

256. Gordon LK, Eggena M, Holland GN, Weisz JM, Braun J. pANCA antibodies in patients with anterior uveitis: identification of a marker antibody usually associated with ulcerative colitis. J Clin Immunol 1998;18: 264-271.

257. Shaarawy M, el-Mallah Y, el-Yamani AM. The prevalence of serum antineutrophil cytoplasmic autoantibodies in preeclampsia and eclampsia. J Soc Gynecol Investig 1997;4: 34-39.

258. Kaplan-Pavlovcic S, Vizjak A, Vene N, Ferluga D. Antineutrophil cytoplasmic autoantibodies in atheroembolic disease. Nephrol Dial Transplant 1998;13: 985-987.

259. Afeltra A, Paggi A, De Rosa FG, Manfredini P, Addessi MA, Amoroso A. Antineutrophil cytoplasmic antibodies in autoinmune thyroid disorders. Endocr Res 1998;24: 185-194.

260. Wichmann I, Sanchez-Roman J, Morales J, Castillo MJ, Ocaña C, Nuñez-Roldan A. Antimyeloperoxidase antibodies in individuals with occupational exposure to silica. Ann Rheum Dis 1996;55: 205-207.

261. Mirapeix E. Anticuerpos anticitoplasmáticos del neutrófilo (ANCA). Su contribución en la patogenia de las vasculitis. Med Clin (Barc) 1998;110: 778-780.

262. Falk RJ, Terrell RS, Charles LA, Jennette JC. Anti-neutrophil cytoplasmic antibodies induce neutrophils to degranulate and produce oxygen radicals *in vitro*. Proc Natl Acad Sci USA 1990;87: 4115-4119.

263. Keogan MT, Rifkin I, Lockwood CM, Brown DL. Antineutrophil cytoplasm autoantibodies increase neutrophil adhesion to cultured human endothelium. Adv Exp Med Biol 1993;336: 115-119.

264. Ewert BH, Jennette JC, Falk RJ. Anti-myeloperoxidase antibodies stimulate neutrophils to damage human endothelial cells. Kidney Int 1992;41: 375-383.

265. Savage CO, Pottinger BE, Gaskin G, Pusey CD, Pearson JD. Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity towards cultured endothelial cells. Am J Pathol 1992;141: 335-342.

266. Vargunam M, Adu D, Taylor CM, Michael J, Richards N, Neuberger J, Thompson RA. Endothelium myeloperoxidase-antimyeloperoxidase interaction in vasculitis. Nephrol Dial Transplant 1995;7: 1077-1081.

267. Ewert BH, Jennette JC, Falk RJ. The pathogenic role of antineutrophil cytoplasmic autoantibodies. Am J Kid Dis 1991;18: 188-195.

268. Casselman BL, Kilgore KS, Miller BF, Warren JS. Antibodies to neutrophil cytoplasmic antigens induce monocyte chemoattractant protein-1 secretion from human monocytes. J Lab Clin Med 1995;126: 495-502.

269. Ralston DR, Marsh CB, Lowe MP, Wewers MD. Antineutrophil cytoplasmic antibodies induce monocyte IL-8 release. Role of surface proteinase-3, α1-antitrypsin, and fcγ receptors. J Clin Invest 1997;100: 1416-1424.

270. Savage COS, Gaskin G, Pusey CD. Anti-neutrophil cytoplasm antibodies (ANCA) can recognize vascular endothelial cell-bound ANCA-associated autoantigens. J Exp Nephrol 1993;1: 190-195.

271. Mayet WJ, Csernok E, Szymkowiak C, Gross WL, Meyer Zum Buschenfelde KH. Human endothelial cells express proteinase 3, the target antigen of anticytoplasmic antibodies in Wegener´s granulomatosis. Blood 1993;82: 1221-1229.

272. Ballieux BEPB, Hiemstra PS, Klar-Mohamad N, Hagen EC, Van Es LA, van der Woude FJ, Daha MR. Detachment and cytolysis of human endothelial cells by proteinase 3. Eur J Immunol 1994;24: 3211-3215.

273. Mayet WJ, Schwarting A, Meyer Zum Buschenfelde KH. Cytotoxic effects of antibodies to proteinase 3 (C-ANCA) on human endothelial cells. Clin Exp Immunol 1993;97: 458-465.

274. Petersen J, Rasmussen N, Szpirt W, Hermann E, Mayet W. T lymphocyte proliferation to neutrophil cytoplasmic antigen(s) in Wegener´s granulomatosis (WG). Am J Kidney Dis 1991;18: 205-209.

275. Brouwer E, Stegeman CA, Huitema MG, Limburg PC, Kallenberg CGM. T-cell reactivity to proteinase 3 and myeloperoxidase in patients with Wegener´s granulomatosis. Clin Exp Immunol 1994;98: 448-453.

276. Griffith ME, Coulthart A, Pusey CD. T cell responses to myeloperoxidase (MPO) and proteinase 3 (Pr3) in patients with systemic vasculitis. Clin Exp Immunol 1996;103: 253-258.

277. King WJ, Brooks CJ, Holder R, Hughes P, Adu D, Savage COS. T lymphocyte responses to anti-neutrophil cytoplasmic autoantibody (ANCA) antigens are present in patients with ANCA-associated systemic vasculitis and persist during disease remission. Clin Exp Immunol 1998;112: 539-546.

278. Van der Wiel BA, Dolman KM, van der Meer-Gerritisen CH, Hack CE, von dem Borne AEGK, Goldschmeding R. Interference of Wegener´s granulomatosis autoantibodies with neutrophil proteinase 3 activity. Clin Exp Immunol 1992;90: 409-414.

279. Dolman KM, Stegeman CA, van der Wiel BA, Hack CE, von dem Borne AEGK, Kallenberg CGM, Goldschmeding R. Relevance of classic anti-neutrophil cytoplasmic autoantibody (c-ANCA)-mediated inhibition of proteinase 3-alpha-1-antitrypsin complexation to disease activity in Wegener´s granulomatosis. Clin Exp Immunol 1993;93: 405-410.

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280. Daouk GH, Palsson R, Arnaout MA. Inhibition of proteinase 3 by ANCA and its correlation with disease activity in Wegener´s granulomatosis. Kidney Int 1995;47: 1528-1536.

281. Segelmark M, Elzouki AN, Wieslander J, Eriksson S. The PiZ gene of α_1-antitrypsin as a determinant of outcome in PR3-ANCA-positive vasculitis. Kidney Int 1995;48: 844-850.

282. Griffith ME, Lovegrove JU, Gaskin G, Whitehouse DB, Pusey CD. C-antineutrophil cytoplasmic antibody positivity in vasculitis patients is associated with Z allele of alpha-1-antitrypsin, and P-antineutrophil cytoplasmic antibody with the S allele. Nephrol Dial Transplant 1996;11: 438-443.

283. Heeringa P, Brouwer E, Cohen Tervaert JW, Weening JJ, Kallenberg CGM. Animal models of anti-neutrophil cytoplasmic antibody associated vasculitis. Kidney Int 1998;53: 253-263.

284. Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology 1998;115: 182-205.

285. Duerr RH, Targan SR, Landers CJ, Sutherland LR, Shanahan F. Anti-neutrophil cytoplasmic antibodies in ulcerative colitis: comparison with other colitides/diarrheal illnesses. Gastroenterology 1991;100: 1590-1596.

286. Cambridge G, Rampton DS, Stevens TRJ, McCarthy DA, Kamm M, Leaker B. Anti-neutrophil antibodies in inflammatory bowel disease: prevalence and diagnostic role. Gut 1992;33: 668-674.

287. Romas E, Paspaliaris B, d'Apice AJF, Elliott PR. Autoantibodies to neutrophil cytoplasmic (ANCA) and endothelial cell surface antigens (AECA) in chronic inflammatory bowel disease. Aust NZ J Med 1992;22: 652-659.

288. Colombel J-F, Reumaux D, Duthilleul P, Noël LH, Gower-Rousseau C, Paris JC, Cortot A. Antineutrophil cytoplasmic autoantibodies in inflammatory bowel diseases. Gastroenterol Clin Biol 1992;16: 656-660.

289. Dalekos GN, Manoussakis MN, Goussia AC, Tsianos EV, Moutsopoulos HM. Soluble interleukin-2 receptors, antineutrophil cytoplasmic antibodies, and other autoantibodies in patients with ulcerative colitis. Gut 1993;34: 658-664.

290. Oudkerk Pool M, Ellerbroek PM, Ridwan BU, Goldschmeding R, von Blomberg BME, Peña AS, Dolman KM, Bril H, Dekker W, Nauta JJ, Gans ROB, Breed H, Meuwissen SGM. Serum antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease are mainly associated with ulcerative colitis. A correlation study between perinuclear antineutrophil cytoplasmic autoantibodies and clinical parameters, medical, and surgical treatment. Gut 1993;34: 46-50.

291. Deusch K, Oberstadt K, Schaedel W, Weber M, Classen M. P-ANCA as a diagnostic marker in ulcerative colitis. Adv Exp Med Biol 1993;336: 527-531.

292. Mulder AHL, Broekroelofs J, Horst G, Limburg PC, Nelis GF, Kallenberg CGM. Antineutrophil antibodies in inflammatory bowel disease recognize different antigens. Adv Exp Med Biol 1993;336: 519-522.

293. Rump JA, Wörner I, Roth M, Schölmerich J, Hänsch M, Peter HH. P-ANCA of undefined specificity in ulcerative colitis: correlation to disease activity and therapy. Adv Exp Med Biol 1993;336: 507-513.

294. Sung JY, Chan KL, Hsu R, Liew CT, Lawton JWM. Ulcerative colitis and cytoplasmic antibodies in Hong Kong Chinese. Am J Gastroenterol 1993;88: 864-869.

295. Broekroelofs J, Mulder AHL, Nelis GF, Westerveld BD, Cohen Tervaert JW, Kallenberg CGM. Anti-neutrophil cytoplasmic antibodies (ANCA) in sera from patients with inflammatory bowel disease (IBD). Dig Dis Sci 1994;39: 545-549.

296. Oudkerk Pool M, Roca M, Reumaux D, Bouma G, Peña AS, Colombel J-F, von Blomberg BME, Meuwissen SGM. The value of pANCA as a serological marker for ulcerative colitis in different european regions. Eur J Gastroenterol Hepatol 1994;6: 399-403.

297. Patel RT, Pall AA, Stokes R, Birch D, Hail C, Adu D, Keighley MRB. Autoantibody prevalence and association in inflammatory bowel disease. Eur J Gastroenterol Hepatol 1994;6: 705-709.

298. Vecchi M, Bianchi MB, Sinico RA, Radice A, Meucci G, Torgano G, Omodei P, Forzenigo L, Landoni M, Arrigoni M, Pozzi C, de Franchis R. Antibodies to neutrophil cytoplasm in Italian patients with ulcerative colitis: sensitivity, specificity and recognition of putative antigens. Digestion 1994;55: 34-39.

299. Lamproye A, Belaiche J, Louis E, Salmon J, Mahieu P. Anticorps anticytoplasme des polynucléaires neutrophiles (ANCA) dans les maladies inflammatoires du tube digestif. Acta Gastroenterol Belg 1994;57: 171-176.

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300. Mulder AHL, Broekroelofs J, Horst G, Limburg PC, Nelis GF. Anti-neutrophil cytoplasmic antibodies (ANCA) in inflammatory bowel disease: characterization and clinical correlates. Clin Exp Immunol 1994;95: 490-497.

301. Sung JY, Chan FKL, Lawton J, Leung JCK, Liew CT, Leung NWY, Hsu R, Lai KN. Anti-neutrophil cytoplasmic antibodies (ANCA) and inflammatory bowel diseases in Chinese. Dig Dis Sci 1994;39: 886-892.

302. Muñoz C, Gómez R, Alcántara M, Martinez JL, Artaza T, Flores V, Tordera FJ, Martinez J. Serum anti-neutrophil cytoplasmic antibodies in ulcerous colitis: clinical utility (resumen). Gut 1994:35 (supl 4): A30.

303. Tural C. Anticuerpos anti-citoplasma de los neutrófilos y enfermedad inflamatoria intestinal (Tesis doctoral). Barcelona: Universitat Autònoma, 1994.

304. Castellino F, Rosina F, Bansi DS, Bauducci M, Touscoz GA, Giorda L, Borghesio E, Bessone MP, Astegiano M, Musso A, Maina AM, Mattalia A, Bonino F, Fleming K, Chapman R, Verme G, Pera A. Anti-neutrophil cytoplasmic antibodies in inflammatory bowel disease: do they recognize different subsets of a heterogeneous disease ? Eur J Gastroenterol Hepatol 1995;7: 859-864.

305. Kossa K, Coulthart A, Ives CT, Pusey CD, Hodgson HJF. Antigen specificity of circulating anti-neutrophil cytoplasmic antibodies in inflammatory bowel disease. Eur J Gastroenterol Hepatol 1995;7: 783-789.

306. Hertervig E, Wieslander J, Johansson C, Wiik A, Nilsson A. Anti-neutrophil cytoplasmic antibodies in chronic inflammatory bowel disease. Scand J Gastroenterol 1995;30: 693-698.

307. Schumacher G, Kollberg B, Sandstedt B, Ljungh A, Nässberger L. Circulating granulocyte antibodies in first attacks of colitis. Scand J Gastroenterol 1995;30: 157-163.

308. Oudkerk Pool M, Bouma G, Meuwissen SGM, von Blomberg BME, van de Merwe JP, Devillé WLJM, Fonk JCM, Peña AS. Serological markers to differentiate between ulcerative colitis and Crohn´s disease. J Clin Pathol 1995;48: 346-350.

309. Kim WH, Choi PM, Landers CJ, Targan SR. Role of antineutrophil cytoplasmic antibodies in an ethnically distinct population: Korean patients with ulcerative colitis. Am J Gastroenterol 1995,90: 1953-1958.

310. Eliakim R, Naparstek Y, Stalnikowicz R, Ulmansky R, Rachmilewitz D. Antineutrophil cytoplasmic antibodies in IBD are the same in Ashkenazi and Sepharadi jews in Israel. J Clin Gastroenterol 1995;20: 82-84.

311. Bansi DS, Chapman RW, Fleming KA. Prevalence and diagnostic role of antineutrophil cytoplasmic antibodies in inflammatory bowel disease. Eur J Gastroenterol Hepatol 1996,8: 881-885.

312. Andreani ML, Frasca G, Brusco G, Borgnino L, Biagi F, Gasbarrini G, Corazza GR. Antineutrophil cytoplasmic antibodies in inflammatory bowel disease: diagnostic tool or research procedure ? Ann Ital Med Int 1996;11: 254-257.

313. Sugi K, Saitoh O, Matsuse R, Uchida K, Nakagawa K, Kozima K, Yoshizumi M, Maemura K, Hirata I, Katsu K. Anti-neutrophil cytoplasmic antibodies (ANCA) in Japanese patients with inflammatory bowel disease (IBD): prevalence and recognition of putative antigens. Gastroenterology 1996;110: A1021.

314. Vasiliauskas EA, Plevy SE, Landers CJ, Binder SW, Ferguson DM, Yang H, Rotter JI, Vidrich A, Targan SR. Perinuclear antineutrophil cytoplasmic antibodies in patients with Crohn's disease define a clinical subgroup. Gastroenterology 1996;110:1810-1819.

315. Habeeb MA, Rajalingam R, Dhar A, Kumar A, Sharma MP, Mehra NK. HLA association and occurrence of autoantibodies in Asian-Indian patients with ulcerative colitis. Am J Gastroenterol 1997,92: 772-776.

316. Freeman H, Roeck B, Devine D, Carter C. Prospective evaluation of neutrophil autoantibodies evaluation in 500 consecutive patients with inflammatory bowel disease. Can J Gastroenterol 1997;11: 203-207.

317. Freeman H. Inflammatory bowel disease with cytoplasmic-staining antineutrophil cytoplasmic antibody and extensive colitis. Can J Gastroenterol 1998;12: 279-282.

318. García Herola A, Nos P, Hoyos M, Hinojosa J, Molés JR, Pascual S, Bustamante M, Sánchez Cuenca JM, Carmona E, Ponce J, Berenguer J. Significado de la determinación de anticuerpos frente al citoplasma de los neutrófilos (ANCA) en la colitis ulcerosa y en la enfermedad de Crohn. Gastroenterol Hepatol 1998,21: 169-173.

319. Halbwachs-Mecarelli L, Nusbaum P, Noël LH, Reumaux D, Erlinger S. Antineutrophil cytoplasmic antibodies (ANCA) directed against cathepsin G in ulcerative colitis, Crohn´s disease and primary sclerosing cholangitis. Clin Exp Immunol 1992,90: 79-84.

320. Ellerbroek PM, Oudkerk Pool M, Ridwan BU, Dolman KM, von Blomberg BME, von dem Borne AEGKr, Meuwissen SGM, Goldschmeding R. Neutrophil cytoplasmic antibodies (p-ANCA) in ulcerative colitis. J Clin Pathol 1994;47: 257-262.

321. Sobajima J, Ozaki S, Okazaki T, Osakada F, Sumita S, Mori K, Nakao K. Anti-neutrophil cytoplasmic antibodies (ANCA) in ulcerative colitis: anti-cathepsin G and a novel antibody correlate with a refractory type. Clin Exp Immunol 1996;105: 120-124.

322. Stoffel MP, Csernok E, Herzberg C, Johnston T, Carroll F, Gross W. Anti-neutrophil cytoplasmic antibodies (ANCA) directed against bactericidal/permeability increasing protein (BPI): a new seromarker for inflammatory bowel disease and associated disorders. Clin Exp Immunol 1996;104: 54-59.

323. Walmsley RS, Zhao MH, Hamilton MI, Brownlee A, Chapman P, Pounder RE, Wakefield AJ, Lokwood CM. Antineutrophil cytoplasm autoantibodies against bactericidal/permeability increasing protein in inflammatory bowel disease. Gut 1997;40: 105-109.

324. Roozendaal C, Zhao MH, Horst G, Lockwood CM, Kleibeuker JH, Limburg PC. Catalase and α-enolase: two novel granulocyte autoantigens in inflammatory bowel disease. Clin Exp Immunol 1998;112: 10-16.

325. Yang P, Bohr J, Tysk C, Danielson D, Järnerot G. Antineutrophil cytoplasmic antibodies in inflammatory bowel disease and collagenous colitis: no association with lactoferrin, β-glucuronidase, myeloperoxidase, or proteinase 3. Inflamm Bowel Dis 1996;2: 173-177.

326. Billing P, Tahir S, Calfin B, Gagne G, Cobb L, Targan S, Vidrich A. Nuclear localization of the antigen detected by ulcerative colitis-asociated perinuclear antineutrophil cytoplasmic antibodies. Am J Pathol 1995;147: 979-987.

327. Vidrich A, Lee J, James E, Cobb L, Targan S. Segregation of pANCA antigenic recognition by DNase treatment of neutrophils: ulcerative colitis, type 1 autoimmune hepatitis, and primary sclerosing cholangitis. J Clin Immunol 1995;15: 293-299.

328. Terjung B, Herzog V, Worman HJ, Gestmann I, Bauer C, Sauerbruch T, Spengler U. Atypical antineutrophil cytoplasmic antibodies with perinuclear fluorescence in chronic inflammatory bowel diseases and hepatobiliary disorders colocalize with nuclear lamina proteins. Hepatology 1998;28: 332-340.

329. Cameron BJ, Bansi DS, Ali R, Chapman RW, Fleming KA. Characterization of the antigen for the anti-neutrophil cytoplasmic antibody specific for primary sclerosing cholangitis (PSC)/ulcerative colitis (UC). Gastroenterology 1998;114: A1218.

330. Mallolas J, Esteve M, Rius E, Cabré E, Gassull MA. Anti-neutrophil antibodies (ANCA) associated to ulcerative colitis interact with a nuclear antigen during the process of apoptosis. Gastroenterology 1998;114: A1031.

331. Eggena M, Targan SR, Iwanczyk L, Vidrich A, Gordon LK, Braun J. Phage display cloning and characterization of an immunogenetic marker (perinuclear anti-neutrophil cytoplasmic antibody) in ulcerative colitis. J Immunol 1996;156: 4005-4011.

332. Cohavy O, Eggena M, Parseghian M, Hamkalo B, Targan SR, Gordon LK, Braun J. Histone H-1, a candidate p-ANCA antigen in ulcerative colitis. Gastroenterology 1997;112: A951.

333. Müller-Ladner U, Gross V, Andus T, Gschwendtner H, Roth M, Caesar I, Schölmerich J, Lang B. Distinct patterns of immunoglobulin classes and IgG subclasses of autoantibodies in patients with inflammatory bowel disease. Eur J Gastroenterol Hepatol 1996,8: 579-584.

334. Seibold F, Weber P, Schöning A, Mörk H, Goppel S, Scheurlen M. Neutrophil antibodies (pANCA) in chronic liver disease and inflammatory bowel disease: do they react with different antigens ? Eur J Gastroenterol Hepatol 1996;8: 1095-1100.

335. Gigase P, de Clerck LS, van Cotthem KA, Bridts CH, Stevens WJ, van Outryve M, Pelckmans PA. Anti-neutrophil cytoplasmic antibodies in inflammatory bowel disease with special attention for IgA-class antibodies. Dig Dis Sci 1997;42: 2171-2174.

336. Esteve M, Mallolas J, Klaassen J, Abad-Lacruz A, González-Huix F, Cabré E, Fernández-Bañares F, Menacho M, Condom E, Martí-Ragué J, Gassull MA. Factors related to the presence of IgA class antineutrophil cytoplasmic antibodies in ulcerative colitis. Am J Gastroenterol 1998;93: 615-618.

337. Proujansky R, Fawcett PT, Gibney KM, Treem WR, Hyams JS. Examination of anti-neutrophil cytoplasmic antibodies in childhood inflammatory bowel disease. J Pediatr Gastroenterol Nutr 1993;17: 193-197.

338. Winter HS, Landers CJ, Winkelstein A, Vidrich A, Targan SR. Anti-neutrophil cytoplasmic antibodies in children with ulcerative colitis. J Pediatr 1994;125: 707-711.

339. Kaneko K, Suzuki Y, Shimizu T, Yamashiro Y, Yabuta K, Lifschitz CH. Anti-neutrophil cytoplasmic antibodies in Japanese children with ulcerative colitis. J Paediatr Child Health 1995;31: 336-338.

340. Olives JP, Breton A, Hugot JP, Oksman F, Johannet C, Ghisolfi J, Navarro J, Cezard JP. Antineutrophil cytoplasmic antibodies in children with inflammatory bowel disease: prevalence and diagnostic value. J Pediatr Gastroenterol Nutr 1997;25: 142-148.

341. Ruemmele FM, Targan SR, Levy G, Dubinsky M, Braun J, Seidman EG. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. Gastroenterology 1998;115: 822-829.

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342. Reumaux D, Colombel JF, Duclos B, Chaussade S, Belaiche J, Jacquot S, Dupas JL, Molis C, Duthilleul P. Antineutrophil cytoplasmic auto-antibodies in sera from patients with ulcerative colitis after proctocolectomy with ileo-anal anastomosis. Adv Exp Med Biol 1993;336: 523-525.

343. Patel RT, Stokes R, Birch D, Ibbotson J, Keighley MRB. Influence of total colectomy on serum antineutrophil cytoplasmic antibodies in inflammatory bowel disease. B J Surg 1994;81: 724-726.

344. Vecchi M, Gionchetti P, Bianchi MB, Belluzzi A, Meucci G, Campieri M, de Franchis R. p-ANCA and development of pouchitis in ulcerative colitis patients after proctocolectomy and ileal pouch anastomosis. Lancet 1994;344: 886-887.

345. Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Antineutrophil cytoplasmic antibody correlates with chronic pouchitis after ileal pouch-anal anastomosis. Am J Gastroenterol 1995; 90:740-747.

346. Aisenberg J, Wagreich J, Shim J, Almer S, Peen E, Heimann T, Gelernt IM, Greenstein A, Rubin P, Harpaz N, Mayer L, Sachar D. Perinuclear anti-neutrophil cytoplasmic antibody and refractory pouchitis. Dig Dis Sci 1995;40: 1866-1872.

347. Zins BJ, Sandborn WJ, Penna CR, Landers CJ, Targan SR, Tremaine WJ, Wiesner RH, Dozois RR. Pouchitis disease course after orthotopic liver transplantation in patiens with primary sclerosing cholangitis and ileal pouch-anal anastomosis. Am J Gastroenterol 1995;90: 2177-2181.

348. Yang P, Öresland T, Järnerot G, Hultén L, Danielsson D. Perinuclear antineutrophil cytoplasmic antibody in pouchitis after proctocolectomy with ileal pouch-anal anastomosis for ulcerative colitis. Scand J Gastroenterol 1996;31: 594-598.

349. Freeman HJ, Roeck B, Devine DV, Carter CJ. Atypical perinuclear antineutrophil cytoplasmic antibodies after colectomy in inflammatory bowel disease. Can J Gastroenterol 1997;11: 305-310.

350. Kaditis AG, Perrault J, Sandborn WJ, Landers CJ, Zinsmeister AR, Targan SR. Antineutrophil cytoplasmic antibodies subtypes in children and adolescents after ileal pouch-anal anastomosis for ulcerative colitis. J Pediatr Gastroenterol Nutr 1998;26: 386-392.

351. Yasuda N, Thomas P, Ellis H, Herbst F, Nicholls J, Ciclitira P. Perinuclear anti-neutrophil cytoplasmic antibodies in ulcerative colitis after restorative proctocolectomy do not correlate with the presence of pouchitis. Scand J Gastroenterol 1998;33: 509-513.

352. Esteve M, Mallolas J, Klaassen J, Abad-Lacruz A, González-Huix F, Cabré E, Fernández-Bañares F, Bertrán X, Condom E, Martí-Ragué J, Gassull MA. Antineutrophil cytoplasmic antibodies in sera from colectomised ulcerative colitis patients and its relation to the presence of pouchitis. Gut 1996;38: 894-898.

353. Aitola P, Miettinen A, Mattila A, Matikainen M, Soppi E. Effect of proctocolectomy on serum antineutrophil cytoplasmic antibodies in patients with chronic ulcerative colitis. J Clin Pathol 1995;48: 645-647.

354. Gionchetti P, Vecchi M, Rizzello F, Ferretti M, Calabresi C, Venturi A, Bianchi MB, Brignola C, Sinico RA, De Franchis R, Miglioli M, Campieri M. Lack of effect of antineutrophil cytoplasmic antibodies associated with ulcerative colitis on superoxide anion production from neutrophils. Gut 1997;40: 102-104.

355. Mizoguchi E, Mizoguchi A, Chiba C, Niles JL, Bhan AK. Antineutrophil cytoplasmic antibodies in T-cell receptor α-deficient mice with chronic colitis. Gastroenterology 1997;113: 1828-1835.

356. Seibold F, Brandwein S, Simpson S, Terhorst C, Elson CO. pANCA represents a cross-reactivity to enteric bacterial. J Clin Immunol 1998;18: 153-160.

357. Targan SR, Landers CJ, Cobb L, MacDermott RP, Vidrich A. Perinuclear anti-neutrophil cytoplasmic antibodies are spontaneously produced by mucosal B cells of ulcerative colitis patients. J Immunol 1995;155: 3262-3267.

358. Shanahan F. Neutrophil autoantibodies in inflammatory bowel disease: Are they important ? Gastroenterology 1994;107: 586-589.

359. Freeman H. Atypical perinuclear antineutrophil cytoplasmic antibodies in patients with Crohn´s disease. Can J Gastroenterol 1997;11: 689-693.

360. Peeters M, Rutgeerts P, Cheyns K, Baert F, D´Haens G, Vlietinck R. Presence of pANCA in Crohn´s patients with "UC-like" clinical phenotype is related to disease activity. Gastroenterology 1997;112: A1060.

361. Jamar-Leclerc Ñ, Reumaux D, Duthilleul P, Colombel JF. Do pANCA define a clinical subgroup in patients with Crohn´s disease ? Gastroenterology 1997;112: 316-317.

362. Shanahan F, Duerr RH, Rotter JI, Yang H, Sutherland LR, McElree C, Landers CJ, Targan SR. Neutrophil autoantibodies in ulcerative colitis: familial aggregation and genetic heterogeneity. Gastroenterology 1992;103: 456-461.

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363. Seibold F, Slametschka D, Gregor M, Weber P. Neutrophil autoantibodies: A genetic marker in primary sclerosing cholangitis and ulcerative colitis. Gastroenterology 1994;107: 532-536.

364. Reumaux D, Colombel J-F, Delecourt L, Noël L-H, Cortot A, Duthilleul P. Anti-neutrophil cytoplasmic auto-antibodies (ANCA) in patients with ulcerative colitis (UC): influence of disease activity and familial study. Adv Exp Med Biol 1993;336: 515-518.

365. Monteleone G, Marasco R, Parrello T, De Medici A, Giglio A, Doldo P, Luzza F, Pallone F. Low prevalence of p-ANCA in unaffected relatives of patients with ulcerative colitis from a mediterranean area. Inflamm Bowel Dis 1996;2: 11-15.

366. Lee JCW, Lennard-Jones JE, Cambridge G. Antineutrophil antibodies in familial inflammatory bowel disease. Gastroenterology 1995;108: 428-433.

367. Bansi DS, Lo S, Chapman RW, Fleming KA. Absence of antineutrophil cytoplasmic antibodies in relatives of UK patients with primary sclerosing cholangitis and ulcerative colitis. Eur J Gastroenterol Hepatol 1996;8;111-116.

368. Yang H, Rotter JI, Toyoda H, Landers C, Tyan D, McElree CK, Targan SR. Ulcerative colitis: a genetically heterogeneous disorder defined by genetic (HLA class II) and subclinical (antineutrophil cytoplasmic antibodies) markers. J Clin Invest 1993;92: 1080-1084.

369. Yang H, Vora DK, Targan SR, Toyoda H, Beaudet AL, Rotter JI. Intercellular adhesion molecule 1 gene associations with immunologic subsets of inflammatory bowel disease. Gastroenterology 1995;109: 440-448.

370. Perri F, Annese V, Piepoli A, Napolitano G, Lombardi G, Ciavarella G, Di Giorgio G, Andriulli A. HLA antigens and pANCA define ulcerative colitis as a genetically heterogeneous disorder. Ital J Gastroenterol Hepatol 1998;30: 56-61.

371. Heresbach D, Alizadeh M, Reumaux D, Colombel JF, Delamaire M, Danze PM, Gosselin M, Genetet B, Bretagne JF, Semana G. Are HLA-DR or TAP genes genetic markers of severity in ulcerative colitis ? J Autoimmun 1996;9: 777-784.

372. Duerr RH, Neigut DA. Molecularly defined HLA-DR2 alleles in ulcerative colitis and an antineutrophil cytoplasmic antibody-positive subgroup. Gastroenterology 1995;108: 423-427.

373. Bioque G, Bouma G, Crusius JBA, Koutroubakis I, Kostense PJ, Meuwissen SGM, Peña AS. Evidence for genetic heterogeneity in IBD: The interleukin-1 receptor antagonist in the predisposition to suffer from ulcerative colitis. Eur J Gastroenterol Hepatol 1996;8: 105-110.

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374. Satsangi J, Landers CJ, Welsh KI, Koss K, Targan S, Jewell DP. The presence of anti-neutrophil antibodies reflects clinical and genetic heterogeneity within inflammatory bowel disease. Inflamm Bowel Dis 1998;4: 18-26.

375. Yang H, Rotter JI. Genetics of inflammatory bowel disease. In: Targan S, Shanahan F, eds. Inflammatory bowel disease: from bench to bedside. Baltimore: Williams & Wilkins, 1994: 32-64.

376. Russel MGVM, Stockbrügger RW. Epidemiology of inflammatory bowel disease: an update. Scand J Gastroenterol 1996;31:417-427.

377. Sandler RS. Epidemiology of inflammatory bowel disease. In: Targan S, Shanahan F, eds. Inflammatory bowel disease: from bench to bedside. Baltimore: Williams & Wilkins, 1994: 5-30.

378. Rotter JI, Yang H, Shohat T. Genetic complexities of inflammatory bowel disease and its distribution among the Jewish people. In: Bonne-Tamir B, Adam A, eds. Genetic diversity among Jews: diseases and markers at the DNA level. New York: Oxford University Press 1992; 395-411.

379. Orholm M, Munkholm P, Langholz E, Nielsen OH, Sorensen TIA, Binder V. Familial occurrence of inflammatory bowel disease. N Engl J Med 1991;324: 84-88.

380. Yang H, McElree C, Roth M-P, Shanahan F, Targan SR, Rotter JI. Familial empirical risks for inflammatory bowel disease: differences between Jews and non-Jews. Gut 1993;34: 517-524.

381. Tysk C, Lindberg E, Jarnerot G, Floderus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. Gut 1988;29: 990- 996.

382. Thompson NP, Driscoll R, Pounder RE, Wakefield AJ: Genetics versus environment in inflammatory bowel disease: results of a British twin study. BMJ 1996;312: 95-96.

383. Parkes M, Satsangi J, Jewell D. Mapping susceptibility loci in inflammatory bowel disease: why and how ? Mol Med Today 1997;3: 546-553.

384. Peña AS, Crusius JBA. Genetics of inflammatory bowel disease: implications for the future. World J Sug 1998;22: 390-393.

385. Satsangi J, Parkes M, Jewell DP, Bell JI. Genetics of inflammatory bowel disease. Clin Sci (Colch) 1998;94: 473-478.

UNIVERSITAT ROVIRA I VIRGILI
MARCADORES INMUNOGENETICOS EN LA ENFERMEDAD INFLAMATORIA INTESTINAL: ESTUDIO SOBRE LOS ANTICUERPOS ANTICITOPLASMA DE NEUTROFILO Y POLIMORFISMOS GENETICOS DE CITOCINAS (IL-1RA, TNFα Y TNFβ) EN UNA POBLACIÓN DE PACIENTES ESPAÑOLES CON COLITIS ULCEROSA Y ENFERMEDAD DE CROHN
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386. Satsangi J, Grootscholten C, Holt H, Jewell DP. Clinical patterns of familial inflammatory bowel disease. Gut 1996;38: 738-741.

387. Bayless TM, Tokayer AZ, Polito JM II, Quaskey SA, Mellits ED, Harris ML. Crohn´s disease: concordance for site and clinical type in affected family members-potential hereditary influences. Gastroenterology 1996;111: 573-579.

388. Polito JM II, Childs B, Mellits ED, Tokayer AZ, Harris ML, Bayless TM. Crohn´s disease: influence of age at diagnosis on site and clinical type of disease. Gastroenterology 1996;111: 580-586.

389. Peeters M, Nevens H, Baert F, Hiele M, De Meyer AM, Vlietinck R, Rutgeerts P. Familial aggregation in Crohn´s disease: increased age-adjusted risk and concordance in clinical characteristics. Gastroenterology 1996;111: 597-603.

390. Colombel JF, Grandbastien B, Gower-Rousseau C, Plegat S, Evrard JP, Dupas JL, Gendre JP, Modigliani R, Bélaïche J, Hostein J, Hugot JP, van Kruiningen H, Cortot A. Clinical characteristics of Crohn´s disease in 72 families. Gastroenterology 1996;111: 604-607.

391. Cottone M, Brignola C, Rosselli M, Oliva L, Belloli C, Cipolla C, Orlando A, De Simone G, Aiala MR, Di Mitri R, Gatto G, Buccellato A. Relantionship between site of disease and familial occurrence in Crohn´s disease. Dig Dis Sci 1997;42: 129-132.

392. Heresbach D, Alizadeh M, Bretagne JF, Dabadie A, Colombel JF, Pagenault M, Heresbach-Le Berre N, Genetet B, Gosselin M, Semana G. TAP gene transporter polymorphism in inflammatory bowel diseases. Scand J Gastroenterol 1997;32: 1022-1027.

393. Bouma G, Poen AC, Garcia-Gonzalez MA, Schreuder GMT, Felt-Bersma RJF, Meuwissen SGM, Peña AS. HLA-DRB1*03, but not the TNFA –308 promoter gene polymorphism, confers protection against fistulising Crohn´s disease. Immunogenetics 1998;47; 451-455.

394. Masuda H, Nakamura Y, Tanaka T, Hayakawa S. Distinct relationship between HLA-DR genes and intractability of ulcerative colitis. Am J Gastroenterol 1994;89: 1957-1962.

395. Futami S, Aoyama N, Honsako Y, Tamura T, Morimoto S, Nakashima T, Ohmoto A, Okano H, Miyamoto M, Inaba H, Naruse T, Nose Y, Kasuga M. HLA-DRB1*1502 allele, subtype of DR15 is associated with susceptibility to ulcerative colitis and its progression. Dig Dis Sci 1995;40: 814-818

UNIVERSITAT ROVIRA I VIRGILI
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396. Bouma G, Oudkerk Pool M, Crusius JBA, Schreuder GMTH, Hellemans HPR, Meijer BUGA, Kostense PJ, Giphart MJ, Meuwissen SGM, Peña AS. Evidence for genetic heterogeneity in inflammatory bowel disease (IBD); HLA genes in the predisposition to suffer from ulcerative colitis (UC) and Crohn´s disease (CD). Clin Exp Inmunol 1997;109: 175-179.

397. Satsangi J, Welsh KI, Bunce M, Julier C, Farrant JM, Bell JI, Jewell DP. Contribution of genes of the major histocompatibility complex to susceptibility and disease phenotype in inflammatory bowel disease. Lancet 1996;347; 1212-1217.

398. Roussomoustakaki M, Satsangi J, Welsh K, Louis E, Fanning G, Targan S, Landers C, Jewell DP. Genetic markers may predict disease behavior in patients with ulcerative colitis. Gastroenterology 1997;112: 1845-1853

399. Sartor RB. The pathogenesis of chronic intestinal inflammation: clues from animal models. Research and Clinical Forums 1996;18: 17-24.

400. Lander ES, Schork NJ. Genetic dissection of complex traits. Science 1994;265: 2037-2048.

401. Hugot JP, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugerie L, Naom I, Dupas JL, Van Gossum AV, Groupe d'Etude Thérapeutique des Affections Inflammatoires Digestives, Orholm M, Bonaiti-Pellie C, Weissenbach J, Mathew CG, Lennard-Jones JE, Cortot A, Colombel JF, Thomas G. Mapping of a susceptibility locus for Crohn's disease on chromosome 16; Nature 1996;379: 821-823.

402. Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP. Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. Nat Genet 1996;14: 199-202.

403. Ohmen JD, Yang HY, Yamamoto KK, Zhao HY, Ma Y, Bentley LG, Huang Z, Gerwehr S, Pressman S, McElree C, Targan S, Rotter JI, Fischel-Ghodsian N. Susceptibility locus for inflammatory bowel disease on chromosome 16 has a role in Crohn's disease, but not in ulcerative colitis. Hum Mol Genet 1996;10: 1679-1683.

404. Parkes M, Satsangi J, Lathrop GM, Bell JI, Jewell DP. Susceptibility loci in inflammatory bowel disease. Lancet 1996;348: 1588.

405. Brant SR, Fu Y, Fields CT, Baltazar R, Ravenhill G, Pickles MR, Rohal PM, Mann J, Kirschner BS, Jabs EW, Bayless TM, Hanauer SB, Cho JH. American families with Crohn´s disease have strong evidence for linkage to chromosome 16 but not chromosome 12. Gastroenterology 1998;115: 1056-1061.

406. Duerr RH, Barmada MM, Zhang L, Davis S, Preston RA, Chensny LJ, Brown JL, Ehrlich GD, Weeks DE, Aston CE. Linkage and association between inflammatory bowel disease and a locus on chromosome 12. Am J Hum Genet 1998;63: 95-100.

407. Curran ME, Lau KF, Hampe J, Schreiber S, Bridger S, Macpherson AJ, Cardon LR, Sakul H, Harris TJ, Stokkers P, Van Deventer SJ, Mirza M, Raedler A, Kruis W, Meckler U, Theuer D, Herrmann T, Gionchetti P, Lee J, Mathew C, Lennard-Jones J. Genetic analysis of inflammatory bowel disease in a large european cohort supports linkage to chromosome 12 and 16. Gastroenterology 1998;115: 1066-1071.

408. Mirza MM, Lee J, Teare D, Hugot JP, Laurent-Puig P, Colombel JF, Hodgson SV, Thomas G, Easton DF, Lennard-Jones JE, Mathew CG. Evidence of linkage of the inflammatory bowel disease susceptibility locus on chromosome 16 (IBD1) to ulcerative colitis. J Med Genet 1998;35: 218-221.

409. Rioux JD, Daly MJ, Green T, Stone V, Lander ES, Hudson TJ, Steinhart AH, Bull S, Cohen Z, Greenberg G, Griffiths A, McLeod R, Silverberg M, Williams CN, Siminovitch KA. Absence of linkage between inflammatory bowel disease and selected loci on chromosomes 3, 7, 12, and 16. Gastroenterology 1998;115: 1062-1065.

410. Toyoda H, Wang SJ, Yang HY, Redford A, Magalong D, Tyan D, McElree CK, Pressman SR, Shanahan F, Targan SR, Rotter JI. Distinct associations of HLA class II genes with inflammatory bowel disease. Gastroenterology 1993;104: 741-748.

411. Sugimura K, Asakura H, Mizuki N, Inoue M, Hibi T, Yagita A, Tsuji K, Inoko H. Analysis of genes within the HLA region affecting susceptibility to ulcerative colitis. Hum Immunol 1993;36: 112-118.

412. De la Concha EG, Fernandez-Arquero M, Santa-Cruz S, Lopez-Nava G, Figueredo MA, Diaz-Rubio M, García-Paredes J. Positive and negative associations of distinct HLA-DR2 subtypes with ulcerative colitis (UC). Clin Exp Inmunol 1997;108: 392-395.

413. Naom I, Lee J, Ford D, Bowman SJ, Lanchbury JS, Haris I, Hodgson SV, Easton D, Lennard-Jones J, Mathew CG. Analysis of the contribution of HLA genes to genetic predisposition in inflammatory bowel disease. Am J Hum Genet 1996;59: 226-233.

414. Reinshagen M, Loeliger C, Kuehnl P, Weiss U, Manfras BJ, Adler G, Boehm BO. HLA class II gene frequencies in Crohn´s disease: a population based analysis in Germany. Gut 1996;38: 538-542.

415. Danzé PM, Colombel JF, Jacquot S, Loste MN, Heresbach D, Ategbo S, Khamassi S, Périchon B, Semana G, Charron D, Cézard JP. Association of HLA class II genes with susceptibility to Crohn´s disease. Gut 1996;39: 69-72.

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416. Sartor RB. Cytokines in intestinal inflammation: Pathophysiological and clinical considerations. Gastroenterology 1994;106: 533-9.

417. Rogler G, Andus T. Cytokines in inflammatory bowel disease. World J. Surg 1998;22: 382-389.

418. McAlindon ME, Mahida YR. Pro-inflammatory cytokines in inflammatory bowel disease. Aliment Pharmacol Ther 1996;10 (suppl.2): 72-74.

419. Pallone F, Monteleone G. Regulatory cytokines in inflammatory bowel disease. Aliment Pharmacol Ther 1996;10 (suppl.2): 75-79.

420. Kagnoff MF. Immunology and inflammation of the gastrointestinal Tract. In: Sleisenger MH,ed. Gastrointestinal and liver disease. Philadelphia: Saunders, 1998: 19-48.

421. Koutroubakis I, Crusius JBA, Peña AS. Immunogenetics of cytokines. Scand J Gastroenterol 1995;30: 1139-1146.

422. Parkes M, Satsangi J, Jewell D. Contribution of the IL-2 and IL-10 genes to inflammatory bowel disease (IBD) susceptibility. Clin Exp Immunol 1998;113: 28-32.

423. Dinarello CA. The interleukin-1 family: 10 years of discovery. FASEB J 1994;8: 1314-1325.

424. Nikolic-Paterson DJ, Lan HY, Atkins RC. Interleukin- receptor antagonist. Sem Nephrol 1996;16: 583-590.

425. Arend WP, Malyack M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: role in biology. Annu Rev Immunol 1998;16: 27-55.

426. Arend WP, Joslin FG, Massoni RJ. Effects of immune complexes on production by human monocytes of interleukin 1 or an interleukin 1 inhibitor. J Immunol 1985;134: 3868-3875.

427. Balavoine JF, de Rochemonteix B, Williamson K, Seckinger P, Cruchaud A, Dayer JM. Prostaglandin E2 and collagenase production by fibroblasts and synovial cells is regulated by urine-derived human interleukin 1 and inhibitor(s). J Clin Invest 1986;78: 1120-1124.

428. Otterness I, Golden H, Brissette W, Seymour P, Daumy G. Effects of continuously administered murine interleukin 1: tolerance, development and granuloma formation. Infection Immun 1989;57: 2742-2750.

UNIVERSITAT ROVIRA I VIRGILI
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429. Rachmilewitz D, Simon PL, Schwartz LW, Griswold DE, Fondacaro JD, Wasserman MA. Inflammatory mediators of experimental colitis in rats. Gastroenterology 1989;97: 326-337.

430. Sartor RB, Holt LC, Bender DE, Murphy ME, McCall RD, Thompson RC. Prevention and treatment of experimental enterocolitis with a recombinant interleukin-1 receptor antagonist. Gastroenterology 1991;100: A613.

431. Cominelli F, Nast CC, Duchini A, Lee M. Recombinant interleukin-1 receptor antagonist blocks the proinflammatory activity of endogenous interleukin-1 in rabbit immune colitis. Gastroenterology 1992;103: 65-71.

432. Cominelli F, Nast CC, Clark BD, Schindler R, Llerena R, Eysselein VE, Thompson RC, Dinarello CA. Interleukin 1 (IL-1) gene expression, synthesis, and effect of specific IL-1 receptor blockade in rabbit immune complex colitis. J Clin Invest 1990;86: 972-980.

433. Ferreti M, Casini-Raggi V, Pizarro TT, Eisenberg SP, Nast CC, Cominelli F. Neutralization of endogenous IL-1 receptor antagonist exacerbates and prolongs inflammation in rabbit immune colitis. J Clin Invest 1994;94: 449-453.

434. Melani L, Hirsch E, Guanzon M, Pizarro TT, Hirsh D, Cominelli F. Deletion of the IL-1 receptor antagonist (IL-1ra) gene increases susceptibility to experimental colitis in mice. Gastroenterology 1997;112: A1040.

435. Mahida YR, Wu K, Jewell DP. Enhanced production of interleukin-1β by mononuclear cells isolated from mucosa with active ulcerative colitis or Crohn´s disease. Gut 1989;30: 835-838.

436. Brynskov J, Tvede N, Vilien M, Andersen CB, Bentzen K. Increased concentrations of interleukin 1β, interleukin 2, and soluble interleukin-2 receptors in endoscopical mucosal biopsy specimens with active inflammatory bowel disease. Gut 1992;33: 55-58.

437. Pullman WE, Elsbury S, Kobayashi M, Hapel AJ, Doe WF. Enhanced mucosal cytokine production in inflammatory bowel disease. Gastroenterology 1992,102: 529-537.

438. Ligumski M, Simon PL, Karmeli F, Rachmilewitz D. Role of interleukin 1 in inflammatory bowel disease: enhanced production during active disease. Gut 1990;31: 686-689.

439. Casellas F, Papo M, Guarner F, Antolín M, Segura RM, Armengol JR, Malagelada JR. Intracolonic release *in vivo* of interleukin-1β in chronic ulcerative colitis. Clinical Science 1995;89: 521-526.

440. Isaacs KL, Sartor RB, Haskill S. Cytokine messenger RNA profiles in inflammatory bowel disease mucosa detected by polymerase chain reaction amplification. Gastroenterology 1992;103: 1587-1595.

441. Gilberts EC, Greenstein AJ, Katsel P, Harpaz N, Greenstein RJ. Molecular evidence of two forms of Crohn disease. Poc Natl Acad Sci USA 1994;20: 12721-12724.

442. Nishiyama T, Mitsuyama K, Toyonaga A, Sasaki E, Tanikawa K. Colonic mucosal interleukin 1 receptor antagonist in inflammatory bowel disease. Digestion 1994;55: 368-373.

443. Casini-Raggi V, Kam L, Chong YJ, Fiocchi C, Pizarro TT, Cominelli F. Mucosal imbalance of IL-1 and IL-1 receptor antagonist in inflammatory bowel disease. A novel mechanism of chronic intestinal inflammation. J Immunol 1995;154: 2434-2440.

444. Dionne S, D´Agata ID, Hiscott J, Vanounou T, Seidman EG. Colonic explant production of IL-1 and its receptor antagonist is imbalanced in inflammatory bowel disease (IBD). Clin Exp Immunol 1998;112: 435-442.

445. Andus T, Daig R, Vogl D, Aschenbrenner E, Lock G, Hollerbach S, Köllinger M, Schölmerich J, Gross V. Imbalance of the interleukin 1 system in colonic mucosa-association with intestinal inflammation and interleukin 1 receptor agonist genotype 2. Gut 1997;651-657.

446. Hyams JS, Fitzgerald JE, Wyzga N, Muller R, Treem WR, Justinich CJ, Kreutzer DL. Relationship of interleukin-1 receptor antagonist to mucosal inflammation in inflammatory bowel disease. J Pediatr Gastroenterol Nutr 1995;21: 419-425.

447. Hendel J, Nielsen OH, Madsen S, Brynskov J. A simple filter-paper technique allows detection of mucosal cytokine levels in vivo in ulcerative colitis. Interleukin-1 and interleukin-1-receptor antagonist. Dig Dis Sci 1996;41: 1775-1779.

448. Saiki T, Mitsuyama K, Toyonaga A, Ishida H, Tanikawa K. Detection of pro- and anti-inflammatory cytokines in stools of patients with inflammatory bowel disease. Scand J Gastroenterol 1998;33: 616-622.

449. Eisenberg SP, Evans RJ, Arend WP, Verderber E, Brewer MT, Hannum CH, Thompson RC. Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. Nature 1990;343: 341-346.

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450. Eisenberg SP, Brewer MT, Verderber E, Heimdal P, Brandhuber BJ, Thompson RC. IL-1 receptor antagonist is a member of the IL-1 gene family. Proc Natl Acad Sci USA 1991;88: 5232-5236.

451. Lennard A, Gorman P, Carrier M, Griffiths S, Scotney H, Sheer D, Solari R. Cloning and chromosome mapping of the human interleukin-1 receptor antagonist gene. Cytokine 1992;4: 83-89.

452. Steinkasserer A, Spurr NK, Cox S, Jeggo P, Sim RB. The human IL-1 receptor antagonist gene (ILlRN) maps to chromosome 2q14-q21, in the region of the IL-1 alpha and IL-1 beta loci. Genomics 1992;13: 654-657.

453. Patterson D, Jones C, Hart I, Bleskan J, Berger R, Geyer D, Eisenberg SP, Smith MF, Arend WP. The human IL-1 receptor antagonist gene (ILlRN) is located in the chromosome 2q14 region. Genomics 1993;15: 173-176.

454. Tarlow JK, Blakemore AIF, Lennard A, Solari R, Hughes HN, Steinkasserer A, Duff GW. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 1993;91: 403-404.

455. Danis VA, Millington M, Hyland VJ, Grennan D. Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. Clin Exp Immunol 1995;99: 303-310.

456. Cominelli F, Pizarro TT. Interleukin-1 and interleukin-1 receptor antagonist in inflammatory bowel disease. Aliment Pharmacol Ther 1996;10 (suppl 2): 49-53.

457. Hurme M, Santtila S. IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. Eur J Immunol 1998;28: 2598-2602.

458. Stokkers PCF, van Aken BE, Basoki N, Reitsma PH, Tytgat GNJ, van Deventer SJH. Five genetic markers in the interleukin 1 family in relation to inflammatory bowel disease. Gut 1998;43: 33-39.

459. Clay FE, Tarlow JK, Cork MJ, Cox A, Nicklin MJH, Duff GW. Novel interleukin-1 receptor antagonist exon polymorphisms and their use in allele-specific mRNA assessment. Hum Genet 1996;97: 723-726.

460. Mansfield JC, Holden H, Tarlow JK, Di Giovine FS, McDowell TL, Wilson AG, Holdsworth CD, Duff GW. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. Gastroenterology 1994;106: 637-642.

461. Duerr RH, Tran T. Association between ulcerative colitis and a polymorphism in intron 2 of the interleukin-1 receptor antagonist gene. Gastroenterology 1995;108: A812.

462. Tountas NA, Kam L, di Giovine FS, Casini-Raggi V, Cominelli F. Genetic association between allele 2 of IL-1 receptor antagonist (IL-1ra) and ulcerative colitis in a Los Angeles based Hispanic population. Gastroenterology 1995;108: A930.

463. Andus T, Caesar I, Vogl D, Schölmerich J, Gross V. Association of HLA-DR15, p-ANCA and IL-1 receptor antagonist allele 2 with ulcerative colitis. Gastroenterology 1995;108: A770.

464. Hacker UT, Gomolka M, Keller E, Eigler A, Folwaczny C, Fricke H, Albert E, Loeschke K, Endres S. Lack of association between an interleukin-1 receptor antagonist gene polymorphism and ulcerative colitis. Gut 1997;40: 623-627.

465. Louis E, Satsangi J, Roussomoustakaki M, Parkes M, Fanning G, Welsh K, Jewell D. Cytokine gene polymorphisms in inflammatory bowel disease. Gut 1996;39: 705-710.

466. Roussomoustakaki M, Satsangi J, Welsh K, Louis E, Fanning G, Targan S, Landers C, Jewell DP. Genetic markers may predict disease behavior in patients with ulcerative colitis. Gastroenterology 1997;112: 1845-1853.

467. Heresbach D, Alizadeh M, Dabadie A, Le Berre N, Colombel JF, Yaouanq J, Bretagne JF, Semana G. Significance of interleukin-1β and interleukin-1 receptor antagonist genetic polymorphism in inflammatory bowel diseases. Am J Gastroenterol 1997;92: 1164-1169.

468. García-Paredes J, Bioque G, Crusius JBA, García-González A, López-Nava G, Díaz-Rubio M, Fernandez-Arquero M, Gómez de la Concha E, Peña AS. The interleukin-1 receptor antagonist gene polymorphism in Spanish IBD patients. Gastroenterology 1996;110: A914.

469. Satsangi J, Jewell DP. Are cytokine gene polymorphisms important in the pathogenesis of inflammatory bowel disease ? Eur J Gastroenterol Hepatol 1996;8:97-99.

470. Perrier S, Coussediere C, Dubost JJ, Albuisson E, Sauvezie B. IL-1 receptor antagonist (IL-1RA) gene polymorphism in Sjogren´s syndrome and rheumatoid arthritis. Clin Immunol Immunopathol 1998;87: 309-313.

471. Carter MJ, Jones S, di Giovine FS, Camp NJ, Lobo AJ, Duff GW. Allele 2 of the interleukin-1 receptor antagonist gene polymorphism is associated with reduced expression of interleukin-1 receptor antagonist in ulcerative colitis. Gastroenterology 1998;114: A947

472. Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. N Engl J Med 1996;334: 1717-1725.

473. Armstrong AM, Gardiner KR, Kirk SJ, Halliday MI, Rowlands BJ. Tumor necrosis factor and inflammatory bowel disease. B J Surg 1997;84: 1051-1058.

474. Sun X, Hsueh W. Bowel necrosis induced by tumor necrosis factor in rats is mediated by platelet-activating factor. J Clin Invest 1988;81: 1328-1331.

475. Bautista AP, Schuler A, Spolarics Z, Spitzer JJ. Tumor necrosis alfa stimulates superoxide anion generation by perfused rat liver and Kupffer cells. Am J Physiol 1991;261: G891-G895.

476. Moser R, Scheliffenbaum B, Groscurth P, Fehr J. Interleukin 1 and tumor necrosis factor stimulate human vascular endothelial cells to promote transendothelial neutrophil passage. J Clin Invest 1989;83: 444-455.

477. Foulkes R, Shaw S. Recombinant human TNF induces a non-constitutive form of nitric oxide (NO)-synthase in the endothelium and smooth muscle. Br J Pharmacol 1992;105: 292P.

478. Neilly PJD, Campbell GR, Anderson NH, Gardiner KR, Kirk SJ, Rowlands BJ. Faecal and systemic tumor necrosis factor in experimental inflammatory bowel disease. Surgical Research Communications 1995;18: 11-17.

479. Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL. Inhibition of Th1 responses prevents inflammatory bowel disease in SCID mice reconstituted with $CD45RB^{high}$ CD4+ T cells. Immunity 1994;1: 553-562.

480. Watkins PE, Warren BF, Stephens P, Ward P, Foulkes R. Treatment of ulcerative colitis in the cottontop tamarin using antibody to tumor necrosis factor alpha. Gut 1997;40: 628-633.

481. Neurath MF, Fuss I, Pasparakis M, Alexopoulou L, Haralambous S, Meyer zum Buschenfelde KH, Strober W, Kollias G. Predominant pathogenic role of tumor necrosis factor in experimental colitis in mice. Eur J Immunol 1997;27: 1743-1750.

482. Kojouharoff G, Hans W, Obermeier F, Mannel DN, Andus T, Schölmerich J, Gross V, Falk W. Neutralization of tumor necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulphate sodium-induced colitis in mice. Clin Exp Immunol 1997;107: 353-358.

483. Braegger CP, Nicholls S, Murch SH, Stephens S, McDonald TT. Tumor necrosis factor alpha in stool as a marker of intestinal inflammation. Lancet 1992;339: 89-91.

484. Cappello M, Keshav S, Prince C Jewell DP, Gordon S. Detection of mRNA for macrophage products in inflammatory bowel disease. Gut 1992;33: 1214-1219.

485. Reinecker HC, Steffen M, Witthoeft T, Pflueger I, Schreiber S, MacDermott RP, Raedler A. Enhanced secretion of tumor necrosis factor-alpha, IL-6, and IL-1-beta by isolated lamina propia mononuclear cells from patients with ulcerative colitis and Crohn´s disease. Clin Exp Immunol 1993;94: 174-181.

486. Murch SH, Braegger CP, Walker SJ, MacDonald TT. Location of tumor necrosis factor-α by immunohistochemistry in chronic inflammatory bowel disease. Gut 1993;34: 1705-1709.

487. Breese EJ, Michie CA, Nicholls SW, Murch SH, Williams CB, Domizio PWS, MacDonald TT. Tumor necrosis factor alpha-producing cells in the intestinal mucosa of children with inflammatory bowel disease. Gastroenterology 1994;106: 1455-1466.

488. Casellas F, Papo M, Guarner F, Antolín M, Armengol JR, Malagelada JR. Intraluminal colonic release of immunoreactive tumor necrosis factor in chronic ulcerative colitis. Clin Sci (Colch) 1994;87: 453-458.

489. Funakoshi K, Sugimura K, Anezaki K, Bannai H, Ishizuka K, Asakura H. Spectrum of cytokine gen expression in intestinal mucosal lesions of Crohn´s disease and ulcerative colitis. Digestion 1998;59: 73-78.

490. Van Dullemen HM, Van Deventer SJH, Hommes DW, Bijl HA, Jansen J, Tytgat GNJ, Woody J. Treatment of Crohn´s disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). Gastroenterology 1995;109: 129-135.

491. Stack WA, Mann SD, Roy AJ, Heath P, Sopwith M, Freeman J, Holmes G, Long R, Forbes A, Kamm MA, Hawkey CJ. Randomised controlled trial of CDP571 antibody to tumor necrosis factor-α in Crohn´s disease. Lancet 1997;349: 521-524.

492. Targan SR, Hanauer SB, Van Deventer SJH, Mayer L, Present DH, Braakman T, DeWoody KL, Schaible TF, Rutgeerts PJ, for the Crohn´s Disease cA2 Study Group. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor α for Crohn´s disease. N Engl J Med 1997;337: 1029-1035.

493. Evans RC, Clarke L, Heath P, Stephens S, Morris AI, Rhodes JM. Treatment of ulcerative colitis with an engineered human anti-TNFalpha antibody CDP571. Aliment Pharmacol Ther 1997;11: 1031-1035.

494. Nightingale SL. From the Food and Drug Administration. JAMA 1998;280: 1128.

495. Wilson AG, di Giovine FS, Duff GW. Genetics of tumor necrosis factor-α in autoimmune, infectious, and neoplastic diseases. J Inflam 1995;45: 1-12.

496. Wilson AG, di Giovine FS, Blakemore, Duff GW. Single base polymorphism in the human necrosis factor alpha (TNFα) gene detectable by NcoI restriction of PCR product. Hum Mol genet 1992;1: 353.

497. Bouma G, Crusius JBA, Oudkerk Pool M, Kolkman JJ, von Blomberg BME, Kostense PJ, Giphart MJ, Schreuder GMTH, Meuwissen SGM, Peña AS. Secretion of tumor necrosis factor α and lymphotoxin α in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. Scand J Immunol 1996;43: 456-463.

498. Louis E, Franchimont D, Piron A, Gevaert Y, Schaaf-Lafontaine N, Roland S, Mahieu P, Malaise M, De Groote D, Louis R, Belaiche J. Tumor necrosis factor (TNF) gene polymorphism influences TNF-α production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. Clin Exp Immunol 1998;113: 401-406.

499. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW: Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. Proc Natl Acad Sci 1997, 94:3195-3199.

500. Kroeger KM, Carville KS, Abraham LJ: The -308 tumor necrosis factor-α promoter polymorphism effects transcription. Mol Immunol 1997, 34:391-399.

501. Brinkman BMN, Zuijdgeest D, Kaijzel EL, Breedveld FC, Verweij C. Relevance of the tumor necrosis factor alpha (TNFα) −308 promoter polymorphism in TNFα gene regulation. J Inflam 1996;46: 32-41.

502. Badenhoop K, Schwarz G, Trowsdale J, Lewis V, Usadel KH, Gale EA, Bottazzo GF. TNF-alpha gene polymorphisms in type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1989;32: 445-448.

503. Messer G, Spengler U, Jung MC, Honold G, Blömer K, Pape GR, Riethmüller G, Weiss EH. Polymorphic structure of the tumor necrosis factor (TNF) locus: an NcoI polymorphism in the first intron of the human TNF-β gene correlates with a variant amino acid in position 26 and a reduced level of TNF-β production. J Exp Med 1991;173: 209-219.

504. Pociot F, Briant L, Jongeneel CV, Mölvig J, Worsaae H, Abbal M, Thomsen M, Nerup J, Cambon-Thomsen A. Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-α and TNF-β by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. Eur J Immunol 1993;23: 224-231.

505. Whichelow CE, Hitman GA, Raafat I, Bottazzo GF, Sachs JA. The effect of TNF*B gene polymorphism on TNF-alpha and beta secretion levels in patients with insulin-dependent diabetes mellitus and healthy controls. Eur J Immunogenet 1996;23: 425-435.

506. Koutroubakis I, Xia B, Crusius JBA, Bioque G, Bouma G, Meuwissen SGM, Manousos ON, Peña AS. Tumor necrosis factor-alpha polymorphism in inflammatory bowel disease. Hell J Gastroenterol 1995,8: 132-135.

507. Heresbach D, Ababou A, Bourienne A, Alizadeh M, Quelvennec E, Pagenault M, Dabadie A, Heresbach-Le Berre N, Campion JP, Launois B, Gosselin M, Genetet B, Bretagne JF, Semana G. Étude du polymorphisme des microsatellites et des gènes du tumor necrosis factor (TNF) au cours des maladies inflammatoires chroniques de l´intestin. Gastroenterol Clin Biol 1997;21: 555-561.

508. Bouma G, Xia B, Crusius JBA, Bioque G, Koutroubakis I, Von Blomberg BME, Meuwissen SGM, Peña AS. Distribution of four polymorphisms in the tumor necrosis factor (TNF) genes in patients with inflammatory bowel disease (IBD). Clin Exp Immunol 1996;103: 391-396.

509. Louis E, Peeters M, Demolin G, Croes F, Dupont P, Rutgeerts P, Belaiche J, and the Belgian IBD Research Group. TNF gene polymorphism may influence disease behavior in Crohn´s disease. Gastroenterology 1998;114: A1027.

510. Bing X, Bouma G, Crusius JBA, Meuwissen SGM, Peña AS. Distribution of an NcoI polymorphism in the lymphotoxin α gene in dutch patients with inflammatory bowel diseases. Chin Med J 1995;108: 739-742.

511. Sugimura K, Asakura H, Mizuki N, Inoue M, Hibi T, Yagita A, Tsuji K, Inoko H. Analysis of genes within the HLA region affecting susceptibility to ulcerative colitis. Hum Immunol 1993;36: 112-118.

512. Bohr J, Tysk C, Yang P, Danielsson D, Järnerot G. Autoantibodies and immunoglobulins in collagenous colitis. Gut 1996;39: 73-76.

UNIVERSITAT ROVIRA I VIRGILI
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513. Quinton JF, Sendid B, Reumaux D, Duthilleul P, Cortot A, Grandbastien B, Charrier G, Targan SR, Colombel JF, Poulain D. Anti-*Saccharomyces cerevisiae* mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. Gut 1998;42: 788-791.

514. Aisenberg J, Zelman G, Bodian C, Sachar D. Serological analysis permits differentiation of Crohn´s disease from ulcerative colitis. Gastroenterology 1998;114: A918.

515. Vecchi M, Bianchi MB, Calabresi C, Meucci G, Tatarella M. Long-term observation of the perinuclear anti-neutrophil cytoplasmic antibody status in ulcerative colitis patients. Scand J Gastroenterol 1998;33: 170-173.

516. Lindgren S, Florén C-H, Lindhagen T, Starck M, Stewenius J, Nässberger L. Low prevalence of anti-neutrophil cytoplasmic antibodies in ulcerative colitis patients with long-term remission. Eur J Gastroenterol Hepatol 1995;7: 563-568.

517. Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Association of antineutrophil cytoplasmic antibodies with resistance to treatment of left-sided ulcerative colitis: results of a pilot study. Mayo Clin Proc 1996;71: 431-436.

518. Elmgreen J, Both H, Binder V. Familial occurrence of complement dysfunction in Crohn´s disease: correlation with intestinal symptoms and hypercatabolism of complement. Gut 1985;26: 151-157.

519. Tysk C, Riedesel H, Lindberg E, Panzini B, Podolsky D, Jarnerot G. Colonic glycoproteins in monozygotic twins with inflammatory bowel disease. Gastroenterology 1991;100: 419-423.

520. Van de Merwe JP, Schroder AM, Wensinck F, Hazenberg MP. The obligate anaerobic faecal flora of patients with Crohn´s disease and their first-degree relatives. Scand J Gastroenterol 1988;23: 1125-1131.

521. Helgeland L, Tysk C, Jarnerot G, Kett K, Lindberg E, Danielsson D, Andersen SN, Brandtzaeg P. IgG subclass distribution in serum and rectal mucosa of monozygotic twins with or without inflammatory bowel disease. Gut 1992;33: 1358-1364.

522. Wayne LG, Hollander D, Anderson B, Sramek HA, Vadheim CM, Rotter JI. Immunoglobulin A (IgA) and IgG serum antibodies to mycobacterial antigens in Crohn´s disease patients and their relatives. J Clin Microbiol 1992;30: 2013-2018.

523. Hollander D, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohn´s disease and their relatives. A possible etiologic factor. Ann Intern Med 1989;105: 883-885.

524. Katz KD, Hollander D, Vadheim CM, McElree C, Delahunty T, Dadufalza VD, Krugliak P, Rotter JI. Intestinal permeability in patients with Crohn´s disease and their healthy relatives. Gastroenterology 1989;97: 927-931.

525. May GR, Sutherland LR, Meddings JB. Is small intestinal permeability really increased in relatives of patients with Crohn´s disease ? Gastroenterology 1993;104:1627-1632.

526. Ruttenberg D, Young GO, Wright JP, Isaacs S. PEG-400 excretion in patients with Crohn´s disease, their first-degree relatives, and healthy volunteers. Dig Dis Sci 1992;37: 705-708.

527. Teahon K, Smethurst P, Levi AJ, Menzies IS, Bjarnason I. Intestinal permeability in patients with Crohn´s disease and their first degree relatives. Gut 1992;33: 320-323.

528. Howden CW, Gillanders I, Morris AJ, Duncan A, Danesh B, Russell RI. Intestinal permeability in patients with Crohn´s disease and their first degree relatives. Am J Gastroenterol 1994;89: 1175-1176.

529. Peeters M, Geypens B, Claus D, Nevens H, Ghoos Y, Verbeke G, Baert F, Vermeire S, Vlietinck R, Rutgeerts P. Clustering of increased small intestinal permeability in families with Crohn´s disease. Gastroenterology 1997;113: 802-807.

530. Korsmeyer SJ, Williams RC Jr, Wilson ID, Strickland RG. Lymphocytotoxic antibody in inflammatory bowel disease. A family study. N Engl J Med 1975;293: 1117-1120.

531. Fiocchi C, Roche JK, Michener WM. High prevalence of antibodies to intestinal epithelial antigens in patients with inflammatory bowel disease and their relatives. Ann Intern Med 1989;110: 786-794.

532. Folwaczny C, Noehl N, Endres SP, Loeschke K, Fricke H. Antineutrophil and pancreatic autoantibodies in first-degree relatives of patients with inflammatory bowel disease. Scand J Gastroenterol 1998;33: 523-528.

533. Seibold F, Mörk H, Tanza S, Müller A, Holzhüter C, Weber P, Scheurlen M. Pancreatic autoantibodies in Crohn´s disease: a family study. Gut 1997;40: 481-484.

534. Biancone L, Monteleone G, Marasco R, Pallone F. Autoimmunity to tropomyosin isoforms in ulcerative colitis (UC) patients and unaffected relatives. Clin Exp Immunol 1998;113: 198-205.

535. Folwaczny C, Noehl N, Tschöp K, Endres SP, Heldwein W, Loeschke K, Fricke H. Globet cell autoantibodies in patients with inflammatory bowel disease and their first-degree relatives. Gastroenterology 1997;113: 101-106.

536. Sendid B, Quinton JF, Charrier G, Goulet O, Cortot A, Grandbastien B, Poulain D, Colombel JF. Anti-Saccharomyces cerevisiae mannan antibodies in familial Crohn´s disease. Am J Gastroenterol 1998;93: 1306-1310.

537. Folwaczny C, Noehl N, Endres SP, Heldwein W, Loeschke K, Fricke H. Antinuclear autoantibodies in patients with inflammatory bowel disease. High prevalence in first-degree relatives. Dig Dis Sci 1997;42: 1593-1597.

538. Napolitano G, Piepoli A, Annese V, Perri F, Astegiano M, Clemente R, Iaquinto V, Cucchiara S, Andriulli A. Antineutrophil cytoplasmic antibody (ANCA) in unaffected relatives in familial inflammatory bowel disease. Gastroenterology 1996;110; A978.

539. Vecchi M, Bianchi MB, Meucci G, Beretta L, Bortoli A, Cesari P, Colombo E, Comin U, Corbellini A, Ferrara A, Gullotta R, Lupinacci G, Politi P, Ranzi T, Ravelli P, Rocca F, de Franchis R. Prevalence of p-ANCA in unaffected family members of Italian ulcerative colitis patients. Gastroenterology 1996;110; A1037.

540. Annese V, Piepoli A, Lombardi G, Napolitano G, Latiano A, Borrelli O, Andriulli A. Antineutrophil cytoplasmic antibody (ANCA) in familial inflamatory bowel disease. Gastroenterology 1997;112: A922.

541. Yang P, Järnerot G, Danielsson D, Tysk C, Lindberg E. P-ANCA in monozygotic twins with inflammatory bowel disease. Gut 1995;36: 887-890.

542. Carter MJ, Jones S, Mansfield JC, di Giovine FS, Camp NJ, Lobo AJ, Duff GW. Further evidence of an association between the alele 2 of the interleukin-1 receptor antagonist (IL-1RA) gene (IL-1RN) polymorphism and ulcerative colitis (UC). Gastroenterology 1998;114: A948.

543. Tountas NA, Yang H, Coulter DL, Rotter JI, Cominelli F. Increased carriage of allele 2 of IL-1 receptor antagonist (IL-1ra) in jewish populations: the strongest known genetic association in ulcerative colitis. Gastroenterology 1996;110: A1029.

544. Bioque G, Crusius JBA, Koutroubakis I, Bouma G, Kostense PJ, Meuwissen SGM, Peña AS. Allelic polymorphism in IL-1β and IL-1 receptor antagonist (IL-1Ra) genes in inflammatory bowel disease. Clin Exp Immunol 1995;102: 379-383.

545. Hacker UT, Bidlingmaier C, Gomolka M, Keller E, Eigler A, Hartmann G, Folwaczny C, Fricke H, Albert E, Loeschke K, Endres S. Inflammatory bowel disease: no association between allele combinations of the interleukin (IL) 1β and IL-1 receptor antagonist gene polymorphisms. Eur J Clin Invest 1998;28: 214-219.

546. Plevy SE, Targan SR, Yang H, Fernandez D, Rotter JI, Toyoda H. Tumor necrosis factor microsatellites define a Crohn´s disease-associated haplotype on chromosome 6. Gastroenterology 1996;110: 1053-1060.

547. Annese V, Piepoli A, Andriulli A, Napolitano G, Biesceglia L, Zelante L, Gasparini P. Polymorphism of motilin gene in patients with Crohn´s disease. Dig Dis Sci 1998;43: 715-719.

548. Lee YT, Sung JY, Poon P, Lai KN, Li PKT. Association of HLA class-II genes and anti-neutrophil cytoplasmic antibodies in Chinese patients with inflammatory bowel disease. Scand J Gastroenterol 1998,33: 623-627.

549. Plevy SE, Taylor K, DeWoody KL, Schaible TF, Shealy D, Targan SR. Tumor necrosis factor (TNF) microsatellite haplotypes and perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) identify Crohn´s disease (CD) patients with poor clinical responses to anti-TNF monoclonal antibody (cA2). Gastroenterology 1997;112: A1062.