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RAPID COMMUNICATION

Association of the HLA-DRB1*0701 allele with perinuclear anti-neutrophil cytoplasmatic antibodies in Mexican patients with severe ulcerative colitis

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Abstract

AIM: To determine the association between the HLA-DRB1 alleles and perinuclear anti-neutrophil cytoplasmatic antibodies (p-ANCA) positive in Mexican patients with ulcerative colitis (UC).

METHODS: Ninety Mexican mestizo patients (45 females) with UC, confirmed by biopsy, were studied. High resolution HLA typing was performed by PCR-SSO reverse dot blot and PCR-SSP. Molecular typing techniques were applied to define HLA-DRB1 alleles. Enzyme-linked immunosorbent assay and immunofluorescence techniques were used to detect p-ANCA.

RESULTS: Forty-eight (53%) UC patients were positive for p-ANCA by ELISA and IF. We found that p-ANCA-positive UC patients had a significantly increased frequency of HLA-DR7 compared with p-ANCA-negative controls (22% *vs* 5.1%; pC = 0.02, OR = 5.2, CI 95%: 1.06-37.82). Disease activity was scored as severe in 20 patients, moderate in 8, mild in 14 and no activity in the remaining 38 patients according to the Truelove and Witts criteria. Subgroup analysis showed a significantly increased frequency of the HLA-DRB1*07 allele in 15 of 20 UC patients with severe activity of UC and p-ANCA positivity [100% *vs* 0%; pC = 0.0000001; OR = 35]. No significant differences were found between p-ANCA positive patients, HLA-DR alleles and other clinical features such as extraintestinal manifestations, proctocolectomy and extension.

CONCLUSION: The HLA-DRB1*07 is associated with p-ANCA positive UC Mexican patients.

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Key words: HLA-DR; p-ANCA; Ulcerative colitis; Mexicans

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INTRODUCTION

Perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) have been found consistently in patients with ulcerative colitis (UC)[1,2] however, their pathogenic role is still uncertain. The prevalence of p-ANCA in UC varies greatly from 39 to 80 percent in the ethnic group studied[2].

A genetic involvement in UC is supported by observations on familial aggregation of the disorder and concordance in monozygotic twins[3]. Considering the central role of the immune system in mediating the tissue damage in IBD, genes that participate in the development and regulation of the immune response, such as the HLA class II genes, are candidates for conferring the genetic susceptibility. Putative associations of UC with the polymorphic genes of the major histocompatibility complex (MHC) located on the short arm of the chromosome 6 suggest a role of these in the genetic susceptibility to develop UC in several populations of different ethnic and geographic background[4].

UC is a clinical and genetic heterogeneous disorder described in several ethnic groups[5-7]. An association between HLA-DR alleles and UC, in particular p-ANCA positive has been reported in patients from Italy[6], China[8] and United States[9].

Mexican mestizo individuals have a proportion of 56% native American Indian genes, 40% Caucasian genes, and 4% African genes[10], thus they are suitable to study the role of ethnicity in the susceptibility to develop UC and

p-ANCA positive in UC. Mestizo represents a complex mixture of European (Caucasian) and American native inhabitants (mongoloid) genetics, and constitutes the core of the Mexican and the Latin American populations.

The purpose of this study was to determine the association between the HLA-DRB1 alleles and p-ANCA positive in Mexican mestizo patients with UC.

MATERIALS AND METHODS

Patients

Ninety Mexican Mestizo patients with ulcerative colitis confirmed by histology were studied. Details of demographic and clinical characteristics of UC were obtained by a questionnaire, review of records and personal interview. No patient had a family history for UC. Disease extension was defined by colonoscopy. Thus, disease was classified either as extensive colitis (inflammation proximal to the splenic flexure) or distal colitis. Disease activity was scored by Truelove and Witts criteria[11].

Blood samples were obtained from 99 Mexican mestizo healthy, unrelated individuals with no family history of UC, ethnically matched to patients with UC as controls. Mexican mestizo individuals included in the present study accounted for 56% native American Indian genes, 40% white genes and 4% Black genes[11].

Methods

HLA typing Genomic DNA was isolated from peripheral venous blood by a modified "salting out" technique[12], precipitated with ethanol and resuspended in sterile distilled water at a final concentration 0.1-1.0 μg/μL before use.

Generic HLA-DRB1 typing was performed by PCR-SSO reverse dot blot hybridization (Amplicor, Hoffmann La Roche, Basel, Switzerland). High resolution HLA typing was performed by dot blot hybridization of amplified DNA with sequence-specific oligonucleotide probes labeled with digoxigenin di-deoxy-uridine-triphosphate (Dig-11-ddUTP). Information about DRB1 sequence was obtained from the 12th International Histocompatibility Workshop[13].

Indirect Immunofluorescence (IFL) Detection of ANCA was done according to the recommendations of the international workshop[14]. Human neutrophils were isolated from peripheral blood from patients and normal healthy volunteers by gradient centrifugation in 2% methylcellulose solution. The slides were fixed in 98% ethanol at 4℃ for 5 min and dried quickly in air. After wetting with phosphate-buffered saline (PBS) in a dilution of 1:40 and diluted two fold until dilution reached 1:320. Following incubation for 1 h at room temperature, the slides were washed three times with PBS and bound antibodies were incubated and detected with fluorescein isothiocyanate (FITC)-conjugated F(ab') rabbit anti-human IgG, diluted 1:100 in PBS for 30 min at room temperature. Subsequently, the slides were washed three times with PBS and covered with glycerin-phosphate-buffered saline. A titer $\geqslant$1:80 was considered positive, based on the results obtained from healthy controls.

Antigen-specific enzyme-linked immunosorbent assays (ELISA). This method was performed to identify the putative antigens recognized by the p-ANCA positive sera. Human Lf, MPO or cathepsin G was diluted to 2 μg/mL in carbonate buffer (pH 9.6) and 100 μL of each solution was placed in the wells of a 96-well microplate and left for 24 h at 4℃. After the plate was washed three times with PBS, 100 μL of diluted sera (1:100 in PBS containing 0.1 % Tween 20 and 0.1 % skim milk) was added to each well, and the plate was incubated for 1 h at 37℃. After washing, the enzyme reaction was performed, and color development was measured with a microplate colorimeter. Optical density values >2 SD were considered positive[15].

Statistical analysis Gene frequencies were compared using a 2×2 contingency table and Chi square test. Odd ratios have been calculated for the disease in carriers of specific alleles. Comparisons of allele frequencies between subgroups were performed using the EPI-INFO statistical package (Version 5.0; USD incorporated 1990, Stone Mountain, Georgia). All p values quoted were corrected by the Bonferroni test for multiple comparisons, while the number of alleles studied was taken into account. Statistical significance was considered when $P<0.05$.

RESULTS

Clinical features

The average disease duration was 7.2±6.6 years. Extensive colitis was present in 60% and distal colitis in 40%. Disease activity was scored as severe in 20 patients (25%), moderate in 8 (10%), mild in 14 (18%) and no activity in the remaining 38 patients (47%). Extraintestinal manifestations were present in 40%: arthritis (14.6%), primary sclerosing cholangitis (10.4%), sachroiliitis (6%), erythema nodosum (6%), ankylosing spondylitis (1.5%) and aphthous ulceration (1.5%). Fourteen patients (15.5%) required colectomy due to refractory medical therapy.

Positive p-ANCA

Positive p-ANCA was detected by IFL and ELISA methods in 48 of 90 patients with UC (53%) whereas c-ANCA was detected in 4 patients with UC. The frequencies of positive p-ANCA were increased significantly in the patients with UC as compared to controls. Titers of p-ANCA ranged 1:80-1:320.

HLA distribution

Patients with positive p-ANCA showed a significantly increased frequency of the HLA-DRB1*07 as compared to p-ANCA negative patients (pC = 0.02, OR = 5.2, CI 95 %: 1.06-37.82) and healthy controls (pC = 0.04, OR = 2.9, CI 95 %: 1.01-20.56). The remaining HLA-DR alleles did not show statistically differences between UC patients with positive p-ANCA and negative p-ANCA as shown in Table 1.

Subgroup analysis showed a significantly increased frequency of the HLA-DRB1*07 allele in patients with severe activity of UC and positive p-ANCA (15/15) as compared to 5 patients with severe disease and negative p-ANCA and no HLA-DRB1*07 [100 % *vs* 0%; pC = 0.0000001;

Table 1 Gene frequencies (g.f.) of HLA-DR alleles in patients with UC and healthy controls

HLA	p-ANCA + N = 96		p-ANCA – N = 84		Healthy controls N = 198	
	n	g.f.	*n*	g.f.	*n*	g.f.
DR7	21	0.218[1,2]	4	0.047	22	0.111
DR2	16	0.166	13	0.154	14	0.090
DR4	14	0.145	17	0.202	47	0.237
DR1	13	0.135	12	0.142	10	0.050
DR8	10	0.104	11	0.130	33	0.165
DR5	8	0.083	11	0.130	20	0.100
DR6	6	0.062	9	0.107	21	0.109
DR3	5	0.052	5	0.059	11	0.055
DR10	3	0.031	2	0.023	1	0.005

N = Number of chromosomes *n*= number of alleles.
[1]p-ANCA positive *vs* negative: pC = 0.02, OR = 5.2, CI 95%: 1.06-37.82.
[2]p-ANCA positive *vs* controls: pC = 0.04, OR = 2.9, CI 95%: 1.01-20.56.

OR=35]. No significant differences were found between p-ANCA positive patients, HLA-DR alleles and other clinical features such as extraintestinal manifestations, proctocolectomy and extension.

Disease activity

Data about disease activity were collected without knowledge of the results of ANCA testing. Interestingly, a significant association was found between severe activity of the disease and positive p-ANCA [75 % *vs* 25 %; pC = 0.00008; OR = 11; CI 95 %: 4.0-34.0]. No association was found between moderate, mild or inactive disease and the presence of p-ANCA (Table 2).

Extension We found that 22 UC patients with extensive colitis (47.8 %) were positive for p-ANCA while 19 patients with distal colitis were positive for p-ANCA (55.8 %). No statistical association was found between p-ANCA positivity and the extension of the disease [*P* = 0.47; OR = 0.72; CI 95 %: 0.27-1.94].

Proctocolectomy Seven patients with proctocolectomy were positive for p-ANCA as compared to 34 patients (51.5 %) who did not undergo the surgical procedure and were p-ANCA positive. No association was found between both groups [*P* = 0.91; OR = 0.94; CI 95 %: 0.26-3.43].

EIMs Futhermore, association was no found between each of the extraintestinal manifestations such as arthritis, primary sclerosing cholangitis, sachroiliitis, erythema nodosum, ankylosing spondilytis or aphthous ulceration and the presence of p-ANCA.

Type of treatment We followed up 80 patients treated with corticosteroids (local and systemic), 5-ASA (local and systemic) and azatioprin, and 10 untreated patients who served as controls. No association was found between p-ANCA positivity and any kind of medical treatment.

The prevalence of p-ANCA was higher in treated patients (45 %) than in untreated patients (6 %), probably reflecting the greater severity of the cases that required surgical treatment. After stratifying patients according to p-ANCA status, prevalence rates were not different as far as age, gender, age at first diagnosis, disease extension, surgery, extraintestinal manifestations were concerned.

No association was found between the number of clinical relapses and p-ANCA positive determination (data not shown).

Table 2 Clinical features of UC patients with positive and negative p-ANCA

Clinical feature	p-ANCA + *n* = 48	p-ANCA - *n* = 42	*P* value
Age at diagnosis (yr)	31 ± 8	33 ± 7	0.34
Gender (F/M)	23/25	22/20	0.62
Disease duration (yr)	7.4 ± 6.3	6.8 ± 6.2	0.45
Clinical relapses	2 ± 1	3 ± 2	0.25
Medical Treatment			
5-ASA	90%	85%	0.75
Corticosteroids	44%	45%	0.90
Azatioprine or 6-MP	24%	22%	0.82
Extensive colitis	29	27	0.47
Distal colitis	19	15	
Colectomy	7	7	0.94
No Colectomy	41	35	
EIMs present	21	25	0.67
EIMs absent	27	17	
Disease activity			
Absent	19	21	0.82
Mild	9	10	0.71
Moderate	5	6	0.65
Severe	15	5	0.000008

[a]OR = 11; CI 95%:4.0-34.0.
n = Number of patients.

DISCUSSION

This study provides evidence of the important role of MHC genes class II in the development of autoantibodies in patients with ulcerative colitis. The relevant finding was the increased frequency of the HLA-DRB1*07 allele in UC patients with positive p-ANCA compared with UC patients with negative p-ANCA. This association has not been reported in other ethnic groups. Several studies on Caucasians have demonstrated the association between the HLA-DR2 and the presence of p-ANCA positive UC patients from United States[16], Germany[17] and Italy[18]. In Chinese patients, a strong association is found between the HLA-DQ alpha 1c allele and the presence of ANCA positive[8].

On the other hand, other studies have not found association of the presence of positive p-ANCA and HLA-DR alleles in Jewish[2], French[18] and Korean[19] UC patients. Previous Mexican studies have reported association between HLA-DR1 and polymorphisms in the promoter region of tumor necrosis factor alpha and the genetic susceptibility to develop UC in this population[7,20].

The HLA-DRB1*07 has not been reported to have an association with positive p-ANCA in patients with UC from other ethnic groups. Interestingly, this allele is associated with chlorpromazine-induced lupus anticoagulant in patients with chronic psychiatric disorders[21]. We also found that the HLA-DR7 was associated with the production of anti-phospholipid antibodies in a group of Mexican mestizo patients with systemic lupus erythematosus[22]. The differences in the associated alleles could also be explained as a result in the genetic variation due to the role

of ethnicity in certain groups.

Finally, the association of the HLA-DRB1*07 allele with p-ANCA may be a result of immunoregulatory mechanisms related to the antigen presentation of protein fragments (autoantigens) to T cells, and may also play an important role in the development of autoimmunity, including the production of several autoantibodies such as lupus anticoagulant and anti-phospholipid antibodies as mentioned above.

This novel association between HLA-DRB1*07 allele and positive p-ANCA in Mexican UC patients suggests that this allele could be a marker of severe activity in patients with UC and plays a role as genetic marker for the production of different kind of autoantibodies in our population.

In conclusion, the HLA-DRB1*07 allele plays a genetic role in the production of p-ANCA in Mexican mestizo patients with ulcerative colitis.

REFERENCES

1 **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

2 **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

3 **Yang H**, Rotter JI. Genetics of inflammatory bowel disease. In: Targan SR, Shanahan F eds. Inflammatory bowel disease: from bench to bedside. Baltimore, MD: Williams and Wilkins, 1994: 32-64

4 **Yamamoto-Furusho JK**. Immunogenetics of chronic ulcerative colitis. *Rev Invest Clin* 2003; **55:** 705-710

5 **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

6 **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

7 **Yamamoto-Furusho JK,** Uscanga LF, Vargas-Alarcon G, Ruiz-Morales JA, Higuera L, Cutino T, Rodriguez-Perez JM, Villarreal-Garza C, Granados J. Clinical and genetic heterogeneity in Mexican patients with ulcerative colitis. *Hum Immunol* 2003; **64:** 119-123

8 **Lee YT,** Sung JJ, Poon P, Lai KN, Li PK. 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

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 **Truelove SC**, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. *Br Med J* 1955; 1041-1048

11 **Bekker-Mendez C,** Yamamoto-Furusho JK, Vargas-Alarcon G, Ize-Ludlow D, Alcocer-Varela J, Granados J. Haplotype distribution of class II MHC genes in Mexican patients with systemic lupus erythematosus. *Scand J Rheumatol* 1998; **27:** 373-376

12 **Miller SA**, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16:** 1215

13 **Charron**: Genetic diversity of HLA functional and medical implication. Proceeding of the 12th International Histocompatibility Workshop and Conference 1997. Paris, France. EDK, Medical and Scientific International Publisher, 1997

14 **Wiik A**. Delineation of a standard procedure for indirect immunofluorescence detection of ANCA. *APMIS Suppl* 1989; **6:** 12-13

15 **Ludemann 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

16 **Yang H,** Rotter JI, Toyoda H, Landers C, Tyran 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

17 **Hirv K**, Seyfarth M, Uibo R, Kull K, Salupere R, Latza U, Rink L. Polymorphisms in tumour necrosis factor and adhesion molecule genes in patients with inflammatory bowel disease: associations with HLA-DR and -DQ alleles and subclinical markers. *Scand J Gastroenterol* 1999; **34:** 1025-1032

18 **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

19 **Myung SJ,** Yang SK, Jung HY, Chang HS, Park B, Hong WS, Kim JH, Min I. HLA-DRB1*1502 confers susceptibility to ulcerative colitis, but is negatively associated with its intractability: a Korean study. *Int J Colorectal Dis* 2002; **17:** 233-237

20 **Yamamoto-Furusho JK,** Uscanga LF, Vargas-Alarcon G, Rodriguez-Perez JM, Zuniga J, Granados J. Polymorphisms in the promoter region of tumor necrosis factor alpha (TNF-alpha) and the HLA-DRB1 locus in Mexican mestizo patients with ulcerative colitis. *Immunol Lett* 2004; **95:** 31-35

21 **Vargas-Alarcon G**, Yamamoto-Furusho JK, Zuniga J, Canoso R, Granados J. HLA-DR7 in association with chlorpromazine-induced lupus anticoagulant (LA). *J Autoimmun* 1997; **10:** 579-583

22 **Granados J,** Vargas-Alarcon G, Drenkard C, Andrade F, Melin-Aldana H, Alcocer-Varela J, Alarcon-Segovia D. Relationship of anticardiolipin antibodies and antiphospholipid syndrome to HLA-DR7 in Mexican patients with systemic lupus erythematosus (SLE). *Lupus* 1997; **6:** 57-62

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