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Effect of the human leukocyte antigen HLA-DRB1 and anti-cyclic citrullinated peptide on the outcome of rheumatoid arthritis patients

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Abstract

Our objective was to determine whether the presence of the human leukocyte antigen HLA-DRB1 locus is associated with production of anti-cyclic citrullinated peptide antibodies (anti-CCP Abs) and to what extent they are associated with increased susceptibility to and severity of rheumatoid arthritis (RA) in Egyptian patients. Twenty-nine RA patients gave informed consent to participate in a case-control study that was approved by the Ain Shams University Medical Ethics Committee. RA disease activity and severity were determined using the simplified disease activity index and Larsen scores, respectively. We used a wide scale national study on the pattern of HLA typing in normal Egyptians as a control study. Anti-CCP Abs and HLA-DRB1 typing were determined for all subjects. The alleles most strongly associated with RA were HLA-DRB1 [*01 , *04 and *06] (41.4%). RA patients with serum anti-CCP Ab titers above 60 U/mL had a significantly higher frequency of HLA-DRB1*01 (58.3%) and HLA-DRB1*04 alleles (83.3%). Significant positive correlations were found between serum and synovial anti-CCP Ab titer, RA disease activity, and severity ($r = 0.87$, 0.66 and 0.63, respectively; $P < 0.05$). HLA-DRB1 SE+ alleles [*01 and *04] were highly expressed among Egyptian RA patients. The presence of these alleles was associated with higher anti-CCP Ab titer, active and severe RA disease. Early determination of HLA-DRB1 SE+ alleles and serum anti-CCP Ab could facilitate the prediction of the clinical course and prognosis of RA when first evaluated leading to better disease control.

Key words: HLA-DRB1; Anti-CCP Ab; Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder in which proliferation of both resident and invading cells leads to chronic joint destruction (1). Susceptibility to RA involves genetic factors and is also modulated by environmental and non-inherited factors (2).

Previous studies have indicated that the risk of RA in siblings of affected individuals is 2-12 times higher than in siblings of unaffected individuals, pointing to a contribution of genetic factors in the pathogenesis of RA (3). An association between RA and the human leukocyte antigen (HLA) complex, also known as the major histocompatibility complex (MHC), has been observed in many different populations, and this is thought to account for approximately one third of the genetic component of RA susceptibility (4).

The greatest effect comes from the *HLA-DRB1* gene, which encodes part of an MHC class II molecule expressed on immune cells such as activated T and B cells and antigen-presenting cells (5). Extensive evidence exists showing an association between certain frequently occurring HLA-DRB1 alleles (*0101, *0102, *0401, *0404, *0405, *0408, *0410, *01001, and *1402) and the susceptibility to and severity of RA (6).

The indicated alleles share a conserved amino acid sequence [QKRAA, QRRAA or RRRAA; also called the shared epitope (SE)] at positions 70-74 in the third hypervariable region of the DRB1 chain. These residues are part of an α helical domain forming one side of the antigen-presenting binding site. According to the SE hypothesis, the SE itself is directly involved in the pathogenesis of RA by allowing the presentation of a peptide to arthritogenic T-cells (2).

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The presence and dose effect of these alleles affect the course and outcome of RA (7). The protein tyrosine kinase non-receptor (*PTPN22*) gene has been confirmed as a second RA susceptibility gene in white Caucasian populations, increasing the risk of RA by 40-70%. Interestingly, the variant has not been found in Far Eastern populations (8). Both the *HLA-DRB1* and *PTPN22* genes are important in immune function and both predispose to other autoimmune diseases, such as type 1 diabetes and systemic lupus erythematosus, as well as RA (9).

In far Eastern populations, variants of a gene coding for an enzyme involved in citrullination, the *PADI4* gene, have been consistently associated with susceptibility to RA. Whether the gene plays a role in susceptibility to RA in Caucasian populations is not known (10).

Over the past years, considerable progress has been made in identifying further RA susceptibility genes as a result of genome-wide association and candidate gene studies. Following a genome-wide association screen of ~2000 UK RA cases and ~3000 controls, an association with a region on the long arm of chromosome 6 (6q23) was confirmed in independent studies from the UK and USA. The closest gene lies some distance away and it is not clear how this particular region predisposes to RA. Another genome-wide study in a US population identified an association with a region on chromosome 9 lying between and encompassing part of the complement 5 (*C5*) and all of the TNF receptor-associated factor 1 (*TRAF1*) genes. This finding has been confirmed in independent studies from the UK, USA, Sweden, and The Netherlands. Work is underway to determine which of the two genes is primarily important in determining susceptibility since both are good candidates. Finally, in following up a region of linkage in US families, an association with the signal transducer and activator of transcription 4 (*STAT4*) gene was identified and has been confirmed in Swedish, Korean and UK populations (11). It is likely that a number of other RA susceptibility genes will emerge as investigations continue.

A prominent feature of RA is the presence of various types of disease-specific and -nonspecific autoantibodies (12). Best known are the rheumatoid factors (RFs), which are antibodies to the FC portion of IgG molecules. Although they are not disease specific, detection of IgM-RF is routinely used in the diagnosis of RA (60-70% sensitivity, 80-90% specificity) (13).

Assays have been recently developed to detect IgG antibodies against protein side chains in which an arginine residue is deaminated to peptidyl citrulline (14). This process is catalyzed by the peptidyl arginine deiminase (PAD) enzymes, which are expressed in RA predominantly in monocytes and macrophages of the synovial membrane (15).

Normally, PAD enzymes are present intracellularly as inactive enzymes (16). Calcium ions are required for activation of the PAD enzymes, but the intracellular calcium concentration in normal cells is much lower than the threshold calcium concentration for PAD activity. During cell death, the integrity of the plasma membrane is lost, causing influx of calcium from the extracellular space and subsequent activation of intracellular PAD. Alternatively, PAD enzymes may leak out of the dying cells, become activated and causing citrullination of extracellular proteins. Many cells in the inflamed synovium have fragmented DNA, which is a sign of apoptosis. PAD enzymes may become activated by intracellular calcium (17).

Although many different citrullinated proteins may be present in the RA synovium, thus far three proteins can be considered as candidate autoantigens in RA: citrullinated fibrin, citrullinated vimentin and citrullinated histones (18).

In RA, early diagnosis and intervention is crucial in preventing irreversible joint damage, and therefore a reliable serological marker is needed. Antibodies (Abs) directed at citrullinated proteins provide this ability. The most sensitive assay for the detection of these antibodies is the determination of the so-called anti-cyclic citrullinated peptide (CCP) by ELISA. The diagnostic value of these antibodies is particularly evident in early disease and they are usually detectable at the onset of the disease or even years prior to the onset of the clinical symptoms (19).

Compared to IgM-RF, anti-CCP Abs have a higher specificity (98%) and a similar sensitivity (68-90%) (13). These antibodies are produced at the site of inflammation in RA mainly in the synovium, suggesting that they might play a role in the disease process (18). Therefore, citrullinated antigens are also expected to be present in the inflamed synovium.

Although citrullination of arginine residues of proteins may result in the formation of epitopes that are targets for Abs, help from T lymphocytes is likely to be required for long-term B cell responses and antibody isotype switching (16).

In mice transgenic for the SE allele HLA-DRB1*0401, it was demonstrated that citrullination of peptides could lead to the activation of CD4+ T cells, most likely as a result of increased binding of these citrullinated peptides to MHC class II molecules (13). There are differences in the strength of association between HLA-DRB1 SE+ alleles and RA in different populations, and reports on HLA association and anti-CCP Abs have been few and conflicting for the Egyptian population.

The aim of the present study was to determine whether the presence of certain HLA-DRB1 alleles is associated with the production of anti-CCP Abs and to determine to what extent certain HLA alleles and anti-CCP Abs can be associated with increased susceptibility to and severity of RA in a group of 29 Egyptian RA patients.

Subjects and Methods

Twenty-nine RA patients randomly recruited in the

internal medicine department and rheumatoid arthritis outpatient specialized clinic at Ain Shams University Hospital participated in the present case-control study. All of them fulfilled the criteria of the American College for Rheumatology for the diagnosis of RA (20). None of the patients were suffering from any other rheumatological disease or other major illness.

Fifteen normal healthy age- and sex-matched volunteers were included in the study as a control group. All subjects gave written informed consent to participate in the study, which was approved by Ain Shams Medical Ethics Committee.

Patients and controls were subjected to the following procedures: full medical history; thorough clinical examination including general, musculoskeletal and other systems; assessment of RA disease activity by the simplified disease activity index score for RA (SDAI) (21); standardized X-rays of both hands and wrists, and assessment of degree of joint damage by the Larsen score (22).

Samples

Venous blood (8 mL) was withdrawn from each subject and 5 mL was placed in EDTA for a complete blood count, for the determination of erythrocyte sedimentation rate (ESR) and for HLA-DRB1 typing. A 3-mL aliquot was placed in a clean flat tube and serum was separated and kept at -20°C until used.

Synovial fluid was aspirated from effusions of large joints (knee joint) in each patient and centrifuged at 3000 *g* for 20 min. The supernatant was separated and stored at -20°C until analysis for anti-CCP Abs.

Clinical and laboratory tests

Complete blood count: [Coulter counter (T660)]. ESR: by the Westergren method. C-reactive protein (CRP): by Avitex (Omega Diagnostics, Ltd., UK). RF: by turbiquant RF (Dad Behring Marburg GmbH D-35041, Germany) using Behring Turbitimer. Polystyrene particles coated with fragments of human gamma globulin form aggregates when mixed with samples. For measurements from day to day (N = 10), the coefficient of variation ranged from 3.7 to 8.2%. Serum and synovial anti-CCP IgG assay: by ELISA (Quanta Lite™ CCP3 IgG ELISA supplied by INOVA Diagnostics, Inc., USA). HRP CCP3 IgG conjugate (goat) anti-human IgG is supplied in the kit. The samples and calibrators were assayed in duplicate and the mean values of all duplicate readings were determined. Results were quantified by drawing 5 point standard curve; using the calibrators supplied in the kit (from A to E) their concentrations were 250, 125, 62.5, 31.25, and 15.62 units, respectively. The mean absorbance of all samples was plotted on the standard curve and their concentrations (in units) were determined. Anti-CCP Ab titer: <20 U/mL was considered negative, a 20-39 U/mL titer weakly positive, a 40-59 U/mL titer moderately positive, and a >60 U/mL titer strongly positive. Between-run assay variation was measured by running duplicates of negative, low positive and strong positive samples in 6 separate assays on 6 different days. CV% (negative, low positive and strong positive) were 0, 4 and 4%, respectively.

HLA-DRB1 typing

DR "low resolution" typing was performed by the polymerase chain reaction with sequence-specific primers (PCR-SSP) using Dynal-SSP (Dynal A.S., Norway).

DNA extraction. DNA was extracted using the GIFXTM genomic blood DNA purification kit (Amersham Pharmacia Biotech Inc., USA).

PCR amplification. Each PCR mixture contained 2-4 allele- or group-specific DR primers and the internal positive control primer pair at a 5-fold lower concentration.

The PCR mixture (10 µL) consisted of 100 ng genomic DNA (50 ng/µL), SSP master mix (containing PCR buffer, dNTP'S, glycerol and cresol red, and 0.5 unit Taq polymerase.

The PCR amplifications were carried out in a Gene Amp PCR system 9600 (Perkin-Elmer Cetus instruments, USA).

Conventionally extracted DNAs were amplified with 31 temperature cycles. Each cycle consisted of denaturation at 94°C for 50 s, annealing at 65°C for 50 s, and extension at 72°C for 20 s.

Detection of PCR products

PCR products were detected by agarose gel electrophoresis. Gels were examined under UV illumination and documented photographically.

*Subtyping of HLA-DRB1*04*. Positive samples were analyzed using Dynal DRB1*04 PCR-SSP subtyping, and the SE alleles were defined as HLA-DRB1*0401 or 0404.

Statistical analysis

Data were analyzed statistically using the SPSS version 12 software as follows: description of quantitative variables as means ± SD and range; description of qualitative variables as number and percentages. The Fisher exact probability test was used instead of the chi-square test. The unpaired *t*-test was used to compare two groups regarding quantitative variables. Spearman rank correlation test was used to correlate categorical parameters. A probability level of $P < 0.05$ was considered to be significant (23).

Results

Twenty-nine RA patients were included in the study. The mean ± SD age was 35.4 ± 9.7 years and all were women. Fifteen age- and gender-matched healthy volunteers (mean age 35.8 ± 10 years) were used as the control group. Variables concerning RA patients are reported in Table 1.

RA patients had significantly higher serum anti-CCP Ab and RF titers than their controls (anti-CCP Ab: 57.7

± 25, 11.1 ± 3.7 µ/mL, RF titer: 49 ± 14, 26 ± 4.4 IU/mL respectively, P < 0.05) while the two groups did not differ significantly in age.

The controls presented in Table 2 were taken from a large-scale national study by Khalil et al. (24) who investigated 541 normal Egyptians for the most frequent HLA-DR alleles. We observed that the alleles most strongly associated with increased susceptibility to RA in our 29 patients were HLA-DRB1*01, *04, [*13, *14(DR6)] (41.4%) and *10 (10.3%) since there was a significant difference between RA patients and controls, whereas the HLA-DRB1*08 allele was not associated with RA susceptibility since it was expressed only among controls (Table 2).

Subtyping of HLA-DRB1*04 and *01 demonstrated that the (SE+) genotypes most strongly associated with RA susceptibility were HLA-DRB1*0101 and *0102 (50%), followed by DRB1*0404 (33.3%) and *0401 (25%).

Our RA patients with serum anti-CCP Ab titers above 60 U/mL presented a higher frequency distribution of the HLA-DRB1*01 and *04 alleles, which was significant only in DRB1*04 (*0401, *0404). In contrast, our RA patients with serum anti-CCP Ab titers below 60 U/mL had a higher frequency distribution of the HLA-DRB1*03, [*11,*12(DR5)] and [*13, *14(DR6)] alleles (Table 3).

There was a significantly higher expression of the HLA-DRB1*01 and *04 alleles (*0401 and *0404) among the RA patients with active disease, while the HLA-DRB1*03, [*11, *12(DR5)] and [*13, *14(DR6)] alleles were expressed more in our RA patients with inactive disease (Table 4).

The HLA-DRB1*04 and *01 (*0401, *0404, *0101 and *0102) alleles were expressed more in our RA patients with erosive arthritis, while HLA-DRB1*03, [*11, *12(DR5)] and [*13, *14(DR6)] alleles were expressed more among our RA patients with non-erosive arthritis (Table 5).

There were significant positive correlations between serum anti-CCP Ab titers and RA disease activity and severity as determined by SDAI score (r = 0.66, P < 0.01; Figure 1) and Larsen score (r = 0.63, P < 0.05). Moreover, there was a significant positive correlation between serum and synovial anti-CCP Ab titers (r = 0.87, P < 0.05).

Table 1. Some characteristics of the 29 rheumatoid arthritis patients.

	Means ± SD	Positive cases	Range
Age (years)	35.4 ± 9.7		21-52
Disease duration (years)	6.9 ± 2.7		1-12
SDAI score	32.7 ± 13		14-52
IgM-RF (IU/mL)	49 ± 14	23 (79%)	15-80
ESR (mm/h)	63 ± 22		25-95
Larsen score	3 ± 0.8		1-4
Serum anti-CCP Ab (U/mL)	57.7 ± 25	18 (62%)	13-110
Synovial anti-CCP Ab (U/mL)	84 ± 27	20 (69%)	25-120

Data are reported as means ± SD or number with percent within parentheses. SDAI score = simplified disease activity index; RF = rheumatoid factor; ESR = erythrocyte sedimentation rate; anti-CCP Ab = anti-cyclic citrullinated peptide antibodies.

Table 2. Frequency distribution of HLA-DR alleles among rheumatoid arthritis (RA) patients and controls.

HLA-DR alleles	RA patients (N = 29)	Controls (N = 541)
DRB1*01	41.4%$^{+}$	5%
DRB1*15, *16(DR2)	6.9%, 3.4%	7%, 4%
DRB1*03	17.2%	19%
DRB1*04	41.4%$^{+}$	16%
DRB1*11, *12(DR5)	13.8%, 3.4%	12%, 2%
DRB1*13, *14(DR6)	27.6%$^{+}$, 13.8%$^{+}$	16%, 5%
DRB1*07	10.3%	6%
DRB1*08	0%$^{+}$	7%
DRB1*10	10.3%$^{+}$	3%

The data for controls were taken from Khalil et al. (24). DR2, DR5, DR6 are equivalent to typing serologically. $^{+}$P < 0.05 compared to controls (Student *t*-test).

Table 3. HLA-DRB1 allele distribution among rheumatoid arthritis patients with serum anti-CCP antibody titers <60 U/mL and >60 U/mL.

HLA-DRB1 alleles	Serum anti-CCP Abs (U/mL)	
	<60 (N = 15)	>60 (N = 14)
*01 (N = 12)	5 (41.7%)	7 (58.3%)
*15, *16(DR2) (N = 3)	3 (100%)	0
*03 (N = 5)	4 (80%)	1 (20%)$^{+}$
*04 (N = 12)	2 (16.7%)	10 (83.3%)$^{+}$
*0401	0	3 (100%)$^{+}$
*0402	1 (100%)	0
*0403	0	1 (100%)
*0404	0	4 (100%)$^{+}$
*0405	1 (100%)	0
*0408	0	1 (100%)
*0409	0	1 (100%)
*11, *12(DR5) (N = 5)	4 (80%)	1 (20%)$^{+}$
*13, *14(DR6) (N = 12)	9 (75%)	3 (25%)$^{+}$
*07 (N = 3)	3 (100%)	0
*10 (N = 3)	3 (100%)	0

Anti-CCP Abs = anti-cyclic citrullinated peptide antibodies. $^{+}$P < 0.05 compared to <60 U/mL (Student *t*-test).

Table 4. HLA-DRB1 allele distribution among rheumatoid arthritis patients with active and inactive disease as determined by simplified disease activity index score.

HLA-DRB1 alleles	Disease activity	
	Inactive (<20) (N = 16)	Active (>20) (N = 13)
*01 (N = 12)	4 (33.3%)	8 (66.7%)+
*15, *16(DR2) (N = 3)	3 (100%)	0
*03 (N = 5)	4 (80%)	1 (20%)+
*04 (N = 12)	3 (25%)	9 (75%)+
*0401	0	3 (100%)+
*0402	0	1 (100%)
*0403	1 (100%)	0
*0404	0	4 (100%)+
*0405	1 (100%)	0
*0408	0	1 (100%)
*0409	1 (100%)	0
*11, *12(DR5) (N = 5)	5 (100%)	0+
*13,*14(DR6) (N = 12)	10 (83.3%)	2 (16.7%)+
*07 (N = 3)	3 (100%)	0
*10 (N = 3)	3 (100%)	0

+P < 0.05 compared to inactive (<20) disease activity (Student *t*-test).

Table 5. HLA-DRB1 allele distribution among rheumatoid arthritis patients with erosive and non-erosive arthritis as determined by Larsen score.

HLA-DRB1 alleles	Larsen score	
	Non-erosive (N = 15)	Erosive (N = 14)
*01 (N = 12)	6 (50%)	6 (50%)
*15, *16(DR2) (N = 3)	2 (66.7%)	1 (33.3%)
*03 (N = 5)	4 (80%)	1 (20%)+
*04 (N = 12)	3 (25%)	9 (75%)+
*0401	0	3 (100%)+
*0402	1 (100%)	0
*0403	0	1 (100%)
*0404	0	4 (100%)+
*0405	1 (100%)	0
*0408		1 (100%)
*0409	1 (100%)	0
*11, *12(DR5) (N = 5)	4 (80%)	1 (20%)+
*13, *14(DR6) (N = 12)	10 (83.3%)	2 (16.7%)+
*07 (N = 3)	3 (100%)	0
*10 (N = 3)	2 (66.7%)	1 (33.3%)

+P < 0.05 compared to non-erosive arthritis by Larsen score (Student *t*-test).

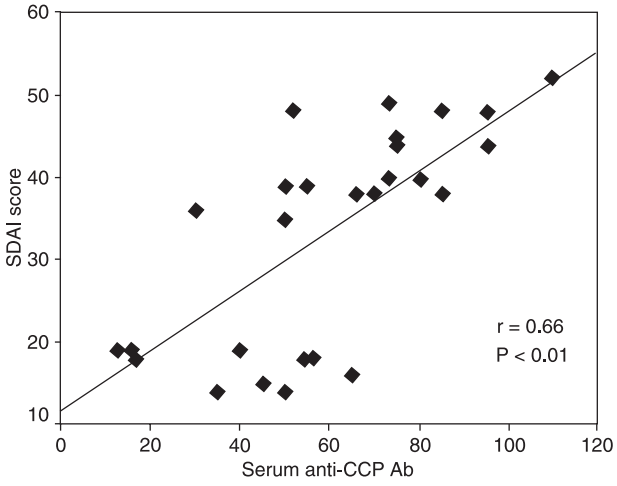

Figure 1. Correlation between serum anti-CCP Ab and SDAI score for 29 patients with rheumatoid arthritis. Anti-CCP Ab = anti-cyclic citrullinated peptide antibody; SDAI = simplified disease activity index score for rheumatoid arthritis (Spearman rank correlation test).

Discussion

HLA-DR genes are the principal genetic factors contributing to RA. To study the genetic and immunologic background of susceptibility to and severity of RA, 29 RA patients were subjected to HLA-DRB1 typing and to testing with anti-CCP Abs.

In the present study, HLA-DRB1 [*15, *16(DR2)], *03, [*11, *12(DR5)] and *07 alleles were found to be the most prevalent alleles among healthy Egyptian subjects. HLA genotyping analysis revealed that certain HLA haplotypes (HLA-DRB1*01, HLA-DRB1*04 [DRB1*0401, *0404] [*13, *14(DR6)] and *10) were associated with clinical RA. Thus, these alleles may be recognized as alleles related to susceptibility to RA. Kaltenhauser et al. (25) obtained similar results showing that the HLA-DRB1*04 group was highly associated with RA.

Roudier (26) reported that the HLA-DRB1 alleles *0401, *0404, *0405 (serologically HLA-DR4), DRB1*0101, *0102 (HLA-DR1) and DRB1* 1001 (HLA-DR10) were associated with RA. HLA-DR genotypes (the two *HLA-DRB1* genes expressed by any individual) determine the risk to develop RA. Double-dose genotypes such as DRB1*0401 and *DRB1*0404 carry a higher risk to develop RA (it is very difficult to find healthy controls with the DRB1*0401/DRB1*0404 genotype), whereas “single-dose” genotypes such as (DRB1*0401/DRB1*07) carry more limited risks (27).

On the other hand, we found that DRB1*08 alleles were present in healthy controls and not in RA patients. This agreed with data reported by van der Helm-van Mil et al. (28) and Barnetche et al. (29) who defined HLA-DRB1*07,

*08, *1201, and *1501 as alleles that protect against RA. These alleles contain, instead of SE, another common anchor region consisting of the amino acids DERAA.

Anti-CCP Abs have recently emerged as sensitive and specific serological markers for RA, providing a superior alternative to the RF test in the laboratory diagnosis of RA (30). Anti-CCP Abs might be positive even before the appearance of the first clinical manifestation of the disease (30). In the present study, measurement of serum anti-CCP Abs in the subjects investigated and synovial anti-CCP Abs in RA patients revealed that serum anti-CCP Abs were significantly higher in RA patients than in controls ($P < 0.05$). Interestingly, there was a significantly higher synovial anti-CCP Ab titer than serum anti-CCP Ab titer in RA patients, with a significant positive correlation between them ($r = 0.87$, $P < 0.05$, Spearman rank correlation test).

A possible explanation is that many different citrullinated proteins are present in the synovium of RA patient, being possible candidates for autoantibody production. The fact that levels of synovial anti-CCP Abs were higher than serum levels suggests diffusion of the locally produced antibodies from the synovium to the periphery (31).

The presence of these Abs in RA patients is probably the result of an abnormal but specific humoral immune response to citrullinated proteins. The subclass distribution of anti-citrullinated protein Abs (predominantly IgG1) is indicative of T cell-dependent antibody production and thus suggests HLA involvement. Vossenaar et al. (16) found that specific HLA haplotypes (HLA-DR4 [DRB1*0401 and DRB1*0404]) confer a genetic predisposition to RA. It was recently shown that citrullinated peptides can be bound much more efficiently by DR4 molecules than by corresponding non-citrullinated peptides (32). This citrulline-specific interaction might be the basis of a citrulline-specific immune response.

In the present study, there was a strong association between HLA-DR4 status and anti-CCP Ab positivity in RA patients. Additionally, we tried to determine the influence of SE alleles on serum titers of anti-CCP antibodies in serum (and hence in the synovial fluid). We observed that the SE-positive alleles DRB1*01, DRB1*04 (*0401, *0404) were associated with production of higher titers (>60 U/mL) of anti-CCP antibodies, which was only statistically significant with HLA-DRB1*04 (*0401, *0404). These results agree with Berglin et al. (33), Kaltenhauser et al. (25), and Hughes et al. (34) who found that the HLA-DRB1*01, DRB1*04 (0401, *0404, and *0405) SE alleles conferred the highest risk for development of anti-CCP Abs in RA patients.

Correa et al. (35) found a highly significant association between anti-CCP and HLA-DRB1*04 and a weaker but still significant association with HLA-DRB1*01. Moreover, they assumed that HLA-DRB1*0401 is not a prerequisite for anti-CCP Ab production, but if HLA-DRB1*0401 was present, 90% of their RA patients were found to have positive anti-CCP Ab titers.

The pathogenetic mechanisms that cause the SE association of anti-CCP Abs are unclear. It has been suggested that the binding of antigenic peptides to MHC class II alleles might be facilitated by citrullination of arginine residues. Thus, it appears that genetic factors (HLA-DR4) are involved in the process that determines whether or not anti-citrullinated protein antibodies are made (4).

Meanwhile, it was found that HLA-DRB1*03, [*11, *12(DR5)], [*13, *14(DR6)] alleles were associated with lower titers of anti-CCP Abs (below 60 U/mL), inactive disease and non-erosive arthritis ($P < 0.05$). This was in accordance with Irigoyen et al. (36) who found that the HLA-DRB1*03 allele was associated with lower titers of anti-CCP Abs even in the presence of the SE+ allele.

When we studied the distribution of HLA-DRB1 SE+ alleles among RA patients with active and inactive disease (measured by SDAI score) we observed that HLA-DRB1*01 and DRB1*04 (*0401, *0404) were significantly more expressed among patients with active disease than among those with inactive disease, who also had significantly higher anti-CCP Ab titers. In contrast, HLA-DRB1*03, [*11, *12(DR5)] and [*13, *14(DR6)] were significantly more expressed among inactive cases who had also significantly lower anti-CCP Ab titers. These findings agree with those reported by Gourraud et al. (7) who found an association between HLA-DRB1*0404 alleles and RA disease activity and severity.

In the present study, there was a highly significant positive correlation between serum anti-CCP Abs and SDAI score, in agreement with Berglin et al. (33) who suggested that repeated measurement of serum anti-CCP Ab titers could be of clinical significance for assessing disease activity and severity. These investigators found that the titers declined in patients with a good therapeutic response and decreased disease activity.

The presence of elevated anti-CCP Ab titers, together with certain HLA-DRB1 SE alleles (DRB1*0401, *0404), increased the relative risk for the development of RA (9). Moreover, these higher titers have been implicated in worse outcomes, particularly increased erosions (36).

In the present study, we found that the HLA-DRB1*04 (*0401, *0404) alleles were significantly expressed among RA patients with erosive arthritis and that there was a significant positive correlation between serum anti-CCP Ab titers and the presence of erosive aggressive disease. This agrees with Gourrand et al. (7) who reported that HLA-DRB1*0401 appeared to be the allele with the highest activity predisposing to erosive RA in Northern European Caucasoids. This also agrees with Kaltenhauser et al. (25), who noticed a faster progression of joint destruction in RA in the presence of high anti-CCP Ab titers and assumed its superiority over RF-IgM to be a prognostic marker of radiologic progression.

Similarly, van Gaalen et al. (13) and Berglin et al. (33) found that the presence of HLA susceptibility alleles and

anti-CCP Abs in high titers heralded a more severe disease course. They concluded that anti-CCP+, SE+ patients at baseline had a significantly higher rate of joint destruction than did all other patients. The rate of joint destruction did not differ between anti-CCP+, SE- patients and anti-CCP-, SE+ patients (P = 0.01) or anti-CCP- and SE- patients (P = 0.09). This means that a higher rate of joint damage was only found when both anti-CCP Abs and SE alleles were present. The possible explanation might be the higher mean ± SD levels of anti-CCP Abs in SE allele-positive patients. Huizinga et al. (37) reported that the number of copies of the SE allele was associated with the presence of erosive disease among patients with positive anti-CCP Abs but not among those with negative anti-CCP Abs.

The correlation between inflammation and joint damage has been extensively studied, especially in terms of the relevance of inflammatory variables such as CRP and ESR. Although there is a link between inflammation and the development of joint damage, it is well established that damage may progress in spite of decreased inflammatory activity, and erosions may develop in patients who have few clinical signs of inflammation. Thus, it has been suggested that pathological processes other than inflammation are involved in the destructive process (38). Lindqvist et al. (39) suggested that a combination of ESR, CRP, HLA-DRB1 SE, RFs, and anti-CCP Abs might provide prognostic information about the development of joint damage in a prospective study of RA patients.

Finally, the established role of anti-CCP Abs in the susceptibility and severity of RA permits the development of therapeutics targeted against these antibodies. Such therapeutics are aimed at reducing the severity of RA course and perhaps may prevent the onset of clinically significant arthritis in genetically susceptible individuals.

Conclusion and Recommendations

HLA-DRB1 SE+ alleles (*01, *04 [*0401, *0404, *0101, and *0102]) were highly expressed in the group of 29 Egyptian RA patients studied. They were strongly associated with the production of high titers of the highly specific anti-CCP Ab, which could be involved in the disease process. The presence of high anti-CCP Ab titers was associated with more active, aggressive and erosive disease as determined by SDAI and Larsen scores.

Early determination of HLA-DRB1 SE+ alleles and anti-CCP Ab titer may facilitate the prediction of disease course and prognosis at the time of initial presentation, being of help for the initial treatment plan directed at the prevention of permanent joint damage.

Given the polymorphic nature of HLA genes, large numbers of RA patients are needed for a better assessment of the role of each allele in the susceptibility to RA and to establish exactly which citrullinated peptide antigens are driving the anti-CCP Ab response in RA patients and how these particular antigens interact with various HLA-DRB1 SE+ alleles. Further studies are needed to highlight the interaction of the non-genetic environmental triggers of RA with the genetic factors, since both may contribute to the immune response to citrullinated proteins, the key step in the pathogenesis of rheumatoid arthritis.

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