Associations of Erosive Arthritis with Anti-Cyclic Citrullinated Peptide Antibodies and MHC Class II Alleles in Systemic Lupus Erythematosus

MADELYNN T. CHAN, PATRICIA OWEN, JULIET DUNPHY, BEVERLEY COX, CHARLOTTE CARMICHAEL, ELEANOR KORENDOWYCH, and NEIL J. McHUGH

ABSTRACT. ***Objective.*** To determine the associations of erosive arthritis (EA) with anti-cyclic citrullinated peptide (anti-CCP) antibodies and major histocompatibility class (MHC) II alleles in systemic lupus erythematosus (SLE).

Methods. One hundred four patients with SLE were evaluated for arthritis and classified as EA, nonerosive arthritis, or no arthritis. EA was further classified as major or minor erosions. Sera from patients and 130 serum controls were tested for anti-CCP2 and rheumatoid factor (RF). Patients and 117 genetic controls were genotyped for HLA-DRB1 and HLA-DQB1. Statistical associations were tested using chi-square tests and odds ratios (OR) with 95% confidence intervals (CI).

Results. Eight patients (8%) were anti-CCP+ and they accounted for 11% (8/71) of patients with synovitis. Twelve patients (11%) had EA. Among patients with synovitis, EA was associated with anti-CCP (OR 28.5, 95% CI 4.7–173.8, p = 0.001), with a weaker association for RF (p = 0.3). Six patients with EA had major erosions and also met criteria for rheumatoid arthritis (RA). Four of these patients (67%) were anti-CCP+. HLA-DQB1*0302 was associated with EA (p = 0.01), with similar trends for HLA-DRB1*0401 and 2 copies of the shared epitope (SE). There were trends for associations of HLA-DQB1*0302 and 2 SE copies with anti-CCP production.

Conclusion. The frequency of EA in SLE is likely to be higher than previously reported. Anti-CCP+ patients with SLE are more likely to have EA. Anti-CCP may be a useful serological marker for EA for patients presenting with synovitis. Anti-citrulline antibodies may have a pathogenic role in the development of major erosions, resulting in clinical features that overlap SLE with RA (rhupus). (First Release Dec 15 2007; J Rheumatol 2008;35:77–83)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS ARTHRITIS GENETIC PREDISPOSITION
ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODY BIOLOGICAL MARKERS

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by the production of specific autoantibodies linked to distinct clinical subsets. For example, anti-double stranded DNA (anti-dsDNA) antibodies crossreacting with alpha-actinin are associated with lupus glomerulonephritis[1], anticardiolipin antibodies are associated with venous thrombosis[2], and anti-Ro/SSA antibodies are associated with subacute cutaneous lupus[3], neonatal lupus, and congenital heart block[4].

Synovitis is a common clinical feature of SLE, with erosive arthritis (EA) affecting 5% of patients[5-7]. It would be useful to have a serological marker that could predict the development of EA, which is generally associated with a worse functional outcome. Recently, the anti-cyclic citrullinated peptide (anti-CCP) antibody was found to be a useful serological marker for rheumatoid arthritis (RA). Anti-CCP antibodies are present years before disease onset[8] and are reported to be highly specific for RA[9]. However, these antibodies are also detected in other autoimmune diseases including psoriatic arthritis (PsA)[10], juvenile idiopathic arthritis (JIA)[11], and SLE[7]. In both RA and PsA, anti-CCP antibodies are associated with radiographic disease progression[10,12].

Clinical subsets of SLE are also influenced by genetic factors. Lupus nephritis is associated with hereditary complement C4A deficiency[13], and with the Fc-γ RIIIA-V/F158 polymorphism[14]. The absence of the major histocompatibility complex (MHC) class II allele HLA-DQB1*0201 is a significant predictor for lupus nephritis[15]. In RA, disease suscepti-

From the Department of Rheumatology, Royal National Hospital for Rheumatic Diseases; and Bath Institute for Rheumatic Diseases, Bath, United Kingdom.

Supported in part by grants from the Royal National Hospital for Rheumatic Diseases and the NHS Executive South West Research and Development Directorate and The Health Foundation.

M.T-L. Chan, MBBS, FRACP, Clinical Research Fellow in Rheumatology; P. Owen, FIBMS, Biomedical Scientist; J. Dunphy, BA(Hons), Biomedical Scientist; B. Cox, PhD, Biomedical Scientist; C. Carmichael, BSc(Hons), Database Manager; E. Korendowych, PhD, MRCP, Consultant Rheumatologist; N.J. McHugh, MD, FRACP, FRCP, FRCPath, Consultant Rheumatologist.

Address reprint requests to Dr. N.J. McHugh, Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath, BA1 1RL, UK.
E-mail: Neil.McHugh@rnhrd-tr.swest.nhs.uk

Accepted for publication September 2, 2007.

bility and severity are associated with several MHC Class II alleles collectively termed the shared epitope (SE)[16,17]. The SE comprises alleles that code for a highly conserved 5-amino acid sequence in the third hypervariable region of the HLA-DRB1 molecule. However, recent evidence suggests that the SE may not be associated with RA specifically, but rather with a phenotype of progressive, erosive disease mediated through anti-CCP production[18].

The aim of our study was to determine the association of EA with anti-CCP antibodies and with MHC Class II alleles in patients with SLE.

MATERIALS AND METHODS

Patients and controls. We studied 104 subjects with SLE from a database of patients attending the Connective Tissue Disease Clinic at the Royal National Hospital for Rheumatic Diseases, a tertiary rheumatology center in the UK. All patients fulfilled the revised American College of Rheumatology (ACR) classification criteria (1997) for SLE[19,20]. Patients were followed for an average of 15 years (range 2–48 yrs). Ethical approval for the study was given by the Bath Local Research Ethics Committee, and written informed consent was given by all participants. Clinical data collected for each patient included the documentation of the presence of joint synovitis at any time in the course of the disease. Patients who experienced joint symptoms had radiographs of hands and feet taken during their routine clinic visits, with radiographs repeated at a minimum of yearly intervals (up to 9-year intervals), according to the treating clinician's decision. Available radiographs were reviewed to determine the presence of joint erosions. Patients with synovitis were designated as having "erosive arthritis" (EA) attributable to an inflammatory arthropathy, or "nonerosive arthritis" (NEA), according to the presence or absence of joint erosions on radiographs. Each patient with EA was then assigned as having major erosions or minor erosions according to the size and extent of the erosions. Patients with EA were also assessed for RA according to ACR criteria[21]. All remaining patients without joint synovitis were designated as having "no arthritis" (NA). Blood samples were collected from all patients for genetic studies and serological tests.

Serum samples from 130 age and sex-matched healthy local blood donors were selected as serum controls. Seventy-six blood samples from the 130 serum controls were available for genotyping and a further 41 samples from sex-matched local blood donors were selected as genetic controls (total n = 117). All serum and genetic controls were British Caucasian in ethnicity.

Autoantibody measurement. Antinuclear antibodies (ANA) were measured by indirect immunofluorescence on Hep-2 cells. Anti-CCP2 IgG antibodies and IgM rheumatoid factor (RF) autoantibodies were measured using commercial ELISA kits (Inova Diagnostics, San Diego, CA, USA) in sera from SLE patients and serum controls. Anti-dsDNA antibodies from SLE serum samples were measured by commercial ELISA kits (Cambridge Life Sciences, Ely, UK). All ELISA tests were performed in duplicate. Absorbances were determined using a commercial microplate photometer (Multiskan Ascent, Labsystems, Turku, Finland). Unequivocal readings of ≥ 25 units (U) for anti-CCP and of ≥ 6 U for RF were assigned positive. Antibodies to extractable nuclear antigens (including U1RNP, Sm, Ro/SSA, and La/SSB) were measured by Ouchterlony double-diffusion.

HLA-DRB1 and HLA-DQB1 genotyping. All 104 SLE and 117 genetic control whole-blood samples were collected into ethylenediamine tetraacetate (EDTA) tubes. Genomic DNA was extracted using a standard salting-out procedure. HLA-DRB1 and -DQB1 alleles were identified from the extracted DNA using a polymerase chain reaction-based method with sequence-specific primers (PCR-SSP), as described[22]. PCR products were analyzed on 2% agarose gel stained with ethidium bromide. The SE alleles were assigned positive if HLA-DRB1*0101, *0102, *0401, *0405, *0408, and *1001 were present[16]. We also investigated the presence of the SLE-associated alleles HLA-DRB1*0301 and HLA-DQB1*0201[22], as well as HLA-DQB1*0302, a genetic marker of disease severity in RA[23].

Statistical analysis. Statistical analysis was performed using SPSS-12 software (SPSS Inc., Chicago, IL, USA). Comparisons were made with 2 × 2 contingency tables using the chi-square test, with odds ratios (OR) and their 95% confidence intervals (95% CI). Where expected numbers for the contingency tables were less than 5, Fisher's exact test was used. For nonparametric comparisons, Mann-Whitney U and Kruskal-Wallis tests were used, with means and interquartile ranges (IQR) quoted. A p value < 0.05 was considered to represent a significant difference between groups, with Bonferroni corrections for multiple comparisons.

RESULTS

Clinical features of SLE patients. We found that 71 of our 104 SLE patients (68%) had experienced synovitis, of whom 12 patients had EA (11%) and 59 had NEA (57%). The remaining 33 patients had NA (32%). Table 1 shows the clinical features of the 3 patient groups. The average age of patients with EA was younger (46 yrs) compared to the other 2 groups (NEA 50 yrs, NA 49 yrs; $p = 0.6$). There was a trend toward longer disease durations for the EA group with a mean of 20 years, compared with 13 years for the others. There were no significant differences in ethnicity among the groups, with 102 patients of British Caucasian descent and 2 of Afro-Caribbean origin. All patients with EA were female, compared with 88% of the NEA group and 80% of the NA group. Patients with EA tended to have more skin involvement, serositis, and hematological disorders than the other groups. No patient with EA had neuropsychiatric disease and only one had renal disease. These differences did not reach statistical significance (Table 1).

Serology of SLE patients. All 104 SLE patients were ANA-positive. Eight (8%) patients were anti-CCP+, compared with 2/130 (1.5%) anti-CCP+ serum controls (OR 5.3, 95% CI 1.1–25.7, $p = 0.02$). There were 18/104 (17%) RF+ patients versus 4/130 (3%) RF+ controls (OR 6.6, 95% CI 2.2–20.2, $p < 0.0001$). Anti-CCP was associated with RF (OR 5.9, 95% CI 1.3–26.2, $p = 0.03$). Table 2 shows the serology results of the 3 patient groups. Among patients with synovitis (comprising 12 EA and 59 NEA), 6/71 (11%) were anti-CCP+ and 5/71 (15%) were RF+. When patients with EA were compared against all other SLE patients, anti-CCP was significantly associated with EA [6/12 (50%) anti-CCP+ patients with EA vs 2/92 (2%) anti-CCP+ all other SLE; OR 45.0, 95% CI 7.4–272.5, $p < 0.0001$]. Anti-CCP remained strongly associated with EA among the 71 patients with synovitis (OR 28.5, $p_{corrected} = 0.01$; Table 2). There was a weaker association of RF with EA ($p = 0.3$). No patient with EA had anti-Ro/SSA or anti-La/SSB antibodies. There were more anti-U1RNP+ patients with EA (58%) compared with the other groups, although this was not statistically significant.

Characteristics of SLE Patients with EA. Table 3 shows the characteristics of the 12 patients with EA. Six patients (50%) had major erosions on radiographs. The earliest erosions occurred after a mean of 11 years for all patients, with no dif-

Table 1. Clinical features of patients with SLE (n = 104).

Feature	Erosive Arthritis, n = 12, n (%) or mean (IQR)	Nonerosive Arthritis, n = 59, n (%) or mean (IQR)	No Arthritis, n = 33, n (%) or mean (IQR)	p (corrected)*
Age, yrs	46 (33–55)	50 (40–59)	49 (38–57)	0.6
Disease duration, yrs	20 (14–23)	13 (9–22)	13 (7–19)	0.01 (0.1)
Race, British Caucasian	12 (100)	57 (97)†	33 (100)	0.6
Sex, female	12 (100)	52 (88)	27 (82)	0.3
Skin involvement	10 (83)	39 (67)	26 (79)	0.4
Serositis	6 (50)	20 (34)	4 (36)	0.5
CNS	0 (0)	8 (14)	8 (24)	0.1
Renal	1 (8)	7 (12)	9 (27)	0.1
Hematological disorder	10 (83)	43 (73)	25 (76)	0.8
Mean no. SLE criteria	5.2 (4–6)	5.8 (4–7)	5.4 (5–6)	0.2

* Comparisons made with all 3 groups. † 2 patients (3%) were of Afro-Caribbean descent. CNS: central nervous system involvement.

Table 2. Serology of patients with SLE (n = 104).

Feature	Synovitis, n = 71						
	Erosive Arthritis, n = 12, n (%)	Nonerosive Arthritis, n = 59, n (%)	OR (95% CI)*	p (corrected)	No Arthritis, n = 33 n (%)	OR (95% CI)†	p (corrected)
Anti-CCP+	6 (50)	2 (3)	28.5 (4.7–173.8)	< 0.0001 (0.001)	0 (0)	—	< 0.0001 (0.001)
RF+	5 (42)	6 (10)	6.3 (1.5–26.2)	0.02 (0.3)	7 (21)	2.7 (0.6–11.0)	0.3
Anti-U1RNP	7 (58)	21 (36)	2.5 (0.7–9.0)	0.2	11 (33)	2.8 (0.7–10.9)	0.2
Anti-dsDNA+	10 (83)	48 (81)	1.1 (0.2–6.0)	1.0	26 (79)	1.3 (0.2–7.6)	1.0
Anti-Sm+	1 (8)	9 (15)	0.5 (0.1–4.4)	1.0	6 (18)	0.4 (0.1–3.8)	0.7
Anti-Ro/SSA+	0 (0)	16 (27)	—	0.06 (0.8)	11 (33)	—	0.2 (0.3)
Anti-La/SSB+	0 (0)	7 (12)	—	0.6	7 (21)	—	0.2

* Comparing erosive arthritis with nonerosive arthritis. † Comparing erosive arthritis with no arthritis. IQR: interquartile range; anti-CCP: anti-cyclic citrullinated peptide; RF: rheumatoid factor.

Table 3. Characteristics of SLE patients with erosive arthritis (n = 12).

Feature	All Erosive Arthritis, n = 12, n (%) or mean (IQR)	Major Erosions, n = 6, n (%) or mean (IQR)	Minor Erosions, n = 6, n (%) or mean (IQR)	OR (95% CI)†	p
Anti-CCP+	6 (50)	4 (67)	2 (33)	4.0 (0.4–44.1)	0.6
Mean anti-CCP value (U)	38 (13–43)	59 (0–98)	17 (0–42)	—	0.4
RF+	5 (42)	3 (50)	2 (33)	2.0 (0.2–20.6)	1.0
Mean RF value (U)	14 (0–19)	10 (0–24)	18 (0–32)	—	0.8
Mean year of earliest erosions	11 (5–17)	12 (4–19)	10 (6–16)	—	0.7
Mean no. ACR SLE criteria	5.2 (4–6)	4.8 (4–5)	5.5 (4–7)	—	0.8
Meeting ACR criteria for RA	7 (58)	6 (100)	1 (17)	—	0.01

† Comparing major erosions with minor erosions. Anti-CCP: anti-cyclic citrullinated peptide, RF: rheumatoid factor.

ferences between those with major erosions and those with minor erosions. Four of the 6 patients with major erosions were anti-CCP+ (67%), whereas only 2/6 (33%) patients with minor erosions were anti-CCP+. Three of the 6 patients with major erosions (50%) were RF+. Patients with major erosions had a higher mean anti-CCP level (59 U vs 17 U; p = 0.4) but a lower mean RF level (10 U vs 18 U; p = 0.8) and fewer ACR features of SLE (mean 4.8 vs 5.5; p = 0.8). All 6 patients with major erosions also met ACR criteria for RA, compared with only 1 patient with minor erosions (p = 0.01).

Characteristics of anti-CCP-positive SLE patients and controls. Table 4 shows the characteristics of the 8 anti-CCP+ SLE patients and 2 anti-CCP+ serum controls. Both serum controls were also genotyped. Six patients (75%) developed

Table 4. Characteristics of anti-CCP-positive SLE patients (n = 8) and controls (n = 2).

Patient	Disease Duration, yrs	Anti-CCP Value, U	RF Value, U	Other Antibodies	HLA-DRB1 Alleles	HLA-DQB1 Alleles	No. of SE Alleles	Type of Arthritis	Other Clinical Features (no. ACR criteria)	Meeting ACR RA Criteria
1	17	> 250	29	ANA, dsDNA, U1RNP	0401, 1001	0302, 0501	2	Erosive (minor erosions)	Hematological disorder, skin (5)	yes
2	20	68	> 100	ANA, dsDNA U1RNP	0101, 1501	0501, 0602	1	Erosive (minor erosions)	Skin (4)	no
3	12	65	> 100	ANA, dsDNA, Ro	0405, 1501	0302, 0602	1	Nonerosive (nondeforming)	Skin (4)	yes
4	22	47	22	ANA, dsDNA	0401H	0301, 0302	2	Erosive (major erosions)	Skin, hematological disorder (5)	yes
5	14	42	0	ANA, dsDNA, Sm, Ro, U1RNP	0302, 1303	0402, 0301	0	Nonerosive (deforming)	Serositis, renal (5)	no
6	35	33	0	ANA, SM, U1RNP	0901, 1303	0303, 0301	0	Erosive (minor erosions)	Skin, oral ulcers, serositis, hematological disorder, renal (8)	yes
7	18	30	7	ANA	0101, 0401	0301, 0302	2	Erosive (major erosions)	Skin, hematological disorder (4)	yes
8	12	26	0	ANA, dsDNA, U1RNP	0403, 1501	0302, 0602	0	Erosive (major erosions)	Skin, hematological disorder (5)	yes
Control 1	ND	196	13	ND	0401, 1401	0301, 0503	1	ND	ND	ND
Control 2	ND	56	9	ND	0401H	0302H	2	ND	ND	ND

U: units, anti-CCP: anti-cyclic citrullinated peptide, RF: rheumatoid factor, SE: shared epitope, H: homozygote, ND: no data.

EA, of whom 4 (50%) had major erosions. All 5 HLA-DQB1*0302 carriers had EA, 4 of whom developed major erosions. Two patients had renal involvement and neither carried the SE nor DQB1*0302. Both these patients had a deforming, Jaccoud-type arthropathy, with one patient developing minor erosions. Both anti-CCP-positive controls were carriers of the SE allele DRB1*0401.

Frequencies of MHC Class II alleles in patients with SLE and in genetic controls. HLA-DRB1*0301 was significantly associated with SLE [39/104 (37%) SLE vs 24/117 (20%) genetic controls; OR 2.3, 95% CI 1.3–4.2, p = 0.005, $p_{corrected}$ = 0.05]. There was a similar trend for HLA-DQB1*0201 [49/104 (47%) SLE vs 41/117 (35%) controls; OR 1.6, 95% CI 1.0–2.8, p = 0.08]. Almost all the SLE group and all the genetic controls were British Caucasian individuals and as expected, the most common SE allele present was HLA-DRB1*0401 [25/104 (24%) SLE vs 21/117 (18%) controls]. There were no differences between patients and controls for the frequencies of the other SE alleles or HLA-DQB1*0302 (results not shown).

Associations of arthritis with MHC Class II alleles. Table 5 shows the associations of arthritis with MHC Class II alleles. HLA-DQB1*0302 was most significantly associated with EA (OR 8.2, p = 0.01). Further, all 6 patients with major erosions were DQB1*0302 carriers [6/6 (100%) vs 20/98 (20%) all other SLE; p < 0.0001, $p_{corrected}$ = 0.001]. There were similar trends toward associations with EA for 2 copies of the SE and HLA*DRB1*0401.

Table 5. Associations of arthritis with MHC class II alleles.

Allele	All Erosive Arthritis, n = 12, n (%)	Major Erosions, n = 6, n (%)	Nonerosive Arthritis, n = 59, n (%)	No Arthritis, n = 33, n (%)	OR (95% CI)†	p (corrected)
DRB1*0301+	2 (17)	1 (17)	24 (41)	13 (39)	0.3 (0.1–1.4)	0.2
DRB1*0401+	6 (50)	4 (67)	13 (22)	6 (18)	3.8 (1.1–13.3)	0.04 (0.5)
DQB1*0201+	4 (33)	2 (33)	28 (48)	17 (52)	0.5 (0.1–1.9)	0.4
DQB1*0302+	8 (67)	6 (100)	11 (19)	7 (21)	8.2 (2.2–30.4)	0.001 (0.01)
SE+	8 (67)	5 (83)	28 (47)	13 (39)	2.5 (0.7–8.80)	0.2
SE, 1 copy	3 (25)	1 (17)	21 (36)	12 (37)	1.2 (0.2–5.5)††	1.0
SE, 2 copies	5 (42)	4 (66)	7 (11)	1 (5)	8.0 (1.8–36.1)††	0.01 (0.1)

† Comparing erosive arthritis with all other groups. †† Using 1 SE copy (total n = 36) or 2 SE copies (total n = 13) compared with no SE copies (total n = 55). MHC: major histocompatibility complex, SE: shared epitope.

Associations of MHC Class II alleles with anti-CCP and RF. We found weak associations of several MHC Class II alleles with anti-CCP production. There were weak associations for HLA-DQB1*0302 (OR 6.0, 95% CI 1.3–27.0, p = 0.02, $p_{corrected}$ = 0.3) and 2 SE copies (OR 5.2, 95% CI 0.9–29.5, p = 0.08), but no association for *DRB1*0401 (OR 2.1, 95% CI 0.4–9.1, p = 0.4). There was a weak negative association for DRB1*0301 with anti-CCP production [0/39 (0%) vs 8/65 (12%); p = 0.02, $p_{corrected}$ = 0.3]. There were no genetic associations with RF production.

DISCUSSION

The frequency of EA in our group (11%) was higher than the prevalence of 4% to 5% reported in previous studies[7,24]. This may reflect the clinical characteristics of our patient population, almost all of whom were British Caucasian (Table 1), whereas in a previous series, 28% were Afro-Caribbean, Asian, or other races. Because radiographs were taken based on clinical suspicion, there may have been a lower detection rate of EA in our cohort than may occur in the SLE population. All patients with EA were women, with a younger mean age than the other 2 groups. Patients with EA had longer disease duration (p = 0.1; Table 1). Only one patient with EA had renal involvement, which is consistent with previous findings that SLE patients with persistent rheumatoid-like arthritis are less likely to develop nephritis[25]. None of these patients had neuropsychiatric manifestations, but they tended to have more skin involvement, serositis, and hematological disorders.

The frequencies of anti-CCP and RF were low in our SLE cohort (8% and 17%, respectively) and higher within the subgroup of patients presenting with synovitis (11% and 25%, respectively). As expected, anti-CCP was strongly associated with RF. A previous series found that 3/231 SLE patients (1%) were anti-CCP1+ and that 2 of these patients had EA[7]. We used the anti-CCP2 ELISA in our study, which has a higher sensitivity than anti-CCP1[26]. This may account for the higher anti-CCP frequency in our study.

Anti-CCP antibodies were reported to be highly specific for RA[9,27,28]. However, anti-CCP is associated with erosive disease not only in RA, but also in PsA[10,12]. Anti-CCP was significantly associated with EA in our SLE cohort, with 6 of the 12 patients with EA (50%) being anti-CCP+ (p = 0.001; Table 2). Although 42% of patients with EA were RF+, RF was also found in 21% of patients with NA. Two previous studies found an association of RF with EA in SLE[7,24]. Our findings suggest that RF is less useful than anti-CCP as a marker of EA in SLE.

Although anti-U1RNP was present more frequently in patients with EA, this was not statistically significant. Anti-U1RNP is the serological hallmark of mixed connective tissue disease (MCTD). Several different patterns of arthritis have been found in MCTD, ranging from NEA to arthritis mutilans[29]. Piirainen reported that anti-U1RNP was associated with progression to EA in patients with MCTD[30]. However, 35% of MCTD patients in that study also met criteria for RA[30]. It is noteworthy that all 6 of our SLE patients with major erosions met ACR criteria for RA. Four (67%) of these patients were anti-CCP+, with a higher mean anti-CCP level (Table 3). Anti-CCP is a serological marker for more severe EA not only in RA, but also in PsA[10] and JIA[11] and it is therefore likely to be a similar marker in SLE. It is also possible that certain serological and genetic markers identify a subset of patients with SLE who have clinical features that overlap with RA, sometimes referred to as rhupus[31].

As we found previously, the most common SLE-associated HLA alleles were HLA-DRB1*0301 and HLA-DQB1*0201[22], which are in linkage disequilibrium. As 67% of our patients with EA were SE carriers, it is not surprising that they were seronegative for anti-Ro/SSA and anti-La/SSB (Table 2), alleles known to be associated with HLA-DRB1*03[32]. The most common SE allele was HLA-DRB1*0401, which we expected in our mainly British Caucasian cohort[33]. HLA-DQB1*0302 had the strongest genetic association with EA in our cohort (OR 8.2, p = 0.01; Table 5) and all 6 patients with major erosions carried DQB1*0302. Weaker associations were seen for HLA-DRB1*0401 and 2 SE copies (Table 5). These associations were similar to the known associations of specific MHC Class II alleles (including the SE and HLA-DQB1*0302[34-36]) with progression of erosions in RA.

A dose-effect of the SE on anti-CCP production is seen in RA populations, and the association of the SE with radiographic disease progression is thought to be an indirect effect mediated by anti-citrulline antibodies[37,38]. Citrullination of arginine-containing residues greatly increases the affinity of the MHC Class II peptide-binding groove for the SE, thereby facilitating antigen presentation and generation of anti-citrulline antibodies[39]. HLA-DQB1*0302 is also associated with anti-CCP production in RA[27] and its association with disease severity may be via similar mechanisms. We observed weak positive associations of DQB1*0302 and 2 SE copies with anti-CCP and a weak negative association of HLA-DRB1*0301 with anti-CCP. Because of the small numbers of anti-CCP+ patients in our study, these findings did not reach statistical significance and larger studies may be able to confirm this effect. HLA-DR3 is associated with anti-CCP-negative RA, which runs a less severe course[40]. The presence of DRB1*0301 in SLE populations may therefore account for the infrequent development of EA, despite the common clinical feature of synovitis.

Arthritis is a common clinical feature of SLE. Our findings suggest that the incidence of EA in SLE is likely to be higher than previously reported. Thus, anti-CCP may be a useful serological marker for EA, particularly among patients with synovitis. Further studies on patients with early SLE may show a predictive role for anti-CCP in the future development of EA. Future studies may also elucidate the mechanisms by which MHC Class II alleles influence anti-CCP production

and the development of a severe arthritis phenotype common to several autoimmune diseases.

REFERENCES

1. Renaudineau Y, Croquefer S, Jousse S, et al. Association of alpha-actinin-binding anti-double-stranded DNA antibodies with lupus nephritis. Arthritis Rheum 2006;54:2523-32.
2. Somers E, Magder LS, Petri M. Antiphospholipid antibodies and incidence of venous thrombosis in a cohort of patients with systemic lupus erythematosus. J Rheumatol 2002;29:2531-6.
3. McCauliffe DP. Cutaneous diseases in adults associated with anti-Ro/SS-A autoantibody production. Lupus 1997;6:158-66.
4. Buyon JP, Winchester RJ, Slade SG, et al. Identification of mothers at risk for congenital heart block and other neonatal lupus syndromes in their children. Comparison of enzyme-linked immunosorbent assay and immunoblot for measurement of anti-SS-A/Ro and anti-SS-B/La antibodies. Arthritis Rheum 1993;36:1263-73.
5. Labowitz R, Schumacher HRJ. Articular manifestations of systemic lupus erythematosus. Ann Intern Med 1971;74:9211-21.
6. Alarcon-Segovia D, Abud-Mendoza C, Diaz-Jouanen E, Iglesias A, De los Reyes V, Hernandez-Ortiz J. Deforming arthropathy of the hands in systemic lupus erythematosus. J Rheumatol 1988;15:65-9.
7. Mediwake R, Isenberg DA, Schellekens GA, van Venrooij WJ. Use of anti-citrullinated peptide and anti-RA33 antibodies in distinguishing erosive arthritis in patients with systemic lupus erythematosus and rheumatoid arthritis. Ann Rheum Dis 2001;60:67-8.
8. Rantapaa-Dahlqvist S, de Jong BAW, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003;48:2741-9.
9. Schellekens GA, Visser H, de Jong BA, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000;43:155-63.
10. Korendowych E, Owen P, Ravindran J, Carmichael C, McHugh N. The clinical and genetic associations of anti-cyclic citrullinated peptide antibodies in psoriatic arthritis. Rheumatology 2005;44:1056-60.
11. Kasapcopur O, Altun S, Aslan M, et al. Diagnostic accuracy of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis. Ann Rheum Dis 2004;63:1687-9.
12. van der Helm-van Mil A, Verpoort K, Breedveld F, Toes R, Huizinga T. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. Arthritis Res Ther 2005;7:R949-R58.
13. Fronek Z, Timmerman LA, Alper CA, et al. Major histocompatibility complex associations with systemic lupus erythematosus. Am J Med 1988;23:42-3.
14. Karassa FB, Trikalinos TA, Ioannidis JPA. The Fc gamma RIIIA-F158 allele is a risk factor for the development of lupus nephritis: A meta-analysis. Kidney Int 2003;63:1475-82.
15. Bastian HM, Roseman JM, G. McGwin J, et al. Systemic lupus erythematosus in three ethnic groups. XII. Risk factors for lupus nephritis after diagnosis. Lupus 2002;11:152.
16. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30:1205-13.
17. Wagner U, Kaltenhäuser S, Sauer H, et al. HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. Arthritis Rheum 1997;40:341-51.
18. Huizinga TWJ, Amos CI, van der Helm-van Mil AHM, et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis Rheum 2005;52:3433-8.
19. Tan E, Cohen A, Fries J, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
20. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
21. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
22. McHugh NJ, Owen P, Cox B, Dunphy J, Welsh K. MHC class II, tumour necrosis factor alpha, and lymphotoxin alpha gene haplotype associations with serological subsets of systemic lupus erythematosus. Ann Rheum Dis 2006;65:488-94.
23. Eberhardt K, Fex E, Johnson U, Wollheim FA. Associations of HLA-DRB and -DQB genes with two and five year outcome in rheumatoid arthritis. Ann Rheum Dis 1996;55:34-9.
24. Richter Cohen M, Steiner G, Smolen JS, Isenberg DA. Erosive arthritis in systemic lupus erythematosus: analysis of a distinct clinical and serological subset. Rheumatology Oxford 1998;37:421-4.
25. Kaplan D, Ginzler EM, Feldman J. Arthritis and nephritis in patients with systemic lupus erythematosus. J Rheumatol 1991;18:233-9.
26. Avouac J, Gossec L, Dougados M. Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review. Ann Rheum Dis 2006;65:845-51.
27. van Gaalen FA, van Aken J, Huizinga TWJ, et al. Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. Arthritis Rheum 2004;50:2113-21.
28. van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: A prospective cohort study. Arthritis Rheum 2004;50:709-15.
29. Bennett RM, O'Connell DJ. The arthritis of mixed connective tissue disease. Ann Rheum Dis 1978;37:397-403.
30. Piirainen HI. Patients with arthritis and anti-U1-RNP antibodies: a 10-year follow-up. Rheumatology Oxford 1990;29:345-8.
31. Panush RS, Edwards NL, Longley S, Webster E. 'Rhupus' syndrome. Arch Intern Med 1988;148:1633-6.
32. Skarsvag S, Hansen KE, Moen T, Eggen BM. Distributions of HLA class II alleles in autoantibody subsets among Norwegian patients with systemic lupus erythematosus. Scand J Immunol 1995;42:564-71.
33. Gorman JD, Lum RF, Chen JJ, Suarez-Almazor ME, Thomson G, Criswell LA. Impact of shared epitope genotype and ethnicity on erosive disease: A meta-analysis of 3,240 rheumatoid arthritis patients. Arthritis Rheum 2004;50:400-12.
34. Vos K, Visser H, Schreuder GMT, et al. Human leukocyte antigen-DQ and DR polymorphisms predict rheumatoid arthritis outcome better than DR alone. Hum Immunol 2001;62:1217-25.
35. Gourraud P-A, Boyer J-F, Barnetche T, et al. A new classification of HLA-DRB1 alleles differentiates predisposing and protective alleles for rheumatoid arthritis structural severity. Arthritis Rheum 2006;54:593-9.
36. Mattey DL, Hassell AB, Dawes PT, et al. Independent association of rheumatoid factor and the HLA-DRB1 shared epitope with radiographic outcome in rheumatoid arthritis. Arthritis Rheum 2001;44:1529-33.
37. van der Helm-van Mil AHM, Verpoort KN, Breedveld FC, Huizinga TWJ, Toes REM, de Vries RRP. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated

peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. Arthritis Rheum 2006;54:1117-21.
38. Mewar D, Coote A, Moore D, et al. Independent associations of anti-cyclic citrullinated peptide antibodies and rheumatoid factor with radiographic severity of rheumatoid arthritis. Arthritis Res Ther 2006;8:R128.
39. Hill JA, Southwood S, Sette A, Jevnikar AM, Bell DA, Cairns E. Cutting edge: The conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC Class II Molecule. J Immunol 2003;171:538-41.
40. Verpoort KN, van Gaalen FA, van der Helm-van Mil AHM, et al. Association of HLA-DR3 with anti-cyclic citrullinated peptide antibody-negative rheumatoid arthritis. Arthritis Rheum 2005;52:3058-62.