Primary research

Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset

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Statement of findings

An inception cohort of 238 patients having peripheral joint synovitis of less than 12 months duration was evaluated clinically and followed prospectively for 1 year to determine the clinical significance of a number of rheumatoid arthritis (RA) associated autoantibodies. Serum samples collected at the time of the initial evaluation were tested for rheumatoid factor (RF) and antibodies to Sa (anti-Sa), RA-33, (pro)filaggrin [antifilaggrin antibody (AFA)], cyclic citrullinated peptide (anti-CCP), calpastatin, and keratin [antikeratin antibody (AKA)]. RF had a sensitivity of 66% and a specificity of 87% for RA. Anti-Sa, AFA, and anti-CCP all had a specificity of more than 90%, but a sensitivity of less than 50% for this diagnosis. Overall, there was a high degree of correlation between AFA, AKA, anti-Sa or anti-CCP, this being highest between anti-Sa and anti-CCP (odds ratio, 13.3; $P<0.001$). Of the 101 patients who were positive for at least one of these four autoantibodies, 57% were positive for only one. Finally, anti-Sa identified a subset of predominantly male RA patients with severe, erosive disease. Anti-Sa, AFA and anti-CCP are all specific for early RA but, overall, have little additional diagnostic value over RF alone. Although these antibodies may preferentially recognize citrullinated antigens, the modest degree of concordance between them in individual patient sera suggests that it is unlikely a single antigen is involved in generating these responses.

Keywords: autoantibodies, early synovitis, human leukocyte antigen, rheumatoid arthritis, spondylarthropathy

ACR = American College of Rheumatology; AFA = antifilaggrin antibody; AKA = antikeratin antibody; APF = antiperinuclear factor; CCP = cyclic citrullinated peptide; CRP = C-reactive protein; ELISA = enzyme-linked immunosorbent assay; RA-1 = calpastatin; RA = rheumatoid arthritis; RF = rheumatoid factor; SE = shared epitope.

Synopsis

Introduction: A spectrum of autoantibodies is now known to be specifically associated with RA. There continues to be uncertainty as to what stage of the disease each of these autoantibodies develop, and whether they are associated with unique clinical features.

Aims: To help address these questions, a spectrum of autoantibodies known to be associated with RA in a cohort of patients with early synovitis was evaluated.

Methods: An inception cohort of 238 patients having peripheral joint synovitis of less than 12 months duration was evaluated clinically then followed prospectively for 1 year. Patients were classified as having RA on the basis of fulfilling the 1987 criteria. Serum samples collected at the time of the initial evaluation were tested for anti-Sa and anti-RA-33 using immunoblotting, and to (pro)filaggrin (AFA), anti-CCP, and calpastatin (anti-RA-1) using enzyme-linked immunosorbent assay techniques. AKA were detected using immunoflurescence on human epidermal tissue. RF was tested by nephelometry. HLA-DRB1 alleles were determined using sequence specific primers. Initial and 1 year radiographs were evaluated for the presence of erosions.

Results: Of the 238 patients with synovitis of recent onset in the cohort, 106 (45%) met RA criteria, 102 (96%) of whom met the criteria on their initial visit. Diagnoses in the remaining patients included 22 (9%) with reactive arthritis, 14 (6%) with psoriatic arthritis or another form of spondylarthropathy, 11 (5%) with another well-defined rheumatic diagnosis, and 85 (36%) with undifferentiated arthritis. The RA patients were significantly older than the nonRA patients (46 ± 13 versus 39 ± 13; $P<0.001$), had higher mean swollen joint count (13.8 ± 9.7 versus 2.3 ± 2.3; $P<0.001$), and higher C-reactive protein (CRP) level (1.9 ± 1.9 versus 1.6 ± 2.4; $P<0.01$). Table 1 summarizes the prevalence of the various RA associated antibodies in patients diagnosed as having RF-positive (RF+) RA, RF-negative (RF–) RA, and nonRA. Regarding the characteristics of these tests, RF had the highest sensitivity at 66%, and all the other antibodies individually were less than 50% sensitive. AFA, anti-Sa, anti-CCP were greater than 90% specific for RA, while RF and AKA were 80–90% specific, and anti-RA-33 and anti-RA-1 was not specific for this diagnosis. The data further indicate that adding any one of AFA, AKA, anti-Sa, or anti-CCP to RF increases the specificity for RA from 80 to 90%. In the absence of RF, the presence of one or more of these antibodies carried a sensitivity of only 31% for RF– RA, with anti-Sa being the most specific at 98%. Overall, there was a high degree of correlation between AFA, AKA, anti-Sa or anti-CCP, this being highest between anti-Sa and anti-CCP (odds ratio, 13.3; $P<0.001$). Despite this high level of correlation, of the 101 patients who were positive for at least one of these four autoantibodies, 57% were positive for only one, suggesting considerable variability in individual reactivity patterns.

RA has been shown in multiple populations to be associated with HLA-DRB1 alleles encoding for the shared epitope (SE). In this study, as illustrated in Table 2, the presence of each of these autoantibodies was significantly associated with having two shared epitope alleles, even when only the RA patients were considered.

Patients with anti-Sa antibodies were predominantly male (61% versus 28%; $P<0.01$), had significantly higher swollen joint counts (18 ± 12 versus 13 ± 9; $P=0.02$), and higher CRP levels (2.6 ± 3 mg/dl versus 1.6 ± 1.4 mg/dl; $P=0.03$) at the initial visit. Despite subsequently being treated with significantly higher doses of prednisone (4.8 ± 6.0 mg/day versus 1.8 ± 3.3 mg/day; $P<0.01$), and more disease modifying antirheumatic drug therapy (1.4 ± 0.8 versus 0.9 ± 0.7 disease modifying antirheumatic drugs; $P<0.01$), the anti-Sa-positive RA patients had a higher frequency of erosions than the rest of the RA patients (60% versus 33%; $P=0.03$). Neither RF nor SE were associated with the disease severity measures, and analyses evaluating all the other autoantibodies failed to reveal a similar trend.

Table 1

Presenting clinical features and prevalence of autoantibodies in rheumatoid factor positive rheumatoid arthritis (RF+ RA), RF-negative RA (RF– RA), and nonRA patients

	RF+ RA ($n=70$)	RF– RA ($n=36$)	NonRA ($n=132$)
Age	47 ± 12	44 ± 14	39 ± 13*
Female	44 (63)	27 (75)	87 (66)
Swollen joint count	13 ± 9	16 ± 10	2 ± 3*
CRP level	1.9 ± 1.9	1.9 ± 2.1	1.6 ± 2.4
Multiple erosions	12 (17)	7 (19)	7 (5)*
RF	70 (100)	0	17 (13)
ANA	25 (36)	10 (28)	30 (23)
ds-DNA	0	0	3 (2)
Anti-SSA	5 (7)	2 (6)	7 (5)
Anti-SSB	2 (3)	1 (3)	2 (2)
Anti-RNP	3 (4)	0	3 (2)
Anti-Sm	0	0	2 (2)
AFA	32 (46)†	3 (8)	9 (7)
Anti-Sa	18 (26)†	5 (14)‡	3 (2)
Anti-CCP	38 (54)†	5 (14)	12 (9)
AKA	26 (37)†	1 (3)	21 (16)
Anti-RA-1	23 (33)	10 (28)	49 (37)
Anti-RA-33	2 (2)	0	1 (1)

All values represent either number of patients (%), or means ± standard deviations. * $P<0.01$ compared with RA patients; † $P<0.01$ compared with RF– RA and nonRA; ‡ $P<0.01$ compared with nonRA. CRP, C-reactive protein; ANA, antinuclear antibodies; AFA, antifilaggrin antibody; RNP, ribonucleoprotein; CCP, cyclic citrullinated peptide; AKA, antikeratin antibody.

Table 2

Association of autoantibodies with shared epitope (SE) alleles

	SE/x	SE/SE	*0401/*0101
All patients			
RF	1.8	3.7*	8.7*
AFA	2.2*	5.1*	9.5*
Anti-Sa	2.0	7.1*	18.9*
Anti-CCP	2.0	5.0*	10.9*
AKA	1.4	3.1*	3.4*
RA patients			
RF	1.8	2.7	5.3
AFA	2.2	4.9*	6.5*
Anti-Sa	1.9	4.0*	10.6*
Anti-CCP	2.0	3.0	7.2*
AKA	1.4	2.9	3.3

Numbers represent odds ratios for having each autoantibody associated with shared epitope alleles. SE/x, SE/SE, DRB *0401/*0101 were each compared with patients with no shared epitope alleles. * $P < 0.05$ by Chi-square after Bonferroni adjustment for multiple comparison. RF, Rheumatoid factor; AFA, antifilaggrin antibody; CCP, cyclic citrullinated peptide; AKA, antikeratin antibody.

Discussion: Despite a well-documented lack of specificity, RF continues to be a central part of the definition of RA, primarily because of its favorable sensitivity profile. In our cohort, RF had a sensitivity of 66%, a specificity of 87%, and an overall accuracy of 78% for the diagnosis of RA. AFA, anti-Sa, anti-CCP were all highly specific for this diagnosis, and when any of them were present in conjunction with RF, the specificity for RA approached 100%. Potentially of more importance to the clinician is the diagnostic value of these antibodies when RF is not detectable. Our data indicate that only 31% of RF– RA patients had any of AKA, AFA, anti-Sa or anti-CCP, and that anti-Sa was the most specific for this diagnosis. This modest level of sensitivity suggests that testing for this spectrum of autoantibodies carries little advantage over RF alone in diagnosing early RA.

AFA, AKA, and antiperinuclear factor (APF) have all been proposed to identify a common antigen present in the skin protein (pro)filaggrin. It has continued to be puzzling why a skin antigen would be targeted relatively specifically in a disorder that is primarily articular. A potential explanation for this may relate to the demonstration that citrulline appears to be an essential constituent of the antigenic determinants recognized by AKA, APF, and AFA. The citrulline rich (pro)filaggrin molecule makes an ideal substrate for detecting this reactivity. Moreover, the Sa antigen, which, unlike (pro)filaggrin, is detectable in rheumatoid synovium, has recently been shown to also be citrullinated. It is thus possible that AKA, AFA, APF, and anti-Sa all recognize one or more citrullinated antigens. Despite this possibility, the modest degree of concordance between them in individual patient sera suggests that it is unlikely that a single antigen is involved in generating these responses.

This study provides evidence suggesting that anti-Sa antibodies appear to be a marker for a subset of early RA patients whose disease may be more severe and erosive. Moreover, it was determined that anti-Sa, AFA, and anti-CCP were all highly associated with SE, particularly two copies. We examined a spectrum of potential RA severity indicators including the number of swollen joints, CRP level, and presence of early radiographic erosions. Our data indicate that anti-Sa was more highly associated with these measures of RA severity than any other parameter, including the most accepted prognostic indicators, RF and SE.

In conclusion, it is demonstrated that antibodies directed against putatively citrullinated antigens including Sa, filaggrin, keratin, and CCP are the most specific for RA, and are detectable early in the disease course. It will be of interest to find out whether the cumulative prevalence of specific autoantibody subsets tends to increase over time, as this would suggest that the mechanisms underlying the development of these reactivities continue to evolve over the course of the arthropathy.

Full article

Introduction

Autoantibodies can be detected in a spectrum of rheumatic diseases where they may be highly associated with distinct clinical syndromes. These are often helpful for diagnosis, and to some extent, prognosis. In RA, RF is detected in 70–80% of patients with established disease, and is an integral part of the definition of this disorder. AKA, APF , AFA, and anti-Sa have all been shown to be associated with RA, and appear to be more specific than RF for this disease [1–9]. Anti-RA-33, [10–12] and anti-RA-1 [13] have also been shown to be prevalent in RA patient populations, but not specific for this disease.

There is an evolving understanding of the antigens to which these RA associated antibodies are directed, although their ultimate pathogenic role, if any, continues to be unclear. AKA, APF, and AFA identify epitopes carried by keratin, profilaggrin and filaggrin, respectively [3,6,14,15]. These proteins follow a common post-translational pathway, which involves a peptidyl-arginine to citrulline deimination. This modification appears to be central to specific antigenicity, giving rise to epitopes recognized by sera from RA patients [16,17]. Anti-Sa antibodies are directed towards an unknown antigen that is abundant in placental tissue and rheumatoid synovium [7]. Interestingly, the Sa antigen also appears to be citrullinated [18]. Anti-RA-33 antibodies recognize the A2 protein, an antigen found in the heterogeneous ribonucleoprotein of the splicosome [19]. Anti-RA-1 antibodies are directed towards domains 3 and 4 of calpastatin, the natural inhibitor of calpains, which

are members of the cysteine proteinases that have been implicated in articular damage [13].

This spectrum of autoantibodies has been evaluated primarily in cohorts of patients with well-established RA. A number of important questions are unresolved regarding the stage of the disease at which each of these antibodies arise, and whether indeed some may antedate the clinical expression of this disorder. The fact that RF, AKA or APF could sometimes antedate the appearance of clinical RA by several years has been reported [20–22]. Anti-Sa, AFA and anti-RA-33 have been reported to be present in early RA patients, although the cohorts studied have generally been small, and the clinical characterization of the patients not detailed [1,8,9,23,24]. Anticyclic citrullinated peptide (anti-CCP) has recently been evaluated in a cohort of patients with early synovitis, and was found to be more specific than RF for early RA, while having comparable sensitivity [25].

Recently, the clinical features and human leukocyte antigen (HLA) associations of a well-characterized cohort of patients with synovitis of recent onset were presented [26]. In the current study, we sought to determine the prevalence and diagnostic value of the RA associated autoantibodies in this heterogeneous cohort of patients with early synovitis. Sera obtained within 12 months of symptom onset were tested for these antibodies, and their presence was related to disease features and subsequent clinical course over a 1 year period. Our data indicate that AFA, anti-Sa, and anti-CCP are all highly specific for early RA, but demonstrate only a modest degree of concordance in the sera of individual patients. Anti-Sa identifies a subset of male RA patients with severe disease.

Methods

Patients

Two hundred and thirty-eight patients were recruited to an early synovitis study at the National Institutes of Health (protocol 94-AR-194). The study patients had persistent synovitis (>6 weeks) of at least one peripheral joint, which had been present for less than 1 year. Patients with traumatic, septic, and crystal induced arthritis were specifically excluded.

Patients underwent a complete clinical evaluation. The number of swollen joints was determined by evaluating for the presence of effusion and/or synovial thickening in 66 peripheral joints (hips were excluded). Patients were then followed clinically over a 1 year period. Routine laboratory data were obtained on each visit, including an evaluation of acute phase reactants. Anteroposterior and lateral radiographs of the hands and feet were either available for evaluation or obtained at the time of assessment. A second set of radiographs was obtained at the 1 year visit. All radiographs were evaluated for the presence of erosions. Although a proportion of the radiographs were felt to have 'possible' or 'questionable' erosions, only patients with definite erosions on either set of radiographs were included in the definition of erosions. At the completion of the year of observation, patients who were diagnosed as having RA had all met the 1987 American College of Rheumatology (ACR) criteria [27] on at least one visit. If a patient had met ACR criteria, but with follow up was unequivocally diagnosed as having another well-characterized rheumatic disease, this individual was not included in the definition of RA.

Detection of autoantibodies

IgM RF was measured by nephelometry, and a level >20 IU/ml was considered positive. Anti-Sa antibodies were detected by immunoblot, as previously described [7]. A partially purified preparation of placental Sa antigen was provided by one of the authors (HAM), and the western blot assay performed blindly by another (GS). RA-33 was detected using immunoblot, as described previously [19]. Filaggrin was obtained from human epidermis and purified using reversed phase high-performance liquid chromatography. After covalently coupling to 96-well plates, AFA were detected by enzyme-linked immunosorbent assay (ELISA) as previously described [1,5]. The cut-off level for positivity (OD, 0.15) was determined on the basis of results of sera from 100 middle-aged (40–65 years) blood donors. Antibodies to CCP were evaluated after generating a cyclic peptide from linear citrulline containing peptides, by substituting serine residues by cysteine. Anti-CCP were detected using ELISA as previously described with the linear peptides [17]. Control peptides had an unmodified arginine rather than citrulline. The cut-off level for positivity (OD, 0.3) was determined on the basis of generating 100% specificity for RA in previous assays using local normal controls. AKA were detected using immunoflurescence. Sections of human breast epidermis were used, and the slides were read independently by two observers (AR and CZ). Any slides in which the observers did not agree were reread, and a consensus agreed. In all cases, the detected autoantibodies were of the IgG class. The results were not corrected for total IgG levels.

HLA typing

HLA-DR typing was done by the molecular polymerase chain reaction-sequence specific primers method using specific oligonucleotide sequences as primers as previously described [28]. Shared epitope (SE) alleles included DRB1*0101, *0102, *0401, *0404, *0405, *0408, *1001, *1402.

Statistical analysis

Patient groups were compared using analysis of variance or the Kruskal–Wallis test for continuous variables, and using the Chi-squared test for proportions. The significance level

for associations between individual autoantibodies and HLA-DRB1 alleles was adjusted for multiple comparisons using the Bonferroni method. Statistical analysis was performed using EpiInfo statistical software (Center for Disease Control, Atlanta, GA, USA: http://www.cdc.gov/epo/epi/epiinfo.htm).

Results

Of the 238 patients with synovitis of recent onset in the cohort, 106 (45%) met RA criteria, 102 (96%) of whom met the criteria on their initial visit. Four patients initially met RA criteria, but were given nonRA diagnoses on follow up. Two of these patients had SLE, and two had psoriatic arthritis. All four presented with RF– polyarthritis. Diagnoses in the remaining nonRA patients included 22 (9%) with reactive arthritis, 14 (6%) with psoriatic arthritis or another form of spondylarthropathy, 11 (5%) with another well-defined rheumatic diagnosis, and 85 (36%) with undifferentiated arthritis. The clinical characteristics of the RF-positive (RF+) RA, RF-negative (RF–) RA and nonRA patients are summarized in Table 1.

Prevalence of the autoantibodies in the patient cohort

Table 1 indicates the prevalence of a spectrum of known autoantibodies and of the RA associated antibodies in patients diagnosed as having RF+ RA, RF– RA, and nonRA. In total, 87 patients were RF-positive, 17 of whom did not meet RA criteria any point during the study period, and were classified as having undifferentiated arthritis. Antinuclear antibodies were detected in 65/238 (27%) patients, and were of comparable prevalence in the patient groups. Less than 10% of the patients in all groups demonstrated antibodies to SSA, SSB, dsDNA, ribonucleoprotein (RNP), and Sm. Of the RA associated antibodies tested, AFA, anti-Sa, anti-CCP, and AKA were all significantly more prevalent in the RF+ RA group compared with either the RF– RA group or the nonRA group. Anti-RA-1 antibodies were detected in 35% of the cohort with no significant difference between the groups. Anti-RA-33 antibodies were detected in three patients in the cohort. Please note, the prevalence of anti-Sa antibodies was significantly higher in the RF– RA group compared with the nonRA group (14% versus 2%; $P<0.01$), but there were no differences in the prevalence of the other autoantibodies between these two patient groups. In total, 90/106 (85%) of the RA patients and 75/132 (57%) of the nonRA patients had at least one of the RA associated antibodies.

Diagnostic value of antibodies for RA

The diagnostic value of the individual antibodies for RA is shown in Table 3. RF had the highest sensitivity at 66%, and all the other antibodies were individually less than 50% sensitive. AFA, anti-Sa and anti-CCP were greater than 90% specific for RA, while RF and AKA were 80–90% specific, and anti-RA-1 was not specific for this diagnosis. The data further indicate that adding any one of AFA, AKA, anti-Sa, or anti-CCP to RF increases the specificity for RA from 80 to 90%. In the absence of RF, the presence of one or more of these antibodies carried a sensitivity of only 31% for RF– RA, with anti-Sa being the most specific at 98%. At the completion of the study, of the 37/132 (28%) nonRA patients positive for one of AFA, AKA, anti-Sa, or anti-CCP, 12 had some form of spondylarthropathy, 4 had a connective tissue disease, and 21 continued with a diagnosis of 'undifferentiated arthritis'.

Table 3

Diagnostic value of individual autoantibodies for rheumatoid arthritis

	Sensitivity	Specificity	PPV	NPV	Accuracy
RF	0.66	0.87	0.80	0.76	0.78
AFA	0.33	0.93	0.79	0.63	0.66
Anti-Sa	0.22	0.98	0.88	0.61	0.64
Anti-CCP	0.41	0.91	0.78	0.66	0.69
AKA	0.26	0.84	0.56	0.58	0.58
RF+ and one or more*	0.50	0.96	0.90	0.70	0.75
RF– and one or more*	0.31	0.73	0.26	0.77	0.63

* Includes antifilaggrin antibody (AFA), anti-Sa, cyclic citrullinated peptide (anti-CCP) and antikeratin antibody (AKA). PPV, positive predictive value; NPV, negative predictive value; RF, rheumatoid factor.

Correlation between antibodies in patient sera

Previous studies have generally suggested a high degree of correlation in RA patient sera between AFA, AKA, anti-CCP, and anti-Sa. We tested the correlation between these antibodies in the patient sera. The correlation was highest between anti-CCP and anti-Sa [odds ratio (OR), 13.3; $P<0.001$), and lowest between anti-Sa and AKA (OR, 2.4; $P=0.09$). In total, 101 patients had at least one of the four antibodies, 64% of whom had RA. Of these 101 patients, 57 were in fact positive for only one antibody (AKA=20, AFA=12, anti-Sa=6, anti-CCP=19). Only seven patients were positive for all four antibodies.

Seropositivity for anti-CCP and AFA was determined on the basis of an ELISA cut-off level as described in the Methods section. The relationship between the titers of these two antibodies, and between each of them and RF, was examined. There was a significant correlation in the titers of anti-CCP and AFA in individual sera ($r=0.46$; $P<0.001$). Titers of both anti-CCP and AFA were not correlated with RF titers. When only anti-CCP-positive patients were examined, the mean CCP titer was significantly higher in the RF-positive patients ($n=44$) compared with the RF-negative patients ($n=10$) (1.07 ± 0.55 versus 0.6 ± 0.34; $P=0.01$). A similar trend was seen with AFA,

Table 4

Sa is associated with severe rheumatoid arthritis (RA)

	Sa+ RA ($n = 23$)	Sa− RA ($n = 83$)	RF+ RA ($n = 70$)	RF− RA ($n = 36$)	SE+ RA ($n = 62$)	SE− RA ($n = 44$)
Male (%)	14 (61)*	23 (28)	27 (39)	10 (28)	24 (39)	13 (30)
Age	48 ± 15	46 ± 13	47 ± 12	44 ± 15	49 ± 13*	42 ± 13
Duration of symptoms (weeks)	36 ± 25	30 ± 18	33 ± 17	29 ± 24	32 ± 15	31 ± 25
Initial visit						
Swollen joints	18 ± 12*	13 ± 9	13 ± 9	16 ± 10	13 ± 9	14 ± 11
Nodules	1 (4)	5 (6)	5 (7)	1 (3)	5 (8)	1 (2)
CRP (mg/dl)	2.6 ± 3*	1.6 ± 1.4	1.9 ± 1.8	1.9 ± 2.1	1.7 ± 1.5	2.1 ± 2.4
On DMARDs (%)	11 (48)	31 (37)	28 (40)	14 (39)	24 (39)	18 (41)
On prednisone (%)	12 (52)	29 (35)	25 (36)	16 (44)	26 (42)	15 (34)
Multiple erosions (%)†	7 (30)	12 (14)	12 (17)	7 (19)	12 (19)	7 (16)
One year visit						
Swollen joints	8 ± 9	8 ± 10	9 ± 11	7 ± 7	10 ± 11*	5 ± 6
CRP (mg/dl)	1.1 ± 0.7	1.2 ± 1.8	1.3 ± 1.9	0.9 ±0.5	1.4 ± 2.1	0.9 ± 0.3
Number of DMARDs	1.4 ± 0.8*	0.9 ± 0.7	0.9 ± 0.7	1.0 ± 0.8	0.9 ± 0.7	1.1 ± 0.8
Prednisone (mg/day)	4.8 ± 6.0*	1.8 ± 3.3	2.7 ± 4.8	2.0 ± 2.5	2.7 ± 4.0	2.1 ± 3.9
Multiple erosions (%)†	12 (60)*	27 (33)	24 (34)	14 (39)	25 (42)	13 (30)
Inactive disease (%)	0	9 (12)	3 (4)	6 (17)	4 (7)	5 (11)

Independent comparisons are made between Sa+ and Sa− RA, RF+ and RF− RA, SE+ and SE− RA. * $P < 0.05$. † One hundred and two out of 106 (96%) of the patients had 1 year radiographs available for analysis. Multiple erosions included all patients with two or more erosions that involved at least two separate joint areas. RF, Rheumatoid factor; SE, shared epitope; CRP, C-reactive protein; DMARD, disease modifying antirheumatic drug.

although this did not reach statistical significance. Of note also, the mean RF titer for RF-positive patients who did not meet RA criteria did not differ from the RF-positive patients who were diagnosed as having RA (202 ± 172 versus 207 ± 169; P = not significant).

RF, Sa, AFA, and CCP are highly associated with shared epitope in early RA

RA has been shown in multiple populations to be associated with HLA-DR alleles encoding for the SE. We sought to determine if specific autoantibodies were particularly associated with SE alleles in our cohort. These data are presented in Table 2. Overall, the SE alleles were highly associated with the diagnosis of RA (OR 3.0; $P < 0.0001$). RF, AFA, anti-Sa, and anti-CCP were all associated with SE alleles, particularly two copies. Interestingly, 6/6 patients with *0101/*0401 were positive for at least one these autoantibodies.

Anti-Sa antibodies identify a male predominant subset of RA patients with severe disease

Table 4 demonstrates that, in comparison with all other RA patients, RA patients with anti-Sa antibodies were predominantly male (61% versus 28%; $P < 0.01$), had significantly higher swollen joint counts (18 ± 12 versus 13 ± 9; $P = 0.02$), and higher CRP levels (2.6 ± 3 mg/dl versus 1.6 ± 1.4 mg/dl; $P = 0.03$) at the initial visit. Despite subsequently being treated with significantly higher doses of prednisone (4.8 ± 6.0 mg/day versus 1.8 ± 3.3 mg/day; $P < 0.01$), and more disease modifying antirheumatic drug therapy (1.4 ± 0.8 versus 0.9 ± 0.7 disease modifying antirheumatic drugs; $P < 0.01$), the anti-Sa-positive RA patients had a higher frequency of erosions than the rest of the RA patients (60% versus 33%; $P = 0.03$). Table 4 further indicates that neither RF nor SE was associated with the disease severity measures, and analyses evaluating all the other autoantibodies failed to reveal a similar trend (data not shown). Of interest, only six RA patients demonstrated the presence of nodules, and this feature was more associated with RF and SE than with anti-Sa. Analysis of the nonRA patients did not reveal any clinically meaningful associations with any of the autoantibodies.

Discussion

A cohort of patients with synovitis of recent onset was evaluated and we sought to determine the prevalence and potential clinical utility of a spectrum of autoantibodies that has been shown to be associated with RA. To date, most of the studies evaluating these antibodies have been in patients with established, well-characterized disease, and their prevalence and diagnostic value in patients with early inflammatory arthritis has not been defined. Our study evaluated patients within a few months of the onset of synovitis, and then followed them for a 1 year period to determine the best clinical diagnosis. In particular, we sought to determine how sensitive and specific each of

the antibodies, alone and in combination, were for early RA. We utilized the accepted definition of RA using the 1987 ACR criteria [27]. Of note, a large epidemiologic study of patients with early inflammatory arthritis found that the proportion of patients who met the ACR criteria increased if the criteria were applied cumulatively over a 5 year period [29]. Although the duration of follow up in this study was shorter, we found that almost all of the patients who had met the RA criteria at the completion of the study period had done so on their initial visit.

Despite a well-documented lack of specificity, RF continues to be a central part of the definition of RA, primarily because of its favorable sensitivity profile. In our cohort, RF had a sensitivity of 66%, a specificity of 87%, and an overall accuracy of 78% for the diagnosis of RA. AFA, anti-Sa and anti-CCP were all highly specific for this diagnosis, and when any of them were present in conjunction with RF, the specificity for RA approached 100%. Potentially of more importance to the clinician is the diagnostic value of these antibodies when RF is not detectable. Our data indicate that only 31% of RF– RA patients had any of AKA, AFA, anti-Sa or anti-CCP, and that anti-Sa was the most specific for this diagnosis. This modest level of sensitivity suggests that testing for this spectrum of autoantibodies carries little advantage over RF alone in diagnosing early RA.

This study provides evidence suggesting that anti-Sa antibodies appear to be a marker for a subset of early RA patients whose disease may be more severe and erosive. These data are consistent with observations in a French RA cohort, where anti-Sa and SE were associated with severe radiographic erosions [9]. Indeed, it was determined that anti-Sa, AFA, and anti-CCP were all highly associated with SE, particularly two copies. We examined a spectrum of potential RA severity indicators including the number of swollen joints, CRP level, and presence of early radiographic erosions. Our data indicate that anti-Sa was more highly associated with these measures of RA severity than any other parameter, including the most accepted prognostic indicators, RF and SE. In particular, there was a significantly higher prevalence of radiographic erosions in the anti-Sa-positive RA patients compared with the rest of the RA population, despite the fact that the anti-Sa-positive patients had received significantly higher doses of prednisone and more disease modifying antirheumatic drug therapy. Interestingly, the anti-Sa-positive subset in this cohort was strikingly predominated by males (61% of Sa-positive versus 28% of Sa-negative RA patients; $P<0.001$). It has recently been shown that, in comparison with female RA patients, male RA patients tended to develop erosions earlier in the disease course [30]. The finding that anti-Sa antibodies are associated both with male gender and with severe early RA further emphasizes the importance of gender differences in the clinical expression of this heterogeneous disease.

AFA, AKA, and APF have been proposed to all identify a common antigen present in the skin protein (pro)filaggrin [1,3–6]. It has continued to be puzzling why a skin antigen would be targeted relatively specifically in a disorder that is primarily articular. A potential explanation for this may relate to the demonstration that citrulline appears to be an essential constituent of the antigenic determinants recognized by AKA, APF, and AFA [16,17]. The citrulline rich (pro)filaggrin molecule makes an ideal substrate for detecting this reactivity. Moreover, the Sa antigen, which, unlike (pro)filaggrin, is detectable in rheumatoid synovium, has recently also been shown to be citrullinated [18]. It is thus possible that AKA, AFA, APF, and anti-Sa all recognize one or more citrullinated antigens. As with previous studies, the current study documents a good overall correlation between these antibodies. Nevertheless, the data clearly indicate that reactivity to CCP does not capture the complete spectrum of AFA, AKA, and anti-Sa reactivity. Indeed, it has been shown that 56% of patients who were positive for one or more of these antibodies were positive for only one, and that only 7% of these patients were positive for all of them. Similarly, previous studies evaluating AKA, APF, and AFA have shown varying degrees of discordance in the seropositivity of individual patient sera [3–5,14]. Moreover, there was a spectrum of reactivity patterns seen when RA sera were tested against various citrulline containing peptides [17]. Together, these data are most consistent with the hypothesis that individual RA patients respond to unique antigenic determinants, that may preferentially be citrullinated, but that are likely distinct from those of targeted by other RA patients.

It was surprising to discover that anti-RA-33 antibodies were all but absent in this cohort of patients, particularly in view of the previously reported prevalence of 20–40% in other RA cohorts [10–12]. This antibody was reported to have a prevalence of only 6% in a Finnish cohort of early RA patients [24]. It should be pointed out that the testing for anti-RA-33, including that performed in the current study, was all performed in the same laboratory (GS). The reasons for this discrepancy are unclear, and may represent inherent differences in the populations studied. Alternatively, it is possible that the development of anti-RA-33 reactivity increases as the disease progresses. Longitudinal follow up of early RA cohorts, such as the present one, will help to further clarify this issue.

In the current study, it was demonstrated that antibodies directed against putatively citrullinated antigens including Sa, filaggrin, keratin, and CCP are the most specific for RA, and are detectable early in the disease course. It will be of interest to find out whether the cumulative prevalence of specific autoantibody subsets tends to increase over time, as this would suggest that the mechanisms underlying the development of these reactivities continue to evolve over the course of the arthropathy.

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