Commentary

Anti-Sa antibodies: prognostic and pathogenetic significance to rheumatoid arthritis

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Received: 22 Feb 2004 Accepted: 5 Mar 2004 Published: 10 Mar 2004

Arthritis Res Ther 2004, **6**:86-89 (DOI 10.1186/ar1171)

See related Research article: http://arthritis-research.com/content/6/2/R142

Abstract

Anti-Sa antibodies are detected in the serum of 20–47% of patients with rheumatoid arthritis. These antibodies have a high degree of specificity for the disease, and appear to identify a subset of early rheumatoid arthritis patients destined to have aggressive and destructive disease. It has recently been confirmed that anti-Sa antibodies are directed to citrullinated vimentin, thus placing them in the anti-citrulline family of autoantibodies. The Sa antigen has previously been shown to be present in synovium. This, along with the demonstration of citrullinated proteins in rheumatoid synovium, suggests that anti-Sa antibodies may play a pathogenetic role in the initiation and/or persistence of rheumatoid synovitis.

Keywords: anti-citrulline antibodies, anti-Sa, autoantibodies, prognosis, rheumatoid arthritis, synovium

Rheumatoid arthritis (RA) is characterized by the development of a persistent, destructive synovitis that targets multiple joints. The joint involvement is often additive over time, and there is an intriguing propensity for symmetry in the way that the joints are affected. The other characteristic feature of this disease is the presence of specific autoantibodies in the sera of most RA patients. Although this has been the strongest line of evidence suggesting that RA is an autoimmune disease, it has proved to be a major challenge to understand how the synovitis and the autoantibody production are related pathogenetically, and whether one is dependent on the other. The study published in the current issue of *Arthritis Research and Therapy* identifying the antigen against which the RA associated anti-Sa antibodies are directed brings us closer to this understanding [1].

Epidemiology of anti-Sa antibodies

Anti-Sa antibodies were originally identified in a French Canadian patient with RA, and this reactivity was found to be highly specific for RA [2]. Subsequent studies have confirmed the high degree of RA specificity, which exceeds 95% in several populations tested [3–6]. The sensitivity of this antibody for RA varies with the stage of the disease tested, ranging from a low of 20–25% in early RA cohorts [5,6] to a high of 47% in patients with more established disease [6].

An association between anti-Sa antibodies and disease severity has also been demonstrated. In an early RA cohort, the anti-Sa-positive subset had a high mean joint count, a high prevalence of early erosions, and a requirement for more aggressive therapy (Table 1) [5]. Interestingly, most of the anti-Sa-positive patients in this cohort were males, a finding that has not been reported in other RA cohorts. The propensity towards progressive erosions in anti-Sa-positive patients was recently confirmed in another early polyarthritis cohort [7]. A study of patients with established RA showed that patients with destructive disease were three times more likely to be anti-Sa positive than patients without destructive disease [6]. Together these data indicate that anti-Sa antibodies are present early in the disease and are markers of an aggressive, destructive form of RA synovitis.

AFA = anti-filaggrin antibodies; AKA = anti-keratin antibodies; CCP = cyclical citrullinated peptide; G6PI = glucose-6-phosphate isomerase; PAD = peptidyl arginine deaminase; RA = rheumatoid arthritis; RF = rheumatoid factor.

Table 1

Anti-Sa-positive patients with early rheumatoid arthritis have more severe disease

Parameter	Sa^+ RA ($n = 23$)	Sa^- RA ($n = 83$)
Rheumatoid factor positive (%)	83	61
Males (%)	61*	17
Swollen joint count	18 ± 12*	13 ± 9
C-reactive protein (mg/dl)	2.6 ± 3*	1.6 ± 1.4
Erosions (%)	60*	33
No. of DMARDs used	1.4 ± 0.8*	0.9 ± 0.7
Prednisone (mg/d)	4.8 ± 6.0*	1.8 ± 3.3
Inactive disease (%)	0	12

*$P < 0.05$ compared with anti-Sa-negative rheumatoid arthritis. DMARD, disease-modifying anti-rheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor. Errors are shown as standard deviations. Data from [5].

Serum reactivity to Sa overlaps considerably but incompletely with anti-cyclical citrullinated peptide (anti-CCP), anti-filaggrin antibodies (AFA), and anti-keratin antibodies (AKA). Data from one early synovitis cohort demonstrated that 23% of the anti-Sa-positive patients were not positive for these other autoantibodies [5]. Similarly, 27% of AFA-positive patients were not recognized by any other assay. It should be noted that the currently available anti-CCP enzyme-linked immunosorbent assay is more sensitive than the original assay used in this study, and it is possible that some of these anti-Sa-positive and AFA-positive patients would be detected by the more sensitive assay. Nevertheless, these data are consistent with the heterogeneity demonstrated in RA sera when tested against a spectrum of citrullinated peptides [8].

Anti-Sa antibodies are directed toward citrullinated vimentin

A body of research has clearly established that the sera of most RA patients have antibodies directed against antigens containing the non-standard amino acid citrulline (reviewed in [9]). Citrulline is generated by the post-translational deimination of arginine residues by peptidyl arginine deiminases (PADs), a family of enzymes whose members have a tissue-specific distribution. Several previously described RA-specific autoantibodies including AKA, AFA, and anti-perinuclear factor have been shown to recognize citrullinated antigens [8]. This has led to the hypothesis that immune responses to endogenous citrullinated antigens are unique to RA and are potentially of pathogenetic significance in this disease.

Although it has been speculated the anti-Sa antibodies might be directed towards a citrullinated antigen, this has not been directly confirmed until now [1]. The results of the Vossenaar paper identify citrullinated forms of vimentin as the major antigen recognized by sera containing anti-Sa reactivity. However, it is apparent from the immunoblotting studies presented that the patterns of reactivity of anti-citrulline and anti-Sa are overlapping but not identical, suggesting that additional reactivity might be present in the anti-Sa-positive sera. Vimentin was identified on the basis of microsequences of two peptides, one which was specific to vimentin (residues 72–86) and the other (residues 283–293) was present in both vimentin and a related neural intermediate filament component, peripherin [10]. Because of the polyclonal nature of the autoimmune sera, and the relative differences in the detection sensitivities of the Western blot and microsequencing, it will be important to demonstrate clearly that vimentin is indeed the only antigen detected in this molecular mass region by sera containing anti-Sa reactivity. It is also important to consider that in cases where post-translational modifications are a component of the autoantigen, the most readily detectable target might not necessarily reflect the biologically relevant antigen, but rather the most abundant species with the given post-translational modification. Thus, although a citrullinated form of vimentin is clearly a major autoantigen recognized by anti-Sa-positive sera, other autoantigens might also be recognized.

Citrullinated autoantigens in RA synovitis

If antibodies against citrullinated antigens do have a role in the pathogenesis of RA synovitis, these antibodies and the antigens they recognize should be present in the synovium. In keeping with this, it has been shown that anti-citrulline antibodies are indeed produced locally in RA synovium by B lymphocytes and plasma cells [11,12], and that these antibodies represent a much larger proportion of the total IgG content of the synovium than of matched peripheral blood [12]. Moreover, rheumatoid factor (RF) synovial tissue explants continue to produce AFA for prolonged periods in culture.

The presence of locally produced anti-citrulline antibodies suggests that one or more synovial antigens stimulate their production. Detailed studies of the synovium of AFA-positive RA patients demonstrated that these antibodies uniquely recognize citrullinated forms of fibrin and fibrinogen in RA synovium [13]. From a pathogenetic viewpoint, this autoantigen would seem more likely to be relevant to RA than filaggrin, which is found in the epidermis and not the synovium. Interestingly, both share the property of being composed of repeating units capable of varying degrees of citrullination. It is of relevance that we have demonstrated, in a histopathologic study of synovium derived from patients with early synovitis, a strong association between the presence of stromal fibrin deposits and the presence of anti-citrulline antibodies in patient sera [14].

Citrullinated vimentin is an appealing possibility as a synovial autoantigen in RA. The Sa antigen, which is derived from placental tissue and is used to detect anti-Sa antibodies by immunoblotting, was originally shown to be present in synovium [2]. Vimentin is an intermediate filament that is widely expressed in mesenchymal cells and macrophages, and is readily detectable in synovium and fibroblast-like synoviocytes [15–17]. The current data suggest that for synovial vimentin to serve as an autoantigen for anti-Sa antibodies, this protein needs to be citrullinated in the synovial microenvironment. *In vivo*, vimentin is usually not in a citrullinated state, and deimination of this protein by PAD is required for the generation of a suitable antigen that would be recognized by anti-Sa antibodies [18]. Although PAD2 and PAD4 are widely expressed in synovium, they seem to require high intracellular calcium concentrations to deiminate cytoplasmic proteins such as vimentin, and the typically low cytoplasmic concentrations of calcium would not allow this to occur [18,19]. Deimination of vimentin by PAD probably occurs in macrophages when they undergo apoptosis, a process that results in an influx of calcium into the cell [18]. On the basis of this, it is proposed that citrullinated vimentin is most probably generated in the RA synovial microenvironment when macrophages and other cells undergo apoptosis. Yet the generally low apoptotic indices found in RA synovitis would suggest that this process occurs at a relatively low rate (reviewed in [20]). In contrast, inadequate clearance of apoptotic material might lead to persistence of the modified vimentin and might in turn promote the development of anti-Sa antibodies. Because it seems, on the basis of the epidemiologic data, that the anti-Sa-positive patients with RA have particularly severe, destructive disease, synovial expression of the Sa antigen needs to be studied systematically in detailed immunohistologic studies.

Do autoantibodies against citrullinated antigens initiate and/or perpetuate RA?

The serum transfer model of Benoist and Mathis has shown that antibodies against the ubiquitous enzyme glucose-6-phosphate isomerase (G6PI) can induce a destructive, RA-like arthropathy in mice from a wide spectrum of genetic backgrounds [21]. The mechanisms by which the synovitis and articular damage occur are still under exploration, but an important role for components of the innate immune system, such as mast cells, has been demonstrated [22]. The relevance of anti-G6PI antibodies in human RA has been debated. Although one study suggested that anti-G6PI antibodies are uniquely present in RA patients [23], these findings have not been confirmed in several other patient cohorts [24]. Notwithstanding this debate, the serum transfer model does indicate that an antibody directed against a ubiquitous antigen can directly precipitate a destructive inflammatory process that specifically targets the joints.

It has been demonstrated that both RF and anti-CCP antibodies are present in the sera of at least some RA patients years before the onset of clinical disease [25]. Data about anti-Sa reactivity in such patients is currently unavailable. The titers of anti-CCP antibodies in these 'preclinical' individuals seem to increase progressively until disease onset. Presumably these responses are sustained by low-level antigenic stimulation until such a point at which a clinical threshold is crossed, and overt synovitis is precipitated. Exposure to new citrullinated synovial antigens expressed during inflammatory 'flares', when apoptotic indices are probably at their highest, would then further stimulate and mature these responses and, importantly, localize them to lymphoid structures in the synovial membrane on a chronic basis.

The spectrum of autoantigens that participate in, and sustain, RA synovitis continues to be explored. Proteomic approaches can be expected to have a significant role in the characterization of these autoantigens. With the use of combined Western blot and in-gel digestion approaches, candidate antigens have been identified in Behcet's disease [26] and anterior uveitis [27]. By using affinity-purified autoantibodies against post-translationally modified proteins, it should be feasible to capture a spectrum of antigens expressing a given modification. This should provide a better understanding of the repertoire of potential target antigens and their possible roles in the pathogenic process.

Competing interests

None declared.

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