Commentary

# Insights into rheumatoid arthritis derived from the Sa immune system

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Received: 12 June 2000
Revisions requested: 27 June 2000
Revisions received: 25 July 2000
Accepted: 28 July 2000
Published: 17 August 2000

*Arthritis Res* 2000, **2**:429–432



## Abstract

The Sa system is a recently described immune system that has a specificity and positive predictive value of nearly 100% for rheumatoid arthritis (RA) in Asia, Europe and the Americas. Its sensitivity of 30–40% suggests that it identifies a subset of RA patients. Anti-Sa antibodies are present from disease onset and are predictive of disease severity. The immune reactants are plentiful in the target tissue: antigen is present in the synovium, IgG antibody in the fluid. Immunologically, Sa is a hapten-carrier antigen in which vimentin is the carrier and citrulline is the hapten. The citrullination of vimentin is closely related to apoptosis, and citrullinated vimentin is extremely sensitive to digestion by the ubiquitous calpains. Nevertheless, Sa is found in only a few cell lines. Calpastatin, the natural specific inhibitor of calpains, is also a RA-associated, albeit non-specific, autoimmune system. Is it possible that calpain-related apoptotic pathways could be prominent in cells containing Sa? The task is to reconcile the specificity of Sa/citrullinated proteins in a multifactorial and polygenic disease such as RA.

**Keywords:** anti-Sa autoantibodies, citrullinated proteins, diagnosis, prognosis, rheumatoid arthritis

## Introduction

The objective of current research activity on RA-associated autoimmune systems is to find a disease-specific marker [1]. It would be simplistic to view that marker as being primarily of importance in clinical diagnosis. Indeed, its net diagnostic contribution beyond that of using the American College of Rheumatology (ACR) criteria with rheumatoid factor is likely to be clinically insignificant in established RA [2–4]. What clinicians really need is a marker that would be a good a priori predictor of future disease severity [5]. In contrast, what researchers are looking for are fresh etiopathogenic clues [6].

## Sa for the clinician

The serum of Mrs Sa… identifies unique tissue-specific banding patterns at approximately 50 kDa in western blots with normal human spleen and placenta, and rheumatoid synovial extracts [7–9]. Until recently (see below), the Sa polypeptides were not found in extracts of a variety of cells of different lineage and were different in western blots from all previously described systems associated with RA including rheumatoid factor, filaggrin and calpastatin [7,9,10]. The components of the Sa system are present at high concentrations in the rheumatoid joint: antigen in the synovium and IgG antibody in the fluid [8,11]. Looking for

ACR = American College of Rheumatology; AKA = anti-keratin antibodies; APF = anti-perinuclear factor; HLA = human leucocyte antigen; PAD = peptidylarginine deiminase; RA = rheumatoid arthritis.

**Table 1**

**Diagnostic significance of anti-Sa in the rheumatic diseases**

| Country | Reference | Total no. of patients | Rheumatoid patients | Sensitivity (%) | Specificity (%) | Positive predictive value (%) |
|---|---|---|---|---|---|---|
| Canada | [7] | 482 | 206 | 43 | 99 | 97 |
| Spain | [17] | 1087 | 360 | 33 | 100 | 99 |
| France | [3] | 403 | 154 | 40 | 92 | n/a |
| Austria | [16] | 247 | 46 | 37 | 98 | n/a |
| China | [18] | 518 | 191 | 32 | 99 | 94 |
| Total | | 2737 | 957 | 37 | 98 | 97 |

The control populations were normal individuals and patients with degenerative arthritis from the communities shown, autoimmune patients with systemic lupus erythematosus, Sjögren and other systemic connective tissue diseases, and patients with chronic arthritis of the spondylarthropathy variants. All RA patients satisfied the ACR criteria, including those with early (less than 1 year) disease. It is important to note that the data on sensitivity, specificity and positive predictive value for RA are valid only for established RA cohorts and would not necessarily apply to early synovitis cohorts, where satisfying the ACR criteria for RA at entry is not a prerequisite. n/a, not applicable.

autoantibodies in sera of 20 pairs of monozygotic twins discordant for RA, anti-Sa antibodies were found only in rheumatoid twins, whereas all the previously mentioned autoantibodies could be found in both rheumatoid and healthy twins [12]. A caveat to that study is the fact that we could test only those sera with the immunofluorescence method for anti-perinuclear factor (APF) and anti-keratin antibodies (AKA). Both tests are plagued with problems of subjective interpretation. The possibility therefore cannot be excluded that our observation could be extended to antibodies against other citrullinated protein or peptide antigens if properly assayed (see below). Overall, those results suggest a closer association of anti-Sa with the disease than with the genes [12]. That last piece of information supports the hypothesis of an environmental trigger.

To submit patients to early aggressive therapy, candidate markers are needed that are both present at disease onset and predictive of disease severity. The shared epitopes of human leucocyte antigen (HLA)-DR alleles have provided epidemiological insight in our understanding of RA but have proved to be of little utility in the clinic because shared epitope genotyping is essentially an a posteriori marker of disease severity [3,13–15]. In contrast, the anti-Sa antibody is present in early disease [4,7,16] and seems to be slightly better than HLA genotyping and other autoantibodies as a predictor of 'erosivity' [3,4]. The anti-Sa antibody sensitivity (approximately 43%), specificity (approximately 99%) and positive predictive value (approximately 97%) for RA have been remarkably reproducible in more than 3000 patients from Europe, America and Asia [3,4,7,16–18] (Table 1).

## Sa for the researcher

In the past 10 years we have purified Sa from human placenta and obtained several amino acid microsequences that all pointed to vimentin as the elusive Sa antigen [15,17]. That was in flagrant contradiction of all the published work on anti-vimentin autoantibodies in human diseases and with the observed RA specificity. All our efforts at cloning Sa with affinity-purified and IgG-adsorbed rheumatoid anti-Sa antibodies to immunoscreen human placental cDNA expression libraries were unsuccessful. As a by-product, we cloned the RA-associated autoantigen, calpastatin (see below) [16]. We were even unable to clone vimentin. We were obviously missing something. Interestingly, the Sa and citrulline-related autoimmune systems (anti-perinuclear-APF, anti-profilaggrin-APF, anti-keratin-AKA, anti-filaggrin-AFA and anti-citrullinated peptides-ACP antibodies) have the same high specificity for RA and early predictive potential for severe RA [3,4, 19–21]. The explanatory breakthrough came from the recent research on filaggrin.

Sa and filaggrin are two different proteins that are both specifically and often (but not always) simultaneously targeted by the same RA sera [3]. A survey of the metabolic handling of filaggrin reveals that it is dephosphorylated, deiminated and twice proteolytically cleaved by enzymes, the latter step being by means of the calcium-dependent cytosolic calpains [1,22–25]. The identification of the citrulline epitopes responsible for the reactivity of RA sera with filaggrin was therefore a logical and major step forward [19,20]. The epitopes are centered on citrulline residues resulting from the deimination of selected arginine residues on filaggrin. That post-translational modification is performed by the calcium-dependent peptidylarginine deiminase (PAD) present in the skin. There are at least four other PAD isoenzymes in other tissues (data from GenBank). We recently identified by western blot an Sa-related, disease-specific, complex banding pattern in ECV 304, a human endothelial cell line, and in human umbilical-vein endothelial cells. Exactly the same multiplicity

of the reaction was seen with a rabbit polyclonal anti-citrulline antibody [26]. We extended that observation to show that bovine albumin, total histones and myelin basic protein could all serve as a carrier of the RA-specific epitopes when they were citrullinated *in vitro* with PAD [27]. We have since cloned vimentin by PCR and citrullinated its expressed recombinant *in vitro*. By differential absorption it proved to be the Sa antigen (Lapointe E, Rochdi MD, Ménard HA, in preparation). Trichohyalin (APF), filaggrin (AFA), keratin (AKA), vimentin (anti-Sa), myelin basic protein and fibrin [28] are all citrullinated *in vivo*. All those proteins can also be citrullinated *in vitro* and function as RA-specific targets, albeit with different sensitivity. The clinical and pathophysiological significance of that phenomenon does not seem to be important *in vivo*, at least in the skin or nervous system in RA. It might be important if the carrier-hapten is generated and accessible to the adaptive immune system in the articular tissues.

## Future research on Sa

Our working hypothesis is that Sa or citrullinated vimentin is the original hapten-carrier immunogen. Classically, antigen-presenting cells will process the original carrier and present its derived peptide in an MHC-restricted fashion at the T cell level and carrier-specific help will then be provided to the hapten-specific B cell. Although the original carrier is important *in vivo*, the carrier is not important in detecting anti-hapten antibodies *in vitro*. That explains why apparently entirely different RA-specific humoral systems were found independently. Many questions now arise, of which the following are a sample. How does citrullination of specific arginine residues come about? Are there cell-specific PADs? How many different PADs are there in each cell type? Vimentin is preferentially citrullinated during apoptosis; that results in protein denaturation and the disorganization of intermediate filaments [29,30]. However, although vimentin is untouched, Sa or citrullinated vimentin is destroyed almost immediately *in vitro* by calpains [17]. Why can it survive as a molecule only in certain cells such as endothelial cells? Where else can it survive? Is that related to its immunogenicity and why? Why is vimentin not co-targeted more often in RA? Is the RA-associated secondary development of antibodies against calpastatin, the natural inhibitor of calpains, a related defence mechanism or a pro-inflammatory amplification loop [31]? Is the extraordinary fine disease specificity of the autoantibodies representing the mythical footprint of the elusive initial causative event(s)? Indeed, several microorganisms have arginine-to-citrulline deiminase activities. Can they also citrullinate proteins? Such a post-translational modification coming from the environment would be a fresh illustration of the 'hit-and-run' theory of autoimmunity.

In autoimmune diseases, 'multifactorial' and 'polygenic' are vogue words. 'Multifactorial' could mean the assault of non-specific environmental factors acting preferentially on specific cells or tissues. That would result in the overproduction of specific endogenous or exogenous arginine deiminases with the over-citrullination or under-citrullination of residues normally or not normally modified by that post-translational pathway. Because citrullination, whether by means of apoptosis or by some other mechanism, is a natural phenomenon, those neo-antigens would need to occur in individuals with the appropriate polygenic background (30 or so genes, as in lupus) that would modulate apoptosis, break tolerance, mount the specific immune response and influence disease expression. Exploring that framework will probably earn great dividends for both patients and science.

## Acknowledgements

This work was supported by the Medical Research Council of Canada and the Arthritis Society.

## References

1. Ménard HA, El-Amine M, Després N: **Rheumatoid arthritis associated autoimmune systems.** *J Rheumatol* 1998, **25**:835–837.
2. Arnett, FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA Jr, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG: **The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis.** *Arthritis Rheum* 1988, **31**:315–324.
3. Hayem G, Chazerain P, Combe B, Elias A, Haim T, Nicaise P, Benali K, Eliaou JF, Kahn MF, Sany J, Meyer O: **Anti-Sa antibody is an accurate diagnostic and prognostic marker in adult rheumatoid arthritis.** *J Rheumatol* 1999, **26**:7–13.
4. Goldbach-Mansky R, Lee J, McCoy A, Hoxworth J, Yarboro C, Smolen JS, Steiner G, Rosen A, Zhang C, Ménard HA, Zhou ZJ, Palosuo T, Van Venrooij WJ, Wilder RL, Klippel JH, Schumacher HR Jr, El-Gabalawy HS: **Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset.** *Arthritis Res* 2000, **2**: 236–243.
5. Kim JM, Weisman MH: **When does rheumatoid arthritis begin and why do we need to know?** *Arthritis Rheum* 2000, **43**:473–484.
6. Bläss S, Engel JM, Burmester GR: **The immunologic homunculus in rheumatoid arthritis.** *Arthritis Rheum* 1999, **42**:2499–2506.
7. Després N, Boire G, Lopez-Longo FJ, Ménard HA: **The Sa system, a novel antigen-antibody system specific for rheumatoid arthritis.** *J Rheumatol* 1994, **21**:1027–1033.
8. Després N, Boire G, Ménard HA, Poole AR, Adams M, Lazure C: **The rheumatoid arthritis specific Sa autoantigen is present in high concentration in the target tissue [abstract].** *Arthritis Rheum* 1992, **35**:S72.
9. Després N: **Immunological and molecular characterization of autoimmune systems associated with rheumatoid arthritis.** PhD Thesis, Université de Sherbrooke; 1995.
10. Després N, Talbot G, Plouffe B, Boire G, Ménard HA: **Detection and expression of cDNA clone that encodes a polypeptide containing two inhibitory domains of human calpastatin and its recognition by rheumatoid arthritis sera.** *J Clin Invest* 1995, **95**:1891–1896.
11. Lapointe E, Després N, Ménard HA: **Clinical and biochemical update on the rheumatoid arthritis-specific Sa system [abstract].** *Arthritis Rheum* 1998, **41**:S349.
12. Hébert A, Ménard HA, Berthelot JM, Williams RC Jr: **Autoantibodies in monozygotic twins discordant for rheumatoid arthritis [abstract].** *Arthritis Rheum* 1996, **39**:S156.
13. Gran JT, Husby G, Thorsby E: **HLA antigens in palindromic rheumatism, nonerosive rheumatoid arthritis and classical rheumatoid arthritis.** *J Rheumatol* 1984, **11**:136–140.
14. Wagner U, Kaltenhauser S, Sauer H, Arnold S, Seidel W, Hantzschel H, Kalden JR, Wassmuth R: **HLA markers and prediction of clinical course and outcome in rheumatoid arthritis.** *Arthritis Rheum* 1997, **40**:341–351.

15. El-Gabalawy HS, Goldbach-Mansky R, Smith II D, Arayssi T, Bale S, Gulko P, Yarboro C, Wilder RL, Klippel JH, Schumacher HR Jr: **Association of HLA alleles and clinical features in patients with synovitis of recent onset.** *Arthritis Rheum* 1999, **42**:1696–1705.
16. Hueber W, Hassfeld W, Smolen JS, Steiner G: **Sensitivity and specificity of anti-Sa autoantibodies for rheumatoid arthritis.** *Rheumatology* 1999, **38**:155–159.
17. Rodriguez-Mahou M: **Cellular localization and distribution in human and animal tissues of a novel antigen (Sa antigen) associated with rheumatoid arthritis: description of a specific diagnostic method.** PhD Thesis, Universidad Complutense de Madrid; 1996.
18. Xu SD, Tang FL, Shi L, Gan X, Shi Y, Cheng L, Li J, Dong Y: **Anti-Sa antibody in Chinese rheumatoid arthritis.** *Chin Med J* 1998, **111**: 204–207.
19. Schellekens GA, de Jong BAW, van den Hoogen FHJ, van de Putte LB, van Venrooij WJ: **Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies.** *J Clin Invest* 1998, **101**:273–281.
20. Girbal-Neuhauser E, Durieux JJ, Arnaud M, Dalbon P, Sebbag M, Vincent C, Simon M, Senshu T, Masson-Bessiere C, Jolivet-Reynaud C, Jolivet M, Serre G: **The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are post-translationally generated on various sites of (pro)filaggrin by deimination of arginine residues.** *J Immunol* 1999, **162**:585–594.
21. Schellekens GA, Visser H, de Jong BAW, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ: **The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide.** *Arthritis Rheum* 2000, **43**:155–163.
22. Resing KA, Al-Alawi N, Blomquist C, Fleckman P, Dale BA: **Independent regulation of two cytoplasmic processing stages of the intermediate filament-associated protein filaggrin and role of Ca2 in the second stage.** *J Biol Chem* 1993, **268**:25139–25145.
23. Senshu T, Kan S, Ogawa H, Manabe M, Asaga H: **Preferential deimination of keratin K1 and filaggrin during the terminal differentiation of human epidermis.** *Biochem Biophys Res Commun* 1996, **225**:712–719.
24. Tarcsa E, Marekov LN, Mei G, Melino G, Lee SC, Steinert PM: **Protein unfolding by peptidylarginine deiminase. Substrate specificity and structural relationships of the natural substrates trichohyalin and filaggrin.** *J Biol Chem* 1996, **271**:30709–30716.
25. Yamazaki M, Ishidoh K, Suga Y, Saido TC, Kawashima S, Suzuki K, Kominami E, Ogawa H: **Cytoplasmic processing of human profilaggrin by active mu-calpain.** *Biochem Biophys Res Commun* 1997, **235**:652–656.
26. Zhou ZJ, Lapointe E, Richard C, Ménard HA: **Autoantibodies to a 53-kDa antigen of endothelial cells are highly specific for rheumatoid arthritis and related to anti-Sa [abstract].** *Arthritis Rheum* 1999, **42**: S89.
27. Lapointe E, Déry U, Vaillancourt F, Ménard HA, Senshu T: **Rheumatoid sera potentially recognize all citrullinated proteins [abstract].** *Arthritis Rheum* 1999, **42**:S86.
28. Masson-Bessière C, Sebbag M, Girbal-Neuhauser E, Vincent C, Serre G: **Synovial target antigens of antifilaggrin autoantibodies are deiminated forms of fibrin alpha and beta chains [abstract].** *Rev Rheum* 1999, **66**:754
29. Asaga H, Yamada M, Senshu T: **Selective deimination of vimentin in calcium ionophore-induced apoptosis of mouse peritoneal macrophages.** *Biochem Biophys Res Commun* 1998, **243**:641–646.
30. Inagaki M, Takahara H, Nishi Y, Sugawara T, Sato C: **$Ca^{2+}$-dependent deimination-induced disassembly of intermediate filaments involves specific modifications of the amino-terminal head domain.** *J Biol Chem* 1989, **264**:18119–18127.
31. Ménard HA, El-Amine M : **The calpain–calpastatin system in rheumatoid arthritis.** *Immunol Today* 1996, **17**:545–547.

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