



Rheumatoid Arthritis : Looking Beyond Rheumatoid Factor

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ABSTRACT

In recent years there is a shift in the paradigm in treatment of rheumatoid arthritis (RA). Early and aggressive use of disease modifying drugs is advocated. Biological therapy has brought a revolution in the treatment of this chronic and disabling disease. The strategy at the present is to diagnose RA early in its course. More specific autoantibodies than rheumatoid factors are available now. One of them, the antibodies to CCP, has proven to be nearly 100% specific for diagnosing RA. This has immense potential in the management of RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic crippling disease that causes significant morbidity and even mortality. The etiology is not known and hence the diagnosis is based on the typical clinical picture of inflammatory symmetrical polyarthritis supported by imaging and serological investigations. Among the serological investigations, rheumatoid factor (RF) is the most widely used. Rheumatoid factor (RF) is an IgM/IgG/IgA autoantibody directed against the Fc portion of human IgG. Commonly it is the IgM RF that is assayed either by latex agglutination or nephelometry. Even though it is present in 60 to 80% of patients with RA, its diagnostic role is limited because of low specificity. It is present in several diseases including autoimmune connective tissue diseases such as primary Sjogren's syndrome (SS), systemic lupus erythematosus (SLE), essential mixed cryoglobulinemia (EMC), infectious diseases like tuberculosis, leprosy and in elderly healthy individuals.¹

In recent years, there has been a shift in the paradigm of treatment of RA. The traditional 'go slow' approach has given way to more aggressive use of disease modifying anti-rheumatic drugs (DMARD) and biological agents early in the course of the disease. 'Early RA' refers to disease duration of less than two years. There has been an emphasis of diagnosing earlier than two years and categorizing the disease as Early or Very Early Rheumatoid Arthritis (VERA). Unfortunately, with less than one year of disease documenting joint damage by radiology is not rewarding. Use of MRI or ultrasound has been widely employed to pick up erosions. A serological marker that would be specific for RA should be useful for clinician to define treatment strategy that would achieve remission and prevent joint damage.

AUTOANTIBODIES OTHER THAN RHEUMATOID FACTOR IN RA

Search for autoantibodies besides RF has led to description of a number of autoantibodies such as antiperinuclear factor, anti-keratin antibodies, antibodies to cyclic citrullinated peptide (CCP), antibodies to Sa, p68 and calpastatin. Of these, anti-CCP antibodies stand out as the most useful clinically, especially in defining RA in early arthritis. We herein describe the important aspects of some of these autoantibodies.

ANTIPERINUCLEAR ANTIBODIES

Nienhuis and Mandema described the antiperinuclear factor (APF) in 1964 as a specific serological marker for RA.² The antigens are located in the keratohyaline granules of the cytoplasm of buccal mucosal cells.³ The prevalence of APF varied widely between 46-86% of patients with RA.^{4,5} The sensitivity of APF in RA was 81%.⁶ Like RF it was not specific for RA and has been found in patients with SLE (21%), systemic sclerosis (26%) and SS (29%), lung cancer and EBV infections.⁶⁻⁸ Further, it was revealed that these autoantibodies to perinuclear factor and profilaggrin were present in the keratohyaline granules.⁶ However, the exact specificities were different as no competitive inhibition was evident between each other. Thus APF and AFA reacted to different epitopes of profilaggrin.

ANTI-KERATIN ANTIBODIES (AKA)

Unlike APF, antibodies to keratin (AKA) have a higher diagnostic specificity for RA. Antibodies to keratin were identified by indirect immunofluorescence (IIF), using cornified epithelium of rat esophagus as substrate. These were so named because keratin was the major protein component of the cornified epithelium. These

were of IgG class and also reacted with the stratum corneum of human epidermis.⁹⁻¹¹ It was later shown that the binding of these antibodies was not inhibited by preincubating the sera with rabbit epidermal cytokeratins antisera, therefore the antigenic components of antikeratin antibodies was not clear. Simon, et al described that the antigenic moiety resides in a protein called the filaggrin. The antigen was a 40 KD isoform of filaggrin involved in the AKA binding of human epidermal cells.¹² Filaggrin is a 37 KD histidine-rich protein involved in cytokeratin aggregation during terminal differentiation in mammalian epidermis. Antifilaggrin antibodies (AFA) were reported in 75 % of RA patients compared to only 7 % of controls.¹² Filaggrin is synthesized in the granular layer of the epidermis as profilaggrin (pF) and undergoes proteolytic cleavage during cell differentiation. The 40 KD isoform probably forms due to destruction of the synovial cells. The highly polymorphic pF gene is located at 1q21. pF repeats are flanked by truncated units fused to non-filaggrin proteins.¹³ As already mentioned, AKA are detected by IIF on rat esophagus epithelium/human epidermis. They can also be demonstrated on rat lip, rabbit and monkey esophagus.¹⁴

The overall diagnostic sensitivity of AKA is 40-55% and the specificity is 95-99%.¹⁴ The negative predictive value of AKA is low. There is no relation to age, gender or duration of disease.¹⁵ AKA predate the appearance of RA.¹⁶ Their presence has been considered a marker of severe disease as well as for the presence of extra-articular manifestations.¹⁷ Moreover high titre AKA has been demonstrated in more than 30% of patients with no RF.¹⁴ Hence RF and AKA can be used to identify different subsets of RA patients.

ANTIBODIES TO CYCLIC CITRULLINATED PEPTIDE (ANTI-CCP)

In spite of the encouraging results obtained with APF and AKA, it was not widely used as a routine diagnostic test for RA. There were problems in availability of buccal smear donors. The indirect immunofluorescent (IIF) technique involved was also very laborious and difficult to standardize. Although immunoblotting technique was developed for APF assay, the results were not uniformly encouraging.^{18,19} Hence, the search for an antigenic peptide component resulted in the discovery of citrulline.

Citrulline residues were identified as essential components of the antigenic determinants recognized by autoantibodies in RA.²⁰ Citrulline is formed during cell differentiation (fig.1) from arginine by the action of peptidyl arginine deiminase (PAD) enzyme and at least five PAD enzymes have been identified so far.²¹ Deimination of arginine leads to a change in molecular mass of one dalton and loss of one positive charge. Citrullination is a potent mechanism involved in post-translational modification of proteins. This is a postulated model for pathogenesis of the humoral response in RA. PAD enzymes are influenced by estrogenic stimulation and this is a possible explanation for the higher prevalence of autoimmune diseases in females.²²

Citrullination has also been found to occur during apoptosis and vimentin is a potent target for the same.²³ During apoptosis, cellular fragments are formed which are normally cleared very

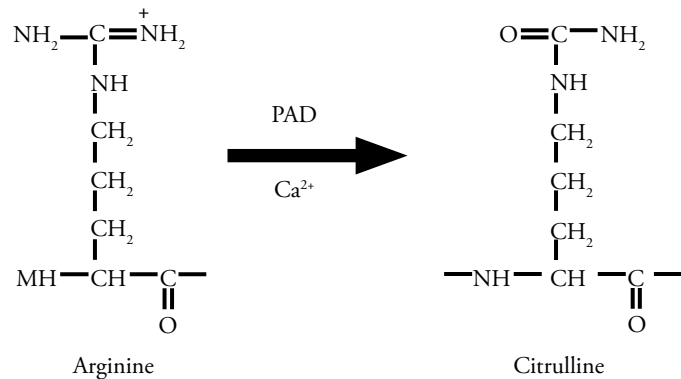


Fig. 1: Peptidyl arginine deiminase (PAD) converts arginine to citrulline

fast. However, in conditions where there is modification of the cellular components, antigen translocation to the membrane surface can lead to loss of intercellular interaction and clearance is affected. These antigens on the surface can act as autoantigens due to sustained exposure. Citrullination of vimentin leads to depolymerization and disruption of the cytoskeletal network. Citrullination also occurs in filaggrin polypeptides and might be involved in the genesis of AKA.¹⁸

The initial ELISA kits developed with synthetic short linear citrulline peptides as antigens failed to give reproducible results. This was ascribed to difficulty in adsorption of the linear peptides to polystyrene plates and loss of the original configuration of the epitope within the solution leading to reduced antigen-antibody affinity. A β -turn conformation is adopted by peptides during antigen-antibody reaction.²⁴ It was demonstrated previously that cyclization of citrulline with cysteine residues led to formation of the β -turn configuration.²⁵ Schellekens GA, et al modified the peptide by converting it into a cyclic form and substituting the serine residues of the original peptide with cysteine.²⁶ The peptides were covalently linked to polystyrene. This improved the antigen-antibody affinity as well as the stability. They used this novel cyclic peptide in 149 RA patients and 337 controls from an early arthritis clinic and reported a sensitivity and specificity of 48% and 96% respectively for anti-CCP ELISA (cut-off 92 units) while those for IgM RF ELISA (cut-off 5 units) were 54% and 91% respectively. When either of the two was considered, the sensitivity rose to 63% and the specificity was 88%. If both were present the specificity was 98%. The positive predictive value if both were present was 91%. Interestingly, they also found an increase in sensitivity of anti-CCP ELISA to 53% while that of IgM RF dropped to 49% over one year follow-up. However, with respect to predictive capability of erosions at two years, both fared equally. Kroot, et al also reported similar findings in a cohort of 273 patients followed up for 6 years.²⁷

In a study on 404 patients followed up for 2 years, there was significant association of APF with erosive disease and activity.²⁸ In their set of experiments they did both IIF for APF and Anti-CCP ELISA. Anti-CCP ELISA positivity was considered an indicator of APF presence as they recognize a subset of APF determinants. Hence the results obtained for APF was a combination of both tests. There was discordance in the results

of the two tests only in 17% of patients and the majority of them had positive IIF and negative ELISA. They demonstrated more erosions in patients with RF+/APF+ status compared to those with RF+/APF-, RF-/APF+ and RF-/APF- patients. Hence APF was useful as a predictor of erosions within RF+ patients. They also showed that median joint counts were significantly higher in APF+ patients compared to those without the corresponding antibodies. There was no such co-relation with RF. It is clear that anti-CCP ELISA is the closest we have reached as far as a specific antibody for RA is concerned. It is also more sensitive than RF in early RA.

OTHER SEROLOGICAL MARKERS IN RA

The other serological markers identified in RA are antibodies to RA 33, Sa, p68, calpastatin, and GPI. A 68k antigen is the target of both T and B cell responses in patients with RA. The p68 antibody assay has a sensitivity of 66% and specificity of 99%.²⁹ This 68k antigen has been demonstrated in almost all tissues assayed with immunoblotting. The reason why such a ubiquitous molecule gives rise to a highly specific immune response in RA is unsorted. The epitope has been identified by alkaline β elimination to be a glycoepitope. It is considered that immune dysregulation involving IL 10 and TGF β might be responsible for the development of antigenicity. The exact nature of the antigen needs to be characterized.

The role of anti-GPI antibodies were first recognized when they were found in high titers in the KRN T cell receptor-transgenic mouse models with severe arthritis. van Gaalen, et al found a significant association of these antibodies in patients with ExRA. In their study on 131 RA patients, they found the presence of anti-GPI antibodies in 5% of uncomplicated RA patients and 4% of controls. Anti-GPI antibodies were present in 92% of patients with Felty's syndrome, 45% of patients with vasculitis and 18% of patients with rheumatoid nodules. GPI is a cytosolic enzyme involved in glycolysis and gluconeogenesis. It has been identified on the surface of blood vessels in RA synovium. It is hypothesized that the anti-GPI antibodies are the result of excessive neutrophil destruction or they may be targeting the vessel wall forming immune complexes in situ. In summary, anti-GPI antibodies can be used for the identification of RA patients with complicated disease especially the Felty's syndrome.

CONCLUSION

A specific biological surrogate marker, antibodies to CCP, is now available that is nearly 100% specific for RA. This has paved the way to diagnose RA very early. This can help in instituting DMARDs and cytokine inhibitors to induce remission early to minimize joint failure.

REFERENCES

1. Smolen JS. Autoantibodies in rheumatoid arthritis. In Manual of Biological Markers of Disease. Eds van Venrooij WJ and Maini RN. 1996. Kluwer Academic publishers, Dordrecht, Netherlands. C1.1/1-C1.1/18.
2. Nienhuis RLF, and Mandema EA. A new serum factor in patients with rheumatoid arthritis. The antiperinuclear factor. *Ann Rheum Dis* 1964;23:302-305.
3. Smit JW, Sondag-Tschroots IRJM, Aaij C, Feltkamp TEW, Feltkamp-Vroom TM. The antiperinuclear factor II. A light microscopical and

immunofluorescent study on the antigenic substrate. *Ann Rheum Dis* 1980;39:381-6.

4. Westgeest AAA, Boerbooms AM Th, Jongmans M, Vandenbrooucke JP, Vierwinden G, Van de Putte LBA. Antiperinuclear factor: indicator of more severe disease in seronegative rheumatoid arthritis. *J Rheumatol* 1987;14:893-7.
5. Sondag-Tschroots IRJM, Aaij C, Smit JW, Feltkamp TEW. The antiperinuclear factor. II. The diagnostic significance of the antiperinuclear factor for rheumatoid arthritis. *Ann Rheum Dis* 1979;38:248-51.
6. Hoet RMA, Boerbooms AM Th, Arends M, Ruiters DJ, van Venrooij WJ. Antiperinuclear factor, a marker autoantibody for rheumatoid arthritis: colocalisation of perinuclear factor and profilaggrin. *Ann Rheum Dis* 1991;50:611-8.
7. Youinou P, Zabba C, Eveillaud C, et al. Antiperinuclear activity in lung carcinoma patients. *Cancer Immunol Immunotherap* 1984;18:80-1.
8. Westgeest AAA, van Loon AM, van der Logt JTM, Van de Putte LBA, Boerbooms AM Th. Antiperinuclear factor, a rheumatoid arthritis specific autoantibody: its relation to Epstein-Barr virus. *J Rheumatol* 1989;16:226-30.
9. Young BJ, Mallya RK, Leslie RD, Clark CJ, Hamblin TJ. Antikeratin antibodies in rheumatoid arthritis. *Br Med J* 1979;2:97-99.
10. Quismorio FP Jr, Kaufman RL, Beardmore T, Mongan S. Reactivity of serum antibodies to the keratin layer of rat esophagus in patient with rheumatoid arthritis. *Arthritis Rheum* 1983;26:494-499.
11. Johnson GD, Carvalho A, Holbrow EJ, Goddard DH, Russel G. Antiperinuclear factor and keratin antibodies in rheumatoid arthritis. *Ann Rheum Dis* 1981;40:263-266.
12. Simon ME, Girbal E, Sebbag M, Gomez-Daudrix V, Vincent C, Salima G, Serre G. The cytokeratin filament-aggregating protein fillaggrin is the target of the so-called "Antikeratin antibodies", autoantibodies specific for rheumatoid arthritis. *J Clin Invest* 1993;92:1387-93.
13. Gan SQ, McBride OW, Idler WW, Markova N and Steinert PM. Organisation, structure and polymorphisms of the human profilaggrin gene. *Biochemistry* 1990;29:9432-9440.
14. Serre G, Vincent C, Filaggrin (keratin) autoantibodies. In Autoantibodies. Eds Peter JB and Schoenfeld Y. Elsevier Science B. V. 1996 pp 271-276.
15. Vincent C, Serre G, Lapeyri F, Fournier B, Ayrolles C, Fournier A, Soleilhavoup JP. High diagnostic value in rheumatoid arthritis of antibodies to stratum corneum of rat oesophagus epithelium, so called 'antikeratin antibodies'. *Ann Rheum Dis* 1989;48:712-22.
16. Kurki P, Aho K, Palosuo T, Heliovaara M. Immunopathology of rheumatoid arthritis. Anti-keratin antibodies precede the clinical disease. *Arthritis Rheum* 1992;35:914-7.
17. Hoet RM, van Venrooij WJ. The antiperinuclear factor (APF) and antikeratin antibodies (AKA) in rheumatoid arthritis. In Rheumatoid arthritis. Eds Smolen JS, Kalden JR, Maini RN. Springer Verlag, Heidelberg. 1992 pp 299-318.
18. Vincent C, Simon M, Sebbag M, Girbal-Neuhausser E, Durieux JJ, Cantagrel A, et al. Immunoblotting detection of autoantibodies to human epidermis filaggrin: a new diagnostic test for rheumatoid arthritis. *J Rheumatol* 1998;25:838-46.
19. Slack SL, Mannik M, Dale BA. Diagnostic value of antibodies to filaggrin in rheumatoid arthritis. *J Rheumatol* 1998;25:847-51.
20. Schellekens GA, de Jong BAW, van den Hoogen FHJ, et al. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 1998;101:273-81.
21. van Venrooij, Purijs GJ. Citrullination: a small change for a protein with great consequences for rheumatoid arthritis. *Arthritis Res* 2000;2:249-251.
22. Takahara H, Kusubata M, Tsuchida M, et al. Expression of peptidylarginine deiminase in the epithelial cells of mouse uterine is dependant on estrogen. *J Biol Chem* 1992;267:520-525.
23. Asaga H, Yamada M, Senshu T. Selective deimination of vimentin in calcium ionophore-induced apoptosis of mouse peritoneal macrophages. *Biochem Biophys Res Commun* 1998;243:641-6.
24. Dyson HJ, Wright PE. Antigenic peptides. *FASEB J* 1995;9:37-42.

25. Dorrow DS, Shi PT, Carbone FR, Minasian E, Todd PEE, Leach SJ. Two large immunogenic and antigenic peptides and the effect of cyclisation. *Mol Immunol* 1985;22:1255-64.
26. Schellekens GA, Visser H, de Jong BAW, van den Hoogen FHJ, et al. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 1998;101:273-81.
27. Kroot E, de Jong BAW, van de Putte LBA, van Venrooij WJ, van Riel PLCM. Serum anti cyclic citrullinated peptide antibody (anti-CCP) levels in patients with rheumatoid arthritis: Correlation with disease activity during five years of follow-up. *Arthritis Rheum* 2000;43:S156.
28. van Jaarsveld CHM, ter Borg EJ, Jacobs JWG, Schellekens GA, Gmelig-Meyling FHJ, van BOOMA-Frankfort C, et al. The prognostic value of the aniperinuclear factor, anti-citrullinated peptide antibodies and rheumatoid factor in early rheumatoid arthritis. *Clin Exp Rheumatol* 1999;17:689-97.
29. Blaß St, Specker Ch, Lakomek HJ, Schneider EM, Schwochau M. Novel 68k autoantigen detected by rheumatoid arthritis (RA) specific autoantibodies. *Ann Rheum Dis* 1995;54:355-60.