

Rethink about the role of rheumatoid factor and anti-citrullinated protein antibody in rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by joint inflammation and extra-articular manifestations. Many questions in the pathogenesis, clinical manifestation, and disease spectrum are answered after the discovery of the first autoantibody namely rheumatoid factor (RF). The finding of the second autoantibody named anti-citrullinated protein antibody (ACPA), which unearths the importance of protein citrullination process. It further provides the insight how immune cells and complement interact to perpetuate the inflammatory response. These two autoantibodies pave the way for our better understanding of RA. This review article focuses on the history, pathophysiology, and clinical association of these two autoantibodies in RA.

Keywords

rheumatoid arthritis • rheumatoid factor • anti-citrullinated protein antibody

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by progressive destructive joint damage and many extra-articular manifestations.^[1–3] Joint involvements are usually symmetric with early morning stiffness. Any joint can be involved, although lumbar spine involvements are rarely reported.^[4, 5] Pulmonary, ocular, and cardiovascular symptoms are common extra-articular manifestations.^[6–10] It is a fact that the natural history of RA is quite variable and its clinical course is fluctuating. If left untreated, most patients have poor prognostic joint outcome and might lead to miserable life quality. In addition to clinical finding, laboratory tools are very useful for the diagnosis of RA. As listed in the ACR/EULAR classification criteria, two autoantibodies namely rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) can be found in more than 60% of RA patients.^[11–14] These two autoantibodies are not only hallmarks of the disease but also suggested to play important role in the pathogenesis of RA.

Pathogenesis of RA is complex and heterologous.^[15–17] During the past decade, attention has been given to genetic and several environmental factors such as cigarette smoking and mucosa diseases. The pathogenesis of RA cannot be fully explained by any single factor. It was suggested that the interaction of these factors occurred long before the

clinical onset of RA. A pre-clinical case maybe symptomless or with very mild arthralgia, but importantly, autoantibodies are present in their serum up to 13 years prior to the clinical disease.^[18, 19] It indicated that in this pre-RA stage, reactivity to some self-antigens might trigger limited inflammatory responses followed by tissue injury. The vicious cycles continue to evolve between inflammation and tissue injury together with the interplay among adaptive and innate cells. After long term relentless inflammation, joint symptoms become more persistent and tissue damage more widespread. Some threshold is crossed and clinically apparent RA develops.^[20] It is likely that different pathways in the initiation of RA may be used to explain the pathogenesis of the disease in different patient groups, for example: sero-positive vs. sero-negative RA.

This review article focuses on the discussion of two important autoantibodies in RA: RF and ACPA. We will illustrate the association of these autoantibodies with RA in three important aspects: historical observation, the role in the pathogenesis, and finally, the clinical association.

Rheumatoid Factor

Historical Observation

As early as 1922, it was found that the serum from patients

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with liver cirrhosis and chronic bronchitis could promote the agglutination of rabbit antibody-sensitized sheep red blood cells. Two decades later, Waaler noted the same observation using serum from patients with RA.^[21] Similar observations were reported and intensive attention was devoted to find the factors causing this phenomenon, although the sero-prevalence rate was different. Due to their high association with RA, the serum factors were christened RF by Pike *et al.*^[22, 23] This factor was considered important not only in differential diagnosis but also in pathogenesis because it might take part in the pathogenesis of RA. Study group from The Rockefeller University in New York found that immune complex (IC) formed by rabbit antibody to human antigen could remove the RF from RA serum. After further observations, they concluded that the gamma globulin components of IC were the target of RF.^[24, 25] Furthermore, subsequent studies revealed that RF was not specific for RA as several infections and disease conditions also elicit RF development. Interestingly, prevalence studies of RF positivity in those infectious conditions highlighted the importance that the requirement of chronic, constant exposure to microbial antigens was needed for the development of RFs. Animal studies also illustrated that the prolong and intensive immunization is the key step in the induction of RF production. A novel hypothesis was proposed that during the long course of antigen challenge, new antigenic determinants on globulin could be elicited.^[26, 27]

RF now is known to belong to the family of immunoglobulins (Igs) that has different isotypes and affinities directed to the Fc portion of IgG. RF is not specific to RA and has been identified in rheumatic, non-rheumatic conditions and even healthy adults.^[28] Genetic and environmental factors contribute to the different worldwide distribution of RF. North American Indians were found to carry highest prevalence of RF in the world. It was reported about 30% of the population are sero-positive.^[29]

The Role in Pathophysiology

It was long believed that RF might play important role in the pathogenesis of RA. It is learned from basic immunology that IC can activate complement and trigger the subsequent inflammation, cytotoxicity, and tissue damage. Early efforts were focused to find the evidence of complement activation and resultant complement end products in the synovial tissue. It was reported that RA patients had lower serum complement level together with lower C1q and C3 in the synovial fluid.^[30, 31] Further observation confirmed the presence of end products of complement cascade, C5a, C5a *des Arg*, and C5b-9 membrane attack complex (MAC) in the joint cavity.^[32, 33] It was proposed that multiple hits exaggerated the immune response to cross the threshold in developing RA after the long term presence of

microbial antigens. The first hit was the induction of RF to IC and the second hit was the activation of complements.

However, RF was found to increase the clearance of IC and the evidence showed that RF-producing B cells may behave as antigen-presenting cells (APCs) and aid the immune response against the infectious antigens.^[34, 35] Other groups believed the net impact of RF production is protective rather destructive. In addition, as mentioned before, RF was widely present in normal healthy adult. No reason to believe that all these normal adults had extensive inflammatory responses uncovered.

Interestingly, RFs found in normal healthy adults are different from those found in RA patients. Both titers and affinity are low and they are likely produced by CD5+ B cells. Compared with those present in RA serum, they show low level of somatic hyper-mutation and maturation affinity, and are usually poly-reactive IgM.^[36] These RFs are reminiscent that low affinity IgM RFs are induced by polyclonal B cell activators such as bacterial lipopolysaccharides and Epstein-Barr virus.^[37, 38] RFs in healthy subjects could be the bystander in the cross-road between inflammation and resolution after an acute infection.

Clinical Association

Although every RF isotype was observed, IgM RFs are the major RF species in RA and are detected in 60–80% of RA patients.^[39] The ACR/EULAR 2010 criteria for the RA diagnosis adopt both RF and ACPA as part of its content. However, RFs were also found in other autoimmune diseases, such as, systemic lupus erythematosus, primary Sjogren's syndrome, and in non-rheumatic conditions such as chronic liver diseases, leprosy and pulmonary tuberculosis as well as in normal healthy subjects as mentioned above. How to make a clinical judgment in a RF positive subject is an important issue. In contrast to RFs found in healthy subjects, RFs in RA patients usually exhibit affinity maturation with high affinity and are at high titers. These RFs are mostly mono-reactive and produced in a T cell-dependent manner by CD5- B2 cells. However, high titer RF, especially for those higher than 100 IU/mL in healthy adults, increases the risk of developing RA.^[40] The importance of isotype distribution for helping the differential diagnosis was illustrated in several reports. The simultaneously presence of three isotypes (IgG, IgA, IgM) are detected in more than 50% of RA patients but found fewer than 5% in other rheumatic diseases. Double positive for IgG and IgA in the absence of IgM is more prevalent in other rheumatic diseases than RA.^[41, 42]

Chronic hepatitis and Sjogren's syndrome are important confounding factors in the diagnosis of RA due to high

prevalence of RF and RA-like inflammatory joint pain in both diseases. It was reported that the prevalence of RF in chronic hepatitis B and C were 20–75% and 40–76% respectively.^[43, 44] Asian countries have higher prevalence of chronic hepatitis than that of RA. By a simple calculation it is found that the most common disease carrying RF in Asian countries is chronic hepatitis rather than RA. Patients with Sjogren's syndrome also had high prevalence of RF and inflammatory joint pain.^[45] These findings make RF a poor diagnostic tool for differentiation between diseases. The isotype distribution is variable among reports. For better differentiation, the use of other biomarkers such as ACPA can be useful, since ACPA is more specific than RF.

RF also can be used as a prognostic factor in RA patients. It is well-known that sero-positive patients have more severe disease: more aggressive form of joint involvement, more extra-articular manifestations.^[46, 47] In many observations, RFs have been shown to be negatively associated with the achievement of joint remission. IgM, in particular, is a risk factor for not achieving remission.^[48] Furthermore, the presence of RF can predict the response to drug therapy, for example, RF and ACPA appear, both associated with better efficacy and lower risk of abatacept discontinuation.^[49, 50] But for conventional DMARD, that data are not very consistent.

Anti-Citrullinated Peptide Antibody

Historical Observation

As mentioned previously, ICs were needed to drive the development of RF. Many antigens were sought in the IC and citrullinated proteins were later found to be a major component of antigens forming the IC. The discovery of ACPA dates back to 1964 when Nienhuis *et al.* found a perinuclear staining pattern by using scraping of mucosa cells as target substrates after the incubation with RA serum.^[51] Subsequently, Young *et al.* discovered anti-keratin antibody using rat esophagus section as an alternative substrate and these antibodies turned out to be identical.^[52–54] Citrullinated filaggrin was finally elucidated to be the so-called perinuclear factor.^[55] This huge step opens the gate for the study of ACPA.

Citrullination is the process in which enzymes called peptidyl arginine deiminases (PADs) deiminate arginine-containing proteins. This natural process occurs in many physiological and pathological conditions including inflammation and oncogenesis.^[56] Till now, there are five PAD enzyme isoforms and PAD2 and PAD4 are the most strongly implicated in RA. Some distinct characteristics of ACPA are different from that of RF including the isotype distribution, N-glycosylation,

somatic hypermutation, B cell activation, and production of plasma cells.^[57] The main isotypes for ACPA are IgG and IgA, which are different from IgM of RF. In contrast to lower level of glycosylation in conventional IgG, more than 90% of ACPA were found to carry glycans and these glycosylation sites were localized predominantly in the variable domain. It was suggested that the hyperglycosylation in variable domain was introduced by sequence of somatic hypermutation.^[58]

In the past decades, more than 100 protein candidates for citrullination had been identified. They contain intracellular and extracellular proteins. Collectively, these citrullinated proteins are termed “RA citrullinome.”^[59, 60] Much effort has been focused on demonstrating the presence of specific citrullinated proteins in the synovial tissue. Unfortunately, none of these citrullinated proteins turned out to be a specific antigen and most ACPA were reactive with many antigens, even cross reactive with protein with carbamylation and acetylation.^[61, 62] Although the targets and magnitude of citrullination may not differ among controls and RA patients in some tissues, it is interesting that the rheumatoid joint is enriched in citrullinated protein. Furthermore, the antigen specificity and avidity can evolve with time. It was demonstrated that repeated somatic hypermutation induced affinity maturation happened in the germinal center to mediate “epitope spreading” and to expand B cell repertoires.^[63, 64]

The Role in Pathophysiology

Genetic and environmental factors are considered to play a major role in the pathogenesis of RA. Compared to normal population, RA patients are identified to carry high prevalence of HLA-DR4 (DRB1*0401 and DRB1*0404), these alleles encode for a specific amino acid sequence known as the shared epitope (SE).^[65]

Smoking is a major environmental risk factor for the development of RA, which carries a very high risk factor up to 2.31.^[66] The production of ACPA is highly associated with smoking and SE. The risk of RA development is 20 times higher when the patients are smokers and carry SE.^[67, 68] Smoking is found to activate the PAD and generate citrullinated neoantigens.^[69] Interestingly, epidemiology study revealed that another mucosa disease, periodontitis caused by pathogens *Porphyromonas gingivalis* which targets individuals predominantly expressing HLA-DRB1*04 alleles highly associated with RA.^[70, 71] *P. gingivalis* expresses high level of PAD to stimulate the citrullination process and further studies showed that the antibodies produced against this bacterium are highly correlated to the titer of ACPA.^[72] Some major source of PADs is intracellular. Through

different cellular death pathways, the leakage of PADs into surrounding tissue induces the generation of citrullinated protein. The most characteristic cellular death pathways, netosis and autophagy, have shown to induce citrullination of tissue protein such as vimentin and alpha-enolase.^[73, 74]

How the interplays between genetic and environmental factors contribute to the break of tolerance is not well known. However, based on the observation that ACPA had high affinity maturation and strong evidence of occurrence of somatic hypermutation before their secretion, it can be said that CD4 T cell plays an important role. The uptakes of IC by antigen presenting cells with subsequent presentation by HLA molecules that contain SE binding motifs activate specific CD4+ T cells. T cells may in turn provide B cell help to the production of ACPA. Finally, these events lead to the perpetuation of immune response. Evidences have shown that both Toll-like receptor 4 and Fcγ receptor mediate the binding of IC to macrophages followed by the activation of inflammatory cascade.^[75] Similar to what RF does, ACPAs are found to activate the complement system, release of C5a and MAC are observed. Both classical and alternative pathways have their roles in the activation of the complement system.^[76]

Recently, the pathogenic role of PAD was highlighted by the finding that PAD inhibitors had therapeutic efficacy in mouse models of inflammatory arthritis (IA). In addition, anti-PAD antibodies are used as possible biomarkers for RA diagnosis and prognosis since anti-PAD2 and PAD4 are found in distinct clinical subsets of patients with RA.^[56]

Clinical Association

As mentioned previously, ACPAs and RF can both be detected in the pre-RA stage. Base on this finding, several models were set to predict the development of RA. Case control studies indicate that elevated ACPA and/or RF tier have high positive predictive value for RA.^[77, 78] In a prospective study in Dutch, 347 patients with ACPA and/or RF positivity and arthralgia but no IA at baseline, 35% developed IA in a median of 12 months. Also, 74% developed IA/RA within 3 years if

patients have higher composite scores.^[79] However, another study revealed that fewer than 50% of ACPA-positive patients with nonspecific musculoskeletal symptoms develop RA after 1 year.^[80] More markers are recruited for the prediction of RA since the presence of ACPAs and/or RF is not sufficient for the prediction of RA development.^[81]

It is well known that ACPA and/or RF have impact on the disease severity and extra-articular involvement. ACPA and RF seemed to be associated with different extra-articular manifestations.^[82, 83] Interestingly, CV events are associated with ACPA. Irrespective of inflammation, autoantibodies might affect cardiac function. Epidemiologic study also shows that ACPA is associated with increased CV death.^[84] Recently, lung tissue was found to have extensive citrullination on RA patients with interstitial lung disease. ACPA was suggested to play role in the process of lung injury.^[85]

Finally, ACPA can be used as a marker for drug response. In AMPLE trial, baseline anti-CCP2 positivity was associated with a better response for abatacept and adalimumab. Higher anti-CCP2 antibody concentrations had better clinical response with abatacept than patients with lower concentrations. This association was not observed with adalimumab.^[86]

Conclusion

RA is a very complex autoimmune disease with variable clinical manifestations. Evidence also identify that several autoantibodies are involved in the pathophysiology of the disease. Two of them, RF and ACPA, are highly prevalent in RA patients. These two autoantibodies provide not only the insights of how immune system is dysregulated but also the clues for clinical diagnosis and managements. Cohort studies reveal that about 60–80% of RA patients are sero-positive. These patients have specific clinical outcome and treatment response. Nowadays, we can use these biomarkers for better care of our patients. Unfortunately, not every RA patient is sero-positive. Further research focused to find more biomarkers will allow us to have a better understanding of the pathogenesis, sub-classification of patients, and algorithm of treatment policy.

Conflict of Interest

No potential conflicts of interest were disclosed.

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