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Review

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Autoantibodies in rheumatoid arthritis: rheumatoid factors and anticitrullinated protein antibodies

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Summary

Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease, characterized by chronic, erosive polyarthritis and by the presence of various autoantibodies in serum and synovial fluid. Since rheumatoid factor (RF) was first described, a number of other autoantibodies have been discovered in RA patients. The autoantigens recognized by these autoantibodies include cartilage components, chaperones, enzymes, nuclear proteins and citrullinated proteins. However, the clinical significances and pathogenic roles of these antibodies are largely unknown except for RF and anticitrullinated protein antibodies (ACPAs), whose clinical usefulness has been acknowledged due to their acceptable sensitivities and specificities, and prognostic values. This review presents and discusses the current state of the art regarding RF and ACPA in RA.

Introduction

Rheumatoid arthritis (RA) is the most common systemic inflammatory autoimmune disease in which joint synovium is primarily affected by a dysregulated immune system. RA is typically associated with serological evidence of systemic autoimmunity as indicated by the presence of autoantibodies in serum and synovial fluid. The first autoantibody in RA, rheumatoid factor (RF), was described by Waaler in 1940,[1] and it was later found to be directed to the Fc region of IgG. Autoantigens targeted by a number of autoantibodies subsequently found in RA display a wide spectrum of cartilage components, stress proteins, enzymes, nuclear proteins and citrullinated proteins (Table 1), which demonstrates that RA is not characterized by only one autoreactivity to a single autoantigen but by accumulated autoreactivities in both B and T cells.[2] The spectrum of these self-antigens and immunologically relevant epitopes probably varies during the disease course, and the set of autoantigens in one individual may differ from that in another.[2] In 1993, Serre *et al.*[3] identified filaggrin as the target antigen of RA-specific anti-keratin antibodies (AKAs). Subsequently, it has been demonstrated that AKAs and other RA-specific autoantibodies known as anti-perinuclear factors (APFs) and anti-Sa antibodies all recognize citrulline-containing peptides/proteins as common antigenic entity,[4–6] and they are collectively termed as anticitrullinated protein antibodies (ACPAs). Currently, only RF and ACPA are utilized in clinical practice because of their diagnostic and prognostic values; the latter, in particular, is highly specific for RA.[7]

Table 1 Well-characterized autoantigens recognized in RA

Cartilage components	Type-II collagen (native and denatured),
Stress proteins	Microbial Hsp65, Bip
Enzymes	α-enolase, glucose-6-phosphate isomerase, calpastatin
Nuclear proteins	RA33/hnRNP A2
Citrullinated proteins	Filaggrin, fibrin, fibrinogen, vimentin, types I and II collagens, α-enolase, synthetic cyclic citrullinated peptides

However, other antibodies may contribute to the pathological processes of RA by immune complex formation and complement fixation. The importance of immune complex formation in the arthritogenic potentials of autoantibodies has been shown in a K/BxN mouse model, in which autoantibodies against glucose-6-phosphate isomerase are key molecular players in disease development.[8] Thus, the capacity of RF to enhance immune complex formation by self-association[9] or with other autoantibodies[10] may not only grant arthritogenicity to RF itself but also potentiate the arthritogenicities of other autoantibodies including ACPA. In this review, we discuss RF and ACPA, the two most remarkable autoantibodies in RA.

Rheumatoid factors

RFs are a family of autoantibodies directed to the Fc portion of IgG. They are locally produced in RA by B cells present in lymphoid follicles and germinal center-like structures that develop in inflamed synovium.[11,12] IgM RFs are the major RF species in RA and are detected in 60–80% of RA patients.[13] RF has been observed in many other autoimmune diseases, such as, in systemic lupus erythematosus, mixed connective tissue disease and primary Sjögren syndrome, as well as in non-autoimmune conditions, such as in chronic infections and old age.[13] However, RFs in RA patients are distinguished from RFs in healthy individuals in that they exhibit affinity maturation, whereas those in healthy individuals are polyreactive and are of low affinity.[14] These observations support the concept that RFs are under strict control to prevent the emergence of high-affinity RF in normal subjects. RF specificity to RA is increased at high titers (e.g. IgM RF $\geqslant$ 50 IU/ml) and with IgA isotypes.[13,15,16] High titer RF and IgA isotypes are also associated with radiologic erosion, extra-articular manifestations and thus, poorer outcomes.[13,16–18] The association between high titer RF status and a poor prognosis indicates that RF may have a role in the pathogenesis of RA. Furthermore, RF has proven to be the most useful disease marker of RA, as included in the American College of Rheumatology classification criteria for RA.[19]

Normally, transient production of low-affinity IgM RF is regularly induced by immune complexes[20] and polyclonal B-cell activators, such as bacterial lipopolysaccharides and Epstein-Barr virus.[21,22] The physiological roles of RFs under normal conditions have been shown (i) to enhance immune complex clearance by increasing its avidity and size, (ii) to help B cells uptake immune complex, and thereby, efficiently present antigens to T cells and (iii) to facilitate complement fixation by binding to IgG containing immune complexes.[23–25] High-affinity and high-titer RFs in RA synovial fluid are believed to exert such functions in a pathogenic manner and thus to potentiate inflammation and antigen trapping in joints. However, there has been no clear evidence that RFs are involved in the initial events triggering the disease process of RA rather than they themselves are triggered by RA.

Accumulated somatic mutations and the presence of isotype switching indicate that RF production is T-cell driven in RA. Although T cells infiltrate RA synovium[26] and contain autoreactive clones,[27] they have been shown to be polyclonal and lack specificity for any particular autoantigen.[27,28] To date, the T-cell clones reactive with autologous IgG have not been detected in RA patients. The ability of RF expressing B cells to take up immune complexes and to present trapped antigens to T cells may enable these cells to bypass the need for specific T cell help and ultimately lead to emergence of autoreactive T cells that can trigger RF synthesis in the absence of an external antigen.[29]

Antibodies to citrullinated protein

The ACPAs are a group of new autoantibodies, which are found in 70–90% of RA patients and have high disease specificity (90–95%).[7,30] Accordingly, they are rarely found in other diseases or in healthy individuals. In general, ACPA has better diagnostic value than RF in terms of sensitivity and specificity. As with RF, they are associated with more erosive RA.[31–33] Although ACPAs are also

referred to as antiperinuclear factor, antikeratin, antifilaggrin and anticyclic citrullinated peptide antibodies depending on the antigens used for their detection, citrulline is a common critical constituent of the antigenic determinant of these antibodies, as its absence leads to a lack of recognition by antibodies.[5,6]

Citrulline is a non-standard amino acid generated by the posttranslational modification of arginine by peptidylarginine deiminase (PADI) enzymes during a variety of biologic processes, which include inflammation. Because PADI mediates citrullination of arginine in the presence of sufficient concentrations of Ca^{2+}, apoptotic granulocytes create an environment for PADI activation, in which cytosolic Ca^{2+} level rises due to caspase-mediated plasma membrane Ca^{2+} pump cleavage.[34] Therefore, when the apoptotic cells are not cleared efficiently as in an inflammatory environment, intracellular citrullinated proteins and/or PADI are released into the extracellular space, where the former are taken up by antigen-presenting cells and the latter induces the citrullination of synovial proteins. ACPAs are locally produced in RA joints, where proteins are citrullinated during the inflammatory process.[35] The major citrullinated protein in the joint was found to be fibrin.[36] Additionally, various other synovial and non-synovial proteins (type II collagen, vimentin, nuclear proteins and stress proteins) have been shown to be targets of citrullination *in vivo*.[37–40] Immune complex formation between ACPAs and citrullinated proteins and subsequent complement fixation were recently demonstrated to occur in RA synovium and are thought to perpetuate RA synovial inflammation, causing a vicious cycle.[41]

Although protein citrullination is a prerequisite to ACPA production, it does not always induce APCA production. For example, in collagen-induced arthritis model, ACPA production was not observed despite abundant citrullinated proteins in the inflamed joints.[42] Therefore, APCA production is thought to be limited to subjects with certain genetic backgrounds, among which RA-shared epitope (SE) located in the HLA-DRB1 gene is the most dominant genetic factor.[43–45] SE, a common region with highly similar sequences among certain HLA-DR class II alleles, is the genetic factor best known to be associated with RA.[46] MHC class II molecules expressing SE can bind and present citrullinated peptides to T cells. Furthermore, it has been shown that conversion of arginine to citrulline in the position that interacts with SE increases peptide–MHC binding affinity.[47] The Dutch observation that SE-encoding HLA alleles are only associated with ACPA-positive RA but not with ACPA-negative RA indicates that these HLA alleles predispose for ACPA positivity rather than for RA.[48] The same relationship between SE alleles and ACPA with respect to RA susceptibility has been confirmed in RA cohorts of North American and Swedish ancestries.[49,50] A subsequent finding that shows a gene–dose effect between SE and ACPA positivity among RA patients further supports that SE predisposes for ACPA production.[49,51]

However, the relationship between SE alleles and ACPA should be interpreted in the context of genetic background, because RA susceptibility and/or disease severity conferred by SE might be modified by the presence of other alleles that are influenced by ethnicity; for example, lack of the association between SE status and ACPA has been reported in Chinese RA patients.[52] Overall, the SE effect on RA must be complex due to several reasons: (i) it exerts indirect effect via ACPA production, (ii) the alleles involve both disease susceptibility and severity, (iii) ethnicity and/or environmental factors are critical determinants for these alleles to manifest as a risk factor for RA susceptibility, RA severity and/or ACPA positivity.[33,48–54] Studies utilizing RA patients of North American and several European ancestries have found that SE alleles are not associated with RA in ACPA negative patients, indicating that these alleles are risk factors for ACPA rather than RA.[48–50] However, the greatly increased odds ratio for RA susceptibility and radiological progression in individuals with the combination of SE gene carriage and ACPA than individuals with either of them suggests a synergistic interaction of SE and ACPA.[43,45] Thus, it remains to be determined, particularly in conjunction with certain ethnic backgrounds, whether SE alleles have any additional roles in RA development and radiological progression rather than simply a risk factor for ACPA positivity.

Smoking has been found to be a risk factor for ACPA positive RA only in SE-positive subjects in European studies that employed Swedish and Dutch RA cohorts.[50,54] Supported by its biological function that generates antigenic stimuli for autoantibody production by inducing PADI4 expression in bronchial macrophages to citrullinate lung proteins,[50,55] smoking is considered to be an important environmental risk factor for ACPA production in susceptible individuals. However, in a more recent study with three large North American RA cohorts, they observed only a weak gene–environmental interaction between SE and smoking in one of the three cohorts,[51] suggesting that other environmental factors are associated with ACPA and RA in these populations.

Although SE alleles are the most well-known genetic risk factor for ACPA positivity, recent genetic analyses have found non-SE-HLA and non-HLA

alleles to predispose for the presence of ACPA, for example 1858C/T allele polymorphism of PTPN22, several single nucleotide polymorphisms of PADI4, 158V/F polymorphism of Fc gamma receptor IIIA gene and others.[52–62] In the meantime, there are not only predisposing factors for ACPA production but also anti-predisposing factors as well, most notably HLA-DR3.[63,64] Interestingly, HLA-DR3 is also known to be a RA susceptibility gene and predisposes for ACPA negative RA.[63,64] The net result of complex interactions between predisposing vs. anti-predisposing genes is thought to determine the final consequence. Studies based on genome-wide scans to uncover other unknown genetic factors are underway.

As with RF, the onset of ACPA positivity and the onset of RA do not always coincide; in some patients, ACPA begins to rise far earlier than the RA symptom onset whereas in others, ACPA sero-conversion occurs after RA onset.[60] Histologic studies have demonstrated extensive synovitis in clinically uninflamed joints,[65] suggesting that a pre-clinical or asymptomatic phase of RA exists. It is unknown whether ACPA and RF precede even the preclinical stage or vice versa. However, the finding that ACPA precedes up to 9 years prior to RA onset[66,67] suggests that the production of these antibodies may be the earliest events in the disease process. Since ACPAs appear early during the course of RA, or even during the preclinical stage,[66–68] their detection is of major interest regarding the identification of RA among recent arthritides. Furthermore, their prognostic values may lead to early aggressive treatment to prevent irreversible joint damage.

Relations between RF and ACPA in RA

Both RF and ACPAs are present during the preclinical stage

It has been demonstrated in studies that retrospectively utilized pre-clinical serum samples that RF and ACPAs are present in the sera of RA patients months to years prior to disease onset.[66,67] These studies showed that the risk of RA development is highest when RFs and ACPAs are present in conjunction. The titers of autoantibodies increased as disease onset was approached and most of the negative to positive sero-conversions occur within 3 years prior to the symptoms onset.[67] However, in certain patients, sero-conversion for either one of the two antibodies continues to occur after RA onset,[60,67] most likely within the first few years after disease onset[60] and the sensitivity and specificity of assays reach a plateau for established RA patients. Because the assays for the autoantibodies were performed on blood samples of only those who developed RA, prospective studies are required to assess the estimated risk of these antibodies.

RF and ACPAs are associated with different clinical features of RA

It is well-known that the extra-articular manifestations of RA are associated with the presence of RF, but this is not the case for ACPA[69,70] although both ACPAs and RFs are associated with more destructive joint pathology. It was demonstrated recently that citrullination occurs in the lung tissues of RA patients with interstitial lung disease (ILD) and in those of idiopathic ILD patients.[71] However, ACPA response was observed in only RA patients. Interestingly, citrullination was found to be mainly localized in intracellular spaces, particularly in macrophages in both groups. These observations raise fundamental questions regarding: (i) whether pulmonary or extra-articular citrullination contributes to ACPA response in RA patients, (ii) whether ACPA is involved in the lung pathology of RA-associated ILD and (iii) whether ACPAs produced against synovial proteins have the same immunological response against citrullinated proteins in the lungs as in joints.

RFs and ACPAs are independently associated with joint erosion

RF, ACPA and shared epitope alleles have been suggested to be associated with aggressive RA phenotypes in prospective studies. RF is an established severity factor for RA, and recent studies have consistently shown an association between ACPA and radiological erosion.[31–33] Several studies have shown that RF and ACPA are independent risk factors of joint erosion.[69,72] To address whether the associations between these factors and radiological erosion are independent of one another, Mewar *et al.*[73] evaluated associations between radiological outcomes and RF, ACPA and SE status simultaneously in longstanding UK RA patients. They found independent associations of both ACPA and RF with erosion, an association between SE alleles and erosion in the RF negative patients, and an association between ACPA status and SE alleles with a gene–dose effect. These observations indicate that the effect of SE may be due to an association with ACPA. However, as mentioned above, this result should be interpreted in the context of a genetic background.

RF decreases more than ACPA during RA treatment

Studies that have evaluated RF titers before and after infliximab therapy have reported a significant decrease in RF levels.[74–76] However, ACPA results during anti-TNF-α therapy are inconsistent. After rituximab therapy, ACPA and RF were found to decrease significantly in treatment responsive patients only, and titers were found to return to previous levels with relapse.[77]

Conclusions

RF and ACPA are two most remarkable autoantibodies in RA and provide different clinical and pathophysiological information. In general, ACPAs are a better diagnostic guide than RF due to their higher sensitivity and specificity for RA. Both RF and ACPA are poor prognostic factors of joint destruction, while RF is also associated with extra-articular manifestations. RFs in RA patients are of high affinity and high titer, which indicates that RF may contribute to disease perpetuation by potentiating immune complex formation and complement fixation. During inflammation, intracellular citrullinated proteins and PADI are released into the extracellular space to provoke ACPA response or further citrullinate synovial proteins. Smoking is thought to be one of such environmental factors that led to inflammation and protein citrullination *in vivo*. The current hypothesis is that genetically susceptible individuals, most notably SE carriers, produce ACPAs against citrullinated proteins, form immune complex in joints with the help of RF and ultimately develop RA. Furthermore, the responses of these two antibodies to treatment vary, which reflects their different mechanisms of involvement in the pathogenesis of RA.

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