

Review

Rheumatoid factors: what's new?

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Rheumatoid arthritis (RA) is a classic example of an autoimmune disorder, with chronic inflammation of the synovial membrane, and deterioration of cartilage and bone in the affected joints. The resultant pain, loss of function and permanent disability are also associated with increased morbidity and mortality. Although the symmetrical joint deformities are a predominant characteristic, the disease has many systemic features. Since the pivotal studies of Waaler [1], serum rheumatoid factor (RF) estimation has been a basic diagnostic aid, varying in popularity with changes in scientific trends and with considerable controversy over its significance. Yet 60 yr later it is still recommended as a prognostic indicator of disease activity and progression in the adult and juvenile forms of arthritis [2, 3]. Science moves in paradigms and for several decades T-lymphocytes have dominated the literature, but with the devotion of persistent researchers, such as Jo Edwards, the B-lymphocyte has moved again to centre stage in the aetiology and progression of RA. Moreover, a recent Delphi panel of senior rheumatologists concluded that the 'highest value ever' for serum RF levels was still the best laboratory indicator of disease severity [4].

New therapies for autoimmune rheumatic diseases

Changes in serum RF levels may be used as indicators of disease activity, but they are also proving useful in monitoring responses to therapy, especially biological disease modifiers [5]. More recently, the therapeutic genetically engineered chimeric monoclonal antibody rituximab was found under trial, in an open-label multicentre study, to provide significant improvement of symptoms in RA patients who were non-responders to methotrexate [6]. The target for rituximab is CD20, a cell surface protein that is present on stages of differentiation from pre-B to mature B-lymphocytes, but not stem cells or end-stage immunoglobulin-secreting plasma cells [7]. This therapy therefore selectively depletes the CD20⁺ B-lymphocyte subpopulation. Rituximab has also been effective in resistant RA when given with haematopoietic stem cell transplantation, providing additional evidence that B-lymphocyte depletion leads to reduced disease activity [8].

Intriguingly, this therapy has worked much better for RF-positive patients. The apoptosis of B-lymphocytes, a therapeutic effect of rituximab, was significant in RF-positive patients compared with those who were RF-negative (Wilcoxon matched pairs, $P=0.0156$). This finding was further emphasized in a recent study in which anti-B-lymphocyte therapy failed to have an impact on symptoms in RA patients who were

RF-negative [9]. Importantly, the therapy acts on the rapidly dividing and mature B-lymphocyte populations whilst leaving the precursor B-lymphocyte untouched.

RF-positive RA and RF-negative RA: are they different disorders?

The occurrence of seronegative RA has frequently proved a stumbling block to the idea that RF is pivotal in the pathogenesis of RA. The evidence that rituximab has significant therapeutic effects only in RF-positive patients adds credence to the role of RF-producing B-lymphocytes in the pathogenesis of RA and would suggest that the mechanism of disease in RF-positive patients differs from that in patients who are RF-negative. This idea that RF-negative RA is different is further supported by genetic studies of a group of RF-negative supposed RA patients who did not have the characteristic shared HLA epitope allele found in RF-positive RA, but instead possessed a separate apoptosis-associated gene (PD-1.3A) [10].

Rheumatoid factors

Antigenic targets (epitopes) and rheumatoid factors

An important antigenic target for RFs is the C γ 2–C γ 3 interface in the Fc region on the immunoglobulin molecule [11], thus making RFs the ultimate autoantibody, since there is no shortage of IgG antigen to form immune complexes. In addition, other potential RF epitopes have been identified via epitope mapping studies [12–14]. The target sites do not seem to have any special autoimmune potential as the main epitopes for autoantibodies appear to be adjacent to, or overlap with, those recognized by antibodies from other species when immunized with human IgG. For instance, epitopes defined by murine anti-IgG antibodies bind at or adjacent to RF antigenic hot spots [15, 16] (Fig. 1).

Certainly, monomeric IgG is a poor activator of RF⁺ B-lymphocytes compared with immune-complexed IgG: the structure of these immune complexes appears to be significant, as the antibodies need to be bound to strictly ordered antigens rather than irregularly arranged, to promote efficient induction of RFs [17].

A question to be asked: 'Is the target for RF the normal immunoglobulin molecule or is it an altered molecule that is the important inducer and binder of RF?' Enzyme modification of antigen has already been established as an important step in coeliac disease—tissue transglutaminase enzyme modification of

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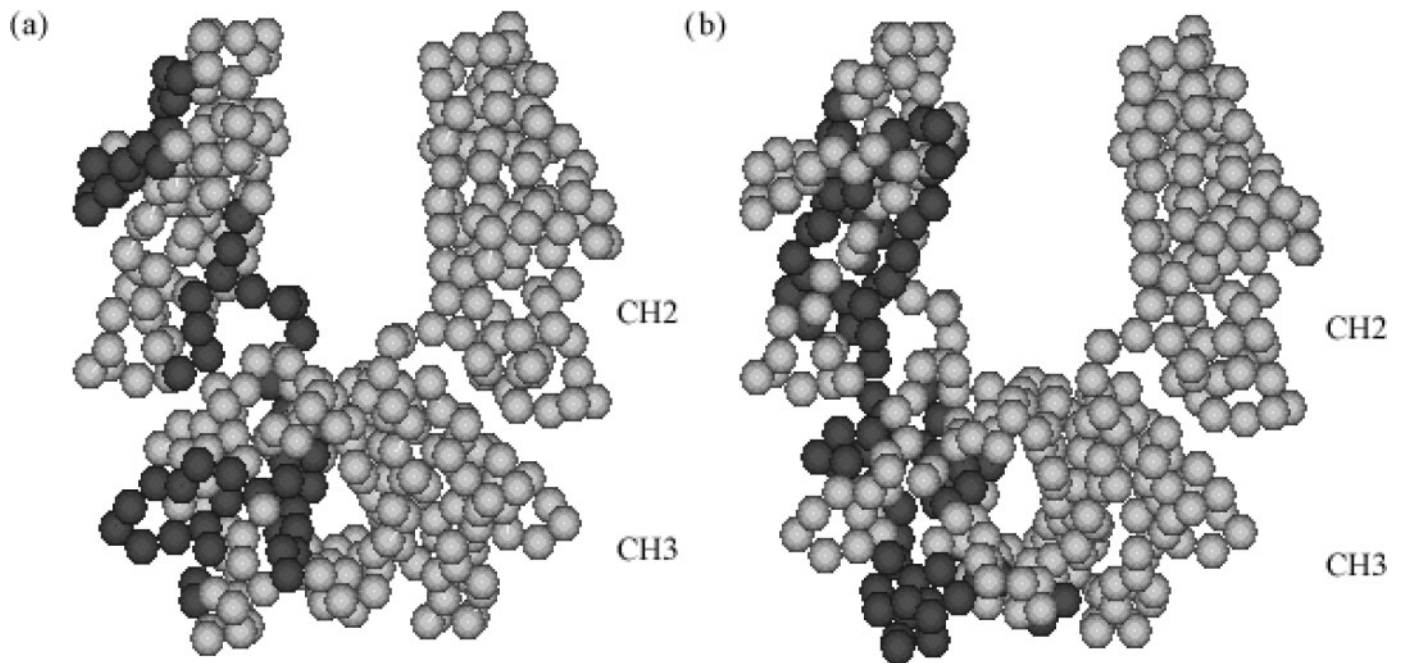


FIG. 1. Molecular models of the Fc region of IgG highlighting the antigenic regions (dark residues) identified by (a) monoclonal antibodies raised by deliberate immunization with IgG [15, 16] and (b) polyclonal and monoclonal rheumatoid factors [12–14].

gliadin is a vital part of the autoimmune process [18]. Perhaps enzyme modification of self antigen may be a significant step in the pathogenesis of other autoimmune diseases. In the case of RA, the detection of agalactosyl immunoglobulins is a characteristic feature. The absent galactose within the Fc region of IgG allows access to RF-binding epitopes that are either exposed, or revealed as a consequence of conformational changes [19]. Importantly, antibodies to these agalactosyl IgG molecules are found to be raised in RA and their estimation is considered a more sensitive indicator of disease activity than conventional RFs [20, 21]. This mirrors the earlier findings of Soltys *et al.* [22], who found that the monoclonal RFs that bound better to agalactosyl IgG composed the high-affinity set of RFs in RA. Other RF-binding epitopes have been shown to be independent of the oligosaccharides [22]. Proteins may be modified by the binding of glucose [e.g. in diabetes, where haemoglobin is glycosylated (HbA1c) and HbA1c levels are used as an indicator of glycaemic control]. Likewise, IgG also may be modified, and autoantibodies against advanced glycosylated end (AGE)-damaged IgG have also been detected and shown to be highly significantly associated with RFs ($P < 0.0001$) and with swollen joint counts ($P < 0.01$) [23].

Cellular production of rheumatoid factors in RA

The microenvironment of the synovial membrane becomes an 'ectopic' secondary lymphoid organ, a site of isotype switching and somatic hypermutation for the RF-positive B-lymphocytes [24]. Williams *et al.* [25] have shown in the autoimmune murine model that somatic hypermutation in autoimmune response happens outside the conventional germinal centres. B-lymphocytes that mutate to autoreactive specificities will have escaped the normal tolerance self-censoring mechanisms that are present in germinal centres. Recent evidence has found that the RFs in RA patients with inflamed joints RA are produced by terminally differentiated CD20⁻CD38⁺ plasma cells in the synovial fluid that are generated by germinal centre-like structures within the synovium [26]. Thus, when giving anti-CD20 therapy (rituximab), which targets the B-lymphocytes at an earlier

developmental stage, one would expect a lag phase between the start of treatment and the onset of disease remission, since the CD20⁻CD38⁺ plasma cells are not eliminated by the treatment, but die eventually of old age.

RF isotypes and specificity

Naturally occurring RFs are detectable in healthy individuals, thus leading to doubt regarding the significance of RFs in disease. These natural RFs are generally polyreactive IgM antibodies of low affinity produced by CD5⁺ B-lymphocytes. They are considered to be part of the normal response, e.g. to lipopolysaccharide or Epstein-Barr virus (EBV) [27, 28], and contribute to the immune complex clearance mechanism by the formation of larger immune complexes that are phagocytosed [29]. The fact that in healthy subjects the RF-positive B lymphocytes coexist with IgG antigen in a non-autoimmune disease state would suggest the existence of a tolerance mechanism. A possibility could be the inhibition of class-switching, so that IgM/IgD RF-positive cells do not switch to being the IgG RF-positive cells that produce high-affinity and pathogenic antibodies via somatic hypermutation [30].

In order to induce B-lymphocytes to switch from producing IgM RFs to secreting IgG RFs or IgA RFs, T-cell help is crucial. It is noteworthy that Lang *et al.* [31] have shown that the response to IgG is HLA-DR-restricted and mediated by T-helper cells (CD4⁺) that are stimulated by IgG, for the initiation and propagation of the immune mechanism of RA [31]. However, it is surprising that this has not been followed up and the experiments repeated to confirm this hypothesis. If the autoimmune mechanism is both a T- and a B-lymphocyte response to IgG, this is highly significant for the development of tolerance-inducing therapies. It has also been proposed that autoreactive T-lymphocytes are stimulated by the presence of IgG aggregates within the joints. Recent evidence has indicated the presence of T-lymphocyte responses to IgG molecules that have been attacked by oxygen free radicals, a pathological mechanism prevalent within the diseased joint [32].

Cytokines, Fc receptors and rheumatoid factors

Rheumatoid factors may be considered somewhat analogous in function to the soluble form of Fc receptors (FcγRs) in specifically cross-linking with immune complexes. FcγRs are normally expressed on selective cell types and, once bound to their targets, the resultant leucocyte activation induces the effector functions of cytokine release, antibody-dependent cytotoxicity and phagocytosis with respiratory bursts. Studies in murine models of FcγR deficiency with collagen-induced arthritis indicate a role for these Fc receptors in inflammation and joint destruction [33]. Studies of allelic forms of FcγR on NK cells in patients with RA and healthy controls have suggested that cellular activation mediated by binding to these receptors could be influential in the disease process of RA [34].

The function of the soluble forms of FcγR remains unclear, although Wines *et al.* [35] have demonstrated inhibition of RF binding to IgG immune complexes by the soluble forms of FcγRIIIa. As to whether the physiological concentrations of soluble FcγR in the synovium are sufficiently high to influence RF binding has yet to be confirmed.

Structure of rheumatoid factors

Surprisingly, all the IgM RFs that have been examined so far have shown unusual molecular structures compared with normal IgM molecules. Analysis of comparative crystallographic data of IgM and IgM RF has exposed differences in the shape, where the IgM RF has an asymmetrical F(ab)₂ region signifying structural differences distinctive from IgM [36]. Likewise, by extrapolating the crystallographic data of the monoclonal human rheumatoid factor, Sutton *et al.* [37] proposed that some RFs are able to simultaneously bind both antigen (environmental or other autoantigens) and the Fc region of IgG via the conventional antigen-binding site and an adjacent binding site. Accordingly, this could lead to high levels of RF synthesis wherever these antibodies are stimulated by an external antigen, and such evidence would point to a possible infectious or environmental trigger for RF-positive RA.

Environmental and genetic triggers of rheumatoid factors

A reasonable predictor of RA risk may be estimated from an analysis of serological and HLA genotype screening. Family studies would point to a genetic influence, but environmental triggers are also fundamental.

Innate immunity and autoimmune disorders

Toll-like receptors are key for the induction of innate response to many environmental pathogens. There is increasing evidence to suggest that the inappropriate stimulation of such receptors is important in autoimmunity (see recent review of Rifkin *et al.* [38]). Moreover, Toll-like receptors are expressed in the synovium of patients with clinically active RA [39]. The crucial importance of Toll-like receptors in RF production has been demonstrated using two forms of immune complex. Rifkin *et al.* [40] showed that in autoimmune mice IgG2a immune complexes were able to stimulate RF production by RF B-lymphocytes. Subsequent work has revealed that such immune complexes contain a nucleosome antigen, and it is this antigen that was key because IgG2a immune complexes made with other antigens had no effect [41]. Detailed analysis of this system has showed that RF⁺ B-lymphocytes require two signals to be triggered—one via the B-lymphocyte receptor (i.e. for the IgG as an antigen) and the other via the Toll-like receptors (stimulated by the nucleosome antigen). Interestingly, a similar role for Toll-like

receptors in stimulating RF⁺ B-lymphocytes has also recently been found in bacterial infections [42] (Toll-like receptors have also been shown to be immunomodulators and pathogenic costimulators of autoreactive B-lymphocytes [43]). This is especially relevant for chronic disease, given the finding of Lanzavecchia's group, who showed that the immunology memory response could be maintained by activating B-lymphocytes via Toll-like receptors alone [44].

Role of virus infections in rheumatoid factor induction

Several viral agents, e.g. EBV, parvovirus B19 and more latterly human endogenous retroviruses (HERVs), have been implicated in the aetiology of chronic autoimmune disorders [45, 46] via excess immune complex production. In particular, HERVs may contribute to the pathogenesis of disease, possibly by acting in concert with other infectious agents [47], or as potential transducing agents that enable other environmental/hormonal triggers to alter the immune system [48]. EBV is a classic example that infects and stimulates B-lymphocytes and may also impact on T-helper cells by transactivating an endogenous viral superantigen [49]. It is noteworthy that RFs targeting the membrane-bound IgG3 have also been shown to induce both EBV and B-lymphocyte activation [50].

Patients with active RA have a lower than normal cellular immune response to an EBV protein that is important for its replication, thereby allowing the virus to replicate, and results in a high EBV load [51], although therapy could also have an impact on this. The virus can continue to replicate even in the face of high antibody responses. This leads to a high antigen load, which, if accompanied by a significantly high antibody response, could easily result in a high level of immune complex formation analogous to an autoimmune response with the resulting stimulation of RF [52].

Rheumatoid factor estimations in diseases other than RA

Rheumatoid factors in cryoglobulinaemia. Mixed cryoglobulinaemia is now known to be associated with hepatitis C infection, and Sansonno *et al.* [53] have shown that these cryoglobulins are formed of hepatitis C virus (HCV) core protein plus IgG antibody, which are then cryoprecipitated by IgM RF. In such patients, B-lymphocyte depletion with rituximab therapy has led to clinical remission of hepatitis C-associated type II mixed cryoglobulinaemia with glomerulonephritis, and signs of systemic vasculitis, where the high levels of RF contribute to the pathology [54].

Other environmental triggers of rheumatoid factors. There are rudimentary data on certain risk factors. Environmental pollutants, silica and blood transfusion have been implicated, but long-term smoking is recognized as a serious factor in RA patients who are RF-positive. It is proposed that high levels of pollutants like smoking and fossil fuels increase the risk of respiratory tract infections, and infectious agents become antigenic targets that trigger autoimmune responses. Long-term smoking, i.e. for more than 20 yr, carries a significant risk of RF-positive RA and the ability to generate detectable RF levels in advance of the clinical presentation of RA. Alternatively, smoking cessation could also reduce the risk of RA [55].

An example of gene–environment interactions is also shown in the significant risk of RA with the shared epitope on HLA-DR that influences at-risk male and female patients who are also smokers [56]. Likewise, particularly in female RA patients with a polymorphism of the enzyme glutathione S-transferase, Matthey *et al.* [57] have hypothesized that a lack of the enzyme that detoxifies smoke-derived by-products has the potential to damage IgG in smokers and could encourage RF production.

Coffee consumption is another suggested risk factor for RA, and its mechanism has been linked with the production of RF [58]. In the Iowa Women's Health Study, the link with RA was with decaffeinated coffee [59]. Since tobacco and significant coffee importation hailed from the New World, could this provide the basis for the hypothesis that the origins of RA were in North America [60]?

Genetics and rheumatoid arthritis

The increased incidence of RA reported in several American Indian and Alaskan populations is well documented and would indicate a strong genetic influence [61]. Population-based studies of the Pima Indians in Arizona have exposed the high incidence of raised RF levels and the subsequent development of RA. However, the decline revealed in recent secular and birth cohort data would implicate environmental influences in early life as significant predictors of the lifelong probability of being RF-positive [62].

Certain HLA-DR4 alleles are associated with RA in white populations in Western Europe and the USA, whilst different alleles are associated with RA in Jewish and Japanese population studies, suggesting that there are interactions of HLA with other genes [63]. Wu *et al.* [64] suggested possible genetic differences between patients with early-onset and those with late-onset RA. They showed a significant association between novel RANKL (receptor activator of NF- κ B ligand; a significant ligand that must be controlled in order to maintain bone homeostasis and prevent bone resorption) genetic polymorphisms and early-onset RA (18–20 yr younger) and patients who were positive for the shared epitope containing DRB1*04 alleles ($P=0.0004$) [64]. Others have classified the HLA-DRB1 alleles with respect to their predisposing, neutral or protective effects in RA [65].

Rheumatoid factors in other forms of rheumatic diseases

Serum RF is sometimes detected in other autoimmune disorders, such as Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE). In primary SS, around 60% of cases are RF-positive, with a higher level of IgA RFs in male compared with female patients [66]. Indeed, disease-related transformation of activated RF-positive B-lymphocyte clones is thought to be instrumental in the aetiology of the lymphoproliferative disorders that develop in around 5% of patients with SS [67]. However, it should be noted that SS patients may be negative for other autoantibodies, e.g. anti-cyclic citrullinated (CCP) antibodies and anti-keratin antibodies [68].

Disease indicators used for rheumatoid arthritis

There is a short window of opportunity in the early onset of RA which, if it is identified and if aggressive treatment is started, could lead to a better prognosis [69]. Hence, understanding the early events and identifying precise markers that predict synovitis in the preclinical stage of RA would aid in the design of therapeutic strategies. Moreover, subjects with raised RF levels who are symptom-free are at significantly increased risk of developing RA [70].

Various methods are available for serum RF estimations but few new methods have been introduced recently. A commercially prepared gelatine-based modification of the Waaler–Rose assay (Serodia-RA), when compared with rate nephelometry at equal specificity, appeared to be more sensitive [71], so could lead to earlier detection of RFs. But a word of caution: by the very nature of RFs, artefacts result and lead to false positives in immunoassays [72]. Likewise, a raised granzyme B level has an early disease association in RA patients who are RF-positive [73].

Rheumatoid factors versus anti-cyclic citrullinated antibodies

A newer diagnostic indicator with high specificity for RA is anti-CCP antibodies (recently reviewed by Vossenaar and van Venrooij [74]), although their presence does not exclude other autoimmune disorders, such as SLE [75]. The anti-CCP antibodies appear in response to an epitope modified by a hormonally controlled enzyme, peptidylarginine deiminase, which converts arginine to citrulline [76]. Citrullinated proteins have been found in intracellular synovial sites in more than 50% of RA samples compared with 5% of controls [77]. It is unlikely that only one single citrullinated antigen is the immune target [78], but it would be interesting to test the efficacy of this enzyme on IgG and subsequent binding of RF. The previously mentioned Delphi panel omitted anti-CCP from their final list of 28 indicators of RA disease severity [4]. Conversely, others have suggested that in patients who have had synovitis for more than 3 months, both anti-CCP and RF estimations are good predictors of patients destined to develop RA [79]. Further, some workers suggest that anti-CCP antibodies are around 20% more sensitive than RFs in the diagnosis of RA [80] and juvenile idiopathic arthritis [81]. Yet there is conflicting evidence as to whether a decrease in anti-CCP and RF levels is detected following anti-TNF- α monoclonal antibody therapy: some studies report decreases in both [82, 83]; others have found a decrease in RF levels only, with anti-CCP levels remaining stable [84], and yet others have shown that further autoantibodies may be induced, e.g. anti-nuclear, anti-double stranded DNA and anti-CCP antibodies [85, 86]. It is suggested that anti-CCP antibodies are particularly helpful in establishing the diagnosis of RA, but RF estimations are better predictors of disease severity [87, 88].

Until a definitive indicator is available, the most useful predictor of joint damage is a combination of various analytes, i.e. anti-CCP, IgA RF, IgM RF, anti-IL-1 α , ESR, C-reactive protein and cartilage oligomeric matrix protein [89]. Detection of IgA RFs and anti-CCP antibodies may be good predictors of RA in the absence of clinical signs and symptoms ([90]; thoughtfully evaluated by Majka and Holers [91]). Further, anti-CCP estimations may be useful in the disease diagnosis of RA patients who are RF-negative or where the diagnosis may be uncertain [3, 92].

In essence, 'anti-CCP and RF are distinct antibody systems yielding different information' [93].

Conclusions

Information on the mechanisms of disease that operate to result in RA has been open yet again to scrutiny. The results in which effective responses occurred preferentially in RF-positive patients treated with rituximab (therapy directed against differentiating pre-B to mature B-lymphocytes) or infliximab (anti-TNF- α monoclonal antibody therapy) add strong support to RF-positive RA being a separate disease from RF-negative RA. With this in mind, attention is being refocused on the role of RF-producing B-lymphocytes in the pathological process. Accordingly, by tailoring therapies to down-regulating these cells, it would be a logical move to refine the development of an effective and RF-specific therapy rather than a non-specific attack on B-lymphocytes. The B-lymphocyte has indeed returned to centre stage in the aetiology and progression of RA, and RF estimations, together with anti-CCP, are still meaningful in the diagnosis and management of the different subsets of RA patients.

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