The pathogenic role of rheumatoid factor in rheumatoid arthritis

Rheumatoid factors (RFs) are the first autoantibodies described in rheumatoid arthritis (RA), which target the Fc region of IgG. Since their discovery, RFs have been the subject of extensive studies not just because of their association with RA, but also because they serve as an excellent model for the regulation and induction of disease-related autoantibodies. Although RFs are normally induced during secondary immune responses, isotype switching and affinity maturation are confined to RA patients and are not observed in normal individuals. Therefore, the emergence of high-affinity RFs, which are believed to depend on T-cell help, is under strict control in normal subjects, whereas these control mechanisms appear to be bypassed in RA. In this article, we provide an overview of the current knowledge of how pathologic RFs are induced and discuss the mechanisms by which RFs contribute to RA disease process.

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The first autoantibody in rheumatoid arthritis (RA), rheumatoid factor (RF), was described by Waaler in 1940, who reported the hemagglutinating activity of a serum from a patient with RA [1]. RFs were named by Pike in 1949 due to their association with RA [2] and were later found to be autoantibodies targeting the Fc region of IgG in the γ2–3 cleft. Although frequently observed in diseased conditions including RA, RFs are also observed in normal subjects [3], which implies that they have both pathological and physiological roles that depend on the circumstances under which they are induced. In this article, we provide an overview of current knowledge regarding the induction of pathologic RFs and discuss the mechanisms by which RFs contribute to the RA disease process.

Characteristics of RFs & RF-producing B cells in normal individuals & in RA patients

Rheumatoid factors are normally produced during secondary immune responses against infections or immunizations [4–6]. This finding is in line with the observation that RFs are frequently found in chronic infectious conditions [7] and that immune-complexed rather than monomeric IgG is an efficient inducer of RF response [8]. In addition, RFs themselves bind with much greater affinity to aggregated IgG or immune complex than to monomeric IgG [9–11]. RFs produced during the secondary immune response are generally polyclonal IgMs of low-affinity, which resemble the characteristics of natural antibodies produced by CD5^{+} B1 cells (B1 cells). B1 cells are a subclass of B cells that spontaneously secrete IgM antibodies (natural antibodies) of low-affinity and polyspecificity. They are different from conventional B2 cells in that they do not undergo somatic hypermutation and memory formation. B-cell subsets capable of secreting RFs are likely to include both B1 and B2 cells, but the culprit subset may be variable depending on the biological condition that induces RF production. B1 cells have been shown to compose a major fraction that secretes large amounts of IgM RFs against the stimulation with *Staphylococcus aureus* [12]; RF secretion from B1 cells was found to occur at comparable levels in RA patients and normal subjects. Thus, it seems that the production of polyclonal low-affinity IgM RFs is not disease specific, but rather a physiological response that may serve in host defense by facilitating the formation and clearance of immune complexes. However, larger numbers of B1 cells are present in RA patients compared with normal subjects [13], and a correlation between peripheral blood B1 cell frequency and RF titer has been reported in RA [14]. Therefore, the significance of these findings remains to be resolved, although these findings may mean that RA patients are more readily triggered to produce RF and, thus, prone to RA development. Alternatively, these could be secondary findings unrelated to disease pathogenesis, because in patients with infectious diseases, RF production is not correlated with the development of arthritis.

Nucleotide sequence analysis of RF genes from normal immunized hosts and RA patients has shown that RFs in normal individuals are frequently encoded by a limited set of IgV genes, do not appear to switch isotype and have few replacement mutations in complementary determinant regions, which results in little affinity maturation [15]. On the other hand, the synovial RFs of RA patients are encoded by a wider range of IgV genes, undergo isotype switching and exhibit extensive nucleotide changes in complementary determinant regions, which lead to affinity maturation [15]. Although the precise phenotypes of RF-producing B cells in lymph nodes and tissue germinal centers (GCs) have not been determined in RA patients, the presence of accumulated somatic mutations and of isotype switching makes it plausible that pathologic RFs are produced by T-cell-driven B2 cells in RA.

How are RFs produced?

The mechanisms responsible for the activation of RF-producing B cells have been elusive for decades. In normal individuals, RF-producing B cells are not activated despite abundant self IgGs. The finding that monomeric IgGs, unlike immune-complexed IgGs, are poor inducers of RF production suggests that effective B-cell receptor (BCR) crosslinking is critical to transfer the B-cell activation signal. However, it has been shown that B-cell activating ability depends not only on the immune complex formation, but also on the nature of antigens contained in the complex in the sense that the ligation of Toll-like receptors (TLRs) is critical to trigger RF production in addition to BCR ligation [16]; both B1 and B2 cells express TLRs, particularly TLR-1 and -9 [17,18]. This finding represents a good example of how TLRs provide the link between innate and adaptive immunity. The interesting aspects of the simultaneous co-ligation of BCR and TLR are that TLRs are expressed on memory B cells and RFs are produced via TLR ligation alone in memory B cells [19]. However, in order to generate high-affinity RFs, particularly of IgG or IgA isotypes, T-cell help might be required.

T-cell requirements for RF production

It has long been believed that T-cell help is crucial for the production of pathologic RFs that have undergone isotype switching and affinity maturation. Studies on transgenic mice expressing human IgM demonstrated that RF-producing B cells are deleted in the absence of T-cell help and that complete B-cell activation involving GC formation only occurs with T-cell help [20]. Another finding that further supports the role of T cells in the production of pathologic RFs is that a critical contact residue of RF from a RA patient was found to be derived from somatic hypermutation [21]. To date, T-cell clones reactive to autologous IgG have not been detected in RA patients, most likely owing to clonal deletion. T cells that infiltrate RA synovium have been shown to be polyclonal and to lack specificity for any particular autoantigen [22,23]. Nevertheless, any T cells that recognize antigen-derived peptides presented by RF-producing B cells have the potential to activate these B cells; the ability of RF-expressing B cells to take up immune complexes and to present trapped antigens to ubiquitous T cells [24] may enable these cells to bypass the need for specific T-cell help, and could, ultimately, lead to the emergence of autoreactive T cells that can trigger RF synthesis. On the contrary, recent studies on the role of TLRs in B-cell activation and differentiation harbor questions whether T-cell help is indispensible (or whether TLR signaling is vital) for the production of pathologic RFs. Emerging data suggest that TLR and BCR co-activation induces isotope switching without T-cell help [25–27], although little is known regarding the effect of TLR signaling in somatic hypermutation and affinity maturation. In addition, TLR engagement seems to provide an essential signal for optimal antibody response even in the presence of T cells [28,29]. Therefore, high-affinity RFs might be triggered in the absence of T-cell help in the initial phase of RA, but their production in established RA is likely to involve both T-cell help and TLR signaling in a synergistic manner.

How is tolerance to self IgG lost in RA?

Evidence shows that RF-producing B cells are highly efficient antigen-presenting cells for multivalent, immune-complexed antigens [24]. Although T cells reactive to human IgG are deleted by a T-cell tolerance mechanism [20], T cells that recognize antigen-derived peptides presented by RF-producing B cells have the potential to activate these B cells. Therefore, the generation of predominantly IgM RFs during secondary immune responses (when Ig isotype switching usually occurs) suggests that

a strict control mechanism prevents the emergence of high-affinity RFs. Affinity-dependent B-cell deletion has been reported to censor autoreactive B cells during GC reaction [30,31]. However, much remains to be determined regarding how high-affinity RF-producing B cells escape tolerance mechanisms.

It has been shown that the synovial membranes of RA patients are extrafollicular secondary lymphoid organs that provide a microenvironment for isotype switching and the somatic mutation of RF-producing B cells [32]. William *et al.* have reported that the somatic hypermutation in an autoimmune response could occur elsewhere to the conventional GCs in an autoimmune murine model [33]. Based on the concept that censoring mechanisms are unique to GCs, these authors proposed that B cells, which have undergone hypermutation outside GCs might escape the self-censoring mechanism that selectively eliminates autoreactive B-cell clones and that they are more prone to be autoreactive. High-affinity RFs are locally produced in RA by B cells in the inflamed synovium [34,35] where lymphoid follicle-like structures are often found [36]. GCs have been found in approximately 25% of the RA synovial samples examined, with the remaining samples showing either diffuse or aggregated infiltrates of T and B cells [36]. Although a substantial amount of evidence indicates that GCs in RA synovium are the primary sites for the affinity maturation of autoantibodies [34,35,37], neither the clinical phenotype of RA nor the presence of RFs is associated with the presence of GCs [38,39]. Therefore, it remains to be resolved whether self-censoring mechanisms in synovial GCs operate in the same manner as in nodal GCs.

Contributions of RFs to the disease process in RA

IgM RFs are the major RF species in RA and are detected in 60–80% of RA patients [40]. Furthermore, RF specificity to RA is increased at high titers (e.g., IgM RF ≥50 IU/ml) and with IgA isotypes [40,41]. High titer RFs and IgA isotypes are also associated with radiologic erosion, extra-articular manifestations and, thus, poorer outcomes [40,42–45]. The association between high titer RF status and a poor prognosis indicates that RFs may have a role in the pathogenesis of RA. In addition, RF has proven to be a useful disease marker of RA, and is included in the 1987 American Rheumatism Association classification criteria for RA [46]

and in the recently proposed American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for RA [47].

The physiological roles of RFs under normal conditions have been shown [48–50]:

- To enhance immune complex clearance by increasing complex avidity and size;

- To help B cells uptake immune complex and, thereby, effectively present antigens to T cells;

- To facilitate complement fixation by binding to IgG-containing immune complexes.

Thus, high-affinity and high-titer RFs in RA synovial fluid are believed to exert such functions in a pathologic manner; the capacity of RFs to enhance immune complex formation [48,51] may not only grant arthritogenicity to RF itself, but also potentiate the arthritogenicities of other autoantibodies, including anticitrullinated protein antibodies (ACPAs). Moreover, several studies have shown that immune complexes isolated from RA patients induce TNF-α or other cytokines from peripheral blood mononuclear cells via Fc-γ receptor IIa engagement [52–54]. In particular, Mathsson *et al.* have demonstrated that both serum and synovial fluid RF levels of RA patients are correlated with polyethylene glycol-precipitated synovial fluid IgG levels, the latter being correlated with TNF-α levels of peripheral blood mononuclear cells induced by polyethylene glycol-precipitated synovial fluid immune complexes; the immune complex-induced TNF-α levels were significantly higher in RF-positive patients than RF-negative patients [54]. They have also shown that serum/synovial fluid ACPA levels were not correlated with serum/synovial fluid polyethylene glycol-precipitated IgG levels or with *in vitro* induced TNF-α levels. On the other hand, immune complexes formed with ACPAs and citrullinated proteins have been shown to induce TNF-α from peripheral blood mononuclear cells or *in vitro* differentiated macrophages [52]. Therefore, both RFs and ACPAs seem to contribute to RA severity.

Although clinical as well as biological evidence indicate that RFs are important players in RA pathogenesis, there has been no clear evidence that RFs are involved in the initial events triggering the disease process of RA rather than themselves being triggered by RA. Unlike the relatively low disease specificity of RF for RA, it has been shown that ACPA production in RA patients is a disease-specific humoral

autoimmune response [55–57]. It has been demonstrated in studies that retrospectively utilized preclinical serum samples that both RF and ACPAs are present in the sera of RA patients months to years prior to disease onset [58–60]. Interestingly, the majority of RF-positive RA patients are also positive for ACPA [55], and considering that immune complexes are strong inducers of RFs, immune complexes containing citrullinated antigens and ACPA might facilitate RF production. In support of this notion, the onset of RF was found by Nielen *et al.* to follow the onset of ACPA by a few years when RF and ACPA titers were serially examined during the preclinical period [60]. Furthermore, RF titers in ACPA-positive RA patients were found to be much higher than those in ACPA-negative patients in their study and *vice versa*. Therefore, a vicious cycle between ACPA and RF is expected; ACPA-containing immune complexes induce RF production and RFs, in return, amplify the inflammatory response by facilitating further immune complex formation, complement fixation, cytokine release and tissue damage. Ultimately, exaggerated inflammation will result in tissue citrullination and more ACPA production.

A number of studies have proved that effective disease-modifying antirheumatic drug therapy can decrease serum RF levels [61]. In particular, reduction of IgM RF levels seems to be correlated with clinical improvement [62–64]. Moreover, TNF-α inhibitors have been shown to decrease serum RF levels [65–68], which was correlated with clinical improvement [65,66,68]. However, pretreatment RF positivity was not different between responders and nonresponders [67]. Rather, high basal levels of IgA RFs but not of ACPAs were associated with poor response to TNF-α inhibitors in a recent prospective study on 131 longstanding RA patients [67]. Unlike RF levels, reduction of anti-cyclic citrullinated peptide levels after disease-modifying antirheumatic drug or
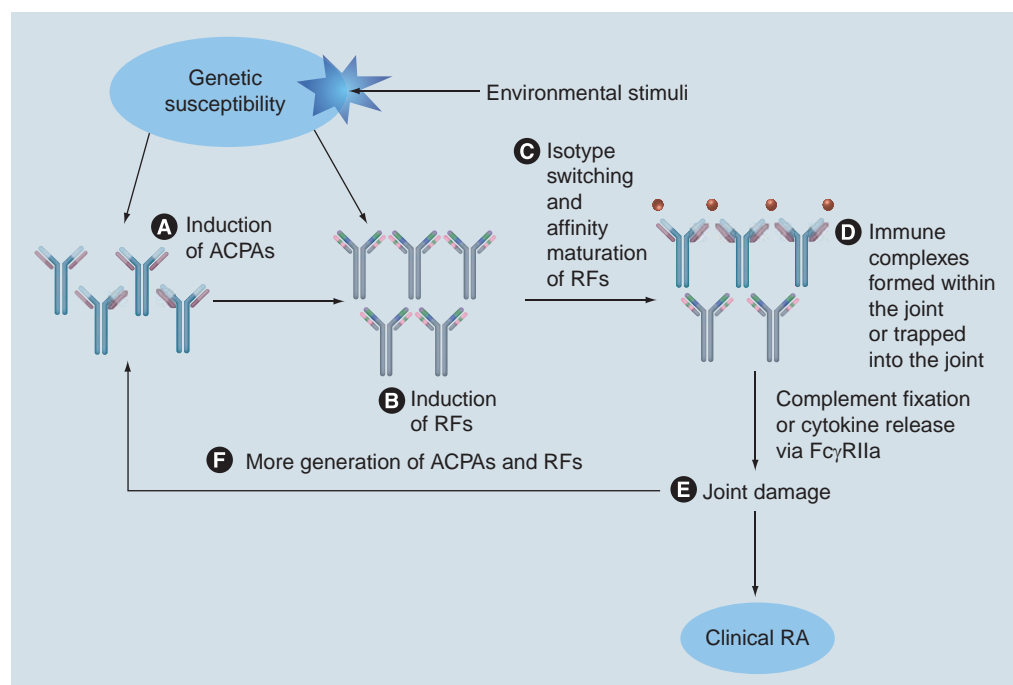

Figure 1. Hypothetical model for rheumatoid factor synthesis and rheumatoid arthritis development. (A) ACPAs are induced in genetically susceptible individuals in response to protein citrullination caused by environmental stimuli. **(B)** RFs may be induced either by ACPA-containing immune complexes (in ACPA-positive and RF-positive patients) or by other inducers (in ACPA-negative and RF-positive individuals). **(C)** RF-producing B cells undergo isotype switching and somatic hypermutation upon chronic exposure to immune complexes. **(D)** Immune complexes, of which formation is facilitated in the presence of RFs, are preferentially formed within the joints where ACPAs and RFs are locally produced. Those formed outside the joints might be trapped into the joints. **(E)** Joint damage/inflammation begins with immune complex deposition, complement fixation and cytokine release. **(F)** Protein citrullination as a result of joint damage drives further ACPA production followed by further RF production. This causes a vicious cycle between joint damage and autoantibody production. RA is diagnosed when joint inflammation results in clinical symptoms. ACPA: Anticitrullinated protein antibody; RA: Rheumatoid arthritis; RF: Rheumatoid factor.

TNF-α treatment has been controversial but reported to occur in early RA patients with less than 1 year of disease duration [64,69], which, however, was not correlated with clinical improvement. The close relationship between serum RF levels (but not ACPA levels) and clinical response to TNF-α therapy might suggest that RFs play a distinguished pathologic role from that of ACPAs. Alternatively, it might suggest that autoantibody-producing cells are differently regulated for RFs and ACPAs; a number of factors are known to be important in plasma cell survival, including cytokines such as TNF-α and the cell adhesion molecule CD44 [70].

Recent advances in RA treatment include the introduction of rituximab, an anti-CD20 monoclonal antibody. CD20 is expressed by B-cell precursors and mature B cells, but not by stem cells or end-stage plasma cells. Rituximab is interesting in that it directly targets autoantibody-producing B cells. Trials of rituximab have shown that the agent is highly effective at reducing RA activity [71–73]. Following B-cell depletion by rituximab, a large and rapid decrease in RF titers was observed, whereas immunoglobulin concentrations remained within normal ranges [73]. Decrease of ACPA levels tended to be delayed and modest compared with that of RF levels [74,75] and decreased levels of RFs and ACPAs were only observed in responders [74]. The different kinetics of RF and ACPA levels during rituximab treatment implies that the production of RFs is more dependent on short-lived plasma cells, while that of ACPAs on long-lived plasma cells. In fact, spontaneous RF response has been shown to occur by continuous generation of short-lived plasmablasts in a transgenic animal model with autoimmune background [76]. The effect of rituximab has been more beneficial in RF-positive than in RF-negative patients [77]. Moreover, RFs have been shown to be a better predictor of a good response to rituximab than ACPAs [78]. These findings provide sound information regarding the actual and critical roles of RFs in the pathophysiology of RA and suggest that RF-producing B cells and their activation mechanisms may be important therapeutic targets in RA.

Conclusion & future perspective

Since RF was first described, substantial evidence has been accumulated from both basic and clinical studies to suggest that RFs are key players in the pathogenesis of RA. This concept has been further corroborated by the results of recent clinical trials adopting B-cell depleting agents. The current hypothesis is that RFs are induced by immune complexes derived from disease-specific autoantibodies, which, in turn, further potentiate immune complex formation, complement fixation, tissue damage and more disease-specific autoantibody production (FIGURE 1). Since simultaneous TLR ligation is required in addition to BCR ligation to activate B cells to secrete RFs, the nature of autoantigens contained in the immune complex might be a critical factor to elicit RF production. Future studies on the mechanisms by which RF-producing B cells are activated and bypass tolerance mechanisms will help refine crucial therapeutic targets.

Executive summary

- The production of the polyclonal low-affinity IgM rheumatoid factor (RF) is not disease specific, but rather a physiological response that may serve host defenses by facilitating the formation and clearance of immune complexes.
- RF-producing B cells secrete RFs when co-ligated on Toll-like receptors together with B-cell receptors, reflecting the link between innate and adaptive immunity in the humoral immune response. T-cell help is believed to be required to enable RF-producing B cells to undergo isotype switching and somatic hypermutation, a process that is responsible for the production of high-affinity pathologic RFs in rheumatoid arthritis (RA). However, the emergence of such RFs is strictly prevented in normal individuals via tolerance mechanisms that are not completely understood.
- Although high-affinity RFs do not seem to trigger RA themselves, they are thought to contribute actively to disease severity and chronicity by enhancing immune complex formation and complement fixation; RFs induced by immune complexes derived from disease-specific autoantibodies further potentiate immune complex formation, complement fixation, tissue damage and more disease-specific autoantibody production. Therefore, RF-producing B cells and their activation mechanisms, including Toll-like receptor ligation may be important targets for RA treatment.

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