HYPOTHESIS

RHEUMATOID ARTHRITIS: THE PREDICTABLE EFFECT OF SMALL IMMUNE COMPLEXES IN WHICH ANTIBODY IS ALSO ANTIGEN

J. C. W. EDWARDS and G. CAMBRIDGE
Rheumatology Unit, University College, London

KEY WORDS: Rheumatoid factor, Immune complexes, CD16, Macrophage, Synovium, B lymphocyte.

The existence of autoantibodies and immune complexes in rheumatoid arthritis (RA) has been recognized for several decades. Antibodies to IgG, or rheumatoid factors (RF), were reported nearly 60 yr ago [1, 2]. A pathogenic role for immune complexes, and those formed by RF of the IgG class in particular, seemed likely in the 1970s, but has become less fashionable, being championed only by a few, notably Roitt, Mannik and Natvig [3–6]. Interest in RF-based immune complexes waned for three reasons. Firstly, there is no obvious reason why these should specifically produce joint disease. Secondly, the relationship between RF and disease is not one to one. Thirdly, the putative pathogenic effects of immune complexes were considered chiefly in terms of complement activation. By 1990, cytokines such as tumour necrosis factor alpha (TNF-α) had come to be considered the more important mediators in the joint, and therapeutic trials using cytokine blockade have reinforced that view [7].

As early as 1983, it had been shown that immune complexes, including those derived from RF, could stimulate macrophages to produce soluble factors (i.e. cytokines) capable of inducing extracellular matrix degradation [8]. It seemed likely that the complexes were binding to IgG Fc receptors (FcγR), but not until 10 yr later had work by Fanger, Lydyard, Huizinga, van de Winkel and others led to a clear understanding of the structure and function of these receptors [9–11]. Moreover, only recently has it been established that synovial intima is a highly specialized immunological microenvironment which includes an unusual pattern of FcγR expression [12, 13].

Armed with this new information, we are now in a position to construct a hypothesis which may resolve the difficulties perceived in the 1980s and point again to a central role for immune complexes, and specifically for IgG RF, in the pathogenesis of RA.

We propose that:

(i) the self-associated dimeric complexes of IgG RF found in the blood of RA subjects are small enough both to evade clearance by complement receptors and to cross endothelium to reach the extravascular space;

(ii) both the synovial and extra-articular features of RA are the predictable outcome of activation of macrophages bearing the receptor FcγRIIIa by immune complexes of this type, with the production of cytokines such as TNF-α, and resulting inflammation;

(iii) in synovium, TNF-α also induces changes in fibroblasts which facilitate the local accumulation of ectopic lymphoid tissue and plasma cells, with the generation of high local levels of RF and the formation of larger immune complex aggregates which induce further inflammatory events, including complement activation.

Perhaps the most important aspect of this hypothesis is that it refocuses attention on the possibility that permanent interruption of autoantibody production might effectively cure the disease. We propose that this might be possible.

Much of the hypothesis is based on conventional immunological dogma. Although large IgM-based immune complexes may form in the circulation of RA, these should bind complement and be cleared via complement receptors. As reviewed by Davies [14], large complexes may cause problems in systemic lupus primarily because the complement system is defective. Kunkel and colleagues identified smaller complexes in the circulation in RA, chiefly in the form of IgG RF self-associating dimers [4]. These small complexes fix complement very poorly [15]. IgG dimers are also small enough to be expected to gain significant access to the extravascular space and allow interaction with tissue macrophages.

Three classes of FcγR on macrophages might bind IgG RF complexes [10]. FcγRI is expressed at rather low levels on macrophages (~10⁴/cell) unless stimulated by, for example, γ-interferon. Interestingly, FcγRI is expressed very weakly by synovial intimal cells in RA. It has a high affinity for free IgG and may be saturated with monomeric IgG under most conditions. Binding of soluble IgG complexes has chieflly been attributed to the other two receptor classes. FcγRII is present at higher levels on all macrophages (~10⁴/cell), but is of low affinity and there is evidence from granulocytes, at least, that binding of very small (dimeric) complexes to FcγRII alone will not activate the cell, and that the presence of FcγRIII is required as well. FcγRIII is of moderate affinity, will bind dimeric complexes and binding of the FcγRIIIa isoform present on macrophages and natural killer (NK) cells
will induce cell signalling, including the production of TNF-α [16]. There are reasons for thinking, therefore, that only those macrophages which express FcγRIIIa as well as FcγRII will produce TNF-α in response to dimeric complexes.

On the above basis, IgG RF may be expected to have a pro-inflammatory effect at sites of FcγRIIIa expression in normal tissues. Macrophages from many tissues will express FcγRIIIa in vitro, but macrophage FcγRIIIa expression in normal tissues in vivo is restricted to specific sites, and notably, synovial intima. Most strikingly, in fetal limbs, synovial intima is the only site of FcγRIIIa expression [13]. The consensus on other tissues is that Kupffer cells, alveolar macrophages (variably), placental Hofbauer cells and macrophage subpopulations in lymphoid organs and bone marrow express FcγRIIIa [17, 18]. We have recently confirmed these findings in normal adult tissues, both in human and marmoset, and also found FcγRIII+ cells in normal pericardium and salivary gland [39]. In dermis, expression is minimal, with the important exception of the sites of mechanical stress where rheumatoid nodules form. At these sites, the majority of macrophages show FcγRIIIa expression [13]. Other tissues show minimal FcγRIIIa expression.

FcγRIIIa expression by macrophages is induced by thrombopoietins, VEGF, TGF-β in vitro [19]. Many cell types produce TGF-β in response to mechanical stress [20] and induction of FcγRIIIa by TGF-β would provide a convenient explanation for the link between mechanical stress and both site and severity of inflammation in RA. The detailed pattern of synovial and dermal lesions [21] suggests that mechanical stresses on soft tissue stretched over bone in a confined space are particularly relevant: in metacarpophalangeal joints, in metatarsophalangeal joints, in metatarsophalangeal joints, where synovium is compressed between bone and ground, and over the olecranon.

The pattern of macrophage FcγRIIIa expression is, therefore, a very reasonable map of pathological changes in RA: synovitis, s.c. nodules at sites of stress, a prominent acute-phase response, suppressed haemopoiesis, lymphadenopathy, pericarditis and alveolitis [21]. The acute-phase response and anaemia of RA are often thought to be secondary to the release of TNF-α and interleukin-6 (IL-6) from joints. However, a direct interaction between immune complexes and FcγRIIIa on Kupffer cells and macrophages in bone marrow, with consequent cytokine-induced stimulation of hepatocytes and suppression of erythropoiesis, would provide an equally good explanation.

The rheumatoid nodule may be seen as the hallmark connective tissue lesion of RA. The architecture of the nodule, with a ring of macrophages surrounding an area of necrosis, is consistent with a self-propagating response to a constant supply of extravascular immune complexes. Macrophages which, under the influence of mechanical stress, express FcγRIIIa, will bind complexes and secrete TNF-α and reactive oxygen species. Ligation of FcγRIIIa is also specifically implicated in release of the potent chemokine, monocyte chemoattractant protein 1 (MCP-1) [22]. These events will lead to local swelling, necrosis and macrophage recruitment. Newly arrived macrophages will respond to increasingly focused local stresses with the expression of FcγRIIIa, amplifying and perpetuating the lesion. As Palmer has proposed [23], the palisade of the nodule may be seen as equivalent to the thickened intima of RA synovium, with surface fibrin and rice bodies in the joint space replacing a focus of fibrinoid necrosis.

The common manifestations of RA are consistent with ligation of macrophage FcγRIIIa, but some features of Felty’s syndrome [21], in which IgG RF levels tend to be very high, suggest additional ligation of FcγRIIIa on other cell types. FcγRIIIa is also present on γδ T cells and large granular lymphocyte (LGL) populations, including CD8+CD57+ T cells and NK cells. FcγRIIIa ligation on LGL can induce proliferation in vitro [24] and, interestingly, a significant proportion of Felty’s syndrome patients have oligo-clonal LGL expansions which may resemble leukaemia [25]. Activation of LGL in bone marrow may contribute to neutropenia, which may also be dependent on binding of complexes to the FcγRIIIb receptor isofrom on granulocytes, with a reduction in neutrophil life span.

Synovitis not only dominates the clinical picture in RA, but differs from other connective tissue lesions in the formation of ectopic lymphoid tissue. This may be explained by another specialized feature of the synovial immunological microenvironment. Synovial fibroblasts, together with bone marrow fibroblasts, differ from fibroblasts from other tissues in that they respond more readily to cytokines with the expression of molecules associated with B-lymphocyte survival and differentiation [12, 26–28]. The expression of vascular cell adhesion molecule-1 (VCAM-1) on subintimal fibroblasts in RA is likely to be crucial, and is consistent with an effect of TNF-α generated in response to FcγRIIIa ligation by immune complexes [12]. The expression of VCAM-1, together with complement decay-accelerating factor and complement receptor 2, allows the tissue to be colonized by B cells, with follicle centre formation and local differentiation into plasma cells. This superior ability of synovial fibroblasts to support B-lymphocyte survival and differentiation has been confirmed in vitro. A significant proportion of the B-cell clones in RA synovium are known to generate RF [3]. High local concentrations of IgG RF have been shown to allow the formation of larger complexes, which will not only interact with Fc receptors, but will also fix complement [29].

The above analysis (summarized in Fig.1) can explain the targeting of joints in RA and the involvement of cytokines. Interest in RF finally waned in the 1980s because, despite many attempts, an RF assay which correlated precisely with clinical disease could not be developed. If pathogenicity of RF is dependent not only on Ig isotype, but also on fine specificities which allow the formation of complexes of very specific characteristics, then a poor correlation with total serum
antibody levels should not be surprising. Inconsistent relationships between antibody titres and clinical disease are familiar even in conditions in which the pathogenicity of autoantibodies is not in doubt. In the antiphospholipid syndrome, it has become clear that pathogenic molecular interactions are dependent on complex steric considerations which may involve not only antigen and antibody, but also other cofactors [30]. Moreover, in seronegative cases, RF may be sequestered within joints much of the time, with infrequent spill-over into the circulation.

The mechanism proposed does not require that the immune complexes should be formed by IgG RF. Any small complexes of similar characteristics might do. It is likely that features such as synovitis and pericarditis in systemic lupus depend on other small complex species. Other small complex species may also be responsible for some seronegative RA. However, complexes in which antibody is also antigen are particularly suited to the genesis of a syndrome in which synovitis is dominant. The fact that antibody is also antigen means that optimal local conditions for complex formation will arise once synovium is colonized by B-cell clones generating IgG RF. These complexes may not only generate inflammation, but also contribute to local B lymphocyte survival by providing the positive signal conveyed by complexed antigen carrying the complement component C3d.

The reasons for persistent production of RF in RA are not clear. The association between RA and MHC Class II allotype suggests that T cells are involved [31]. T-cell responses to Fc peptides are not present, but as Thompson et al. [5] argued, help may come from T cells recognizing foreign antigens endocytosed in the form of immune complexes by B cells of RF specificity. This mechanism could also be fuelled by T-cell responses to IgG Fab peptides, which have been found to be elevated in RA (C. J. Elson, personal communication). It is also possible that the Fc binding peptides present in RF Fab regions mimic peptides in bacterial IgG binding proteins, such as staphylococcal protein A, to which T-cell responses are likely to be Class II restricted.

A more central role for T cells in RA has been fashionable in Europe for some time, despite the evidence that T cells in RA synovium are, if anything, hyporesponsive [32, 33]. T cells extracted from RA synovium may show a ‘TH1’ phenotype [34]. The mechanism proposed does not require that the immune complexes should be formed by IgG RF. Any but this does not alter the fact that B-cell survival and differentiation are occurring in the tissue, and that whatever T cells are present are providing the help required.

IgG RF may also promote survival of B cells of RF specificities in follicle centres by coating them with IgG RF polymers associated with C3d. The detailed arguments involved are beyond the scope of this article, but it seems likely that B-cell clones which produce IgG RF can be self-perpetuating. There is also evidence from Natvig’s group that the RF produced in rheumatoid joints do not derive from Ig genes used by B-cell clones generating IgG RF. These complexes may not only generate inflammation, but also contribute to local B lymphocyte survival by providing the positive signal conveyed by complexed antigen carrying the complement component C3d.

The reasons for persistent production of RF in RA are not clear. The association between RA and MHC Class II allotype suggests that T cells are involved [31]. T-cell responses to Fc peptides are not present, but as Thompson et al. [5] argued, help may come from T cells recognizing foreign antigens endocytosed in the form of immune complexes by B cells of RF specificity. This mechanism could also be fuelled by T-cell responses to IgG Fab peptides, which have been found to be elevated in RA (C. J. Elson, personal communication). It is also possible that the Fc binding peptides present in RF Fab regions mimic peptides in bacterial IgG binding proteins, such as staphylococcal protein A, to which T-cell responses are likely to exist. These responses are likely to be Class II restricted.

A more central role for T cells in RA has been fashionable in Europe for some time, despite the evidence that T cells in RA synovium are, if anything, hyporesponsive [32, 33]. T cells extracted from RA synovium may show a ‘TH1’ phenotype [34], but this does not alter the fact that B-cell survival and differentiation are occurring in the tissue, and that whatever T cells are present are providing the help required.

IgG RF may also promote survival of B cells of RF specificities in follicle centres by coating them with IgG RF polymers associated with C3d. The detailed arguments involved are beyond the scope of this article, but it seems likely that B-cell clones which produce IgG RF can be self-perpetuating. There is also evidence from Natvig’s group that the RF produced in rheumatoid joints do not derive from Ig genes used by ‘physiological’ IgM RF, but from unrelated Ig genes [6]. This is consistent with the idea that pathogenic autoantibodies such as IgG RF develop by chance mutations within germinal centres during responses to unrelated antigens [35]. This suggests that if B cells of pathogenic RF specificity are destroyed, the chance of them reappearing may be no greater than that of de novo appearance on the same genetic background.

Selective destruction of B-cell clones of RF specificity poses major problems. Attempts to encourage these clones to die using modified antigen (IgG) would have to compete with 50–100 g of native IgG. An alternative
strategy may be simpler: to kill all B cells. If B-cell clones with specificities for non-self-antigens develop from clones with germ line sequences by sequential affinity-based selection under the control of corresponding T-cell responses, but pathogenic IgG RF-producing clones do not, then the logical thing to do is destroy all mature B cells. This should allow anti-non-self B-cell clones, but not pathogenic IgG RF-producing clones, to re-emerge. This may well be what happens when subjects with RA treated with high-dose cyclophosphamide prior to bone marrow transplantation go into long-term remission [36]. Recent reports indicate that destruction of mature B cells can be achieved with an anti-B-cell (CD20) antibody with minimal unwanted effects [37, 38], since B cells are produced rapidly and Ig levels are maintained in the short term.

Several detailed aspects of the hypothesis need to be tested, but means of testing are available. The precise role of FcγRIIIa in the cytokine response to immune complexes of different sizes and steric characteristics needs to be documented. The relationship between the clinical evolution of RA and circulating IgG RF dimers capable of inducing TNF-α release from FcγRIIIa+ macrophages in vitro needs to be reassessed. The link between mechanical stress, growth factor production and FcγRIIIa expression needs to be clarified. The ultimate test of the hypothesis is the efficacy of destruction of RF-producing B-cell clones by anti-CD20 antibodies and/or other agents. The chance that RF B-cell clones can be abrogated permanently is uncertain, but perhaps for the first time there is a strategy that would logically lead to disease cure. We propose that it is worth trying.

ACKNOWLEDGEMENT

The authors would like to acknowledge the contribution of Dr J. Highton, who suggested how the elements of the hypothesis might fit together.

REFERENCES


