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REVIEW ARTICLE

The role of collagen antibodies in mediating arthritis

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Abstract This review examines evidence that rheumatoid arthritis (RA) depends on autoimmunity to articular collagen, and mechanisms whereby autoantibodies to type II collagen contribute to disease development. Three major autoantigenic reactants have been identified in RA; the corresponding autoantibodies are rheumatoid factor (RF), antibodies to citrullinated peptide antigens (ACPA), citrullinated peptides (anti-CCP), and anti-type II collagen (anti-CII). Both RF and ACPA are well-validated and predictive markers of severe erosive RA, but cannot be linked to pathogenesis. By contrast, in various animal species immunized with CII there occurs an erosive inflammatory arthritis resembling that seen in human RA, together with antibodies to CII with an epitope specificity similar to that in RA. We discuss the well-known role of immune complexes in the induction of inflammation within the joint, and present recent data showing, additionally, that antibodies to CII cause direct damage to cartilage in vitro. The close resemblances between human RA and collagen-induced arthritis in animals suggest that autoimmunity, and particularly autoantibodies to CII, are important for both the initiation and perpetuation of RA in a dual manner: as contributors to the inflammation associated with immune complex deposition, and as agents with direct degradative effects on cartilage integrity and its repair.

Keywords Collagen antibodies · Collagen antibody-induced arthritis · Collagen induced arthritis · Pathogenic antibodies · Rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) has been considered as an autoimmune disease for more than 40 years [1], on the basis that it fulfilled markers for autoimmune pathogenesis. These included hypergammaglobulinemia associated with active disease; serum autoantibodies; deposition of gamma globulin in rheumatoid (synovial) lesions and particularly rheumatoid nodules; lymphoid infiltrations in synovial villi of affected joints; a clinical response to corticosteroids; and clustering within a patient and/or relatives with other autoimmune diseases, notably Sjögren's disease and systemic lupus erythematosus.

The hallmark of RA is an erosive inflammation, historically evident as infiltration of lymphocytes and granulocytes into an affected joint, neovascularization of the synovial lining surrounding the joint, proliferation of synovial fibroblasts and macrophages, and the development of inflammatory tissue (pannus) over the surface of the articular cartilage and eroding the subchondral bone. This proliferative process is clinically evident as swelling, erythema and pain in multiple joints, and there is progression to joint destruction, with bone erosions and loss of architecture. Many different cells participate—macrophages, dendritic cells, fibroblast-like synoviocytes, mast cells,

eosinophils, neutrophils, and T and B lymphocytes—and there is production of signature antibodies, cytokines, chemokines, and enzymes such as metalloproteinases, serine proteinases, and aggrecanases. Such mediators interact to facilitate disease progression, leading to digestion of the extracellular matrix and loss of articular integrity. There is systemic inflammation as well, with pronounced vascular lesions. Notably, there is substantial curtailment of life expectancy [2]. Most of the successful new biologic therapies, such as Infliximab (anti-TNFα) and Etanercept (recombinant TNFα receptor) are antagonistic to cytokines or cell surface receptors that are involved in these inflammatory pathways, and these therapies are also being applied successfully to other inflammatory diseases. Nonetheless, "inflammatory arthritis" is not synonymous with "rheumatoid arthritis", and there is increasing recognition that treatments designed specifically to reduce inflammation do not necessarily prevent the initiation of inflammation or ongoing cartilage breakdown [3], and the clinical success of such treatments depends on the initial degree of inflammation [4].

The pro-inflammatory cytokines that feature in RA are products mostly of macrophages and fibroblasts, whereas T and B cells are likely to have a more immunoregulatory function, although they could also be directly proinflammatory. Indeed the recent successful use in RA of therapies that target the CD20 molecule on B cells (Rituximab) [5–7] has generated renewed interest in the role of B cells overall in the developmental and effector stages of RA, reviewed by Edwards and Cambridge [8]. The early demonstration of IgG-reactive rheumatoid factors in serum, and in affected joints in RA, together with the presence of "ectopic" lymphoid follicles with germinal centres containing large numbers of plasma cells within the pannus suggest that autoantibodies are integral to disease development. Among the various autoantibodies that have been identified in human RA, the three most prevalent are the time-honoured rheumatoid factor (RF), which react with an epitope on the Fc region of IgG, the long-studied autoantibodies to type II collagen (CII) and, most recently recognized, the autoantibodies to citrullinated proteins (ACPA). However no indisputably causal relationship between any of these autoantibodies and the development of disease has ever been demonstrated. Both RF and ACPA are certainly well validated as markers of disease activity; they are both predictive and associated with erosive arthritis. ACPA has a sensitivity of 50–70% and a high specificity, and is highly correlated with classical RA [9, 10], whereas RF is more common, but less specific, since it is demonstrable in other diseases. In the case of ACPA, we await discovery of the identity of the true initiating as well as target antigens [11].

Our particular interest in autoimmune aspects of RA is the production of autoantibodies to CII. These have repeatedly been reported to occur in RA, but the antibody assay is notoriously difficult to standardize and so no routinely applicable tests for anti-CII are clinically available. However, as has been suggested recently [12], antibody assays to dominant CII epitopes might be clinically useful in the prognosis and diagnosis of arthritis. Moreover, in the light of recent evidence that monoclonal antibodies (mAb) to CII cause cartilage damage in vitro, as discussed herein, a reassessment of the pathogenic role of autoantibodies to CII in RA is clearly warranted.

Autoimmunity to collagen in rheumatoid arthritis

The idea that the pathogenesis of rheumatoid arthritis involved autoimmunity to collagen was introduced by Steffen [13], based on studies in RA of autoantibodies to cartilage collagen and collagen–anti-collagen immune complexes in the serum and synovial fluids. These early studies of autoimmunity to collagen were performed before there was clear distinction between type II collagen in articular cartilage, first described by Miller [14], and type I collagen in skin and bone, a problem that made the interpretation of some earlier studies difficult. Nonetheless, serum and synovial fluid of patients with RA have repeatedly been shown to display antibody [15–17] and T cell reactivity to CII [18–21] and autoantibodies to other cartilage collagens [22] as well. In early RA, serum autoantibodies to CII are present in up to 70% of cases [15, 16, 23, 24], but there is a rapid decline in the frequencies and levels of such antibodies among patients with erosive disease [25], possibly because these antibodies become sequestered in immune complexes within the joint as the cartilage is progressively eroded, thus exposing collagen. These antibodies precede radiological changes [25] or the appearance of RF [24], and their frequency has been correlated in RA with the presence of HLA alleles that confer susceptibility to disease [26].

Long ago, Witebsky et al. [27] enunciated "postulates" to establish the autoimmune nature of a given disease: autoantibodies or autoreactive cells are demonstrable; the autoantigen can be identified; and immunization with this autoantigen induces an equivalent autoimmune response accompanied by a disease similar to the human counterpart in one or another experimental animal. In the case of RA, CII is the only nominal autoantigen that can fulfill these postulates, if we note in particular that immunization of various species of animal with native (helical) CII in Freund's adjuvant induces, in each species tested, a similar autoimmune response and also a disease similar to human RA, whether in mice [28], rats [29], or primates [30]. Moreover, epitopes of CII recognized by autoantibodies [12, 31, 33] and by T lymphocytes [34, 35] in rats and mice are shared with those identified in human RA.

Autoimmunity to collagen in collagen-induced arthritis in animals

Collagen-induced arthritis (CIA) in animals certainly simulates human RA in its pathological expressions, immunoreactivity to collagen II and epitopes thereof, and genetic predisposition, which is dependent on particular risk and protective alleles of the MHC [36–40]. As in RA, CIA is characterized by synovial hyperplasia, infiltration of cells of the immune system, and marginal erosion and cartilage destruction with disruption of joint architecture. Moreover, induction of CIA depends on an immune response to epitopes of collagen that are represented on syngeneic (autologous) collagen, i.e. it is a true autoantigen. Although CIA is usually (and best) induced using heterologous (xenogeneic) collagen, it can be induced in mice with syngeneic collagen; in this case there is a relapsing-remitting course that even more closely simulates human RA than does the usually monophasic CIA induced by immunization with heterologous collagen [41, 42]. As for human RA, susceptibility to CIA is associated with specific MHC class II haplotypes, and in the mouse model the specific class II gene (coding for the A^q molecule) responsible for disease susceptibility has been identified [36]. The relevance of CIA to human RA is greatly enhanced by experiments wherein strains of mice that are not susceptible to CIA are rendered susceptible by transgenic introduction of RA-susceptibility HLA (MHC) class II alleles [43–46]. Importantly, the arthritis-associated MHC class II molecules in both mouse and man bind a peptide from the collagen molecule that presents the same potentially glycosylated lysine side chain to T cells [35, 47, 48].

Moreover, although major susceptibility genes for CIA are associated with MHC class II haplotypes, CIA (like RA) is polygenic, and genetic analysis has shown associations with genes outside the MHC in both mice and rats [49, 50]. MHC associations are particularly related to T and B cell responses to antigens, but other genes may affect the development of inflammation or a propensity to autoimmunity, and these same genes may have effects in various different diseases. Although most of the particular genes have not yet been identified, Griffiths and Remmers [50] described 20 independent quantitative trait loci (QTL) regulating the severity of CIA in different strains of rats; of these, 11 colocalized to genomic regions linked with other autoimmune disease models—of diabetes, multiple sclerosis or lupus in mice or rats—and several colocalized with QTLs modulating other models of arthritis. The locations of most of these QTLs identified as being important for the severity of CIA in rats were in regions where the homologous region in humans contained previously identified putative RA-associated loci. These studies, and similar studies in mice, suggest the presence of highly conserved disease pathways and possibly general mechanisms involved in attacking specific tissues operating in different autoimmune diseases controlled by clusters of genes across species [51, 52].

CIA in all species is accompanied by high levels of antibodies to native triple helical CII, with immunodominant B and T cell epitopes located at various regions of the CII molecule. The importance of both T cells [53] and B cells [54] in CIA are now very well established [55]. Panels of mAb to CII have been derived from mice immunized with CII [56–60], and the major epitope regions have been identified within peptides derived by cyanogen bromide (CB) cleavage of the triple helix, CB11 (aa 124–402), CB8 (aa 403–551) and CB10 (aa 552–897), with a major immunodominant epitope C1 located within CB11 [59, 61], a region that also contains a major T cell epitope [62, 63].

Various mAbs have been derived from mice immunized with CII, and for several epitopes have been closely mapped by use of chimeric molecules in which peptide sequences of CII have been inserted into recombinant collagen X that retains the triple helical structure of collagen but is unreactive with the mAb to CII [59]. In this way, epitope regions have been identified along the CII molecule: these share a common amino acid motif, a triplet of arginine–glycine–hydrophobic amino acid, including the previously described C1 epitope (aa 359–369), recognized by mAb CIIC1, J1 (aa 551–564) recognized by mAb M2139, U1 (aa 494–504), recognized by mAb UL1, and the F4 epitope (aa 932–936) recognized by mAb CIIF4 [32, 59, 61]. These epitopes map to regions within the collagen fibrils that are surface-exposed and accessible for antibody binding [59]. Antibodies from rats immunized with CII [31, 32], and humans with RA [32, 33], likewise react with the same epitopes as those recognized by murine mAbs.

A functional B lymphocyte response is essential for the development of CIA, since T cell recognition of peptides of collagen II alone is insufficient [39] and B-cell-deficient mice are not susceptible [54]. By contrast, arthritis can be transferred passively to naive animals with CII-reactive serum [64, 65], and by mAb to CII [57, 66–68], demonstrating a pathogenic role for antibodies in mediating the joint inflammation. Notably, a similar arthritis has been transferred to mice using an IgG fraction from a patient with seronegative RA but with high levels of autoantibody to CII [69]. Recent studies also demonstrated the arthritogenicity of plasma or serum from patients with active RA in FcγRIIb-deficient mice, and an IgG-rich fraction was identified as the pathogenic factor [70].

Collagen antibody-induced arthritis (CAIA) induced by the passive transfer of arthritogenic antibodies is severe, of rapid onset (within days or even hours of transfer), and histologically resembles post-immunization disease [56].

CAIA also resembles human RA by reason of bone erosions, influx of neutrophils and deposition of IgG and complement (C3) on the articular cartilage. The disease can be transferred by a single CII mAb [67], but the ensuing arthritis is usually not persistent and is less severe than arthritis after active immunization with CII. However transfer of a combination of mAbs to CII does induce a severe arthritis [33, 57, 71, 72]. Interestingly, single anti-CII mAbs also induce relapses in mice with chronic arthritis that have previously been immunized with CII [60, 73]. The development of both CIA [74–77] and CAIA [78, 79] requires complement, so that arthritogenic mAbs need to be of the complement-fixing subtypes, IgG2a, and IgG2b, although not all such mAbs to CII are arthritogenic (see below). Moreover, complete and specific cleavage of IgG2a antibodies by IdeS from *S. pyogenes* absolutely blocked arthritis in IgG2a-induced CAIA, significantly prevented IgG2a antibody-induced relapses in mice that had chronic arthritis, and delayed the onset and reduced the severity of arthritis in classic CIA [73]. Unlike active CIA, CAIA is not MHC-dependent, but the process involves both complement and Fc receptor binding [40]. An absence of activating Fcγ receptors rendered the mice CAIA-resistant, whereas a deficiency of the inhibitory FcγRIIb exacerbated the antibody-induced disease [67, 80]. Interestingly, carbohydrate moieties present on the Asn 297 of the CH2 domain of arthritogenic antibodies were found to be important in FcγR binding and stable immune complex formation [73]. On this basis, CAIA can be regarded as a good example of immune complex-mediated arthritis, as described below.

Immune complex-mediated arthritis: a prototypical inflammatory process in vivo

Autoantibodies as constituents of immune complexes have a pivotal role in triggering inflammation in various autoimmune diseases [81–83]. Immune complexes that are readily demonstrable in the articular tissues of most patients with RA are likely to be important for pathogenesis by initiating and maintaining the inflammatory cascade and the resultant destruction of cartilage. The deleterious effects of the deposition of immune complexes in articular tissues have been studied in many experimental models of arthritis. These include (1) direct injection of immune complexes of lysozyme–antilysozyme [84], (2) passive transfer of antibodies to a naturally occurring autoantigen in the joint, for example to glucose-6-phosphate isomerase (G6PI), which is a ubiquitous cytoplasmic enzyme that is associated with spontaneous arthritis in the transgenic K/BxN mouse [85], or to CII [67, 68], in which CII-reactive T cells prolonged the disease induced by antibodies [86], or (3) intra-articular injection of an antigen, e.g. methylated BSA, into mice previously immunized with mBSA [87], a process that also requires a T cell response. Although procedural details among these models vary, in each case it is the formation and deposition of immune complexes that induces inflammation and consequential cartilage damage, a process that involves the binding of "inflammatory" Fc-receptors onto intra-articular cells and then complement activation, whether by the classical or the alternative pathway.

Thus, complement fragments binding to immune complexes, tissue damage, and FcγR crosslinking cumulatively activate mononuclear leukocytes in situ that in turn release proinflammatory cytokines, so recruiting neutrophils and macrophages. These phagocytes then become further activated, creating a proinflammatory cytokine milieu that affects resident cell populations of synoviocytes and chondrocytes, and release of tissue-degrading enzymes that cause cartilage damage. Fc receptors in articular cells provide a link between the humoral and cellular immune systems [82, 88], and FcRs play an important role in the efficient uptake, processing and presentation of antigenic peptides by phagocytic cells to responder T cells [89], either in regional lymph nodes or in ectopic lymphoid follicles in pannus. Alternatively, Fc receptors may play an immunomodulatory role through the downregulation of the inflammatory response that follows engagement of the inhibitory FcγRIIB [84].

The effector cytokines in the antibody-induced arthritis are TNFα and interleukin-1β [90, 91], and the effector cells are neutrophils and macrophages [68, 92], whereas mast cell-deficient mice developed arthritis with CII-specific antibodies [93]. Importantly, not only pro-inflammatory cytokines but also the anti-inflammatory cytokines IL-4 and IL-10 promoted the antibody-induced disease [91, 94, 95]. Depletion of IL-4 suppressed the disease, which was abrogated by IFN-γ neutralization, and mice predisposed to produce Th2 cytokines developed more severe arthritis than mice biased towards Th1 cytokines [91]. Also, although IFN-β has been shown to downregulate proinflammatory cytokines, and it has been considered for therapeutic use in RA, IFN-β deficient mice developed more severe and prolonged CAIA [96]. Similarly, TGF-β type I receptor kinase inhibitor has been shown to prevent CAIA [97]. All of these studies demonstrate the requirement for critical evaluations of cytokine neutralization therapies at different phases of the disease in RA patients.

Such mechanisms are implicated in human RA as well, since immune complexes containing antibodies to CII or RF [98] occur in the rheumatoid joint and, in vitro, immune complexes containing antibodies to CII can induce the production by peripheral blood monocytes of inflammatory cytokines including TNFα, interleukin-1 beta (IL-1β) and

interleukin-8 [99, 100]. In RA, acute inflammation, with high C-reactive protein and ESR at disease onset, has been linked to high serum levels of antibodies to CII, and the induction of inflammatory cytokines by immune complexes binding Fcγ IIA [101]. The Fcγ IIA receptor does not occur in rodents, yet mice with trangenic expression of the human Fcγ IIA receptor develop a spontaneous autoimmune disease with features of RA that include joint erosions and the development of pannus [88].

Thus, to sum up, immune complex-mediated arthritis is a prototypic inflammatory process characterized by the release of proinflammatory cytokines and the activation of degradative enzymes. This concept has long dominated perceptions of the nature of human RA as a progressive inflammatory synovitis, and such perceptions have been amplified by the successful use of powerful biologic therapies that quash inflammation, so much so that attention has been diverted from mechanisms that operate at the onset of RA. Also, the question as to whether autoantibodies have any direct role in the initiation or perpetuation of arthritis other than promoting the formation of immune complexes remains neglected. We address this question in the next section.

Direct antibody-mediated effects on cartilage collagen

It is not always the case that antibodies to CII derived from mice with CIA are arthritogenic on passive transfer. Thus, some mouse strains that are resistant to the induction of CIA produce high levels of CII-specific antibodies that demonstrably bind to cartilage antigen [102] yet do not initiate arthritis [103]. This is attributable in part to the isotype/subclass of the antibodies produced, but also important may be differences in the topological distribution of epitopes of collagen II that are antigenic for individual strains of mice [104]. The mAb CIIF4 is of particular interest since it is strongly reactive with CII and with cartilage both in vitro and in vivo; it is of the same IgG2a subtype and is derived from the same mouse strain (DBA/1) as the mAb CIIC1 that is arthritogenic, yet it does not transfer arthritis to naïve animals [12, 33]. In fact, CIIF4 is actually protective in that it reduces or even prevents the development of arthritis when transferred to naïve mice together with normally arthritogenic mAbs [33]. This observation that only some but not all autoantibodies to CII can transfer arthritis indicates that arthritogenic autoantibodies could have direct pathogenic effects on cartilage independent of the presence of inflammation.

This is an appropriate point at which to review the properties of articular cartilage, a relatively acellular tissue in which a small number of chondrocytes maintain an abundant extracellular matrix. This cushions the ends of bones and allows for almost frictionless articular movement. These characteristics of cartilage are dependent on the interactions of molecules in the matrix, which comprises a highly organized network of fibrils of CII and other less abundant collagens such as collagens IX and XI, in which negatively charged proteoglycans, hyaluronan, and numerous minor but highly important components that interact to maintain the integrity of the fibrillar network are entrapped. The chondrocytes are entirely responsible for the development and maintenance of articular cartilage and, in contrast to other tissues in which cells are in close contact, cartilaginous cell–cell interactions are minimal, and signaling occurs via cell–matrix interactions. In adult cartilage chondrocytes are relatively inert, and matrix turnover and synthesis occurs only slowly. In particular, the collagen network, when damaged, is not readily replaced. Thus, "vulnerable" epitopes could involve regions on the CII molecule that interfere with these intracartilaginous interactions, either among chondrocytes, or with other matrix components.

Native CII, a well-studied autoantigenic molecule, contains several molecularly defined epitope regions that are relatively short, retain the triple-helical native conformation, and confer antigenicity on the intact CII molecule. Various mAbs to CII have been tested in vitro, to examine their effects on either pre-existing cartilage or the synthesis of new cartilage, as might occur after cartilage damage in vivo. The mAbs thus used, shown in Table 1, engage epitopes that have been defined, and antibodies to these have known and measured arthritogenicity on transfer to naïve mice. These include three potently arthritogenic mAbs—CIIC1, M2139 and UL1—and one non-arthritogenic and protective mAb, CIIF4 [12, 33]. The arthritogenic mAbs each cause damage to cartilage explants cultured with the mAb in vitro, marked by loss of proteoglycan from sites on the surface of the cartilage at which the mAb penetrates, accompanied by progressive denaturation and loss of collagen from the surface, and even complete matrix destruction [12, 105] (Fig. 1). However, in terms of cartilage damage, the differences between these arthritogenic mAbs only seem to be one of degree.

The same arthritogenic mAbs also modify the production of matrix by chondrocytes in vitro, but here there are differences in effect between the antibodies, as judged by the quantity and quality of matrix produced, and also the morphology of the chondrocytes (Table 1). Thus, collagen fibrils produced in culture with CIIC1 are thin and irregular, whereas fibrils in culture with M2139 are thick and aggregated [106]. However, the morphology of chondrocytes cultured with CIIC1 is normal [106, 107], whereas the morphology of chondrocytes cultured with either M2139 [106] or UL1 [12] is quite abnormal. By contrast, the mAb CIIF4, which is not arthritogenic in vivo, has no

Table 1 Details of mAbs to CII and their effects in vivo and in vitro

mAb	CIIC1	UL1	M2139	CIIF4
IgG subclass	IgG2a	IgG2b	IgG2b	IgG2a
Epitope location [59]	CB11	CB8	CB10	CB9
Amino acids	356–369	494–504	551–564	926–936
Sequence [32, 59]	A**RGL**T	LVGPRGE**RGF**P	MPGER**RGAAGIAGPK**	H**RGF**T
Binding site	Chondroadherin [106]	Integrin [12, 32]	Collagen IX/integrin [106]	Stromelysin [33]
Arthritogenic in vivo	Yes	Yes	Yes	No
Antibodies in human arthritis	Yes, RA>OA	Yes, severe RA	Yes in RA, but less frequent	Yes, OA>RA
Effects in vitro				
Chondrocyte cultures [12, 106, 107]				
Chondrocytes	Normal	Vacuolated	Pleomorphic	Normal
Collagen fibrils	Thin	Normal	Thick, aggregated	Normal
Matrix synthesis	Increased	Normal	Normal	Normal
Explant cultures [12, 105]				
PG loss at surface	Yes	Yes	Yes	No
Collagen denaturation	Yes	Yes	Yes	No
Collagen loss	Yes	Yes	Yes	No

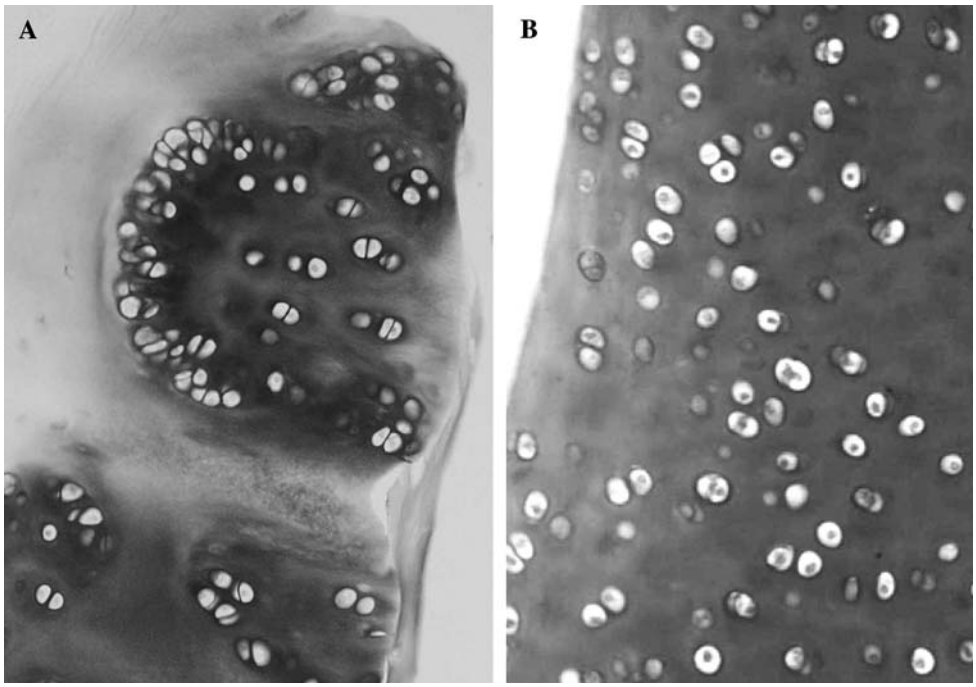

Fig. 1 Illustrating the destructive effects of arthritogenic mAb to CII on bovine cartilage stained with toluidine blue after seven days in culture with the mAb M2139 that is arthritogenic in vitro (**a**) or cultured with the non-arthritogenic mAb CIIF4 (**b**). Cartilage cultured with M2139 shows loss of the normal architecture, with clumping of chondrocytes, and patchy loss of matrix between the cell clumps, whereas cartilage cultured with CIIF4 shows normal architecture, with cells distributed individually in an evenly stained matrix

apparent effect on either pre-existing cartilage, or on the production of matrix by chondrocytes [106]. These observations suggest that arthritogenic autoantibodies target one or another of "vulnerable" epitopes on collagen II with ensuing direct effects on cartilage synthesis and stability.

Interactions between cells and matrix, or between matrix components, depend on precise regions of the collagen sequence, but the exact locations of many of these sites on CII are yet to be defined. Nonetheless, there is increasing evidence that the arthritogenic mAbs do indeed bind to sites of important interactions. In the case of CIIC1, the epitope is close to the binding site of chondroadherin [106], and interaction between CII and chondroadherin is known to influence fibrillogenesis [108]. In the case of M2139, the epitope is in the region of CII that is involved in binding CII to type IX collagen (CIX) [59, 109], so that binding of M2139 to this region of CII would readily interfere with CII–CIX interactions. CIX belongs to the group of fibril-associated collagens with interrupted triple helices (FACIT), and occurs on the surface of collagen fibrils,

functioning as a spacer separating the CII fibrils and preventing lateral fusion; hence it is important in the control of the thickness and stabilization of CII fibrils [109, 110]. As evidence for the role of CIX in maintaining cartilage stability, there is the observation that mice that lack CIX develop mild osteoarthritis of the knee joints [111, 112], and there is in humans a form of multiple epiphyseal dysplasia (EDM2) that has been linked to the *col9a2* gene, with development of irregular epiphyses, and osteoarthritis in the knee joints [113, 114]. Following immunization with CII, mice deficient in CIX develop more severe arthritis than controls, and arthritis passively induced by mAbs to CII occurs earlier in the paws and also involves the knee joints, suggesting that a lack of CIX in cartilage results in increased vulnerability of collagen to antibody binding [115].

Epitopes recognized by mAbs UL1 and M2139 include the collagen sequences GFOGER and GMOGER (O is hydroxyproline), which represent major binding sites for $\alpha1\beta1$ and $\alpha2\beta1$ integrins on collagen [116, 117]. The β_1 integrins, of which the $\alpha1\beta1$ and $\alpha2\beta1$ integrins are the best characterized in cartilage, are widely distributed cellular receptors for collagens, mediating a wide range of cellular activities, and the extracellular matrix profoundly influences chondrocytes via signaling pathways that involve integrins [118, 119]. Moreover, loss of contact between cells and matrix can cause major morphological changes [118] and could explain the histologic atypia of chondrocytes observed in studies in vitro with the two mAbs M2139 and UL1 that affect integrin-binding sites.

The lack of effect of the mAb CIIF4 both in vitro and in vivo calls for consideration. The epitope for mAb CIIF4 lies at the extreme end of the CII triple helix, and within the assembled collagen fibrils it is believed to lie close to the cleavage site of matrix metalloproteinase 3 (MMP3, stromelysin-1) [33]. MMP-3 is associated with cartilage degradation, whether in osteoarthritis or rheumatoid arthritis [120, 121]. MMP-3 primarily targets proteoglycans and is not a conventional collagenase, but it acts as a telopeptidase by cleaving CII at a site inside its NH_2-telopeptide crosslinking residue [122]. Therefore, as such, MMP-3 plays an essential role in the degradation of not only aggrecan but also of collagen fibrils in the cartilage [121]. By blocking cleavage of CII, CIIF4 could well block some of the damaging effects of MMP3 on cartilage; note that collagen loss may be permanent whereas aggrecan loss is temporary.

Taken together, these studies suggest that antibodies to CII, whether in CIA or human RA, have adverse effects other than the generation of immune complexes. That is, the data indicate there is actual interference with the integrity of the matrix by the arthritogenic mAbs. Such direct damage to the matrix could then lead to further exposure of collagen II, and release of cartilage breakdown products, thus enhancing the production and exposure of immune complexes to cells capable of producing an inflammatory response. Moreover, the chondrocytes within the cartilage damaged by arthritogenic antibodies in culture may themselves contribute to inflammation through the production of IL-1 [123, 124] with augmented attraction of inflammatory cells and fibroblastic synoviocytes into the destructive pannus of CIA and RA [125].

The role of rheumatoid factor

An important consideration when comparing human RA and CIA is the role of RF in each disease. Rheumatoid factor was first described in RA [126] and is a well-validated marker of disease severity in RA. Until the recent discovery of antibodies to citrullinated proteins, it was the only autoantibody included in the American Rheumatism Association (ARA) criteria for the diagnosis of RA [127]. RF also occurs in animals with CIA, in both rats [128] and mice [129–131]. In CIA, as in human RA, it may be particularly associated with more severe disease, as observed in HLA-DQ8-transgenic mice that lack CD8 T cells [132], or in mice that lack the CC chemokine receptor 2 (CCR2) [133]. Also, RF-like activity has been associated with one of the CII-specific arthritogenic mAbs [134].

Nonetheless, RF is not specific for RA, and occurs in various infectious diseases [135], other autoimmune diseases, most notably Sjögren's syndrome [136], and (transiently) in healthy individuals following immunization [137, 138]. In animals, RF can be readily induced by immunization with immune complexes, including in mice immunized with complexes of CII-anti-CII [131], and it has been proposed that it plays an important immunoregulatory role [138]. In the context of autoimmunity to CII, it is interesting that RF production is particularly associated with antibody responses to highly ordered repetitive patterns of epitopes, such as occur in viral envelopes, or bacterial antigens, and also in various autoantigens such as CII or DNA [139]. By crosslinking Fc regions of antibodies, RF effectively increases the net avidity and specificity of antibodies of low affinity, and by forming immune complexes and efficiently activating complement, RF may play an essential role in removing pathogens [138]. In the case of a response to an autoantigen such as CII, the same characteristics would enhance an ongoing autoimmune response. Thus, in RA, RF production could occur as a normal response to the highly ordered immune complexes of CII and antibody within the joint, but once produced, immune complexes containing RF may contribute directly to the induction of immune complex-mediated arthritis.

The citrullination effect: relevance to collagen

Still to be considered is the potential effect of citrullination of CII in creating an "RA antigen". We note that autoantibodies to citrullinated sequences of CII have been reported in patients with RA [140, 141], and fragments of citrullinated CII as well as immune complexes containing autoantibodies and citrullinated CII have been detected within affected joints [140].

Undoubtedly, the recognition in RA of autoantibodies that are specifically reactive with citrullinated proteins has been a most important advance, practically and theoretically. ACPA are now fully validated as specific diagnostic markers for RA; such antibodies are not detectable in other rheumatic disorders, and are predictors of the early development of erosive arthritis [9, 10]. The reactivity of ACPA depends entirely on the presence of the modified amino acid citrulline in the antigenic protein, and this in turn results from deimination of arginine residues by the enzyme peptidylarginine deiminase (PAD). Within joints, an isoform of PAD (PAD-4) is primarily expressed in macrophages and granulocytes, and is inducible by non-specific inflammation [140]. Although ACPA in RA react with various citrullinated proteins, including keratin, filaggrin or fibrin, no particular initiating antigen has been identified so far. It is likely that PAD, which is locally expressed within the inflamed synovium, can engage and citrullinate any protein with accessible arginines, and in fact commercial high-performance anti-CCP assays use a generic cyclic citrullinated peptide that confers maximum reactivity. Interestingly, the gene for PAD-4 has been identified as a candidate RA-susceptibility gene [142], with particular genotypes that show increased stability of mRNA being associated with RA [143].

Current evidence from experiments based on CIA indicate that, whereas ACPA alone are not pathogenic, they do have potent activity as disease amplifiers. Thus, in a recent study of CIA [144], mice immunized with CII produced not only anti-CII but also antibodies to citrullinated proteins, with both being detectable prior to frank joint inflammation. Moreover, in the same study, mice rendered tolerant to a citrullinated peptide and immunized for CIA developed a less severe form of CIA and, in a transfer model, antibodies to citrullinated fibrinogen enhanced arthritis when co-administered with anti-CII. By contrast, administration of a mAb reactive with citrullinated proteins did not induce arthritis, and this mAb ACPA bound only to elements in inflammatory pannus, and not to normal synovial tissue.

Our view is that exposure to arthritogenic mAbs, and the consequent damage to the matrix, may well lead to abnormal exposure of CII moieties on the cartilage surface: such regions would then become accessible to modification by PAD, thus generating citrullinated CII (CCII) neoepitopes with a greatly enhanced immunogenicity and thereby eliciting a generic ACPA response. However, we see this as occurring independently of the immune response to natural epitopes of CII, as discussed above. Assuming that antibodies to CCII react with the same arginine-containing regions that are reactants for autoantibodies to unmodified CII, there would be a "double source" for immune complex formation, with augmentation of the disruptive effects on cartilage stability caused by specifically arthritogenic antibodies to CII.

In conclusion

RA is a complex disease, clinically, pathologically and genetically, and may well be an end result of several initiating causes and differing immunogenetic anomalies. As described above, various models have been developed, each providing insights into one or another particular facet of the "entire" disease. Both innate and adaptive immune responses must be implicated and among these there may well be multiple autoantigenic pathways. Or, stated otherwise, "researchers have dismantled the pathogenic subunits of RA, adding gene to gene, molecule to molecule, and pathway to pathway in an ever more complex scheme of dysfunction" [145]. Clearly present knowledge precludes any definitive statement or unifying theory on the pathogenesis of RA. We have concentrated on evidence that autoimmunity to articular collagen is an important component in many patients—evidence that is surprisingly neglected in contemporary treatises on the pathogenesis of RA.

To reassemble the evidence, (1) both T and B lymphocytes that are reactive with CII occur in RA, (2) the animal model of RA of collagen-induced arthritis is induced in many species by immunization with CII and shows MHC associations that are similar to those occurring in RA; (3) experimentally, arthritis can be transferred by antibodies to CII, whether from mice with CIA, or from a patient with RA; and (4) there is now evidence from our laboratories that autoantibodies to CII have direct degradative effects on cartilage and chondrocytes, and interfere with resynthesis of damaged cartilage. This review provides a synthesis of our studies that provide strong evidence for a dual pathogenic role of anti-CII: first by contributing to the pro-inflammatory immune complex-based response that has been amply described and illustrated by CIA and CAIA, and second via an inflammation-independent reactivity that is damaging to CII and thereby to the entire assembly of molecules of articular cartilage.

In fully established RA, the contribution to the pathogenesis of autoimmunity to CII could appear minor when

lined up against the direct effects of the many other components of the inflammatory milieu. Nonetheless, autoantibodies to CII are present very early in the development of RA, and precede the appearance of rheumatoid factor, and possibly antibodies to the citrullinated proteins that depend on inflammation for their generation. Moreover, as evidence that a component of cartilage itself provides the stimulus for inflammation, there is the observation that synovitis is reduced after cartilage removal [146].

In closing, autoimmunity, and particularly autoantibodies to CII, is seen as having an important role in both the initiation and perpetuation of RA, not only by contributing to the inflammation associated with immune complexes, but also by direct degradative effects and likely interference with repair. Complete treatment and eradication of the damaging effects of RA in the long term must take both processes into account.

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