Editorial

Collagen of Articular Cartilage: The Neglected Autoantigen of Rheumatoid Arthritis

> If we knew the antigenic determinants towards which the forbidden clones...in rheumatoid arthritis are directed, major therapeutic approaches would be in sight.
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> I.R. Mackay, F.M. Burnet, *Autoimmune Diseases*[1]

The lead quotation comprises the last 3 lines of a text on autoimmune disease published 45 years ago. Since then an immense literature has accumulated on various immunological aspects of rheumatoid arthritis (RA), including an article from Finland in this issue of *The Journal* describing the association between RA and immune responses to the minor articular collagen type IX (CIX)[2]. While most scholars accept that RA amply fulfils the markers and criteria for an autoimmune disease, the actual identity of a provocative autoantigen(s) remains an enigma. However, given the abundance of collagen in the primary site of the disease in RA, and the proven autoantigenicity of the collagen molecule, this could be expected to be high on the list of suspects. Yet, curiously, there appear expert reviews covering the pathogenesis of RA with barely a reference to autoimmunity to collagen. Perhaps the striking successes of the new biological therapies, whether anti-phlogistic or B cell-depleting, transcend the need to consider "causes" in favor of modes of generation, and action, of inflammatory immune complexes as mediators of disease. In this editorial, and as outlined in more detail elsewhere[3], we draw attention to evidence that articular cartilage and particularly collagen is a significant autoantigen for pathogenesis of RA.

Articular Cartilage: an Immunologically Interesting Tissue

Articular cartilage is a paucicellular tissue in which a few highly specialized cells, chondrocytes, sustain an abundant and functionally important matrix. This matrix cushions the ends of bone against high compression stresses, and provides for virtually frictionless movement across smooth articular surfaces: Picture the skillful skier gliding down a steep bumpy black diamond run. Structurally, the articular cartilage matrix consists of an intricately organized network of fibrils of type II collagen (CII), and other less abundant collagens type IX and type XI, that entraps negatively charged matrix components, proteoglycans, predominantly aggrecan, and hyaluronan. Chondrocytes are responsible for the production and maintenance of this fibrillar network but, unlike parenchymal cells elsewhere, cell–cell interaction is minimal, and signaling occurs via cell–matrix interactions. In adult cartilage, chondrocytes are relatively inert, and matrix turnover and synthesis occur only slowly. CII constitutes the dominant fibrillar collagen of articular cartilage and CIX, which is structurally and antigenically similar, has binding sites for CII. CIX belongs to the group of fibril-associated collagens with interrupted triple helices (FACIT collagens), and is located on the surface of fibrils, where it functions as a spacer that separates the fibrils to prevent their lateral fusion[4].

Articular cartilage is immunogenic. As evidence for this, there is the natural occurrence in RA of immune responsiveness of T cells and B cells to various cartilage components, and also the occurrence of equivalent immune reactivities after deliberate immunization of animals with cartilage or CII. However, although other constituents of cartilage can elicit immune responses, CII is the dominant element, and immunization with CII in animals can induce inflammatory articular disease.

Rheumatoid Arthritis as Immune-Complex-Mediated Arthritis

Rheumatoid synovitis is prototypically an immune-complex-mediated inflammatory disease. The complex machinery of immune complex disease, involving Fcγ-receptor cross-linking, complement activation, leukocyte influx, and release of proinflammatory cytokines, is seen to be the essence of RA[5]: our primary concern is the origin of the antigenic moiety of the complexes in the first place. There

See Serum antibodies against intact human CIX are elevated at onset of RA but are not related to development of erosions, *page 745*

could be several sources. Type II collagen is certainly one of these, as judged by analysis of complexes extractable from rheumatoid joints[6]. Other antigenic sources could be the Fc piece of IgG that is reactive with rheumatoid factor, and citrullinated proteins as identified by assays *in vitro* for antibodies to cyclic citrullinated peptides (CCP). Another experimentally verified source of inflammatory immune complexes in joints is glucose 6 phosphate isomerase, a ubiquitous cellular enzyme shown to be the provocative autoantigen in the spontaneously occurring model of RA in the K/BxN mouse strain[7]. Whether a counterpart of this murine arthritis occurs in humans remains uncertain. RA can have various extraarticular expressions and, while these could similarly be explained by systemic deposition of immune complexes, the antigenic component is unclear. We submit that CII and its analogs, CIX and CXI, are suspect sources, whatever else may become swept up into the conflagration. The earliest research on autoimmunity to collagen in RA was reported in the 1960s by Steffen[8], with descriptions of autoantibody to collagen, and collagen-anti-collagen immune complexes in serum and synovial fluids. Since then there have been numerous reports on the occurrence in blood and synovial fluids of anti-CII reactivity, but frequencies among different laboratories were inconsistent for RA, other rheumatic diseases, and healthy subjects, and results were far more divergent than for interlaboratory assay differences among other autoantibody reactivities. The Monash Laboratory experience has been that while immunoassays for anti-CII are technically capricious and difficult to standardize, sensitivity and specificity do have indicative value for the diagnosis of RA, with assays proving more sensitive in early-stage than in late-stage disease, due perhaps to antibody being absorbed from serum by damaged cartilage, and with cleavage polypeptides of CII such as CB10, derived by cleavage with cyanogen bromide (CB), giving more discriminatory results than intact CII[9]. In any event, a view emerged that an anti-CII immune response would more likely be a consequence than a cause of cartilage destruction in RA, and interest lapsed in this response as a diagnostic marker or a pathogenetic process.

Collagen-Induced Arthritis (CIA): a Telling Experimental Model

Observations on autoimmunity to CII in RA in the 1960–70 era led to studies on effects of immunization of animals with articular cartilage, initially reported in rats and then in mice, and thereafter in other species including primates[10]. "Clinically" and histologically, the features of CIA resemble those of RA but 2 particular features require comment: first, induction of disease required use of adjuvant, now recognized as a potent activator of the innate immune system, and for which there is no actual counterpart in human experience; second, CIA, like its neurological counterpart, experimental autoimmune encephalomyelitis (EAE), is usually a monophasic disease although, as in EAE, a relapsing form of CIA can be elicited when mice are immunized with syngeneic rather than (as usual) xenogeneic CII[3]. Experimental immunization with CIX elicits an immune response but not actual arthritis[3], of interest in relation to the observations in humans from the Finnish study[2].

Susceptibility to RA and CIA is genetically based, being dictated predominantly by alleles of the major histocompatibility complex, and particularly compelling heuristically is the fact that equivalent class II elements operate in both diseases, and transgenic introduction of human HLA-DR susceptibility alleles into non-CIA-susceptible strains of mice overcomes their resistance to induction of CIA[11]. Further, CII-immunized mice develop high levels of autoantibodies to CII, which are pathogenic as judged by the occurrence, after passive transfer to naive mice, of what is known as collagen-antibody-induced arthritis (CAIA)[3]. Finally, as outlined below, monoclonal antibodies to CII have been derived from mice immunized with CII that have enabled a precise definition of epitope sites relevant to CIA on native CII[12], and these sites correspond to epitope sites that are engaged by the autoantibodies to CII detectable in human RA[12-14].

Epitopes on the Collagen Type II Molecule

Definition of epitopes on CII has been accomplished using monoclonal antibody (mAb) derived from mice with CIA by testing these mAb against chimeric CII molecules in which peptide sequences of CII were inserted into recombinant CX that retains the triple helical structure of native CII, but is nonreactive with anti-CII[12]. The several epitope sequences thus identified share the common amino acid motif of a triplet of arginine-glycine-X, where X is a hydrophobic residue. Notably, these epitopes map to regions within the collagen fibrils that are surface-exposed and hence accessible for antibody binding and, as mentioned, these are "generic" epitopes, since they are engaged also by sera of rats immunized with CII and humans with RA. Finally, and most interestingly, there is evidence that anti-CII independently has deleterious effects on cartilage architecture, both on the synthesis of new matrix, as shown by studies on the effects of CII epitope-specific mAb on cultured chondrocytes[15,16] and on preexisting matrix using cartilage explants[16,17]. Thus, after exposure to mAb known to be arthritogenic *in vivo*, cartilage explants showed clear degradative effects, as judged by loss of proteoglycan and collagen from sites on the surface of the cartilage at which the mAb penetrated. The same arthritogenic mAb were shown to affect matrix synthesis in chondrocyte cultures, with alterations in the morphology of collagen fibrils in the matrix, and altered appearances of chondrocytes. Functionally the epitopes for the arthritogenic mAb were close to regions of interactions between CII and other cartilage components that are essential for cartilage stability.

Interestingly, mAb CIIF4 proved to be nonarthritogenic

and, when administered in combination with other arthritogenic mAb, was actually protective *in vivo*[16]. The epitope for CIIF4 is at the extreme end of the CII triple helix and close to the cleavage site of matrix metalloproteinase 3 (MMP3, stromelysin-1), which mediates cartilage degradation. Hence interference of CIIF4 with MMP3 might block damaging effects of MMP3 on cartilage. Presently, all these effects of antibodies on cartilage have been obtained using murine mAb, and although the same epitopes are recognized by anti-CII in human sera, the relative importance of antibodies of each specificity in human RA remains to be determined.

Citrullination of Collagen Peptides

The citrullination of proteins results in reactants for immune responses that are highly relevant to the prediction and diagnosis of RA, and may be a source of harmful immune complexes. The enzyme responsible for deimination of arginines (citrullination), peptidyl arginine deaminase, would have access to the arginine-containing CII epitopes on the surface of the fibril, so potentially generating citrullinated CII neoepitopes. It is not credible that citrullination explains the entire immunogenicity of CII in RA, or CIA, but it is certainly possible that CII is among the proteins in inflamed joints from which citrullinated epitopes are derived. Were this to be the case, articular cartilage would fire a double-barreled immunopathic shot: as a source of autoantibody to native CII, and as a contributor to an anti-CCP response, and with both conceivably entering the immune complex inflammatory pathway.

Conclusions

There is a wealth of evidence that CII is an immunogenic molecule and hence a likely source of inflammatory immune complexes that drive the articular pathology of RA, on a suitable genetic background. While the complex expressions of RA, articular and extraarticular, preclude nomination of CII as the only driver, its abundance in the joint and relative accessibility render it a strong candidate. If indeed the "Holy Grail" of immunotherapy, for transplant retention and abrogation of autoimmunity, is restoration of natural immune tolerance, the therapy of RA may well have an important goal beyond the current successes of antiinflammatory biologicals and B cell-depleting antibodies, namely reestablishment of failed tolerance to native articular collagens.

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