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MINIREVIEW

Immunity to Type II Collagen in Rheumatoid Arthritis: A Current Appraisal[1] (41848)

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". . . in this iconoclastic view, pannus is the result, rather than the cause, of cartilage injury" (1).

This review will briefly analyze current concepts regarding the pathogenesis of rheumatoid arthritis (RA) with major emphasis devoted to the hypothesis that anti-self reactivity specific for the cartilage type (native type II) of collagen is a major effector mechanism in the disease. The intent is to update more extensive reviews of immunity to collagen in the rheumatic diseases (2–7).

The Synovium in Health and Disease. The relatively few studies of the histology of the human synovium in various disease states suggest that this membrane has a limited capacity to react to immunologic, viral, bacterial, physical, or metabolic insults. Perhaps it is not too simplistic to envision two basic kinds of immunologically mediated synovitis in humans. The first type would consist of pannus formation and mononuclear inflammatory cell infiltration that culminates in articular cartilage and bone destruction. This situation is represented by the disease termed, nosologically, RA. The second type would be comprised, predominantly, of polymorphonuclear-leukocyte infiltration and subsynovial neovascularization in the absence of pannus formation and connective-tissue destruction. This process is exemplified by the diarthrodial-joint lesion in systemic lupus erythematosus. Proliferative synovitis will be the focus of this review.

The older morphologic studies of the normal human synovium have been supplemented by recent investigations of the synovium of the knee in healthy young-adult rats using light and transmission electron microscopy (8, 9). These pursuits have confirmed the existence of the dichotomous synovial cell types proposed by Barland *et al.* (10), i.e., the type A phagocytic appearing cell and the type B synoviocyte, the principal function of which is apparently the secretion of joint lubricants.

In addition to this reaffirmation, these animal analyses have prompted an awareness that the normal synovium is not, universally, a one- to two-cell-layer-thick lining. Areas in the rat knee contiguous to cartilage can attain a thickness of five to eight cell layers in the absence of inflammatory-cell infiltrates (8). Whether this occurrence is a premorbid event, is indicative of microtrauma-induced injury or merely delimits areas providing the bulk of hyaluronate synthesis is unknown. It is equally uncertain whether there is a counterpart of this regional synovial thickening in human diarthrodial joints.

A final insight afforded by these ultrastructural studies of the normal rat synovium is the frequent occurrence of pentalaminar inclusions, resembling viral particles, within the lysosomes of synoviocytes (8). The existence of inclusion bodies has been reported in human synoviocytes as well (1), but their potential role in health and disease is conjectural.

Current Concepts Regarding the Pathogenesis of RA. In this review, only notions regarding the pathogenesis of RA for which substantive supporting data exist will be mentioned. Although an exogenous-agent etiology continues to be an attractive conjecture (11), the absence of direct evidence of viral involvement in RA precludes its further discussion. RA was, historically, perhaps the first disease perceived as an expression of autoimmunity (due to the recognition of rheumatoid factor). In the past decade, RA has begun to be further appreciated as an autoimmune or, at least, an immunologically mediated disease.

Initial evidence favored RA representing an intraarticular immune complex induced process (1). The profound decreases in complement levels in the synovial fluid of patients with RA (12, 13) and the hints that antigen–antibody containing material had been isolated (14, 15) provided provocative indications of the validity of this theory.

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But the majority of the work accomplished since that time has been consistent with the possibility that RA is a disease propagated by delayed-type hypersensitivity (16). Initial evidence for the concept was afforded by the recognition that immunocompetent cells could passively transfer disease in the model of inflammatory synovitis induced in rats with complete Freund's adjuvant (adjuvant arthritis) (17, 18). The rapid amelioration of joint inflammation in RA during thoracic-duct drainage (19), the recognition that the vast majority of lymphocytes within the rheumatoid synovium are, phenotypically, T cells (20, 21), the identification of lymphokine-like substances in the synovial fluid of patients with RA (22), and the suggestions that total-lymphoid irradiation can selectively suppress T-cell but not B-cell processes in concert with disease improvement (23–25) have fostered the theory that RA is a T-cell-produced disorder. Recently, immunohistochemical techniques have provided a compelling hint that the rheumatoid synovium is the site of antigen-presenting macrophages contiguous to activated T cells (26); the lesion in its entirety resembling a delayed-type hypersensitivity response to relevant-antigen challenge.

Thus, the intraarticular events of RA could prove to be subserved partially by T cells and their soluble mediators. However, it is equally probable that at least some of the extraarticular manifestations of RA, such as vasculitis, are B-cell and complement provoked. This global envisioning of the pathogenesis of RA would integrate the immunologically mediated features of the disease. If the evidence for immunologic mediation of RA is accepted, then the search for candidate autoantigens in the disease becomes a paramount issue.

History of the Theory that Type II Collagen Functions as an Autoantigen in RA. The hypothesis that collagen functions as an autoantigen in RA may have been at least partially formulated before the era of rheumatoid factor (27–31). Initial studies of the immunological properties of collagen used type I and found that it possessed antigenicity (29, 31, 32–39). The isolation of type II collagen from cartilage in 1969 (40) and type III collagen from several human tissues in 1974 (41) demonstrated that these three so-called interstitial collagens actually comprised a family of biochemically distinct macromolecules providing support for different tissues (42). This discovery prompted additional interest in the possible role of these collagens as type-specific autoantigens in rheumatoid arthritis (43–46). In attempting to raise reference antisera, it was found that native type II collagen is arthritogenic in the rat (47). Later, it was recognized that type II collagen-induced arthritis can also occur in mice (48). This review will now describe the histologic and immunologic features of collagen arthritis and relate them to those observed in adjuvant arthritis, since these two models continue to be the most frequently studied experimental counterparts of RA.

The Morphology of Experimentally Induced Synovitis in the Rat. Serial morphologic studies of the peripheral joint lesion in collagen (8) and adjuvant (49–52) arthritis of rats have failed to show any distinguishing feature. In both diseases, the initial lesion that can be discerned histologically occurs within the synovium and consists of two simultaneous events: (i) synoviocyte disruption and a change from a flattened to a rounded cellular appearance and, (ii) intrasynovial edema and fibrin deposition within and upon the surface of the synovium and articular cartilage (8). Shortly thereafter, evidence of synovial hyperplasia, intra- and subsynovial neovascularization, and pannus extending over the surface of the normally denuded cartilage is observed. Mitotic events in cells that appear to have originated within the synovium (as opposed to being macrophage-like elements carried to the synovium in the blood after arising in the bone marrow) are occasionally seen. Pannus formation continues for several days in the absence of inflammatory-cell infiltration, but by the time the lysosomes of synoviocytes become filled with debris, polymorphonuclear leukocytes and mononuclear-inflammatory cells appear in this granulation tissue. Coincident with the ingress of these inflammatory cells is the inception of cartilage and bone destruction. Microabscess-like areas comprised of either polymorphonuclear leukocytes or mononuclear-inflammatory cells are often juxtaposed with the erosive lesions in these models. A final inflammatory stage of experimentally induced synovitis in the rat is a persistent, hyperplastic synovium packed with dense infiltrates of mononuclear cells and

granulomatous areas (8). Immunofluorescence techniques show a paucity of immunoglobulin and complement deposited in the arthritic synovium (8). As in the RA synovium (20, 21), T cells predominate in collagen arthritis.

Little is known concerning the truly inceptual event in RA, although the evidence of synovial hyperplasia, neovascularization (53), and T-cell infiltration (54) in the early stages of RA appears convincing. In established disease, there is a close histologic resemblance in the experimental models to the biopsy picture of RA. The occasional occurrence of auricular chondritis in collagen-immunized (4, 55–57) or irradiated (58) rats resembling the lesion in relapsing polychondritis (59) should also be noted, as well as the report that immunization with type II collagen can induce endolymphatic hydrops, a lesion resembling that occurring in Meniere's disease, in guinea pigs (60).

Immunologic Aspects of Experimental Arthritis. Readily detectable humoral and cellular responses to collagen develop around the inception of collagen arthritis in rats (52, 61–66). Paralleling the exceptional arthritogenicity of this protein, type II collagen-induced immunity in both rats (46, 61, 66) and mice (43, 68) is antigen specific. In one study, evidence favoring an antibody-mediated pathogenesis of collagen arthritis in mice was afforded by a peaking of antibody titers to collagen coincident with the onset of arthritis (68). Thymidine-incorporation assays indicated that there was a transient cellular sensitization to type II collagen that occurred prior to the beginning of arthritis (68), further implying that antibodies rather than T-cell immunity to collagen were critical to the inception of this disease. However, murine experiments in the reviewer's laboratory, using a radiometric ear assay to measure delayed-type hypersensitivity to collagen, have shown that *in vivo* evidence of cellular sensitivity to collagen develops later, around the time that arthritis emerges. These contrasting findings render it impossible to conclude, at present, from studies of afferent sensitization to collagen whether antibody- or cellular-mechanisms play a role in the pathogenesis of collagen arthritis in either rats or mice.

Investigations using cobra-venom factor to induce transient complement depletion (69, 70) and antigen-presenting cells to suppress collagen arthritis (71) or induce delayed-type hypersensitivity in the absence of antibody responses to collagen (72) have suggested that antibody responses to collagen are involved in the induction of collagen arthritis in rats. However, nonspecific effects of cobra venom (73) and the theoretical possibility that T suppressor cells influencing arthritis were primed by the antigen-presentation protocol (74) urge caution in the interpretation of these data. It should also be pointed out that environmental manipulation can affect the morphologic manifestations of collagen arthritis (75, 76) and could have influenced the results obtained in these studies. Finally, evidence suggesting that collagen arthritis in rats is more complex than simply an antibody-mediated disease has been obtained in studies using psychological stress (75, 76), passive transfer of the phenomenon of collagen-specific suppression of disease (77), and administration of eicosapentaenoic acid (78), dimethyl sulfoxide (79), and retinoic acid (80). The observation that T-cell-deficient (nude) rats are refractory to the induction of collagen arthritis (81) indicates that type II collagen is a T-cell-dependent immunogen. This finding also suggests a T-cell dependency for this disease. Nonetheless, it is possible that the resistance found in this nude-rat strain is conferred by non-T-cell-mediated genetic mechanisms that, at present, have not been elucidated. Thus, these studies have not provided definitive evidence regarding the role of B and T cells in the primary pathogenesis of collagen arthritis.

What is known about the possible role of collagen immunity in adjuvant arthritis? Cellular sensitivity to collagen in this model has been found in some (33, 52, 82) but not all (83) studies. Only extremely preliminary and indirect indications exist, at present, that collagen immunity is involved in any aspect of the primary pathogenesis of this disease (82–84), and evidence to the contrary has also been reported (85, 86).

Insights Afforded by Passive Transfer Experiments. Much more convincing data regarding the pathogenesis of collagen arthritis and adjuvant arthritis has been provided by passive transfer experiments (87–90). These studies have strongly implied that both collagen and adjuvant arthritis are immunolog-

ically mediated processes. However, transfer experiments in neither model have shown definitively the relative roles of T- and/or B-cell pathways in the primary disease.

Collagen arthritis was first transferred by intravenously injected spleen and lymph node cells from rats immunized with type II collagen (87). More recent work has demonstrated that synovitis can be induced in rats (88, 90) or mice (89) by intravenous injection of IgG antibodies specific for native type II collagen. Unanswered questions exist, at present, regarding the results of all of these experiments.

Cell transfer produced a lesion that resembled a milder form of the primary disease, i.e., substantial pannus formation, cartilage and bone erosion, and predominantly mononuclear inflammatory cell infiltration. Moreover, the disease persisted until the latest time of evaluation, 24 days after cell transfer. No antibodies to collagen were detected in the sera of the arthritic recipients (87), suggesting that antibody production by the recipient did not explain the induction of synovitis in this system. However, no evidence has been reported, at present, that collagen arthritis can be passively transferred by T cells, and subsequent attempts using T-cell-enriched fractions or thoracic-duct lymphocytes have been negative in the reviewer's laboratory. Therefore, cell transfer of collagen arthritis has not provided conclusive evidence of T-cell involvement in collagen arthritis.

Similarly, serum transfer experiments have failed to furnish irrefutable evidence that collagen arthritis is a B-cell-mediated disease. Several serum-transfer protocols have given negative results (72, 87). Subsequent work has shown that synovitis can be induced by this system, but disease in the recipient occurs only when large amounts of immunoglobulin-containing material is injected (88–90). Moreover, the lesion created by antibody has been extremely transient, resolving in, at most, a week or 10 days (88–90). Although cartilage destruction can occur (88–90), other morphologic aspects of the synovitis induced by antibody appear to differ from the primary disease. Although one group reported some pannus formation in rats (88) and mice (89), another laboratory has concluded that intravenous injection of affinity-purified IgG antibody to type II collagen induces polymorphonuclear-leukocyte infiltration but not pannus in rats (90). An articular disease characterized by polymorphonuclear-leukocyte dominance would not recapitulate the chronic proliferative synovitis observed after cell transfer (87), in primary collagen arthritis (8), or in RA. Additional work in this area is, therefore, required.

At present, the chief importance of cell and serum transfer of collagen arthritis is in showing that products of B cells, and perhaps T cells, reactive to heterologous type II collagen, can induce synovitis. The exact relationship of these data to collagen arthritis and RA is unknown.

Although there is no direct evidence that T-cell sensitization to collagen can induce arthritis, indications do exist that T cells can incite a proliferative synovitis in rats. Again, these data must be interpreted with restraint. Syngeneic T cells from rats with adjuvant arthritis stimulated with concanavalin A *in vitro* can induce synovitis following intravenous injection (91). However, it has not been demonstrated that the transferred disease represents the complete counterpart of adjuvant arthritis, since T cells not exposed to concanavalin A are ineffectual in disease transfer (91). Likewise, in vitro propagated T cells from rats with adjuvant arthritis, reactive to both mycobacterial and homologous type II collagen (82) or exclusively mycobacterial (92) antigenic stimulation, are capable of inducing inflammatory synovitis in irradiated recipients. These data also provide an indication that T cells can produce experimental synovitis. However, spontaneous arthritis can occur, on rare occasions, in rats, and total lymphoid irradiation can induce an inflammatory synovitis as well (58). For these reasons, the possibility exists that the irradiation regimen was involved in the induction of arthritis in these propagated T-cell transfers. Currently, the only immunologic moiety that is clearly capable of inducing joint inflammation would be IgG antibody to native type II collagen. Further work with T cells is required before this claim can be convincingly asserted.

Afferent Sensitization to Collagen in RA. It is evident that some patients with RA have antibodies to the interstitial collagens in their sera (32, 35, 93–105) and that a proportion exhibit cellular sensitivity to collagen (24, 26,

98, 102, 104–111). Antibodies to collagen are also reported to be present in the synovial fluid of some patients with this disease (36, 97). These humoral (96, 98, 103) and cellular (26, 98) reactivities appear to be specific for collagenous determinants. However, the titers of such serum antibodies are low (98, 102, 103), and they do not correlate with signs or symptoms of RA (98, 103). Moreover, collagen reactivity is not restricted to RA; being present in a variety of diseases characterized by inflammation of connective tissue (34, 37–39, 94, 99, 102–104, 108, 110–115), as well as on rare occasions in normal subjects (104, 110). These data tell the investigator little concerning possible collagen-induced effector mechanisms in RA.

Immunogenetic Regulation of Collagen Immunity in Rodents. The immune response to collagen in rats (55, 116) and mice (117, 118) appears to be, as is the case with most protein immunogens (117), regulated by immune-response (Ir) genes. In addition, susceptibility to collagen arthritis in rats (55, 116) and mice (118) and to adjuvant arthritis (119) is influenced by genetic mechanisms as well. But immunogenetic pathways that control collagen reactivity in humans have not been convincingly identified. Preliminary indications that HLA-DR4 governs one's ability to respond, cellularly, to collagen (110, 111) have failed to receive support from the findings in other studies (104, 107). *In vitro* assays of cellular immunity to protein antigens continue to be relatively imprecise, and this fact mandates additional work on this problem.

Speculations Regarding the Possible Role of Type II Collagen-Specific Autoantibodies and T-Cell Factors in the Pathogenesis of RA. Based on the available evidence, several theories regarding the possible role of collagen immunity in the pathogenesis of RA can be proposed. The morphologic features, mimicking those occurring in RA, that can be created by sensitization to a constituent of cartilage or by adjuvant injection argue that further studies of experimentally inducible synovitis are of potential merit.

The first speculation relates to epitope-specific collagen reactivities being critical to the pathogenesis of rodent and human disease. Lessons in human lupus and myasthenia gravis illustrate this reasoning. Autoimmune diseases represent states in which a panoply of autoantibodies and sensitized lymphocytes exist (120). Antibodies against denatured DNA are found in many diseases but increasing evidence suggests that exquisitely specific anti-native DNA responses are a major effector mechanism in lupus (120). In myasthenia gravis, a number of anti-self reactivities exist, but it now appears that anti-acetylcholine receptor reactivities are the process intimately involved in the pathogenesis of the disease (121). A few patients with RA that possess high titers of antibodies specific for native type II collagen have been identified (103). All of these data are consistent with the possibility that critical epitopes on type II collagen, a probably complex multideterminant protein antigen (4), may function in the primary pathogenesis of RA. If preliminary evidence from a leukapheresis trial suggesting that RA is a heterogeneous disorder (122) is correct, then certain patients with RA in which anti-collagen reactivity is a fundamental pathogenetic mechanism may exist.

The second speculation relates to collagen antibodies versus anti-collagen T-cell factors in the pathogenesis of RA. A variety of antigen-specific T-cell-derived inducer and suppressor factors have been identified in rodent systems (74). If RA is a T cell mediated disease, and if autoimmunity to collagen is involved, then a unifying pathway would be provided by a type II collagen-specific arthritogenic lymphokine functioning in the human disease. Evaluating the effect of collagen-sensitized T-lymphocyte products on cultured synovial cells is experimentally feasible and could lead to insights in this area.

Finally, the rigorous test of whether collagen immunity is involved in RA must await trials involving antigen-specific immunosuppression (4, 5). Protocols capable of achieving antigen-specific attenuation of experimental arthritis in the rat (71, 77, 123) have been identified. Adaptations of these techniques may someday be evaluated in the human disease.

Conclusion. Studies of the immunologic properties of native type II collagen in the past decade have delineated a new field in connective-tissue disease. The opinion that cartilage injury may not be merely the result of pannus but that the origin of synovial hyperplasia could, in some way, be related to car-

tilage is no longer iconoclastic. The recent advances in the field of collagen immunology have provided data which are clear cut, but the proper interpretation of the experimental results is, at present, only partially evident. In the near future, additional work may answer the questions posed in this review; out of controversy could come certainty.

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