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Immunogenicity of Xenogeneic Cartilage Matrix Components in a Rabbit Model

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Purified xenogeneic cartilage matrix components, including proteoglycan subunits, chondroitin 4 sulfate, and chondroitin 6 sulfate, were inoculated into the knee joint of rabbits, and local as well as systemic responses were evaluated. Proteoglycan was associated with synovial hyperplasia and infiltrates of eosinophils and lymphocytes and with rising titers of anti-proteoglycan antibodies in a tanned sheep rbc hemagglutination assay over a six-week period of weekly intra-articular injections and observations. Chondroitin sulfates failed to evoke detectable changes in the joint or serum. Immunogenicity of cartilage matrix components may play a role in allogeneic and xenogeneic osteochondral grafts, and it is also possible that autogenous matrix immunogens exist and contribute to progression of degenerative joint disease. The immunogenicity of allogeneic and autogenous cartilage matrix components remains undefined.

The histologic features and metabolic state of osteoarthritic articular cartilage have been described in detail [1-4], but the etiology and pathophysiology of most degenerative joint disease remain unknown. Initially, trauma was felt to be the primary cause and, indeed, many animal models of osteoarthritis are based upon this concept [5,6]. Chrisman [7] has presented evidence that biochemical insults, particularly catabolic enzyme activity, also play a dominant role in the advancement of degenerative process. More recently, there is support for the addition of immunologic factors that, along with mechanical and biochemical events, comprise a vicious cycle responsible for progression of the osteoarthritic lesion. It is the existence and potential role of matrix immunogens that is the subject of this report.

Hulten and Gellerstedt [8] demonstrated that homogenates of articular cartilage were capable of producing a synovitis, presumably based upon a chemically induced inflammatory reaction. They reported in 1940 that intra-articular injections of either autologous or allogeneic hyaline cartilage, crude in preparation by today's standards, caused a synovitis in the rabbit. No changes, however, were observed in the cartilage by light microscopic evaluation of hematoxylin- and eosin-stained sections following the infrequent dosage schedule of once or twice a month for half a year. Lloyd-Roberts [9] noted fragments of bone and cartilage phagocytized by synovium

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in arthritic human hips and postulated the synovial inflammation and capsular fibrosis that accompanies degenerative joint disease was a result of this debris.

Chrisman, Fessel, and Southwick [10] addressed the same hypothesis by injecting homogenates of autologous costal cartilage into canine knee joints on a weekly basis for one to 12 months. Some animals followed for six months or longer developed marginal osteophytes and subchondral cyst formation in addition to chronic synovitis. In order to define the relative contributions of the various matrix components to this joint reaction, George and Chrisman [11] separately injected purified autologous collagen, proteoglycan, purified chondroitin sulfate A (derived from calf nasal cartilage), or chondroitin sulfate C (derived from shark) into the knee joints of rabbits for 10 to 15 weeks prior to sacrifice and evaluation. Synovitis, characterized by hypertrophy of the lining cell layer and a cellular infiltrate, was seen most strikingly after proteoglycan treatment, followed in severity by chondroitin sulfate C, and, to a lesser degree by chondroitin sulfate A, with no reaction attributed to the collagen preparation. Parenthetically, it was not known at that time that skin collagen (type I), the source used in this experiment and which failed to cause a reaction, differed from cartilage collagen (type II). It is now recognized that type II collagen of cartilage origin under proper circumstances is, indeed, antigenic [12]. The authors speculated that the synovitis and arthritis represented a chronic chemical inflammation resulting from excessive concentrations of the polysaccharide fraction of cartilage debris. Thus, the traditional role of mechanical injury in osteoarthritis was revised to include chemically mediated events. The protection offered by salicylates as noted by Simmons and Chrisman [13] supported this view.

More recently it has been speculated that cartilage matrix components [14], chondrocytes [15,16] or both may evoke specific immune responses that also play a role in the pathophysiology of degenerative joint disease, particularly if these potential sources of immunogens were sequestered from the host under normal circumstances. This concept of cartilage behaving as an immunologically privileged site with normally hidden immunogens has potential relevance to our understanding of inflammatory arthritis, articular cartilage transplantation, and chondrogenic malignancies.

The present study was undertaken to evaluate the immunogenicity of matrix components in a xenogeneic circumstance. In particular, we chose to assess the ability of purified bovine nasal proteoglycan and chondroitin sulfate A and chondroitin sulfate C of shark origin to evoke a systemic humoral response in rabbits following intra-articular inoculation.

MATERIALS AND METHODS

Groups of four to six mature New Zealand white rabbits received weekly intra-articular injections of the knee for six weeks with 4 mg of either proteoglycan subunit (fraction AlD of bovine nasal cartilage), chondroitin sulfate A (bovine nasal cartilage), chondroitin sulfate C (shark), or saline as a control. Sera were collected prior to initiation of the immunization schedule and weekly thereafter until sacrifice one week after the sixth injection. Synovial tissue was removed from the treated knees for histologic evaluation, and paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin.

Sera were stored frozen at $-20°C$ until evaluated with a standard passive tanned sheep red blood cell microhemagglutination assay [17]. The same matrix components used for inoculation served as antigens in the assay, and sera were absorbed against sheep red blood cells and bovine serum albumin prior to evaluation.

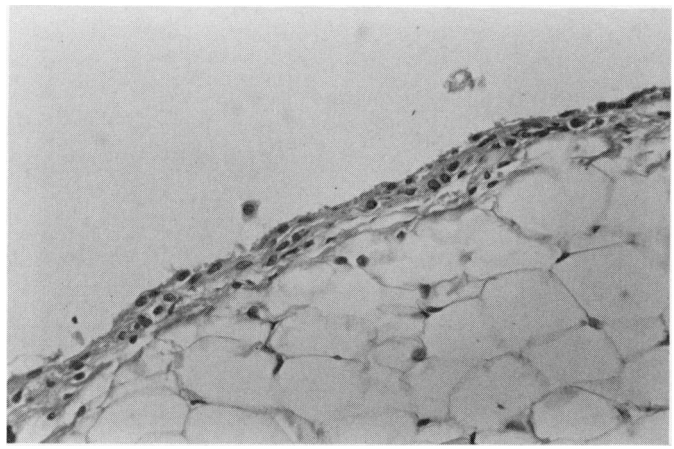

FIG. 1. Synovium from rabbits receiving intra-articular saline as a control was histologically normal.

RESULTS

Histology

No abnormalities were noted in synovial tissue removed from rabbit knees injected with either chondroitin sulfates A or C. Proteoglycan, on the other hand, was associated with a significant inflammatory response in all four rabbits evaluated after the six-week period of treatment. This tissue response was characterized by synovial thickening and cellular infiltrates, including eosinophils and lymphocyte clusters (Figs. 1 and 2).

Humoral Antibody

Chondroitin sulfates A and C failed to evoke a detectable response in this model, but proteoglycan caused rising titers of hemagglutinating antibodies in all four rabbits (Fig. 3).

DISCUSSION

Proteoglycan subunits of bovine nasal cartilage origin caused a local synovitis and appearance of systemic hemagglutinating antibodies following six weekly intra-articular injections of this purified matrix component in rabbits. Xenogeneic chon-

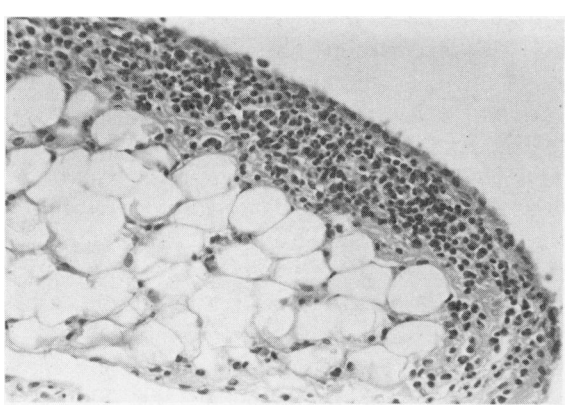

FIG. 2. Synovium from rabbits receiving intra-articular xenogeneic proteoglycan was characterized by thickening and infiltration of eosinophils and lymphocytes.

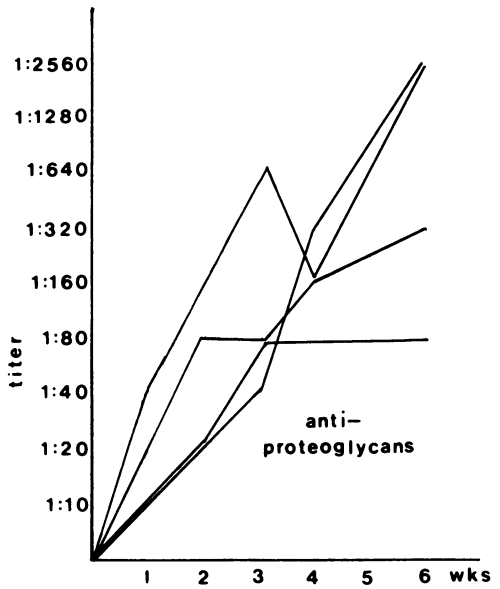

FIG. 3. Rising titers of anti-proteoglycan antibody were identified in all four animals evaluated over six weeks by a microhemagglutination assay.

droitin sulfates A and C failed to cause a detectable joint reaction (by gross and histologic examination) or immune response in this same model.

Previous studies of cartilage immunogenicity inspired by its significance as a transplantable tissue have, for the most part, focused attention on cell-surface transplantation antigens. Elves [15] has demonstrated that sheep chondrocytes possess histocompatibility antigens identical to those found on the host's peripheral lymphocytes. He further speculated that residual matrix on cartilage cells following isolation procedures may mask these antigenic sites. Langer and Gross [16] described the ability of syngeneic rat chondocytes to evoke a positive response in a leukocyte migration inhibition assay, suggesting sequestered chondrocytes were capable of causing autoimmune responses. Similarly, they demonstrated that intact articular cartilage transferred between disparate strains of inbred rats were not immunogenic, again using leukocyte migration inhibition as an indicator, but slices of articular cartilage or isolated chondrocytes did cause a positive response. The presence of humoral antibody was evaluated with a chromium-release assay and gave similar patterns of reactivity. The implication of these data was that cartilage matrix imposes both an afferent and efferent block to the immune response, preventing antigen from being recognized and not allowing antibodies, if formed, to reach chondrocytes within the matrix. This is consistent with the theoretical "pore" size of articular cartilage described by Maroudas and associates [18].

More recently, Yablon and co-workers [19,20] have demonstrated the ability of matrix components to act as potential immunogens in a canine distal femoral osteochondral allograft transplantation model. Rabbit anti-dog proteoglycan and link protein were used in direct immunofluorescent tests to demonstrate these matrix components in synovial tissue six weeks after transplantation of articular surfaces, and revealed the presence of immune complexes in these tissues by 12 weeks following the surgical procedure. Skin tests and lymphocyte migration inhibition assays became positive by the twenty-sixth week of observation reflecting the presence of cell-mediated responses, and circulating humoral antibody to proteoglycan and link protein was detected by radioimmunoassay at this same point in time. These im-

munologic findings coincided with abnormal histologic features in the synovium at 26 weeks (primarly hypercellularity) and the appearance of pannus and cartilage degeneration.

There has recently been interest in evaluating the matrix components of malignant cartilage tumors. Rosenberg and colleagues [21,22] have demonstrated abnormalities in keratan sulfate content and chain length of proteoglycans isolated from human chondrosarcomas. Mankin and co-workers [23] have also identified qualitative and quantitative changes in proteoglycan from chondrosarcomas, including the presence of an uncharacterized "excess protein" in these tumors. These biochemical alterations may serve diagnostic or prognostic roles in the future.

The majority of attention to the immunogenicity of matrix components has been prompted by interest in animal models of arthritis. The original observations of Chrisman, Fessel, and Southwick [10] and George and Chrisman [11] have already been mentioned. Also, as previously stated, type II collagen has been demonstrated to be immunogenic under proper circumstances and the detection of anticollagen antibodies in various disease states, including arthritis, has been exemplified by the work of Trentham [12]. Poole et al. [24] described rheumatoid-like changes in knee joints of approximtely 50 percent of rabbits with serum sickness induced with bovine serum albumin. The histologic description of synovial lining cell hyperplasia and lymphocyte accumulations, in the presence of immune complexes, is essentially the same as described by Chrisman [10,11] in his previous work.

Poole, Rosenberg, and co-workers [25,26] presented data supporting the immunogenicity of purified xenogeneic proteoglycan subunits and link protein following systemic challenge, the two components being immunologically distinct, and the lack of cross-reactivity between bovine proteoglycan and human proteoglycan subunits was also demonstrated. Similar conclusions were reported by Wieslander and Heinegard [27], again using purified bovine proteoglycan and link protein in a rabbit model and evaluating the response with rocket and crossed immunoelectrophoresis. Glant and co-workers [28] evaluated collagen-free cartilage extracts which they characterized as proteoglycan with some glycoprotein contamination, obtained from bovine, porcine, and human cartilage and injected into the foot pad as well as intramuscularly into rabbits. Using a tanned sheep red blood cell hemagglutination assay, they found humoral antibodies as early as nine days following inoculation with rising titers thereafter. Immunodiffusion assays became positive after approximately six weeks, revealing at least two separate species of proteoglycan. Skin tests were positive after the first week and an additional indicator of cell-mediated immunity, lymphocyte migration inhibition, was also positive shortly after exposure to these antigens. Glant et al. [28] suggested that the core protein of proteoglycan (along with glycoprotein) accounted for the immunogenicity of hyaline cartilage and further stated that glycosaminoglycans were not only devoid of immunogeneic properties but were also capable of masking antigenic sites of other matrix subunits.

While not strictly an arthritic disorder, Marshall et al. [29] have described the concept of "chemical radiculitis" that they postulate occurs when glycoprotein is liberated from nuclear material of intervertebral discs through a ruptured annulus and gains access to nerve roots. They used an indirect immunofluorescent test in which the patient's serum was reacted with fluorescenated nuclear proteins and then layered over tissue sections of nucleus pulposis. Increasing titers were found in some patients after the third week of symptoms.

In summary, our current study in a small group of animals demonstrates both a

local and systemic response to intra-articular xenogeneic purified proteoglycan subunits. With the exception of the study by Yablon, all previously reported investigations regarding the immunogenicity of matrix components were also carried out in xenogeneic systems, and none of these past reports evaluated an intra-articular route of immunization followed by assessment of systemic responses. This study serves to re-emphasize that cartilage matrix components appear to be an important additional source of immunogenicity that may be of significance in osteochondral allograft recipients. A pathogenic role may also exist in arthritis, as may a diagnostic and/or prognostic function in cartilage tumor-bearing patients. The immunogenicity of *allogeneic* matrix components must now be explored, and the possibility of autoimmunization cannot be dismissed without further investigation.

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