TYPE II COLLAGEN-INDUCED ARTHRITIS IN RATS

Passive Transfer with Serum and Evidence That IgG Anticollagen Antibodies Can Cause Arthritis*

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Collagen-induced arthritis is an animal model of polyarthritis that can be induced in susceptible rats by immunization with native type II collagen (1). The development of arthritis is associated with high levels of both cell-mediated and humoral immunity to type II collagen (2) and the arthritic response appears to be due to collagen immunity. This animal model is of importance because collagen immunity has also been described in association with human rheumatoid arthritis (RA)[1] (3-6) and other rheumatic diseases (7, 8). In addition, the histopathology of collagen-induced arthritis resembles that seen in human RA in that the lesion is one of synovial proliferation that progresses to pannus formation and results in marginal erosions with extensive destruction of cartilage. Radiographs of affected rat joints also show erosive changes similar to those seen in human RA (1).

The mechanism by which arthritis develops in this model is unclear. It has been reported (9) that arthritis can be transferred from immunized arthritic and nonarthritic donor rats to nonimmunized syngeneic recipients with pooled spleen and lymph node cells. However, the specific cell type responsible for transfer of disease has not been identified; furthermore, neither cellular sensitivity nor antibodies to collagen could be detected in the arthritic recipients. We have been impressed with the invariable association of arthritis with high antibody levels to collagen and decided to attempt to transfer disease using serum. Early serum from rats at the time of onset of disease was chosen because of the marked difference in anticollagen antibody levels in arthritic as compared with nonarthritic donors (10) and the suggestion that early serum was essential to transfer of experimental autoimmune thyroiditis in rabbits (11). This serum, when fractionated with 50% ammonium sulfate and concentrated, proved to be effective in transferring disease. Having successfully transferred disease, we addressed the questions of (a) whether the serum factor could reproduce all of the histopathologic findings present in actively immunized arthritic rats, and (b) what component of serum was responsible for development of arthritis.

[1] *Abbreviations used in this paper:* RA, rheumatoid arthritis; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunoassay; OPD, ortho-phenylenediamine.

Materials and Methods

Animals. Female Wistar outbred rats (100–150 g) were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass., housed in metal cages, and fed a standard rodent chow diet with water *ad libitum.*

Antigens and Immunization Methods. Native type II collagen was isolated by limited pepsin digestion of fetal bovine articular cartilage obtained fresh from a local slaughter house and from a rat chondrosarcoma known to produce type II collagen (12). The tumor was the kind gift of Dr. George Martin (National Institutes of Health). Purification of collagen from both sources was performed in the same manner. Articular cartilage or tumor was dissected free of surrounding connective tissue and bone, minced with a sharp scalpel, and homogenized in 4 M guanidine hydrochloride with a homogenizer (model SD-45 with G-450 generator; Tekmar Co., Cincinnati, Ohio) kept cold with an ice bath. After extraction for 24 h at 4°C, the supernatant fluid was collected by centrifugation and discarded, and the precipitate was washed thoroughly with cold distilled water. The precipitate was subjected to limited pepsin digestion and the solubilized type II collagen was purified as previously described (13). Amino acid analysis, uronic acid assay, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed the purified type II collagens to be free of contaminating noncollagenous protein, proteoglycan, and type I collagen, respectively. The collagen was stored lyophilized in a vacuum desiccator at 4°C until used.

In experiments using radiolabeled collagen, bovine type II collagen was acetylated as previously described (10), except that [^{3}H]acetic anhydride (5,000–10,000 mCi/mmol; Research Products International Corp., Mt. Prospect, Ill.) was used instead of [^{14}C]acetic anhydride in order to obtain labeled collagen with higher specific activity. The resulting labeled collagen had a specific activity of 6.7×10^5 dpm/mg.

For immunization, type II collagen was dissolved in 0.01 N acetic acid at a concentration of 2 mg/ml by stirring overnight at 4°C. An emulsion was made with incomplete Freund's adjuvant and collagen solution at a ratio of 6:4 using a VirTis homogenizer (model 23; VirTis Co., Inc., Gardiner, N. Y.) at high speed for 2 min. Wistar rats were given 0.1 ml of cold emulsion by intradermal injection of one hind footpad and received a booster injection in the tail 1 wk later using another identically prepared emulsion. This immunization procedure results in an incidence of arthritis of ~80% with a fairly uniform time of onset 12–16 d after the initial injection. The incidence and severity of arthritis and antibody levels are all greater than those achieved with the single injection we have previously reported (10).

Preparation of Serum Concentrate. Rats immunized with type II collagen were observed daily for the development of arthritis. Blood was obtained from nonarthritic rats by cardiac puncture and exsanguination of rats lightly anesthetized with ether on the day arthritis developed or on day 21. It was allowed to clot for 2 h at room temperature and the serum was recovered by centrifugation. Arthritic and nonarthritic sera were pooled separately. A gamma globulin concentrate was obtained by adding an equal volume of saturated solution of ammonium sulfate to the pooled sera. The resulting precipitate was recovered by centrifugation at 15,000 *g* for 30 min. Distilled water was added to the precipitate dropwise so that it was resolubilized in a minimal volume. After dialysis against several changes of phosphate-buffered saline (PBS) for 24 h, the volume of the solubilized concentrate was adjusted to 50% of the starting serum volume with PBS. The concentrate was then sterilized by filtration through a 0.22-μm microporous filter. This concentrate was either administered immediately to rats intravenously through a tail vein or stored frozen at −80°C until used.

Immunoassay of Antibodies to Collagen. Sera from rats not undergoing cardiac puncture were obtained by retro-orbital bleeding using a capillary tube. An enzyme-linked immunoassay (ELISA) technique, adapted from the methods described by Engvall and Perlman (14), was used to measure antibody titers. A similar method for measuring antibodies to collagen has been described previously (15). Round-bottomed 96-well microtiter plates (Nunc, Neptune, N. J.) were coated with collagen as follows: collagen was dissolved in 0.4 ionic strength phosphate buffer, pH 7.6, by stirring overnight at 4°C. The collagen concentration was adjusted to 5 μg/ml based on specific absorbance at 230 nm as detected by spectrophotometry. Each well received 100 μl of collagen solution and the plates were incubated at 4°C overnight. The coating solution was emptied by inverting the plate and sharply snapping the wrist to remove

remaining droplets. To reduce nonspecific adsorbtion, 100 μl of 0.15 M NaCl, 0.02 M phosphate, pH 7.4 (PBS), containing 0.5% ovalbumin (Grade V; Sigma Chemical Co., St. Louis, Mo.) was added and incubation was continued for 1 h. Three additional washes were performed with PBS containing 0.05% Tween 20 (PBS-Tween) and the plates were used immediately.

For antibody assay, sera were diluted with PBS-Tween containing 0.5% ovalbumin, and aliquots of 100 μl were added to the type II collagen-coated microtiter plates. After incubation at 4°C overnight, the plates were washed three times with PBS-Tween, 100 μl of a 1:1,000 dilution of rabbit anti-rat IgG peroxidase conjugate (Cappel Laboratories, Inc., Cochranville, Pa.) was added, and incubation was continued for 4 h at room temperature. Excess conjugate was removed by washing three times with PBS-Tween and the amount of conjugate specifically bound was determined by adding orthophenylenediamine (OPD) (Sigma P 3888; Sigma Chemical Co.) as a substrate. After incubation at room temperature for 1 h, the reaction product was measured by absorbance at 492 nm using an automated device (Dynatech Laboratories, Inc., Alexandria, Va.). Optimal dilution of the conjugate was determined based on "checkerboard" titration under actual assay conditions.

Substrate was prepared by dissolving 40 mg of OPD in 100 ml of 0.024 M citric acid, 0.05 M dibasic sodium phosphate, pH 5.0, and adding 40 μl of 30% H_2O_2. Because OPD is light sensitive, substrate was always prepared immediately before use.

Collagenase Digestion. To determine whether immune complexes were involved in the development of arthritis, serum concentrate was subjected to collagenase digestion to remove the collagenous component of the presumed complex. Serum concentrate was dialyzed against 0.01 M Tris, 0.15 M NaCl, pH 7.4, and made to contain 4 mM $CaCl_2$. Purified bacterial collagenase (kindly provided by Dr. Carlo Mainardi, Memphis Veterans' Administration Medical Center, Memphis, Tenn.) was added at a concentration of 10 U/ml and the solution was incubated for 90 min at 37°C. The reaction was terminated by addition of 10 mM EDTA and 5 mM phenanthroline (final concentrations). To ensure that the collagenase was active and capable of digesting collagen from immune complexes, an aliquot of immune serum was preincubated with 50 μg/ml of native type II collagen and then subjected to a similar collagenase digestion. The antibody titer of the immune serum was determined before addition of collagen, after addition of collagen, and after collagenase digestion.

Affinity Chromatography. Because immune complexes were not detected in serum concentrate, further purification of the active principle was carried out by a combination of affinity and gel filtration chromatography. Native type II collagen was bound to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N. J.) by the following procedure. Collagen was dissolved in 0.4 ionic strength phosphate buffer, pH 7.6, at a concentration of 2 mg/ml by stirring overnight at 4°C. CNBr-activated Sepharose was prepared by washing 1 g of the dry beads with 200 ml of 1 mM HCl in a scintered glass funnel over a 15-min period. The moist beads were added directly to the collagen solution and the suspension was gently shaken overnight at 4°C using a rotator. 1 ml of 1 M ethanolamine was added and incubation was continued for 1 h. The Sepharose conjugate was then washed alternately with PBS and 0.2 M glycine-HCl, pH 2.8, for three cycles. The quantity of collagen bound to the beads was determined by hydrolyzing a 100-μl aliquot and performing an analysis of hydroxyproline content as previously described (16). Under the conditions used, ~0.6–1.0 mg of collagen/ml of Sepharose was bound. Finally the conjugated collagen-Sepharose was equilibrated with PBS and 3 ml of gamma globulin concentrate was added. After incubation overnight at 4°C, the suspension was placed in a 1- × 6-cm polypropylene column and washed with PBS until no protein could be detected in the eluate by spectrophotometry. All of the wash volume was collected (PBS eluate). Protein bound to the affinity column was eluted with 0.2 M glycine-HCl, pH 2.8 (acid eluate), and dialyzed exhaustively against PBS. Both eluates were separately concentrated to 2 ml by pressure dialysis using a concentration cell equipped with a YM 30 membrane (Amicon Corp., Lexington, Mass.) and either applied to a column for gel filtration or sterilized by microporous filtration before being given to recipient rats by intravenous injection into the tail.

Gel Filtration Chromatography. The acid eluate from the collagen-Sepharose affinity column was applied to a column (2.5 × 100 cm) of Biogel A5m (BioRad Laboratories, Richmond, Calif.) that had been equilibrated with PBS and calibrated using purified rat IgM and IgG

(Cappel Laboratories, Inc.). A drop of tritiated water was mixed with each sample to serve as an internal marker for the column volume. The column effluent was continuously monitored at 280 nm and fractions of 5 ml were collected. Fractions were pooled as indicated, concentrated by pressure dialysis as described above, sterilized, and given to recipient rats by intravenous injection into the tail.

Immunoelectrophoresis and Immunodiffusion. Immunoelectrophoresis was performed on agarose-coated glass microscope slides using a Gelman electrophoresis apparatus and standard techniques (17). Briefly, Noble Agar (Difco Laboratories, Detroit, Mich.) was dissolved in High Resolution Buffer (Gelman Sciences Inc., Ann Arbor, Mich.) at a concentration of 1.5%. 3 ml of agar gel were applied to each clean 25- × 75-mm glass slide. Wells were punched in the agar and filled with 5–7 μl of sample. After the antigen solution had diffused into the well, electrophoresis was performed at room temperature and 4 mA for 60 min. 1-mm troughs were removed and antisera were added. Precipitin lines were permitted to develop over 48 h in a humidified chamber at 4°C.

Ouchterlony immunodiffusion was performed in 1% Noble Agar (Difco Laboratories) dissolved in 0.1 M sodium acetate, pH 7.4. 1-mm holes separated by 4 mm were punched using a template. Holes were filled with antigen or antisera at room temperature and permitted to develop overnight.

Inhibition Studies. The specificity of the purified transfer antibody was examined by testing the ability of various collagens to inhibit its binding to bovine type II collagen-coated microtiter plates. Each collagen tested was dissolved in 0.1 N acetic acid at a concentration of 1 mg/ml and dialyzed against PBS at 4°C. When denatured collagen was used, an aliquot of the respective native collagen was placed in a boiling water bath for 10 min to assure thorough heat denaturation. Appropriate dilutions of each inhibitor were added to aliquots of purified transfer antibody and incubated for 30 min at 4°C before testing for binding in the standard ELISA system.

Results

Passive Transfer of Arthritis. Gamma globulin concentrate of sera obtained from rats at the time of onset of collagen-induced arthritis transferred disease to 100% of naive recipients if given in sufficient concentration (Table I). Control gamma globulin concentrate obtained from animals immunized with incomplete Freund's adjuvant-acetic acid emulsion was not effective in transferring disease. In addition, sera from rats immunized with type II collagen, which did not develop arthritis, would not

TABLE I

Passive Transfer of Arthritis

Donor source	Recipients Arthritic/total
Immunized with type II collagen	
Arthritic (2.5 ml)	12/12
Arthritic (2.0 ml)	3/4
Arthritic (1.5 ml)	0/4
Nonarthritic (2.5 ml)	0/4
Immunized with incomplete Freund's adjuvant-buffer (2.5 ml)	0/4
Nonimmunized (2.5 ml)	0/4

Donor rats were immunized with native type II collagen and bled on the day definite arthritis was first detected. Rats not developing arthritis and those receiving incomplete Freund's adjuvant-buffer were bled on day 21 and their sera were pooled separately. An equal volume of saturated solution of ammonium sulfate was added to each pooled serum and the precipitate was redissolved in water, dialyzed against PBS, adjusted to 1:2 the original serum volume, sterilized, and administered intravenously to nonimmunized recipients in the amounts shown in parentheses.

transfer disease when 2.5 ml of the concentrate was given to nonimmunized recipients. Quantities larger than 2.5 ml could not be given because administration of higher volumes resulted in an excessive rate of mortality in the recipients.

Clinical Course and Histopathology of Passively Transferred Disease. Arthritis in the recipient rats developed within 18–72 h after transfer. In the initial experiments, 67% (10/15) of the recipients that developed arthritis did so by 24 h, with an additional 27% developing disease by 48 h, and another 7% by 72 h. In addition, all of the rats that became arthritic by 24 h had bilateral hindpaw involvement. An example of one of these rats is shown in Fig. 1. Of those animals that became arthritic between 24 and 72 h, two had bilateral and three had unilateral hindpaw disease. We have not observed forepaw lesions in any of the recipients. Furthermore, disease has been less severe than in the immunized donors. It has consisted primarily of redness and swelling of the ankle joint and did not extend into the small joints of the digits. In most of the arthritic recipients, the redness and swelling abated over a period of 3–5 d (mean duration of arthritis, 4.7 ± 1.6 SD). No chronic lesions or permanent deformity were noted.

The histopathology of passively transferred arthritis was similar to that seen in early disease of immunized donors. The lesion was one of synovial proliferation, infiltration of subsynovial tissue with neutrophils and mononuclear cells, and exuda-
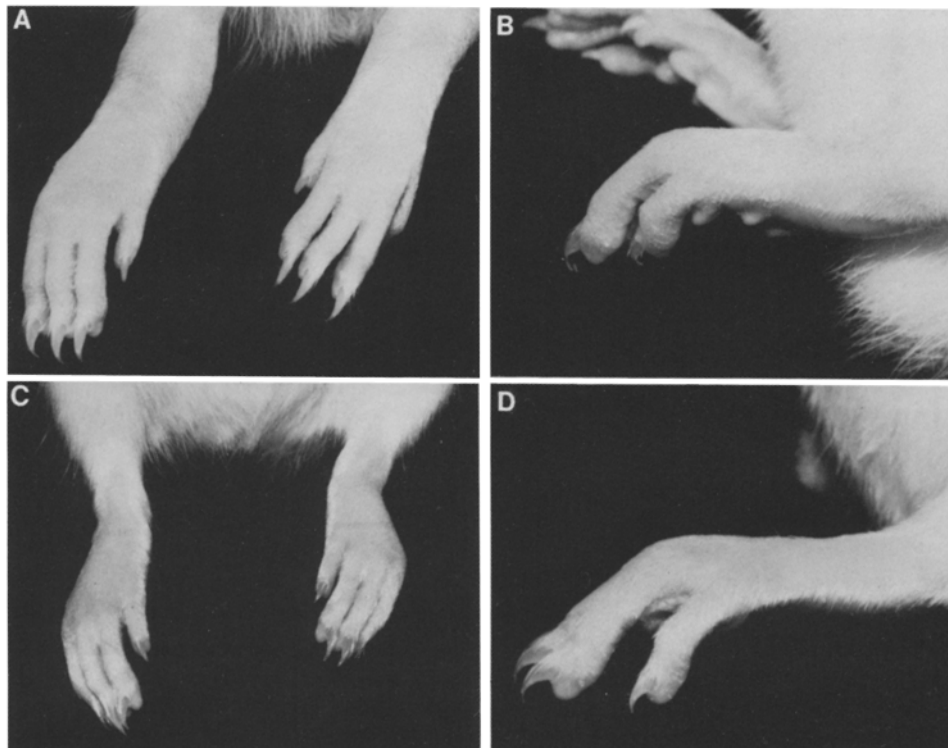

Fig. 1.   Hind limbs from a rat that developed bilateral arthritis after transfer of serum concentrate from an arthritic donor. Note the swelling of both ankles (A) as compared with a normal rat (C) and increased thickness of the dorsum of the foot (B) as compared with a normal rat (D). Photograph of the arthritic rat was taken 24 h after onset, 48 h after transfer.
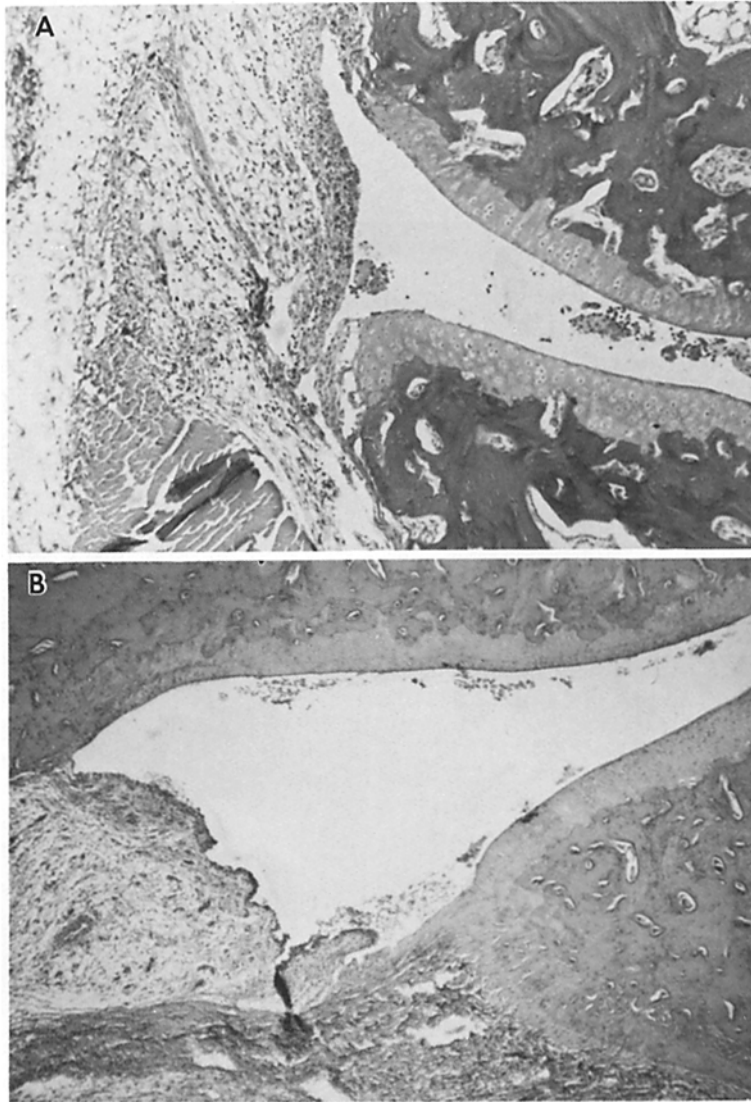
FIG. 2.   Histology of the arthritis developing 24 h after passive transfer (A) as compared with that developing 14 d (B) after immunization with type II collagen. Both joints were obtained on the day of onset. Hematoxylin and eosin × 80 (A).

tion of cells into the synovial space (Fig. 2). An early marginal erosion was seen in one of the joints examined histologically 24 h after the onset arthritis (Fig. 3). Although the most frequent cell found in the synovial space was the neutrophil, a substantial mononuclear cell infiltration was seen in the synovial tissue. Vasculitis was not prominent in either the immunized donors or in the recipients.

Humoral Immunity to Collagen in Recipients.   Recipients were bled at intervals after transfer and antibody levels to type II collagen were measured. During the first several hours after transfer, the levels were stable but they rapidly declined between 24 and 48 h and then more slowly declined over the next several days (Fig. 4). Low
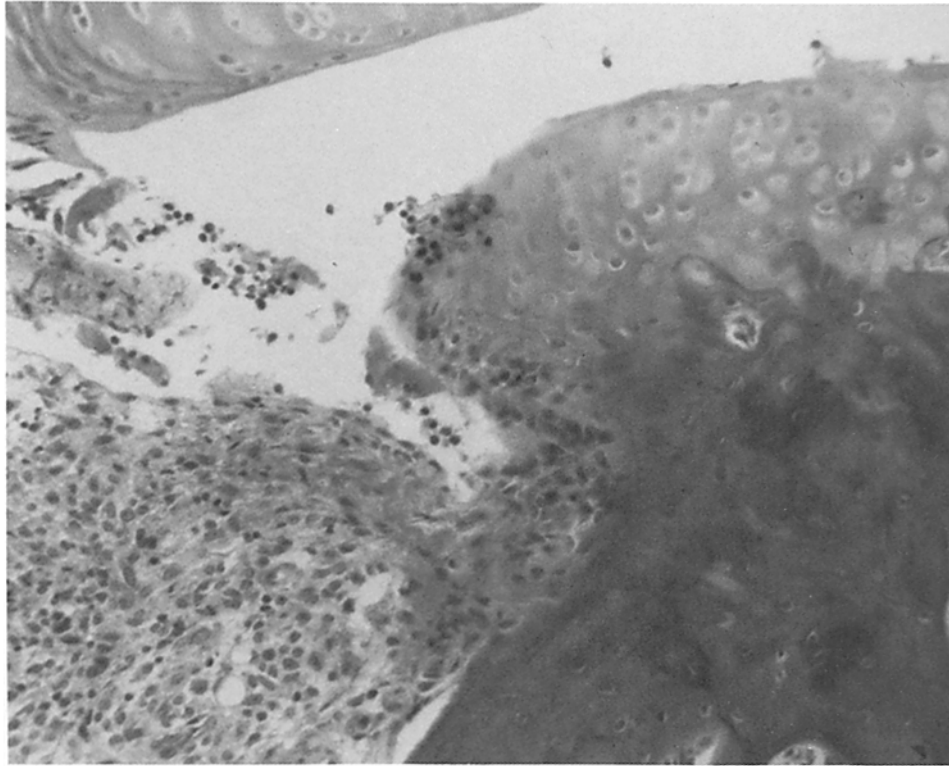
FIG. 3. A marginal erosion developing in a rat with arthritis after passive transfer of serum from a donor that had collagen-induced arthritis. Hematoxylin and eosin × 200.
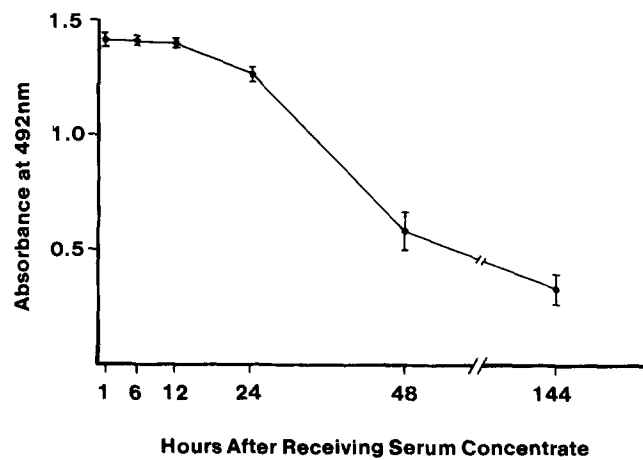

Hours After Receiving Serum Concentrate

FIG. 4. Detection of antibodies (± SEM) to collagen in four rats that received serum concentrate from arthritic donors. All had developed arthritis first detectable at 24 h and reaching maximal severity at 48 h. Antibody levels were measured by ELISA on a 1:1,000 dilution of serum. Normal serum run simultaneously showed 0.1 U of absorbance.
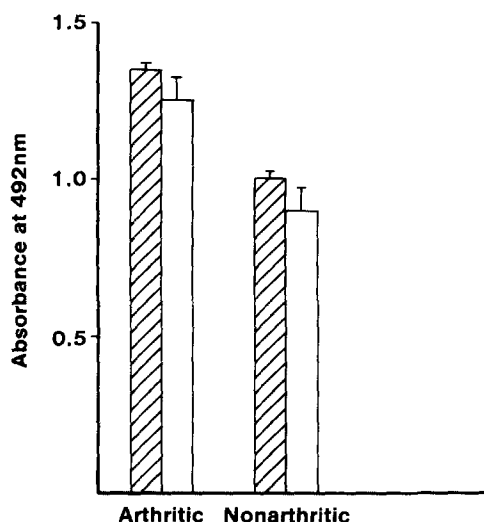
Fig. 5.   Antibody levels in donor and recipient rats (24 h after transfer). A 1:1,000 dilution was used for all sera tested. The mean ± SEM for replicate samples of pooled donor serum is shown by shaded bars. The mean ± SEM for four recipients is shown by an open bar. Each recipient received 2.5 ml of donor serum concentrate.

Table II

Antibody Levels by ELISA after Collagenase

Sample tested	Absorbance	
	1:500	1:5,000
Serum	1.402	0.136
Serum + collagen	0.287	0.068
Serum + collagen + collagenase	1.304	0.110
Serum + collagenase	1.345	0.112

The pooled serum used in transfer experiments was assayed for collagen binding after preincubation with 50 μg/ml collagen or 10 U/ml collagenase or both sequentially. The reaction was terminated by adding EDTA and phen-anthroline to each tube. Aliquots diluted in PBS 1:500 and 1:5,000 were then placed on collagen-coated microtiter plates and binding was measured by ELISA.

but detectable levels were still present after 7 d, when arthritis had clinically resolved. Pooled sera from arthritic and nonarthritic donor rats were also examined for antibody levels and these were compared with levels in the sera obtained from recipients 24 h after transfer. The antibody levels in recipients of sera from arthritic donors was higher than that attained in recipients of sera from nonarthritic donors. These experiments therefore did not determine whether the difference between arthritic and nonarthritic rats represented a qualitative or merely a quantitative difference in the antibodies produced (Fig. 5).

Evidence against an Immune Complex Etiology.   Because in initial experiments only serum concentrate from rats with acute arthritis was capable of transferring disease, and because the earliest lesion detected was one of synovial hypertrophy and synovitis, consideration was given to the possibility that the arthritis was caused by deposition of a circulating immune complex in the synovial tissue. To test that hypothesis,

gamma globulin concentrate was incubated with bacterial collagenase in an attempt to destroy the collagenous portion of the presumed complex. After incubation with 10 U of collagenase for 90 min at 37°C, the concentrate fully retained its ability to transfer disease. To prove that the collagenase was active and capable of digesting collagen bound to antibody, collagen was added to an aliquot of the concentrate and the antibody titer was determined by ELISA. After addition of collagen to the antisera, there was substantial reduction of the detectable antibody titer. Digestion with collagenase exactly as performed in the aliquot used to transfer disease, completely restored the antibody titer (Table II).

In a separate experiment, six rats were immunized with collagen that had been acetylated with [^{3}H]acetic anhydride. Four of these rats developed arthritis. Sera (~3 ml each) was obtained from the arthritic rats on the day arthritis was first detected and from the nonarthritic rats on day 21. Each of the sera was separately subjected to 50% ammonium sulfate precipitation and the precipitate was assayed for the presence of ^{3}H. There was no increase in detectable radioactivity above background in any of the sera.

Finally, aliquots of arthritic sera were tested for Clq binding activity (kindly performed by Dr. Tom Lawley, National Institutes of Health). All of the arthritic and normal sera showed <10% binding. When collagen was added to an aliquot of immune serum and Clq binding was measured, the level increased to 28%. Normal serum heated to 60°C for 10 min to cause aggregation of IgG showed 34% binding.

Isolation and Purification of the Active Principle. Because no evidence of immune complexes was detected in arthritic rat sera, the gamma globulin concentrate was absorbed with a native bovine type II collagen affinity column to determine whether the active principle would bind to collagen. The PBS eluate, when concentrated to a volume of 2.5 ml and administered to a naive rat, could not transfer disease. An acid eluate was also obtained, dialyzed against PBS, and concentrated to 2.5 ml. The acid eluate was fully active in transferring disease. This result was verified on two additional attempts. Finally, the acid eluate from several affinity column runs was pooled and subjected to gel filtration on Biogel A5m. The elution profile is shown in Fig. 6. Two major protein containing peaks were detected by absorbance at 280 nm. These coincided with the IgM and IgG molecular weight markers. In addition, a variable small peak was detected in the void volume. Fractions were pooled as indicated and pool III was found to transfer disease. None of the other pooled fractions could transfer disease. The active principle therefore eluted as a single peak coincident with the IgG marker.

It was confirmed by immunoelectrophoresis that the major protein in the purified active fraction was actually IgG (Fig. 7). Ouchterlony gel diffusion was also performed and showed a band of identity between the active fraction and purified IgG. No IgM was detected using μ chain-specific antisera and normal serum as a control. There was only one band present when the purified fraction was tested against rabbit anti-rat whole serum. These data confirmed that the component of arthritic serum responsible for transfer was an IgG anticollagen antibody.

Specificity of the Purified Transfer Antibody. Inhibition studies were performed using the purified antibody preparation. Aliquots containing 1 μg of antibody/ml were preincubated with native and denatured bovine and rat type II collagen for 30 min and then assayed for binding to bovine type II collagen-coated plates in our standard
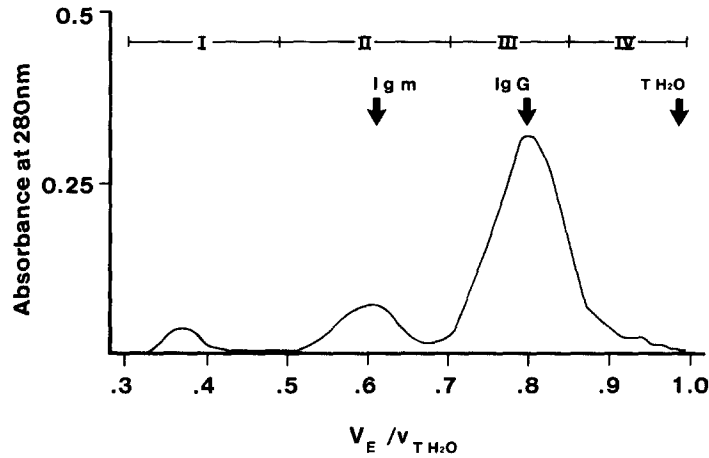
Fig. 6.   Elution profile of the acid eluate from the type II collagen affinity column. The elution volume (V_E) is given relative to tritiated water (V_{TH_2O}) and the elution volumes of calibration proteins-purified rat IgM and IgG are marked by vertical arrows. A 2.5- × 100-cm column packed with Biogel A5m was used. Fractions were pooled as indicated and concentrated by pressure dialysis. Only pool III would transfer arthritis.
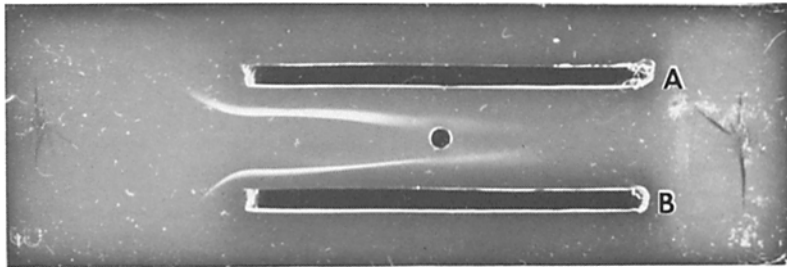

Fig. 7.   Immunoelectrophoresis of the purified transfer material. Purified transfer material was placed in the well and electrophoresis was performed. Rabbit anti-rat IgG, γ chain specific, was placed in trough A and rabbit anti-rat whole serum was placed in trough B. Although the precipitin line seen against the anti-IgG is heavier, a pattern typical for rat IgG developed with no additional lines seen against anti-rat whole serum.

ELISA assay. There was substantial cross-reactivity of the homologous native collagen with the bovine type II collagen used for immunization (Fig. 8). Neither of the denatured collagens was an effective inhibitor.

Discussion

There is considerable evidence that collagen-induced arthritis in rats results from the development of an immune response specific for native type II collagen. Several laboratories (18, 19) have confirmed the observation that immunization of rats with native type II collagen but not denatured collagen or other types of collagen results in the development of inflammatory arthritis. In addition, it has been reported that some strains of mice are also susceptible to type II collagen-induced arthritis (20). We have now shown that purified IgG antibody to native collagen can transfer arthritis in rats. This passively transferred disease has all of the histopathologic characteristics of the early lesions of animals that develop disease as a result of immunization,
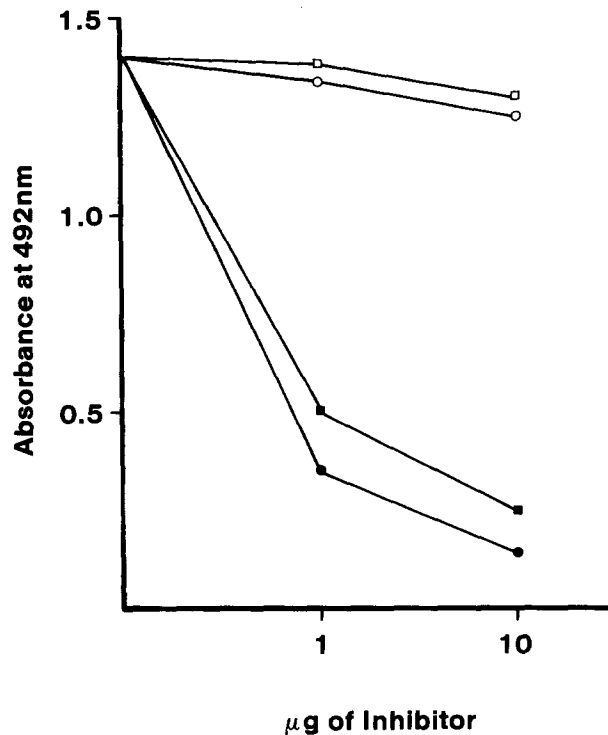
Fig. 8. Inhibition studies. Purified transfer antibody, 1 μg/ml, was incubated with native bovine type II collagen (●), denatured bovine type II collagen (○), native rat type II collagen (■), or denatured rat type II collagen (□), and assayed for binding to native bovine type II collagen by ELISA.

including synovial proliferation, subsynovial infiltration of neutrophils and mononuclear cells, exudation of cells into the synovial space, and development of marginal erosions. It has, however, been less severe than disease in immunized animals and was relatively transient. This may be related to the use of a single injection of serum concentrate to transfer disease. The effect of multiple serial injections has not been investigated. It is also possible that cellular mechanisms may contribute to the disease seen in immunized rats.

Other authors have reported that disease can be transferred with cells from immunized syngeneic donors (9). However, they used a relatively large number (6 X 10^8–10 X 10^8) of pooled lymph node and spleen cells, including B cells. Because neither cellular sensitivity nor antibodies to collagen were detected in their arthritic recipients and because the transferred cell responsible for disease develoment was not identified, it is uncertain whether the passively transferred disease they describe results from a mechanism similar to that we have reported or from another mechanism. The authors in fact suggest that viable B cells may have been transferred and could have produced antibodies in the recipients. Two additional differences in their studies as compared with ours were (a) they found that disease could be transferred from either arthritic or nonarthritic donors, and (b) only 40% of recipients developed arthritis. Because the arthritis incidence in their immunized donors was also 40%, it appeared that some rats were protected or actively suppressed disease development. Our studies

suggest that most or all Wistar outbred rats will develop disease if given sufficient antibody of the requisite type.

The question of whether cellular sensitivity to collagen is sufficient to initiate arthritis or whether it will intensify or perpetuate disease has not yet been answered. Consideration was given to the possibility that the crude serum concentrate transferred in our experiments contained anti-idiotype antibodies or other mediators of cellular reactivity that could trigger a rapidly developing cellular response. Other investigators have shown that T helper cells may be triggered by anti-idiotype antibodies (21). However, lympocyte proliferation in response to collagen, a measure of cell-mediated immunity, was not detected in the arthritic recipients in our studies, whereas strong reactivity was readily detected in immunized donors (data not shown). Furthermore, it is unlikely that the immunoglobulin purified by collagen affinity chromatography contained anti-idiotype because anti-idiotype raised in a syngeic system is usually binding site specific (22) and epitope inhibitable (23). The most important mechanisms of arthritis development in the rats that received serum in our studies appeared to be humorally mediated and independent of cellular sensitivity to collagen.

There is circumstantial evidence that IgG anticollagen antibodies are also critical to the development of collagen-induced arthritis in mice. We have shown that arthritis in DBA/1 mice is temporally associated with high levels of IgG antibody specific for native type II collagen (24). IgM antibody levels and cellular sensitivity to collagen were declining in these mice when arthritis occurred. Furthermore, only the antibodies were specific for native collagen. Cellular sensitivity was triggered by the primary structural and not conformational determinants on the collagen molecule. When the susceptibility of various mouse strains to collagen-induced arthritis was tested, only mice that developed high levels of antibodies to native type II collagen developed arthritis (25), a relationship similar to that found among various strains of rats tested for susceptibility to collagen-induced arthritis (26).

High levels of antibodies reactive with type II collagen are not the only determinant of arthritis development, however. It has been reported (2) that although the mean antibody level in arthritic rats is higher than in nonarthritic, some individual nonarthritic rats also have high antibody levels. In addition, we have previously shown (24) that DBA/1 mice, which are susceptible to collagen-induced arthritis, develop high anticollagen antibody titers when immunized with denatured type II collagen. These antibodies cross-react with native collagen but the mice do not develop arthritis (24). Finally, some mouse strains, notably B10.S and B10.D2, are high antibody responders when immunized with native type II collagen but are not susceptible to collagen-induced arthritis (25). Although anti-type II collagen antibodies are always present in arthritic rats or mice, the reverse finding is not universal.

Because antibody levels were not the only determinant of disease development, and because the arthritic lesion was one of synovial hypertrophy, whereas type II collagen is located in the cartilage and is not known to be present in synovial tissue, consideration was given to the possibility that circulating immune complexes were responsible for the development of arthritis. Several experiments were designed to test that hypothesis. All of these experiments consistently failed to demonstrate the presence of circulating immune complexes. The arguments against a circulating immune complex etiology can be summarized as follows: (a) Digestion of serum concentrate with bacterial collagenase failed to prevent transfer of disease. This could

not have been due to protection of the collagen by binding to antibody because control studies demonstrated that the collagen in collagen-antibody complexes formed in vitro were susceptible to collagenase. (b) When isotopically labeled collagen was used to immunize rats, no label could be detected in acute sera or in arthritic joints. (c) Direct testing of sera from acutely arthritic rats by CIq binding assay failed to demonstrate the presence of immune complexes. However, complexes formed in vitro by the addition of collagen to immune serum were readily detected when assayed simultaneously. (d) It was unlikely that collagen-immune complexes would bind to an affinity column, be eluted, and reformed in the eluate. (e) By molecular seive chromatography the active component of serum had a molecular weight identical to monomeric IgG, whereas immune complexes would be expected to be of substantially higher molecular weight.

We therefore postulate that the arthritis observed after passive transfer was caused by circulating IgG antibodies that localized to the joint by combining with autologous collagen. The feasibility of this occurring was demonstrated by inhibition studies, which showed considerable cross-reactivity between the bovine collagen used for immunization and rat type II collagen. Proof of this hypothesis, however, will depend upon identification of specific antibody in the joints of arthritic rats. The reason that the early lesion appears to be one of synovitis, when in fact the inciting antigen has been identified in cartilage but not synovium, remains unresolved. In addition, the presence of high antibody levels in the absence of arthritis among some immunized animals needs further investigation. It is likely that the antibodies produced after immunization with a protein as complex as collagen are heterogeneous and have specificities for more than one epitope. Perhaps antibodies to only one or a limited number of these epitopes are arthritogenic. All of the rats that produce sufficient quantities of the critical antibody develop arthritis. In support of this possibility is the finding that serum concentrate would transfer disease to essentially all of the recipients, which suggests that all individual rats were susceptible to disease development.

These data also have relevance to human disease. They demonstrate that histologic changes similar to those associated with rheumatoid arthritis can be induced in rats by passive transfer of IgG anti-type II collagen antibodies. In particular, marginal erosions, long an unresolved dilemma in studies of human arthritis, can apparently be induced in rats solely by anticollagen antibodies. Because it has been reported by several investigators that antibodies to type II collagen are present in rheumatoid sera and synovial fluid (8, 27), it is tempting to speculate that pathophysiologic mechanisms similar to those in collagen-induced arthritis may be involved in RA. However, the precise specificity of the antibodies detected in RA is unknown. Most studies of RA have emphasized that stronger reactivity was detected in response to denatured collagen than to native collagen (8) and that the reaction was not necessarily specific for type II collagen (28). However, two reports of inhibition studies performed on RA sera selected for strong collagen reactivity suggested that at least some patients may have antibodies that are specific for type II collagen and react preferentially with conformationally dependent determinants (29, 30). Our data indicate that only antibodies of a particular, though as yet only partially characterized, specificity may be responsible for development of disease in rats and that in contrast to the reactivity usually detected in RA, they are highly specific for native type II collagen. Further

studies are needed to characterize anticollagen reactivity in both rodents and man before analogies can be drawn.

Summary

We have found that serum from rats with type II collagen-induced arthritis, when fractionated with 50% ammonium sulfate and concentrated, would transfer arthritis to nonimmunized recipients. The arthritis in recipients developed within 18–72 h and displayed all of the major histopathologic characteristics of the early lesion in immunized animals but was transient and less severe. Although consideration was given to the possibility that a circulating immune complex was involved, no evidence of such a complex was detected.

Further fractionation of the serum yielded an IgG anticollagen antibody that was fully active in transferring disease. The antibody's reaction was inhibited by the native bovine type II collagen used for immunization of donors and the antibody strongly cross-reacted with homologous type II collage but not with denatured collagen.

These studies demonstrate that arthritis in rats can be induced with anti-type II collagen antibodies and suggest that an autoimmune process is involved. Because antibodies to collagen have also been detected in human rheumatic diseases, further investigation of the characteristics of collagen antibodies capable of inducing arthritis seems warranted.

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