SERUM TRANSFER OF COLLAGEN-INDUCED ARTHRITIS IN MICE*

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In 1977, Trentham et al. (1) described a model of polyarthritis induced in rats by immunization with type II collagen. The lesions identified on histopathologic examination resembled those of human rheumatoid arthritis (RA)[1]. In addition, the arthritis was associated with high levels of both cellular and humoral immunity to collagen (2). Since collagen-related autoimmunity has also been described in individuals with RA (3–6), Trentham proposed that the same pathophysiologic mechanism responsible for collagen-induced arthritis in rats might cause some of the lesions in RA (7). Type II collagen is limited in its bodily distribution primarily to cartilage (8), so an immune response to this specific collagen could account for the predilection of RA to involve diarthroidal joints. However, the earliest lesion of collagen-induced arthritis is synovial proliferation in the absence of apparent cartilage abnormality.

To determine the precise sequence of events leading to experimental arthritis, detailed investigations of the immune response to type II collagen in rats were undertaken. Kinetic studies showed rising levels of both cellular and humoral immunity to collagen at the time arthritis appeared (9). Apparently, the disease could be transferred with either cells (10) or with an immunoglobulin concentrate (11) from collagen-immunized donors.

In addition to rats, some mice were susceptible to collagen-induced arthritis (12) but, according to one report (13), only strains of the H-2^q major histocompatibility haplotype. In humans, the major histocompatibility haplotype HLA-DR4 is also associated with immune responsiveness to collagen (14) and with RA (15), providing additional evidence that collagen-induced arthritis is a relevant model for the study of RA. Unfortunately, no simple correlation is obvious between the immune response to collagen and collagen-induced arthritis in mice. Although strains that are susceptible to arthritis have concomitantly high anti-collagen responses, many resistant strains have equally high responses. Either the immune response to collagen is qualitatively different in the latter stains, or other factors in the host milieu are independent determinants of susceptibility to arthritis.

The present studies were undertaken to determine if arthritis could be trans-

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; CFA, complete Freund's adjuvant; CII, chick type II collagen; ELISA, enzyme-linked immunoassay system; ICF, incomplete Freund's adjuvant; OPD, orthophenylenediamine; PBS, phosphate buffered saline; PBS-Tween; phosphate buffered saline containing 0.05% Tween 20; RA, rheumatoid arthritis.

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ferred with a serum concentrate from immunized donor DBA/1 mice to unimmunized recipients, to identify host factors important for disease susceptibility and to clarify the reasons for prominent synovitis in the absence of apparent cartilage damage. We found that a concentrate of immune sera could induce arthritis, that susceptibility to transferred arthritis was independent from the recipients' H-2 haplotype, that transferred anti-type II collagen antibodies localized both to the joints and to other cartilaginous tissues, and that IgG and C3 deposited on the articular surface of cartilage caused synovitis even though the cartilage appeared normal by routine histologic examination.

Materials and Methods

Animals. DBA/1, B10.D2, C57BL, and BALB/c mice were bred and maintained in the mouse colony of the Scripps Clinic and Research Foundation. Both males and females were used, all between 8 and 12 wk old at the time of immunization or transfer of serum. Wistar/Furth rats (125–150 g) that originated from the Microbiological Associates' colony were obtained from Harlan-Sprague-Dawley (Walkersville, MD). All of the animals were housed in plastic cages with absorbent bedding. They were fed a standard rodent chow diet and water *ad libitum*.

Collagen. Native type II collagen was obtained from chick sternae or bovine articular cartilage. The methods of preparation and the criterion for purity were described previously (16).

Immunization. Collagen for immunization was dissolved in 0.01 N acetic acid by stirring overnight at 4°C at a concentration of 1 mg/ml. Mice were primed by injection at the base of the tail with 25 μg of chick type II collagen emulsified in complete Freund's adjuvant. Freund's adjuvant was made by pulverizing lyophilized heat-killed mycobacteria (strains C, DT, and PN, Ministry of Agriculture, Fisheries, and Food, Weybridge, Surrey, England) with a mortar and pestle. The ground mycobacteria were added to incomplete Freund's adjuvant at a concentration of 2 mg/ml. An emulsion was formed by slowly adding an equal volume of collagen solution to the adjuvant and mixing with a blade type emulsifier at high speed (Sorval Omnimixer, Dupont Co., Wilmington, DE). The mice received an intraperitoneal booster injection of 25 μg of collagen without adjuvant 21 d after the initial injections.

Rats were immunized with 50 μg of bovine type II collagen emulsified with incomplete Freund's adjuvant injected intradermally into the right hind footpad and were given a booster injection of an identical emulsion 7 d later. All of the solutions and emulsions were maintained at 4°C or in an ice bath to prevent denaturation of the collagen before immunization.

Evaluation of Arthritis. Mice were observed daily for the presence of arthritis. Severity was graded visually by using a method adapted from that previously described for rats (17), and swelling was measured with constant tension calipers (Starrett Co., Athol, MA) at the dorsum of the foot, ankle, and wrist.

Histological Evaluation. Mice were killed at intervals, and tissues for routine histological examination were placed in Bouin's solution for 48 h, decalcified for 2–4 d in decalcifying solution (Scientific Products, McGraw Park, IL) and imbedded in paraffin. Finally, sections were cut and stained with hematoxylin and eosin. Sections to be studied with immunofluorescence were prepared by a method to be described elsewhere.[2] Briefly, the limbs were decalcified in a solution of 0.05% *N*-ethylmaleimide and 7% ethylenediaminetetra-acetic acid, pH 7.0, to which 55 g of ammonium sulfate per 100 ml was added. After decalcification was complete, the limbs were placed in the same buffer without ammonium sulfate for 30 min and then frozen onto a cork support by using Tissuetech O.T.C. compound (Miles Laboratories, Naperville, IL). Sections were cut 4 μm thick and stained with

[2] Stuart, J. M., M. West, J. H. Slack, C. B. Wilson. A simplified method for preparation of small joints for immunofluorescence studies. Manuscript in preparation.

fluorescein-conjugated goat anti-mouse $F(ab')_2$. This method preserves immunoglobulin within the joint for immunofluorescence study after sectioning of the frozen decalcified tissues.

Preparation of Serum Concentrate for Transfer Studies. Immune sera for transfer were obtained from DBA/1 mice 5 wk after primary immunization with collagen. The sera were pooled, and an equal volume of saturated solution of ammonium sulfate was added to precipitate the immunoglobulin. The precipitate was collected by centrifugation, redissolved in a minimal volume of water, and dialyzed against 0.15 M NaCl and 0.01 M sodium phosphate (PBS) at pH 7.2. The volume was then adjusted to ½ that of the original serum, and the concentrate was sterilized by microporous filtration through a 0.45-μm disposable filter unit. The concentrate was either used immediately or frozen at −80°C until needed.

Serum concentrate from rats was prepared as previously described (11).

Immunoassay of Antibodies to Collagen. An enzyme-linked immunoassay system (ELISA) was used to quantitate antibody to collagen. This was performed essentially as previously described (18) except for minor modifications. Immulon II plates (Dynatech, Alexandria, VA) were coated with collagen at a concentration of 10 μg/ml in 0.4 ionic strength phosphate buffer, pH 7.6, by incubation at 4°C overnight. Plates were washed with PBS containing 0.05% Tween 20 (PBS-Tween) and incubated with serum-diluted PBS-Tween containing 1% bovine serum albumin (BSA) for 4 h at room temperature. After washing with PBS-Tween, conjugate diluted 1:1,000 in PBS-Tween-BSA was added to each well. The conjugates used in these experiments consisted of affinity-purified peroxidase goat anti-mouse IgG (Tago, Burlingame, CA) and peroxidase rabbit anti-rat IgG (Cappel Laboratories, Cochranville, PA). The plates were developed with orthophenylenediamine (OPD) as the substrate. 40 mg of OPD was dissolved in 100 ml of citrate-phosphate buffer, pH 5.0, to which 40 μl of 30% H_2O_2 was added immediately before use. The absorbance of each well was determined by using a microELISA reader (Model 600, Dynatech Laboratories, Alexandria, VA) at a test wavelength of 492 nm and a reference wavelength of 595 nm. Results are reported as units of absorbance. Dilutions of affinity purified anticollagen antibodies and normal sera were included in all runs to provide standard reference values.

Isotopic Labeling of Anticollagen Antibodies. Anticollagen antibodies were absorbed from immune DBA/1 serum onto native chick type II collagen coupled to Sepharose. While still attached to the collagen-Sepharose, they were labeled with ^{125}I by using lactoperoxidase. Chick type II 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. This was coupled to CNBr-activated Sepharose 4B as previously described (11). After washing with PBS, 40 μl of collagen-Sepharose was added to an equal volume of immune serum and incubated at 4°C overnight. The Sepharose was then washed three times by suspending it in 4 ml of PBS, centrifuging at 400 *g* for 2 min, aspirating the supernatant fluid and repeating the procedure. 50 μl of 0.5 M phosphate buffer, pH 7.2, and 50 μl of Enzymobeads (Bio-Rad Laboratories, Burlingame, CA) were then added to the Sepharose. 1 mCi of ^{125}I in 25 μl of 0.1 M NaOH was neutralized with an equal volume of 0.1 M HCl and added to the Sepharose suspension. Finally, 20 μl of 1% β-D-glucose was added and the reaction allowed to proceed for 20 min at room temperature. The reaction mixture was recovered in 1.4 ml of 0.2 M glycine-HCl, pH 2.3, and placed on a PD-10 column that had been equilibrated with PBS. The reaction vial was washed with an additional 1 ml of glycine-HCl and the desorbed, ^{125}I-labeled antibody recovered by washing the column with 3 ml of PBS. A total of 250 μCi of ^{125}I was incorporated into TCA-precipitable protein.

Control immunoglobulin from sera of normal DBA/1 mice was labeled in an identical fashion except that absorption was accomplished with protein A-Sepharose and ^{131}I was used to permit differential identification of the labeled preparations. Use of this method permitted isolation of isotopically labeled anticollagen antibodies and normal immunoglobulin that had been subjected to identical conditions of pH and oxidation.

Unimmunized DBA/1 mice were given approximately equal quantities of isotopically labeled anticollagen antibodies and normal immunoglobulin by intravenous injection. At

intervals, these mice were bled and killed; then the quantities of both ^{131}I and ^{125}I in various tissues were determined. The hind limbs were severed proximal to the ankle, the forelimbs proximal to the wrist, and the internal organs individually removed. Each of the limbs and internal organs was placed in a 12 mm × 75 mm glass tube and counted in a gamma scintillation counter. After correction for background and channel overlap, the quantity of both isotopes in each tissue was determined, and the results were reported as the percentage of the isotope detected compared to the total injected or as the ratio of ^{125}I to ^{131}I in a given tissue compared to that in serum. The latter ratio provided an indication of whether either isotope was selectively concentrated compared to its blood level.

Results

Passive Transfer of Arthritis. Sera were obtained from the blood of ~200 DBA/1 mice 5 wk after primary immunization with native chick type II collagen, a time when both arthritis and peak levels of IgG anticollagen antibodies occur. All mice were bled whether arthritic (~60%) or not and the blood was pooled. An immunoglobulin concentrate was obtained from these sera, dialyzed against PBS, sterilized, and administered to unimmunized DBA/1 recipients. Although concentrate from 3 ml of serum would transfer arthritis occasionally, concentrate from 2 ml given on 2 consecutive days reliably transferred arthritis to 100% of the recipients (Table I). Recipients of control sera from mice immunized with complete Freund's adjuvant and buffer alone did not develop arthritis. Shown in Fig. 1 is the arthritic hind limb from a mouse 48 h after passive transfer of serum. In previous studies (10, 11), arthritis induced in rats by injecting either immune cells or serum was limited to the hind limbs. However, in mice, this disease was often evident in or limited to the forelimbs.

Histopathologically, the transferred arthritis resembled early disease in immunized donors (Fig. 2). There was synovial proliferation with early pannus formation, subsynovial infiltration of inflammatory cells, exudation of cells into the joint space, widening of the space between the cartilage surfaces with excessive convolution of the synovium, and deposition of a fibrinlike material on the surface of the articular cartilage in some areas.

Time Course of Transferred Arthritis. In all cases, the transferred arthritis was

TABLE I

Passive Transfer of Arthritis in DBA/1 Mice

Donor immunized with:*	ml of Concentrate given to recipients‡		Recipients arthritic/total
	Day 0	Day 1	
Unimmunized	0.75	0.75	0/4
CFA alone	0.75	0.75	0/6
CII + CFA	0.75	0.75	6/6
CII + CFA	1.0	None	1/4

* DBA/1 donors were immunized with complete Freund's adjuvant (CFA) either with or without 100 μg of chick type II collagen (CII), those receiving collagen were given an additional 100 μg of collagen intraperitoneally 21 d later and were bled 4 wk after the primary immunization.

‡ Sera from immunized donors were pooled, the immunoglobulin precipitated with ammonium sulfate and the precipitate redissolved so that each 3 ml of sera yielded 1 ml of concentrate.

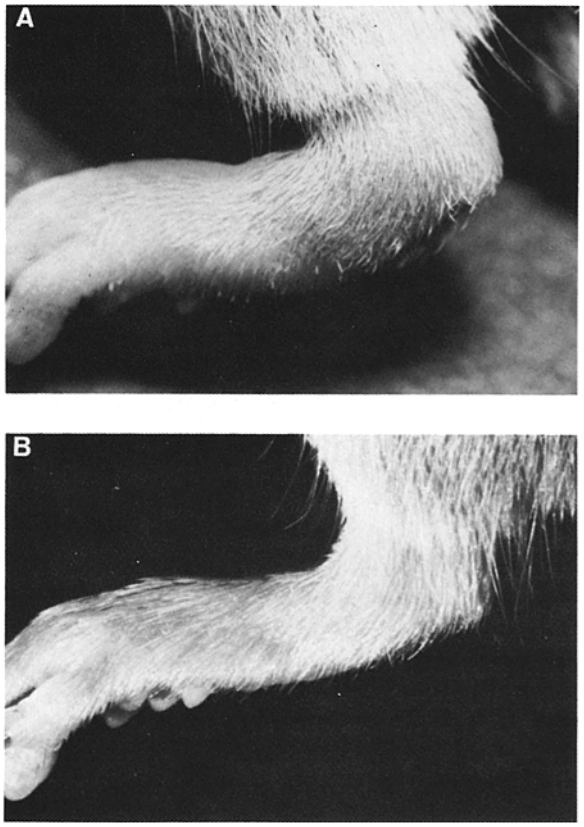

FIGURE 1. Shown is the hindpaw of a mouse with passively transferred arthritis (*A*) 48 h after intravenous injection of immunoglobulin concentrate and a normal hindpaw (*B*) for comparison. Note swelling of the ankle with loss of bony landmarks and increased thickness of the dorsum from the affected foot.

transient. Disease was first evident 24–48 h after the second injection of serum concentrate and reached maximal severity within the next 24 h. During the following 4–5 d, the arthritis gradually resolved (Fig. 3), and joints examined histopathologically 2 wk after transfer were free from inflammation or permanent damage. Serial bleedings of transfer recipients showed that the exogenous anticollagen antibody rapidly left their sera and was undetectable within 2 wk.

Identification of IgG in Joints of Mice After Passive Transfer of Serum. Sections of arthritic joints from transfer recipients were stained with fluorescein labeled antimouse IgG and C3, both of which subsequently localized to the articular surface of the cartilage (Fig. 4). No staining was evident in the synovial tissue. Staining tended to be most intense at the periphery of the cartilage and decreased to a thin line near the center of the weight-bearing portion of the joint. In sections from normal joints of unimmunized control mice, the entire articular surface was negative for specific fluorescence. Occasionally, cells in the bone marrow became stained with anti-mouse IgG in both normal joints and arthritic joints.

Distribution of Isotopically Labeled Antibody to Type II Collagen After Administration

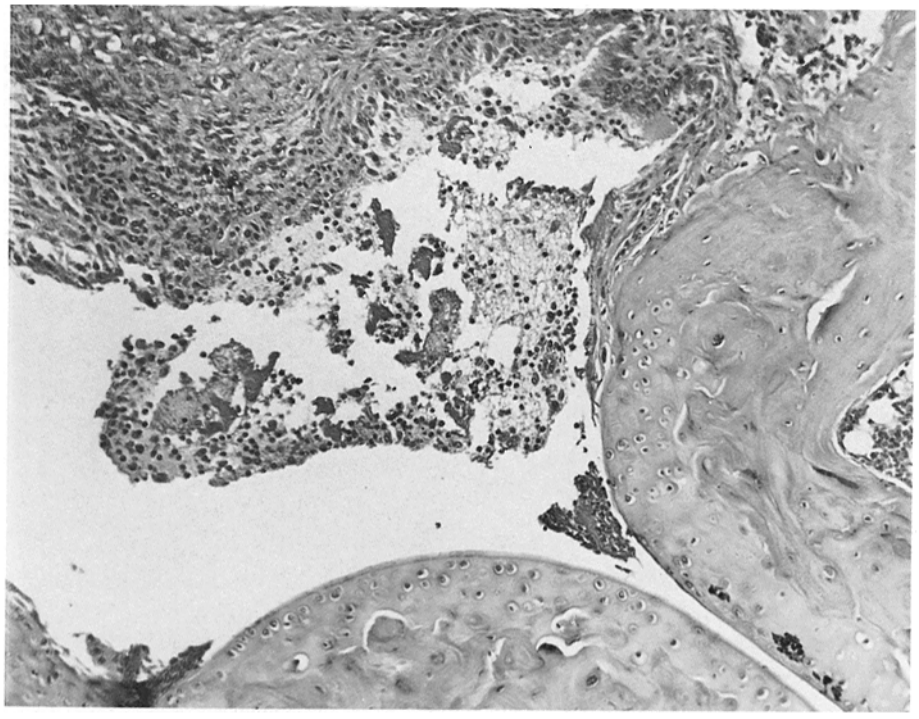

FIGURE 2. Photomicrograph of an arthritic joint from a mouse with passively transferred arthritis 72 h after injection of immunoglobulin concentrate.

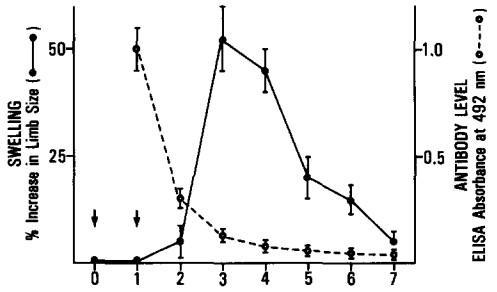

FIGURE 3. Time course of passively transferred arthritis in a representative mouse after injection of concentrate from 2 ml of serum from immunized donors on 2 consecutive days (↓). Swelling was measured at the ankle and dorsum of the foot using a constant tension calipers (Mean ± 1 SD). Anticollagen antibody levels were measured by ELISA using a 1:1,000 dilution of serum.

to Unimmunized Recipients. ^{125}I-labeled anticollagen antibodies and ^{131}I-labeled normal immunoglobulin were administered intravenously to unimmunized mice. At various intervals these recipients were sacrificed, dissected, and tested for the quantity of each label in the limbs, ears, and internal organs. The ^{125}I label concentrated selectively in the limbs within ½ h and peaked at 1 h after administration of the antibody (Fig. 5). By 24 h, 81% of the ^{125}I-labeled anticollagen antibodies disappeared from the serum compared with only 46% of the ^{131}I-labeled nonspecific immunoglobulin. If the distribution of anticollagen an-

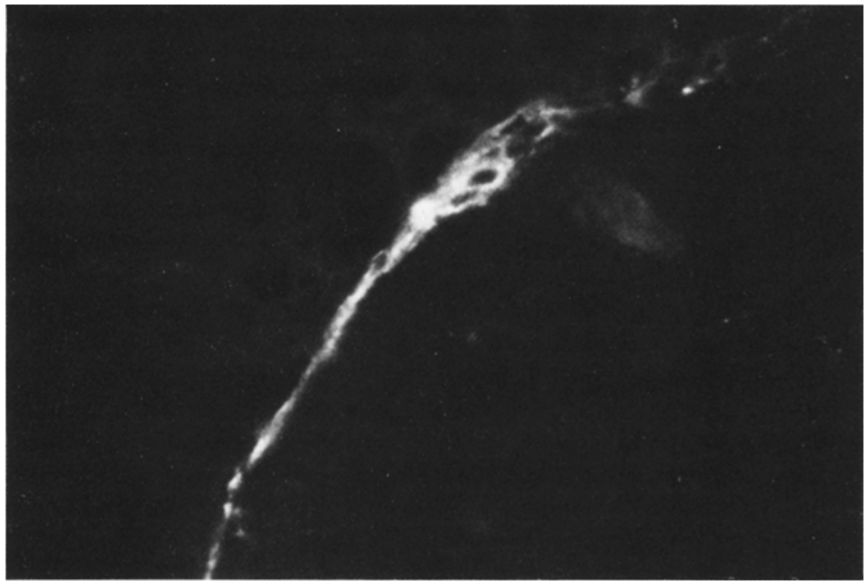

FIGURE 4. Immunofluorescence after reaction of fluorescein conjugated rabbit anti-mouse C3 with a section from the hindpaw of a mouse with passively transferred arthritis. Reaction with anti-mouse IgG showed an identical pattern. There were immune complexes on the articular surface of the cartilage in a smooth linear pattern. A normal joint showed no fluorescence with either conjugate. Absorbtion of the anti-IgG and anti-C3 conjugates with purified IgG or preformed immune complexes, respectively, eliminated the specific fluorescence.

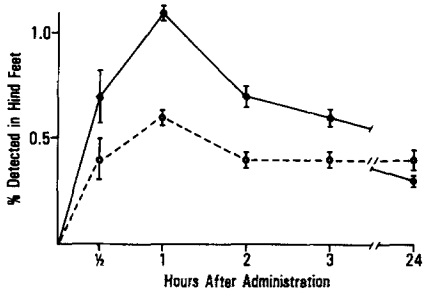

FIGURE 5. Time course of the accumulation of ^{125}I-labeled anticollagen antibodies (●) and ^{131}I-labeled normal immunoglobulin (○) in the hind limbs of mice receiving approximately equal quantities of both intravenously. Each data point represents the mean ± standard deviation of both hind limbs from two mice.

tibodies and control immunoglobulin was determined solely by circulation to particular tissues, then their ratio in those tissues and in serum should have been the same. However, at 1 h after administration, the ^{125}I label settled selectively in the limbs, ears, eyes, trachea, and intestines. Other internal organs including the heart, lungs, kidneys, spleen, and liver did not attract this disproportionate amount of ^{125}I (Table II).

Transfer of Disease Does Not Depend on the H-2 Haplotype. Arthritis in mice immunized with chick type II collagen has been linked to the major histocompatibility complex and specifically to the H-2^q haplotype (13). Strains with other

TABLE II

Distribution of Isotopically Labeled Anticollagen Antibodies (^{125}I) as Compared to Normal IgG (^{131}I) 1 H After Administration

Organ	Ratio $^{125}I/^{131}I$	Concentration index*	% of Total‡	
			^{125}I	^{131}I
Blood	0.68	—	49.0	60.0
Hind feet	1.35	2.0	1.1	0.6
Fore feet	1.35	2.0	0.5	0.3
Ears	1.31	2.0	0.2	0.1
Eyes	1.99	2.9	0.2	0.1
Trachea	1.34	2.0	1.7	1.0
Spleen	0.45	0.7	0.4	0.8
Liver	0.53	0.8	7.6	11.5
Lungs	0.85	1.2	1.9	1.8
Kidneys	0.57	0.8	2.7	3.8
Heart	0.65	1.0	1.1	1.3
Intestine	2.27	3.3	15.5	5.4

* Concentration index = ^{125}I to ^{131}I ratio in organ divided by the ^{125}I to ^{131}I ratio in serum. A number >1 would indicate relative concentration of anticollagen antibody as compared to its level in serum.

‡ Quantity of each isotope detected as a percentage of the total administered.

TABLE III

Transfer of Arthritis to Several Different Strains

Recipient strain	Recipient H-2 haplotype	Antibody response to immunization with type II collagen*	Arthritis after passive transfer of serum‡ (arthritic/total)
C57BL/6	b	++	3/4
B10.D2	d	+++	4/4
BALB/c	d	++	2/4
C3H/He	k	+	3/4
DBA/1	q	+++	4/4

* Relative antibody levels achieved in each recipient strain after immunization with chick type II collagen in complete Freund's adjuvant (reference 13, unpublished observation). None of these strains developed arthritis as a result of immunization except DBA/1.

‡ Transfer serum concentrate was obtained from immunized DBA/1 donors. Each unimmunized recipient received the immunoglobulin concentrate from 2.25 ml of serum on each of the 2 consecutive days.

haplotypes did not develop arthritis despite the high levels of antibodies to collagen some attained. To determine whether resistance to arthritis results from failure of an effector mechanism, from active suppression, or from qualitative differences in the immune response of these strains, an attempt was made to transfer arthritis to mice of several haplotypes. Table III illustrates that all of the strains tested were susceptible to arthritis passively transferred by antibodies to collagen, although there was minor variation in incidence and severity.

Lesion Formation Does Not Depend on the Species in Which the Immunizing Serum

Originated. Having demonstrated that serum concentrate from DBA/1 mice transferred arthritis to other strains, we decided to test the ability of serum concentrate from rats with collagen-induced arthritis to transfer this disease to mice. Immunoglobulin concentrate prepared from sera of rats immunized with bovine type II collagen was administered intravenously to unimmunized DBA/1 recipients. This rat immunoglobulin concentrate passively transferred arthritis as well as that from mice (Table IV). Volumes equivalent to those effective in the homologous system were used, but the quantities of immunoglobulin were about twofold greater because the concentration of circulating nonspecific IgG in the rats used for transfer was greater. Unimmunized rats had ~12 mg/ml of IgG as compared with ~5 mg/ml in DBA/1 mice. Quantitation by ELISA showed comparable titers of specific anticollagen IgG in sera from the arthritic rats and mice used as transfer donors.

Transfer of Disease to Arthritis-resistant B10.D2 Mice Requires Less Serum Concentrate After Priming. Immunization of B10.D2 mice with type II collagen results in an anticollagen response that is comparable in magnitude to that of DBA/1 mice; however, the B10.D2 strain does not become similarly arthritic. Since we have now shown that arthritis can be passively transferred to the latter strain, either the immune response is qualitatively different from that of susceptible strains or the immunization induces a suppressor mechanism that prevents arthritis. To test the latter possibility, we used B10.D2 mice either not immunized but given transfer serum, or immunized with type II collagen and 4 wk later given arthritogenic doses of serum concentrate from immunized DBA/1 mice. Arthritis developed in the immunized B10.D2 mice with even greater severity than in those that were not immunized (Table V), thereby precluding the possibility of suppression. Therefore, we decided to lower the amount of serum concentrate injected to 50% of the dose required for reliable transfer of arthritis in unimmunized DBA/1 or B10.D2 recipients. This reduced dosage induced arthritis in 100% of the immunized B10.D2 recipients. Thus, prior immunization of this "nonresponder" strain decreased their requirement for transfer concentrate to induce arthritis. However, in none of the mice tested was the disease as severe

TABLE IV

Transfer of Arthritis to DBA/1 Mice Using Serum Concentrate from Rats Immunized with Bovine Type II Collagen (BII)

Donors immunized with:*	ml of Concentrate given to recipients‡		Recipients arthritic/total
	Day 0	Day 1	
ICF only	0.75	0.75	0/4
ICF + BII	0.75	0.75	5/6
ICF + BII	1.00	None	1/6

* Donor Wistar/Furth rats received 100 μg of collagen emulsified in incomplete Freund's adjuvant (ICF) intradermally in the footpad, a repeat injection of an identically prepared emulsion 7 d later and were bled 16 d after the initial injection.

‡ Sera from immunized donors were pooled; the immunoglobulin was precipitated with ammonium sulfate and the precipitate redissolved so that each 3 ml of sera yielded 1 ml of concentrate.

TABLE V
Effect of Priming on Ability of DBA/1 Sera to Transfer Arthritis to B10.D2 Mice*

Recipient	ml of Concentrate‡ given to recipients		Arthritic/ total	Mean severity§
	Day 0	Day 1		
Unprimed	0.75	0.75	4/4	3.25 ± 1.0
Primed	0.75	0.75	4/4	6.25 ± 1.3
Unprimed	0.75	None	0/4	0
Primed	0.75	None	4/4	5.5 ± 1.3

* B10.D2 were primed by immunization with 25 μg of chick type II collagen in complete Freund's adjuvant 4 wk before administration of transfer concentrate. These mice were not susceptible to arthritis as a result of priming without serum transfer.

‡ Sera from immunized donors were pooled; the immunoglobulin was precipitated with ammonium sulfate, and the precipitate redissolved so that each 3 ml of sera yielded 1 ml of concentrate.

§ Mice were graded for arthritis severity daily by visually scoring each limb from 1–4 (maximum severity = 16). Calculations are based on highest score achieved during the course of observation.

as that in immunized DBA/1 mice. The usual consequence of arthritis in DBA/1 mice after immunization was ankylosis and permanent deformity. These sequelae were not seen even in immunized B10.D2 mice after passive transfer.

Discussion

Collagen-induced arthritis in mice can be passively transferred with an immunoglobulin concentrate from immunized donors. Histopathologically, the arthritis resembled that induced by immunization with collagen. Synovial proliferation, subsynovial infiltration of inflammatory cells, early pannus formation, destruction of cartilage, and marginal erosion were all characteristic of the transferred disease. Previously, collagen-induced arthritis was also transferred to rats by using serum, and anticollagen antibody proved to be responsible for the lesion (11). Other investigators, who depleted complement in rats by using cobra venom factor, found that the onset of arthritis was delayed until the complement levels returned toward normal (19). These data support the concept that arthritis in rats and mice is caused by similar mechanisms and that humoral immunity plays an important role.

One characteristic of collagen-induced arthritis is synovial proliferation, which can be detected before changes in the articular cartilage are evident. Since type II collagen is not known to be present in synovium, the reason for this involvement is uncertain. Our studies clearly demonstrated deposition of IgG and C3 on the articular surface in the absence of detectable IgG in synovial tissue. By light microscopy the cartilage appeared normal, while the synovial tissue became inflamed. The IgG apparently localized to the cartilage by direct binding, forming immune complexes in situ that activated complement. Synovitis may have resulted from (*a*) complement-mediated chemotaxis of synovial cells, (*b*) activation of synovial cells via their Fc receptors (20), or (*c*) activation of synovial cells by the products of polymorphonuclear leukocytes (21). These data support

the hypothesis that deposition of anticollagen antibodies on the cartilage surface can initiate arthritis and may be responsible for synovial proliferative changes in the absence of other inciting events, and that this can occur without cartilage damage evident by light microscopy.

The passively transferred arthritis was transient. This was expected, since anticollagen activity rapidly disappeared from the serum after transfer. Isotopically labeled anticollagen antibodies selectively localized to the joints within 30 min of intravenous administration but by 24 h had virtually disappeared both from sera and limbs. Thus, antibodies readily bound to intact cartilage but apparently were rapidly catabolized after binding. The isotopically labeled immunoglobulin had been exposed to conditions of low pH and oxidation during labeling and may have partially denatured, accounting in part for its rapid disappearance. However, after administration of unlabeled concentrate, anticollagen activity in serum also declined rapidly, confirming that antibody localized to the cartilage quickly after intravenous administration of the concentrate.

Only a small percentage of the ^{125}I-labeled anticollagen antibodies administered was detected in each limb. But the amount of cartilage present in the samples was also small compared with that in the entire body. The limbs were removed just proximal to the ankles and wrists so that the knees, hips, elbows, and shoulders, which contain large amounts of cartilage, all remained with the carcass. Anti-type II collagen antibodies also bound to cartilage in locations other than joints. Proportionately more activity was actually seen in the trachea than in joints. Some activity was even detected in the eye, where type II collagen is present as a constituent of the vitreous (22).

When collagen-induced arthritis in rats was first described, only lesions of joints were reported (1). As established later, some rats also developed lesions of the external ear caused by chondritis of the underlying cartilage plate (23). In addition, some rats had decreased hearing and vestibular dysfunction from inflammation in the inner ear (24, 25). The precise pathophysiology of these lesions has yet to be identified and they are unknown in mice. The present studies suggest that anticollagen antibodies are specifically concentrated in diverse places within the body wherever type II collagen is located. The potential for destructive lesions of other cartilaginous tissues in mice needs further study to determine whether lesions may exist elsewhere or, if not, why no lesions develop in spite of apparent antibody binding.

A relatively large concentration of ^{125}I was also noted in the intestines. This was probably caused by preferential catabolism of anticollagen antibody and excretion of the label into the feces. Such preferential destruction could have resulted from greater denaturation of anticollagen antibodies or from their rapid catabolism after specific binding, activation of complement, and involvement in an inflammatory process. For two reasons, preferential destruction probably was not caused by greater denaturation of the specific antibodies. First, both specific antibody and normal immunoglobulin were labeled simultaneously in the same buffers so that both were exposed to identical conditions of pH and oxidation. Second, if the anticollagen antibodies were nonspecifically removed by the reticuloendothelial system, then relatively more ^{125}I than ^{131}I should have been found in the liver and spleen. Since this was not the case, the faster catabolism

of anticollagen seems more likely related to its specific binding.

Susceptibility to arthritis induced by immunizing mice with collagen, as opposed to transferring the disease with antibody to collagen, is linked to the H-2 major histocompatibility complex (13). All susceptible strains developed high levels of specific immunity after immunization with type II collagen. However, resistant strains varied in their anticollagen response after immunization; in some, antibody titers against collagen were as high as those of arthritis-susceptible strains. For example, the response of resistant B10.D2 mice was quantitatively equal to that of susceptible DBA/1 mice. The failure of this strain to become arthritic cannot result from either intrinsic host factors that reduce susceptibility or the induction of nonspecific suppressor mechanisms by immunization, since both naive and collagen-immunized B10.D2 mice became arthritic after passive transfer. Evidently, the response to immunization is qualitatively different, and B10.D2 mice may lack a critical antibody. This means there is a diversity of anticollagen antibodies, some of which are arthritogenic, whereas others are not.

The situation must be more complex, however, than the simple presence or absence of a particular antibody. Immunization of B10.D2 mice did not prevent transfer of arthritis, but rather heightened susceptibility. The cause could have been either induction of low levels of arthritogenic antibody in this strain or a capacity for other anticollagen antibodies and cellular mechanisms to lower the threshold of disease production and to aggravate the lesion, once initiated. Perhaps only a few epitopes on type II collagen are accessible on cartilage in vivo. Once cartilage is damaged, other epitopes are exposed that then participate in the inflammatory reaction. For example, the proliferative response of cells to collagen is primarily directed against determinants on the denatured molecule (18, 26). Such determinants may be concealed in normal, intact cartilage.

The ability of serum concentrate from arthritic rats to transfer arthritis to mice is interesting for at least two reasons. It establishes that transfer is not species specific and that arthritis may result from the same mechanisms in both species. In addition, the rats were immunized with bovine type II collagen because of its effectiveness in inducing disease in this species. However, DBA/1 mice are relatively resistant to induction of arthritis by bovine collagen; chick collagen is much more effective. A possible explanation is that multiple epitopes are present on both chick and bovine type II collagen. A response to the arthritogenic epitope(s) may be influenced by responses to other epitopes on the same molecule causing different overall responses by the DBA/1 mice and Wistar/Furth rats used in these experiments. An alternative explanation would be that arthritis is caused by the sum of responses to several epitopes and that mice and rats respond to overlapping, but not identical, subsets of epitopes. Comparison of the reactivity of mouse and rat antisera against chick and bovine collagen, respectively, for reactivity with mouse type II collagen would help to clarify this point.

We have not attempted to address the role of cellular immunity to collagen in arthritic animals after immunization. The acute lesions, including synovitis, seem to be inducible by humoral immunity alone. We have shown that arthritis after passive transfer is caused by binding of antibody to the articular surface of joint cartilage and that susceptibility to arthritis depends on particular antibodies in

the transfer concentrate, not upon the host milieu. Nevertheless, the host's immune status can affect disease severity and the threshold of susceptibility. From these data we postulate that at least three stages mark the development of collagen-induced arthritis. First is a preclinical accumulation of antibody on the joint surface, second is an acute inflammatory reaction occurring when the antibody reaches a critical level, and third is a chronic inflammatory reaction resulting from exposure of denatured collagen and influx of cells following the second stage. Although this postulate is somewhat speculative, it is consistent with our data and provides testable predictions.

Summary

Immunization of DBA/1 mice with native chick type II collagen resulted in development of polyarthritis 4–5 wk later. Sera of these mice contained high levels of anticollagen antibodies, and immunoglobulin concentrates of their sera transferred arthritis to unimmunized recipients. Histopathologically, this passively transferred arthritis resembled the early disease of immunized donors. Immunofluorescence studies demonstrated the deposition of IgG and C3 on the articular surface but not in synovial tissue of arthritic joints. Transferred, isotopically labeled anticollagen antibodies rapidly localized to the limbs and to other cartilage-containing tissues. When transfer concentrate was administered to arthritis-resistant strains, they also developed arthritis. Indeed, immunoglobulin concentrates from rats with collagen-induced arthritis transferred arthritis to naive mice. The amount of concentrate required for transfer to B10.D2 resistant mice was reduced by immunizing them with collagen 4 wk before transfer. Although susceptibility to arthritis from immunization is H-2 linked, these studies clearly demonstrate that passive transfer of arthritis depends upon injection of specific antibody and not on other host factors.

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