TYPE II COLLAGEN-INDUCED ARTHRITIS IN MICE

I. Major Histocompatibility Complex (I Region) Linkage and Antibody Correlates*

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The induction of immunological reactivity to type II collagen, a major component of cartilage, has recently been used to establish an experimental model of arthritis in the rat (1). Trentham and colleagues (1) have shown that the intradermal injection of collagen in Freund's complete or incomplete adjuvant elicits an arthritogenic reaction, specifically with native type II collagen. Arthritis occurs in 40% of the rats injected, and this arthritis could be passively transferred by the transplantation of immunocompetent cells (2). There was also a correlation between the humoral response to type II collagen and the occurrence of arthritis (3).

The relationship between the collagen arthritis model in the rat and rheumatoid arthritis (RA)[1] is unclear. The histology demonstrated in the experimental model (1) was very similar to that seen in RA, and x-rays taken of the joints of affected rats show erosive damage as seen in human rheumatoid disease (4). Autoimmunity to type II collagen has been observed in RA, with circulating antibodies in the majority of patients (5, 6) and an abnormal cellular response assessed by leukocyte inhibition factor production (7). It is conceivable that an abnormal immune response to type II collagen could be relevant in the joint inflammation found in RA.

It has been shown that antigens of the major histocompatibility complex (MHC) may be related to both the susceptibility to disease (8, 9) and the control of the immune response (10, 11). Because human lymphocyte antigens influence susceptibility to various human arthritides, it is particularly relevant to examine the genetic control of the immune response to type II collagen in the experimental model of arthritis. However, the MHC of rats is not as well documented as that of mice. Recent evidence has shown that type II collagen arthritis may be induced in mice (12).

This paper reports on the incidence of type II collagen arthritis in various strains of mice and maps the susceptibility to the disease within the I region of the MHC. The humoral immune response to type II collagen was also investigated, and strains susceptible to type II collagen arthritis correlate well with high antibody responders to the same antigen.

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; CFA, complete Freund's adjuvant; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; RA, rheumatoid arthritis.

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Materials and Methods

Mice. Mice were bred and maintained in the Mouse Immunogenetics Colony, Mayo Clinic, with the exception of DBA/1J mice, which were obtained from The Jackson Laboratory, Bar Harbor, Maine.

Collagen. Native type II collagen was purified from lathyritic chick preparations by the method of Cremer et al. (13).

Induction of Arthritis. Native type II collagen was solubilized in 0.1 M acetic acid at 1 mg/ml, and emulsified in complete Freund's adjuvant (CFA). Mice were primed with 100 μg of type II collagen in CFA injected intradermally at four to six sites on the back. Control mice were primed with acetic acid emulsified in CFA. Mice were challenged after 21 d with 100 μg of type II collagen in 100 μl acetic acid, injected intraperitoneally. Control mice received 100 μl of 0.1 M acetic acid alone, intraperitoneally.

Assessment of Arthritis

MEASUREMENT OF JOINT THICKNESS. Mouse joints were measured using a constant tension caliper (Schelltaster, Syitem, Kröplin, Germany). All mice were measured before the start of the experiment to obtain base-line readings at three joints on the hind limb (paw thickness, ankle width, and knee width) and two joints on the fore limb (paw thickness and elbow width).

All mice of a strain with any visual appearance of arthritis were measured daily to observe the progress of joint swelling during the course of the disease, and to measure the variation in measurement of normal joints.

CLINICAL EVALUATION. All mice were examined daily for the initial visual appearance of arthritis: redness and swelling of a fore or hind paw. Dates of onset of the disease after immunization were recorded for individual mice.

An arthritic index of the disease was developed, based upon the visual appearance of a limb and the caliper measurements. Limbs were graded 0–3, representing increased joint swelling, erythema, and visible joint distortion. Changes in the number of affected limbs and the arthritis index for each limb were recorded on a daily basis.

HISTOLOGICAL EVALUATION. Mice were killed at various intervals during the experiment. Histological sections were prepared by the Pathology Department, Mayo Clinic. Limbs were dissected out for histology, and the clinical joint assessment was coded for blind observation. Joints were decalcified in Surgipath Decal (Medical Industries, Inc., Northbrook, Ill.) for 3–4 d and embedded in paraffin blocks. Sections of 6-μm thickness were cut along a longitudinal axis at a number of varying depths for each joint, mounted, and stained with hematoxylin and eosin before grading. The histological grading system was based upon (*a*) inflammation (*b*) erosion and pannus formation, and (*c*) ankylosis.

Antibody Quantitation. Mice were bled for sera on at least three occasions during the experiments: (*a*) day 7, primary response; (*b*) day 28, secondary response; and (*c*) bleed out.

Sera were tested for antibodies to type II collagen using a solid-phase radioimmunoassay, developed from the method of Clague et al. (14).

Polystyrene tubes (LP3 Luckhams, Sussex, England) were coated with type II collagen by the addition of 3 μg of collagen solubilized in 20 μl of 0.1 M acetic acid, followed by 500 μl of phosphate-buffered saline (PBS). After overnight incubation at 4°C and washing three times with 1 ml PBS, the remaining protein binding sites were blocked by the addition of 500 μl of 0.1% bovine serum albumin (BSA) in PBS for 4 h at room temperature. The tubes were washed three times with 1 ml PBS before the addition of 10 μl of mouse test sera and 500 μl of 0.1% BSA/PBS. The preparation was incubated overnight at 4°C, then washed three times with 1 ml PBS. Bound IgG was measured by the addition of iodinated (^{125}I) *Staphylococcus* protein A, 0.025 μg in 500 μl BSA/PBS for 45 min at 4°C. The tubes were washed three times with 1 ml PBS and bound ^{125}I-*Staphylococcus* protein A measured in a gamma counter (Beckman Instruments, Inc., Fullerton, Calif.).

The quantity of IgG anti-type II collagen antibody was expressed as micrograms per milliliter by comparison against a purified guinea pig anticollagen IgG control.

Results

Characteristics of Type II Collagen-induced Arthritis. The intradermal injection of 100 μg of type II collagen in CFA, followed by a further 100 μg injected intraperitoneally after 21 d, induced an arthritis in a variable percentage of mice of susceptible strains.

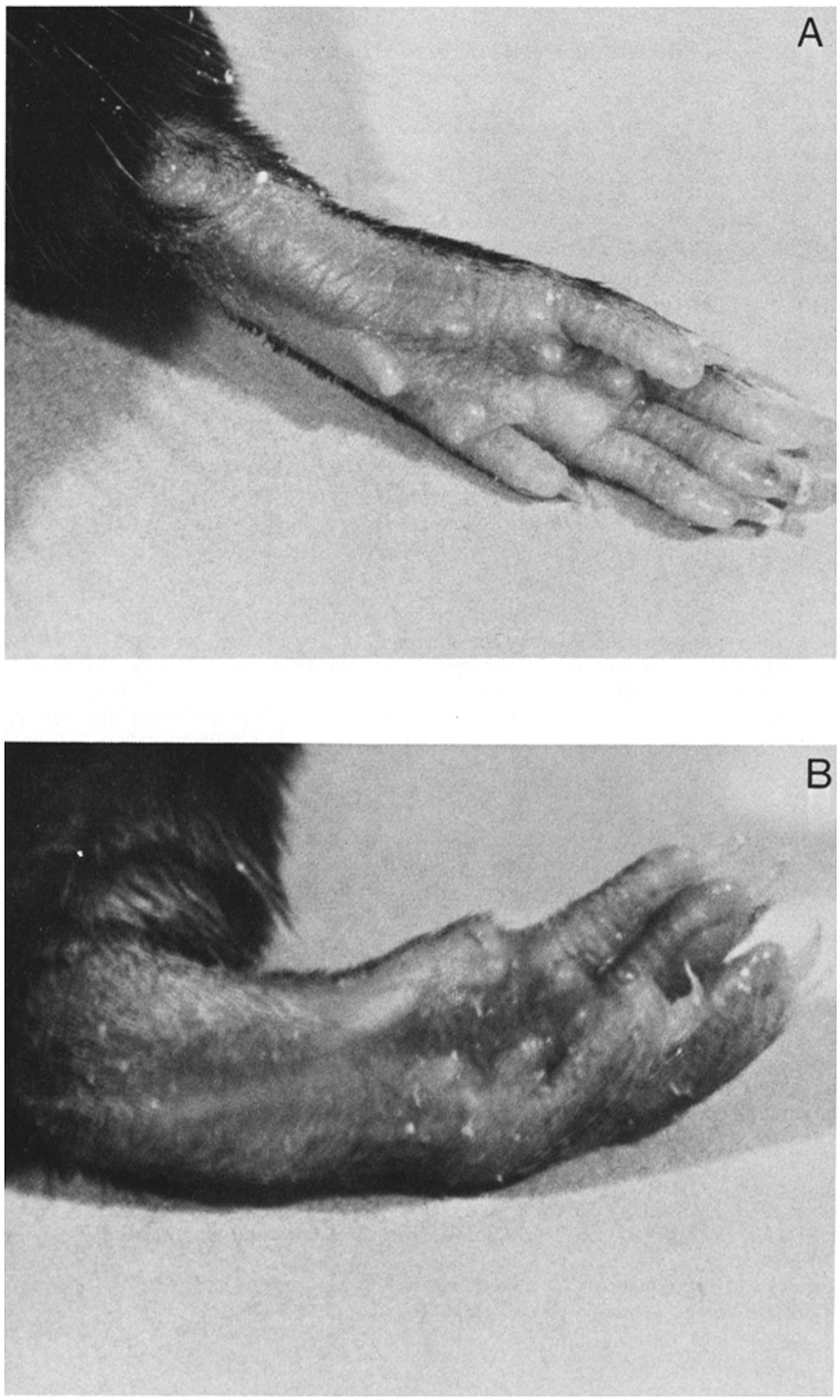

FIG. 1. The appearance of a normal mouse hindlimb (A) in contrast to the appearance of mouse type II collagen arthritis (B). The arthritic paw was graded 3 by the arthritic index.

The initial signs of collagen arthritis were swelling and erythema in one or more paws. The disease would progress at a variable rate, and other limbs could become involved at later intervals. Increases in paw thickness (arthritis/preonset) were recorded from 1.16–2.25, with a mean of 1.78 at peak disease. The variation in clinical limb score from 0–3 is exemplified by Fig. 1 A and B. The number of limbs involved and the stage of the disease allowed a clinical score of 0–12.

The date of onset of collagen arthritis was extremely variable: from 19 d after the initial injection (preboost) to 112 d, with a mean of 46.8 d. The date of peak disease was also variable, with a mean of 10.8 d after onset.

No involvement of vertebral joints or other extraarticular manifestations was observed in type II collagen arthritis in mice. No control mice receiving adjuvant and acetic acid alone were observed to suffer any form of arthritis.

Histological Appearance of Collagen Arthritis. Results of the histological section are shown in Figs. 2 and 3. Fig. 2 shows a section through a mouse foot. The section reveals the tarsal area with the articular surface of the tarsal bones covered with hyaline cartilage that is of uniform thickness and has a smooth surface. The synovial lining is barely perceptible and is one to two cell layers thick. Soft tissues surrounding the joint are normal.

Fig. 3 shows the grading system used in this study. In some sections the only abnormality seen was mononuclear cell proliferation in the superficial layers of the synovium-stage I (Fig. 3 A). This could be seen to progress to pannus formation with damage to the superficial layers of the cartilage—stage 2 (Fig. 3 B). Fig. 3 C shows

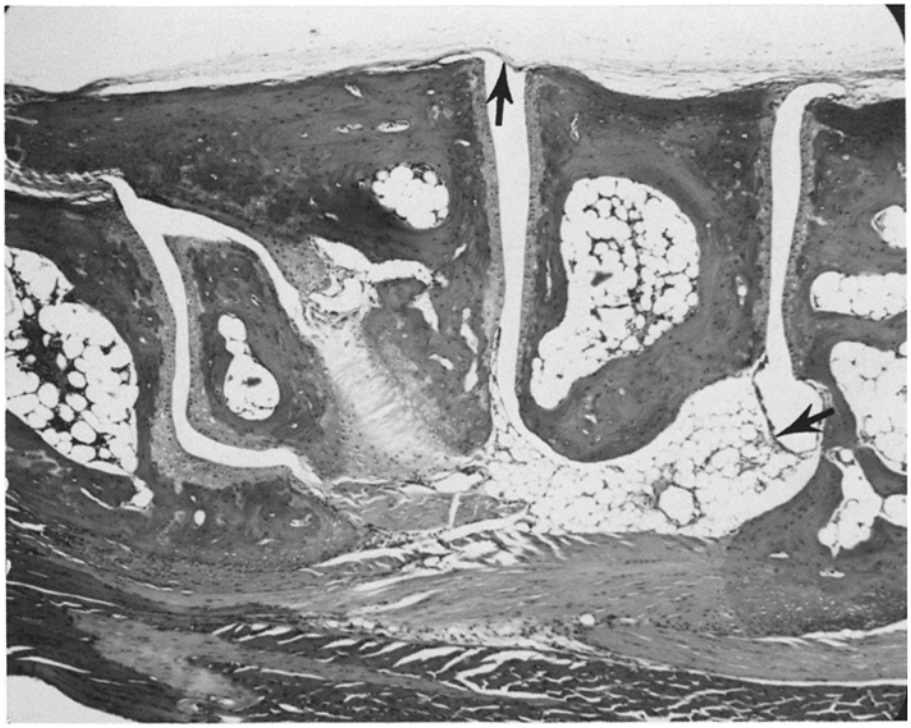

FIG. 2. Cross-section of the foot from a normal mouse. Hemotoxylin and erosion × 64. Arrows point to normal synovial lining.

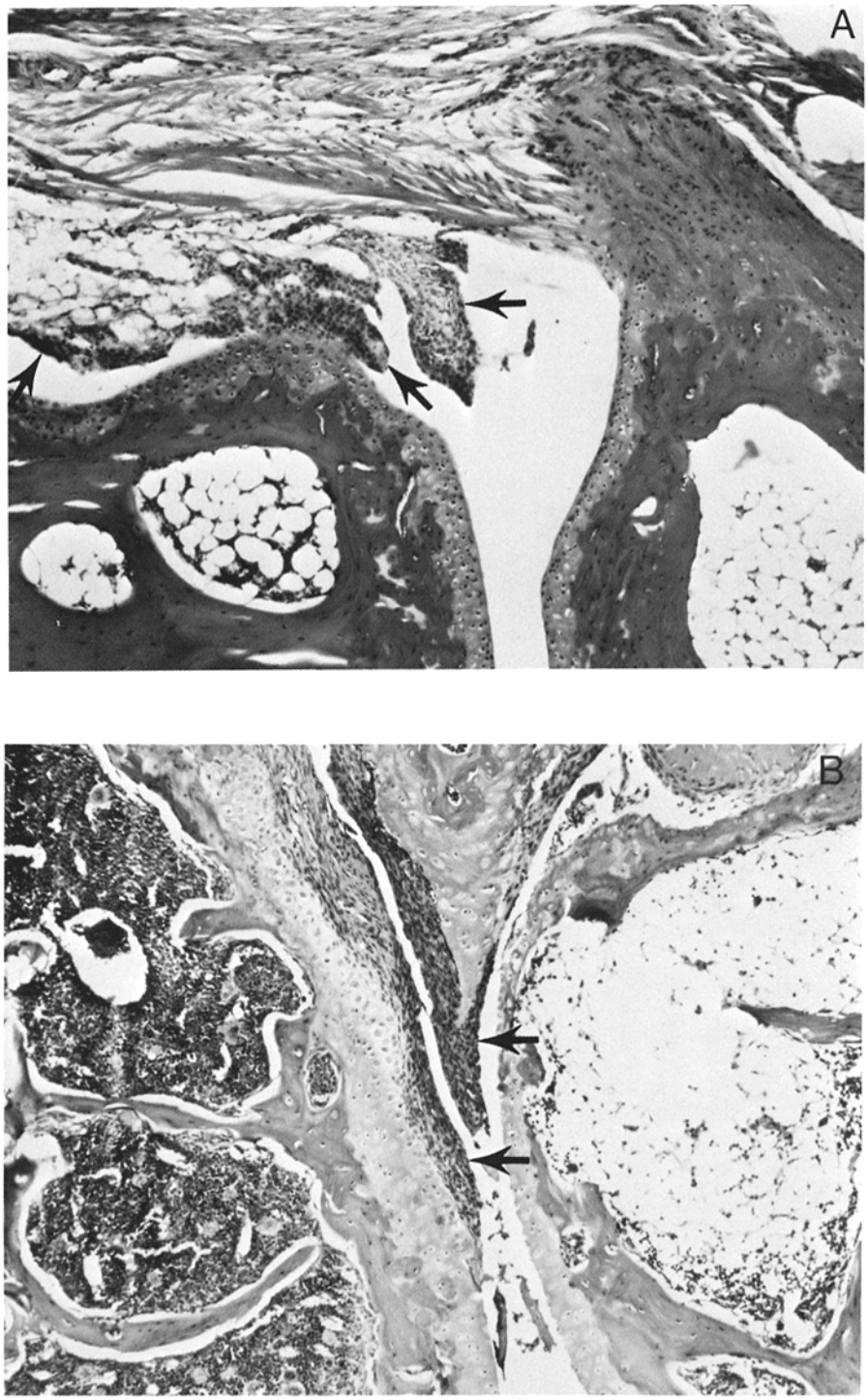

FIG. 3. Sections through various joints of mice with collagen type II-induced arthritis. (A) Mononuclear cell infiltrate in synovial lining × 100 (arrows). (B) Pannus formation with superficial cartilage damage × 100 (arrows). (C) Subchondral bone erosion × 64 (arrows). (D) Complete joint destruction with replacement by mononuclear cell infiltrate × 64.

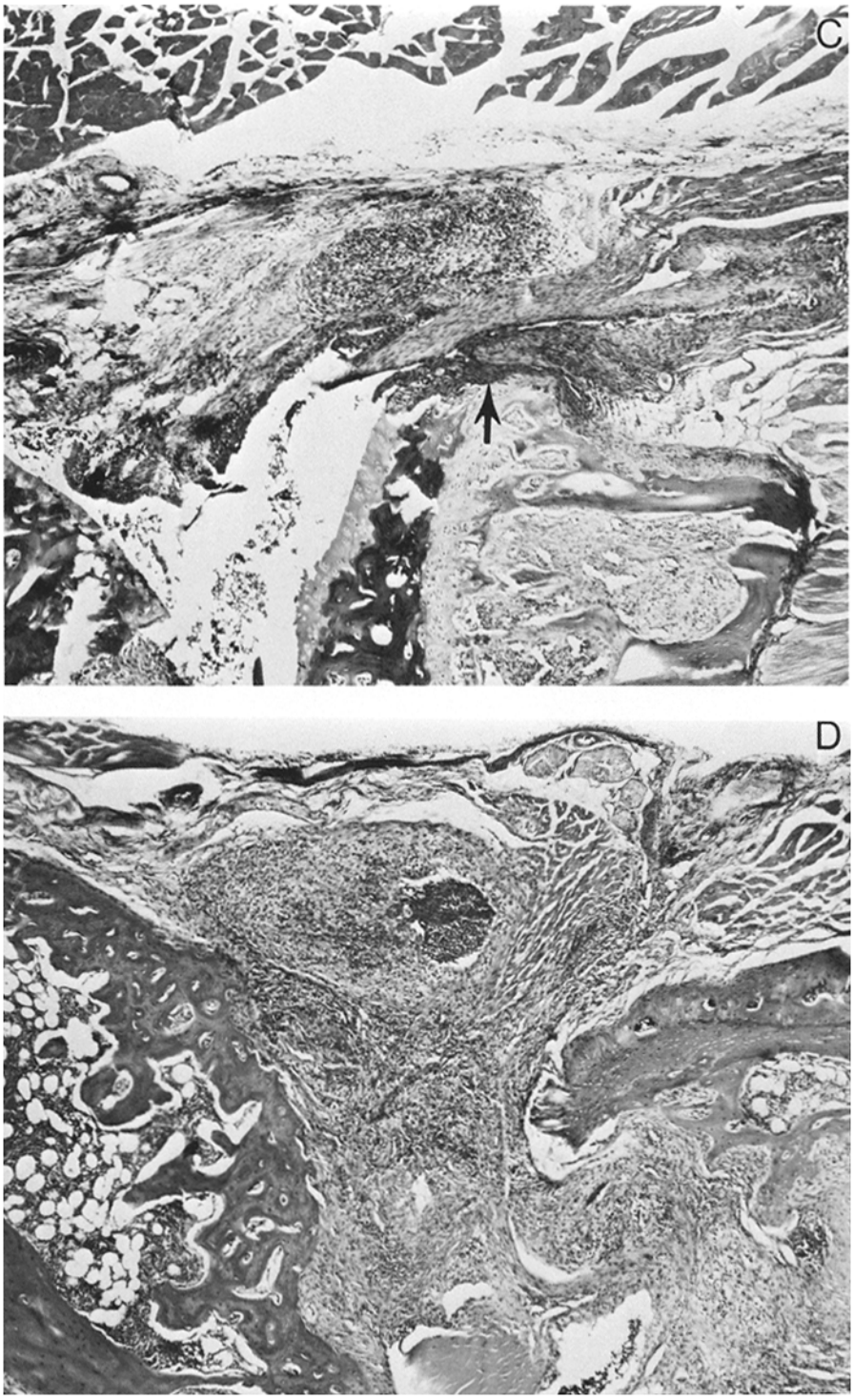

FIG. 3 C and D

stage III of the inflammatory process with development of bone erosions. Stage IV (Fig. 3 D) demonstrated severe osteolysis, with complete disorganization of the joint space and replacement with mononuclear cells, destruction of cartilage, subchrondral bone, and in some areas, development of fibrous or bony ankylosis.

In each animal the range of involvement varied from normal joints to stage I–IV destruction. There was good correlation between the clinical score and the histological score (Table I) except for B10.S animals that clinically were normal but revealed some mononuclear cell hyperreactivity in the synovial lining. There was no pannus identified and no cartilage or bone destruction noted.

Strain Differences in Susceptibility to Type II Collagen Arthritis. Two categories of mice were studied to investigate genetic differences in susceptibility to type II collagen arthritis (Table II): first, inbred mice of known H-2 type with varying background genes, and second, inbred mice of varying H-2 haplotypes congeneic to the B10 background.

Only mice bearing the H-2^q haplotype (B10.Q, B10.G, and DBA/1J) were suscep-

TABLE I

Histological Appearance of Mouse Joint Sections

	Histological grade (range)
CBA/N	0
DBA/1J	1–4
B10.Q	1–3
B10.F	0
B10.WB	0
B10.S	0–1
B10.AQR	0
B10.T(6R)	1–4
B10.BYR	0
B10.DA	1–4
B10.AKM	0

TABLE II

Incidence of Type II Collagen-induced Arthritis in Independent Haplotype Mice

Strain	H-2 haplotype	Arthritis incidence
DBA/1J	q	3/7
CBA/N	k	0/6
B10	b	0/5
B10.D2	d	0/8
B10.MB	f	0/6
B10.WB	j	0/12
B10.K	k	0/6
B10.BR	k	0/12
B10.F	p	0/8
B10.G	q	5/12
B10.Q	q	15/20
B10.RIII	r	0/12
B10.S	s	0/18
B10.PL	u	0/5
B10.SM	v	0/10

TABLE III

Incidence of Type II Collagen-induced Arthritis in Recombinant Haplotype Mice

Strain	Type	Arthritis incidence
B10.AQR	$K^qI^kD^d$	0/10
B10.T(6R)	$K^qI^qD^d$	10/20
B10.BYR	$K^qI^kD^b$	0/12
B10.DA	$K^qI^qD^s$	15/26
B10.AKM	$K^kI^kD^q$	0/7
B10.MBR	$K^bI^kD^q$	0/6

TABLE IV

Incidence of Type II Collagen-induced Arthritis in (Resistant × Susceptible)F_1 Mice

Strain	H-2 haplotype	Arthritis incidence
B10.Q	q	15/20
B10.M	f	0/6
B10.A(4R)	h4	0/5
(B10.Q × B10.M)F_1	q/f	3/4
(B10.Q × B10.A(4R))F_1	h4/q	1/6

TABLE V

Strain Differences Observed in Type II Collagen Arthritis-susceptible Mice

Strain	Incidence	Mean			
		Onset	Limbs involved	Clinical score	Collagen AB
		d			
B10.Q	15/20 (75%)	47.1	2.33	5.54	557 ± 84
B10.G	5/12 (41.7%)	49.3	1.20	2.00	337 ± 136
B10.DA	15/26 (57.7%)	45.0	2.73	6.60	404 ± 77
B10.T(6R)	10/20 (50%)	44.2	1.90	3.80	368 ± 54
B10(Q × M)F_1	3/4 (75%)	46.0	2.66	6.33	634 ± 154
B10(Q × A(4R))F_1	1/6 (16.7%)	51	2	5	373
DBA/IJ	3/7 (42.9%)	57.7	2.33	5.53	426 ± 116

tible to collagen arthritis. No sex differences were observed with respect to disease in the B10.Q or B10.G mice, with only male DBA/IJ mice being investigated.

Gene Mapping of Susceptibility of Type II Collagen Arthritis. Recombinants within the H-2 complex (Table III) were used to map susceptibility to collagen arthritis to the I^q region. Strains bearing K^q alone (B10.AQR, B10.BYR) were resistant, whereas strains with K^qI^q (B10.DA, B10.T[6R]) were susceptible to collagen arthritis. The involvement of genes to the right of the I region was eliminated by the resistance of B10.AKM and B10.MBR, which bear D^q. The susceptibility genes could not be mapped to a specific I subregion because intra-I region recombinants involving H-2^q are not available.

Type II Collagen Arthritis in the (Resistant × Susceptible)F_1 Animals. Two crosses involving B10.Q and a resistant strain were investigated, (B10.Q × B10.M) and

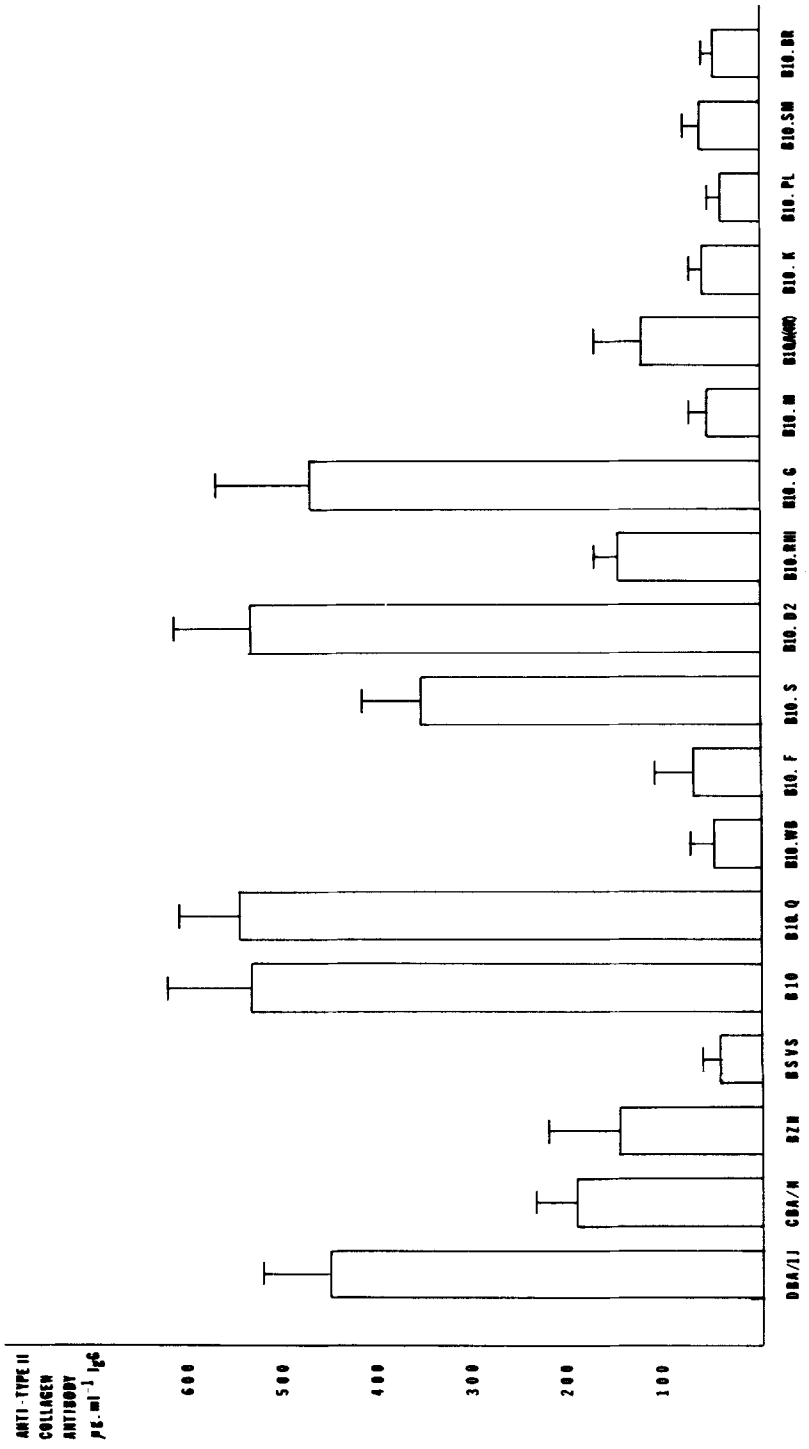

FIG. 4. Anti-type II collagen antibody levels in independent haplotype mice.

(B10.Q × B10.A[4R]) (Table IV). The susceptibility to collagen arthritis was dominant in the F_1 animals, with a similar incidence of arthritis as that of the parental (B10.Q) strain observed in the (B10.Q × B10.M)F_1, but a low incidence of the disease in the (B10.Q × B10.A[4R])F_1 (Table IV). Further crosses are under investigation to study the incidence of arthritis in F_1, F_2, and backcross mice.

Strain Differences in the Incidence and Appearance of Type II Collagen Arthritis. The incidence, onset, and gradation of collagen arthritis for each susceptible strain are summarized in Table V. The incidence of arthritis in B10.Q mice was 75%.

No significant differences were seen in the time of disease onset between strains. However, comparison of the maximum clinical score observed between strains revealed a significant variation in disease severity between B10.Q and B10.G, both strains of which carry the H-2^q haplotype. Table V shows B10.G to have a lower disease incidence, fewer involved limbs, and a lower clinical score than B10.Q. Independent *t* tests showed significant differences in the comparison of arthritis mice of the two strains ($P < 0.05$), and when the strains were compared overall (which tests for the low incidence in B10.G), the difference was highly significant ($P < 0.01$). A similar difference was seen in the comparison of B10.G with B10.DA; however, no other differences between strains were observed.

The Genetic Control of Anti-Type II Collagen Antibody. The antibody response to type II collagen in all independent haplotypes investigated is shown in Fig. 4. Strains were classified as low responders (0–100 μg/ml), intermediate responders (100–300 μg/ml), and high responders 300+ μg/ml). Mouse strains susceptible to type II collagen arthritis (B10.Q, B10.G, DBA/1J, B10.T[6R], B10.DA, and F_1 crosses of B10.Q) were all high responders to type II collagen. High antibody responses were also seen in B10, B10.D2, and B10.S mice, however, there was a significant association ($\chi^2 = 10.65$, $P < 0.005$) between high anti-type II collagen antibody levels and susceptibility to collagen arthritis. Antibody titers in F_1 animals were similar to the higher responder (B10.Q) parental titer. Low antibody responses were associated with the H-$2^{j,p,q,k,u,v,k}$ haplotypes, intermediate responses were associated with the H-2^r haplotype, and high responses were associated with the H-$2^{q,s,d,b}$ haplotypes. Intermediate responses were seen in the recombinant strains B10.AQR, B10.BYR, B10.MBR, B10.AKM, and B10.A(4R), suggesting possible influence of H-2D end in anti-type II collagen antibody response. Anticollagen antibody titers in control animals were found to be 0–12 μg/ml.

Relationship between Anti-Type II Collagen Antibody and Type II Collagen Arthritis. The antibody levels found in arthritic mice (Table V) are not significantly different from the mean antibody levels in comparable strains (Fig. 4).

Discussion

These data indicate that the intradermal injection of native chick type II collagen in CFA may induce arthritis in ~50% of mice of susceptible strains.

The arthritis was characterized by initial redness and swelling of one or more limbs, which resulted in increases of paw thickness of 30–120%. Later clinical characteristics of the disease included gross joint deformation and total loss of joint mobility. The time-course of the disease was extremely variable, with disease onset varying from 19–112 d after the primary injection. Also, the involvement of other limbs after the onset was unpredictable. Mice with single limb involvement appeared most likely to

experience disease recession; however, no spontaneous exacerbations were observed in any animal after recession. The examination of sections of affected joints revealed a good correlation between the clinical score and the histological score in type II collagen arthritis. No clinical or histological evidence of arthritis was seen in control animals receiving CFA and acetic acid alone.

The clinical and histological appearance of collagen arthritis in the mouse is essentially similar to that described in the rat (1), however, the median onset date was later in mice, and additional limbs showed involvement at periods >48 h. Peak severity of the disease was also attained after a longer period in the mouse.

These results are in agreement with the findings of Courtenay et al. (12), who also demonstrated a further increase in the disease time-course by using incomplete adjuvant.

Because the induction of arthritis is dependent upon the intradermal injection of type II collagen in adjuvant, it is conceivable that the delayed development of the disease in mice may be related to the lack of arthritogenic activity of adjuvant in mice. Also, because CFA is nonarthritogenic in mice, it is apparent that the type II collagen presented in this manner is responsible for the disease.

The initial experiments of this study indicated that only mice bearing the H-2^q haplotype were susceptible to type II collagen arthritis. The influence of non-MHC genes upon susceptibility to type II collagen arthritis is unclear because DBA/1J mice showed a similar incidence and course of the disease as did B10.Q mice, whereas B10.G mice, with similar background and MHC genes as B10.Q mice, showed a lower incidence and less severe course of collagen arthritis. This may indicate that the susceptibility gene of collagen arthritis may be linked to the MHC genes responsible for the expression of H-2^q antigens, and the different origin of MHC regions in B10.Q and B10.G may have influenced the action of the disease susceptibility gene to some extent. Investigation of (B10.G × B10.Q)F_1 animals is at present underway to study this phenomena. The use of recombinant congenic mouse strains further mapped the susceptibility to be controlled within the I^q region. This genetic specificity of collagen arthritis was not observed by Courtenay et al. (12), however, this may indicate that the response to bovine collagen may differ in genetic control to the response to chick collagen. The humoral (15, 16) and cellular (17) immune response to bovine collagens is under the control of H-2-linked Ir genes. We can postulate that Ia antigens on macrophages of I^q strains can effectively present type II chick collagen to trigger anti-self response. On the other hand, $Ia^{d,b,s}$ may be presenting the chick type II collagen in a manner in which only heterologous antibodies are generated.

It has also been proposed that the native type II collagen molecule contains at least two antigenic determinants (18). It is conceivable that immunization with extracted type II collagen could elicit an immune response against collagen determinants that are hidden within the cartilage of the joint. Such an immune response should be detectable by the in vitro assay against the immunizing agent, extracted collagen, but the response should not be pathogenic due to the inaccessibility of the determinant in vivo. However, should the response be directed against collagen determinants that are expressed on joint cartilage, it is possible that autoimmune damage could result. Thus, the immune response seen in I^q haplotype mice may indicate a specific response against type II collagen antigenic determinants expressed within the joint. Alternatively, it is conceivable that the immune response of I^q haplotype mice to chick type

II collagen cross-reacts with autologous mouse collagen and results in an autoimmune disease, whereas the response in other strains is confined to determinants specific for chick type II collagen. Thirdly, it may be that the immune response to collagen is against a common antigen determinant in all mouse strains, whereas the response of I^q haplotype mice is higher than other strains. A more aggressive response by I^q haplotype mice might result in damage to the joint cartilage and lead to inflammation and arthritis. However, high antibody responses were observed in three strains not expressing I^q. The level of antibody in B10.D2 ($H\text{-}2^d$) was equivalent or higher than that observed in all the arthritis-susceptible (I^q) strains. Thus, antibody titer as measured by RIA is not an accurate prediction for the onset of arthritis. Similarly, the antibody responses within a susceptible strain were not indicative of the development of the disease. Arthritic mice overall showed a tendency to produce higher anticollagen antibody titers than nonarthritic mice, but arthritis was observed in animals with low antibody titers, particularly in B10.G mice. Studies to determine the role of the cellular response to type II collagen in arthritis and its relationship to disease susceptibility are being conducted at present.

The relationship of type II collagen arthritis in mice to RA would be worth exploring. The histological data in particular indicate that the features observed within the joint correspond to the histological appearance of RA. It is particularly relevant to examine any relationship that the $H\text{-}2^q$ antigens of mice may have to the expression of HLA-DRw4 in man, either in structural similarity or linkage to the control of immune responses. It is known that 70% of RA patients have circulating antibodies to type II collagen (5), and recent work by Solinger and colleagues (19) indicates that individuals with HLA-DRw4 have an abnormal leukocyte response to collagen.

Type II collagen arthritis in mice may prove a valuable model for the study of histocompatibility-linked arthritis in man.

Summary

A model of arthritis was established by the injection of type II collagen into mice. Only mice bearing the $H\text{-}2^q$ haplotype were susceptible to the disease. Susceptibility was further mapped by the use of recombinant strains to the I^q locus. Type II collagen arthritis was observed in the (resistant × susceptible)F_1 cross.

Mice strains were designated high, intermediate, or low responders with respect to the anti-type II antibody levels measured by radioimmunoassay. Arthritis-susceptible strains were all classified as high antibody responders.

The clinical and histological appearance of type II collagen arthritis in the mouse indicates that it may be a good animal model for the investigation of various immunogenetic traits in rheumatoid arthritis.

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