

Human HLA-DR β Gene Hypervariable Region Homology in the BioBreeding BB Rat: Selection of the Diabetic-resistant Subline as a Rheumatoid Arthritis Research Tool to Characterize the Immunopathologic Response to Human Type II Collagen

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Summary

Collagen arthritis (CA), an autoimmune model of rheumatoid arthritis (RA), has been studied in various animals. However, it has not been studied in an animal with a genetic background relevant to RA. We selected rats from a diabetic-resistant (DR) subline of the diabetic BB rat because they have an autoimmune disease-prone background, but not the immunodeficiencies of the diabetic BB rat, and the third hypervariable region (HVRIII) of the BB RT1.D β gene appeared to encode a nucleotide sequence of the human HLA DR β gene, which has been reported to be associated with susceptibility to RA. We synthesized oligonucleotide primers flanking the RT1.D β HVRIII, cloned polymerase chain reaction-amplified DNA into M13mp18, and confirmed the presence of the susceptibility sequence (SS) (RRRAA) by the dideoxy sequencing method in a colony of DR BB/Wor-UTM rats. When immunized with human type II collagen (CII) in incomplete Freund's adjuvant (IFA), arthritis developed rapidly by day 10 with 100% incidence. Light and electron microscopy revealed an unusually severe and aggressive, bidirectional pattern of cartilage resorption by synovial and subchondral mononuclear and multinucleated inflammatory cells. These findings coincided with a predominant humoral response to the cyanogen bromide (CB) 11 fragment of the human CII molecule by the pathogenic IgG2a isotype. This study provides further support to the role of CA as a relevant RA model, the specific roles of the CB11 fragment as a major site of arthritogenic epitopes, and of antibody mechanisms in the pathogenesis of CA. Furthermore, the identification of an RA SS in an immune response gene of the DR BB rat presents a novel opportunity to determine with an animal model the role of other antigens as well as this SS in RA.

Recent DNA sequence studies have established a molecular basis for the association between rheumatoid arthritis (RA)¹ susceptibility and HLA class II immune response (Ir) genes (1). These studies indicate that the association is linked specifically to the polymorphic, third hypervariable region (HVRIII) of the DR β gene. Those DR β alleles associated with RA are marked by HVRIII, which code for a five-member amino acid sequence between residues 69 and

75 of the β chain referred to as the RA susceptibility sequence (SS). The SS is QRRAA for DR1-Dw1, QKRAA for DR4-Dw4, QRRAA for DR4-Dw14, QRRAA for DR4-Dw15, and RRRAA for DRw10 (1).

In reviewing a discussion by Todd et al. (1) on the subject of Ir gene polymorphism in insulin-dependent diabetes mellitus (IDDM), we observed that the RA SS appeared to be encoded by the HVRIII in the RT1.D β gene of the diabetic BB rat. Numerous examples of homology between human, rat, and mouse class II Ir genes have been recognized previously (2). The assignment of rat RT1.D and RT1.B, and mouse MHC I-E and I-A genes as homologues of HLA-DR and DQ Ir genes, are representative examples. These examples are thought to be based upon framework homologies in which

¹ Abbreviations used in this paper: CA, collagen arthritis; CB, cyanogen bromide; CII, type II collagen; DR BB, diabetic-resistant BioBreeding; HVRIII, third hypervariable region; IDDM, insulin-dependent diabetes mellitus; Ir, immune response; RA, rheumatoid arthritis; SS, susceptibility sequence.

nucleotide sequences are shared between human, rat, and mouse Ir β genes. In contrast to these examples, our observation of the RRRAA sequence in the BB RT1.D β gene appeared to represent a novel example of HVR homology in which a nucleotide sequence implicated as a susceptibility sequence in RA is present within the HVRIII of a rat Ir β gene.

Collagen arthritis (CA) is an autoimmune disease model of RA (3). CA susceptibility is regarded as polygenic and the full expression of the autoimmune disease process is thought to represent the interaction of multiple class II MHC Ir and nonMHC genes (4–6). In the investigation conducted herein, we selected the diabetic-resistant (DR) subline of the diabetic BB rat to study CA. This subline was derived by Dr. Arthur A. Like from a family of diabetic BB rats (7). It has the autoimmune disease-prone genetic background of the diabetic BB rat (8), but unlike the diabetic BB rat, this subline does not develop any of the immunological or pathological problems associated with the diabetic condition (9). Moreover, as we observed in the article by Todd et al. (1) and confirmed in the investigation conducted herein, the RT1.D β gene in this subline carries an RA SS, and thus, this subline also has an Ir gene background relevant specifically to RA.

Materials and Methods

Source of DR BB Rats. DR BB/Wor-UTM rats were obtained from the University of Tennessee, Memphis breeding colony. This colony was established in 1984 by Dr. Solomon S. Solomon from DR BB/Wor breeding stock supplied by Dr. Arthur A. Like, Worcester, MA. In the current studies, we used 45–55-d-old females.

Sequencing the HVRIII of the DR BB/Wor-UTM-RT1.D β Gene. Genomic DNA was extracted from freshly prepared liver tissue by established methods (10). The extracted DNA was diluted to 1 ng/ μ l and digested with BamHI. A 1- μ l aliquot of the digested DNA was used as a template for amplification by the PCR (11). Oligonucleotides complementary to regions flanking the BB RT1/D β HVRIII were synthesized on a DNA synthesizer (model 380A; Applied Biosystems, Foster City, CA). These oligonucleotides were designed so as to include not only the complementary regions corresponding to the deduced amino acid positions 14–20 and 88–94 (12), but also unique restriction sites for HindIII and XbaI at their respective 5' ends. The structures were 5' GAC GAA GCT TGA GTG TCA TTT CTA CAA CGG G 3' and 5' CAG TTC TAG ATC TCC GCG GCA CAA GGA ATC T 3'.

PCRs contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 15 mM MgCl₂, 0.01% gelatin, 200 μ M dATP, 200 μ M dCTP, 200 μ M dGTP, 200 μ M dTTP, 1.0 μ M of each primer, 1 ng template DNA, 2.5 U Taq polymerase (Perkin Elmer Cetus, Norwalk, CT). The PCR profile was comprised of 30 cycles of 30-s melting (95°C) and annealing (50°C) steps, and a 1-min polymerization (72°C) step. A 263-bp amplified product was extracted with phenol/chloroform and precipitated with ethanol. The final pellet was redissolved in 10 mM Tris, pH 8, 0.1 mM EDTA and digested with HindIII and XbaI. Digested DNA was subjected to electrophoresis on a 3% Nusieve agarose gel, visualized by ethidium bromide staining, excised from the gel, and purified using a GeneClean Kit (Bio 101).

The purified DNA was ligated into M13mp18 that had been predigested with HindIII and XbaI. Plaques containing inserts were identified by blue/white selection. Positive plaques were selected

for DNA extraction and sequenced using Sequenase enzyme (U.S. Biochemical Corp., Cleveland, OH) and the M13 universal primer for dideoxy chain termination sequencing.

Immunizing DR BB/Wor-UTM Rats with Human CII. Human CII was extracted and purified from articular cartilage by standard limited pepsin digestion/salt precipitation methods (13). Rats were immunized with 0.2 ml of a water in oil emulsion containing 2 mg/ml native human CII in IFA (Difco Laboratories Inc., Detroit, MI). This emulsion was prepared by homogenizing one part native human CII (4 mg/ml in 0.1 M acetic acid) into one part IFA at 4°C. The emulsion was injected intradermally at the base of the tail. We also conducted comparative studies using native bovine CII prepared from fetal cartilage by identical methods.

Determining the Incidence and Clinical Severity of the Arthritis. The incidence of arthritis was determined by comparing the number of rats with hindpaw redness and swelling to the total number of rats immunized with human CII. Clinical severity was determined by comparing the volume (ml) of arthritic hindpaws to the volume (ml) of nonarthritic hindpaws. Hindpaw volumes were determined by taring a beaker of mercury to zero on a digital balance, dipping each hindpaw into the mercury to the level of the lateral malleolus, recording the displaced weight in grams, and dividing this number by the density of mercury.

Determining the Isotype and Epitope Specificity of the Antibody Response. An ELISA was used to determine the isotype and epitope specificity of the antibody response to native human CII or renatured cyanogen bromide (CB) peptides of human CII. The renatured CB peptides were prepared by methods described previously in detail (13). All steps in the ELISA were done under 4°C conditions. Serum samples were diluted in ELISA buffer (0.1 M Tris, 0.15 M sodium chloride, 0.1% Tween-20, pH 7.5) and incubated on microtiter plates (polyvinyl SeroCluster Plates, Costar, Data Packaging, Cambridge, MA) coated with either 10 μ g native human CII/ml 0.145 M potassium phosphate, pH 7.6, for isotype analysis or 10 μ g CB8, 9-7, 10, 11, and 12/ml 0.145 M potassium phosphate buffer, pH 7.6, for analyses of epitope specificity. Human CII or CB peptide coating was done overnight. All subsequent steps used 1-h incubations. The wells were blocked with 1% human albumin (Buminate 25%, Baxter Healthcare Corp., Glendale, CA) in ELISA buffer before the addition of the sera. For isotype analysis, the plates were developed for rat IgM, IgG1, 2a, 2b, 2c, and IgA by stepwise incubation with biotinylated mouse monoclonal isotype reagents (Zymed Labs, South San Francisco, CA) and peroxidase-conjugated ExtraAvidin (Sigma Chemical Co., St. Louis, MO). For analysis of epitope specificity, the plates were developed for rat IgG2a only, because of the predominance of the IgG2a re-

DR BB/Wor-UTM RT1.D β (u) Allele	β Chain Residue										
	69	HVRIII						75	79		
	GAG	CGG	AGG	CGG	GCC	GCG	GTG	GAC	ACG	TAC	TGC
	E	R	R	R	A	A	V	D	T	Y	C
Human HLA DR β Alleles Associated with RA (1)											
HLA DR1-Dw1	E	Q	R	R	A	A	V	D	T	Y	C
HLA DR4-Dw4	E	Q	K	R	A	A	V	D	T	Y	C
HLA DR4-Dw14	E	Q	R	R	A	A	V	D	T	Y	C
HLA DR4-Dw15	E	Q	R	R	A	A	V	D	T	Y	C
HLA DRw10	E	R	R	R	A	A	V	D	T	Y	C

Figure 1. The nucleotide sequence and deduced amino acid sequence of the HVRIII of the RT1.D β (u) allele in the DR BB/Wor-UTM rat. Comparison with the β chain HVRIII sequences for those human HLA DR β alleles associated with RA reveals that the RA SS located between residues 69 and 75 is encoded by the RT1.D β (u) allele in the DR BB/Wor-UTM rat.

sponse (see Results). OD values for isotype and epitope analyses were determined at 490 nm with an automated ELISA reader (Molecular Devices, Menlo Park, CA).

Determining the Histopathologic Nature of the Arthritis. Standard light and electron microscopic methods were used as described in previous studies (14). In brief, the ankles were skinned; fixed in 3% glutaraldehyde, 0.1 M cacodylate, pH 7.4, for 3 d; decalcified for 2 wk in 10% EDTA, 0.05 M Tris, pH 7.4, at 4°C; cut sagittally; and decalcified for another 2 wk. For light microscopy, the tissues were embedded in paraffin, cut at 5 μ m, and stained with 1% toluidine blue. For electron microscopy, the tissues were embedded in Araldite, cut at 1 μ m, and stained with 1% toluidine blue. These thick sections were examined by light microscopy to select areas for thin sections. Thin sections were stained in Reynolds lead-citrate and examined in an electron microscope (1200; Jeol USA, Inc., Peabody, MA).

Results

Sequence of the HVRIII of the DR BB/Wor-UTM RT1.D β Gene. As shown in Fig. 1, the amino acid sequence of the HVRIII between residues 69 and 75 of the RT1.D β chain was RRRAA as deduced from the nucleotide sequence of PCR-amplified DNA cloned into M13mp18 and sequenced by the dideoxy method. These results are in complete agreement with those reported by Todd et al. (1) for BB rats obtained from the Worcester colony as well as by Holowachuk and Greer (15) for BB rats from the Toronto colony. As Fig. 1 also shows, this sequence is a member of the RA SSSs as revealed by comparison with the deduced amino acid sequences of HVRIII of human HLA DR β alleles associated with RA.

Incidence and Severity of Arthritis in Human CII Immunized DR BB/Wor-UTM Rats. The incidence of CA after immunization with native human CII in the DR BB/Wor-UTM rat was 100% in three separate studies (5 out of 5, 5 out of 5, and 6 out of 6). The arthritis was evident clinically by day 10 in 15 out of 16 rats; the other rat developed arthritis on day 14. The arthritis in the hindpaws was bilateral in 15 out of 16 cases. The arthritic swelling in either hindpaw amounted to an approximate doubling in hindpaw volume as measured by mercury displacement. The means (range; *n*) for left normal and arthritic hindpaws were 1.4 (0;3) and 2.7 (2.5–3.1; 6) indicating an increase in arthritic swelling of 94%. Values for right normal and arthritic hindpaws were 1.5 (1.4–1.5; 3) and 2.8 (2.1–3.0; 6), indicating an increase in arthritic swelling of 84%. Similar results were observed in three comparative studies with native bovine CII in IFA (3 out of 3, all on day 10; 5 out of 5, 4 on day 10, 1 on day 13; 5 out of 5, all on day 10).

Isotype and Epitope Specificity of the Antibody Response to Human CII. The isotype and epitope specificity of serum antibody responses in human CII immunized, DR BB/Wor-UTM rats were determined within 48 h of the onset of arthritis which was day 10 for the data shown in Table 1. Isotype analysis revealed a predominance of IgG2a antibody to human CII. The ELISA values in optical density units for pooled serum were 0.218 for IgM, 0.023 for IgG1, >3.000 for IgG2a, 0.024 for IgG2b, 0.079 for IgG2c, and 0.023 for IgA. As a result of the IgG2a predominance, the epitope

specificity analysis shown in Table 1 was confined to an analysis of the IgG2a isotype only. As the data shows, CB11 was the immunodominant epitope for the IgG2a antibody response in arthritic BB rats immunized with human or bovine CII. In contrast, this immunodominant CB11 response was relatively suppressed in nonarthritic Brown-Norway rats immunized with native human CII for comparative purposes.

Histopathologic Nature of the Arthritis. Light microscopic analysis of ankle joints from arthritic DR BB/Wor-UTM rats revealed a bidirectional pattern of cartilage erosion. As shown in Fig. 2, the arthritis began as a proliferative synovitis with pannus formation at the joint margins. Cartilage erosion occurred in the outer (unmineralized) layer of cartilage in association with this synovial tissue. Cartilage erosion also occurred in the inner (mineralized) layer of cartilage. However, the erosions in this layer were associated with a marked inflammatory cell reaction originating from within the subchondral compartment. As shown in Fig. 3 by electron microscopy, both mononuclear and multinucleated inflammatory cell types were identified at sites of erosion occurring as a result of this reaction.

Discussion

In previous CA studies with DBA/1 mice, we demonstrated that CA susceptibility correlates with an antibody response that is directed predominantly against epitopes within the CB11 fragment of the CII molecule (16), and that is restricted in its expression to a pathogenic isotype such as IgG2a (6). These studies and those more recently reported by Myers et al. (17) clearly support this response as a major arthritogenic response in the DBA/1 mouse model of CA.

One of the major questions raised by these studies has been whether this arthritogenic response is peculiar to the I-Aq Ir gene background of DBA/1 mice. Their Ir gene background is unusual in that only one of the two Ia isotypes is expressed; i.e., I-A, the mouse homologue of human DQ (18). The I-E isotype, the mouse homologue of human DR, is not expressed. Two other related questions raised in these studies have been whether this response is peculiar to the use of chick CII and/or to the use of CFA. Precedents exist for an effect by the species source of CII on CA susceptibility (19) and for an effect by mycobacterial antigens in CFA on the quality of immune responses in general (20).

Our aim in the present study was to select a strain of animal with a genetic background relevant to RA, and to use it as an RA research tool to study CA, specifically its arthritogenic response to CII. Our selection of the DR BB rat was based upon the following reasoning. (a) Unlike DBA/1 mice, DR BB rats express both Ia isotypes; i.e., RT1.B and RT1.D, the rat homologues of human DQ and DR, respectively (2, 21). (b) The DR BB RT1.D β Ir gene carries a polymorphic sequence RRRAA implicated as an SS in RA. As shown in Fig. 1 for the DR BB/Wor-UTM rat, this SS was present in the correct location of the Ia β gene, i.e., between codons 69 and 75 of the HVRIII, and in the correct Ia isotype, i.e., RT1.D, the rat homologue of human HLA DR. And (c), the DR BB rat which was derived from a family

Table 1. The Epitope Specificity of the IgG2a Antibody Response to Renatured Human CII CB Peptides in DR BB/Wor-UTM Rats With Collagen Arthritis

Source of serum	Anti-human CII CB peptide ELISA values					Total
	CB8	CB9-7	CB10	CB11	CB12	
Arthritic DR BB rats						
Human CII Immunized (1:900)						
1	0.192	0.346	0.106	0.532	0.122	1.298
2	0.017	0.397	0.203	0.564	0.204	1.385
3	0.124	0.386	0.226	0.650	0.230	1.616
4	0.294	0.496	0.246	0.741	0.292	2.069
5	0.255	0.563	0.310	0.714	0.296	2.138
Bovine CII Immunized (1:900)						
1	0.108	0.338	0.112	0.560	0.148	1.266
2	0.162	0.390	0.133	0.626	0.180	1.491
3	0.156	0.412	0.256	0.713	0.301	1.838
4	0.222	0.492	0.180	0.704	0.242	1.840
5	0.401	0.460	0.318	0.688	0.140	2.007
Nonarthritic Brown-Norway rats						
Human CII Immunized (1:300)						
1	0.054	0.106	0.082	0.130	0.042	0.414
2	0.052	0.154	0.107	0.095	0.056	0.464
3	0.193	0.175	0.078	0.137	0.065	0.648
4	0.118	0.542	0.096	0.241	0.074	1.071
5	0.196	0.374	0.186	0.370	0.147	1.273

ELISA plates were coated with renatured CB peptides prepared from human CII as described in detail previously (13), and developed after incubation with individual sera using biotinylated mouse monoclonal anti-rat IgG2a and ExtraAvidin peroxidase. The results are shown in optical density units for sera from arthritic DR BB rats immunized with human CII or bovine CII and diluted 1:900 in ELISA buffer. For comparative purposes, ELISA results also are shown for sera from nonarthritic Brown-Norway rats (Harlan, Indianapolis, IN) immunized with human CII and diluted 1:300 in ELISA buffer. The bold numbers indicate the predominant IgG2a anti-human CII CB peptide value for each rat. Controls consisted of normal sera assayed on CB peptide-coated wells or immune sera assayed on uncoated wells. The results in either case were <0.100 optical density units (data not shown).

of diabetic BB rats (7), has the autoimmune disease-prone genetic background (8), but not the immunodeficiencies of the diabetic BB rat (9). This autoimmune disease-prone genetic background may consist of various non-Ir genes which may be common to all HLA class II-linked autoimmune diseases such as RA and IDDM (1), and which may be responsible for promoting the full expression of the autoimmune mechanisms in these diseases as well as in animal models of these diseases (6, 22).

When immunized with CII in the present study, we found the DR BB rat to be markedly susceptible to CA. Arthritis developed rapidly by day 10 with an incidence of 100%. This marked susceptibility contrasts with that reported previously in other rat strains studied by us and others in which arthritis typically develops later between days 12–24 and occurs with a much lower incidence usually between 40 and 80% (3, 23–25). Our epitope and isotype analysis of the humoral response at the onset of arthritis revealed an immunodominant

IgG2a antibody response to the CB11 fragment of human CII after immunization with either human or bovine CII without mycobacteria. The arthritogenic antibody response as defined in previous studies (6, 16) appears, therefore, to be peculiar neither to DBA/1 mice, to the use of chick CII, nor to the use of CFA. Moreover, in our comparative study with the CA-resistant BN rat, this immunodominant IgG2a response to CB11 was relatively suppressed. These results provide further support to the proposed role of CB11 as a major site of arthritogenic epitopes in CA. Since rat IgG2a, like mouse IgG2a, is a pathogenic isotype (26), these results also provide further support to the proposed role of antibody mechanisms in the pathogenesis of CA (14).

In completing these immunological studies, we considered it important to verify the histopathological nature of CA in the DR BB rat. In so doing, we observed that while the nature of its antibody response did parallel that of DBA/1 mice, the nature of its histopathological response did not par-

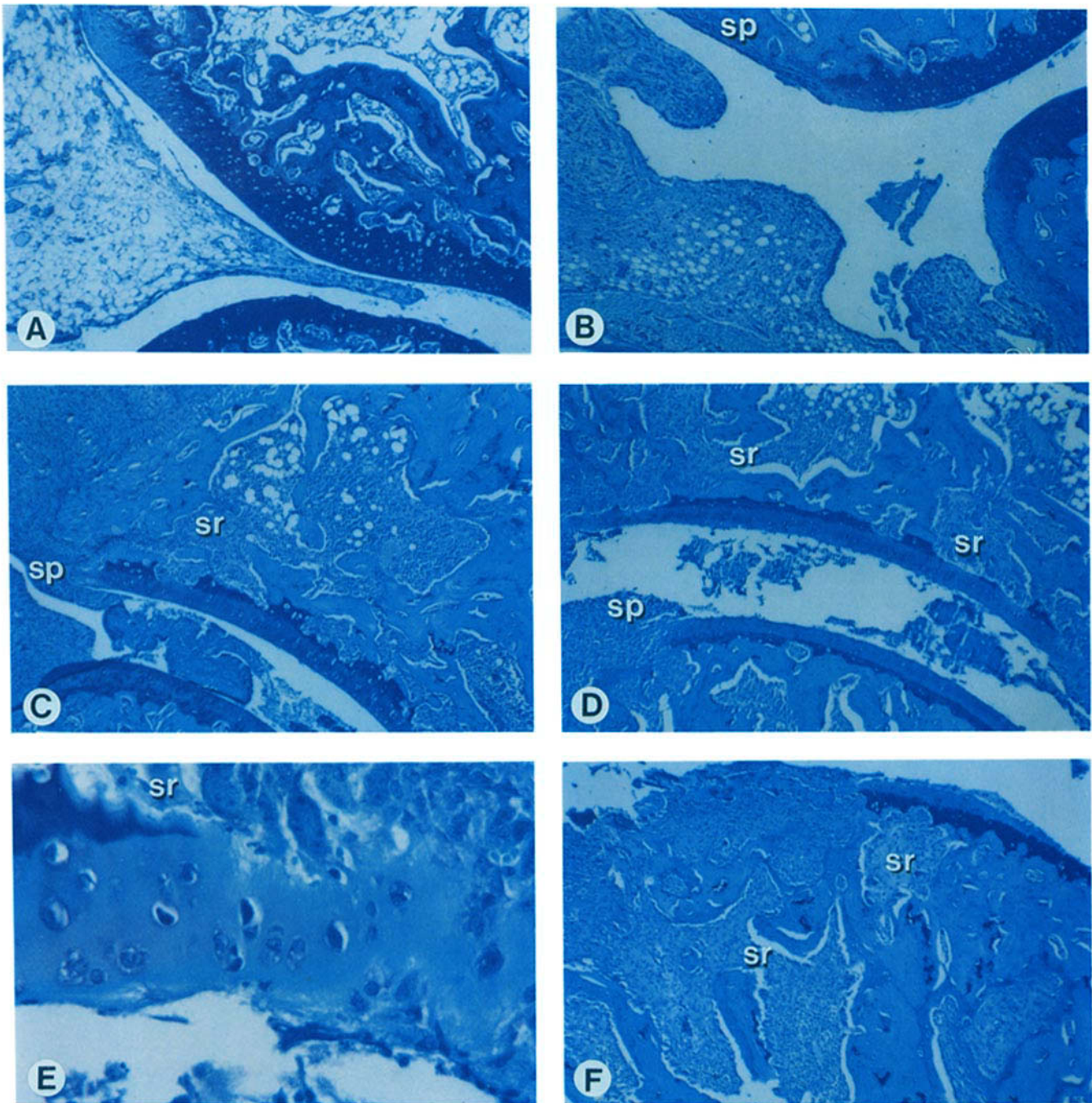


Figure 2. Development of a bidirectional pattern of cartilage resorption in the tibia-tarsal joints of DR BB rats with CA. Light micrographs were taken of toluidine blue-stained sections from normal (A) and arthritic DR BB rats 10 (B), 13 (C), 21 (D and E), and 30 (F) days after immunization with human CII in IFA. As these micrographs show, cartilage resorption occurred at the joint margins in association with the synovial pannus (*sp*) in *b-d*, and at central regions of the articular cartilage in association with a subchondral inflammatory reaction (*sr*) in *c-f*. The micrograph in *E* shows that this subchondral inflammatory reaction in *D* (lower right area of tibia) at higher magnification.

allel that of DBA/1 mice (5, 27). In fact, it did not parallel that of other strains of CA-susceptible rats studied previously by us and others (3, 23–25). The significant, bilateral swelling observed in the ankles of DR BB rats was associated initially with the cartilage-erosive, synovial reaction typical of that reported previously in other animals. However, in addition to this reaction, we also observed the development of promi-

nent inflammatory reactions originating from subchondral regions and involving both mononuclear inflammatory cells and multinucleated giant cells. These reactions appeared to be responsible for much of the erosions occurring in the subchondral bone and in the central regions of the articular cartilage.

We know from recent passive transfer studies that humoral

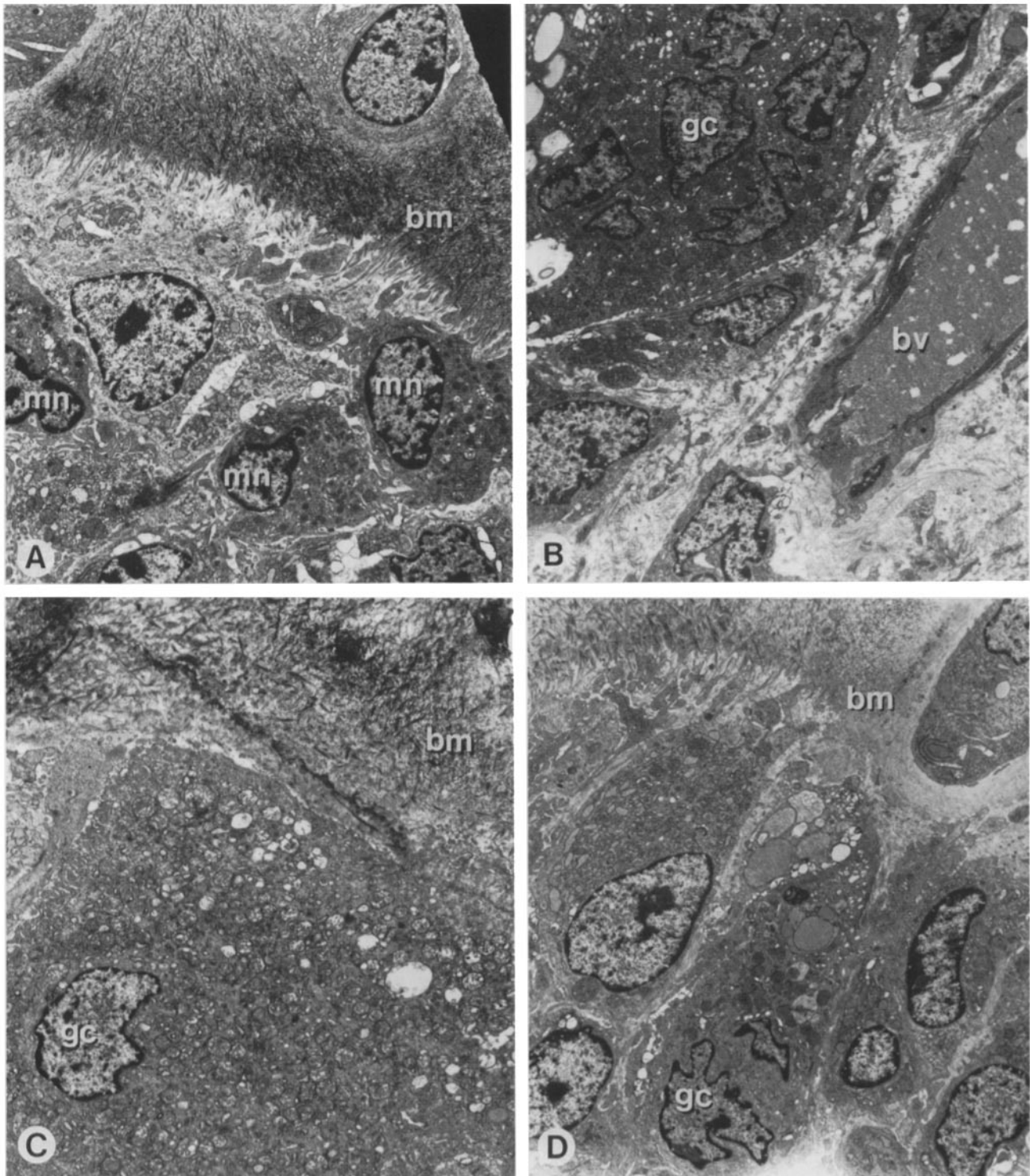


Figure 3. Identification of mononuclear (*mn*) inflammatory cells and multinucleated giant cells (*gc*) associated with the subchondral inflammatory reaction. Electron micrographs of tibia-tarsal sections from arthritic DR BB rats 21 d after immunization with human CII in IFA revealed the presence of mononuclear macrophage-like cells in *A* as well as giant cells adjacent to a subchondral blood vessel (*bv*) in *B*, and adjacent to sites of bone matrix (*bm*) resorption in *C* and *D*. The giant cell in *C* contained several nuclei, only one of which is shown in this micrograph. The morphology of the giant cells in *C* and *D* are consistent with that of cells actively degrading the bone matrix. In this regard, note in *C* the penetration of this giant cell into the subchondral bone and in *D* the presence of large, endocytotic digestive vacuoles in this giant cell and the marked reduction in the collagen fibril size and overall density of the bone matrix immediately adjacent to this giant cell.

mechanisms alone are sufficient to provoke the cartilage-erosive, synovial reaction observed in CA (14). However, we do not know presently whether these mechanisms alone are sufficient to also provoke the subchondral inflammatory cell reactions observed in DR BB rats with CA. These reactions could reflect the production of antibodies with additional arthritogenic specificities either to different epitopes within CB11 and/or to epitopes within other CB peptides. In regard to this latter possibility, we would like to note that while the major CB peptide specificity of the antibody response in DBA/1 mice and DR BB rats is similar, the fine specificity of this response in these two animals is different. Thus, in DBA/1 mice the predominant specificity is to CB11 followed by CB10 and 8, and then with a much lesser degree of specificity to the other peptides (16). In DR BB rats, the predominant specificity also is to CB11, but is followed closely by CB9-7, and then with a much lesser degree to the other peptides (Table 1). This difference in fine specificity, related undoubtedly to the different Ir gene backgrounds of these animals (28), draws attention to the potential role of CB9-7 as an additional site of arthritogenic epitopes. It is relevant perhaps to note that the antibody response to CB9-7 in the CA resistant BN (RT1ⁿ) rat was weak compared to that in the CA-susceptible DR BB (RT1^u) rat as judged by comparing CB9-7 ELISA values for sera diluted 1:300 and 1:900, respectively (Table 1). Regardless of whether the aggressive, bidirectional pattern of cartilage resorption in the DR BB rat is related to humoral mechanisms involving antibodies with additional arthritogenic specificities or is related to other non-RT1 class II-linked mechanisms unique to the genetic background of the DR BB rat, the results presented herein clearly establish it as a characteristic histopathologic feature of CA in the DR BB rat.

We would like to point out that this bidirectional pattern is not unique to DR BB rats with CA. Bromley et al. (29) have reported that the resorption of articular cartilage in RA also exhibits a bidirectional pattern involving mononuclear inflammatory cells arising from the synovial tissues, and both mononuclear and multinucleated giant cells arising from the subchondral compartment. This bidirectional pattern could be caused in part by cartilage immune complex mechanisms similar to those described previously in passive transfer studies with CII antibodies from animals with CA (30). In support of this suggestion, recent clinical studies of CII antibodies from patients with RA show that (a) while the prevalence of CII antibodies in RA sera is low, the prevalence of CII antibodies in RA cartilage is high (13, 31); (b) epitope analysis reveals a predominance of CII antibodies directed against the CB11 fragment (13); (c) IgG isotype analysis reveals a

predominance of the pathogenic IgG3 subclass (32); and (d) in vitro experiments with a human cartilage immune complex model implicate several potentially important pathogenic mechanisms by which these antibodies could contribute to the resorption of articular cartilage in RA (33).

The bidirectional resorption of articular cartilage in RA represents a serious stage of this disease. The clinical studies cited above not only serve to support the role of autoimmune mechanisms involving CII in this stage of the disease, but also serve to raise several important questions relating ultimately to the goal of developing more effective strategies for treating this stage of the disease (34). These questions include, (a) What is the structure of the relevant arthritogenic CII epitope(s) that initiates this autoimmune response; (b) what is the structure of the T cell receptor(s) that responds to it; and (c) what are the humoral and/or cellular effector mechanisms that mediate the cartilage and bone destructive consequences of this response? We suggest that the DR BB rat model of CA presents a novel opportunity to begin to answer these questions. As discussed herein, the DR BB/Wor-UTM rat has an Ir gene background relevant specifically to RA, and CA in this rat is associated with a similar bidirectional pattern of cartilage resorption and humoral response to human CII.

Finally, in addition to CII, a variety of other antigens have been proposed as candidate arthritogens in RA (35). To date, however, none of these other antigens has been examined for arthritogenicity in an animal with a genetic background relevant specifically to RA. Furthermore, while a molecular basis for the association between RA susceptibility and HLA class II Ir genes has been determined, a functional basis has not been determined. We do not know how the presence of the RA SS on a number of otherwise divergent HLA class II haplotypes confers susceptibility to the development of RA (1). Two well-defined hypotheses have been described: altered antigen presentation and altered T cell reactivity (36). In the former, the RA SS is thought to confer to the Ia α/β antigen-presenting receptor increased binding and presentation to T cells of some as yet unidentified arthritogenic peptide(s). In the latter, the RA SS itself is viewed as an arthritogenic epitope for a population of autoreactive T cells arising as a result of molecular mimicry between the RA SS on the HLA DR β chain and the nuclear capsid antigen of EBV (36). Considering the presence of the RA SS on the DR BB RT1.D β chain, we also suggest that the DR BB rat presents a novel opportunity to test with an animal model the proposed role of these other antigens as well as these proposed roles of the SS in RA.

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References

1. Todd, J.A., H. Acha-Orbea, J.I. Bell, N. Chao, Z. Fronck, C.O. Jacob, M. McDermott, A.A. Sinha, L. Timmerman, L. Steinman, and H.O. McDevitt. 1988. A molecular basis for MHC class II-associated autoimmunity. *Science (Wash. DC)*. 240:1003.
2. Howard, J.C. 1983. The major histocompatibility complex of the rat: a partial review. *Metabolism*. 32:41.
3. Trentham, D.E., A.S. Townes, and A.H. Kang. 1977. Autoimmunity to type II collagen: an experimental model of arthritis. *J. Exp. Med.* 146:857.
4. Griffiths, M.M., and C.W. DeWitt. 1981. Immunogenetic control of experimental collagen-induced arthritis in rats. II. ECIA susceptibility and immune response to type II collagen (CALF) are linked to RT1. *J. Immunogenet. (Oxf.)*. 8:463.
5. Wooley, P.H., H.S. Luthra, J.M. Stuart, and C.S. David. 1981. Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. *J. Exp. Med.* 154:688.
6. Watson, W.C., and A.S. Townes. 1985. Genetic susceptibility to murine collagen II autoimmune arthritis. Proposed relationship to the IgG2 autoantibody subclass response, complement C5, major histocompatibility complex (MHC) and non-MHC loci. *J. Exp. Med.* 162:1878.
7. Butler, L., D.L. Guberski, and A.A. Like. 1983. Genetic analysis of the BB/W diabetic rat. *Can. J. Genet. Cytol.* 25:7.
8. Like, A.A., E.J. Weringer, A. Holdash, P. McGill, D. Atkinson, and A. Rossini. 1985. Adoptive transfer of autoimmune diabetes mellitus in BioBreeding/Worcester (BB/W) inbred and hybrid rats. *J. Immunol.* 134:1583.
9. Rossini, A.A., S. Slavin, B.A. Woda, M. Geisberg, A.A. Like, and J.P. Mordes. 1984. Total lymphoid irradiation prevents diabetes mellitus in the BioBreeding/Worcester (BB/W) rat. *Diabetes*. 33:543.
10. Greenberg, M.E. 1988. *Current Protocols in Molecular Biology*. Greene Publishing Associates & Wiley-Interscience, New York. 2.2.1-2.2.3.
11. Bell, J. 1989. The polymerase chain reaction. *Immunol. Today*. 10:351.
12. Chao, N.J., L. Timmerman, H.O. McDevitt, and C.O. Jacob. 1989. Molecular characterization of MHC class II antigens (β 1 domain) in the BB diabetes-prone and -resistant rat. *Immunogenetics*. 29:231.
13. Terato, K., Y. Shimozuru, K. Katayama, Y. Takemitsu, I. Yamashita, M. Miyatsu, K. Fujii, M. Sagara, S. Kobayashi, M. Goto, K. Nishioka, N. Miyasaka, and Y. Nagai. 1990. Specificity of antibodies to type II collagen in rheumatoid arthritis. *Arthritis Rheum.* 33:1493.
14. Watson, W.C., P.S. Brown, J.A. Pitcock, and A.S. Townes. 1987. Passive transfer studies with type II collagen antibody in B10.D2/old and new line and C57Bl/6 normal and beige (Chediak-Higashi) strains: evidence of important roles for C5 and multiple inflammatory cell types in the development of erosive arthritis. *Arthritis Rheum.* 30:460.
15. Holowachuk, E.W., and M.K. Greer. 1989. Unaltered class II histocompatibility antigens and pathogenesis of IDDM in BB rats. *Diabetes*. 38:267.
16. Terato, K., K.A. Hasty, M.A. Cremer, J.M. Stuart, A.S. Townes, and A.H. Kang. 1985. Collagen-induced arthritis in mice. Localization of an arthritogenic determinant to a fragment of the type II collagen molecule. *J. Exp. Med.* 162:637.
17. Myers, L.K., J.M. Stuart, J.M. Seyer, and A.H. Kang. 1989. Identification of an immunosuppressive epitope of type II collagen that confers protection against collagen-induced arthritis. *J. Exp. Med.* 170:1999.
18. Tacchini-Cottier, F.M., and P.P. Jones. 1988. Defective E β expression in three mouse H-2 haplotypes results from aberrant RNA splicing. *J. Immunol.* 141:3647.
19. Wooley, P.H., H.S. Luthra, M.M. Griffiths, J.M. Stuart, and C.S. David. 1985. Type II collagen-induced arthritis in mice. IV. Variations in immunogenic regulation provide evidence for multiple arthritogenic epitopes on the collagen molecule. *J. Immunol.* 135:2443.
20. Leclerc, C., F. Audibert, and L. Chedid. 1978. Influence of synthetic adjuvant (MDP) on qualitative and quantitative changes of serum globulins. *Immunology*. 35:963.
21. Holowachuk, E.W., M.K. Greer, and D.R. Martin. 1988. Elevated mRNA levels of major histocompatibility complex class II genes in lymphocytes of autoimmune BB rats. *Diabetes*. 37:1637.
22. Colle, E., R.D. Guttmann, T.A. Seemayer, and F. Michel. 1983. Spontaneous diabetes mellitus syndrome in the rat. IV. Immunogenetic interactions of MHC and non-MHC components of the syndrome. *Metabolism*. 32:54.
23. Stuart, J.M., M.A. Cremer, A.H. Kang, and A.S. Townes. 1979. Collagen-induced arthritis in rats. Evaluation of early immunologic events. *Arthritis Rheum.* 22:1344.
24. Terato, K., R. Hashida, K. Miyamoto, T. Morimoto, Y. Kato, S. Kobayashi, T. Tajima, S. Otake, H. Hori, and Y. Nagai. 1982. Histological, immunological, and biochemical studies on type II collagen-induced arthritis in rats. *Biomed. Res.* 3:495.
25. DeSimone, D.P., D.B. Parsons, K.E. Johnson, and R.P. Jacobs. 1983. Type II collagen-induced arthritis. A morphologic and biochemical study of articular cartilage. *Arthritis Rheum.* 26:1245.
26. Farkas, A., G. Medgyesi, G. Fust, K. Miklos, and J. Gergely. 1982. Immunogenicity of antigen complexed with antibody. I. Role of different isotypes. *Immunology*. 45:483.
27. Holmdahl, R., L. Jansson, E. Larsson, K. Rubin, and L. Klareskog. 1986. Homologous type II collagen induces chronic and progressive arthritis in mice. *Arthritis Rheum.* 29:106.
28. McDevitt, H.O., and B. Benacerraf. 1969. Genetic control of specific immune responses. *Adv. Immunol.* 11:31.

29. Bromley, M., H. Bertfield, J.M. Evanson, and D.E. Woolley. 1985. Bidirectional erosion of cartilage in the rheumatoid knee joint. *Ann. Rheum. Dis.* 44:676.
30. Watson, W.C., A.S. Townes, and A.H. Kang. 1986. Immunopathogenic mechanisms in type II collagen autoimmune arthritis. *Pathol. Immunopathol. Res.* 5:297.
31. Jasin, H.E. 1985. Autoantibody specificities of immune complexes sequestered in articular cartilage of patients with rheumatoid arthritis and osteoarthritis. *Arthritis Rheum.* 28:241.
32. Watson, W.C., M.A. Cremer, P.H. Wooley, and A.S. Townes. 1986. Assessment of the potential pathogenicity of type II collagen autoantibodies in patients with rheumatoid arthritis. Evidence of restricted IgG3 subclass expression and activation of complement C5 to C5a. *Arthritis Rheum.* 29:1316.
33. Watson, W.C., and A.H. Kang. 1988. Cartilage bound autoantibodies to type II collagen in rheumatoid arthritis: activators of complement and inflammatory cells and proposed role as "receptors" for rheumatoid factor. *Proc. 6th SEAPAL Congr. Rheum.*, 1st, Tokyo. 809:153.
34. Scott, D.L., B.L. Coulton, D.P.M. Symmons, and A.J. Popert. 1987. Long term outcome of treating rheumatoid arthritis: results after 20 years. *Lancet.* 1108.
35. Bennett, J.C. 1989. The etiology of rheumatic diseases. In *Textbook of Rheumatology*. 3rd ed. W.N. Kelley, E.D. Harris, S. Ruddy, and C.B. Sledge, editors. W.B. Saunders, Co., Philadelphia. 138-147.
36. Roudier, J., G. Rhodes, J. Petersen, J.H. Vaughan, and D.A. Carson. 1988. The Epstein-Barr virus glycoprotein gp110, a molecular link between HLA DR4, HLA DR1, and rheumatoid arthritis. *Scand. J. Immunol.* 27:367.