HLA-DQ8 Transgenic Mice Are Highly Susceptible To Collagen-induced Arthritis: A Novel Model For Human Polyarthritis

By Gerald H. Nabozny,* Jeanine M. Baisch,* Shen Cheng,* Dominic Cosgrove,§ Marie M. Griffiths,‖ Harvinder S. Luthra,‡ and Chella S. David*

From the Departments of *Immunology and §Rheumatology, Mayo Medical School, Rochester, Minnesota 55905; ‡Center for Hereditary Disorders, Boys Town National Research Hospital, Omaha, Nebraska 68131; and ‖Research Service, Veteran’s Affairs Medical Center, and Department of Medicine, Division of Rheumatology, University of Utah, Salt Lake City, Utah 84132

Summary

Genetic studies have indicated that susceptibility to rheumatoid arthritis (RA) maps to the HLA-DR locus of the major histocompatibility complex. Strong linkage disequilibrium between certain HLA-DQ genes and HLA-DP genes associated with RA, however, suggests that HLA-DQ molecules may also play a role in RA susceptibility. To examine the role of HLA-DQ molecules in arthritis, we generated transgenic mice expressing the DQA1*0301 and DQB1*0302 genes from an RA predisposing haplotype (DQ8/DR4Dw4). The transgenes were introduced into mouse class II-deficient H-2Ab ~ mice, and their susceptibility to experimental collagen-induced arthritis was evaluated. The HLA-DQ8+, H-2Ab ~ mice displayed good expression of the DQ8 molecule, while no surface expression of endogenous murine class II molecules could be detected. The DQ8 molecule also induced the selection of CD4+ T cells expressing a normal repertoire of Vβ T cell receptors. Immunization of HLA-DQ8+, H-2Ab ~ mice with bovine type II collagen (CII) induced a strong antibody response that was cross-reactive to homologous mouse CII. Also, in vitro proliferative responses against bovine CII, which were blocked in the presence of an antibody specific for HLA-DQ and mouse CD4, were detected. Finally, a severe polyarthritis developed in a majority of HLA-DQ8+, H-2Ab ~ mice, which was indistinguishable from the disease observed in arthritis susceptible B10.T(6R) (H-2Aq) controls. In contrast, HLA-DQ8-, H-2Ab ~ fullsibs did not generate CII antibody and were completely resistant to arthritis. Therefore, these results strongly suggest that HLA-DQ8 molecules contribute to genetic susceptibility to arthritis and also establish a novel animal model for the study of human arthritis.

It is widely accepted that a strong genetic component contributes to the susceptibility or resistance to certain human autoimmune diseases (1). Attempts to identify the particular genes involved in these disorders has been an area of major focus for many laboratories, and inroads have been clearly made. Among the numerous genes studied, one group that has garnered much attention are the genes encoding the class I and class II molecules of the HLA complex. Located on the short arm of chromosome 6, the primary function of HLA class I and II molecules is to bind and present processed antigenic peptides to T cells bearing receptors specific for the peptide–HLA complex. This presentation event plays a pivotal role in shaping the cellular immune repertoire and dictating the nature and scope of the immune response against a given antigen (2).

A role for HLA molecules in the etiology of autoimmune disease derives from genetic studies showing a clear association between the presence or absence of certain HLA class I or II alleles, as well as increased or decreased susceptibility to a particular autoimmune disorder. A disease with a strong autoimmune foundation and HLA class II association is rheumatoid arthritis (RA)1. In Caucasians, genetic studies initially showed a high prevalence of the HLA-DR4Dw4 subtype among RA patients (3). Work using different ethnic groups, however, has implicated other

1Abbreviations used in this paper: CIA, collagen-induced arthritis; CII, type II collagen; RA, rheumatoid arthritis.
HLA-DR alleles in RA susceptibility. Subsequent analysis of the HLA-DR alleles associated with RA revealed that a large proportion of RA patients who express the Dw1, Dw4, Dw14, Dw15, or Dw16 haplotype bear a similar amino acid sequence within the 67-74 region of the HLA-DRB1 molecule (4, 5). This similarity prompted investigators to put forth the “shared epitope” hypothesis for RA susceptibility (6). The hypothesis states that a critical element in conferring an increased risk for RA susceptibility is possession of this common epitope within the HLA-DRB1 molecule. Whether strictly accurate or not, this hypothesis has had a tremendous influence in accelerating progress toward the molecular definition of RA susceptibility. However, the functional role for the “shared epitope” in RA remains obscure.

In general, HLA genes are inherited as a haplotype with a low recombination frequency between loci (7). Also, linkage disequilibrium between certain DQB genes and particular HLA-DR genes have revealed, for some disorders, a much stronger association at the DQ than DR locus. For instance, a strong association of HLA-DQ8 and DQ6 with susceptibility and protection, respectively, in type I diabetes has been demonstrated (8, 9). Also, HLA-DQ polymorphisms have been shown to play a role in influencing autoantibody levels in primary Sjogren’s syndrome (10) and myasthenia gravis (11). Most recently, Welsh et al. (12) demonstrated an association between susceptibility to the disease alopecia areata and HLA-DQ8. In Caucasoids, the DQB0301 (DQ7) allele has been shown to be associated with a majority of HLA-DR4 alleles (6), while DQB0302 (DQ8) is in linkage disequilibrium with DR4 among the Asian population (13). More importantly, data exists showing an increased frequency of a particular DQ allele, such as DQ7, in RA patients (14, 15). Also, an interesting study analyzing Indian patients with RA showed that 100% of the patients possessed the DQ8 allele versus 33.3% in normal subjects (13). On balance, these data support a role for HLA-DQ alleles in genetic predisposition to RA.

To better understand the role of HLA molecules in autoimmunity disease, researchers have developed transgenic animals expressing disease-associated HLA gene products. Success using this approach was illustrated by the development of a transgenic rat model for HLA-B27–associated spondyloarthropathy (16). Initial studies with transgenic mice expressing human class II genes demonstrated that the HLA class II molecules are functional as shown by the in vitro proliferative responses in mice bearing disrupted murine class II gene products should, in theory, lead to the preferential development of a population of human class II–restricted T cells. With this hypothesis in mind, therefore, we introduced a transgene encoding the α and β chains of the HLA-DQB1*0302, DQA1*0301 molecule (HLA-DQ8) into mouse class II–deficient H-2Abb mice. The HLA-DQ8 molecule was chosen because of its reported association with various autoimmune disorders, such as RA (13). Given the presence of local immune reactivity against type II collagen (CII) within inflamed synovial tissue of some RA patients (25), we evaluated the susceptibility of HLA-DQ8+,H-2Abb mice to the experimental disease collagen-induced arthritis (CIA), a model that bears many similarities to human RA (26). We report here that expression of the HLA-DQ8 molecule in murine class II–deficient H-2Abb mice leads to the selection and restoration of a peripheral CD4+ T cell compartment. Moreover, immunization of HLA-DQ8+,H-2Abb mice with CII induced a vigorous anti-CII response that culminated in a severe inflammatory polyarthritis in a majority of the animals. These studies are the first report demonstrating the induction of a pathogenic immune response in human class II transgenic mice, and they establish a unique animal disease model to dissect the role of HLA class II molecules in human polyarthritis.

Materials and Methods

Mice. All mice used in this study were bred and maintained in the pathogen-free Immunogenetics Mouse Colony of the Mayo Clinic. Generation of B10.M-DQ8 transgenic mice was achieved as follows: briefly, cosmids H11A and X10A, which contain the DQA*0301 and DQB*0302 genes, respectively, were provided by Dr. Jack Strominger (Harvard University, Cambridge, MA). Clone H11A is a 30-kb DNA fragment containing the DQA*0301 gene and the DQB*0302 gene with a truncated promoter. Clone X10A is a 38-kb DNA fragment containing the center, DQB*0302 gene (27). The cosmids inserts were released by Sall digestion, purified, and microinjected into (CBA/J × B10.M)F2 embryos, as previously described (28). Transgene-positive founders were identified by Southern blot analysis of tail DNA and subsequently mated to B10.M mice. The HLA-DQ8 transgenes were introduced into H-2Abb mice as described in Fig. 1. Mouse class

28 Experimental Arthritis in HLA-DQ8-transgenic Mice
II-deficient H-2Abβ mice were kindly provided by Drs. Diane Mathis and Christophe Benoist (INSERM, Salzburg, France). Mice of both sexes were used in this study, and they were 8–12 wk old at the start of the experiment.

**Flow Cytometry.** Analysis of HLA-DQ8, murine class I, and class II expression on PBL was achieved as follows: mice were bled via the tail artery and the white cell fraction was isolated by centrifugation over a Ficoll-Hypaque gradient. After extensive washing in PBS containing 1% BSA and 0.1% sodium azide (PBS/BSA), the cells were incubated with one of the following mAbs: IVD12, anti-HLA-DQ (29); AF6-120, anti–H-2Abβ (30); 7–16.7 anti–H-2Aαβ (kindly provided by Dr. David McKean, Mayo Clinic, Rochester, MN); Y17, anti–H-2Eβb (31); and 28–14–8S, anti–H-2Dβ (32). After a 30-min incubation, the cells were washed in PBS/BSA and then incubated with an FITC-conjugated goat Fab’2 fragment specific for mouse IgG (Accurate Chemical & Science Corp., Westbury, NY). The cells were subsequently washed and fixed with 1% formalin before analysis. To determine the level of CD4+ and Vβ TCR–positive cells, the mice were killed and the peripheral lymph nodes were removed and homogenized to dislodge the cells. The lymph node cells (LNC) were then extensively washed with PBS/BSA, and ~10^6 cells were incubated with one of the following Vβ TCR–specific mAbs: KT4, rat anti–Vβ4 (33); MR9–4, mouse anti–Vβ8.1.2 (34); F23.2, mouse anti–Vβ8.2 (35); 14–2, rat anti–Vβ14 (36); and KM 114, rat anti–CD44 (37). After a 30-min incubation, the cells were washed then incubated with FITC-conjugated Fab’2 fragments specific for either mouse or rat IgG or rat IgM (Accurate Chemical). After 30 min, the cells were washed and then incubated with a 1:1 mixture of PE- and red 613-conjugated mAb specific for mouse CD4 and CD8, respectively (GIBCO BRL, Gaithersburg, MD). Finally, the samples were washed and fixed with 1% formalin. Both single- and three-color fluorescent analysis were performed using a FACS® vantage flow cytometer (Becton Dickinson & Co., Mountain View, CA).

**Induction of CIA.** Highly purified native bovine and mouse CII were isolated as described elsewhere (38). Lyophilized bovine CII was dissolved overnight at 4°C in 0.01 N acetic acid then emulsified at a 1:1 ratio with CFA (Mycobacterium tuberculosiis strain H37 Ra; Difco Laboratornes, Detroit, MI). The animals were then immunized with 100 µl of 100 mg bovine CII per animal. Animals were subsequently injected with 100 µl of 100 mg bovine CII emulsified in IFA. The mice were carefully monitored for three to four times per week for the onset and progression of CIA from the beginning of the experiment until its termination at 12 wk after immunization. The severity of arthritis was evaluated as previously described (39) based on a grading system for each paw as follows: 1 = redness or swelling in paw or toes; 2 = severe swelling and/or joint deformity; 3 = joint ankylosis. The score per paw was summed to give a maximal possible score of 12 per animal. The mean CIA score per group was determined using arthritic animals only.

**Anti-CII ELISA.** The level of IgG antibody reactive against bovine and mouse CII was determined using a highly sensitive ELISA technique (40). Briefly, day 35 sera from bovine CII-immunized mice was diluted in PBS containing 0.05% Tween 20 and 0.2 M NaCl (PNT). Microtiter wells were coated with either bovine or mouse CII dissolved in KPO4 buffer, pH 7.6, at 300 µl per well (20 µg/ml of CII) overnight at 4°C. After washing with PNT, the wells were blocked with 1% BSA in PNT. Duplicate serial fourfold dilutions of sera (1:100 to 1:6,400) were then added to the wells and incubated at 4°C overnight. The wells were washed, incubated with a peroxidase-conjugated goat anti-mouse IgG (Organon Teknika Corp., West Chester, PA), and the color was developed using O-phenylenediamine. The amount of total IgG anti-CII antibody was calculated by comparing OD values with a high titer standard sera arbitrarily determined to contain 100 CII antibody U/ml sera.

**In Vitro LNC Proliferation.** Lyophilized bovine CII was dissolved in 0.1 N acetic acid overnight at a concentration of 2 mg/ml and then emulsified 1:1 with CFA. Mice received an intradermal injection of 100 µl of cold emulsion at the tail base and 50 µl in each hind footpad for a total of 200 µg bovine CII per mouse. 10 d later, the animals were killed, and the draining LNC were isolated and suspended to a concentration of 10^6/ml in RPMI 1640 medium (GIBCO BRL) supplemented with 5% heat-inactivated horse serum, 25 nM Hepes buffer, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. 100 µl of the cell suspension, containing 10^6 cells, were added per flat-bottom microtiter wells (Corning Glassware, Corning, NY), and they were subsequently challenged with 100 µl media alone or 50 µg/ml heat-denatured (45°C for 5 min) bovine CII. For in vitro–blocking studies, 20 µl per well of serial fivefold diluted (1:10 to 1:1,250) culture supernatant containing mAb specific for HLA-DQ (IVD–12), H-2Aαβ (7–17.7), H-2Eβb (Y17), mouse CD4 (GK 1.5), mouse CD8 (53.7.72), control mouse IgG (M40.5, anti–HLA-A, -B, and –C), or rat IgG (M5/114, anti–H-2Abβ) were added to the LNC in the presence of 50 µg/ml bovine CII. The cells were incubated 48 h at 37°C with 5% CO2 and 95% air and then pulsed with 1.8 µCi of [3H]thymidine during the final 18 h of culture. The cells were harvested on glass fiber filters, and the extent of [3H]thymidine uptake was determined using a liquid scintillation counter (model 3801; Beckman Instruments, Palo Alto, CA).

**Histologic Evaluation.** Mice were killed at the end of the experiment and histological sections of the hind limbs were prepared by the Pathology Department of the Mayo Clinic. Limbs were dissected, and the joints were decalcified for 3–4 d and then embedded in paraffin blocks. Sections that were ~6 µm thick were cut for each joint at differing intervals, mounted, and stained with hematoxylin and eosin before analysis.

**Statistical Analysis.** Statistical differences in the mean arthritic severity and mean day of CIA onset between groups were determined using the nonparametric Mann-Whitney U test.

**Results**

**Introduction and Expression of the HLA-DQ8 Molecule in H-2Abβ Mice.** In an effort to understand the role of HLA class II molecules in RA, we introduced the RA-associated HLA-DQB1*0302 and DQA1*0301 genes (HLA-DQ8) into mouse class II–deficient H-2Abβ mice. Fig. 1 illustrates the strategy to derive the HLA-DQ8+,H-2Abβ line. Briefly, B10.M (H-2β2) mice bearing a transgene encoding the DQ0301 α and DQ0302 β chain genes of the HLA-DQ8 molecule were mated with H-2Abβ mice (41). The offspring were screened for HLA-DQ8 expression by flow cytometric analysis of PBL using the HLA-DQ specific mAb IVD12. The HLA-DQ8+,H-2Abβ progeny were intercrossed, and segregation of the H-2Abβ and H-2Abα gene was monitored via fluorescent analysis using the H-2Abβ-specific mAb 3F-12 and the H-2Abα-specific mAb AF6–120. The offspring that typed as HLA-DQ8+,H-2Abβ/0 and HLA-DQ8+,H-2Abα/0 were selected and intercrossed.
to develop the HLA-DQ8+,H-2Ab0 and HLA-DQ8-,H-2Ab0 lines.

Fig. 2 A shows that transgenic HLA-DQ8+,H-2Ab0 mice expressed the HLA-DQ8 molecule on ~25% of the PBL population, with a maximum level of 40% in some animals. Given the presence of intracytoplasmic H-2A\textsubscript{a} and H-2E\textsubscript{b} chains in H-2Ab0 mice (41, 42), it was possible that hybrid A\textsubscript{a}-DQ8\textsubscript{b} or DQ8\textsubscript{a}-E\textsubscript{b} molecules were also present in the HLA-DQ8+,H-2Ab0 line. To eliminate this possibility, PBL from HLA-DQ8+,H-2Ab0 mice were analyzed for surface expression of the H-2A\textsubscript{a} and H-2E\textsubscript{b} molecule. Use of the H-2A\textsubscript{a}-specific mAb 7-16.17 did not detect expression of H-2A\textsubscript{a} in HLA-DQ8+,H-2Ab0 animals (Fig. 2 B). Surface expression of the H-2E\textsubscript{b} molecule using the H-2E\textsubscript{b}-specific mAb Y17 was similarly undetected. The Y17 mAb, however, reacted strongly with PBL from positive control B10.Ea\textsuperscript{k} transgenic mice, which express the H-2E\textsubscript{b} chain due to the presence of the H-2E\textsubscript{a}\textsuperscript{b}.

Figure 1. Schematic illustration of the generation of HLA-DQ8+,H-2Ab0 mice. Segregation of the HLA-DQ8 transgene was monitored by flow cytometric analysis of PBL using the HLA-DQ8-specific mAb IVD12. Segregation of the mutant H-2A\textsubscript{a} gene was also evaluated by flow cytometry by monitoring the expression of the H-2A\textsubscript{a} and H-2Ab0 molecules using the mAbs 3F-12 and AF6-120, respectively.

Figure 2. Analysis of HLA-DQ8 and murine MHC expression in transgenic HLA-DQw8+,H-2Ab0 mice. PBL from HLA-DQ8+,H-2Ab0 mice, B10, HLA-DQ8-,H-2Ab0, and B10.Ea\textsuperscript{k} animals were analyzed by flow cytometry for surface expression of the molecules HLA-DQw8 (A), H-2A\textsubscript{a} (B), and H-2E\textsubscript{b} (C). The methodology and antibodies used for analysis are described in detail in Materials and Methods.
molecule (Fig. 2 C). As expected, HLA-DQ8+,H-2Ab0 animals did not express the H-2Aα chain, and expression of the MHC class I molecule D b was present at a level similar to HLA-DQ8+,H-2Ab0 and B10 mice (data not shown). Thus, cell surface expression of the DQ8 molecule requires both the DQ0301 α and DQ0302 β chains.

Selection of Peripheral CD4+ T Cells in HLA-DQ8+,H-2Ab0 Mice. A hallmark feature of class II-deficient H-2Ab0 mice is a paucity of peripheral CD4+ T cells. In general, H-2Ab0 animals contain <5% CD4+ cells within the lymph nodes, and the majority of these cells express the CD44 (Pgp-1) antigen (41). To determine if expression of the human HLA-DQ8 molecule in H-2Ab0 mice induces the selection of CD4+ T cells, LNC from HLA-DQ8+,H-2Ab0 and negative littermate HLA-DQ8-,H-2Ab0 mice were analyzed for the expression of CD4 and CD44 molecules. As a comparative control, mouse class II-sufficient B10.T(6R) mice, which bear the collagen arthritis susceptible H-2A1 haplotype (48), were also studied. Table 1 shows that HLA-DQ8+,H-2Ab0 mice displayed a threefold increase in the level of CD4+ LNC versus HLA-DQ8-,H-2Ab0 animals. Moreover, the frequency of double-positive CD4/CD44 (Pgp-1) cells in the HLA-DQ8+,H-2Ab0 line closely resembled mouse class II-sufficient B10.T(6R) mice. Similar to previous reports (41), ~75% of the CD4+ cells in HLA-DQ8+,H-2Ab0 mice expressed the CD44 molecule. Single positive HLA-DQ8α or HLA-DQ8β transgenic mice did not display an increase in CD4+ LNC, thereby illustrating the importance of appropriate HLA-DQ8 α and β pairing in the restoration of the CD4+ T cell compartment. Analysis of Vβ TCR expression within the CD4+ population showed that HLA-DQ8+,H-2Ab0 mice expressed a variety of Vβ TCRs. In addition, distinct differences in the level of some CD4+/Vβ TCR+ cells, such as Vβ5 and Vβ8.2, was detected between HLA-DQ8+,H-2Ab0 mice and transgene-negative littermates (Table 1). On balance, these data suggest that expression of the HLA-DQ8 molecule in H-2Ab0 mice induces the selection of CD4+/Vβ TCR+ cells, which are distinct from the small population of CD4+ lymphocytes normally present in class II-deficient H-2Ab0 mice.

Production of CII Antibody in HLA-DQ8+,H-2A1 Mice. Typically, murine CIA is induced in susceptible strains of mice bearing the H-2α or H-2β haplotype after immunization with CII in CFA (39, 43). Both humoral and cellular immune responses against the CII molecule is essential for the development of severe chronic arthritis (44) and induction of CIA is critically dependent upon the presence of CII-specific CD4+, TCR αβ+ T cells (45, 46).

Given the putative association of the HLA-DQ8 allele in certain RA populations (13), it was possible that the HLA-DQ8+,H-2Ab0 mice possessed the potential to mount a pathogenic immune response against CII, a molecule implicated in RA (25, 47). Therefore, HLA-DQ8+,H-2Ab0 animals, along with transgene-negative littermates, positive control B10.T(6R) and negative control H-2Ab0 mice, were immunized with bovine CII in CFA and monitored for the generation of a CII-specific antibody. Analysis of sera 35 d after immunization by ELISA revealed that HLA-DQ8+,H-2Ab0 mice mounted a strong IgG antibody response against bovine CII (Fig. 3). The level of bovine CII antibody was comparable to arthritis-susceptible B10.T(6R) controls, and no CII reactivity was detected in sera from HLA-DQ8-,H-2Ab0 littermates or H-2Ab0 animals. Moreover, HLA-DQ8+,H-2Ab0 sera was highly cross-reactive against mouse CII. Like the reactivity against bovine CII, the level of mouse CII-reactive antibody was similar to B10.T(6R) sera, and the extent of cross-reactivity in both strains was >50%. Although many mouse strains of various H-2 haplotypes can mount antibody responses against a heterologous CII species, strong reactivity against homologous mouse CII is limited to strains that bear a CII-susceptible H-2 haplotype (48). Thus, the generation of mouse CII reactive antibody in HLA-DQ8+,H-2Ab0 mice suggested that these animals may have the potential to develop collagen arthritis.

In Vitro Proliferative Response of HLA-DQ8+,H-2Ab0 LNC against Bovine CII. To further explore the immune response of HLA-DQ8+,H-2Ab0 mice against bovine CII, in vitro LNC proliferative responses were assessed. Fig. 4 A shows that LNC from bovine CII–immunized HLA-DQ8+,H-2Ab0 mice mounted a detectable proliferative response against bovine CII in vitro (stimulation index >2.5), which is comparable to control B10.T(6R) mice. Also, addition of mAb specific for the human HLA-DQ or mouse CD4 molecules inhibited the anti–bovine CII response by >90%
Bovine CI!

Figure 3. Measurement of CII antibody in HLA-DQ8+,H-2Ab0 mice. Transgenic HLA-DQ8+,H-2Ab0 mice, negative littermate HLA-DQ8+,H-2Ab0 animals, as well as control H-2Ab0 and B10.T(6R) mice, were immunized on day 0 with 100 μg bovine CII in CFA, and they were boosted with 100 μg bovine CII in IFA on day 28. Sera were collected on day 35, and the level of IgG antibody specific for bovine and mouse CII determined by ELISA. The data were obtained using 5–15 animals per group.

(Fig. 4 B). No inhibition was observed in cultures containing mAb specific for mouse H-2A, b, H-2E, b, or CD8. These data indicate that CD4+ T cells recognize and proliferate to bovine CII presented by the HLA-DQ8 molecule.

Development of Arthritis in HLA-DQ8+,H-2Ab0 Mice. To determine if the immune response against bovine CII was arthritogenic, bovine CII-immunized HLA-DQ8+,H-2Ab0 mice, HLA-DQ8-,H-2Ab0 littermates, B10.T(6R) animals, and H-2Ab0 mice were monitored for the onset and development of CIA. As shown in Table 2, experiment 1, HLA-DQ8+,H-2Ab0 mice were highly susceptible to CIA. Among the 18 animals tested, 12 developed a noticeable arthritis in ~5 wk after immunization which progressed to severe arthritis and persisted until the termination of the experiment. In general, both HLA-DQ8+,H-2Ab0 and B10.T(6R) animals developed severe inflammation, swelling, and joint deformity in afflicted limbs (Fig. 5, B and C). Histologic examination of arthritic hind limbs showed that the nature of the inflammatory infiltrate was similar in both strains; a marked synovitis consisting of synovial cell hyperplasia, infiltration of mononuclear cells, and erosion of articular cartilage and subchondral bone was observed (Fig. 5, E and H versus F and I). Transect-negative HLA-DQ8+,H-2Ab0 littermates showed no signs of clinical arthritis and histologic evidence of synovial inflammation was not detected (Fig. 5, A, D, and G). Serum analysis revealed that both arthritic and nonarthritic HLA-DQ8+,H-2Ab0 and B10.T(6R) mice possessed the bovine CII–specific IgG antibody that was cross-reactive against mouse CII. Also, no significant correlation in the level of CII antibody and the development or severity of CIA was detected in either the HLA-DQ8+,H-2Ab0 or B10.T(6R) strains (data not shown).

To confirm our observations, a second HLA-DQ8+,H-2Ab0 line that expresses the HLA-DQ8 molecule on ~15% of PBL were immunized with bovine CII and monitored for CIA. Once again, a majority of HLA-DQ8+,H-2Ab0 mice developed CIA (Table 2, experiment 2). Interestingly, the onset of clinical arthritis was significantly earlier in this HLA-DQ8+,H-2Ab0 line compared to B10.T(6R) mice. Likewise, the severity of CIA was significantly greater in arthritic HLA-DQ8+,H-2Ab0 animals. Transect-negative HLA-DQ8+,H-2Ab0 littermates did not develop CIA, and antibody against bovine CII was not detected (data not shown). Therefore, these findings demonstrate that expression of the DR4-linked HLA-DQ8 molecule in class II—
Table 2. Susceptibility to CIA in HLA-DQ8+, H-2Ab− Mice

| Strain                  | Percent HLA-DQ8+ cells in PBL (x ± SD) | Clinical arthritis (positive/total) | Percent | Day of onset (x ± SE) | Arthritis score (x ± SE) |
|-------------------------|----------------------------------------|------------------------------------|---------|-----------------------|--------------------------|
| **Experiment 1**        |                                        |                                    |         |                       |                          |
| HLA-DQ8+, H-2Ab− (line 1) | 30.8 ± 4.9                             | 12/18                              | 67      | 39 ± 3               | 6.7 ± 0.8                |
| HLA-DQ8−, H-2Ab0        | NA*                                   | 0/10                               | 0       | —                    | —                        |
| H-2Ab0                  | NA                                     | 0/10                               | 0       | —                    | —                        |
| B10.T(6R)               | NA                                     | 17/23                              | 74      | 41 ± 4               | 5.8 ± 0.8                |
| **Experiment 2**        |                                        |                                    |         |                       |                          |
| HLA-DQ8+, H-2Ab− (line 2) | 15.4 ± 4.8                             | 5/7                                | 71      | 25 ± 1|                      | 9.0 ± 0.6                |
| HLA-DQ8−, H-2Ab0        | NA                                     | 0/5                                | 0       | —                    | —                        |
| B10.T(6R)               | NA                                     | 8/10                               | 80      | 43 ± 4|                      | 5.6 ± 1.2                |

*Mice were immunized with 100 μg bovine CII in CFA on day 0 and boosted with 100 μg bovine CII in IFA on day 28. All animals were monitored regularly for the onset and development of CIA until the termination of the experiment at 12 wk after immunization.

1 Mean arthritic score was calculated at the end of the study using arthritic animals only.

1 Not applicable.

*P < 0.05

deficient H-2Ab− mice confers susceptibility to induction of CIA.

Discussion

The data presented here comprise the first report demonstrating the induction of a pathogenic autoimmune response in mice expressing a human MHC class II molecule. By introducing an HLA-DQ8 transgene into murine class II-deficient H-2Ab− mice, we observed that this human class II molecule induced selection of a CD4+ T cell population and conferred susceptibility to the RA-like disease CIA. Our findings establish a novel animal model to study autoimmune arthritis, as well as a means to functionally address a possible role for HLA-DQ molecules in genetic predisposition to RA.

Previous studies suggested that the inability of the mouse CD4 coreceptor to interact efficiently with human class II would limit the experimental usefulness of HLA class II transgenic mice. The results in this study clearly demonstrate that mouse CD4 coreceptors can sufficiently interact with the HLA-DQ molecule in the absence of endogenous mouse class II. Thus, the interaction between mouse CD4 and human class II appears to be affinity oriented, and the absence of mouse class II molecules within the thymus of HLA-DQ8+, H-2Ab− mice successfully forces the selection of HLA-DQ8 restricted CD4+ cells. Although the work of Vignali et al. (21) suggested a species-specific barrier in the binding of CD4 and class II molecules, it is possible that the structural constraints that hinder binding between murine CD4 and human class II may be limited to HLA-DR, and not HLA-DQ molecules. Altmann et al. (20), however, recently reported that HLA-DR1−transgenic mice mount equipotent HLA-DR1−restricted T cell responses to the 139-154 peptide of human myelin basic protein. Therefore, together with our observations, it is clear that in certain cases murine CD4 can efficiently interact with HLA-class II molecules. Previous studies have identified regions within the β2 domain of the MHC class II β chain as putative CD4 interaction sites (49, 50). Analysis of these interaction sites within the β2 domain (amino acid residues 110, 141, and 142) show that this region of the HLA-DQ8 chain is identical to the mouse H-2Aβ molecule, while the HLA-DRβ chain differs at residues 110 and 141 (David, C. S., unpublished observations). Clearly, studies examining the processes mediating the selection of CD4+ T cells in HLA-DQ8+, H-2Ab− mice may identify additional regions on MHC class II molecules involved in CD4 binding or shed light on the role of CD4 signalling in T cell development.

The level of peripheral CD4+ T cells in HLA-DQ8+, H-2Ab− mice was lower than mouse class II−sufficient, CIA-susceptible B10.T(6R) mice. The frequency of CD4+ cells in HLA-DQ8+, H-2Ab− animals, however, was threefold higher than HLA-DQ8+, H-2Ab− littermates. Moreover, the CD4+ populations were distinct between the two lines based on expression of the CD44 molecule. When immunized with bovine CII, strong antibody responses against CII were measured in HLA-DQ8+, H-2Ab− mice. In addition, in vitro proliferative responses against bovine CII that were effectively inhibited by mAbs specific for HLA-DQ and mouse CD4 were detected. These results strongly suggest that the response against bovine CII is mediated by CD4+ T cells restricted by the HLA-DQ8 molecule. Of
Figure 5. Clinical and histologic presentation of collagen arthritis in HLA-DQ8+, H-2Ab~ mice. (A–C) The appearance of a normal rear paw from a bovine CII-immunized HLA-DQ8+, H-2Ab~ mouse (A) contrasted with arthritic paws from an HLA-DQ8+, H-2Ab~ animal (B) and a positive control B10.T(6R) mouse (C). (D–I) Cross-sections of the hind foot from an arthritis-resistant HLA-DQ8+, H-2Ab~ mouse (D and G) compared with an arthritic joint from HLA-DQ8+, H-2Ab~ (E and H) and B10.T (6R) animals (F and I). D and G illustrate normal synovial lining, while E, F, H, and I show regions of mononuclear cell infiltration of the synovium with pannus formation and cartilage and subchondral bone erosions. The magnifications of each section are noted in the lower right-hand corner. The areas of tissue illustrated in G, H, and I are higher magnifications of the boxed areas in D, E, and F, respectively.

paramount importance was the finding that the anti-CII response was arthritogenic. A severe inflammatory polyarthritis developed in a majority of bovine CII-immunized HLA-DQ8+, H-2Ab~ mice. The histological changes in the arthritic lesions were indistinguishable from CIA-susceptible B10.T(6R) animals. The cellular infiltrate consisted primarily of mononuclear cells and synoviocytes with few polymorphonuclear cells, and they closely resembled the lesions found in RA. This observation indirectly suggests that the mechanisms underlying the arthritic process in
HLA-DQ8+H-2Abb mice are similar to those responsible for prototypical CIA. Also, the susceptibility of HLA-DQ8+H-2Abb mice to CIA is reproducible as illustrated by the development of arthritis in a second group of HLA-DQ8+,H-2Abb animals, which expressed a lower level of HLA-DQ8+ cells in PBL. Interestingly, this line of mice developed an accelerated and more severe CIA versus B10.T(6R) controls. Whether this difference was merely an exceptional observation or a distinct characteristic of this particular HLA-DQ8+,H-2Abb line requires further investigation. Nonetheless, the data clearly show that expression of the HLA-DQ8 molecule confers susceptibility to CIA. Finally, preliminary studies indicate that polymorphisms within the HLA-DQ molecule dictates susceptibility to CIA; bovine CII immunized HLA-DQ6+,H-2Abb mice mount weak responses to CII and do not develop CIA. Interestingly, this line of mice (Nabozny, G.H., and C.S. David, unpublished observations). Given the lack of association between HLA-DQ6 and human RA, such data is of interest.

To date, identification of the antigens and/or agents responsible for the manifestations in RA have not been unequivocally demonstrated. Candidate antigens include tissue proteoglycans (51), heat shock proteins, (52) as well as CII (53). The relative role for immunity against CII in RA pathogenesis remains controversial; however, extant in the literature are reports clearly showing the presence of CII reactive T cells (54), B cells (25, 47), and antibody (53) within inflamed synovium of RA patients. Such anti-CII immunity is not observed in patients with other inflammatory joint diseases such as psoriatic arthritis (25). In addition, CII-reactive antibody from RA patients can passively induce an inflammatory arthritis in naive mice, thus illustrating its arthritogenic potential (55). Our finding that the HLA-DQ8 molecule can restrict an arthritogenic CII response is intriguing and, at the very least, points to a possible role for CII as an antigenic target in RA. Clearly, identification of the precise regions on CII responsible for CIA in the HLA-DQ8+,H-2Abb strain may provide insight concerning the contribution of CII immunity in arthritis.

The successful induction of arthritis in mice expressing an HLA-DQ molecule raises questions regarding the role of HLA-DQ alleles in human arthritis. Considered sine qua non for RA susceptibility, a majority of recent research efforts have focused upon understanding the significance of the HLA-DR-associated "shared epitope" in RA. In contrast, less extensive studies have dealt with HLA-DQ associations in this disease. Preliminary studies using HLA-DR4 human CD4 double-transgenic mice suggests that these animals can be induced to mount HLA-DR4-restricted responses against CII, but fail to develop arthritis (56). Recently, our laboratory has hypothesized a more prominent role for HLA-DQ alleles in RA (57). The generation of CIA in the HLA-DQ8+,H-2Abb transgenic mice provides a useful tool to test such an hypothesis. It is possible that our findings may serve as a model for a subset of HLA-DQw8+ RA patients with strong CII immunity or as a broader model for RA in general. Regardless, our results suggest that a more thorough examination regarding a potential contribution for HLA-DQ alleles in RA is warranted. Quite possibly, an extended haplotype consisting of a particular HLA-DQ and HLA-DR molecule may ultimately be shown to be a necessary factor for the induction and progression of this disease.

The potential research opportunities available via the use of HLA-DQ8+,H-2Abb mice are exciting. The use of these animals as a novel model to study autoimmune arthritis is obvious. In addition to CII, other potential autoantigens and arthritogens such as bacterial and viral superantigens, heat shock proteins, and extracellular matrix proteins can be tested in these mice. Also, this strain provides a useful vehicle to assess the therapeutic efficacy of human HLA class II-blocking agents in the modulation of a pathogenic in vivo immune response. Likewise, identification of TCR usage against CII in HLA-DQ8+,H-2Abb mice may open the door towards identifying homologous human TCRs important in RA. Finally, introduction of additional HLA-DQ or HLA-DR molecules in these mice will provide an opportunity to study the interactions and influences of multiple human class II alleles on a defined immune response. Hopefully, our findings have laid the foundation for an exciting and fruitful new era in human autoimmune disease research.

We are indebted to Dr. Jack Strominger for providing the HLA-DQ8 cosmids and Priess cell lines, and to Drs. Christophe Benoist and Diane Mathis for providing the mouse class II-deficient H-2Abb mice. We also thank Suresh Savarirayan for producing the transgenic mice, Julie Hanson and her crew for excellent mouse breeding and husbandry, and Michele Smart and Karen Hodgson for technical assistance.

These studies were supported by National Institutes of Health (NIH) grants AR30752 and AI14764. G. H. Nabozny is the recipient of a postdoctoral fellowship from the Arthritis Foundation. J. M. Baisch was supported by NIH training grant CA09127. The laboratory of Dr. Marie M. Griffiths is supported by research funds from the Department of Veteran's Affairs.

Address correspondence to Dr. Chella S. David, Department of Immunology, Mayo Graduate School of Medicine, Rochester, MN 55905. Dr. J. M. Baisch's present address is Gene Screen Inc., 2600 Stemmons Freeway, Suite 133, Dallas TX 75207. Dr. S. Cheng's present address is DEKALB Poultry Research Inc., 3100 Sycamore Road, DeKalb, IL 60115. Dr. G. H. Nabozny's present address is Boehringer Ingelheim, Inc., 900 Ridgebury Road, Ridgefield, CT.
References

1. Seldin, M.F. 1992. New approaches to the genetics of autoimmune disease. Concepts Immunopathol. 8:18–37.
2. German, R.N. 1993. Antigen processing and presentation. In Fundamental Immunology. W.E. Paul, editor. Raven Press Ltd., New York. pp. 629–676.
3. Stastny, P. 1978. Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. N. Engl. J. Med. 298:869–872.
4. Gregersen, P.K., M. Shen, Q-L. Song, P. Merryman, S. De- gant, T. Seki, J. Macciari, D. Goldberg, H. Murphy, J. Schwenzer, et al. 1986. Molecular diversity of HLA-DR4 haplotypes. Proc. Nat. Acad. Sci. USA. 83:2642–2646.
5. Wilkens, R.F., G.T. Nepom, C.R. Marks, J.W. Nettles, and B.S. Nepom. 1991. Association of HLA-Dw16 with rheumatoid arthritis in Yakima Indians. Further evidence for the “shared epitope” hypothesis. Arthritis Rheum. 34:43–47.
6. Gregersen, P.K., J. Silver, and R.J. Winchester. 1987. The molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum. 30:1205–1209.
7. Begovich, A.B., G.R. McClure, V.C. Suraj, R.C. Helmsch, N. Fildes, T.L. Bugawan, H.A. Erlich, and W. Klint. 1992. Polymorphism, recombination, and linkage disequilibrium within the HLA class II region. J. Immunol. 148:249–258.
8. Todd, J.A., J.I. Bell, and H.O. McDevitt. 1987. HLA-DR beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature (Lond.) 329:599–604.
9. Baish, J.M., T. Weeks, R. Giles, M. Hoover, P. Stastny, and J.D. Capra. 1990. Analysis of HLA-DR4 genotypes and susceptibility in insulin-dependent diabetes mellitus. N. Engl. J. Med. 322:1836–1841.
10. Harley, J.B., M. Reichlin, F.C. Arnett, E.L. Alexander, W.B. Bias, and T.T. Provost. 1986. Gene interaction at HLA-DQA and HLA-DQB loci enhances autoimmune production in primary Sjogren’s syndrome. Science (Wash. DC). 232:1145–1147.
11. Bell, J., L. Rassenti, S. Smoot, K. Smith, C. Newby, R. Hohlfeld, K. Toyka, H. McDevitt, and L. Steinman. 1986. HLA-DR beta-chain polymorphism linked to myasthenia gravis. Lancet. i:1058–1060.
12. Welsh, E.A., H.H. Clark, S.Z. Epstein, J.D. Reveile, and M. Duvic. 1994. Human leukocyte antigen-DQB1*03 alleles are associated with alopecia areata. J. Invest. Dermatol. 103:758–763.
13. Tanega, V., N.K. Mehra, A.N. Chandreshkeran, R.K. Ahuja, Y.N. Singh, and A.N. Malaviya. 1992. HLA-DR4-DQ8, but not DR4-DQw7, haplotypes occur in Indian patients with rheumatoid arthritis. Rheumatol. Int. 11:251–255.
14. Single, D.P., M. D’Souza, D. Reid, W.G. Benson, Y.B. Kasam, and J.D. Adachi. 1987. HLA-DR beta-chain polymorphism in HLA-DR4 haplotypes associated with rheumatoid arthritis. Lancet. i:1118–1120.
15. Lanchbury, J.S., L.I. Sakkas, S.G. Marsh, J.G. Bodmer, K.I. Welsh, and G.S. Panayi. 1989. HLA-DR beta 3.1 allele is a determinant of susceptibility to DR4-associated rheumatoid arthritis. Hum. Immunol. 26:59–71.
16. Hammer, R.E., S.D. Marka, J.A. Richardson, J-P. Tang, and J.D. Taurog. 1990. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2m: an animal model of HLA-B27-associated human disorders. Cell. 63:1099–1112.
17. Lawrance S.K., L. Karlson, J. Price, V. Quaranta, Y. Ron, J. Sprent, and P.A. Peterson. 1989. Transgenic HLA-DR alpha faithfully reconstitutes IE-controlled immune functions and induces cross-tolerance to E alpha in E alpha 0 mutant mice. Cell. 58:583–594.
18. Zhou, P., G.D. Anderson, S. Savarirayan, H. Inoko, and C.S. David. 1991. Thymic deletion of V beta 11+, V beta 5+ T cells in H-2E negative, HLA-DR alpha beta+ single transgenic mice. J. Immunol. 146:854–859.
19. Yamamoto, K., Y. Fukui, Y. Esaki, T. Inamitsu, T. Sudo, K. Yamane, N. Kamikawaji, A. Kimura, and T. Sasazuki. 1994. Functional interaction between human histocompatibility leukocyte antigen (HLA) class II and mouse CD4 molecule in antigen recognition by T cells in HLA-DR and DQ transgenic mice. J. Exp. Med. 180:165–171.
20. Aitken, D.M., D.C. Douek, A.J. Frater, C.M. Hetherington, H. Inoko, and J.I. Elliot. 1995. The T cell response of HLA-DR transgenic mice to human myelin basic protein and other antigens in the presence and absence of human CD4. J. Exp. Med. 181:867–876.
21. Vigani, D.A.A., J. Moreno, D. Schiller, and G.J. Hammerling. 1992. Species-specific binding of CD4 to the beta-domain of major histocompatibility complex class II molecules. J. Exp. Med. 175:925–932.
22. Woods, A., H.Y. Chen, M.E. Trumbauer, A. Sirotina, R. Cummings, and D.M. Zaller. 1994. Human major histocompatibility complex class II restricted T cell responses in transgenic mice. J. Exp. Med. 180:173–181.
23. Fugger, L., S.A. Michle, I. Rulifson, C.B. Lock, and G.S. McDevitt. 1994. Expression of HLA-DR4 and human CD4 transgenes in mice determines the variable region beta-chain T cell repertoire and mediates an HLA-DR restricted immune response. Proc. Natl. Acad. Sci. USA. 91:6151–6155.
24. Yeung, R.S.M., J.M. Penninger, T.M. Kundig, Y. Law, K. Yamamoto; N. Kamikawaji, L. Burkly, T. Sasazuki, R. Fla- vell, P.S. Ohashi, and T.W. Mak. 1994. Human CD4 major histocompatibility complex class II restricted T cell responses in transgenic mice. J. Exp. Med. 180:1911–2012.
25. Tarkowski, A.T., L. Klareskog, H. Carlsten, P. Herberts, and W.J. Koopman. 1989. Secretion of antibodies to types I and II collagen by synovial tissue cells in patients with rheumatoid arthritis. Arthritis Rheum. 32:1087–1093.
26. Courtney, J.S., M.J. Dallman, A.D. Dayan, A. Martin, and B. Mosedale. 1980. Immunization against heterologous type II collagen induces arthritis in mice. Nature (Lond.). 283:666–668.
27. Okada, K., J.M. Boss, H. Prentice, T. Spics, R. Mengler, C. Auffray, J. Lillie, D. Groossberger, and J.L. Strominger. 1985. Gene organization of DC and DX subregions of the human major histocompatibility complex. Proc. Natl. Acad. Sci. USA. 82:3410–3414.
28. Wei, B.-Y., J. Martin, S. Savarirayan, R. Little, and C.S. David. 1990. Transgenic mice and mutants in MHC research. I.K. Egorov and C.S. David, editors. Springer-Verlag, Berlin. pp. 237–246.
29. Giles, R.C., G. Nunez, C.K. Hurley, A. Nunez-Roldan, R. Winchester, P. Stastny, and J.D. Capra. 1983. Structural anal-
ysis of a human I-A homologue using a monoclonal antibody that recognizes an MB3-like specificity. J. Exp. Med. 157: 1461–1470.

30. Loken, M.R., and A.M. Stall. 1982. Flow cytometry as an analytical and preparative tool in immunology. J. Immunol. Methods. 50:R85.

31. Lerner, E.A., L.A. Matis, C.A. Janeway, Jr., P.P. Jones, R.H. Schwartz, and D.B. Murphy. 1980. Monoclonal antibody against an Ig product? J. Exp. Med. 152:1085–1101.

32. Ozato, K., T.H. Hansen, and D.H. Sachs. 1980. Monoclonal antibodies to mouse MHC antigens. II. Antibodies to the H-2Ld antigen, the products of a third polymorphic locus of the mouse major histocompatibility complex. J. Immunol. 125: 2473–2477.

33. Tomonari, K., E. Lovering, and S. Spencer. 1990. Correlation between the Vp4 CDB T-cell population and the H-2d haplotype. Immunogenetics. 31:333–339.

34. Kanagawa, O., Y. Utsunomiza, J. Bill, E. Palmer, M.W. Moore, and F.R. Carbone. 1991. Conformational difference of T cell antigen receptors revealed by monoclonal antibodies to mouse Vp6 T cell receptor for antigen determinants. J. Immunol. 147:1307–1314.

35. Kappler, J.W., U. Staerz, J. White, and P.C. Marrack. 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. Nature (Lond.). 322:35–40.

36. Liao, N.-S., J. Maltzman, and D.H. Raulet. 1989. Positive selection determines T cell receptor Vp14 gene usage by CD8+ T cells. J. Exp. Med. 170:135–143.

37. Miyake, K., K.L. Medina, S.I. Hayashi, S. Ono, T. Hamaoka and P.W. Kincade. 1990. Monoclonal antibodies to Pgp-1/CD44 block lymphohemopoiesis in long term bone marrow cultures. J. Exp. Med. 171:477–489.

38. Griffiths, M.M., E.J. Eichwald, J.H. Martin, C.B. Smith, and C.W. DeWitt. 1981. Immunogenetic control of experimental type II collagen induced arthritis. Arthritis Rheum. 24:781–789.

39. 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. 154:688-700.

40. Griffiths, M.M., G.H. Nabozny, J. Hanson, D.S. Harper, S. McCall, K.G. Moder, H.S. Luthra, and C.S. David. 1994. Collagen-induced arthritis and TCRs in SWR and B10.Q mice expressing and MHC class II-deficient mice. J. Exp. Med. 171:477–489.

41. Cosgrove, D., D. Gray, A. Dierich, J. Kaufman, M. Lemeur, C. Benoist, and D. Mathis. 1991. Mice lacking MHC class II molecules. Cell. 66:1051–1066.

42. Grubbs, M.J., R.S. Johnson, V.E. Papaioannou, and L.H. Gl inorder cell of CD4 T cells in major histocompatibility complex class II–deficient mice. Science (Wash. DC). 253:1417–1420.

43. Wooley, P.H., H.S. Luthra, M.M. Griffiths, J.M. Stuart, A. Huse, and C.S. David. 1985. Type II collagen-induced arthritis in mice IV. Variations in immunogenetic regulation provide evidence for multiple arthritogenic epitopes on the collagen molecule. J. Immunol. 135:2443–2451.

44. Seki, N., Y. Sudo, T. Yoshioka, S. Sugihara, T. Fujitsu, S. Sakuma, T. Ogawa, T. Hamaoka, H. Senoh, and H. Fujiwara. 1988. Type II collagen-induced murine arthritis. I. Induction and perpetuation of arthritis require synergy between humoral and cell-mediated immunity. J. Immunol. 140:1477–1484.

45. Ranges, G.E., S. Siriram, and S.M. Cooper. 1985. Prevention of type II collagen-induced arthritis in mice by treatment with anti-L3T4. J. Exp. Med. 162:1105–1110.

46. Moder, K.G., H.S. Luthra, R. Kubo, M. Griffiths, and C.S. David. 1992. Prevention of collagen induced arthritis in mice by treatment with an antibody directed against the T cell receptor a6 framework. Autoimmunity. 11:219–224.

47. Ronnelid, J., J. Lysholm, A. Engstrom-Laurent, L. Klareskog, and B. Heyman. 1994. Local anti-type II collagen antibody production in rheumatoid arthritics synovial fluid. Evidence for an HLA-DR4-restricted IgG response. Arthritis Rheum. 37:1023–1029.

48. Holmdahl, R., L. Klareskog, M. Andersson, and C. Hansen. 1986. High antibody response to autologous type II collagen is restricted to H-2d. Immunogenetics. 24:84–89.

49. Konig, R., L-Y. Huang, and R.N. Germain. 1992. MHC class II interaction with CD4 mediated by a region analogous to the MHC class I binding site for CD8. Nature (Lond.). 356:796–798.

50. Brown, J.H., T.S. Jardetzky, J.C. Gorga, L.J. Stern, R.G. Urban, J.L. Strominger, and D.C. Wiley. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature (Lond.). 364:33–39.

51. Glant, T., J. Csongor, and T. Szucs. 1980. Immunopathologic role of proteoglycan antigens in rheumatoid joint diseases. Scand. J. Immunol. 11:241–252.

52. Strober, S., and J. Holoshitz. 1990. Mechanisms of immune injury in rheumatoid arthritis: evidence for the involvement of T cells and heat shock protein. Immunol. Rev. 118:233–255.

53. Terato, U., Y. Shinozuru, K. Katayama, Y. Takemitsu, I. Yamashita, M. Miyata, K. Fujii, M. Sagara, S. Kobayashi, M. Goto, et al. 1990. Specificity of antibodies to type II collagen in rheumatoid arthritis. Arthritis Rheum. 33:1493–1500.

54. Londei, M., C.M. Savill, A. Verhoef, F. Brennan, Z.A. Leech, V. Duance, R.N. Maini, and M. Feldmann. 1989. Persistence of collagen type II specific T-cell clones in the synovial membrane of a patient with rheumatoid arthritis. Proc. Nat. Acad. Sci. USA. 86:636–640.

55. Wooley, P.H., H. S. Luthra, S.K. Singh, A.R. Huse, J.M. Stuart, and C.S. David. 1984. Passive transfer of arthritis to mice by injection of human anti-type II collagen antibody. Mayo Clinic Proc. 59:737–743.

56. Sonderstrup-McDevitt, G., M. Congia, A. Cope, N. Hain, J. Rothbard, and L. Fugger. 1995. Mice transgenic for HLA-DR4 and human CD4: an animal model to explore the immunogenic T cell epitopes of self antigens in human autoimmune disease. J. Cell. Biochem. 21A:C2-483. (Abstract).

57. Zanelli, E., M.A. Gonzalez-Gay, and C.S. David. 1995. Could HLA-DR1 be the protective locus in rheumatoid arthritis? Immunol. Today. 16:274–278.