

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

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## 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-DR 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<sup>0</sup> mice, and their susceptibility to experimental collagen-induced arthritis was evaluated. The HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> 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<sup>+</sup> T cells expressing a normal repertoire of V<sub>β</sub> T cell receptors. Immunization of HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> 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<sup>+</sup>, H-2Ab<sup>0</sup> mice, which was indistinguishable from the disease observed in arthritis susceptible B10.T(6R) (H-2A<sup>q</sup>) controls. In contrast, HLA-DQ8<sup>-</sup>, H-2Ab<sup>0</sup> 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)<sup>1</sup>. 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

Drs. Nabozny and Baisch made equal contributions to this study.

<sup>1</sup>Abbreviations 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-DR $\beta$ 1 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-DR $\beta$ 1 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 autoimmune 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 spondyloarthritis (16). Initial studies with transgenic mice expressing human class II genes demonstrated that the HLA class II molecules are functional as shown by the intrathymic deletion of T lymphocytes bearing certain V $\beta$  T cell receptors (17, 18). However, the consistent generation of human class II-restricted immune responses in these transgenic mice has been limited (19, 20). A possible reason for such poor responsiveness may be an inefficient interaction between the mouse CD4 coreceptor and the human class II molecule (21). Recent attempts to circumvent this problem have led to the generation of enhanced human class II-restricted immune responses. For instance, Woods et al. (22) constructed a human-mouse chimaeric HLA-DR4/H-2E transgene containing the mouse CD4-binding domain, while Fugger et al. (23) recovered human class II-

restricted responses in HLA-DR4 transgenic mice coexpressing the human CD4 molecule. Similarly, Yeung et al. (24) showed that expression of the human CD4 and HLA-DQ6 molecules in mice bearing disrupted murine CD4 and CD8 genes reconstituted the human class II-restricted limb of the immune system. In all cases, however, the ability of these mice to mount a protective or pathologic response against a known pathogen or autoantigen is unknown. Likewise, deciphering the functional role of HLA molecules in these animals may prove problematic given the dominant expression of endogenous mouse class II molecules.

An alternative approach to generate human class II-restricted responses in mice is to express the HLA class II transgene in animals rendered deficient for mouse class II expression through gene targeting. Expression of the human class II molecule in the absence of mouse 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  $\alpha$  and  $\beta$  chains of the *HLA-DQB1\*0302*, *DQA1\*0301* molecule (HLA-DQ8) into mouse class II-deficient H-2Ab<sup>0</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> 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-2Ab<sup>0</sup> mice leads to the selection and restoration of a peripheral CD4<sup>+</sup> T cell compartment. Moreover, immunization of HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> 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 in the center, *DQB\*0302* gene (27). The cosmid inserts were released by Sall digestion, purified, and microinjected into (CBA/J  $\times$  B10.M)F<sub>2</sub> 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-2Ab<sup>0</sup> mice as described in Fig. 1. Mouse class

II-deficient H-2Ab<sup>0</sup> mice were kindly provided by Drs. Diane Mathis and Christophe Benoist (INSERM, Salsbourg, 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-2A<sup>b</sup> (30); 7-16.7 anti-H-2A<sub>α</sub><sup>b</sup> (kindly provided by Dr. David McKean, Mayo Clinic, Rochester, MN); Y17, anti-H-2E<sub>β</sub><sup>b</sup> (31); and 28-14-8S, anti-H-2D<sup>b</sup> (32). After a 30-min incubation, the cells were washed in PBS/BSA and then incubated with an FITC-conjugated goat Fab'<sub>2</sub> 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<sup>+</sup> and V<sub>β</sub> 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<sup>6</sup> cells were incubated with one of the following V<sub>β</sub> TCR-specific mAbs: KT4, rat anti-V<sub>β</sub>4 (33); MR9-4, mouse anti-V<sub>β</sub>5.1.2 (34); F23.2, mouse anti-V<sub>β</sub>8.2 (35); 14-2, rat anti-V<sub>β</sub>14 (36); and KM 114, rat anti-CD44 (37). After a 30-min incubation, the cells were washed then incubated with FITC-conjugated Fab'<sub>2</sub> 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<sup>®</sup> 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 tuberculosis* strain H37 Ra; Difco Laboratories, Detroit, MI). The animals were subsequently immunized with 100 μl (100 mg bovine CII) of the emulsion at the tail base. 28 d later, the animals received a booster injection of 100 μg bovine CII emulsified in IFA. The mice were carefully monitored 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 KPO<sub>4</sub> buffer, pH 7.6, at 300 ml 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.

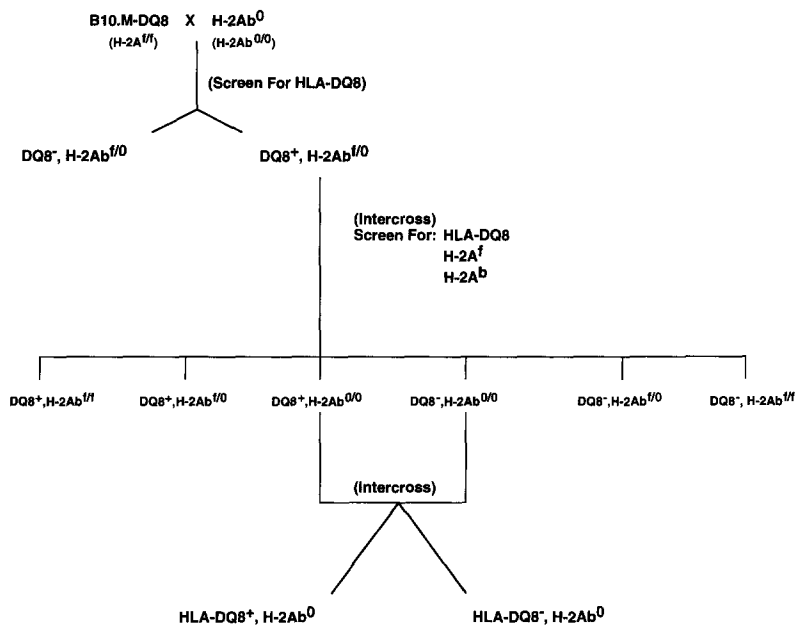
**In Vitro LNC Proliferation.** Lyophilized bovine CII was dissolved in 0.1 N acetic acid overnight at a concentration of 2 mg/ml and then emulsified 1:1 with CFA. Mice received an intradermal injection of 100 μl of cold emulsion at the tail base and 50 μl in each hind footpad for a total of 200 μg bovine CII per mouse. 10 d later, the animals were killed, and the draining LNC were isolated and suspended to a concentration of 10<sup>7</sup>/ml in RPMI 1640 medium (GIBCO BRL) supplemented with 5% heat-inactivated horse serum, 25 mM Hepes buffer, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. 100 μl of the cell suspension, containing 10<sup>6</sup> 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-2A<sub>α</sub><sup>b</sup> (7-17.7), H-2E<sub>β</sub><sup>b</sup> (Y17), mouse CD4 (GK 1.5), mouse CD8 (53.7.72), control mouse IgG (MB40.5, anti-HLA-A, -B, and -C), or rat IgG (M5/114, anti-H-2A<sup>b</sup>) 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% CO<sub>2</sub> and 95% air and then pulsed with 1.8 μCi of [<sup>3</sup>H]thymidine during the final 18 h of culture. The cells were harvested on glass fiber filters, and the extent of [<sup>3</sup>H]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 was determined using the nonparametric Mann-Whitney U test.

## Results

**Introduction and Expression of the HLA-DQ8 Molecule in H-2Ab<sup>0</sup> 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-2Ab<sup>0</sup> mice. Fig. 1 illustrates the strategy to derive the HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> line. Briefly, B10.M (H-2<sup>f</sup>) mice bearing a transgene encoding the *DQ0301* α and *DQ0302* β chain genes of the HLA-DQ8 molecule were mated with H-2Ab<sup>0</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> progeny were intercrossed, and segregation of the H-2Ab<sup>0</sup> and H-2Ab<sup>f</sup> gene was monitored via fluorescent analysis using the H-2A<sup>f</sup>-specific mAb 3F-12 and the H-2A<sup>b</sup>-specific mAb AF6-120. The offspring that typed as HLA-DQ8<sup>+</sup>,H-2Ab<sup>0/0</sup> and HLA-DQ8<sup>-</sup>,H-2Ab<sup>0/0</sup> were selected and intercrossed

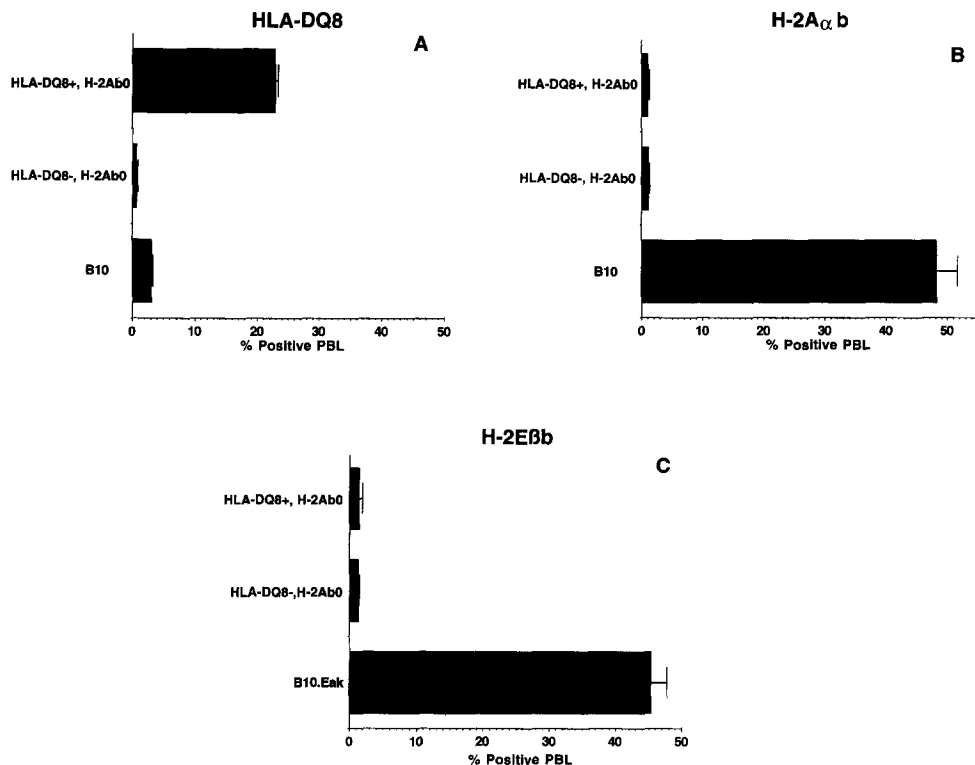


**Figure 1.** Schematic illustration of the generation of HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> mice. Segregation of the HLA-DQ8 transgene was monitored by flow cytometric analysis of PBL using the HLA-DQ-specific mAb IVD12. Segregation of the mutant H-2A <sub>$\beta$</sub> <sup>0</sup> gene was also evaluated by flow cytometry by monitoring the expression of the H-2A<sup>I</sup> and H-2A<sup>b</sup> molecules using the mAbs 3F-12 and AF6-120, respectively.

to develop the HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> and HLA-DQ8<sup>-</sup>, H-2Ab<sup>0</sup> lines.

Fig. 2 A shows that transgenic HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> 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 <sub>$\alpha$</sub> <sup>b</sup> and H-2E <sub>$\beta$</sub> <sup>b</sup> chains in H-2Ab<sup>0</sup> mice (41, 42), it was possible that hybrid A <sub>$\alpha$</sub> <sup>b</sup>-DQ8 <sub>$\beta$</sub>  or DQ8 <sub>$\alpha$</sub> -E <sub>$\beta$</sub> <sup>b</sup> molecules were also present in the HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> line. To eliminate this

possibility, PBL from HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> mice were analyzed for surface expression of the H-2A <sub>$\alpha$</sub> <sup>b</sup> and H-2E <sub>$\beta$</sub> <sup>b</sup> molecule. Use of the H-2A <sub>$\alpha$</sub> <sup>b</sup>-specific mAb 7-16.17 did not detect expression of H-2A <sub>$\alpha$</sub> <sup>b</sup> in HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> animals (Fig. 2 B). Surface expression of the H-2E <sub>$\beta$</sub> <sup>b</sup> molecule using the H-2E <sub>$\beta$</sub> <sup>b</sup>-specific mAb Y17 was similarly undetected. The Y17 mAb, however, reacted strongly with PBL from positive control B10.Ea<sup>k</sup> transgenic mice, which express the H-2E <sub>$\beta$</sub> <sup>b</sup> chain due to the presence of the H-2E <sub>$\alpha$</sub> <sup>k</sup>



**Figure 2.** Analysis of HLA-DQ8 and murine MHC expression in transgenic HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> mice. PBL from HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> mice, B10, HLA-DQ8<sup>-</sup>, H-2Ab<sup>0</sup>, and B10.Ea<sup>k</sup> animals were analyzed by flow cytometry for surface expression of the molecules HLA-DQw8 (A), H-2A <sub>$\alpha$</sub> <sup>b</sup> (B), and H-2E <sub>$\beta$</sub> <sup>b</sup> (C). The methodology and antibodies used for analysis are described in detail in Materials and Methods.

**Table 1.** Selection of Peripheral CD4<sup>+</sup> T Cells in HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> Mice\*

Strain	Percent CD4 (x ± SD)	Percent CD4/CD44 (x ± SD)	Percent CD4/Vβ LNC (x ± SD)				
			Vβ4	Vβ5.1.2	Vβ6	Vβ8.2	Vβ14
HLA-DQ8 <sup>+</sup> , H-2Ab <sup>0</sup>	11.2 ± 2.1	46.8 ± 0.6	6.6 ± 1.6	6.5 ± 0.5	7.2 ± 1.6	8.0 ± 0.1	7.7 ± 1.6
HLA-DQ8 <sup>-</sup> , H-2Ab <sup>0</sup>	3.7 ± 0.3	75.0 ± 3.2	5.4 ± 2.6	9.3 ± 1.7	7.1 ± 2.9	14.4 ± 0.7	6.5 ± 2.4
B10.T(6R)	29.5 ± 2.1	36.6 ± 4.4	6.5 ± 0.4	5.2 ± 0.6	4.4 ± 0.5	12.9 ± 0.4	5.4 ± 0.9

\*Lymph node cells were removed and analyzed by flow cytometry as described in Materials and Methods. The frequency of CD4<sup>+</sup>/Vβ TCR-positive cells was calculated from the gated CD4<sup>+</sup> population shown in the first column. The data are presented as the mean percent positive cells ± SD of three animals per group.

molecule (Fig. 2 C). As expected, HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> animals did not express the H-2A<sub>β</sub><sup>b</sup> chain, and expression of the MHC class I molecule D<sup>b</sup> was present at a level similar to HLA-DQ8<sup>-</sup>,H-2Ab<sup>0</sup> and B10 mice (data not shown). Thus, cell surface expression of the DQ8 molecule requires both the DQ 0301 α and DQ 0302 β chains.

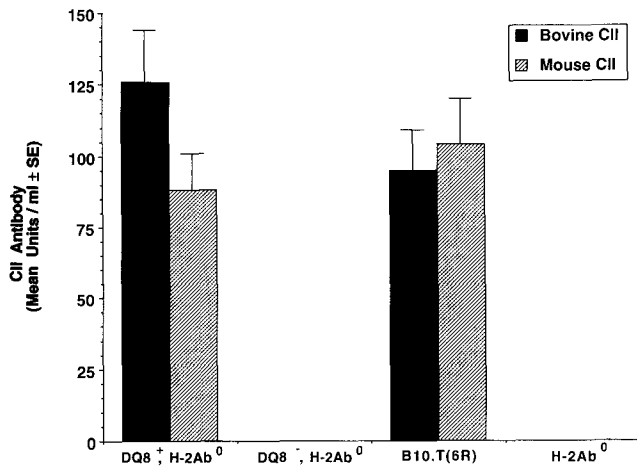
*Selection of Peripheral CD4<sup>+</sup> T Cells in HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> Mice.* A hallmark feature of class II-deficient H-2Ab<sup>0</sup> mice is a paucity of peripheral CD4<sup>+</sup> T cells. In general, H-2Ab<sup>0</sup> animals contain <5% CD4<sup>+</sup> cells within the lymph 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-2Ab<sup>0</sup> mice induces the selection of CD4<sup>+</sup> T cells, LNC from HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> and negative littermate HLA-DQ8<sup>-</sup>,H-2Ab<sup>0</sup> 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-2A<sup>g</sup> molecule (39), were also studied. Table 1 shows that HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice displayed a threefold increase in the level of CD4<sup>+</sup> LNC versus HLA-DQ8<sup>-</sup>,H-2Ab<sup>0</sup> animals. Moreover, the frequency of double-positive CD4/CD44 (Pgp-1) cells in the HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> line closely resembled mouse class II-sufficient B10.T(6R) mice. Similar to previous reports (41), ~75% of the CD4<sup>+</sup> cells in HLA-DQ8<sup>-</sup>,H-2Ab<sup>0</sup> mice expressed the CD44 molecule. Single positive HLA-DQ8<sub>α</sub> or HLA-DQ8<sub>β</sub> transgenic mice did not display an increase in CD4<sup>+</sup> LNC, thereby illustrating the importance of appropriate HLA-DQ8 α and β pairing in the restoration of the CD4<sup>+</sup> T cell compartment. Analysis of V<sub>β</sub> TCR expression within the CD4<sup>+</sup> population showed that HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice expressed a variety of V<sub>β</sub> TCRs. In addition, distinct differences in the level of some CD4<sup>+</sup>/V<sub>β</sub> TCR<sup>+</sup> cells, such as V<sub>β</sub>5 and V<sub>β</sub>8.2, was detected between HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice and transgene-negative littermates (Table 1). On balance, these data suggest that expression of the HLA-DQ8 molecule in H-2Ab<sup>0</sup> mice induces the selection of CD4<sup>+</sup>,V<sub>β</sub>TCR<sup>+</sup> cells, which are distinct from the small population of CD4<sup>+</sup> lymphocytes normally present in class II-deficient H-2Ab<sup>0</sup> mice.

*Production of CII Antibody in HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> Mice.* Typically, murine CIA is induced in susceptible strains of

mice bearing the H-2<sup>g</sup> or H-2<sup>e</sup> 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<sup>+</sup>, TCR αβ<sup>+</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> mice possessed the potential to mount a pathogenic immune response against CII, a molecule implicated in RA (25, 47). Therefore, HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> animals, along with transgene-negative littermates, positive control B10.T(6R) and negative control H-2Ab<sup>0</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> 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<sup>-</sup>,H-2Ab<sup>0</sup> littermates or H-2Ab<sup>0</sup> animals. Moreover, HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> 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 CIA-susceptible H-2 haplotype (48). Thus, the generation of mouse CII reactive antibody in HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice suggested that these animals may have the potential to develop collagen arthritis.

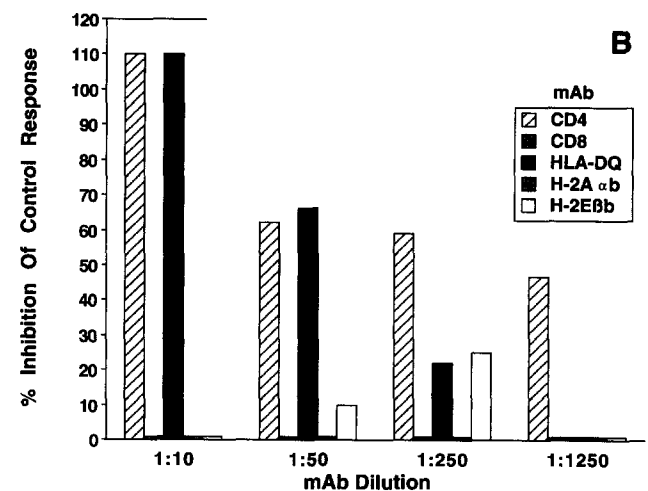
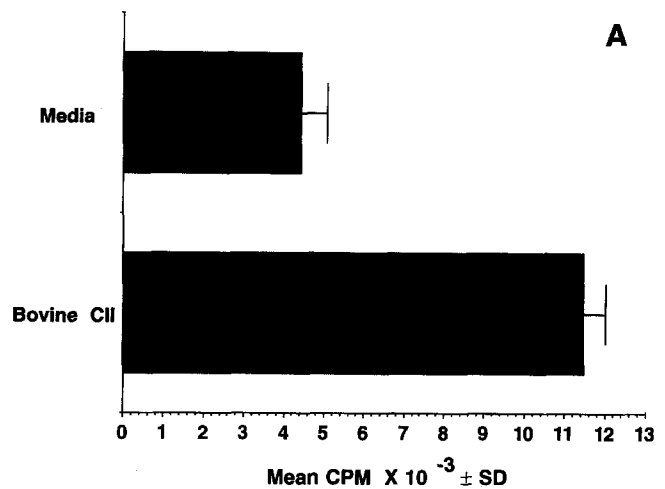
*In Vitro Proliferative Response of HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> LNC against Bovine CII.* To further explore the immune response of HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice against bovine CII, in vitro LNC proliferative responses were assessed. Fig. 4 A shows that LNC from bovine CII-immunized HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> 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%



**Figure 3.** Measurement of CII antibody in HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice. Transgenic HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice, negative littermate HLA-DQ8<sup>-</sup>,H-2Ab<sup>0</sup> animals, as well as control H-2Ab<sup>0</sup> 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<sub>α</sub><sup>b</sup>, H-2E<sub>β</sub><sup>b</sup>, or CD8. These data indicate that CD4<sup>+</sup> T cells recognize and proliferate to bovine CII presented by the HLA-DQ8 molecule.

**Development of Arthritis in HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> Mice.** To determine if the immune response against bovine CII was arthritogenic, bovine CII-immunized HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice, HLA-DQ8<sup>-</sup>,H-2Ab<sup>0</sup> littermates, B10.T(6R) animals, and H-2Ab<sup>0</sup> mice were monitored for the onset and development of CIA. As shown in Table 2, experiment 1, HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> 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). Transgene-negative HLA-DQ8<sup>-</sup>,H-2Ab<sup>0</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> or B10.T(6R) strains (data not shown).



**Figure 4.** In vitro LNC proliferative response of HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice to bovine CII. (A) Adult HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice were immunized in the tail base and hind footpads with a total of 200 μg bovine CII in CFA. 10 d later, the mice were killed and the draining LNC were cultured in vitro in the presence or absence of bovine CII. (B) LNC from A were cultured in the presence of bovine CII and mAb specific for HLA-DQ, H-2A<sub>α</sub><sup>b</sup>, H-2E<sub>β</sub><sup>b</sup>, mouse CD4, mouse CD8, or control IgG. The percent inhibition of the bovine CII response was calculated as follows:  $[1 - (\Delta\text{cpm in the presence of mAb} / \Delta\text{cpm in the presence of control mAb})] \times 100$ .

To confirm our observations, a second HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> mice developed CIA (Table 2, experiment 2). Interestingly, the onset of clinical arthritis was significantly earlier in this HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> line compared to B10.T(6R) mice. Likewise, the severity of CIA was significantly greater in arthritic HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> animals. Transgene-negative HLA-DQ8<sup>-</sup>,H-2Ab<sup>0</sup> 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<sup>+</sup>, H-2Ab<sup>0</sup> Mice\*

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

\*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.

<sup>‡</sup>Mean arthritic score was calculated at the end of the study using arthritic animals only.

<sup>§</sup>Not applicable.

<sup>||</sup>P < 0.01

<sup>¶</sup>P < 0.05

deficient H-2Ab<sup>0</sup> mice confers susceptibility to induction of CIA.

## Discussion

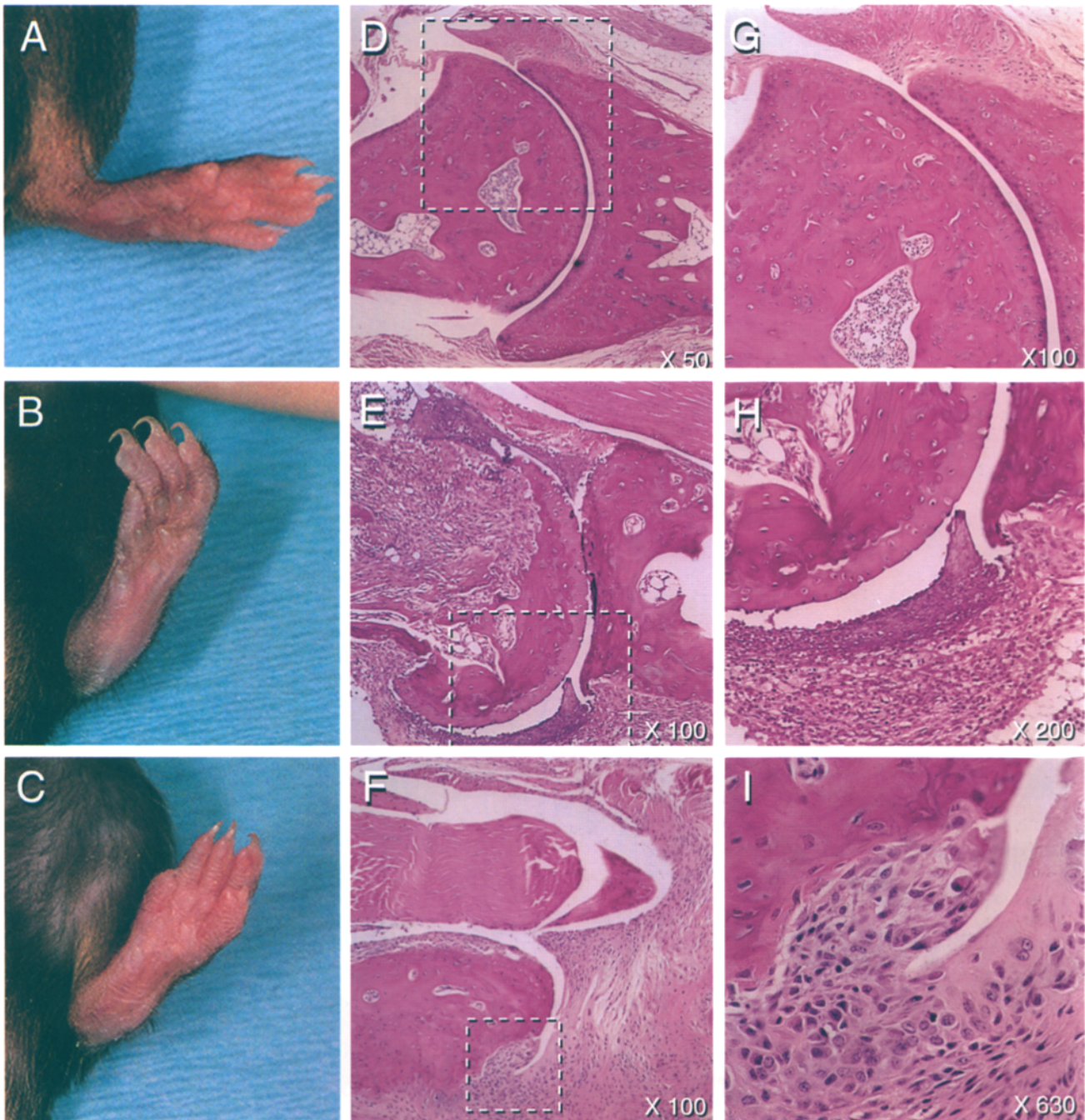
The data presented here comprise the first report demonstrating the induction of a pathogenic autoimmune response in mice expressing a human MHC class II molecule. By introducing an HLA-DQ8 transgene into murine class II-deficient H-2Ab<sup>0</sup> mice, we observed that this human class II molecule induced selection of a CD4<sup>+</sup> 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<sup>+</sup>, H-2Ab<sup>0</sup> mice successfully forces the selection of HLA-DQ8 restricted CD4<sup>+</sup> 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, re-

cently 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 β<sub>2</sub> domain of the MHC class II β chain as putative CD4 interaction sites (49, 50). Analysis of these interaction sites within the β<sub>2</sub> domain (amino acid residues 110, 141, and 142) show that this region of the HLA-DQ8β 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<sup>+</sup> T cells in HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> 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<sup>+</sup> T cells in HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> mice was lower than mouse class II-sufficient, CIA-susceptible B10.T(6R) mice. The frequency of CD4<sup>+</sup> cells in HLA-DQ8<sup>+</sup>, H-2Ab<sup>0</sup> animals, however, was threefold higher than HLA-DQ8<sup>-</sup>, H-2Ab<sup>0</sup> littermates. Moreover, the CD4<sup>+</sup> 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<sup>+</sup>, H-2Ab<sup>0</sup> 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<sup>+</sup> T cells restricted by the HLA-DQ8 molecule. Of





**Figure 5.** Clinical and histologic presentation of collagen arthritis in HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice. (A–C) The appearance of a normal rear paw from a bovine CII-immunized HLA-DQ8<sup>-</sup>,H-2Ab<sup>0</sup> mouse (A) contrasted with arthritic paws from an HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> 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<sup>-</sup>,H-2Ab<sup>0</sup> mouse (D and G) compared with an arthritic joint from HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> (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<sup>+</sup>,H-2Ab<sup>0</sup> mice. The histological changes in the arthritic lesions were indistinguishable from CIA-suscepti-

ble 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<sup>+</sup>,H-2Ab<sup>0</sup> mice are similar to those responsible for prototypical CIA. Also, the susceptibility of HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> mice to CIA is reproducible as illustrated by the development of arthritis in a second group of HLA-DQ8<sup>+</sup>,H-2Ab<sup>0</sup> animals, which expressed a lower level of HLA-DQ8<sup>+</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> mice mount weak responses to CII and do not develop CIA (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<sup>+</sup>,H-2Ab<sup>0</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> 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<sup>+</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> 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<sup>+</sup>,H-2Ab<sup>0</sup> 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.

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