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HLA-DQ6/8 Double Transgenic Mice Develop Auricular Chondritis Following Type II Collagen Immunization: A Model for Human Relapsing Polychondritis¹

David S. Bradley,* Pritam Das,* Marie M. Griffiths,† Harvinder S. Luthra,† and Chella S. David^{2*}

We have generated transgenic (tg) mice expressing HLA-DQ8 $\alpha\beta$ (*DQA1*0301/DQB*0302*) or HLA-DQ6 $\alpha\beta$ (*DQA1*0103/DQB1*0601*) molecules lacking endogenous murine class II expression ($A\beta 0$) to investigate the ability of these HLA class II to present type II collagen (CII) and induce collagen-induced arthritis. The DQ8 $\alpha\beta$ tg mice responded strongly to CII, developing severe arthritis, while DQ6 $\alpha\beta$ tg mice were nonresponsive to CII. The addition of the mixed haplotype DQ8 $\alpha\beta$ molecule did not significantly influence CII reactivity. To examine the interaction of DQ6 $\alpha\beta$ and DQ8 $\alpha\beta$ molecules in vivo, we generated double tg DQ6 $\alpha\beta$ /8 $\alpha\beta$ ($A\beta 0$) mice expressing both the α - and β -chains of DQ6 and DQ8 molecules by mating DQ6 $\alpha\beta$ ($A\beta 0$) and DQ8 $\alpha\beta$ ($A\beta 0$) tg mice. CII-immunized DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice developed severe experimental polychondritis, exhibiting both polyarthritis and auricular chondritis. The clinical, serologic, and histologic manifestations of experimental polychondritis are similar to those symptoms in human relapsing polychondritis. The susceptibility of DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice compared with resistance in the parental strains suggests that expression of both the DQ6 $\alpha\beta$ and DQ8 $\alpha\beta$ tgs, unique to the DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg strain, is important in susceptibility to experimental polychondritis. The DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice provide a model to investigate putative autoantigens and the mechanisms of pathogenesis involved in relapsing polychondritis as well as the influence of the expression of multiple HLA class II molecules on the disease process. *The Journal of Immunology*, 1998, 161: 5046–5053.

Relapsing polychondritis (RP)³ is a human autoimmune disease of unknown etiology. Jaksch-Wartenhost described this disease in 1923 as a polychondropathy (1). Pearson et al. (2) first used the term RP in 1960, describing an individual with inflammation of the outer ear, polyarthritis, and collapse of the bridge of the nose due to the absence of cartilaginous tissue in the nasal septum. RP is most common between 40 and 50 yr of age, and susceptibility is equivalent between males and females (3). There is no known correlation between the expression of specific HLA-A or -B loci and a predisposition to RP (4). Lang et al. have shown an association between RP and HLA-DR4, a twofold greater expression of at least one DR4 allele in RP patients, compared with healthy controls (5, 6). Interestingly, however, there was no predominance of any of the DR4 subtypes among RP patients. At least 33% of RP patients also display an additional preexisting autoimmune disease (3). The onset of RP is

associated with a significant increase in the rate of mortality, one-fourth of RP patients die of RP-associated symptoms within 5 yr of diagnosis (3).

Auricular chondritis is the most common clinical manifestation of RP, present in at least 30% RP patients at the initial diagnosis and eventually displayed in 85% of all RP cases (3, 7). Auricular chondritis is characterized by recurrent incidence of acute pain, erythema, and swelling of the cartilaginous portions of the outer ear, which resolves within weeks. During the acute phase, there is extensive mononuclear infiltrate containing lymphocytes, macrophages, and eosinophils accompanied by depositions of Ig and the C3 component of complement along the fibrocartilaginous line (8). One or more inflammatory episodes result in the destruction of the cartilaginous tissues of the outer ear, which is then replaced with fibrous tissue (9), often described as a “cauliflower” ear.

Another common feature of RP is polyarthritis, developing in >50% of patients over the course of RP (6, 10). Other manifestations include nasal chondritis, which results in the classical saddle nose deformation following the destruction of the cartilaginous tissues in the nasal bridge (3), ocular inflammation (7), laryngotracheal disease (3), and renal involvement (11). Involvement of the cartilaginous tissues of the respiratory tract is the most critical manifestation in RP. Much of the increased mortality associated with RP is due to airway collapse with respiratory obstruction (3).

Both anti-type II collagen (anti-CII)-specific Abs (12, 13) and cell-mediated immunity (14) have been observed in RP patients. The anti-CII Abs have been shown to be specific for native CII and unable to recognize denatured CII (12, 15), and correlation between anti-CII Ab levels and disease activity has been found (12). Anti-CII Abs detected in RP patients have a different specificity than anti-CII Abs produced during rheumatoid arthritis (RA). The anti-CII Abs produced during RP recognize predominately the cyanogen bromide (CB) fragment 9/7 of human CII, while

Departments of *Immunology and †Rheumatology, Mayo Clinic and Medical School, Rochester, MN 55905; and ‡Research Service, Veteran's Affairs Medical Center, and Department of Medicine, Division of Rheumatology, University of Utah, Salt Lake City, UT 84132

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² Address correspondence and reprint requests to Dr. Chella S. David, Department of Immunology, Mayo Clinic, Rochester, MN 55905. E-mail address: chella.david@mayo.edu

³ Abbreviations used in this paper: RP, relapsing polychondritis; CII, type II collagen; RA, rheumatoid arthritis; CB, cyanogen bromide; tg, transgenic; CIA, collagen-induced arthritis; LNC, lymph node cell; SI, stimulation index; AU, antibody units.

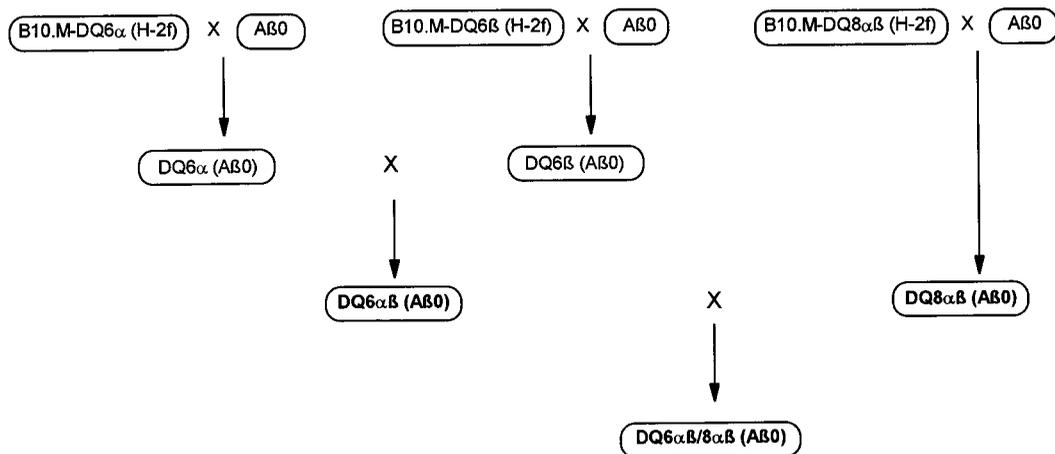


FIGURE 1. Schematic of breeding strategy used for the derivation of the HLA-DQ tg mice. The DQ transgenes were microinjected into (CBA/J × B10.M)F₂ (43) and backcrossed onto a B10. M (H-2^{f/f}) background. The B10.M-DQ tg mice were mated with class II-deficient mice (Aβ0) as depicted. The transgene-positive mice were determined by PCR of peripheral blood with DQ allele-specific oligonucleotide primers, and expression of the DQ molecules was determined by flow cytometry of PBLs. The absence of H-2 class II expression on the cell surface was also confirmed by flow cytometry.

RA-associated Abs have a more diverse pattern of specificity, with CB 11 and CB 8 being the most commonly recognized fragments (13). Polychondritis has also been reported in CII-immunized rats, with 10% of the experimental rats developing auricular chondritis (16–18). Disease was shown to be driven by a mononuclear infiltration leading to destruction of the cartilage and was associated with systemic humoral and cellular CII-specific responses. These observations indicate an autoimmune aspect of RP and suggest a role for CII as a putative autoantigen.

We have recently generated transgenic (tg) mice expressing HLA-DQ6αβ (*DQA1*0103/DQB1*0601*) or HLA-DQ8αβ (*DQA1*0302/DQB1*0301*) molecules, both lacking endogenous class II expression (Aβ0), to investigate the role of human class II molecules in the induction of arthritis using an animal model of RA, collagen-induced arthritis (CIA). Bovine CII-immunized DQ6αβ tg mice were resistant to arthritis (19), while the DQ8αβ tg mice developed severe arthritis and strong CII-specific B and T cell responses (20). Addition of the DQ6β-chain (*DQB1*0601*) to the DQ8αβ tg mice tempered the strong DQ8αβ-mediated CII-specific response, but did not block CIA (19). None of these tg strains displayed other characteristics of RP.

Here we describe the generation of double tg mice expressing both HLA-DQ6 and HLA-DQ8 molecules on a murine class II-deficient background (DQ6αβ/8αβ tg mice). Following bovine CII immunization, unlike the parental strains, DQ6αβ/8αβ tg mice developed auricular chondritis and severe polyarthritis, accompanied by strong anti-CII-specific humoral and cellular immune responses. The observation of polychondritis in DQ6αβ/8αβ tg mice but not in the parental strains indicates that expression of all four human class II chains is required for the additional disease phenotype. Certain human diseases have been associated with the expression of certain combinations of HLA class II alleles. It has been postulated that this may be due to the formation of mixed haplotype molecules, produced by transcomplementation of HLA class II molecules; however, a role for such hybrid class II molecules in pathogenesis has not been shown definitively. The DQ6αβ/8αβ tg mice provide a novel model to examine the immunologic and pathogenic aspects of RP as well as to investigate how the expression of multiple human class II molecules may affect susceptibility to disease.

Materials and Methods

Mice

All mice were bred in our pathogen-free facility and maintained in our clean conventional area of the Immunogenetics Mouse Colony at the Mayo Clinic (Rochester, MN). The generation of mice expressing HLA-DQ6α (*DQA1*0103*), DQ6β (*DQB1*0601*), DQ8α (*DQA1*0301*), and DQ8β (*DQB1*0302*) transgenes was achieved by mating HLA-DQ6αβ tg (Aβ0) (19) and HLA-DQ8αβ tg (Aβ0) mice (20). The generation of the parental strains has been described previously and is outlined in Figure 1. The presence of the transgenes was determined by PCR, and surface expression was determined by flow cytometry. Experimental mice were between 6 and 12 wk of age when immunized with CII, and there was an equal representation of both sexes in each experimental group.

Flow cytometry

The expression of cell surface markers were assessed by flow cytometry as previously described (19). Briefly, PBLs were isolated from whole blood, washed extensively, and incubated with mAbs specific for HLA-DQα (IVD12) (21), HLA-DQ6β (L227) (22), H-2A_α^b (7-17.7) (23), or H-2E_β^b (Y-17) (24). The PBLs were washed again and incubated with FITC-conjugated goat F(ab')₂ specific for mouse IgG (Accurate Chemical and Science, Westbury, NY). In those preparations in which CD4 expression was measured, PBL preparations were incubated with phycoerythrin-conjugated CD4 mAb. All preparations were fixed in 1% formalin and analyzed using a FACS Vantage flow cytometer (Becton Dickinson, Mountain View, CA).

Immunization with CII

Pure native type II bovine collagen (CII) was obtained by extensive and multiple step purification described previously in detail (16). Polychondritis was induced by intradermal immunization at the base of the tail of 100 μg of pure native bovine CII emulsified 1/1 in CFA (*Mycobacterium tuberculosis* H37 Ra; Difco, Detroit, MI), and mice were boosted on day 28 with 100 μg of native bovine CII emulsified in IFA. The mice were observed at least three times a week, for 16 wk following CII immunization, for the development of auricular chondritis and/or polyarthritis. The severity of clinical arthritis was assessed as previously described (25), with each paw scored as follows: 0 = normal, no inflammation; 1 = swelling of digits; 2 = severe swelling of the foot or ankle and/or joint deformity; or 3 = ankylosis of the ankle joint. Each mouse had a possible arthritic score of 0 to 12.

CII-specific T cell proliferation analysis

The ability of DQ-expressing APCs to induce a CII-specific response from DQ6αβ/8αβ T cells was assessed by an in vitro proliferation assay. Briefly, lymph node cells (LNCs) were purified from mice primed with bovine CII (200 μg of bovine CII emulsified in CFA 1/1 intradermally at

the base of the tail and the hind footpads), resuspended in complete medium (RPMI 1640, Life Technologies, Grand Island, NY; 5% heat-inactivated horse serum, 25 mM HEPES, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin), added in triplicate to flat-bottom microtiter plates (Corning, Corning, NY) at 1×10^6 cells/well, and challenged with 5 µg/well of heat-denatured bovine CII. The cells were incubated at 37°C in 5% CO₂ for 48 h, pulsed with [³H]thymidine (1.8 µCi), incubated for an additional 18 h, and harvested; then [³H]thymidine uptake was measured as counts per minute in a liquid scintillation counter (Beckman, Palo Alto, CA). The proliferative responses are displayed as a stimulation index (SI) to appropriately compare the proliferative ability of T cells from strains of mice with different intrinsic background counts per minute: SI = [(mean cpm of triplicate cultures with denatured bovine CII) ÷ (mean cpm of triplicate cultures with medium alone)]. SI values ≥ 2.5 were considered a significant proliferative response. The influence of various cell populations on the proliferative response was determined by the addition of mAb specific for HLA-DQ α (IVD12), HLA-DQ β (L227), H-2A_b (7-17.7), H-2E β (Y-17), CD4 (GK1.5), and CD8 (53.7.72) or an irrelevant isotype-matched mAb specific for HLA-A, -B, and -C (MB40.5; 20 µl of culture supernatant/well) to cultures of LNCs stimulated with bovine CII. The efficacy of blocking is reported as the percent inhibition: $1 - [(\Delta\text{cpm of cultures} + \text{experimental mAb}) \div (\Delta\text{cpm of cultures} + \text{irrelevant control mAb})] \times 100$.

Anti-CII Ab analysis

Levels of anti-bovine and anti-mouse CII IgG were measured by ELISA as described previously (26) in sera obtained 35 and 84 days following CII immunization. Briefly, microtiter plates were coated with bovine or mouse CII (6 µg/well in KPO₄, pH 7.6), incubated overnight at 4°C, washed extensively, and blocked with 1% BSA in PBS/0.05% Tween-20. Serum was added in fourfold dilutions (1/100 to 1/65,000) in duplicate and incubated overnight at 4°C. Serial dilution of a high titer anti-CII IgG-positive serum were run in parallel with all ELISAs for standardization. The plates were washed again, peroxidase-conjugated goat anti-mouse IgG (Organon Teknica, West Chester, PA) was added, and overnight incubation was performed at 4°C. The plates were washed, *O*-phenylenediamine was added, and the colorimetric change was measured at 410 nm. Anti-CII IgG levels were calculated from the OD of the high titer standard sera, arbitrarily determined to equal 100 Ab units (AU)/ml.

The specificity of the anti-bovine CII Abs was determined by a similar ELISA. Microtiter plates were coated with one of the following CB fragments of bovine CII: CB8, CB10, CB11, or CB12, and the level of CB-specific anti-CII IgG is expressed as a percentage of the total CII Ab response: $[1 - (\text{OD of the CB-specific IgG}) \div (\text{OD of total anti-bovine CII IgG})] \times 100$.

Histologic assessment

Outer ears exhibiting clinical chondritis were surgically removed from anesthetized mice at various time points following the onset of disease, embedded in paraffin, sectioned (6 µm), and mounted. Sections were stained with hematoxylin and eosin or toluidine blue. Hind limbs were removed and dissected from euthanized mice at the culmination of the experiment. The joints were decalcified in EDTA for 4 days and embedded in paraffin. Sections of 6 µm were cut, mounted, and stained with hematoxylin and eosin. All stained sections were assessed for histopathologic changes.

In addition, chondritic ears obtained as described above, fixed in OTC, (Sakura Fine Technical Co., Torrance, CA) sectioned, and mounted for immunostaining. Sections were incubated with biotinylated anti-mouse CD3- ϵ (145-2C11) (27, 28), CD4 (H129.19) (29, 30), CD8 (53-6.7) (31), B220/CD45R (RA3-6B2) (32), or CD11b/Mac1- α_M (M1/70) (33, 34) mAb. Cells expressing these surface markers were visualized with avidin-horseradish-peroxidase. Stained sections were evaluated in a blinded manner and scored as follows for the percentage of mononuclear cells present: -, no positive cells detected; \pm , <5% positive; +, 5 to 10% positive; ++, 11 to 20% positive; +++, 21 to 50% positive; and +++, >50% positive.

Statistical analysis

The significance of differences in incidence of peak arthritic severity and onset of clinical arthritis were compared using the nonparametric Mann-Whitney *U* test. Only arthritic mice were considered for comparison of severity and onset of disease. Ab levels were compared by Student's *t* test.

Table I. MHC class II expression on HLA-DQ transgenic LNCs^a

Mouse Strain	% MHC Class II Expression			
	HLA-DQ α	HLA-DQ β	H-2A α ^b	H-2E β ^b
DQ6 α β /8 α β (A β 0) ^b	32.5	46.3	— ^c	—
DQ6 α β (A β 0)	39.4	51.9	—	—
DQ8 α β (A β 0) ^b	38.1	7.2	—	—
DQ neg (A β 0) ^d	—	—	—	—
B10 (H-2 ^b)	—	—	43.7	—
B10.E α^k (H-2A ^b /E α^k E β^b)	—	—	41.0	32.4

^a LNCs were purified, labeled with MHC class II specific mAbs, and detected with a species-specific FITC-conjugated mAb by flow cytometry. The percentage expression is obtained from LNCs pooled from two mice/strain and are representative of at least three separate assays for each strain.

^b Presence of DQ8 β gene was confirmed by PCR.

^c — level of detection was less than or equal to background staining.

^d Transgene negative littermates of each strain were assessed with none of the strains expressing murine or human class II molecules on the surface of LNCs.

Results

Double tg HLA-DQ6 α β /8 α β mice

To assess the influence of coexpression of HLA-DQ6 and DQ8 molecules on CII-induced autoimmune responses, HLA-DQ6 (A β 0) tg mice (19) were mated with HLA-DQ8 (A β 0) tg mice (20) as delineated in Figure 1. Offspring of this combination, DQ6 α β /8 α β tg mice, contained transgenes coding DQ6 α , DQ6 β , DQ8 α , and DQ8 β , as determined by PCR (data not shown), and expressed HLA-DQ molecules on the surface of PBLs (Table I). All the mice used in these studies lack the expression of endogenous class II molecules, similar to the class II knockout mice (35).

The DQ6 α β /8 α β tg mice expressed 7.9% CD4⁺ T cells in the periphery, compared with 8.9% in DQ8 α β tg mice and 5.7% in DQ6 α β tg mice, based on mean expression on PBLs of three mice per strain assessed individually by flow cytometry. The CD4⁺ T cell population was polyclonal in nature as demonstrated by the expression of a variety of V β TCR families on DQ6 α β /8 α β tg PBLs (data not shown).

Development of polyarthrititis

Polyarthrititis. Cohorts of DQ6 α β /8 α β tg, DQ8 α β tg, DQ6 α β tg, and CIA prototypic B10.T(6R) (H-2q) mice were immunized with bovine CII in CFA and boosted 28 days later with bovine CII in IFA. The DQ6 α β /8 α β tg mice developed polyarthrititis with an incidence comparable to that in CIA-susceptible parental DQ8 α β tg mice (71 and 79%, respectively; Table II). The mean days of onset were also similar (day 36 in DQ6 α β /8 α β tg mice and day 39 in DQ8 α β mice). The peripheral joints of all four limbs of DQ6 α β /8 α β tg mice were capable of exhibiting severe clinical arthritis, with a representative arthritic rear limb illustrated in Figure 2B. There was no preferential development of polyarthrititis based on sex. Histologic analysis demonstrated that the inflammation was accompanied by a mononuclear infiltrate, pannus formation, and the destruction of the cartilage (Fig. 2D) as seen in polyarthrititis associated with RP, RA, and CIA. The DQ6 α β tg mice were resistant to CIA, with only one of the mice developing clinical arthritis.

Auricular chondritis. Interestingly, approximately 22% of the CII-immunized or one-third of the arthritic DQ6 α β /8 α β tg mice developed auricular chondritis (Table II). Neither parental strain, DQ6 α β tg or DQ8 α β tg mice, had clinical or histologic involvement of the outer ear following CII immunization (data not shown). Acute bilateral chondritis was detected in DQ6 α β /8 α β tg mice with a mean day of onset of 51 days after initial bovine CII immunization, with incidence occurring equally between male and

Table II. Summary of polyarthritides in DQ6 α β/8 α β mice

Mouse Strain	Auricular Chondritis		Polyarthritides		
	% Incidence (chondritic/total)	Day of onset \pm SE ^a	% Incidence (arthritic/total)	Day of onset \pm SE ^a	Mean peak severity \pm SE ^a
DQ6 α β/8 α β tg	25.3 (21/83)	51 \pm 12	71.4 (59/83)	36 \pm 3	6.3 \pm 0.4
DQ6 α β/8 α β tg neg	0.0 (0/19)	—	0.0 (0/19)	—	—
DQ6 α β tg	0.0 (0/12)	—	8.3 (1/12)	52	3
DQ8 α β tg	0.0 (0/14)	—	78.6 (11/14)	39 \pm 4	5.9 \pm 0.9
B10.T (6R)	0.0 (0/38)	—	57.9 (22/38)	44 \pm 2	4.0 \pm 0.3

^a Day of onset of auricular chondritis and polyarthritides as well as the severity of arthritis was calculated only from affected mice displaying the pertinent symptom.

female mice. The outer ears exhibited swelling and erythema (Fig. 3B), which then progressed to the classic cauliflower ear (Fig. 3C) within 4 wk of the onset. Histologic examination revealed disruption of the cartilaginous layer detectable within 1 wk of onset, which progressed to almost complete destruction by 4 wk (Fig. 4B). Toluene blue staining of chondritic ear sections confirmed the depletion of cartilage in DQ6 α β/8 α β ears (data not shown). At 1 wk after the onset of clinical auricular chondritis, there was a significant infiltration of mononuclear cells along the fibrocartilaginous line, which consisted of approximately 10% CD4⁺ and 5% CD8⁺ T cells and >50% macrophages (Table III).

CII-specific proliferative response in HLA-DQ6 α β/8 α β tg mice

The ability of DQ6 α β/8 α β tg mice to recognize and present bovine CII was assessed *in vitro* using LNCs from CII-primed DQ6 α β/8 α β tg mice. As shown in Figure 5, the DQ6 α β/8 α β T cells mount a significant response to denatured bovine CII challenge. As we have previously shown, DQ8 α β T cells also respond strongly to bovine CII (20), at levels comparable to those in

DQ6 α β/8 α β T cells, while DQ6 α β T cells do not respond (19), indicating that the DQ6 α β molecule is unable to recognize and/or present bovine CII. Coincubation with anti-CD4 and anti-DQ mAb demonstrated that the bovine CII response is CD4 and DQ restricted in DQ6 α β/8 α β tg mice (Fig. 6). Blocking of CD8, HLA-DQ6 β , H-2A α , or H-2E β molecules did not significantly affect the proliferative response.

Anti-CII IgG levels in HLA-DQ6 α β/8 α β tg mice

Following bovine CII immunization, DQ6 α β/8 α β tg mice developed high levels of anti-bovine CII IgG, significantly higher than anti-bovine CII levels in DQ8 α β tg and B10.T(6R) mice (212 AU/ml compared with 123 AU/ml and 71 AU/ml for DQ6 α β/8 α β tg, DQ8 α β tg, and B10.T(6R) mice, respectively; Fig. 7). Bovine CII immunization also induced a strong anti-mouse CII IgG response in DQ6 α β/8 α β tg mice, similar to that in DQ8 α β tg and B10.T(6R) mice. The DQ6 α β/8 α β negative littermates produced neither an anti-bovine nor an anti-mouse CII Ab response.

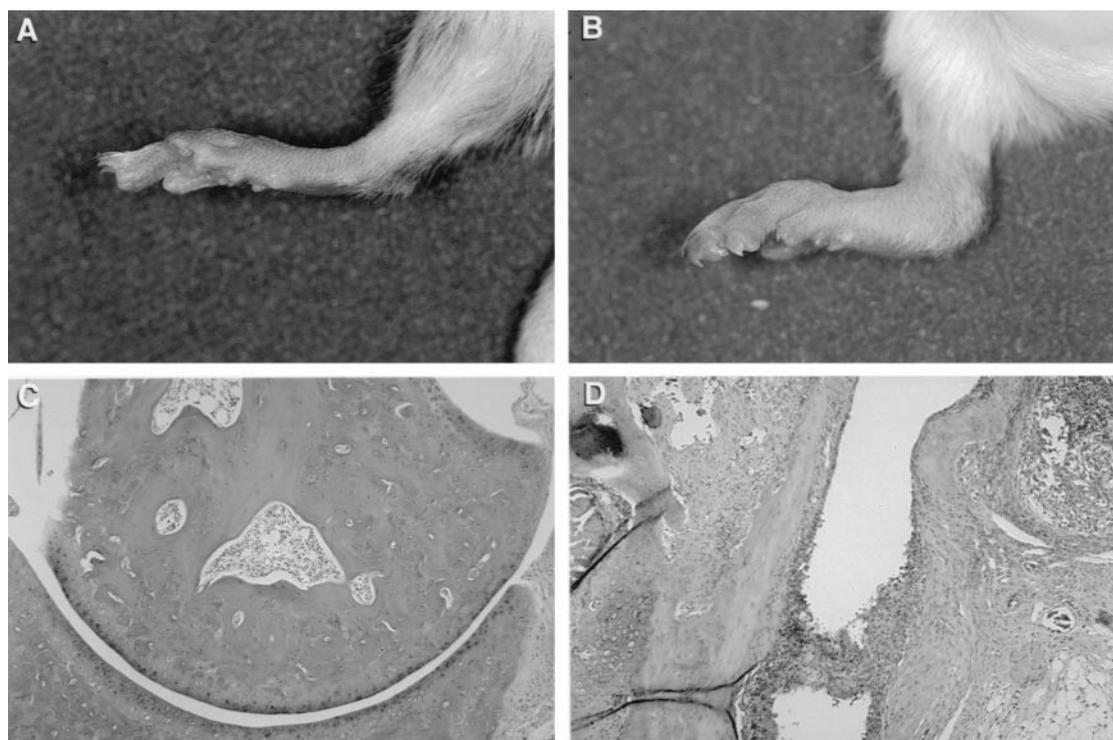


FIGURE 2. Representative polyarthritic hind limb from a HLA-DQ6 α β/8 α β tg mouse. A normal (A) and an arthritic hind limb (B) from DQ6 α β/8 α β tg mice are shown. Both individual digits as well as the ankle joint are involved in polyarthritides induced in DQ6 α β/8 α β tg mice (B). Sections from a normal and an arthritic DQ6 α β/8 α β tg hind limb stained with hematoxylin and eosin are shown in C and D, respectively. During polyarthritides there are significant mononuclear infiltrate, cartilage destruction, pannus formation, and bone destruction (D). Micrographs are at \times 50 magnification.

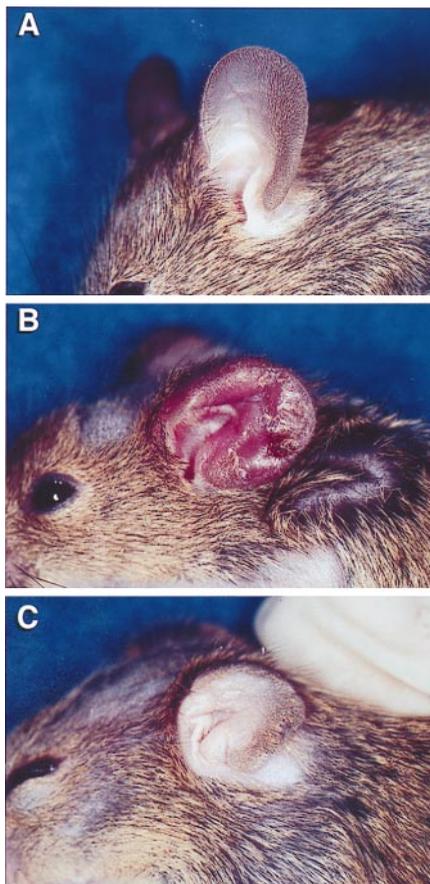


FIGURE 3. Representative chondritic ears from HLA-DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice. *A*, A normal age-matched ear; *B*, a highly inflamed chondritic ear 1 wk after disease onset and (*C*) a chondritic ear at 4 wk after disease onset, demonstrating the cauliflower ear prototypic of human RP following the destruction of the cartilaginous tissues. All ears are from DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice.

The largest percentage of the anti-CII IgG response was specific for the CB10 fragment of bovine CII (Fig. 8), unlike the H-2^d restricted B10.T(6R) response, which was predominately specific for the CB11 fragment.

Discussion

We have described the generation of double tg mice containing both DQ6 $\alpha\beta$ (*DQA1*0103/DQB1*0601*) and DQ8 $\alpha\beta$

Table III. Mononuclear cells localized in the outer ear during auricular chondritis in HLA-DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice^a

Surface Molecule Expression	Normal Ears (nondiseased)	Acute Chondritic Ears (1 wk postonset)	Chronic Chondritic Ears (8 wk postonset)
CD3	–	++	–
CD4	–	+	–
CD8	–	+	–
B220	–	+/-	–
CD11b/Mac 1	–	++++	++++

^a Sections of chondritic and normal DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg ears were incubated with mAbs specific for the indicated cell surface molecules and scored for the presence of positive cells as follows: –, no positive cells detected; +/-, <5% positive; +, 5–10% positive; ++, 11–20% positive; +++, 21–50% positive; and +++++, >50% positive cells.

(*DQA1*0301/DQB1*0302*) genes in the absence of endogenous murine class II expression. The expression of these four human class II chains renders these mice susceptible to auricular chondritis following heterologous CII immunization, which is lacking in both parental strains, DQ6 $\alpha\beta$ tg and DQ8 $\alpha\beta$ tg mice, and in DQ6 β 8 $\alpha\beta$ tg mice as we have described previously (19). Like DQ8 $\alpha\beta$ tg and DQ6 β 8 $\alpha\beta$ tg mice, DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice develop a strong cellular and humoral CII-specific response. The CII-specific IgG is predominately directed at the CB10 fragment in the DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice, as in DQ8 $\alpha\beta$ tg and DQ6 β 8 $\alpha\beta$ tg mice (19, 20), suggesting that this region of CII contains at least one or more arthritogenic epitopes and possibly the chondritic epitopes as well. These strains also develop polyarthritis approximately 5 wk after bovine CII immunization. Conversely, bovine CII-immunized DQ6 $\alpha\beta$ tg mice do not produce a detectable CII-specific T cell response, have a much weaker anti-CII Ab response (19), and do not develop either polyarthritis or auricular chondritis.

DQ6 $\alpha\beta$ tg and DQ8 $\alpha\beta$ tg mice have similar, but not identical, backgrounds, containing contributions from background genes derived from C57BL/6, 129, and B10.M strains. The principle difference among these strains is the expression of specific HLA-DQ alleles in the absence of endogenous murine class II molecules. The control mice are full siblings lacking the DQ6 $\alpha\beta$, DQ8 $\alpha\beta$, or both transgenes. Thus, the expression of both DQ6 $\alpha\beta$ and DQ8 $\alpha\beta$ molecules is probably responsible for the development of polychondritis. Heterozygosity at class II has been closely associated with predisposition to lupus in (NZB \times NZW)_{F1} mice. There is evidence of the expression of both H-2A α^d /A β^z and H-2E α^d /E β^z mixed haloptype molecules in the (NZB \times NZW)_{F1} strain (36, 37). Although transcomplementation of HLA class II molecules, i.e., DQ molecules forming in trans from DQ α and

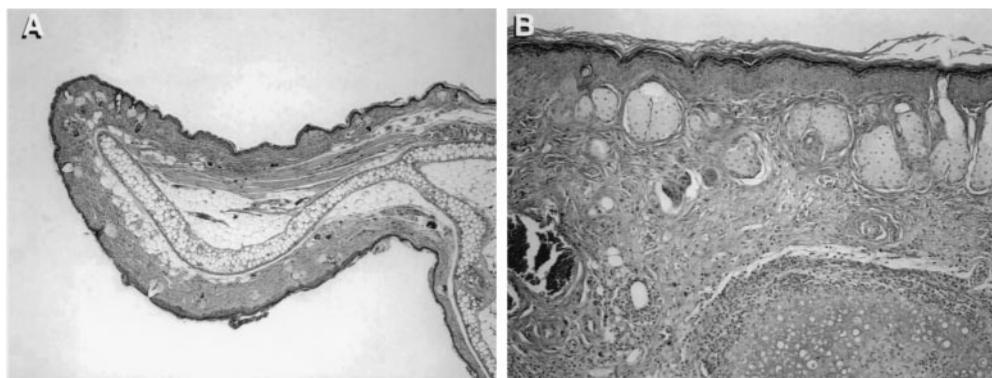


FIGURE 4. Histologic sections of chondritic HLA-DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg ears. Sections of normal ear (*A*) and chondritic ear 4 wk after the onset of disease (*B*) stained with hematoxylin and eosin. The cartilage layer is significantly disrupted by 4 wk with an overwhelming mononuclear infiltrate (*B*). Micrographs are at $\times 50$ magnification.

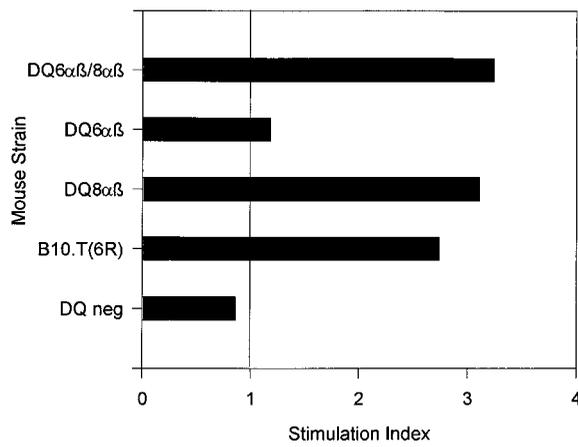


FIGURE 5. CII-specific T cell proliferation in HLA-DQ tg mice. Three mice per strain were primed with bovine CII, LNCs were purified and challenged in vitro with bovine CII, and CII-specific proliferation was measured by [³H]thymidine incorporation. Proliferation is displayed as a standardized SI. The results are representative of three separate assays. Background/medium only: DQ6αβ/8αβ = 1553 cpm; DQ6αβ = 724 cpm; DQ8αβ = 1768 cpm; B10.T(6R) = 2380 cpm; DQ tg neg = 1106 cpm.

DQβ of different haplotypes has been suggested in human celiac disease (38) and insulin-dependent diabetes (39–41), characterization of this phenomenon is very difficult in humans. The potential formation of mixed haplotype DQ molecules in the DQ6αβ/8αβ tg mice would be the most likely explanation for susceptibility to auricular chondritis. In addition, expression of the mixed haplotype DQ8αβ molecule does not result in this disease phenotype; therefore, the likely candidate would be the DQ6αβ molecule. We are currently generating these animals in our laboratory for further studies.

Although CII is a putative autoantigen in both RP and RA, the Ag specificity appears to be different. Similarly, DQ8αβ tg mice

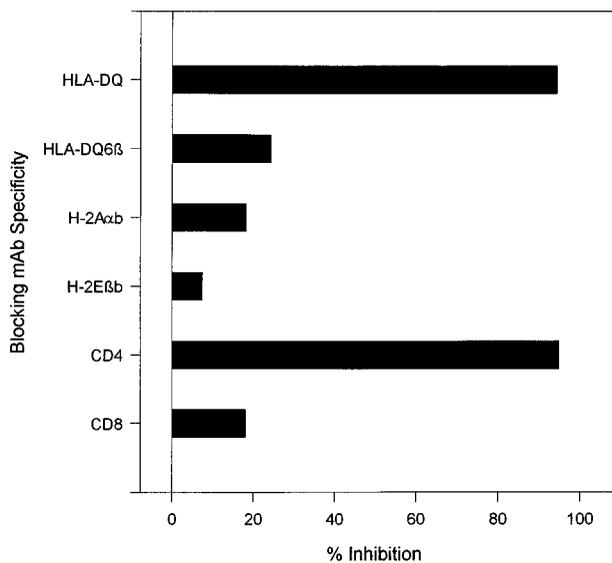


FIGURE 6. Inhibition of bovine CII-induced T cell proliferation in HLA-DQ6αβ/8αβ tg mice. LNCs of bovine CII-primed DQ6αβ/8αβ tg mice were challenged with bovine CII in the presence of various mAb as described in Figure 5. Inhibition of proliferation is displayed according to the following calculation: [(proliferation in the presence of an irrelevant mAb) – (proliferation with cell surface-specific mAb)/proliferation in the presence of an irrelevant mAb] × 100.

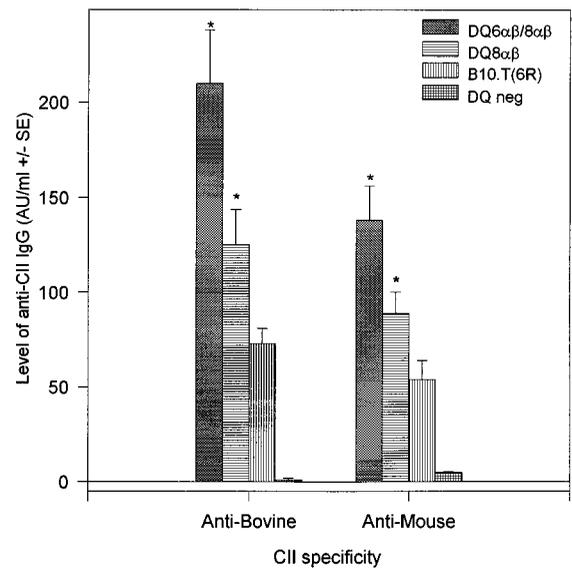


FIGURE 7. CII-specific IgG levels in HLA-DQ6αβ/8αβ tg mice. Anti-CII IgG levels were determined in sera obtained at 35 and 84 days after CII immunization and were measured by ELISA using CII-coated microtiter plates. Abs are displayed as AU per milliliter ± SE, calculated by comparison with the OD of a high titer anti-CII IgG-positive sera, arbitrarily set equal 100 AU/ml. *, *p* < 0.005.

develop an RA-like polyarthritis, while DQ6αβ/8αβ tg mice develop both polyarthritis and auricular chondritis, resembling a RP phenotype. It is likely that mixed haplotype DQ6αβ/8αβ molecules are able to present other CII epitopes causing the stimulation of another subset of autoreactive T cells homing to the ear. Alternatively, a new epitope presented by these molecules may stimulate autoreactive T cells cross-reactive with another collagen present in the outer ear, such as type IX or type XI collagen. Preliminary

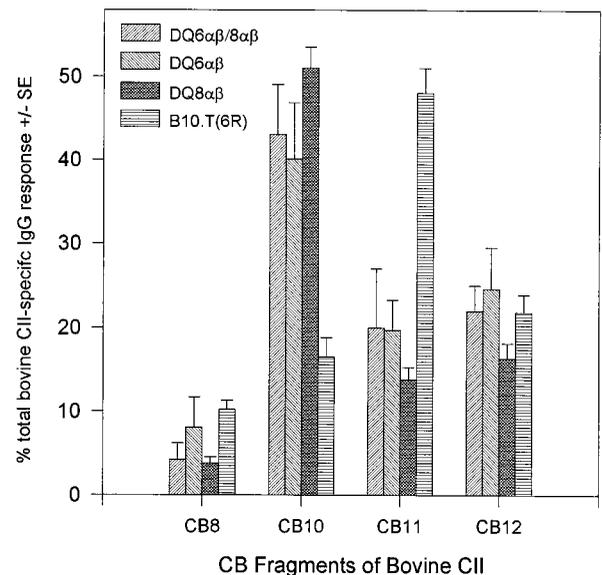


FIGURE 8. Specificity of CII-specific IgG in HLA-DQ6αβ/8αβ tg mice. The specificity of the anti-bovine CII IgG was determined by ELISA, as described in Figure 7, in sera collected on day 35. The percentage of the total anti-CII response specific for the individual bovine CB fragments was calculated as follows: [1 – (OD of anti-CB-specific response/OD of total anti-bovine CII response)] × 100. Values are displayed as the mean percentage ± SE.

studies indicate that CII-immunized DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice do express anti-type IX collagen Abs that are lacking in the DQ8 $\alpha\beta$ tg mice following CII immunization (data not shown). Type IX and XI collagen-specific humoral and cellular immune responses have also been detected in RP patients (15, 42). There are regions of homology between type II and XI collagen molecules, making such cross-reactivity between these two molecules conceivable.

Both the polyarthritis and the auricular chondritis that develop in DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice mirror similar manifestations in RP. The polyarthritis involves a massive infiltration of mononuclear cells, the destruction of cartilage, and pannus formation. The auricular chondritis is bilateral, with erythema and swelling of the outer ear corresponding to a massive infiltration of mononuclear cells. The cartilaginous tissues are destroyed, resulting in a shrunken ear that resembles the characteristic cauliflower ear of RP. While the polychondritis is not cyclical in the DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice, the histopathologic phenomenon appears to be similar to the relapsing events occurring in RP that ultimately result in the same disease outcome. Therefore, the DQ6 $\alpha\beta$ /8 $\alpha\beta$ tg mice provide an excellent tool to investigate the immunologic events that may be involved in RP. In addition, the potential for the formation of mixed haplotype DQ molecules in these mice provides a model to investigate their influence on pathogenesis. A number of connective tissue diseases have been difficult to characterize due to the overlapping manifestations. Investigation of the potential of mixed haplotype molecules may also be insightful as to the role of HLA class II expression during these diseases.

Some linkage studies in RP have implicated HLA-DR4 genes in the disease, but the results are not conclusive. HLA-DQ8 is one of the DQB1*03 genes in linkage disequilibrium with the DRB1*04 genes. If RP is mediated by a unique DQ8/6 "hybrid" molecule, linkage studies will implicate the DQ8/DR4 haplotype in some individuals and not in others. We are currently generating DQ/DR double tg mice to further elucidate genetic predisposition to RP. Several other autoimmune diseases in which HLA class II linkage analysis has been controversial could involve unique mixed haplotype class II molecules. Utilization of tg mice expressing the various combinations of HLA DQ and DR α and β genes could unravel the genetic predisposition of those diseases.

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