Modulation of HLA-DQ-restricted collagen-induced arthritis by HLA-DRB1 polymorphism

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Abstract

Mouse class II-deficient HLA-DQB1*0302, DQA1*0301 (DQ8) transgenic mice are susceptible to severe collagen-induced arthritis (CIA), an animal model for rheumatoid arthritis. To examine whether polymorphism at the DRB1 locus can modulate DQ-restricted arthritis, we generated double-transgenic (DR/DQ) mice. HLA-DRB1*1502 (DR2) and DRB1*0301 (DR3) were introduced separately into CIA susceptible DQ8.Aβo transgenic mice to generate DQ8/DR2.Aβo and DQ8/DR3.Aβo mice. The HLA-DR molecules in these mice were found to be functional on the basis of their positive/negative selection of the Vβ T cell repertoire. Introduction of the DR2 gene led to a significant decrease in disease incidence in DQ8.Aβo mice, while the DR3 transgene had no effect. In vitro T cell proliferative responses against bovine CII collagen in primed mice were higher in DQ8/DR3 mice compared with DQ8/DR2 mice. Cytokine analysis showed a Th2 profile in DQ8/DR2 mice, while DQ8/DR3 mice showed a Th1 profile. These results suggest that DRB1 polymorphism can modulate the disease.

Introduction

Rheumatoid arthritis (RA) is a severe autoimmune inflammatory disease leading to destruction of joints. About 1% of the population of the US is affected. Although the etiology is unknown, a strong genetic component contributes to the susceptibility and resistance to the disease. Initial genetic studies showed a high prevalence of the DR4 (DW4) subtype among RA patients (1). Extensive documentation exists on the association of HLA-DR4 with susceptibility to RA in most population studied. Among the 22 defined serological subtypes of the DR4 group, DRB1*0401 and DRB1*0404 are dominant alleles in RA patients, while DRB1*0405 predisposes to RA in Japanese. Subsequent analysis in different ethnic groups showed other alleles like DR1, DR6 and DR10 also to be associated with disease (2–4). Gregersen et al. (5) put forth a ‘shared epitope’ hypothesis for predisposition to RA. According to the hypothesis, the HLA-DRB1 alleles sharing the motif L/Q/K/A and L/Q/R/A at position 67, 70, 71 and 74 are implicated in the disease, while the motif I/D/E/A (DRB1*0402) is not. In some populations protection to RA has been associated with DR2 (DRB1*1502) (6–8). Limited studies on the role of HLA-DQ in RA show conflicting results on the association of DQB1 alleles, DQB1*0301 and DQB1*0302 with RA (8–11).

The target autoantigen is unknown for RA. Since the disease results in damage to cartilage, collagen type II (CII) which constitutes the major collagen content of articular cartilage is a strong candidate as an autoantigen. Recently it has been shown that multiple peptides of CII can bind to HLA-DQ molecules of a RA susceptible HLA haplotype (12). On the contrary, fewer collagen peptides bound to DR molecule. DRB1*0401-restricted T cell response in DR4 transgenic mice recognized a single peptide which is conservative in bovine CII and human CII (13).

Collagen-induced arthritis (CIA) is an experimental model of autoimmune inflammatory polyarthritis with features similar to RA. H2-A molecules which have the highest sequence homology with DQ among HLA class II molecules are involved in susceptibility to CIA (14). Recent studies using mouse class II-deficient HLA-DQ8.Aβo transgenic mice exhibited severe arthritis after immunization with type II collagen (15). Introduction of the H2-E molecule (homologue of HLA-DR) protected DQ8.Aβo mice against arthritis (16). Polymorphism...
of H2-E has been shown to protect modulation in CIA (17). Further, DRB1*1502 (DR2) protected H-2^d mice from CIA (18).

To simulate more closely a human class II haplotype we generated double transgenic (DR/DQ) mice. We have developed mice expressing DRB1*1502, DQA1*0301, DQB1*0302 (DQ8/DR2) and DRB1*0301, DQA1*0101, DQB1*0302 (DQ8/DR3). This was done by mating HLA-DR3 and -DR2 transgenic mice with DQ8^o mice. The HLA-DR2 and DR3 were chosen because DR2 is known to be protective while DR3 is neutral in RA in most of the populations studied. We report here that DRB1 polymorphism modulates DQ8-restricted CIA. While DR2 protects DQ8^o mice against arthritis, DR3 does not influence CIA. This is the first report demonstrating a role for DRB1 polymorphism in experimental arthritis using double-transgenic/knockout mice. This offers a unique animal disease model which simulates human haplotype and can shed light on the role of both DR and DQ molecules in RA.

Methods

Generation of DQ8/DR2 (DQA1*0301, DQB1*0302, DRB1*1502)^o and DQ8/DR3 (DQA1*0301, DQB1*0302, DRB1*0301, DQA1*0301, DQB1*0302, DRB1*0301, DQA1*0103).^o transgenic mice

DQ8/DR2 mice were generated by mating transgenic mice expressing the DR2 (DRB1*1502) gene (18) with transgenic mice expressing the DQ8 (DQA1*0301, DQB1*0302) genes (15). The offspring expressing the DQ8 and DR2 molecules were intercrossed to produce a line. All the parental mice used were homozygous for A^o, thus the new line was DQ8/DR2.A^o. The segregating littersmates were used as controls. DQ8/DR3.A^o mice were produced by mating transgenic mice expressing the DR3 (DRA1*0101, DRB1*0301) gene (19) with DQ8^o mice to generate the line of double transgenics. Negative littersmates were used as controls. All mice used in this study were bred and maintained in the pathogen-free Immunogenetics Mouse Colony of Mayo Clinic. Mice of both sexes were used in this study and they were 8–12 weeks old at the start of the experiment.

Flow cytometry

Expression of HLA-DR, HLA-DQ, CD4, TCR V^β chains, and mouse H2-A and H2-E molecules on peripheral blood lymphocytes (PBL) was analyzed by flow cytometry using mAb L227 (anti-DR), IVD12 (anti-DQ), Y-17 (anti-H2E^β), 14.4.4s (anti-E^α) or lyt2 (anti-CD8) as described earlier (15). The splenic B cells and macrophages were analyzed by using mAb B20.6 (anti-V^β2), KT4-10 (anti-V^β1), MR9-8 (anti-V^β5.1,2), MR9-8 (anti-V^β5.1), 44-22-1 (anti-V^β6), TR310 (anti-V^β7), KJ-16 (anti-V^β8.1,2), F23.2 (anti-V^β8.2), MR10-2 (anti-V^β9), KJ11 (anti-V^β11), 14.2 (anti-V^β14), KJ23a (anti-V^β17), HB163 (anti-A^β), 14.4.4s (anti-E^α) and Y17 (anti-E^β) as described earlier (15). The splenic B cells and macrophages were analyzed by using mAb B220 (PharMingen, San Diego, CA) and Mac1 (PharMingen) respectively.

Induction of CIA

Bovine CII was isolated as described elsewhere (14). Double-transgenic mice (8–12 weeks old) along with negative littermates were immunized. Lyophilized bovine CII dissolved in 0.01 N acetic acid overnight at 4°C at a concentration of 2 mg/ml was emulsified 1:1 with complete Freund’s adjuvant H37Ra (Difco, Detroit, MI). All mice were immunized with 100 μl of the emulsion intradermally at the base of the tail. Animals received a booster of 100 μg of CII emulsified with incomplete Freund’s adjuvant 28 days later. Mice were monitored for the onset and progression of CIA from 3 to 12 weeks post-immunization. The arthritic severity of mice was evaluated as described previously with a grading system for each paw of 0–3 (20). The mean arthritic score was determined using arthritic animals only.

Anti-collagen antibodies

Mice were bled on day 35 post-immunization, and the levels of anti-mouse CII and anti-bovine CII antibodies in serum were determined using a standard ELISA technique (21).

In vitro T cell proliferation

Mice were immunized with 200 μl of bovine CII emulsified 1:1 with CFA (200 μg), intradermally at the base of the tail (100 μl) and in each hind foot pad (50 μl). Ten days post-immunization, draining popliteal, caudal and lumbar lymph nodes were removed and prepared for in vitro culture. Lymph node cells (LNC) (1 x 10^6) were challenged by adding 100 μl of medium (negative control) or concanavalin A (2 μg, positive control) or native collagen. For inhibition experiments, 20 μl (= 5 μg of antibody) of culture supernatants containing mAb GK1.5(anti-CD4), IVD12 (anti-HLA-DQ), L227 (anti-DR), Y-17 (anti-H2E^β), 14.4.4s (anti-E^α) or lyt2 (anti-CD8) was added to the cells challenged in vitro with CII at 50 μg/ml. The cells were incubated for 48 h at 37°C and then pulsed with [^3H]thymidine. Eighteen hours later, the [^3H]thymidine uptake was determined by scintillation counting. Results are calculated as Δc.p.m. (mean c.p.m. of triplicate cultures containing antigen – mean c.p.m. of medium).

Transgenic and negative littersmates were also tested for T cell response to self peptide DW3 and DW12 (65-79) for DQ8/DR3 and DQ8/DR2 respectively, and DQ8 (30-60 and 60–90) peptides for both transgenics. DW10 peptide (65–79) was also used as a control. DW10 is known to be a good binding peptide which induces a strong in vitro proliferative response for DQ8.A^o mice (22). The mice were primed with 200 μg of peptide and challenged in vitro with 20 μg of the peptide. To see if the response was restricted by either DR or the DQ molecule, blocking studies using L227 and IVD12 antibodies were done.

Measurement of cytokines

Capture ELISA was done for measuring cytokines IFN-γ and IL-4 using kits (Genzyme). Briefly, plates were coated with capture antibody (monoclonal rat anti-mouse IL-2/IL-4 or hamster anti-mouse IFN-γ) overnight at 4°C. Plates were blocked for non specific antibodies by using blocking buffer (PBS 0.01 M, 4% BSA). Samples and diluted standards were incubated for one hour at 37°C and then the biotinylated secondary antibody was added. Plates were washed with buffer (PBS, 0.05% Tween 20) and horseradish peroxidase conjugated streptavidin was used as a detection reagent. The TMB substrate system (Sigma) was used as a substrate. The reaction was stopped using 2 N phosphoric acid. Plates were read at 450 nm. A standard curve was plotted from the mean
absorbance for each standard and antibody levels in samples were calculated from the standard curve.

IgG subtypes

Bovine CII-specific IgG subtypes IgG1, IgG2a, IgG2b and IgG3 were measured in sera of immunized and non-immunized mice by indirect capture ELISA. Briefly, plates were coated with bovine CII overnight for samples and goat anti-mouse IgG for standards and then blocked using blocking buffer (TBS, 0.05% Tween 20, 2% BSA). Test sera and standards (purified mouse IgG subtypes, Southern Biotechnology Associates, Birmingham, AL) were diluted and plated in coated plates for 1 h at 37°C. After washing, goat anti-mouse IgG subtypes conjugated with alkaline phosphatase was added for another hour. Detection was carried out with substrate p-nitrophenyl phosphate. The reaction was stopped with 2 N NaOH, and absorbance was read at 419 and 490 nm dual wavelengths in an ELISA reader. The concentration of IgG subtypes were tested in sera 35 days post-immunization.

Histology

Thymus removed from 8-week-old DQ8/DR3 and DQ8/DR2 transgenic mice was embedded in OCT compound and frozen. DQ8.α7o mice were used as control. Sections (5 μm) of the thymus were incubated with biotinylated L227 (anti-DR) antibody. Streptavidin–horseradish peroxidase was used for detection. Control thymus did not show any staining with biotinylated antibody.

Statistical analysis

The difference in the incidence of arthritis between groups was analyzed using the χ²-test with Yates’ correction. Antibody levels and mean scores for arthritic mice were compared using Student’s t-test.

Results

Expression of HLA-DR, DQ and TCR Vβ in double-transgenic mice

The HLA-DQ8 was expressed in 25–40% PBL in both DQ8\DR2 as well as DQ8\DR3 mice. Surface expression of DR molecules using L227 showed 15–20% for DR2 and 20–25% for DR3 in PBL (Fig. 1). Through flow cytometry, Vβ5 and Vβ11 were found to be deleted in DRDQ transgenics and B10.Eeκ mice but not in DQ8.α7o mice (Table 1). The Vβ T cell repertoire for DQ8\DR3 mice was normal and comparable with B10.Eeκ mice. In DQ8\DR2 mice, an increased Vβ8 with a deletion of Vβ6 bearing T cells was found in the CD4+ and CD8+ PBL. A similar profile was also seen in the thymus and LNC in these mice (data not shown).

Analysis of distribution of DR molecules was done in the thymus of both transgenics using biotinylated anti-DR antibody. Differences in the distribution were seen between the DQ8\DR2 and DQ8\DR3 mice (Fig. 2). DQ8\DR3 mice show expression of DR in thymic cortex as well as medulla. On the other hand, in DQ8\DR2 mice expression of DR was limited to the medulla and was much lower than in DQ8\DR3 mice. The expression of DQ8 in thymus was similar in both transgenics irrespective of the DR molecule (data not shown). Expression of DR and DQ was tested on splenic B cells and macrophages from animals at the termination of experiments (after 12 weeks of immunization). Only DQ8\DR3 mice were studied because of the low incidence of arthritis in DQ8\DR2 mice. An interesting observation was low expression of HLA-DR in B cells of arthritic mice, P = 0.05 (Table 2 and Fig. 3). A similar phenomenon was also seen for macrophages although the difference between arthritic and non-arthritic mice was not significant. Expression of HLA-DQ8 was similar in B cells and macrophages irrespective of the disease status (data not shown).

DRB1 polymorphism modulates DQ8-restricted CIA

Double-transgenic mice DQ8\DR2, DQ8\DR3 and their negative littermates were immunized with bovine CII, and monitored for onset and progression of disease. The incidence of arthritis in DQ8\DR2 mice at 12 weeks post-immunization was significantly lower than DQ8 mice (χ² = 13.6, P < 0.001) (Table 3). On the other hand, there was a marginal difference in DQ8\DR3 mice compared with DQ8 which was not statistically significant. The negative littermates showed no arthritis except two H2-E+ Aβ o mice showed mild arthritis. Mice carrying only DR3 showed a low incidence and less severe disease. All arthritic mice developed anti-collagen antibodies to bovine CII and mouse CII. Analysis of sera from 35 days post-immunized mice revealed marginally lower levels of antibodies to both bovine CII and mouse CII in DQ8\DR2 and DQ8\DR3 mice which was not statistically significant difference. DQ8\DR2 Aβ o mice had lower antibody levels than DQ8.α7o (P < 0.05).

In vitro response to bovine CII

To evaluate whether the response to bovine CII was DR- or DQ-restricted in double transgenics and study the effect of DRB1 polymorphism on the immune response of DQ8 to bovine CII, the DQ8\DR2 and DQ8\DR3 mice were immunized with bovine CII and 10 days later sacrificed for in vitro T cell proliferation studies. Draining LNC were isolated and challenged in vitro with bovine CII alone or in presence of specific antibodies to CD4, DR and DQ molecules. The DQ8\DR3 mice mounted a stronger response to bovine CII compared to DQ8\DR2 mice (Fig. 4). Blocking the response with GK1.5 (anti-mCD4) showed 75% inhibition. Using an anti-DQ antibody resulted in an inhibition of 60–70%. However, L227 (anti-DR antibody) inhibited only 25% of the response in DQ8\DR2 mice, while in DQ8\DR3 mice there was an inhibition of 65%. Anti-CD8 antibody did not inhibit the response to bovine CII in both transgenics.

In vitro response to self HV3 peptides

To see if these mice could present self antigens, DW12\DW3 HV3 (65–79) and DQ8 (30–60 and 60–90) peptides were used to immunize the DQ8\DR2 and DQ8\DR3 mice (Fig. 5). DW10 was used as a control peptide for both transgenics. No proliferation was seen for the self peptide as well as control peptide for both transgenics although DQ8\DR2 mice did show a mild proliferation (SI = 2.0) with DW12 and DQ8\DR3 with DQ8 (60–90) (SI = 2.2). Both transgenics showed
Fig. 1. Surface expression of DRB1*1502, DRB1*0301 and DQB1*0302 and Aβ in double-transgenic and control mice was analyzed by flow cytometry using specific antibodies. The methodology and antibodies are described in Methods.

Table 1. Selection of peripheral Vβ T cell repertoire in CD4⁺ T cells in DQ8/DR2, Aβo, DQ8/DR3, Aβo and control transgenic mice

<table>
<thead>
<tr>
<th>Vβ</th>
<th>B10.Edk</th>
<th>DQ8/DR2</th>
<th>DQ8/DR3</th>
<th>DQ8</th>
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<tr>
<td>2</td>
<td>4.6 ± 0.4</td>
<td>3.5 ± 0.3</td>
<td>3.1 ± 0.6</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>8.5 ± 1.1</td>
<td>10.2 ± 2.4</td>
<td>7.4 ± 1.2</td>
<td>6.6 ± 0.6</td>
</tr>
<tr>
<td>5.1.2</td>
<td>1.9 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>7.5 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>0.08 ± 0.0</td>
<td>0.6 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>6.8 ± 0.8</td>
</tr>
<tr>
<td>6</td>
<td>10.1 ± 1.4</td>
<td>0.3 ± 0.1</td>
<td>12.8 ± 2.5</td>
<td>7.4 ± 1.2</td>
</tr>
<tr>
<td>7</td>
<td>5.2 ± 0.2</td>
<td>4.9 ± 0.4</td>
<td>4.8 ± 1.0</td>
<td>9.1 ± 1.1</td>
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<tr>
<td>8</td>
<td>12.6 ± 2.0</td>
<td>18.8 ± 1.9</td>
<td>12.7 ± 1.3</td>
<td>10.3 ± 0.5</td>
</tr>
<tr>
<td>8.2</td>
<td>10.2 ± 0.8</td>
<td>8.4 ± 0.5</td>
<td>8.9 ± 0.3</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>9</td>
<td>7.8 ± 1.0</td>
<td>5.5 ± 0.7</td>
<td>4.3 ± 0.6</td>
<td>6.7 ± 1.1</td>
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<td>11</td>
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<td>0.3 ± 0.2</td>
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<tr>
<td>14</td>
<td>15.2 ± 2.4</td>
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<td>7.8 ± 1.3</td>
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<td>17</td>
<td>2.4 ± 0.4</td>
<td>2.5 ± 0.5</td>
<td>2.2 ± 0.2</td>
<td>5.6 ± 1.0</td>
</tr>
</tbody>
</table>

Vβ TCR were done by flow cytometry using peripheral blood mononuclear cells. The frequency of CD4⁺ Vβ TCR was calculated from the gated CD4⁺ population. The data are presented as the mean percent positive ± SD of three animals per group.

Table 1. Selection of peripheral Vβ T cell repertoire in CD4⁺ T cells in DQ8/DR2, Aβo, DQ8/DR3, Aβo and control transgenic mice

Table 1. Selection of peripheral Vβ T cell repertoire in CD4⁺ T cells in DQ8/DR2, Aβo, DQ8/DR3, Aβo and control transgenic mice

Proliferation with DQ (30–60) peptide although DQ8/DR2 mice had a higher response (data not shown).

DQ8/DR2 mice have higher IL-4 levels

Culture supernatants from bovine CII primed mice showed that DQ8/DR2 mice produced a strong Th2 type of cytokine, IL-4, with or without in vitro challenge with bovine CII. Interestingly the production of IL-4 was seen only after bovine CII immunization as unimmunized naive mice had no detectable levels. Cytokines from CIA⁻ and CIA⁺ DQ8/DR2 mice tested after 12 weeks of immunization showed production of IL-4 and IFN-γ in both, although the levels of IL-4 were lower and IFN-γ higher for CIA⁺ mice. DQ8/DR3 mice on the other hand, produced significant amounts of IFN-γ in comparison to DQ8/DR2 (Fig. 6). Unimmunized mice produced very low levels of IFN-γ when challenged with bovine CII (data not shown). Anti-DR and anti-DQ antibodies led to reduced production of cytokines. When challenged with self Dw (65–79) peptide, both transgenics had very low levels of IFN-γ and undetectable levels of IL-4. On the other hand, DQ peptides produced higher IFN-γ levels in DQ8/DR3 mice when compared with DQ8/DR2 mice.
Table 2. Expression of DR and DQ molecules in antigen-presenting and peripheral blood cells in arthritic and non-arthritic DQ8\DR3 transgenic mice

<table>
<thead>
<tr>
<th>Mice tested</th>
<th>CIA status</th>
<th>DR</th>
<th>DQ</th>
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<tr>
<td></td>
<td></td>
<td>B cells (%)</td>
<td>PBL (%)</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>66.8 ± 12</td>
<td>20.4 ± 5.5</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>42.3 ± 8.3</td>
<td>15.3 ± 3.5</td>
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*P = 0.05, percent B cells expressing DR molecule in CIA+ versus CIA− mice.

All mice used were Aβo.

IgG Subtypes

Since various IL regulate the production of different IgG subtypes, bovine CII-specific subtypes were studied in sera of mice immunized with bovine CII to support the cytokine data. Both transgensics showed the presence of bovine CII-specific IgG1, IgG2a and IgG2b in immunized mice (Fig. 7). A high variability of all IgG subtypes was observed in CIA+ mice. A comparison of IgG subtypes in DQ8\DR2 and DQ8\DR3 arthritic mice showed a significant higher levels of IgG1 in the former (P < 0.001), while DQ8\DR3 mice had higher levels of IgG2b (P < 0.05). In non-arthritic mice levels of all the IgG subtypes were lower than arthritic mice; however, no significant differences were observed in DQ8\DR2 and DQ8\DR3 mice.

Discussion

This is the first report demonstrating a modulatory role of DRB1 in the DQ-restricted pathogenic autoimmune response using double-transgenic/knockout mice. Introduction of the HLA-DQ8 transgene in H-2Aβ0 mice confers susceptibility to CIA, an RA like disease. We mated the DR2 (DRB1*1502) and the DR3 (DRB1*0301) transgenic mice with DQ8.Aβ0 mice to generate double transgenic mice expressing both the HLA-DR and DQ genes and thus simulate a human haplotype. Our findings establish a unique model to study the role of DR as well as DQ genes in the genetic predisposition to RA. About 20–25% of PBL show positivity for the DR molecule and 30–45% are positive for the DQ molecule in the double-transgenic mice. Expression of the DR molecule leads to deletion of certain Vβ T cells similar to the H2-E molecule for the selection of TCR. An increase in CD4 cells in the transgenic mice over Aβo and a unique Vβ profile of the T cell repertoire.
DRB1 polymorphism modulates DQ8-restricted CIA

Fig. 3. Surface expression of HLA-DR on B cells in CIA+ (a) and CIA- (b) mice analyzed by flow cytometry. The cells are gated on B cells stained with B220-phycoerythrin and analyzed for DR-FITC. Littermates negative for transgene were used as a control.

Fig. 4. In vitro LNC proliferative response of transgenic mice to bovine CII. LNC from 10 days immunized mice were cultured in vitro with CII alone and also in the presence of mAb specific for HLA-DQ (IVD12), HLA-DR (L227), mouse CD4 (GK1.5) and mouse CD8 (lyt2). The background counts for immunized mice with media alone were 5230 ± 1024 for DQ8, 4678 ± 1332 for DQ8\DR2 and 7082 ± 2154 for DQ8\DR3 mice.

shows a DQ/DR-mediated thymic education. DQ8\DR3 and DQ8\DR2 mice show certain differences in Vβ profile. DQ8\DR2 mice show partial deletion of Vβ6. Since these mice are MTV-7+ it is possible that DR2 is able to present a different superantigen leading to negative selection of Vβ6. Alternatively, Vβ6 cells may be the self-reactive T cells which are regulated by the presentation of a different self antigen by the DR2 molecule. There is an increased population of Vβ8 T cells in DQ8\DR2 mice suggesting a positive selection of this subset in the context of DR2. There is a differential expression of DR molecules in the thymus of both transgenics (Fig. 2). DR3 has a good expression in both the cortex and medulla, while DR2 is expressed predominantly in the medullary region.

A recent study has shown that differential expression of Eα peptide in the thymus of mice can regulate negative and positive selection (23). It is possible that a different TCR repertoire is being selected in both transgenics because of the differential expression of DR which may lead to a difference in peripheral peptide presentation. However, the present data does not answer if this could be a plausible explanation for the difference in susceptibility to disease in these mice.

The expression of the DR2 gene in DQ8.Aβ0 mice led to a significant decrease in the incidence of arthritis, although severity and onset of the disease was not affected. On the other hand, DR3 does not influence the incidence of arthritis in DQ8.Aβ0 mice. Recent studies have shown that DQ8 can bind multiple CII peptides while DR4 of the RA susceptible HLA haplotypes DR4\DQ4 binds only a limited number of collagen-derived peptides (12). Even though DR molecules bind a few CII peptides with high affinity, there was no difference between the susceptible and non-susceptible haplotypes. Using overlapping peptides spanning the CB11 fragment of human type II collagen in DQ8.Aβ0 transgenic mice, the DQ8 molecule was found to bind several CII epitopes (unpublished observation). Also studies using Hsp65 peptides in Aβ0\DR3 transgenic mice show that similar peptides are restricted by HLA-DR in mice and human (24). The above studies show that HLA molecules can present antigens in transgenic mice in a similar manner as in human. Few peptides from bovine CII collagen which are homologous to human CII have been shown to bind DR4 (13). It is possible that DR3 can present some of the collagen peptides since DQ8\DR3 mice give a higher response to bovine CII than DQ8 mice and also an anti-DR antibody can reduce the response to bovine CII in DQ8\DR3 mice. On the other hand, DR2 may present only a few CII peptides, since in DQ8\DR2 mice only a mild inhibition is seen with the anti-DR antibody. Since the major response to CII is DQ and CD4 restricted, the DR molecules may be involved in shaping the T cell repertoire rather than presenting arthritogenic peptides. Thus, the DRB1 polymorphism may determine protection, exacerbation or neutrality in the disease.

Mice with low expression of DR on antigen-presenting cells
were more likely to get arthritis. It is possible that in mice with good expression of the DR molecule, processed DR peptides are binding to the DQ8 molecule preventing the presentation of CII peptides. DW10 and DW2 peptides show good T cell proliferative responses in vitro in DQ8.Aβo mice indicating good binding of these peptides, while DW3 peptide does not trigger any T cell proliferation in an in vitro culture with primed DQ8.Aβo cells (22). Neither of the double transgenics shows proliferation to DW10 as well as self peptide. It is possible that in DQ8/DR2 mice DW peptide binds DQ8 in thymus which leads to negative selection of self-reactive T cells. In DQ8/DR3 mice, DW3 does not bind DQ8 strongly, which could lead to the positive selection of autoreactive T cells. It could be that this difference in selection of T cells leads to differential cytokine profile in both the transgenics when

**Fig. 5.** In vitro LNC proliferative response of transgenic mice to peptides Eβ (65–79), DQ8 (60–90), self Dw peptide (65–79); DW12 in DQ8/DR2 and DW3 in DQ8/DR3 mice, and control peptide DW10 (65–79). DQ8.Aβo mice did not give any response to DW3 peptide (data not shown).

**Fig. 6.** Cytokine analysis for T-h1 (IFN-γ) and T-h2 (IL-4) response from culture supernatants of LNC in the presence or absence of bovine CII from mice immunized with bovine CII. Aβo mice did not show detectable levels of IFN-γ and IL-4.

**Fig. 7.** IgG subtypes IgG1, IgG2a, and IgG2b in DQ8/DR2.Aβo and DQ8/DR3.Aβo arthritic mice. IgG subtypes were done from sera collected from mice after 5 weeks of immunization with bovine CII. Significant differences in the levels of IgG1 (P = 0.006) and IgG2b (P = 0.05) were found between the two transgenic groups.

**Table 3.** Collagen induced arthritis in DQB1*0302, DRB1*0301.Aβo and DQB1*0302, DRB1*1502.Aβo transgenic mice and littermates using bovine CII

<table>
<thead>
<tr>
<th>Mice</th>
<th>Incidence (%)</th>
<th>Onset (days)</th>
<th>Score (Mean ± SD)</th>
<th>Antibodies to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Mean ± SD)</td>
<td></td>
<td>Bovine CII</td>
</tr>
<tr>
<td>DQ8/DR3</td>
<td>12/21 (57.1)</td>
<td>42 ± 9</td>
<td>4 ± 3.2</td>
<td>30.9 ± 2.8</td>
</tr>
<tr>
<td>DQ8/DR2^a</td>
<td>2/11 (18.1)</td>
<td>35 ± 5</td>
<td>5 ± 2</td>
<td>22.5 ± 3.1</td>
</tr>
<tr>
<td>DQ8</td>
<td>34/44 (77.3)</td>
<td>40 ± 5</td>
<td>5.7 ± 2.3</td>
<td>42.6 ± 5.4</td>
</tr>
<tr>
<td>DR3</td>
<td>4/19 (21.0)</td>
<td>45 ± 13</td>
<td>1.5 ± 0.5</td>
<td>17.9 ± 2.3</td>
</tr>
<tr>
<td>E+</td>
<td>2/30(6.6)</td>
<td>65</td>
<td>2</td>
<td>6.9 ± 2.2</td>
</tr>
<tr>
<td>DR2</td>
<td>0/9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβo</td>
<td>0/10</td>
<td></td>
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</tr>
</tbody>
</table>

^aDQ8 versus DQ8/DR2, χ² = 11.1, P < 0.001.

All mice were immunized with 100 µg of bovine CII in complete Freund’s adjuvant on day 0 and boosted with 100 µg bovine CII in incomplete Freund’s adjuvant on day 28. Mean arthritic score was calculated at the termination of experiment at 12 weeks post-immunization using only the arthritic mice. The antibody levels are described as mean ± SD (U/ml) for arthritic mice only.
immunized with bovine CII. In peripheral blood, DR3 probably can also present some CII peptides resulting in a low incidence of CIA. The DR3 gene has been linked to RA in the Arab population (25).

An in vitro challenge with bovine CII in primed mice leads to production of high levels of IFN-γ in DQ8/DR2 mice while DQ8/DR2 mice produce low levels of IFN-γ and high amounts of IL-4 in the presence or absence of bovine CII. Since both transgenics develop antibodies to bovine CII, it is possible that in DQ8/DR2 mice T cells secreting Th1 cytokines switch to Th2-producing cells. A possible explanation comes from the fact that CII*” mice produce IFN-γ and also low amounts of IL-4, while CII-type mice have higher amounts of IL-4. Alternatively, one cell might be producing both Th1 as well as Th2 cytokines at different times. Intracellular staining of IFN-γ and IL-4 in CD4 memory cells from primed mice challenged with bovine CII might answer the question. We are already working in this direction to find out if the production of cytokines in these mice switches at some time during the disease process or whether one cell is producing both cytokines in primed mice. There is a study showing modulation of cytokine profile to HSP-reactive CD4+ T cells by HLA-DR polymorphism (26).

References


