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Expression of the H2-E molecule mediates protection to collagen-induced arthritis in HLA-DQ8 transgenic mice: role of cytokines

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Abstract

Transgenic mice expressing DQA1*0301 and DQB1*0302 (HLA-DQ8) molecules in class II-deficient Ab° mice are susceptible to collagen-induced arthritis (CIA). To evaluate the role of the H2-E molecule (a homolog of HLA-DR) in DQ-restricted arthritis, the H2-E gene was introduced into DQ8.Ab° mice to generate DQ8/E$^+$.Ab° mice. Expression of the E molecule protects DQ8.Ab° mice against arthritis. *In vitro* studies using draining lymph nodes from mice primed with bovine type II collagen (BII) showed that the response to BII in both transgenics is DQ and CD4 restricted. Challenge with BII *in vitro* leads to production of high levels of IFN-γ in DQ8 and IL-4 in DQ8/E$^+$ mice. We have hypothesized that the H2-E molecule modulates the T cell repertoire and changes the cytokine balance, resulting in protection of disease.

Introduction

Rheumatoid arthritis (RA) is a chronic immuno-inflammatory disorder of unknown etiology with a characteristic pattern of joint disease and extra-articular complications.

Genetic studies in various ethnic groups have shown predisposition to RA linked to the class II HLA-DRB1 locus. A large proportion of RA patients carry alleles sharing a sequence homologous to DRB1*0401/0404 at the hypervariable (HV) 3 region of the DRB1 chain (67–74). Gregersen *et al*. (1) proposed that the molecular basis for susceptibility lies in sequences 67, 70, 71 and 74 of the DRB1 chain. Studies in some populations have shown that DR2 confers protection in RA (2,3). The association of RA with DQ3 (DQB1*0301 and DQB1*0302) has shown conflicting results in various studies. Although DQ7 has been associated with severity of disease (4,5), an increased frequency of DQ8 in RA has been shown in some populations (3,6).

Collagen-induced arthritis (CIA) is an animal model of autoimmune inflammatory polyarthritis, sharing similarities with RA. Susceptibility to CIA in mice is influenced by the H2-A locus within the MHC and is restricted to haplotype H2^q and H2^r (7). Studies in this laboratory have shown that polymorphism of the class II Eb gene determines protection to CIA in H2^q mice (8,9). Introduction of the HLA-DR2 (DRB1*1502) gene in H2-A^q mice also protects against CIA (10). Recently we have shown that transgenic mice expressing DQA1*0301, DQB1*0302 (DQ8) in class II-deficient Ab° mice are susceptible to CIA. A severe arthritis was seen in DQ8.Ab° mice compared to negative littermates (11). To evaluate if the H2-E molecule (a homolog of HLA-DR) could protect against a DQ-restricted arthritis, the H2-E genes were introduced into DQ8.Ab° mice to generate DQ8.Ab°/E$^+$ mice. We report here that expression of the E molecule in CIA susceptible DQ8.Ab° mice protects mice against arthritis probably by modulating the T cell repertoire.

Methods

*Generation of DQ8 (DQA1*0301, DQB1*0302).Ab° and DQ8/E$^+$.Ab° transgenic mice*

DQ8.Ab° mice were generated using cosmids H II A X10A, generously provided by J. Strominger as described earlier (11–13). The DQ8.Ab° mice were mated with H2-E$^+$.Ab° mice to generate DQ8/E$^+$.Ab° mice. The H2-E$^+$.Ab° mice were previously generated by introduction of the E_α^k transgene into

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Ab° mice (14). The E_α^k molecule pairs with the E_β^b molecule expressed in Ab° mice to generate a functional H2-E molecule. 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 CD4, HLA–DQ, TCR V_β chains, and mouse H2-A and H2-E molecules on peripheral blood lymphocytes (PBL) was analyzed by flow cytometry using mAb GK1.5 (anti-CD4), IVD12 (anti-DQB1), PK136 (anti-NK1.1), MR9-4 (anti-V_β5.1), KJ-16 (anti-V_β8.1.2), 44-22-1(anti-V_β6), KT11 (anti-V_β11), 25-5-16 (anti-Ab), 7-16.17(anti-A_α) and Y17 (anti-E_β^b) as described previously (9,11).

Induction of CIA

Bovine type II collagen (CII) and chick CII were isolated as described elsewhere (7). Lyophilized 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 (CFA; Difco, Detroit, MI). All mice (8–12 weeks) were immunized with 100 μl of the emulsion intradermally at the base of the tail. Animals received a boost of 100 μg of CII emulsified in incomplete Freund's adjuvant 28 days later. Mice were monitored for the onset and progression of CIA for 3–12 weeks after immunization. The severity of arthritis in these mice was evaluated as described previously, with a grading system for each paw of 0–3 (15). 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, anti-chick CII and anti-bovine CII in serum were determined using a standard ELISA technique (16). The amount of total 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 T cell proliferation

Mice were immunized with 200 μl of bovine CII emulsified 1:1 with CFA (200 μg), 100 μl intradermally at the base of the tail and 50 μl in each hind foot pad. Ten days after immunization draining popliteal, caudal and lumbar lymph nodes (LN) were removed and prepared for culture. LN cells (1×10^6) were challenged *in vitro* by adding 100 μl of medium (negative control), concanavalin A (2 μg, positive control) and native collagen. For inhibition experiments, 20 μl (5 μg of antibody) of culture supernatants containing mAb GK1.5 (anti-CD4), IVD12 (anti-HLA-DQ), Y-17 (anti-H2-E_β^b) or lyt2 (anti-CD8) was added to the cells challenged *in vitro* with CII at 50 μg/ml. Eighteen hours later, tritium incorporation 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 littermates were tested for T cell responses to the E_β^b peptide (amino acids 60–90). The mice were primed with 200 μg of peptide and tested *in vitro* after challenge with 20 μg of the peptide. For blocking NK1.1-positive cells, PK136 (anti-NK1.1) was used.

Measurement of cytokines

Capture ELISA was done for measuring cytokines IFN-γ, IL-2 and IL-4 using kits (Genzyme, Cambridge, MA). 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 (0.01 M PBS, 4% BSA). Samples and diluted standards were incubated for 1 h at 37°C and then the biotinylated second antibody was added. Plates were washed with buffer (PBS, 0.05% Tween 20) and horse-radish peroxidase-conjugated streptavidin was used as detection reagent. 3,3,5,5′-Tetramethylbenzidine (TMB substrate system; Sigma Bio Sciences, St Louis, MO) 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 the concentration in samples was 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 were 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 the coated plates for 1 h at 37°C. After washing, goat anti-mouse IgG subtypes conjugated with alkaline phosphatase were added for a further 1 h. 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 the samples was calculated from the standard curve. IgG subtypes were estimated in sera from mice before immunization, 5 weeks post-immunization and at the termination of the experiment at 12 weeks.

Statistical analysis

The difference in the incidence of arthritis between groups was analyzed using the χ^2-test with Yates' correction. Antibody levels and mean scores for arthritic mice were compared using Student's *t*-test.

Results

Expression of HLA-DQ8, H2-E, CD4 and TCR V_β in DQ8.Ab° and DQ8/E^+.Ab° transgenic mice

Both transgenics expressed HLA-DQ8 in 25–40% of the PBL which restored CD4 expression to a substantial level (Fig. 1). Surface expression of the H2-E_β^b molecule, using H2-E_β^b-specific mAb Y17, was undetectable in DQ8.Ab° mice while DQ8/E^+.Ab° mice reacted as strongly as positive control E^+.Ab° mice. An increased number of NK1.1 cells was found in DQ8/E^+ mice (4.5 ± 1.2) when compared to DQ8 (0.8 ± 0.2), while E^+Ab° mice had 1.0 ± 0.4. By flow cytometric analysis, deletion of V_β5.1.2- and V_β11-

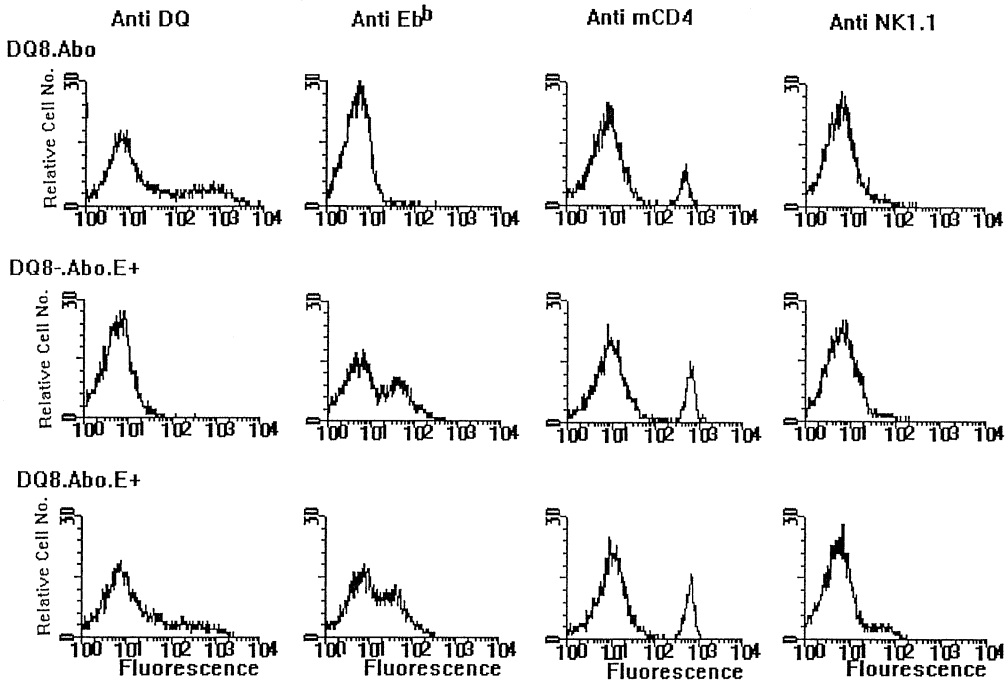

Fig. 1. Expression of HLA-DQ8, H2-E molecule, CD4 and NK1.1$^+$ cells in PBL of transgenic mice DQ8.Ab°, DQ8/E$^+$.Ab° and E$^+$.Ab° by flow cytometry using specific antibodies. The methodology and antibodies used are described in Methods.

Table 1. V_β repertoire in peripheral CD4$^+$ and CD8$^+$ T cells in DQ8/E$^+$.Ab° and DQ8.Ab° mice

V_β	DQ8.Ab°		DQ8/E$^+$.Ab°		E+.Ab°	
	CD4	CD8	CD4	CD8	CD4	CD8
V_β5.1.2	10.6 ± 2.8	17.9 ± 0.8	2.4 ± 0.4	4.4 ± 1.5	0.9 ± 0.02	3.1 ± 0.9
V_β6	12.1 ± 1.1	6.9 ± 1.4	16.3 ± 0.9	10.4 ± 0.5	15.1 ± 0.2	9.1 ± 0.5
V_β8.1.2	12.7 ± 2.0	12.1 ± 1.2	10.6 ± 0.8	17.8 ± 1.6	8.9 ± 0.5	16.2 ± 2.8
V_β11	11.2 ± 3.5	7.2 ± 0.6	1.6 ± 1.3	2.9 ± 0.2	0.8 ± 0.1	2.2 ± 0.2

The frequency of V_β TCR$^+$ cells was calculated from gated CD4$^+$ and CD8$^+$ populations. The data are presented as the mean percent positive cells ± SD of three animals per group.

bearing T cell populations was found in CD4 and CD8 subsets for DQ8/E$^+$ and E$^+$.Ab° mice only (Table 1).

Presence of the H2-E molecule protects CIA-susceptible DQ8.Ab° mice against arthritis

To investigate the effect of the H2-E molecule on susceptibility to CIA, DQ8 and DQ8/E$^+$, mice along with negative littermates were immunized with bovine and chick CII, and monitored for onset and progression of CIA for at least 12 weeks. The incidence of arthritis in DQ8/E$^+$ mice at 12 weeks after immunization was significantly lower than in DQ8 mice immunized with bovine CII ($\chi^2 = 20.08$) as well as chick CII ($\chi^2 = 5.01$) (Table 2). The negative littermates did not show any arthritis, while in the E$^+$.Ab° group only three mice developed mild arthritis with a score of 1–2. Among the DQ8 mice, noticeable arthritis was seen within 4–5 weeks with >50% of the total arthritic mice having an onset at 4 weeks. On the other hand, DQ8/E$^+$ mice developed arthritis at ~5–7 weeks post-immunization. The mean severity score was much milder for DQ8/E$^+$ mice when compared to DQ8 mice, $P < 0.001$.

Anti-CII antibodies are depressed in DQ8/E$^+$ mice

To evaluate the differences in humoral response in these mice, anti-CII antibodies were measured in mice immunized with bovine CII in CFA. The inhibitory effect of the E_β^b transgene in DQ8 mice was apparent in the development of antibodies against bovine, chick and mouse CII. Analysis of sera from animals 35 days after immunization revealed a strong IgG antibody response to bovine CII in both groups of transgenics which developed arthritis, although it was significantly higher in DQ8 (30.6 ± 6.4 U/ml) as compared to DQ8/E$^+$ (13.3 ± 4.6 U/ml), $P < 0.0001$. A similar result was seen for antibodies against mouse CII in these mice. Mice which had transient arthritis developed low levels of antibodies. There was a 57 and 50% reduction in the mean units of

Table 2. CIA in DQ8/E$^+$.Ab° and DQ8.Ab° mice and negative littermates using bovine and chick type II collagen

Strain	Incidence (%)	Onset (days) (mean ± SD)	Score (mean ± SD)	Antibodies to	
				bovine CII/chick CII (U/ml)	mouse CII (U/ml)
Bovine CII					
DQ8.Ab°	34/44 (77.3)	35 ± 6	6.2 ± 2.1	30.6 ± 4.4	28.8 ± 5.1
DQ8/E$^+$Ab°[a]	12/44 (27.3)	44 ± 8	3.8 ± 1.8	13.3 ± 4.6	14.1 ± 3.8
E$^+$.Ab°	2/30 (6.6)	65		27.8 ± 3.2	6.9 ± 2.2
Ab°	0/17				
Chick CII					
DQ8.Ab°	9/10 (90)	32 ± 7	6.3 ± 2.4	26.0 ± 6.4	25.3 ± 5.5
DQ8/E$^+$Ab°[b]	5/14 (35.7)	54 ± 10	5.1 ± 2.5	12.1 ± 6.7	13.2 ± 4.6
E$^+$.Ab°	1/11	35	6	22.6	10.88
Ab°	0/6				

[a] $\chi^2 = 20.08$, $P < 0.0001$.
[b] $\chi^2 = 5.01$, $P < 0.02$.
For antibodies to bovine CII and chick CII, DQ8 versus DQ8/E$^+$, $P < 0.001$.
Mice were immunized with 100 μg bovine or chick CII in CFA on day 0 and boosted with 100 μg 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 the arthritic mice only. The antibody levels are described as mean ± SD (U/ml) for CIA$^+$ mice only.

bovine CII- and mouse CII-reactive antibodies respectively in DQ8/E$^+$ mice as compared to DQ8 mice. Similar results were observed for chick CII immunized DQ8 and DQ8/E$^+$ mice ($P < 0.001$, Table 2).

In vitro proliferative response of DQ8/E$^+$.Ab° and DQ8.Ab° mice to bovine CII

To evaluate the effect of the H2-E molecule on the immune response of DQ8 mice against bovine CII, LN cells from bovine CII immunized mice were used for *in vitro* proliferation. DQ8/E$^+$ mice mounted a strong detectable response when challenged *in vitro* with bovine CII (Fig. 2a). The response was comparable in both transgenics (stimulation index of 2.5–3). The response could be inhibited with a mAb specific for HLA-DQ (65–85%) or mouse CD4 (40–50%) (Fig. 2b). Addition of an unrelated antibody, i.e. L227 (anti-HLA-DR) and Y17 (specific for E_β^b), did not affect the response, indicating that bovine CII is being presented by the DQ molecule in DQ8/E$^+$ mice.

In vitro response to self E_β^b HV3 peptide

To evaluate if the self peptide E_β^b can be presented by DQ8/E$^+$ mice, LN cells from mice immunized with the peptide were challenged *in vitro* with the E_β^b (60–90) peptide comprising HV3. While DQ8 mice could present the peptide, DQ8/E$^+$ and E$^+$.Ab° mice showed very poor stimulation to the peptide. Studies using PK136 (anti-NK1.1) with E_β^b peptide in culture showed an increase in proliferation (double) to the peptide in DQ8/E$^+$ mice while there was no significant difference in DQ8 mice (Fig. 3). Thus the DQ8 molecule is capable of presenting E_β^b peptide, but the presence of the H2-E molecule induces tolerance.

Increased production of IL-4 in DQ8/E$^+$ mice

DQ8 and DQ8/E$^+$ were immunized with 200 μg of bovine CII emulsified in CFA at the base of tail and hind paws. Mice were sacrificed 10 days later and LN cells were used to study cytokines. IFN-γ, IL-2 and IL-4 were tested for T_h1 or T_h2

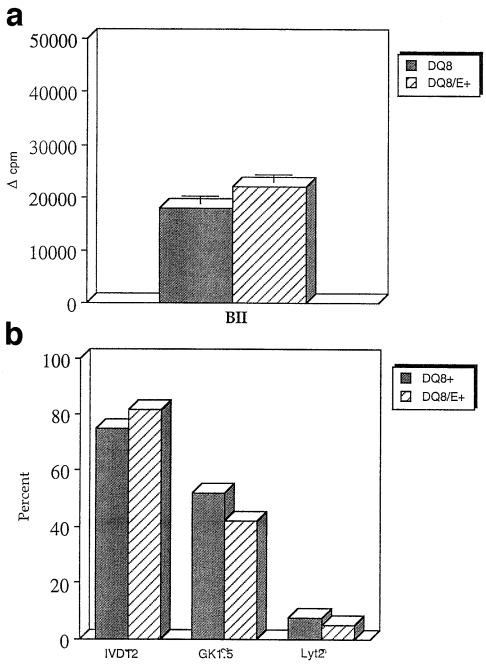

Fig. 2. (a) *In vitro* LN cell proliferative response of HLA-DQ8. H2-Ab° and DQ8/E$^+$.H2-Ab° mice to bovine CII. The results are expressed as Δc.p.m. The value of Δc.p.m. for Ab° mice challenged with bovine CII was 1265 ± 135. (b) For inhibition studies, LN cell were cultured in the presence of bovine CII and mAb specific for HLA-DQ, mouse CD4 and mouse CD8. The percent inhibition of the bovine CII response was calculated as follows: [1 – (Δc.p.m. in the presence of mAb/Δc.p.m. in the presence of control mAb)]×100.

cytokines. There was an increased production of IFN-γ when LN cells were challenged *in vitro* with bovine CII which was reduced drastically when anti-CD4 and anti-DQ antibodies were used in the assay (Fig. 4). This increase was significantly higher in DQ8 compared to DQ8/E$^+$ mice. On the other hand, a significant increase in IL-4 was observed in DQ8/E$^+$ mice

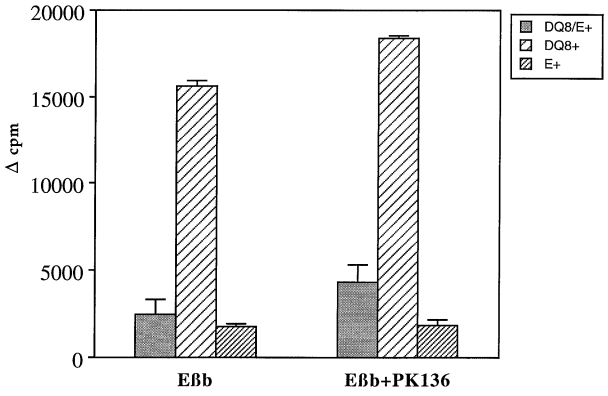

Fig. 3. *In vitro* LN cell proliferative response of HLA-DQ8.H2-Ab°, DQ8/E^+.H2-Ab° and E^+.Ab° mice to E_β^b peptide (60–90). The Δc.p.m. for Ab° mice challenged with E_β^b peptide was 562 ± 163. LN cells were cultured in the presence of peptide and mAb PK136 (anti-NK1.1).

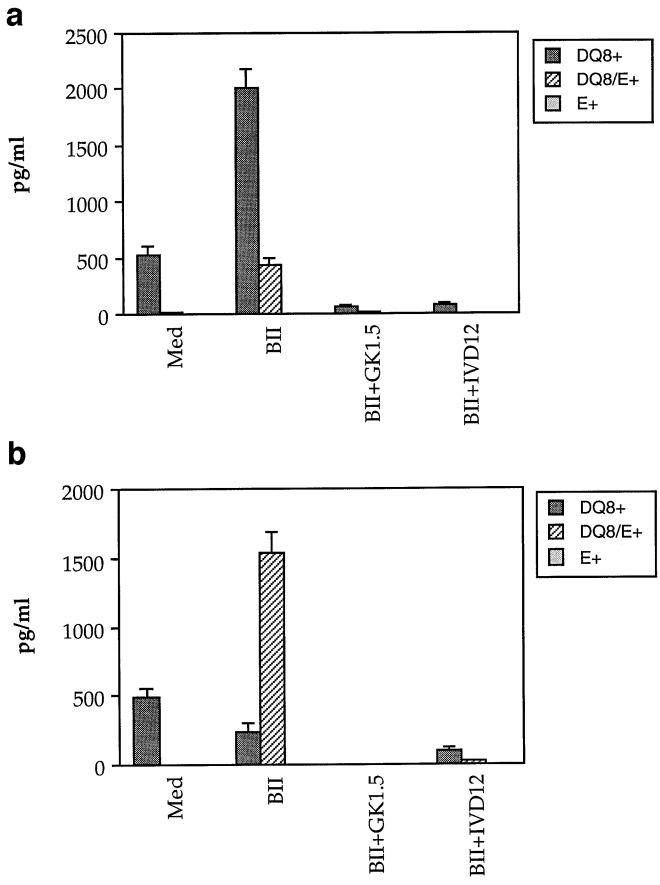

Fig. 4. (a) IFN-γ levels and (b) IL-4 levels in culture supernatants of LN cells from bovine immunized DQ8.Ab°, DQ8/E^+.Ab° and E^+.Ab° mice. mAb to mouse CD4 (GK1.5) and HLA-DQ (IVD12) were used to see if inhibition of response to bovine CII changed the cytokine profile. E^+.Ab° mice did not have any detectable levels of IFN-γ and IL-4. Presence of the GK1.5 abrogated any detectable levels of IL-4 in all transgenic mice.

subsequent to challenge with bovine CII *in vitro*. The control E^+.Ab° mice did not show any cytokine production. In a pilot study, the sera of mice immunized with bovine CII showed the presence of IL-4 as well as IL-2 (data not shown).

IgG subtypes

Since different IgG subtypes regulate the production of various ILs, bovine CII-specific IgG subtypes were studied in sera of mice immunized with bovine CII to support the cytokine data. Both DQ8 and DQ8/E^+ mice showed the presence of bovine CII-specific IgG1 and IgG2a/2b in sera from immunized mice (Fig. 5a and b). A high variability of all IgG subtypes was observed in CIA^+ mice. Mice with severe arthritis had higher IgG2a levels as compared to mice with milder arthritis within one group. Unimmunized mice did not show any IgG antibodies in their sera. Similarly, negative control Ab° and Ab°.E^+ mice did not have any IgG1 while very low levels of IgG2a and IgG2b were present at termination of the experiment. A comparison of IgG subtypes at different time intervals in CIA^+ versus CIA^- mice showed an increase in all IgG subtypes in CIA^+ mice as compared to CIA^- mice. Although the DQ8.Ab° mice had higher levels of all IgG subtypes studied, the difference in IgG1 and IgG2a levels between DQ8 and DQ8/E^+ was significant only in CIA^- mice. Analysis of sera from all the mice on day 0 did not show the presence of any IgG subtypes except a very low level of IgG2a in DQ8 mice which developed CIA after immunization (data not shown).

Discussion

Expression of the HLA-DQ8 transgene in class II-deficient H2-Ab° mice renders them susceptible to the RA-like disease CIA. We report here an HLA-DQ-restricted pathogenic autoimmune response in HLA-DQ8.Ab° transgenic mice can be prevented by the expression of the H2-E molecule (a homolog of HLA-DR). A protective role of E_β^d and DRB1*1502 in the CIA susceptible $H2^q$ haplotype has been documented (8–10). Introduction of E_α^k gene in DQ8.Ab° mice resulted in expression of the H2-E molecule ($E_\alpha^k E_\beta^b$). About 50–60% of the peripheral cells were positive for E_β^b by flow cytometry using an anti-Eb antibody Y17. Analysis of the V_β TCR repertoire in PBL demonstrated deletion of V_β5.1.2 and V_β11 in DQ8/E^+ mice only. This shows that thymic education of T cells is normal in these mice. Expression of the E_β^b molecule in CIA susceptible DQ8 mice led to a reduction in the incidence and severity of the disease. Measurement of antibodies to mouse CII and bovine CII also showed decreased levels of IgG in the sera of DQ8/E^+ mice. We found an increase in the occurrence of NK1.1 cells in DQ8/E^+ mice. These cells might play a role in protection although the present data does not explain how this subset of NK1.1 cells can lead to less susceptibility to disease.

Recently, it has been shown that ligand motifs for H2-E molecules are allele specific (17). The peptide motifs indicate a very high structural similarity of mouse class II H2-E molecules with human DR molecules. The motifs of H2-E molecules can be aligned to pockets predicted by the crystal structure of the HLA-DR1 molecule. These findings make our present study more relevant since it simulates the human situation where both DR and DQ are expressed in one individual. At HV3 E_β^b resembles DRB1*1502 which is known to be protective in RA,

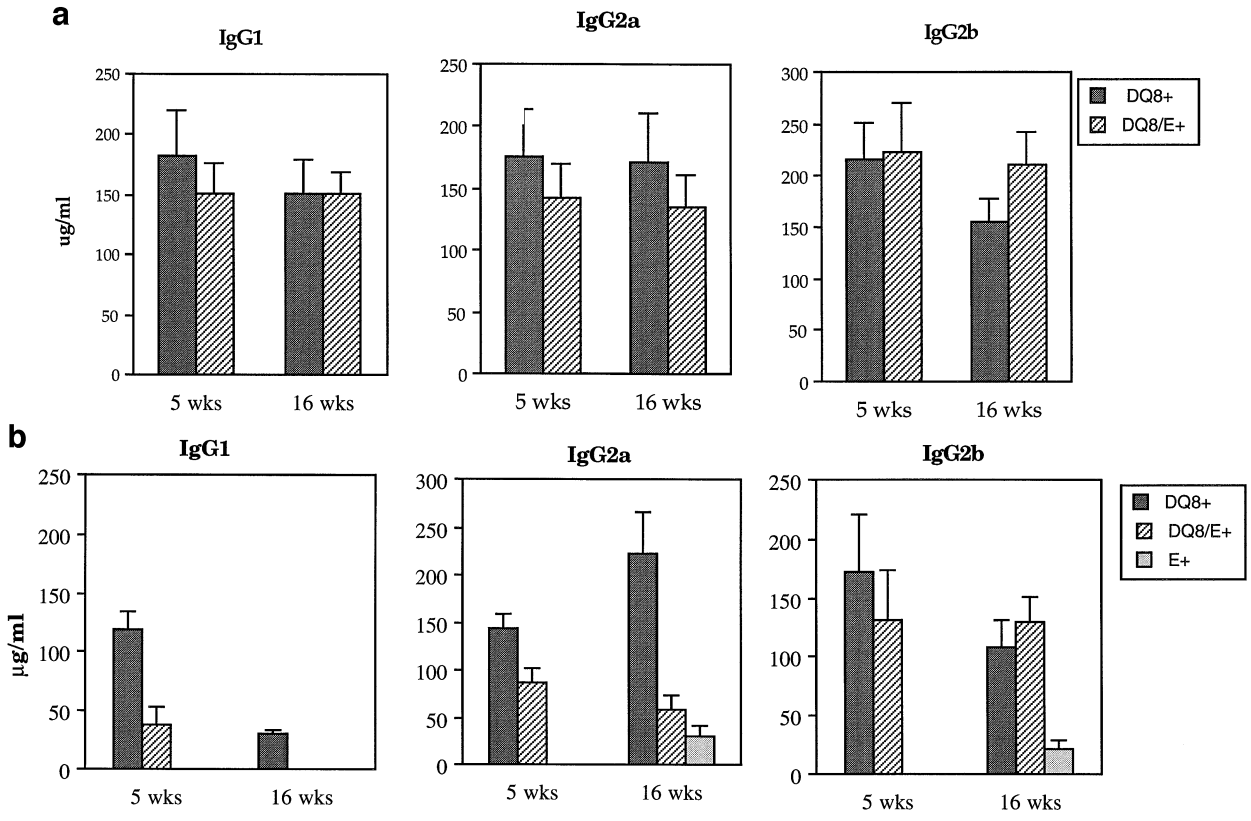

Fig. 5. IgG subtypes IgG1, IgG2a and IgG2b in (a) CIA$^+$ and (b) CIA$^-$ DQ8.Ab° and DQ8/E$^+$.Ab° mice along with negative littermates. IgG subtypes were done prior to immunization (0 week), post-immunization at week 5 and at the termination of experiment. No IgG subtypes were detected in transgenic mice at 0 week in both CIA$^+$ and CIA$^-$ mice except IgG2a which was detected in small amounts in DQ8.Ab° mice which developed CIA after immunization.

except at position 74 where alanine is replaced by glutamic acid. DRB1*0803, shown to be negatively associated with RA in the Japanese population, has negative charge at position 70 (Asp). It is possible that the overall charge in pocket 4 is similar for E_β^b and DRB1*0803. Alternatively, since this difference lies in P4, it might not alter the peptide binding specificity although avidity might differ. In human RA, individuals with DRB1*1502 have a low incidence of arthritis comparable to the lower number of DQ8/E$^+$ mice which develop arthritis.

We found that DQ8/E$^+$ mice were not able to present the E_β^b peptide while DQ8 mice could. It is possible that in DQ8/E$^+$ mice the presentation of the self H2-E peptide by the DQ molecule negatively selects T cells in thymus which are self reactive, thereby altering the T cell repertoire. On the other hand, the H2-E molecule may be positively selecting T cells which are not self reactive. Recently, an invariant chain peptide was eluted from DRB1*0402 but not from DRB1*0401 and *0404 molecules (18). Likewise, it can be postulated that E_β^b can bind the naturally processed (tolerogenic) peptide modulating the T cell repertoire, thus being unable to contribute to the loading of arthritogenic peptide(s).

The present results suggest that the cytokines produced by T cells may be central to the disease process. The cytokines and IgG subtypes in sera and culture supernatants of LN cells from bovine CII immunized DQ8 and DQ8/E$^+$ mice show the presence of both T_h1 and T_h2 types of response. The T_h1 and T_h2 responses are ameliorated when an anti-CD4 antibody is used, indicating the response to be CD4 restricted. Since CD4$^+$ T cells are known to be abundant in the infiltrates of the synovium, the local production of the cytokines may be important in the tissue damage in the joint. A considerably higher IFN-γ production by DQ8 mice might be responsible for the T cell-mediated severe inflammatory disease observed in these mice. IFN-γ is spontaneously produced by bovine CII immunized DQ8 mice while in DQ8/E$^+$ mice an *in vitro* challenge with bovine CII is required for IFN-γ production. The presence of IL-4 might be responsible for the autoantibody production in these mice. On the other hand, lower IFN-γ and higher IL-4 levels in DQ8/E$^+$ mice might be the reason for reduced severity and incidence of disease in DQ8/E$^+$ mice. Our IgG subtypes results support this data indirectly. IL-10 (T_h2 cytokine in mice) has been suggested as a switch factor for naive B cells to secrete IgG1 and IgG3 (19). We found elevated levels of IgG1 in bovine CII immunized mice. However, in CIA$^-$ mice IgG1 levels were lower when compared to CIA$^+$ mice at the termination of the experiment (16–20 weeks). The reverse was seen for IgG3 (data not shown). While IgG3 could be detected in CIA$^+$ DQ8.Ab° mice, the levels were lower than CIA$^-$ mice. On the other hand, only CIA$^+$ DQ8/E$^+$ mice showed the presence of IgG3 at 5 weeks after immunization which decreased as the time progressed. IgG2a/2b, indicative of a T_h1 response,

were present in both arthritic and CIA$^-$ mice, although the levels of the former were much lower in CIA$^-$ mice.

Thus one probable mechanism by which expression of the H2-E molecule protects DQ-restricted arthritis could be by modulating the T cell repertoire and altering the balance of cytokines produced by autoreactive T cells. Since NK1.1 cells are a major source of IL-4 upon priming, in DQ8/E$^+$.Ab° mice an increase in these cells could be responsible in some way for deviating the immune response. While autoantibody production is similar in both groups, T cell activity decreases in DQ8/E$^+$ mice. Recently, using transgenic and non-transgenic NOD/LT mice, it was shown that expression of H2-E on antigen-presenting cells may block insulin-dependent diabetes mellitus by altering the balance of cytokines produced by β cell autoreactive T cells (20). Our results with cytokines and IgG subtypes indicate that both T_h1 and T_h2 responses are present in bovine CII immunized arthritic mice. The T_h1 cytokines may be responsible for the inflammatory response, while T_h2 may actually lead to autoantibody production. Thus cytokines may be central to the disease process by regulating autoantibody production and contributing to disease directly or indirectly.

These findings support our hypothesis that predisposition to human RA is determined by an extended HLA-DQ/DR haplotype, rather than purely by the DRB1 'shared epitope'. While the HLA-DQ molecule may be responsible for the T_h1/T_h2-mediated inflammatory response and antibodies, the HLA-DR molecules may contribute to a primarily T_h2-mediated antibody response. The role of the 'shared epitope' may be in shaping the T cell repertoire. Further studies with our 'humanized' mouse model of CIA may shed some new light on this mystery.

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Abbreviations

CFA	complete Freund's adjuvant
CIA	collagen-induced arthritis
CII	type II collagen
HV3	hypervariable
LN	lymph node
PBL	peripheral blood lymphocyte
RA	rheumatoid arthritis

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