Expression and Function of Transgenic HLA-DQ Molecules and Lymphocyte Development in Mice Lacking Invariant Chain

Govindarajan Rajagopalan, Michele K. Smart, Christopher J. Krco and Chella S. David

*J Immunol* 2002; 169:1774-1783; doi: 10.4049/jimmunol.169.4.1774

http://www.jimmunol.org/content/169/4/1774

---

**References**

This article cites 58 articles, 25 of which you can access for free at: http://www.jimmunol.org/content/169/4/1774.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Expression and Function of Transgenic HLA-DQ Molecules and Lymphocyte Development in Mice Lacking Invariant Chain

Govindarajan Rajagopalan, Michele K. Smart, Christopher J. Krco, and Chella S. David

Invariant chain (Ii) is a non-MHC-encoded molecule, which plays an accessory role in the proper assembly/expression of functional MHC class II molecules and thereby plays an important role in Ag processing/presentation.

In wild-type mice (14), the level of surface expression of DQ8 molecules is comparable with that of DQ8.Ii−/− mice. However, in mice expressing Ii, the level of surface expression of DQ8 molecules was increased in DQ8.Ii−/− mice, DQ8.Ii−/− APCs were capable of presenting Ag to primed T cells from wild-type DQ8 mice as well as to a specific T cell hybridoma. Differentiation of mature B cells was also affected to a certain extent in DQ8.Ii−/− mice. The Journal of Immunology, 2002, 169: 1774–1783.
Although several studies have addressed the accessory role of the Ii in the proper assembly and expression of murine class II molecules, the extent of Ii participation in the expression of HLA class II (especially DQ8), is not fully known. Understanding the expression and function of DQ8 is crucial not only because it is strongly associated with certain autoimmune diseases such as type 1 diabetes and celiac disease (31), this would also shed some light on the interaction between Ii and DQ8 from the in vivo standpoint. As allelotypic variations exist within the murine class II molecules on Ii dependence, the role of Ii in assembly, expression, and function of a human class II molecule was determined using HLA-DQ8 and -DQ6-transgenic mice with disrupted Ii gene.

Materials and Methods

Mice

BALB/c and C57BL/10 (B10) mice originally came from The Jackson Laboratory (Bar Harbor, ME). Generation of HLA-DQ8-transgenic mice lacking endogenous class II molecule (Aβ) has been described earlier (32, 33). HLA-DQ8.Ii−/− mice were generated as follows. The Ii KO mice on B10.D2 background (produced by Viville et al., Ref. 15; and obtained through J. Miller, University of Chicago, Chicago, IL) were crossed with HLA-DQ8-transgenic mice. The heterozygous offspring were intercrossed and the Ii−/− littermates expressing DQ8 were selected and intercrossed for several generations to obtain DQ8.Ii−/− mice. HLA-DQ8 mice with and without Ii are hereafter referred to as DQ8.Ii+/− and DQ8.Ii−/−, respectively. Lines lacking the HLA-DQ8 transgene and Ii were also maintained in the mouse colony. Similarly, mice transgenically expressing HLA-DQ6 (32) were bred to Ii−/− mice to generate Aβ.DQ6.Ii−/− mice. The HLA-transgenic mice do not express any endogenous mouse class II Ags due to introduction of the inactivated H2-Aβ gene at mating to the Aβ- bearing mice (32). Mice bred in the barrier facility were moved to conventional facility following weaning and used at 8–12 wk of age unless specifically mentioned.

Abs and reagents

The following Abs were used for FACS analysis. Anti-TCR Vβ8.1, 2, 3 (F23.1), anti-HLA-DQ (IVD12, HB-14), and L227 (HB-96) were obtained from the manufacturer’s protocol. Enriched CD3+ T cells obtained as above were cultured (5 × 10^6 cells/well) with equal numbers of irradiated APCs from either DQ8.Ii−/− or DQ8.II−/− mice in the presence or absence of varying concentrations of SEB for a total of 72 h, and cell proliferation was determined by thymidine incorporation.

Peptide immunization and lymphoproliferation assay

A previously described peptide (P44) encompassing aa 554–573 from human type II collagen, which is highly immunogenic in DQ8 mice (33), was emulsified in CFA and injected s.c. at the base of the tail and footpads such that each mouse received 100 μg of the peptide. Seven days later, mononuclear cell suspensions were prepared from draining lymph nodes and cultured in either medium alone or different concentrations of P44 in 96-well flat-bottom tissue culture plates in triplicates (1 × 10^5 cells/well) for a total of 48 h. Cell proliferation was determined by thymidine incorporation as described above.

Ag presentation assays

HLA-DQ8-transgenic mice were immunized with P44 and lymph node mononuclear cells were isolated 7 days later as described above. CD3+ T cells from pooled lymph node mononuclear cells were enriched by depleting B cell and macrophages using columns as described earlier. The enriched CD3+ T cells (5 × 10^6 cells/well) were cultured with equal numbers of irradiated splenocytes from either DQ8.Ii−/− or DQ8.II−/− mice as APCs for a total of 72 h in the presence of Con A or various concentrations of P44. Cell proliferation was determined by thymidine incorporation as described above.

Cytokine analysis

Splenocytes from naive mice were cultured in 24-well plates for 72 h with SEB (1 μg/ml) or left unstimulated. Cytokines present in the culture supernatants were quantified by sandwich ELISA as described earlier (34). Cytokines present in the sera were also determined by routine sandwich ELISA as per published protocol (34).

Induction of toxic shock with SEB

Mice received two i.p. injections of SEB (100 μg in 200 μl of PBS) 48 h apart and were closely monitored for the symptoms of shock. In the t-galactosamine (t-gal)-sensitized toxic shock model, mice were injected i.p. with 20 mg of t-gal (Sigma-Aldrich) followed by SEB (20 μg/mouse), each in 200 μl of PBS.

Estimation of serum IgG levels

Mice were bled at different time points and the concentration of serum IgG was estimated by standard ELISA. Briefly, ELISA plates were coated with goat anti-mouse IgG (10 μg/ml) in sodium bicarbonate buffer as the capture Ab overnight at 4°C. Following blocking with 3% BSA for 2 h, adequately diluted serum samples were added to the wells in duplicates and incubated further for 2 h. Following a washing step, peroxidase-conjugated goat anti-mouse IgG was added as the detection Ab. Mouse IgG captured on the plates was revealed by adding the substrate tetramethylbenzidine. Concentration of IgG present in the sera was extrapolated from the standard curve generated using the purified mouse IgG (Sigma-Aldrich).
FUNCTIONALITY OF TRANSGENIC HLA-DQ IN Ii KO MICE

Statistics
The statistical significance of the results was determined by using GraphPad Prism software (version, 3.0a; GraphPad, San Diego, CA).

Results
Expression of DQ8 in naive Ii-deficient mice
Analysis of the splenocytes from DQ8.Ii−/− mice by flow cytometry using the Ab IVD12 revealed reduced surface expression of HLA-DQ8 when compared with the wild-type DQ8-transgenic mice (Fig. 1, a–c). Screening with other HLA class II-specific Abs such as IVA12 (HB145, anti-DR, -DP, -DQ; ATCC) and 93.F10 (HB-180, anti-DR, -DQ; ATCC) by flow cytometry also showed similar results (data not shown). Therefore, for further analysis only IVD12 was used. Even the percentage of cells expressing DQ8 was reduced in Ii−/− mice (Fig. 1d). Expression of DQ8 remained at low levels when mice of different age groups were tested, indicating that expression of DQ8 in Ii-deficient mice does not change with age (data not shown). Similarly, we could not find any difference in the expression profile of DQ8 between the mice raised in the conventional animal facility vs the ones reared in the barrier facility (data not shown). This again implies a minimal influence of the environment on DQ8 expression in Ii−/− mice. Moreover, culturing splenocytes from DQ8.Ii−/− mice with LPS did not result in increased surface expression of class II molecules (data not shown).

T cell development in Ii-deficient mice
As mice lacking functional Ii have reduced surface expression of class II molecules, we studied the development of CD4+ T cells in these mice. As predicted, DQ8-transgenic mice lacking Ii had significantly reduced numbers of mature CD4+ T cells in the periphery with a slight increase in CD8+ T cells (Fig. 2a). Defective CD4+ T cell maturation in Ii−/− mice was persistent and did not improve with age (Fig. 2, a and b, and additional data not shown).

SEB-induced proliferation of splenocytes from DQ8.Ii−/− mice was severalfold higher than that of DQ8.Ii+/+ mice at all doses of SEB tested (Fig. 3a). We also compared the proliferative responses of splenocytes from DQ8.Ii−/− mice with that of B10 mice to both SEB and to a nonspecific mitogen, Con A. As shown in Fig. 3b, the proliferative response of DQ8.Ii−/− splenocytes to SEB was significantly higher than that of B10 mice and this difference was more pronounced at lower dose of SEB. This response was DQ8 dependent as the proliferative response was almost absent in Ii−/− mice lacking the DQ8 transgene (Fig. 3b). Defective proliferation of B10 splenocytes was specific for SEB as they proliferated very efficiently to Con A. This is because HLA class II can present SEB more efficiently than H2-Aβ (35).

In some experiments, irradiated splenocytes from DQ8.Ii−/− mice were used to present SEB to enriched CD3+ splenocytes from either B10 mice or from mice transgenically expressing TCR Vβ8 (Fig. 4). When CD3+ splenocytes from B10 mice were used as responders, defective SAg presentation by DQ8.Ii−/− mice was more apparent at lower concentrations of SEB (Fig. 4a). The same was true when the enriched CD3+ T cells from mice expressing TCR Vβ8 on CD4+ T cells were used (Fig. 4b). However, when the enriched CD3+ T cells from mice expressing TCR Vβ8 on CD8+ T cells were used as responders, very little proliferation could be observed with DQ8.Ii−/− APCs (Fig. 4c). The results indicate that 1) DQ8.Ii−/− mice are compromised in their ability to respond to the bacterial SAg SEB both in terms of cell proliferation and cytokine production when compared with their Ii-sufficient counterpart and 2) DQ8.Ii−/− mice still express residual levels of DQ8 and are thus capable of inducing proliferation of responder cells in response to SEB (which is superior to murine class II, H2-Aβ).

SEB-induced expansion of T cells in vivo
To study in vivo T cell activation in SEB-primed Ii-deficient and -sufficient DQ8-transgenic mice, splenocytes were collected at different time points and analyzed by flow cytometry. The percentage of total CD4+ and CD8+ T cells remained at low levels when mice of different age groups were tested (Fig. 5). SEB caused massive activation resulting in expansion of both CD4+ and CD8+ T cells bearing TCR Vβ8 family in HLA-DQ8.Ii−/− transgenic mice. There was a 5-fold increase in both CD4+ and CD8+ T cells bearing TCR Vβ8 by day 3 after
SEB injection. The number of TCR Vβ8-bearing CD4+ cells remained elevated (4-fold) even by day 7, while the TCR Vβ8-bearing CD8+ T cell numbers were only slightly above the pre-treatment values at day 7. As DQ8.Ii−/− mice have very low CD4+ T cell numbers, there was not a significant expansion of CD4+ T cells in these mice at any time point. However, there was a definite and comparable increase in TCR Vβ8-bearing CD8+ cells in these mice at day 3 followed by a deletion in TCR Vβ8-bearing CD8+ cells at day 7 similar to that seen in DQ8.Ii+/+ mice. The results indicate that even though DQ8.Ii−/− mice express very low levels of surface DQ8, leading to improper positive selection of CD4+ T cells, they express adequate levels of DQ8 to present the SAg SEB and to drive the expansion of SEB-reactive CD8+ T cells. Proliferation of splenocytes from DQ8.Ii−/− mice in response to SEB and the ability of APCs from DQ8.Ii−/− mice to activate T cells bearing TCR Vβ8 support this observation.

**SEB-induced toxic shock**

We have previously shown that HLA-DQ8-transgenic mice are susceptible to SEB-induced toxic shock even without sensitization with β-gal (34). Because DQ8.Ii−/− mice expressed very low levels of surface DQ8 and had fewer CD4+ T cells in the periphery, we wondered whether the absence of Ii rendered HLA-DQ8-transgenic mice resistant to SEB-induced shock. Although all of the DQ8.Ii+/+ mice succumbed following the second SEB injection, none of the DQ8.Ii−/− mice died (Table I). We then studied SEB-induced mortality in β-gal-sensitized mice. As shown in Table I, 50% of the DQ8.Ii−/− mice were still susceptible to SEB-induced toxic shock while none of the Ii−/− mice lacking the HLA class II transgene (Abo.DQ8-.Ii−/−) and none of the B10 mice succumbed, indicating clearly that DQ8 is important for this process.

**SEB-induced in vivo cytokine production was determined by measuring serum TNF-α and IFN-γ by ELISA. We could not detect any TNF-α even in sera of DQ8.Ii+/+ mice that succumbed to SEB-induced shock (data not shown), whereas we could detect very high levels of IFN-γ in these sera at 3 h following SEB injection (Fig. 6). DQ8.Ii−/− mice had significantly less IFN-γ in the sera than Ii-sufficient mice, implying a possible pathogenic role for IFN-γ in SEB-induced lethal shock.**

**Response to P44**

We also tested the ability of DQ8.Ii−/− mice to mount a T cell response to P44, which has been shown by us previously to be immunogenic in DQ8 mice (33). Although lymph node cells from peptide-immunized DQ8.Ii+/+ could proliferate efficiently upon restimulation with the same peptide, no response could be elicited in cells obtained from DQ8.Ii−/− mice immunized with the same peptide, indicating severe defects in mounting a CD4+ T cell-dependent immune response (Fig. 7a). However, irradiated splenocytes from naïve DQ8.Ii−/− mice were capable of presenting peptides to T cells obtained from DQ8 mice immunized with P44 (Fig. 7b). We also studied the ability of splenocytes from Ii-sufficient and -deficient mice to present peptide to a peptide-specific T cell hybridoma. Splenocytes from Abo.DQ8.Ii+/+ mice could efficiently present peptide to the T cell hybridoma at 18 h. However, by 36 h, Abo.DQ8.Ii−/− splenocytes could induce IL-2 production though not to the same extent as Abo.DQ8.Ii+/+ splenocytes (Fig. 7c). The results indicate that even though Ii deficiency results in reduced expression of DQ8 (which leads to poor
positive selection of CD4+ T cells and compromises the ability to mount a CD4+-mediated T cell response), the Ag-presenting capacity of the APCs is still maintained albeit in reduced efficiency.

**Effect of Ii deficiency on DQ6 expression and function**

We also studied the role of Ii on expression/function of another related HLA class II molecule, DQ6. Expression of DQ6 was analyzed using two different Abs, IVD12 and L227. (We have observed that L227 can recognize the DQ6-*0601 chain encoded by DQ6 and can thus be used to monitor surface expression of DQ6.) Irrespective of the Ab used, DQ6.Ii mice always showed poor expression of DQ6 when compared with DQ6.Ii mice (Fig. 8), thus proving that even DQ6 is dependent on Ii for its expression. As a reflection of poor DQ6 expression, CD4+ T cell development was also compromised in DQ6.Ii mice (Fig. 8b). Poor proliferative response to SEB underscores these defects (Fig. 8c). Overall, it appears that Ii is necessary for proper expression of HLA-DQ6 and -DQ8 molecules.

**B cell maturation in Ii-deficient mice**

Because the Ii has been shown to play an important role in maturation of B cells, we estimated the percentage of mature B cells in peripheral lymphoid organs of DQ8.Ii-deficient mice. DQ8.Ii mice had significantly reduced numbers of CD23 high (Fig. 9a) and IgD high cells (Fig. 9b) in the spleen and the difference...
was more pronounced in older mice. Similar results were obtained when lymph nodes from these mice were analyzed. However, mature B cells were not completely absent in Ii-deficient mice. Although DQ8.Ii−/− mice had comparable numbers of B220+ cells at 10 wk of age, indicating that there was no defect as such in the development of B cell precursors, at 40 wk of age there was a 2-fold reduction in B220+ cells, implying that B cell development could be compromised in older DQ8.Ii−/− mice (Fig. 9c). (B220 is a pan-B cell marker expressed from pro-B cell to mature B cell stages.) Furthermore, as an indicator of mature B cell function, the serum IgG level in naive DQ8.Ii−/− mice was quantitated at two time points. DQ8.Ii−/− mice consistently had significantly less serum IgG levels when compared with the wild-type DQ8-transgenic mice (Fig. 9d). Immunizing DQ8.Ii−/− mice with a known Ag in CFA also did not result in an increase in CD23+, IgM+, or IgD+ cells (Fig. 10, a–d) nor an increase in CD4+ T cells in the draining lymph nodes (Fig. 10, e and f).

Discussion

Processing of exogenous Ag and presentation by class II molecules is a complex process involving the coordinated participation of several molecules (7, 8, 36). The most important of all is the MHC class II molecule which finally presents the peptide fragments generated from the processed Ag to CD4+ T cells. Invariant chain is another molecule which plays an essential role in this process (37, 38). It acts as a chaperone in assembly, maturation, and expression of MHC class II molecules (39, 40). Allelic variations between different MHC class II molecules for Ii dependence exist. Although class II molecules of H-2d and H-2k haplotypes are relatively independent of Ii, H-2k+ allele shows strong dependence on Ii. Mice lacking the functional Ii have reduced expression of surface class II molecules which is associated with functional defects such as defective CD4+ T cell selection (27), poor responses to exogenous Ags/SAgs (20), viral Ags (41), and also autoantigens (42, 43).

The phenotype of HLA-DQ8.Ii−/− mice was similar to that of H-2b mice lacking functional Ii in that surface expression of fully mature HLA-DQ8 was significantly reduced. Surface expression of DQ8 was determined by flow cytometry using the Ab IVD12 (44). There exists a possibility that in the absence of Ii, the DQ8 αβ heterodimer might exist in different conformation and hence IVD12 is not able to detect them, but the normal level of DQ8 is still expressed in DQ8.Ii−/− mice. However, this is unlikely as the expression profile remained similar even when other Abs were used for screening. Moreover, defective expression of DQ6 as assessed by two different Abs underscores the importance of Ii on expression of HLA-DQ molecules (DQ6 and DQ8). CD4+ T cell numbers are also significantly reduced in these mice. Positive selection of CD4+ T cells in the thymus depends on the presence of functional class II molecules. Significantly reduced numbers of CD4+ T cells both in the thymus of DQ8.Ii−/− (not analyzed for

### Table I. SEB-induced toxic shock

<table>
<thead>
<tr>
<th></th>
<th>AbC. DQ8.li−/+</th>
<th>AbC. DQ8.li−/−</th>
<th>AbC. DQ8.Ii−/−</th>
<th>B10</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEB (2 × 100 μg)</td>
<td>6/6*</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>n-gal (20 mg)</td>
<td>3/4</td>
<td>3/6</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>SEB (20 μg)</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* Mortality.
DQ6.II mice clearly indicate a significant reduction in the functional class II molecules in DQ6-deficient mice. It is of interest to note that HLA-DM molecules are capable of chaperoning DR molecules from ER through Golgi even in the absence of Ii in some cell lines (45, 46). However, this does not seem to be an efficient pathway in vivo in mice. This could be due to differences between the mouse H-2M molecules and human HLA-DM or it could be due to inherent differences in the class II molecules in question, DR vs DQ8.

We further studied the functionality of DQ8/DQ6 in DQ-deficient mice by testing its ability to present the bacterial SAg SEB. SAg, a family of microbial proteins, are strong polyclonal activators of T and B lymphocytes. The T cell SAg differ from mitogens in that T cell activation by SAg is MHC class II dependent and TCR mediated.

They differ from conventional Ags in that T cell activation by SAg is MHC dependent, peptide nonspecific, and CD4/CD8 coreceptor independent. Although a processed nominal Ag is presented either by MHC class I or class II molecules to CD8+ and CD4+ T cells, respectively, SAg in their native form bind to MHC class II molecules outside the peptide-binding groove and vigorously activate both T cell subsets bearing certain TCR Vβ families (reviewed in Ref. 47). Because class II molecules are mandatory for eliciting a response to SAg, the poor proliferative response of splenocytes from DQ6/DQ8.II mice further confirms that there are defects in expression of the fully functional class II molecules. It is of interest to note that in spite of the reduced expression of class II molecules in DQ8.II mice, the response to SEB was significantly higher than that of wild-type B10 mice expressing normal levels of class II and CD4+ T cells. This is due to the fact that HLA class II molecules present SEB more efficiently than their murine counterpart (35).

Defective T cell response in DQ6/DQ8.II mice to SEB could be attributed to either low CD4+ T cell numbers (the responders) or to reduced expression of surface class II molecules (the presenters) and most likely the combination of both the defects. Less efficient presentation of SEB by DQ8.II APCs to either T cells from B10 mice or from TCR-transgenic mice expressing Vβ8 on CD4+ T cells reveal defective functioning of MHC class II molecules (DQ8) in the absence of Ii. DQ8.III mice were also less efficient in activating T cells from TCR-transgenic mice expressing Vβ8 on CD4+ T cells. Comparatively, the defects in presentation of SEB by DQ8.II APCs were more pronounced when CD8+ T cells were used as responders. This could be due to possible differences in the responder cell types (CD4+ vs CD8+ T cells). Because the nature of the T cell response to SAg depends on several factors such as TCR Vβ usage (47), TCR Vα usage (48), ligand density (49), the peptide repertoire bound by class II molecules, etc. (50, 51), it is beyond the scope of the present investigation to explore these differences. The main conclusion from this set of experiments is that in the absence of Ii, the efficiency of presentation of SAg by DQ8 is reduced but not completely lost.

Even though DQ8 molecules in Ii+ mice were less efficient in inducing proliferation of CD8+ T cells expressing the LCMV-specific TCR Vβ8 transgene, in vivo administration of SEB resulted in an increase in TCR Vβ8-expressing CD8+ T cells in DQ8.III mice which was comparable to that seen in DQ8.II mice. However, the CD4+ T cell population did not show any significant change in the DQ8.III mice. This clearly indicates that even the reduced levels of surface DQ8 is still sufficient to drive proliferation of TCR Vβ8+CD8+ T cells in Ii−/− mice. Activation of CD8+ T cells by the residual DQ8 might explain the proliferation and cytokine production still seen in DQ8.III cultures.

SAg-induced toxic shock is the major pathology seen mainly in humans resulting from massive T cell activation and concomitant cytokine production (52). Mice are generally considered to be resistant to SEB-induced shock because injection of SEB alone is rarely lethal in mice, except at very high doses and only in certain mouse strains and not in others (53). We have shown previously (34) and also in the present study that DQ8-transgenic mice are highly susceptible to SEB-induced toxic shock even without β-gal sensitization, and the pathology is probably due to high production of IFN-γ. However, DQ8.II+ mice were completely resistant to this procedure. We could consistently detect very high levels of IFN-γ in the sera of DQ8.II+ mice but not in DQ8.II− mice following injection with SEB. Protection of DQ8.II− mice from SEB-induced shock is probably due to low levels of surface DQ8 and low numbers of CD4+ T cells which are the major producers...
of IFN-γ following activation with SEB. However, pretreatment with β-gal, which sensitizes the hepatocytes to apoptosis induced by TNF-α (53), rendered ~50% of DQ8.Ii−/− mice still susceptible to SEB-induced shock while B10 mice and Ii−/− mice lacking DQ8 were completely resistant (Table I). Mortality in β-gal-sensitized DQ8.Ii−/− mice could be due to cytokines produced by CD8 T cells, which as discussed earlier, are activated by the low levels of DQ8 still expressed in these mice.

DQ8.Ii−/− mice closely resembled Ii KO mice expressing endogenous H-2K class II molecules even with respect to mounting an immune response to peptide Ags (21). Priming with an immunogenic peptide elicited poor secondary T cell responses in DQ8.Ii−/− mice. Interestingly, the comparable efficiency with which the irradiated DQ8.Ii−/− APCs presented the peptide to primed T cells from wild-type DQ8 mice indicates that even in the absence of Ii, low levels of DQ8 reach the cell surface and are still capable of presenting exogenously added peptides (4, 21, 45, 54). The ability of Abo.DQ8.Ii−/− APCs to activate the T cell hybridoma by presenting the specific peptide further supports this hypothesis. The time delay in activating the T cell hybridoma effectively by DQ8.Ii−/− APCs is probably due to low levels of surface DQ8 molecules in Abo.DQ8.Ii−/− mice and hence it might take a longer time to achieve the ligand density/threshold to activate the hybridoma. As discussed earlier, SEB-induced proliferation of DQ8.Ii−/− splenocytes in vitro and the comparable expansion of CD8 T cells expressing TCR V8 following in vivo administration of SEB in DQ8.Ii−/− mice lend support to this hypothesis.

Another interesting concept that emerged from previous studies of Ii-deficient mice is its role in maturation of B lymphocytes. Ii-deficient mice have defective differentiation of B cells from the immature stage to the mature stage (28, 29). This results in elevated numbers of immature B cells which are characterized by low levels of surface IgD and CD23. Recently, Matza et al. (30) have elucidated the role of the Ii in B cell maturation. They have shown that the Ii induces B cell maturation by up-regulating transcription mediated by the NF-κB p65/RelA pathway. However, the entire

FIGURE 9. B cell development in DQ8. Ii−/− mice. Spleens from naive DQ8.Ii−/− and DQ8.Ii−/− mice were collected and analyzed by flow cytometry for the presence of mature B cells. Representative histograms depict IgD expression (f) or CD23 expression (k). Cells were gated for high or low expression and the percentage of cells falling in these regions is indicated. The percentage of B220+ cells in spleens is also shown (c). DQ8.Ii−/− and DQ8.Ii−/− mice were bled at the indicated time points and serum IgG was estimated by ELISA (d). Each bar represents the mean ± SD of at least five mice.

FIGURE 10. B cell and T cell numbers in immunized mice. DQ8.Ii−/− and DQ8.Ii−/− mice were immunized with P44 as described in Materials and Methods and draining lymph nodes were collected 7 days later. The percentages of CD23 (a and b), IgM (c and d), IgD+ cells (e and f), and CD4+CD8+ T cells (g and h) were enumerated by flow cytometry. A representative result from three similar results is given. Numbers indicate percentage of cells falling within respective gates. Average RBC-depleted cell yield from immunized DQ8.Ii−/− and DQ8.Ii−/− mice were 75 ± 8.7 × 10⁶ and 30 ± 1.7 × 10⁶, respectively.
molecular event involved in this process is still undefined. Even in the present investigation, we observed that naïve DQ8.1Il showed similar defects in B cell maturation when compared with DQ8.1Il-/+ mice and the serum IgG levels were significantly low but not absent in these mice. Similar results have been reported earlier. Infection of Il-/- with I-A g7 and that Il might be crucial for effector molecules in Ii-deficient mice. Infection of Ii-deficient mice shows similar defects in B cell maturation when compared with Ii+/+ mice. In addition, our study confirms the partial defects in B cell maturation in Il-deficient mice.

Acknowledgments

We thank Julie Hansen and her crew for mouse husbandry. We thank Dr. Jim Miller (University of Chicago, Chicago, IL) for providing us the Ii/H11002 transgenic murine accessory molecules, Ii and H-2M. Thus, in conclusion, this recent study has shown that nonobese diabetic mice express very high levels of I-Ag7 associated with CLIP, implying a strong interaction of Il with I-A g7 and that Il might be crucial for effector molecule in lacking the p41 isoform of invariant chain. Immunology 3:58-53.

References