Expression of the B7.1 Costimulatory Molecule on Pancreatic β Cells Abrogates the Requirement for CD4 T Cells in the Development of Type 1 Diabetes

Evis Havari, Ana Maria Lennon-Dumenil, Ludger Klein, Devon Neely, Jacqueline A. Taylor, Marcia F. McInerney, Kai W. Wucherpfennig and Myra A. Lipes

*J Immunol* 2004; 173:787-796; doi: 10.4049/jimmunol.173.2.787
http://www.jimmunol.org/content/173/2/787
Expression of the B7.1 Costimulatory Molecule on Pancreatic β Cells Abrogates the Requirement for CD4 T Cells in the Development of Type 1 Diabetes

Evis Havari,* Ana Maria Lennon-Dumenil,† Ludger Klein,‡ Devon Neely,* Jacqueline A. Taylor,* Marcia F. McInerney,* Kai W. Wucherpfennig,‡ and Myra A. Lipes 2*

Although HLA-DQ8 has been implicated as a key determinant of genetic susceptibility to human type 1 diabetes, spontaneous diabetes has been observed in HLA-DQ8 transgenic mice that lack expression of murine MHC class II molecules (mII−/−) only when the potent costimulatory molecule, B7.1, is transgenically expressed on pancreatic β cells. To study the contribution of HLA-DQ8 to the development of diabetes in this model, we crossed RIP-B7.1mII−/− mice with a set of transgenic mouse lines that differed in their HLA-DQ8 expression patterns on APC subpopulations, in particular dendritic cells and cortical thymic epithelial cells. Surprisingly, we found that even in the absence of HLA-DQ8 and CD4 T cells, a substantial fraction of the RIP-B7.1mII−/− mice developed diabetes. This disease process was remarkable for not only showing insulitis, but also inflammatory destruction of the exocrine pancreas with diffusely up-regulated expression of MHC class I and ICAM-1 molecules. Expression of HLA-DQ8 markedly increased the kinetics and frequency of diabetes, with the most severe disease in the lines with the highest levels of HLA-DQ8 on cortical thymic epithelial cells and the largest numbers of CD4 T cells. However, the adoptive transfer of diabetes was not HLA-DQ8-dependent and disease could be rapidly induced with purified CD8 T cells alone. Expression of B7.1 in the target tissue can thus dramatically alter the cellular and molecular requirements for the development of autoimmunity.


Type 1 diabetes in humans and NOD mice is an autoimmune disease in which self-reactive T cells mediate the destruction of insulin-producing pancreatic β cells. Although the mechanisms of disease complexity are diverse, genes in the MHC play a primary role in conferring disease susceptibility in both humans and NOD mice. The human MHC class II molecule, HLA-DQ8 (DQA1*0301/DQB1*0302), and the NOD mouse homologue, I-Ag7, have been implicated as the most important contributors to MHC-associated disease susceptibility. Because of the presence of other HLA class II alleles in humans and the strong linkage disequilibrium across the MHC class II region, the mechanism(s) by which HLA-DQ8 molecules confers susceptibility to diabetes have been difficult to study. For this reason, several groups have generated transgenic mice that express only HLA-DQ8 and are deficient in murine class II molecules (mII−/−) (1–5). However, despite the striking functional and structural similarities between HLA-DQ8 and I-Ag7 (6, 7), none of the HLA-DQ8mII−/− transgenic mice developed spontaneous diabetes, even when backcrossed onto the NOD strain (3, 5). In retrospect, this has been largely attributed to the technical constraints inherent in backcrossing the Aβ mutation, which was originally made in an embryonic stem cell line of 129/Sv origin (8), onto the NOD strain where an extended region of the 129/Sv haplotype, including flanking genes known to inhibit diabetes (9), were inadvertently introgressed into the NOD background.

It has recently been shown that HLA-DQ8mII−/− mice that express the costimulatory molecule, B7.1 (CD80), on pancreatic β cells develop spontaneous diabetes, even when they are on the autoimmune-resistant B6 background (2). Diabetes was only observed in HLA-DQ8, but not in control HLA-DQ6 transgenic mice, indicating that the development of disease was not merely due to the expression of a human MHC class II molecule (2). However, the mechanism(s) by which HLA-DQ8 mediates diabetes susceptibility in the RIP-B7.1 model is not known.

The close similarities between HLA-DQ8 and I-Ag7 have suggested that diabetes is caused by similar Ag presentation events in humans and NOD mice (7). The primary function of MHC class II molecules is to bind and present peptides to CD4 T cells. MHC class II-mediated Ag presentation events could be critical at three discrete steps in the development of diabetes: CD4 T cell repertoire selection, priming of naive islet-reactive CD4 T cells, and establishment of insulitis (10). Although the precise APC subtypes that mediate autoimmune disease in NOD mice are not fully identified, strong evidence suggests that the activation of islet-specific CD4 T cells that precedes diabetes in NOD mice occurs in the draining pancreatic lymph nodes, rather than in the target organ (11, 12). Dendritic cells (DCs), the most potent APC subtype, have been postulated to be responsible for the presentation of islet-derived Ags to naive T cells (Ref. 13, and references therein). Accordingly, the removal of pancreatic lymph nodes has been

9 Joslin Diabetes Center, Boston, MA 02215, and Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School; 3Department of Pathology, Harvard Medical School, 4Department of Cancer Immunology and AIDS, Dana Farber Cancer Institute, and Department of Neurology, Harvard Medical School, Boston, MA 02215. E-mail address: myra.lipes@joslin.harvard.edu

1 This work was supported by National Institutes of Health Grant PO1 AI39619 (to K.W.W. and M.A.L.), the Iacocca Foundation (to M.F.M), Juvenile Diabetes Research Foundation (to M.A.L., K.W.W., and A.L-D.), and the Irvington Institute for Immunological Research (to L.K.).

2 Address correspondence to Dr. Myra Lipes, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215. E-mail address: myra.lipes@joslin.harvard.edu

3 Abbreviations used in this paper: mII−/−, murine MHC class II Aβ−/− deficient; TEC, thymic epithelial cell; cTEC, cortical TEC; mTEC, medullary TEC; DC, dendritic cell; insB, insulin B chain; HEL, hen egg lysozyme; EpCAM, epithelial cell adhesion molecule.
shown to prevent the development of diabetes in NOD mice (14). It has been postulated that DCs might likewise play a critical role in initiating diabetes in the HLA-DQ8 RIP-B7.1mII \textsuperscript{-/-} transgenic model (2). On the other hand, the B7.1 molecule is one of the most potent costimulators known, and in vitro and in vivo studies have shown that islet β cells that are engineered to express B7.1 acquire APC function (15). Because β cells do not express MHC class II molecules (16, 17), it thus remained possible that the autoimmune disease might be caused by CD8 T cells that directly recognize B7.1 and autoantigens presented by class I molecules on the target tissue.

To distinguish between these two hypotheses, we crossed RIP-B7.1mII \textsuperscript{-/-} mice with a set of HLA-DQ8mII \textsuperscript{-/-} transgenic mouse lines that are noteworthy in displaying different levels of HLA-DQ8 expression on different APC subpopulations, in particular DCs and cortical thymic epithelial cells (cTECs). We show that even though expression of HLA-DQ8 greatly accelerated the development of diabetes in this model, CD4 T cells were not essential, because diabetes occurred in the absence of CD4 T cells and disease could be readily transferred with purified CD8 T cells alone. Expression of a costimulatory molecule in the target organ can thus dramatically alter the cellular and molecular requirements for the initiation of autoimmunity. Expression of B7.1 was also required in the effector phase of the disease, indicating that sustained expression of costimulatory molecules in the target organ may be important in chronic inflammatory diseases.

**Materials and Methods**

*Mice and monitoring of diabetes*

The generation of the three HLA-DQ8 lines (70, 275, and 73) on the murine MHC class II mutant (I-A\textsuperscript{B-/-}, hereafter, mI-A\textsuperscript{B-/-}) C57BL/6 (B6) background have been described previously (5). RIP-B7.1mII \textsuperscript{-/-} B6 mice (2) were kindly provided by Dr. L. Wen (Yale Medical School, New Haven, CT). The HLA-DQ8 transgenic lines were individually crossed with RIP-B7.1mII \textsuperscript{-/-} B6 transgenic mice (2) to generate double-transgenic (70, 275, and 73) \times RIP-B7.1mII \textsuperscript{-/-} mice. The HLA-DQ8 RIP-B7.1 \textsuperscript{mII/-}, HLA-DQ8 RIP-B7.1 \textsuperscript{mII-/-}, and HLA-DQ8 RIP-B7.1 \textsuperscript{mII+/+} mice derived from this breeding were used as control groups. All the mice used in this study were littermates derived from the same breeders. Mice were screened for HLA-DQ8, mI-A\textsuperscript{B-/-}, and RIP-B7.1 transgenic and knockout mutations by PCR or flow cytometric analysis as described (5, 18).

Diabetes was assessed by weekly screening of urine for glucose with Diastix strips (Bayer, Heidelberg, Germany). A Diastix reading above 250 mg/dl was considered indicative of diabetes and was confirmed by glucose testing with a Glucometer Elite meter (Bayer). Animals with two consecutive blood glucose measurements above 300 mg/dl were considered diabetic.

The mice were housed in specific pathogen-free facilities, and all experiments were undertaken in accordance with approved Joslin Diabetes Center Animal Care and Use Committee protocols (Boston, MA).

*T cell assays*

Line (70, 275, and 73) mI-A\textsuperscript{B-/-} mice were immunized s.c. with HSV-2 VP16 or insulin peptides, OVA or hen egg lysozyme (HEL), and emulsified in CFA. Draining popliteal lymph nodes were pooled 8–10 days later from two mice, and lymph node cells (5 \times 10\textsuperscript{5}) were cultured in AIM-V medium (Invitrogen Life Technologies, Carlsbad, CA) containing 2 mM l-glutamine and 5 \times 10\textsuperscript{-5} M 2-ME without or with increasing concentrations of peptides or Oas for 3 days at 37°C, 5% CO\textsubscript{2}, [\textsuperscript{3}H]Thymidine was added for the last 18 h of culture. The results are expressed as cpm (\times 10\textsuperscript{4}), the mean ± SEM of triplicate wells. HLA-DQ8 blocking studies were performed with the mAb 9.3.F10 (anti-DQ/DR-specific) or L243 (anti-DR-specific), both from American Type Culture Collection, Manassas, VA). Cell killing experiments were performed with irradiated splenocytes from lines 70 or 73 as APCs (5 \times 10\textsuperscript{5} cells per well) in a T cell proliferation assay with lymph node cells (2.5 \times 10\textsuperscript{5} cells per well) from line 70 mice that had been restimulated in vitro with HSV-2 VP16 peptide.

*Bone marrow DCs*

Bone marrow cells were cultured for 6 days in medium containing 200 U/ml GM-CSF (PeproTech, Rocky Hill, NJ) as described (19), followed by exposure to 1 μg/ml LPS (Sigma-Aldrich, St. Louis, MO) for ~22 h (20). Prestim blocking was performed with the mAb 2.4G2. Staining reagents included: anti-CD11c-PE, anti-CD8-PE, and Leu10-FITC; biotin was visualized with streptavidin-CyChrome (all from BD Pharmingen, San Diego, CA).

**Preparation and staining of thymic stromal cells**

Thymic stromal cells were prepared as described (21). Cells were stained with IVD12-biotin, washed, and incubated with anti-CD45 CyChrome (BD Pharmingen), anti-epithelial cell adhesion molecule (EpCAM)-FITC (Clone G8.8), Ly51 PE (BD Pharmingen), and streptavidin-allophycocyanin. cTECs were identified according to the surface phenotype CD45\textsuperscript{−}, EpCAM\textsuperscript{+}, and Ly51\textsuperscript{−}, while medullary TECs (mTECs) were identified as CD45\textsuperscript{−}, EpCAM\textsuperscript{−}, and Ly51\textsuperscript{+}. For analysis of thymic DCs, the light density fraction was stained with IVD12-biotin, washed, and incubated with anti CD11c-PE and streptavidin-allophycocyanin.

**Adoptive transfer experiments**

Recipients (5- to 7-wk-old) were irradiated (750 rad) 1 day before the adoptive transfer. Donors consisted of diabetic (70, 275, or 73) RIP-B7.1mII \textsuperscript{-/-} mice. For whole spleenocyte transfers, single-cell suspensions were depleted of erythrocytes in hypotonic NH\textsubscript{4}Cl and injected i.v. into the recipients (2 \times 10\textsuperscript{7} cells/recipient). CD4 and CD8 T cell subset depletions were performed on erythrocyte-depleted splenocytes using either mouse CD4 (L3T4) or mouse CD8a (Ly-2) microbeads (both from Miltenyi Biotech, Munchen, Germany) and injected i.v. at 1–2 \times 10\textsuperscript{7} cells/recipient. CD8 T cells were purified with the CD8\textsuperscript{+} T Cell Isolation Biotin Cocktail (Miltenyi Biotech). The resulting populations, confirmed by flow cytometry to be >95% pure, were injected i.v. at 0.9–8.0 \times 10\textsuperscript{7} cells per recipient. The recipient mice were monitored for glycosuria three times weekly, and the development of diabetes was confirmed by blood glucose measurement. The mice were sacrificed after the onset of diabetes or at the termination of the experiments 60 days after the adoptive transfer.

**Histology**

Pancreas tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and stained for H&E and stained for HLA class II (CD44) expression using indirect immunohistochemistry as described (22) using anti-H-2K\textsuperscript{b}, -ICAM-1, -CD4, -CD8 (all from BD Pharmingen), glucagon (Biogenesis, Kingston, NH), or IVD12 mAbs.

**Statistical analysis**

Statistical analysis was performed with the Student t test. Data are shown as means ± SEM. Diabetes incidence in the different groups was analyzed by the Kaplan-Meier method and compared with the log-rank test.

**Results**

**Differential expression of HLA-DQ8 on APC subpopulations in three lines of transgenic mice**

Although several groups have generated mice that are transgenic for HLA-DQ8, only single lines were reported and the HLA-DQ8 expression patterns on different subpopulations of APCs, including DCs and TECs, were not reported (1, 3, 4, 23). It is well known that it is difficult to reproduce “normal” levels of MHC class II expression, i.e., similar to those of murine MHC class II genes, using human genomic clones as murine transgenes. For this reason, we generated and carefully characterized the HLA-DQ8 expression patterns in three lines of transgenic mice.

Flow cytometric analysis of splenic B220\textsuperscript{+} cell populations with the framework HLA-DQ mAb, Leu10, revealed that HLA-DQ8 was expressed at similar levels on B cells in 70 and 275 mice (10 ± 1.4% positive cells (n = 13), compared with 12 ± 2.4% (n = 8), respectively (p = NS)), with higher amounts in line 73 mice (31 ± 6.3% positive cells, n = 10) (p < 0.001 compared with line 70, Fig. 1a). In contrast, immunohistochemical analysis of frozen thymic sections showed that all three lines expressed HLA-DQ8 in a characteristic reticular pattern in the thymic cortex (c) with denser staining in the medulla (m), with line 73 and 275 exhibiting similar amounts of staining in the cortex that was greater than that seen in line 70 (Fig. 1b). Lines 73 and 275 mice displayed ~3-fold more peripheral CD4 T cells than did 70 mice.
with mean CD4:CD8 ratios in peripheral lymphocytes of 0.69 ± 0.18 (n = 16) and 0.60 ± 0.18 (n = 10) in lines 73 and 275 respectively (p = NS), compared with a CD4:CD8 ratio of 0.18 ± 0.01 (n = 18) in line 70 (p = < 0.001, compared with lines 73 or 275). As expected, mII`/mII` mice that lacked HLA-DQ8 had minimal peripheral CD4 T cells with a mean CD4:CD8 ratio of 0.05 ± 0.004 (n = 25, p = < 0.001, compared with line 70). These results suggested that the size of the restored peripheral CD4 T cell compartment in each line (Fig. 1c) correlated with the level of HLA-DQ8 expressed in the thymic cortex (Fig. 1b).

**HLA-DQ8-restricted T cell responses following immunization**

To investigate the ability of each of the lines to mount HLA-DQ8-restricted T cell responses, mice were immunized with the HSV-2 VP16 (HSV, residues 433–445: DMTPADALDDFDL) (24) and insulin B chain (insB, residues 9–23: SHLVEALYLVCGERG) peptides that are bound by HLA-DQ8. The crystal structure of HLA-DQ8 was determined as a complex with this insulin peptide (7), and the peptide has been shown to be recognized by T cells from HLA-DQ8-positive diabetic patients (25). Primary assays revealed robust (10- to 20-fold above baseline) proliferative responses in line 70 against both HSV and insB peptides (Fig. 2). Line 275 also showed good dose-dependent proliferative responses following peptide immunization (i.e., ~7- to 10-fold above baseline), but the magnitude of these responses was not as great as in line 70. Proliferative responses were specifically blocked by the addition of the anti-HLA-DQ/DR mAb, 9.3.F10, but were not affected by the addition of control mAb against HLA-DR (L243) (Fig. 2). Unexpectedly, despite exhibiting the greatest numbers of peripheral CD4 T cells, line 73 mice did not mount detectable proliferative responses to either the HSV or insB peptides (Fig. 2). Line 73 mice also showed severely attenuated responses to immunization with complex proteins such as OVA and HEL (Fig. 2). This did not reflect a generalized T cell unresponsiveness because...
T cells from 73 mice displayed normal in vitro proliferative responses to mitogens such as Con A (data not shown). Cell-mixing experiments demonstrated that the inability of line 73 to mount primary T cell responses resided in its APC compartment: in vitro proliferative responses of T cells from HSV-immunized line 70 mice were greatly diminished when irradiated splenocytes from line 73, rather than from line 70, were used as APCs (Fig. 2). The impaired Ag presentation capabilities of line 73 were particularly surprising in view of its severe autoimmune phenotype when it was crossed with RIP-B7.1 mice (see below). These seemingly paradoxical findings stimulated a more detailed analysis of the HLA-DQ8 expression patterns on APC subpopulations in the different lines.

Expression of HLA-DQ8 on mature DCs

In view of the pivotal role of DCs in initiating primary immune responses (26) and their known potency in T cell stimulation assays compared with resting B cells (27), we compared HLA-DQ8 expression on bone-marrow-derived DCs from line 70, 275, and 73 mice following exposure to LPS, a known potent stimulus for DC maturation (19, 20). Three-color FACs analysis revealed that CD11c+ DCs from all three lines displayed high levels of the expected repertoire of costimulatory markers including CD40, CD80, and CD86 (data not shown). When mature (CD11chigh, CD86high) DCs were gated and examined for class II surface expression, we found that whereas HLA-DQ8 was expressed on ~50% of mature DCs from line 70, expression was almost undetectable on mature DCs from line 73 (Fig. 3a). Similar findings were seen on freshly isolated mature splenic DCs from line 70 and 73 mice that received in vivo treatment with Flt3 ligand (data not shown). Surprisingly, despite their ability to mount significant responses to peptide immunization, line 275 mice also showed very low levels of HLA-DQ8 expression on mature DCs (Fig. 3a). The lack of HLA-DQ8 expression on mature DCs from line 73 provides a plausible explanation for its inability to respond to immunization. The slightly greater, but still barely detectable, amount of class II molecules is required on mature DCs for the generation of primary T cell responses.

Expression of HLA-DQ8 on thymic cell subpopulations

To further characterize the cell-type-specific HLA-DQ8 expression patterns in the thymus, we enriched stromal cell subpopulations to high purity by a combination of density fractionation and cell sorting (Fig. 3b) (21). Identification of the four major thymic APC subsets, cTECs (CD45lowLy51+G8.8+), mTECs (CD45lowLY51−G8.8+), DCs (CD11c−/CD11b−), and macrophages (CD11c−CD11b+), was based on surface markers expressed by the respective subpopulations. Staining of each of these subpopulations with IVD12 (Fig. 3b) revealed that HLA-DQ8 was
expressed more abundantly on cTEC from line 275 and 73 mice, compared with line 70, whereas, for thymic DCs, this picture was reversed with HLA-DQ8 expression greater on line 70 than 275 and 73 mice. Thus, our HLA-DQ8 lines showed opposite patterns of DQ8 expression in cTECs and DCs, the principal mediators of positive and negative selection, respectively. These findings suggested that the CD4 T cell repertoire differed both quantitatively and qualitatively in each of the three HLA-DQ8 lines. Thus, the HLA-DQ8 expression levels did not precisely match those of murine MHC class II molecules in the three transgenic lines. Interestingly, a side-by-side analysis of the HLA-DQ8 line of Wen and colleagues, which was made from the identical cosmid clone insert (23), demonstrated that the HLA-DQ8 expression patterns in this line also did not match those for murine MHC class II: HLA-DQ8 was expressed at very low levels on cTECs, but expression was high on thymic and peripheral DCs (data not shown), likely explaining the reduced numbers of peripheral CD4 T cells previously observed in these mice (23). These results were initially surprising because at the time that these transgenic mice were constructed, it was thought that this 33-kb cosmid clone insert would contain ample flanking sequences to confer coordinate HLA-DQ8 expression on all APC subtypes. However, recent studies have suggested that distal “far upstream” regions of class II genes, situated up to 20 kb upstream of the promoter, may be required for position-independent and copy-number-dependent expression of MHC class II genes in transgenic mice (28).

All three double-transgenic HLA-DQ8×RIP-B7.1mII−/− mouse lines develop accelerated diabetes

To examine the impact of these different HLA-DQ8 expression patterns on the development of diabetes, each of the lines was crossed with RIP-B7.1mII−/− B6 mice (2) to generate double-transgenic (70, 275, and 73)×RIP-B7.1mII−/− mice. Because the expression patterns of HLA-DQ8 in each of these lines was distinct, comparison of the kinetics of diabetes in these double-transgenic lines could enable us to determine what APC subset (B cells, DCs, or cTECs) was most critical for disease development. We found no evidence to suggest that the RIP-B7.1 transgene changed HLA-DQ8 expression in any of the three lines in the thymus, on splenic B cells, or CD4:CD8 ratios, consistent with previous observations (29).

Surprisingly, all three RIP-B7.1 lines (70, 275, and 73) developed a rapid onset and high incidence of diabetes, with the fastest kinetics occurring in line 73 mice with >80% of the mice developing diabetes, starting as early as 9 wk of age (Fig. 4). Immunohistochemical analysis showed that the numbers of CD4 T cells in the insulitis lesions closely paralleled the size of the peripheral CD4 T cell compartment in the different lines, with line 73 and 275 showing considerably more abundant numbers of CD4 T cells than line 70 mice (data not shown). We also considered the possibility that the particularly rapid progression to diabetes in line 73 mice might be due to reduced levels of regulatory T cells. Of interest, we found that the percentage of CD4+CD25+ T cells in 73 mice (7.8 ± 0.52%, n = 5) was very similar to wild-type (i.e., MHC class II-sufficient) B6 mice (7.8 ± 0.60%, n = 6). These findings...
are consistent with other studies that have shown that CD25+CD4+ T cells are induced by MHC class II molecules expressed by cTECs and that MHC class II expression in the periphery is not required for the survival of this cell population (30, 31). However, we cannot rule out the possibility that line 73 (and perhaps also line 275) mice exhibit reduced levels of other subsets of regulatory T cells.

**Single-transgenic RIP-B7.1mII−/− mice develop inflammatory destruction of both the endocrine and exocrine pancreas**

Similar to previous reports (2, 15), we found that the incidence of insulitis and diabetes was very low in RIP-B7.1 mice that expressed I-Aα molecules (mII+; Figs. 4 and 5a), with only 1 of 32 mice developing diabetes after 9 mo of age (data not shown), suggesting that CD4 T cells selected on “normal” mouse MHC class II protect from diabetes. This was also to be expected because it has been shown that the expression of B7.1 alone does not usually lead to diabetes (15). Therefore, it came as a surprise that a significant percentage (40%) of the control RIP-B7.1mII−/− mice that did not express the HLA-DQ8 transgene or a murine MHC class II molecule developed diabetes (Fig. 4). Histological analysis of the pancreas of RIP-B7.1mII−/− mice was remarkable in revealing not only insulitis (Fig. 5b, arrowhead) but also severe mononuclear cell infiltration of the exocrine tissue (arrows), which was largely destroyed and replaced by fat (asterisk), consistent with pancreatitis. Because the B7.1 molecule was expressed only in pancreatic β cells, these findings raised the possibility that the pancreatitis might be due to the underlying MHC class II deficiency. Histological examination of the pancreas of mII−/− mice that did not express the B7.1 transgene confirmed this possibility: severe pancreatitis was present with diffuse interstitial lymphocytic infiltrates (Fig. 5c, yellow arrows) and destruction of large regions of the exocrine tissue (Fig. 5c, asterisk). However, although the infiltrates were often in direct contact with islets, the islets remained intact (Fig. 5c, arrowheads). HLA-DQ8 RIP-B7.1 mice showed both insulitis (Fig. 5d, arrowhead) and pancreatitis (Fig. 5d, arrows), but the extent of exocrine damage was not as severe as that seen in the absence of MHC class II molecules.

Pancreatitis has been previously observed in mII−/− B6 mice and was shown to be mediated by CD8+ T cells (32). Because chronic pancreatitis in humans is characterized by up-regulation of MHC class I Ags (33), and the level of MHC Ag expressed on islets of RIP-B7.1 is known to play a critical role in the development of diabetes in RIP-B7.1 transgenic mice (15, 34), we next examined MHC class I expression in the pancreata of RIP-B7.1mII−/− mice. As expected, the pancreas of healthy control B6 mice expressed only low levels of MHC class I (Fig. 6). In contrast,
contrast, class I expression was markedly up-regulated in the pancreas of prediabetic RIP-B7.1mII−/− mice, with further diffuse up-regulation in diabetic RIP-B7.1mII−/− mice (Fig. 6). Moreover, there was a striking coordinate up-regulation of expression of ICAM-1, in the pancreas of RIP-B7.1mII−/− mice with the patterns mirroring those of MHC class I (Fig. 6). Of interest, previous studies have shown that coexpression of B7.1 and ICAM-1 could synergize in converting fibroblasts into potent APCs that could activate naive CD8 (35) and CD4 cells (36, 37). Taken together, these findings raised the possibility that the chronic inflammatory reaction in the pancreas of the RIP-B7.1mII−/− mice might lead to a CD8 rather than a CD4 T cell-driven disease process.

Diabetes in HLA-DQ8 RIP-B7.1mII−/− mice is primarily CD8 T cell-mediated

To examine the relative roles of CD8 and CD4 T cells in the disease pathogenesis, we established an adoptive transfer system where splenocytes from diabetic line 70, 275, or 73 mice were introduced into young (5- to 7-wk-old) irradiated line 70 RIP-B7.1mII−/− recipients. We found that transfer of 2 × 10^7 bulk splenocytes from the HLA-DQ8 RIP-B7.1mII−/− mice resulted in a rapid induction of diabetes with all (17 of 17) recipients developing diabetes by 28 days after transfer (Fig. 7a); at this age, spontaneous diabetes was never normally observed in line 70 mice (Fig. 4). Use of other HLA-DQ8 RIP-B7.1mII−/− donors as recipients did not influence the frequency or kinetics of disease transfer (data not shown). In contrast, regardless of the severity of the diabetes in the donors, diabetes could not be transferred into HLA-DQ8mII−/− mice that lacked B7.1 (p < 0.0001). To our surprise, despite the marked acceleration of diabetes in the presence of HLA-DQ8, depletion of CD4 T cells from diabetic splenocytes, either by magnetic beads or cell sorting, had no effect on the kinetics of adoptive transfer of diabetes with all (7 of 7) mice developing diabetes within 25 days after transfer (Fig. 7a). In contrast, depletion of CD8 T cells significantly reduced the incidence of disease transfer (4 of 10 diabetic mice) and delayed disease onset (p < 0.0001 compared with mice that received bulk splenocytes) (Fig. 7a). In further support of these findings, depletion of CD4 T cells from diabetic donors led to a significantly reduced incidence of diabetes transfer (4 of 10 diabetic mice) and delayed disease onset (p < 0.0001 compared with mice that received bulk splenocytes) (Fig. 7a).
of the critical role for CD8 T cells in this disease process, diabetes could be rapidly induced with purified CD8 T cells derived from diabetic HLA-DQ8 RIP-B7.1 mice with all (5 of 5) recipients developing disease (Fig. 7a), starting at 11 days post-transfer. As expected, in contrast to the islet infiltrates of the diabetic donor mice that contained both CD4 T cells and CD8 T cells (Fig. 7c), the infiltrated islets of the diabetic recipients contained predominantly CD8 T cells (Fig. 7c). These studies demonstrate that CD8 T cells alone are sufficient for the induction of diabetes when the B7.1 molecule is expressed by β cells. Consistent with the lack of an HLA-DQ8-dependent effector mechanism, we found that splenocytes from HLA-DQ8° RIP-B7.1 diabetic mice induced diabetes with similar efficiency in HLA-DQ8° RIP-B7.1mII/− mice and HLA-DQ8° RIP-B7.1mII/− recipients (Fig. 7b).

Discussion
These results demonstrate that expression of HLA-DQ8 can significantly accelerate the development of diabetes in RIP-B7.1 transgenic mice, but that expression of a human or murine MHC class II molecule is not essential when the B7.1 costimulatory molecule is expressed on β cells. Surprisingly, diabetes was observed in RIP-B7.1 mice that did not express MHC class II molecules, indicating that the expression of B7.1 on the target cells profoundly changes the cellular and molecular requirements for the development of the disease. Consistent with these findings, disease could be efficiently transferred with purified CD8 T cells and was not dependent on MHC class II molecules. Importantly, disease was only transferred from RIP-B7.1 transgenic mice to recipients who also expressed B7.1 on β cells, indicating that the continued presence of costimulatory activity on the target cells is also required in the effector stage of the disease. Therefore, this model differs dramatically from the conventional NOD mouse model where CD4 T cells are essential for the initiation of diabetes (38).

These results were unexpected in view of previous reports demonstrating that although islets from RIP-B7.1 transgenic mice exhibit APC function that can be demonstrated both in vivo and in vitro, the expression of B7.1 on islets alone is not normally sufficient to lead to autoimmunity (15). Islet autoimmunity would not be expected to occur because while naïve autoreactive T cells normally exist in the periphery, they cannot usually access nonlymphoid tissues (39). However, it is also known that B7.1 can transform nonprofessional APCs, such as islets, into competent APCs that trigger autoimmunity when additional factors are present such as local inflammation (15), up-regulated MHC Ag expression (15, 34), or high numbers of potentially autoreactive T cells (40). We hypothesize that in the RIP-B7.1mII/− mice, the combination of an initiating event that induces local inflammation and lymphocytic infiltration (i.e., chronic pancreatitis due to MHC class II deficiency) and the ability of the islets to present self peptides in an immunogenic form to T cells (i.e., via the aberrant expression of B7.1 along with up-regulated class I and ICAM-1 expression) provides sufficient signals to trigger diabetes.

These results also clearly demonstrate that HLA-DQ8-restricted CD4 T cells significantly accelerate diabetes in this model system. Surprisingly, the most severe disease was observed in line 73 where expression of HLA-DQ8 was very low on mature DCs and was associated with an impaired ability to prime immune responses. Therefore, a question that arises is how are autoreactive CD4 T cells activated to accelerate diabetes in this setting? It is possible that B cells may serve as relevant APCs. B lymphocytes have been shown to contribute to diabetes in NOD mice as a subset of APCs with a preferential ability to process and present certain B cell Ags to autoreactive MHC class II-restricted CD4 T cells (41, 42). Support for an important role for B lymphocytes as APCs has also been provided by other reports showing resistance to diabetes in NOD mice expressing H-2K° MHC class II molecules on all APC except B lymphocytes (43), and that NOD mice lacking B cells are protected from diabetes (44, 45). In addition, or alternatively, it is possible that in RIP-B7.1mII/− mice, the presence of chronic pancreatitis creates a proinflammatory milieu favoring either the differentiation of destructive Th1 CD4 T cells, which could then directly attack the target tissue, or the recruitment of virus-specific and autoreactive T cells, as has been proposed to occur in diabetes induced by Coxsackie B4 virus infection, which destroys the exocrine pancreas, but not the islets (46, 47). The initiation of an autoimmune response against islets may thus occur as part of a “bystander” mechanism, a model that is consistent with findings in other transgenic models and human diseases (48).

Of interest, when bred onto the NODmII/− background, all three HLA-DQ8 lines developed spontaneous myocarditis in the absence of transgenic expression of a costimulatory molecule in the target organ, with line 73 and 275 again developing the most severe autoimmune disease (5). However, it should be noted that HLA-DQ8 was expressed at significantly higher levels on all APC subsets examined, including mature DCs, on the NOD compared with B6 background (5); the basis for this effect remains to be determined. Our hypothesis is that HLA-DQ8 shapes the T cell repertoire in the thymus such that significant numbers of CD4 T cells specific for islet (and myocardial) Ags are selected that promote organ-specific autoimmunity (5).

The results of this study contrast with the report of Wen and colleagues (2), which suggested that diabetes in HLA-DQ8 RIP-B7.1mII/− mice was primarily CD4 T cell-mediated. We confirm the development of type 1 diabetes in this model system, but find that expression of HLA-DQ8 only increases the incidence and severity of the disease. As discussed above, we attribute the relatively high incidence of diabetes (~40%) in the RIP-B7.1mII/− mice that lack expression of human or murine MHC class II molecules to the presence of exocrine pancreas inflammation in a setting where islets are rendered immunogenic via the expression of B7.1 (15). MHC class II-deficient B6 mice have been previously shown to develop autoimmune pancreatitis that is CD8 T cell-mediated (32), and we also observed severe pancreatitis in NODmII/− mice (5). Wen et al. (29) also observed the development of diabetes in RIP-B7.1mII/− mice, but at a very low incidence (1 of 15 mice, 6.7%). It is possible that as yet unidentified environmental factors influence the development of pancreatitis and diabetes in the different colonies; the effect of environmental factors on incidence of diabetes is well known in NOD mice. It is also possible that the histopathological features of pancreatitis (Fig. 5) could have been missed if studies were performed on frozen tissue sections (2, 48), which can lack the detailed morphology of paraffin-embedded tissues. Our studies also clearly demonstrate that the transfer of diabetes in HLA-DQ8 RIP-B7.1 mice was primarily mediated by CD8 T cells and was not dependent on expression of HLA-DQ8. Wen et al. (2) also observed diabetes in 25% of recipients that did not express HLA-DQ8, but did not observe diabetes following transfer of purified CD8 T cells. However, only three mice were analyzed in the latter experiment, and adoptive transfer experiments with purified CD4 T cells were not reported. Our findings are consistent with a previous study that showed that although CD4 T cells are normally essential for the spontaneous development of diabetes in NOD mice, this requirement could be circumvented in CD4-deficient NOD mice by the transgenic expression of B7.1 on β cells (38) and another study that showed that diabetes in RIP-B7.1-glycoprotein B6 mice could be prevented with treatment with anti-CD8, but not anti-CD4, mAbs (49). These considerations should be kept in mind when the
RIP-B7.1 model system is used to study the mechanisms by which MHC class II molecules confer susceptibility to type 1 diabetes. Inflammation-induced abnormal expression of molecules involved in Ag recognition has been postulated to play a role in a number of autoimmune diseases, either by triggering the initial autoimmune reaction or by perpetuating it through epitope spreading (50). This is well illustrated by Thielier’s disease of the CNS, where viral infection induces inflammation and elicits the recruitment of cytokine-producing, viral-specific, and autoreactive T cells that are responsible for the disease chronicity (51). In addition, several reports have noted that B7.1 is expressed in the CNS in experimental allergic encephalomyelitis and multiple sclerosis (52). Sustained expression of B7.1 (and B7.2) may thus be an important factor in the maintenance of other chronic inflammatory conditions.

It has been shown in a number of transgenic models that autoimmune diabetes may result from nonspecific inflammation of pancreatic β cells, either as a result of cytokine production (53), viral infection (46, 54), or the provision of costimulatory activity (47). The presence of autoreactive T cells has been shown to be sufficient for the development of an insulin-dependent form of diabetes, whose onset closely correlates with the selection of CD4 T cells from young nonobese diabetic (NOD) islets that can transfer rapid onset of diabetes in NOD mice in the absence of CD4 cells. J. Exp. Med. 183:67.


