Autoantigen-Independent Deletion of Diabetogenic CD4⁺ Thymocytes by Protective MHC Class II Molecules

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Autoantigen-Independent Deletion of Diabetogenic CD4+ Thymocytes by Protective MHC Class II Molecules

Dennis Schmidt, Abdelaziz Amrahi, Joan Verdaguer, Sonny Bou, and Pere Santamaria

Some MHC class II genes provide dominant resistance to certain autoimmune diseases via mechanisms that remain unclear. We have shown that thymocytes bearing a highly diabetogenic, I-A\textsuperscript{g7}-restricted β-cell-reactive TCR (4.1-TCR) undergo negative selection in diabetes-resistant \(H\text{-}2\text{e}^{7/6}\) mice by engaging several different antidiantibiotic MHC class II molecules on thymic (but not peripheral) hemopoietic cells, independently of endogenous superantigens. Here we have investigated 1) whether this TCR can also engage protective MHC class II molecules (I-Ab\textsuperscript{b}) on cortical thymic epithelial cells in the absence of diabetogenic (I-A\textsuperscript{g7}) molecules, and 2) whether deletion of 4.1-CD4\textsuperscript{+} thymocytes in I-A\textsuperscript{b}-expressing mice might result from the ability of I-A\textsuperscript{b} molecules to present the target β-cell autoantigen of the 4.1-TCR. We show that, unlike I-A\textsuperscript{g7} molecules, I-A\textsuperscript{b} molecules can restrict neither the positive selection of 4.1-CD4\textsuperscript{+} thymocytes in the thymic cortex nor the presentation of their target autoantigen in the periphery. Deletion of 4.1-CD4\textsuperscript{+} thymocytes by I-A\textsuperscript{b} molecules in the thymic medulla, however, is a peptide-specific process, since it can be triggered by hemopoietic cells expressing heterogeneous peptide/I-A\textsuperscript{b} complexes, but not by hemopoietic cells expressing single peptide/I-A\textsuperscript{b} complexes. Thus, unlike MHC-autoreactive or alloreactive TCRs, which can engage deleting MHC molecules in the thymic cortex, thymic medulla, and peripheral APCs, the 4.1-TCR can only engage deleting MHC molecules (I-A\textsuperscript{b}) in the thymic medulla. We therefore conclude that this form of MHC-induced protection from diabetes is based on the presentation of an anatomically restricted, nonautoantigenic peptide to highly diabetogenic thymocytes. The Journal of Immunology, 1999, 162: 4627–4636.

Genetic susceptibility and resistance to autoimmunity are primarily determined by highly polymorphic genes of the class II region of the MHC (1). Much of what is currently known about the MHC-linked susceptibility and resistance to autoimmunity has been learned from studies of insulin-dependent diabetes mellitus (IDDM),\textsuperscript{3} a spontaneous autoimmune disease that results from selective destruction of the insulin-producing pancreatic β-cells by T lymphocytes (2, 3). The diabetes-prone nonobese diabetic (NOD) mouse, which spontaneously develops a form of diabetes resembling human IDDM, is homozygous for a unique MHC haplotype (\(H\text{-}2\text{e}^7\)) that carries a nonproductive \(I\text{-}Ea\) gene and encodes an \(I\text{-}A\alpha^9\)/\(I\text{-}A\beta^3\) heterodimer with unique molecular structure and biochemical behavior (4, 5). Studies of NOD mice expressing non-NOD H-2 haplotypes or MHC transgenes have shown that MHC class II molecules play a direct role in providing either susceptibility or resistance to spontaneous IDDM (2, 6). The mechanisms underlying the associations between MHC class II polymorphism and autoimmune disorders such as IDDM, however, remain unclear.

MHC molecules play a crucial role in instructing T cells maturing in the thymus to discriminate between self and nonself and in presenting Ags to mature T cells in the periphery (7, 8). During T cell development, CD4\textsuperscript{+}CD8\textsuperscript{–} thymocytes bearing TCRs capable of recognizing self peptide/MHC complexes on cortical thymic epithelial cells above certain affinity/avidity thresholds mature into CD4\textsuperscript{+}CD8\textsuperscript{–} or CD4\textsuperscript{–}CD8\textsuperscript{–} cells and exit the thymic cortex toward the thymic medulla (positive selection) (9–16). High affinity/avidity engagement of self peptide/MHC complexes by positively selected thymocytes, usually on bone marrow-derived APCs of the thymic medulla, leads to thymocyte death (negative selection) or functional unresponsiveness (anergy) (14–22). These processes ensure that the only T cells exiting the thymus are those capable of recognizing foreign, but not self, Ags in the context of self MHC.

It has been hypothesized that the ability of specific MHC molecules to afford autoimmune disease susceptibility or resistance is a function of their ability to promote the thymic selection or deletion of pathogenic T cells, respectively (23–26). The observation that murine and human prodiabetogenic MHC class II molecules form unstable complexes on the surface of APCs has provided circumstantial support to this theory (5, 27); however, direct evidence has been lacking until recently. We found that thymocytes bearing a diabetogenic, I-A\textsuperscript{g7}-restricted, β-cell-reactive TCR (4.1) undergo negative selection in \(H\text{-}2\text{e}^7/6\), \(H\text{-}2\text{e}^7/6\), \(H\text{-}2\text{e}^7/5\), and \(H\text{-}2\text{e}^7/5\) non-TCR transgenic mice (29), the diabetes resistance afforded by the MHC-induced deletion of thymocytes in 4.1-TCR transgenic mice was found to reside in the bone marrow (28). These results provided a compelling explanation of how protective MHC class II alleles carried on one haplotype can override the susceptibility to autoimmune diabetes provided by pathogenic alleles carried on a second haplotype.

\textsuperscript{3}Abbreviations used in this paper: IDDM, insulin-dependent diabetes mellitus; NOD, nonobese diabetic; RAG, recombination-activating gene; CLIP, class II-associated invariant chain peptide.

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\textsuperscript{3}Abbreviations used in this paper: IDDM, insulin-dependent diabetes mellitus; NOD, nonobese diabetic; RAG, recombination-activating gene; CLIP, class II-associated invariant chain peptide.
The TCR:peptide/MHC interactions that drive the positive selection of thymocytes in the thymic cortex are usually less specific than those driving their subsequent negative selection in the thymic medulla (22, 30–41). As a result, MHC molecules restricting the negative selection of thymocytes bearing MHC-autoactive or MHC-alloreactive TCRs in the thymic medulla can also usually restrict the thymocytes’ prior positive selection in the thymic cortex (22, 32, 36, 37); I-A\(^b\)-autoactive TCRs, for example, undergo unopposed positive selection in thymus expressing I-A\(^b\) molecules only in the cortex, but undergo sequential positive and negative selection in thymus expressing I-A\(^b\) molecules in both cortex and medulla (22). We thus hypothesized that when expressed only on cortical thymic epithelial cells, MHC molecules capable of triggering the deletion of 4.1-thymocytes in the thymic medulla (i.e., I-A\(^b\)) would actually be able to promote the positive selection of these thymocytes. Since the 4.1-TCR does not cross-react with these MHC molecules on peripheral APCs, and thymocyte deletion is almost invariably target Ag or superantigen dependent, we also hypothesized that negative selection of 4.1-thymocytes in 4.1-NOD.H-2\(^{k+}\) mice would be triggered by \(\beta\)-cell autoantigen/I-A\(^b\) complexes ferried from the periphery to the thymic medulla by bone marrow-derived APCs.

Here, we have tested these hypotheses by following the fate of the 4.1-TCR in 4.1-TCR transgenic mice selectively expressing either a protective MHC transgene (I-A\(^a\)) on cortical thymic epithelial cells or wild-type (nontransgenic) I-A\(^a\) molecules on radioresistant thymic epithelial cells, 2) by investigating whether I-A\(^b\) molecules can present the 4.1-TCR’s target autoantigen to 4.1-CD4\(^+\) T cells, and 3) by investigating whether 4.1-thymocytes can engage I-A\(^b\) molecules bound to single peptides on bone marrow-derived APCs. We have found that I-A\(^a\) molecules delete 4.1-thymocytes in the thymic medulla in a peptide-dependent manner, but to our surprise we restrict neither the positive selection of 4.1-thymocytes in the thymic cortex nor the presentation of their target autoantigen in the periphery. Pathogenic MHC class II molecules (I-A\(^b\), on the other hand, can efficiently restrict the positive selection of 4.1-CD4\(^+\) T cells in the thymus and their autoantigen-driven activation in the periphery. Thus, unlike MHC-autoactive or alloreactive TCRs, which can engage deleting MHC molecules in the thymic cortex, thymic medulla, and peripheral APCs, the 4.1-TCR can only engage deleting MHC molecules (I-A\(^a\)) in the thymic medulla. These observations establish the existence of a mechanism of MHC-induced protection from autoimmunity that is based on the presentation of an anatomically restricted, nonautoantigen-derived peptide to highly pathogenic TCRs.

### Materials and Methods

**Mice**

NODLt mice were purchased from The Jackson Laboratory (Bar Harbor, ME). RAG-2\(^{-/-}\) NOD have been described previously (42). C57BL/6/I-A\(^B\) mice were purchased from Taconic Laboratories (Germantown, NY). C57BL/10/J-2\(^\text{R}^{+}\) mice were provided by W. Licker and L. Peterson (Merck Research Laboratories, Rahway, NJ). 4.1-NOD mice, expressing a transgenic, I-A\(^a\)-restricted, \(\beta\)-cell-reactive TCR derived from a CD4\(^+\) T cell clone (NY4.1) that was isolated from pancreatic islets of a diabetic NOD mouse have been described previously (28). 4.1-NOD (4.1-TCR-transgenic, I-A\(^d\)/I-A\(^B\)) mice were outcrossed with K14-I-A\(^B\)/I-A\(^b\) mice (K14-I-A\(^B\)-transgenic, I-A\(^d\)/I-A\(^b\); a gift from T. Laufer and L. Glimcher, Harvard School of Public Health, Harvard University, Boston, MA) (22) or wild-type C57BL/6 mice (I-A\(^d\)/I-A\(^B\)); The Jackson Laboratory) to generate 4.1/K14-I-A\(^b\)/I-A\(^b\) (4.1-TCR\(^+\)), K14-I-A\(^b\), I-A\(^d\)/I-A\(^b\), I-A\(^d\)/I-A\(^b\), and 4.1-I-A\(^d\)/I-A\(^b\) mice (4.1-TCR\(^+\), I-A\(^d\)/I-A\(^b\), and I-A\(^b\)/I-A\(^b\), respectively. 4.1/K14-I-A\(^b\) mice were then intercrossed to generate I-A\(^d\), 4.1-I-A\(^d\)/I-A\(^b\), 4.1-I-A\(^d\)/I-A\(^b\), 4.1-I-A\(^d\)/I-A\(^b\), and 4.1/K14-I-A\(^b\) mice (see Table I for MHC genotypes and phenotypes of each mouse strain). 4.1-NOD mice were also crossed with A\(^d\)E\(_{\text{g7}}\) mouse (I-A\(^d\), I-A\(^B\)), I-A\(^b\)/I-A\(^b\); A\(^d\)E\(_{\text{g7}}\) mice expressing the I-A\(^B\)-E\(_{\text{g7}}\)-transgene; a gift from P. Marrack, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO) (32) or H-2 M\(^{t+}\) mice (I-A\(^d\), I-A\(^B\), H-2 M\(^{t+}\); a gift from L. Van Kaer, Vanderbilt University School of Medicine, Nashville, TN (43). The 4.1-TCR transgenic F1 mice resulting from these crosses were intercrossed to generate 4.1-I-A\(^d\)/I-A\(^b\) and 4.1-I-A\(^d\)/I-A\(^b\) mice, respectively. The mice were screened for inheritance of transgenes (4.1-I-A\(^d\), 4.1-I-A\(^b\), I-A\(^b\)), mutated genes (I-A\(^d\), I-A\(^b\)), and MHC genotypes by PCR of tail DNA. The sequences of the primers used for genotyping were as follows: 4.1-I-A\(^b\), 4.1-I-A\(^d\), I-A\(^b\), I-A\(^d\), and I-A\(^d\)/I-A\(^b\). The sequences of the primers used for genotyping were as follows: 4.1-I-A\(^b\), 4.1-I-A\(^d\), I-A\(^b\), I-A\(^d\), and I-A\(^d\)/I-A\(^b\).

**Abs and flow cytometry**

The anti-CD8a mAb-secreting hybridoma 53-6.7 was obtained from the American Type Culture Collection (Manassas, VA). The hybridoma 30-2, secreting the anti-I-A\(^d\)/CLIP mAb, was a gift from A. Rudensky (University of Washington, Seattle, WA). The mAbs anti-Lyt-2 (CD8a)-phycocerythrin (53-6.7), anti-L3T4-FITC (IM7), anti-L3T4-biotin (CD4; H129.19), anti-V\(_{\text{J1}}\)-FITC (R3S-15), anti-H-2K\(^d\)-FITC (SF1-1.1), and anti-I-A\(^b\)-biotin (I-A\(^b\)-2D0.1) were all purchased from Pharmingen (San Diego, CA). Streptavidin-PerCP was obtained from Becton Dickinson (San Jose, CA) and was used to reveal biotin-coupled staining. Thymocytes and splenocytes (RBC-depleted) were analyzed by three-color flow cytometry using a FACScan (Becton Dickinson) (28).

**Proliferation assays**

Splenocytes were depleted of CD8\(^+\) T cells using anti-CD8 mAb (53-6.7)-coated magnetic beads (44). Splenocytes were also used to prepare pure CD4\(^+\) T cells using the MACS microbead purification system (Miltenyi Biotec, Auburn, CA). Pancreatic islets were isolated by collagenase digestion of the pancreas, purified by Ficoll density purification and hand picking, and disrupted into single cell suspensions by trypsin treatment (44). CD4\(^+\) T cells were adjusted to 2 \times 10^6 cells/100 \mu l of complete medium (RPMI 1640 medium containing 10% heat-inactivated FBS (Life Technologies, Grand Island, NY), 50 U/ml penicillin, 50 \mu g/ml streptomycin (Flow Laboratories, McLean, VA), and 50 \mu M 2-ME (Sigma, St. Louis, MO)) and incubated in triplicate with gamma-irradiated (3000 rad) islet cells (10^3 well) in 96-well tissue culture plates for 3 days at 37°C in 5% CO\(_2\). Irradiated NOD splenocytes (10^5 well) were used as feeder cells in some experiments. Cultures were pulsed with 1 \mu C of [3H]thymidine during the last 18 h of culture, harvested, and counted. Specific proliferation was calculated by subtracting background proliferation (counts per minute of cultures containing only islet cells with/without feeders and counts per minute of cultures of T cells alone) from islet cell-induced proliferation (counts per minute of cultures containing T cells and islet cells).

**Radiation bone marrow chimeras**

Bone marrow chimeras were generated following standard protocols (45). Briefly, recipient mice (I-A\(^d\)\(^+/\)), I-A\(^d\)\(^+/\)), or I-A\(^b\)\(^+/\)) mice were first treated with two doses of 500 rad 3 h apart from a 137Cs source (Gammar, Atomic Energy of Canada, Ottawa, Canada) and then transfused with bone marrow cell suspensions (8–10 \times 10^6 cells/mouse) from donor mice (4.1-I-A\(^d\)\(^+/\)), 4.1-I-A\(^b\)\(^+/\)), 4.1-I-A\(^b\)\(^+/\)), or 4.1-I-A\(^b\)\(^+/\)). Chimeric mice were sacrificed 6–8 wk after bone marrow transplantation.

**Statistical analyses**

Statistical analyses were performed using Mann-Whitney U and \(\chi^2\) tests.
Results

Positive selection of 4.1-thymocytes in I-A\textsuperscript{b7}\textsuperscript{a} mice expressing deleting I-A\textsuperscript{b} molecules exclusively on cortical thymic epithelial cells

Our first set of experiments focused on confirming that 4.1-thymocytes can undergo positive selection in I-A\textsuperscript{b7} hemizygous mice, and that I-A\textsuperscript{b} molecules (capable of inducing complete 4.1-thymocyte deletion in H-2\textsuperscript{b7} mice (28)) can only trigger the deletion of 4.1-thymocytes when expressed on bone marrow-derived APCs, but not when expressed on cortical thymic epithelial cells. Our first approach involved following the fate of 4.1-thymocytes in I-A\textsuperscript{b7} transgenic I-A\textsuperscript{b} mice expressing an I-A\textsuperscript{b} transgene under the control of the K14 (keratin) promoter (referred to as K14 mice). K14 mice express deleting I-A\textsuperscript{b} molecules (transgenic I-A\textsuperscript{b} chains complexed with endogenous I-A\textsuperscript{a} chains) exclusively on cortical thymic epithelial cells (22) (Table 1).

The thymocyte cell number and the thymic and splenic cytofluorometric profiles of either I-A\textsuperscript{b7} hemizygous or I-A\textsuperscript{b7} homozygous 4.1-TCR-positive/K14-negative offspring from 4.1/K14-I-A\textsuperscript{b70} × 4.1/K14-I-A\textsuperscript{b70} intercrosses (designated 4.1-I-A\textsuperscript{b70} and 4.1-I-A\textsuperscript{b70} mice, respectively) were virtually identical (Table I and Fig. 1). This indicated that positive selection of 4.1-thymocytes does not require expression of two copies of the I-A\textsuperscript{a} and I-A\textsuperscript{b} genes. Subsequent cytofluorometric studies of thymic 4.1-I-A\textsuperscript{b70} mice expressing the K14-I-A\textsuperscript{b} (4.1/K14-I-A\textsuperscript{b70} mice) revealed that, unlike I-A\textsuperscript{b} molecules expressed on bone marrow-derived APCs (28), I-A\textsuperscript{b} molecules expressed on cortical thymic epithelial cells cannot trigger the deletion of 4.1-thymocytes (Table I and Fig. 2): the thymic 4.1-I-A\textsuperscript{b70} and 4.1/K14-I-A\textsuperscript{b70} mice contained significantly more thymocytes ($p < 0.0001$), greater percentages of CD4$^+$CD8$^-$V\textbeta$^{11+}$ thymocytes ($p < 0.0009$), and lower percentages of CD4$^+$CD8$^+$ thymocytes ($p < 0.002$ and $p < 0.009$, respectively) than the thymus of 4.1-I-A\textsuperscript{b70} mice (Figs. 1A and 2A). As expected, the spleens of 4.1-I-A\textsuperscript{b70} and 4.1/K14-I-A\textsuperscript{b70} (but not control K14-I-A\textsuperscript{b70}) mice also had significantly more CD4$^+$V\textbeta$^{11+}$ T cells than the spleens of deleting 4.1-I-A\textsuperscript{b70} mice ($p < 0.003$ and $p < 0.017$, respectively; Fig. 2B).

Next, we investigated whether the CD4$^+$CD8$^-$V\textbeta$^{11+}$ T cells maturing in 4.1/K14-I-A\textsuperscript{b70} mice were responsive to stimulation with cell-autologous antigen in vitro. To do this, we compared the proliferative activity of splenic CD4$^+$ T cells from 4.1-I-A\textsuperscript{b70}, 4.1-I-A\textsuperscript{b70}, 4.1-I-A\textsuperscript{b70}, 4.1/K14-I-A\textsuperscript{b70}, and K14-I-A\textsuperscript{b70} mice (Table I) in response to stimulation with NOD islet cells (I-A\textsuperscript{b767}; Fig. 3). As expected on the basis of the cytofluorometric studies shown above, the splenic CD4$^+$ T cells of 4.1/K14-I-A\textsuperscript{b70}, but not K14-I-A\textsuperscript{b70} or 4.1-I-A\textsuperscript{b70}, mice proliferated at least as well as the splenic CD4$^+$ T cells of 4.1-I-A\textsuperscript{b767} and 4.1-I-A\textsuperscript{b70} mice in response to islet stimulation. This indicated that in addition to not being able to trigger 4.1-thymocyte deletion, cortical thymic I-A\textsuperscript{b} molecules cannot anergize 4.1-thymocytes.

It was possible, however, that the inability of cortical I-A\textsuperscript{b} molecules to delete 4.1-thymocytes in 4.1/K14-I-A\textsuperscript{b70} mice resulted from these mice expressing low levels of transgenic I-A\textsuperscript{a} molecules on cortical thymic epithelial cells compared with wild-type I-A\textsuperscript{a} molecules in H-2\textsuperscript{b7} mice. To rule this out, we transfused bone marrow from 4.1-I-A\textsuperscript{b70} into lethally irradiated I-A\textsuperscript{b70} mice (expressing I-A\textsuperscript{b} molecules only on radioresistant thymic epithelial cells) or into I-A\textsuperscript{b70} mice (as controls) and followed the fate of the 4.1-TCR in the chimeras. As shown in Fig. 4 (middle and left panels), the cytofluorometric profiles of these two chimeras, which

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Table 1. MHC class II genotype, phenotype, and outcome of thymocyte selection in 4.1/K14 mice

<table>
<thead>
<tr>
<th>Name</th>
<th>Genotype</th>
<th>Site of thymic expression</th>
<th>4.1-Thymocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α locus</td>
<td>β locus</td>
<td>Transgene</td>
</tr>
<tr>
<td>4.1-I-A\textsuperscript{b70}</td>
<td>I-\textalpha\textsuperscript{a}</td>
<td>I-\textalpha\textsuperscript{b}</td>
<td>–</td>
</tr>
<tr>
<td>4.1-I-A\textsuperscript{b70}</td>
<td>I-\textalpha\textsuperscript{a}</td>
<td>I-\textalpha\textsuperscript{b}</td>
<td>–</td>
</tr>
<tr>
<td>4.1/K14-I-A\textsuperscript{b70}</td>
<td>I-\textalpha\textsuperscript{a}</td>
<td>I-\textalpha\textsuperscript{b}</td>
<td>–</td>
</tr>
<tr>
<td>4.1/I-A\textsuperscript{b70}</td>
<td>I-\textalpha\textsuperscript{a}</td>
<td>I-\textalpha\textsuperscript{b}</td>
<td>–</td>
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<tr>
<td>4.1/I-A\textsuperscript{b70}</td>
<td>I-\textalpha\textsuperscript{a}</td>
<td>I-\textalpha\textsuperscript{b}</td>
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<td>4.1-I-A\textsuperscript{b70}</td>
<td>I-\textalpha\textsuperscript{a}</td>
<td>I-\textalpha\textsuperscript{b}</td>
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<td>4.1-I-A\textsuperscript{b70}</td>
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<td>I-\textalpha\textsuperscript{b}</td>
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<td>4.1-I-A\textsuperscript{b70}</td>
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<td>I-\textalpha\textsuperscript{b}</td>
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<tr>
<td>4.1-I-A\textsuperscript{b70}</td>
<td>I-\textalpha\textsuperscript{a}</td>
<td>I-\textalpha\textsuperscript{b}</td>
<td>–</td>
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</tbody>
</table>

$^a$4.1-thymocyte positive selection precedes negative selection.
$^b$Thymocyte cell death in these animals is due to massive developmental arrest of 4.1-thymocytes.
expressed wild-type MHC class II molecules, were virtually identical and were similar to those corresponding to 4.1/K14-I-\(\text{A}^\text{b}\)0/0 mice.

Taken together, these results demonstrated that the 4.1-TCR undergoes unopposed positive selection in I-\(\text{A}^\text{d}\) hemizygous mice and that splenocytes from 4.1-I-\(\text{A}^\text{d}\)/0 and 4.1-I-\(\text{A}^\text{d}\)/0 mice are similar to those corresponding to 4.1/K14-I-\(\text{A}^\text{b}\)0/0.

I-\(\text{A}^\text{b}\) molecules on cortical thymic epithelial cells cannot induce the positive selection of 4.1-TCR cells

Having established that I-\(\text{A}^\text{b}\) molecules on cortical thymic epithelial cells cannot delete 4.1-thymocyte, we proceeded to investigate whether cortical I-\(\text{A}^\text{b}\) molecules could actually induce their positive selection. This was first investigated by comparing the fate of 4.1-thymocytes in 4.1-TCR-transgenic I-\(\text{A}^\text{b}\)/0/I-\(\text{A}^\text{b}\)0 mice expressing or lacking the K14 transgene (4.1/K14-I-\(\text{A}^\text{b}\)0/0 and 4.1-I-\(\text{A}^\text{b}\)0/0, respectively; Table I). Surprisingly, these studies revealed that cortical I-\(\text{A}^\text{b}\) molecules could not positively select 4.1-thymocytes; thymi from both 4.1/K14-I-\(\text{A}^\text{b}\)0/0 and 4.1-I-\(\text{A}^\text{b}\)0/0 mice had very few CD4+CD8- cells and numerous CD4-CD8+ cells (Fig. 5A, two left panels), probably reflecting the massive developmental arrest of 4.1-CD4+CD8+ thymocytes in the absence of selecting MHC class II molecules. The absence of 4.1-thymocyte positive selection in these mice was not the result of a general inability of K14 transgene-encoded I-\(\text{A}^\text{b}\) molecules to drive the positive selection of thymocytes; as shown in Fig. 5 (two right panels), non-TCR transgenic K14-I-\(\text{A}^\text{b}\)0/0 mice were efficient positive selectors of CD4+CD8- T cells; these mice had more thymocytes (\(p < 0.0001\)) and greater percentages of CD4+CD8- thymocytes (Fig. 5A) and splenic CD4+ T cells (Fig. 5B) than I-\(\text{A}^\text{d}\)0/0 mice (\(p < 0.0012\), which do not express MHC class II molecules (Table I). Furthermore, 4.1/K14-I-\(\text{A}^\text{b}\)0/0 mice, which express I-\(\text{A}^\text{b}\) molecules in the thymic cortex and thus can select non-TCR transgenic thymocytes, had more thymocytes than 4.1-I-\(\text{A}^\text{b}\)0/0 mice (\(p < 0.0001\); Fig. 5A). Non-TCR transgenic K14-I-\(\text{A}^\text{b}\)0/0 mice also had more thymocytes than I-\(\text{A}^\text{d}\)0/0 mice (61 ± 13 vs 32 ± 5 × 105; \(p < 0.001\)).

To confirm that the lack of 4.1-thymocyte positive selection in 4.1/K14-I-\(\text{A}^\text{b}\)0/0 mice was not due to low levels of expression of transgenic I-\(\text{A}^\text{b}\) molecules on thymic cortical epithelial cells, we followed the fate of the 4.1-TCR in lethally irradiated H-2\(\text{d}\)/0 mice that had been reconstituted with 4.1-I-\(\text{A}^\text{d}\)/0/0 mice (28), which chimera expressed wild-type I-\(\text{A}^\text{b}\) molecules only on radioresistant thymic epithelial cells. As shown in Fig. 4 (right panels), the cytofluorometric profiles of thymi from these chimera were very similar to the cytofluorometric profiles of thymi from 4.1/K14-I-\(\text{A}^\text{b}\)0/0 mice. This confirmed that cortical I-\(\text{A}^\text{b}\) molecules cannot drive the positive selection of 4.1-thymocytes.

A small percentage of CD4+CD8- cells (B) from 4.1-I-\(\text{A}^\text{d}\)/0 and 4.1-I-\(\text{A}^\text{d}\)/0 mice, however, did appear to mature in 4.1/K14-I-\(\text{A}^\text{b}\)0/0 mice (Fig. 5B). Proliferation assays employing splenic CD4+ T cells from 4.1/K14-I-\(\text{A}^\text{d}\)/0, 4.1/K14-I-\(\text{A}^\text{d}\)/0, or K14-I-\(\text{A}^\text{d}\)/0 mice as responders and irradiated NOD islet cells and splenocytes (as a source of antigen and APCs, respectively) revealed that, unlike the CD4+ T cells from K14-I-\(\text{A}^\text{d}\)/0 mice, the CD4+ T cells from 4.1/K14-I-\(\text{A}^\text{d}\)/0 mice contained some \(\beta\)-cell-reactive cells (Fig. 6A). These cells, however, displayed significantly lower islet reactivity (Fig. 6A), contained fewer \(\beta\) cells (\(p < 0.006\)), and expressed lower levels of the transgenic TCR \(\beta\)-chain (\(\beta\)11 mean fluorescence intensities, 61 ± 13 vs 78 ± 14, respectively; \(p < 0.006\)) than the CD4+ T cells from 4.1/K14-I-\(\text{A}^\text{d}\)/0 mice (compare middle panel of Fig. 2B with the second panel of Fig. 5B). This suggested that the few islet-reactive CD4+ T cells that matured in 4.1/K14-I-\(\text{A}^\text{b}\)0/0 mice were actually selected on endogenous TCRs (i.e., the I-\(\text{A}^\text{b}\)-autoimmune TCRs that are found in K14-I-\(\text{A}^\text{b}\)0/0 mice (22)) rather than on the transgenic 4.1-TCR itself.

I-\(\text{A}^\text{b}\) molecules cannot restrict recognition of the target \(\beta\)-cell autoantigen by 4.1-CD4+ T cells

Our next set of experiments was aimed at determining whether the I-\(\text{A}^\text{b}\)-induced deletion of 4.1-thymocytes in H-2\(\text{d}\)/0 mice might be triggered by the target autoantigen of the 4.1-TCR. Since purified splenic CD4+ T cells from 4.1-NOD mice engage their target autoantigen on a resident, highly stimulatory intraislet APC population in the context of I-\(\text{A}^\text{d}\) (28), we first asked whether I-\(\text{A}^\text{b}\) molecules expressed on intraislet APCs from I-\(\text{A}^\text{b}\)-deficient mice could restrict the recognition of this autoantigen by 4.1-CD4+ T cells from 4.1-I-\(\text{A}^\text{d}\)/0 mice. We also asked whether I-\(\text{A}^\text{b}\) molecules on such intraislet APCs could trigger the proliferation of the I-\(\text{A}^\text{b}\)-autoreactive CD4+ T cells that are selected in K14-I-\(\text{A}^\text{b}\)0/0 mice (22) and, possibly, 4.1/K14-I-\(\text{A}^\text{b}\)0/0 mice. This was done by determining whether purified splenic CD4+ T cells from each of these three types of mice (4.1-I-\(\text{A}^\text{d}\)/0, 4.1/K14-I-\(\text{A}^\text{b}\)/0, and K14-I-\(\text{A}^\text{b}\)0/0) could proliferate in response to I-\(\text{A}^\text{d}\)/0 (Fig. 6B), I-\(\text{A}^\text{b}\)/0 (Fig. 6C), or
I-A<sup>0</sup> islet cells (Fig. 6D; from insulitis-free RAG-2<sup>−/−</sup> NOD, C57BL/6, and C57BL/6. I-A<sub>b</sub>b<sup>/−</sup> mice, respectively). As shown in Figs. 6 B–D, the CD4<sup>+</sup>T cells of 4.1-I-A<sup>g7/b</sup> mice contained cells capable of recognizing islet Ag in the context of I-A<sup>g7</sup>, but not in the context of I-A<sub>b</sub> (proliferation in response to I-A<sup>0</sup> islet cells was similar to proliferation in response to I-A<sub>b</sub/b<sup>/−</sup> islet cells). Lack of proliferation in response to I-A<sub>b</sub/b<sup>/−</sup> islet cells was due to an inability of I-A<sub>b</sub> molecules to present β-cell autoantigen, since the CD4<sup>+</sup>T cells of 4.1-I-A<sup>g7/b</sup> mice proliferated well in assays employing islet cells from H-2<sup>g7</sup>-congenic C57BL/10. H-2<sup>g7</sup> mice (Fig. 6E), which are also insulitis free and diabetes resistant. These I-A<sup>g7</sup>-dependent responses were not the result of I-A<sup>g7</sup> autoreactivity, since 4.1-CD4<sup>+</sup>T cells from 4.1-NOD mice did not proliferate against I-A<sup>g7/g7</sup> splenocytes in the absence of islet Ag (data not shown). In contrast, the CD4<sup>+</sup>T cells from K14-I-A<sup>0</sup> and, to a lesser extent, 4.1/K14-I-A<sup>0</sup> mice proliferated vigorously in response to I-A<sub>b</sub>b<sup>/−</sup> islet cells (Fig. 6, B and C) and only slightly in response to I-A<sup>g7/g7</sup> islet cells, confirming the presence of I-A<sub>b</sub>-expressing APCs in I-A<sub>b</sub>b<sup>/−</sup> islets. These responses were the result of I-A<sub>b</sub> autoreactivity, since they were not observed in assays employing islet cells, where I-A molecules are absent (I-A<sup>0</sup> islet cells; Fig. 6D).

Taken together, these results demonstrated that 1) I-A<sub>b</sub> molecules on intraislet APCs cannot present the putative β-cell autoantigen recognized by the 4.1-TCR, but are highly stimulatory for I-A<sub>b</sub>-autoreactive CD4<sup>+</sup>T cells; 2) I-A<sub>b</sub> molecules on intraislet APCs cannot stimulate 4.1-CD4<sup>+</sup>T cells; and 3) the few β-cell-reactive CD4<sup>+</sup>V<sub>β11</sub> T cells that mature in 4.1/K14-I-A<sup>0</sup> mice are actually selected on endogenous, I-A<sub>b</sub>-autoreactive, TCRs. Deletion of the 4.1-TCR by I-A<sub>b</sub> molecules on hemopoietic cells is peptide dependent

The inability of cortical I-A<sub>b</sub> molecules to trigger the positive selection of 4.1-thymocytes and the inability of the 4.1-TCR to recognize its target Ag in the context of I-A<sub>b</sub>, or I-A<sup>a</sup> molecules as allogeneic indicated that the MHC class II promiscuity of the diabetogenic 4.1-TCR is a phenomenon that is restricted to the thymic medulla. In turn, these results suggested that the ability of different antidiabetogenic MHC class II molecules (i.e., I-A<sup>a</sup>) to trigger 4.1-thymocyte deletion cannot be accounted for by their ability to present the target β-cell autoantigen of the 4.1-TCR in the thymic medulla. How, then, can a single, nonalloreactive TCR engage so many different MHC class II molecules selectively on thymic (but not peripheral) hemopoietic cells? We entertained two alternative possibilities to explain this phenomenon: 1) the 4.1-TCR undergoes thymocyte deletion by reacting with residues shared by cross-reactive MHC class II molecules on the surface of

**FIGURE 2.** Absence of 4.1-thymocyte deletion in 4.1-TCR transgenic mice selectively expressing deleting MHC class II molecules (I-A<sup>a</sup>) on cortical thymic epithelial cells. A and B, CD4, CD8, and V<sub>β11</sub> cytofluorometric profiles of thymocytes (A) and splenocytes (B) from 4.1-I-A<sup>g7</sup>, 4.1/K14-I-A<sup>g7</sup>, and K14-I-A<sup>g7</sup> mice. See Fig. 1 for details. The numbers of thymocytes in 4.1/K14-I-A<sup>g7</sup>, 4.1-I-A<sup>g7</sup>, and K14-I-A<sup>g7</sup> mice were 23 ± 6 × 10<sup>6</sup>, 6 ± 1 × 10<sup>6</sup>, and 60 ± 7 × 10<sup>6</sup>, respectively. Data shown are average values of 4–12 mice/group (6–8 wk old). See text for statistical values.

**FIGURE 3.** Splenic CD4<sup>+</sup>T cells from 4.1-I-A<sup>g7</sup> and 4.1/K14-I-A<sup>g7</sup> but not 4.1-I-A<sup>g7</sup> or K14-I-A<sup>g7</sup> mice are islet reactive. Proliferation of splenic CD4<sup>+</sup>T cells from 4.1-I-A<sup>g7</sup>, 4.1-I-A<sup>g7</sup>, 4.1/K14-I-A<sup>g7</sup>, K14-I-A<sup>g7</sup>, and 4.1-I-A<sup>g7</sup> mice in response to NOD islet cells. Cultures of 2 × 10<sup>5</sup> splenic CD4<sup>+</sup>T cells (CD8<sup>−</sup>T cell depleted) were incubated with irradiated NOD islet cells and splenocytes (10<sup>5</sup> cells/well) for 3 days, pulsed with [<sup>3</sup>H]thymidine, and harvested. Bars show the SEMs.
a specialized thymic APC regardless of the nature of the peptides bound to these molecules (i.e., deletion is peptide independent); or 2) the 4.1-TCR undergoes thymocyte deletion by engaging a peptide that binds efficiently to protective, but not pathogenic, MHC class II molecules and that is exclusively presented in sufficiently high quantities by APCs of the thymic medulla, but not by peripheral or intraislet APCs (i.e., deletion is peptide dependent).

To distinguish between these two alternative possibilities, we followed the fate of the 4.1-TCR in radiation bone marrow chimeras bearing thymic epithelial cells expressing the selecting I-A^d^7 molecule and bone marrow-derived APCs expressing single peptide/I-A^b^ complexes. Our first experiments were performed with irradiated NOD mice (H-2^g7^) that had been transfused with marrow from 4.1-A^bE^pLi^0/0^ mice (invariant chain-deficient

**FIGURE 4.** 4.1-thymocyte development in radiation bone marrow chimeras expressing I-A^b^ molecules on thymic epithelial cells. Bone marrow cells (7–12 × 10^6^) from 4.1-I-A^d^7/g^7^ mice were transfused into the tail veins of lethally irradiated I-A^d^7/g^7^ (A), I-A^d^7/b^ (B), or I-A^b^/b^ (C) mice. The thymocyte cytofluorometric profiles of the radiation bone marrow chimeras were analyzed 6–8 wk after transplantation (see Fig. 1). The cells lying in the center of the right panel correspond to dead thymocytes displaying nuclear autofluorescence. The numbers of thymocytes in I-A^d^7/g^7^, I-A^d^7/b^, and I-A^b^/b^ hosts were 6 ± 1 × 10^6^, 10 ± 4 × 10^6^, and 1 ± 1 × 10^6^, respectively. Results shown are average values from two to eight mice per group.

**FIGURE 5.** Developmental arrest of 4.1-thymocytes in I-A^d^7-negative 4.1-TCR transgenic mice expressing deleting MHC class II molecules only on cortical thymic epithelial cells. A and B, CD4, CD8, and Vβ11 cytofluorometric profiles of thymocytes (A) and splenocytes (B) from 4.1-I-A^0/0^, 4.1/K14-I-A^0/0^, I-A^0/0^, and K14-I-A^0/0^ mice. See Fig. 1 for details. The numbers of thymocytes in 4.1-I-A^0/0^, 4.1/K14-I-A^0/0^, I-A^0/0^, and K14-I-A^0/0^ mice were 6 ± 2 × 10^6^, 22 ± 3 × 10^6^, 55 ± 14 × 10^6^, and 108 ± 13 × 10^6^, respectively. Data are average values from 7–13 mice/group. See text for statistical comparisons.
chimeras had significantly fewer thymocytes and greater percent-
1 CD4 
E invariant chain-derived CLIP peptide) (43, 46, 47). Unlike 4.1-

ments in irradiated NOD mice (4.1-TCR transgenic H-2 b/b mice expressing single-peptide/I-A b complexes on hemopoietic cells therefore demonstrated that the I-A b-induced negative selection of 4.1-thymocytes in 4.1-H-2 b mice is a peptide-dependent process.

Discussion

The studies presented here were based on our serendipitous discovery that thymocytes bearing the highly diabetogenic, I-A b-restricted β-cell-reactive 4.1-TCR undergo deletion in diabetes-resistant NOD.H-2 b mice by engaging antidiabetogenic MHC class II molecules (I-A a, I-E b and/or I-A b, I-A a, I-A b, and/or I-E b) on bone marrow-derived APCs (28). Since the diabetes resistance afforded by protective MHC class II molecules in non-TCR transgenic mice also resides in the bone marrow (29), this observation provided an explanation of how protective MHC molecules might afford resistance to specific autoimmune diseases (28). The present study was initiated to further understand why the 4.1-TCR deletes in the presence of antidiabetogenic MHC class II molecules. We hypothesized that 1) the 4.1-TCR would engage deleting MHC class II molecules not only on thymic hemopoietic cells, but also on cortical thymic epithelial cells, as is the case for most MHC-alloreactive or MHC-autoantigene TCRs; and 2) the MHC-induced deletion of this TCR would be autoantigen dependent. Our results show that this is not the case; the I-A b reactivity of this pathogenic autoreactive TCR is restricted to the thymic medulla and, although peptide-dependent, cannot be accounted for by the ability of I-A a molecules to present the 4.1-TCR’s target autoantigen. It thus appears that the 4.1-TCR can engage two or more different peptide/MHC complexes in at least two different anatomical regions within individual mice: cortical thymic epithelial cells and intraislet APCs (in the context of I-A a) and thymic hemopoietic cells (in the context of I-A b; Fig. 8). On the basis of these unexpected findings, we conclude that this form of MHC-induced protection from autoimmunity is based on the presentation of an anatomically restricted, nonautoantigen-derived peptide to highly pathogenic thymocytes.

It could be argued that the inability of the cortical thymic epithelial cells of 4.1/K14-I-A a mice to support the positive selection of the 4.1-TCR may have been the result of low levels of expression of the K14-I-A b transgene. We believe, however, that this is highly unlikely, since 4.1-thymocytes also failed to undergo positive selection in 4.1-I-A a/I-E b/chimeras (but not in 4.1-I-A a/I-E b/chimeras), which express wild-type I-A b molecules on radioreistant thymic epithelial cells. This ability of the 4.1-TCR to engage I-A b molecules only in the thymic medulla and in a peptide-dependent manner suggests that the 4.1-TCR does not undergo negative selection by reacting primarily with MHC (as opposed to peptide) residues, as may be the case for some self-MHC-restricted or MHC-alloreactive TCRs (32, 37, 41, 48–51). We do not yet know whether deletion of the 4.1-TCR in the thymic medulla is due to recognition of different peptides bound to different deleting MHC class II molecules (one peptide per MHC molecule) or to recognition of a single peptide that can bind to all these MHC molecules. It appears, however, that this peptide(s) is not the diabetogenic, I-A a binding peptide that the 4.1-TCR recognizes in the periphery; mature 4.1-CD4+ T cells proliferated efficiently in response to intraislet APCs expressing I-A a molecules, but not in response to intraislet APCs expressing I-A b molecules. Since the 4.1-TCR cannot engage deleting MHC class II

4.1-TCR transgenic mice expressing I-A b molecules covalently linked to the I-Eo52-68 peptide) (32). NOD mice transfused with marrow from 4.1-H-2 b or 4.1-H-2 b mice were used as controls for positive and negative selection, respectively. Cytomfluorometric studies of thymocytes (Fig. 7A) and proliferation assays employing splenic CD4+ T cells from these chimeras (Fig. 7B) indicated that the 4.1-TCR underwent positive selection in 4.1-H-2 b/NOD and 4.1-A b, I-E b/I-E b NOD chimeras, but not in 4.1-H-2 b/NOD chimeras. As shown in Fig. 7A, 4.1-H-2 b/NOD chimeras had significantly fewer thymocytes and greater percentages of CD4+ CD8- thymocytes than 4.1-H-2 b/NOD and 4.1-A b, I-E b/I-E b NOD chimeras (p < 0.01). Since the expression levels of I-A a molecules in A b, I-E b NOD mice are significantly lower than those in wild-type H-2 b mice (32), there was still the possibility that 4.1-thymocytes did engage the transgenic I-A b/I-Eo52-68 complex on hemopoietic cells of the 4.1-A b, I-E b/NOD chimeras, but with an avidity that was too low to result in their negative selection. To address this possibility, we repeated these experiments in irradiated NOD mice (H-2 b) that had been transfused with marrow from 4.1-H-2 M b mice (4.1-TCR transgenic H-2 b mice almost exclusively expressing I-A b molecules bound to the invariant chain-derived CLIP peptide) (43, 46, 47). Unlike 4.1-A b, I-E b NOD mice, 4.1-H-2M b mice express even greater levels of total I-A b molecules than 4.1-H-2 b mice (Fig. 7B), which are efficient deleters of 4.1-thymocytes. As shown in Fig. 7A, 4.1-thymocytes underwent unopposed positive selection in these chimeras. The absence of 4.1-thymocyte negative selection in I-A b+/+ mice expressing single-peptide/I-A b complexes on hemopoietic cells therefore demonstrated that the I-A b-induced negative selection of 4.1-thymocytes in 4.1-H-2 b mice is a peptide-dependent process.

FIGURE 6. Islet and MHC auto/alloreactivity in 4.1-TCR-transgenic mice expressing deleting or selecting MHC class II molecules on cortical thymic epithelial cells. In vitro proliferation of purified splenic CD4+ T cells from 4.1/K14-I-A a NOD, K14-I-A a NOD, K14-I-A a NOD, and/or 4.1/K14-I-A a NOD mice in response to RAG-2−/− NOD (A and B), C57BL/6 (C), and C57BL/6/I-A b NOD cells (D; all insulitis free) in the presence (A) or the absence (B–D) of exogenous APCs. E. Proliferation of purified splenic CD4+ T cells from 4.1-I-A b NOD mice against C57BL/6 NOD mice expressing I-A b NOD (also insulitis free) in the absence of exogenous APCs. Proliferation assays were performed as described in Fig. 3, except that CD4+ T cells were purified using the MACS purification system (>94% purity).
molecules on peripheral or intraislet APCs, this peptide(s) may only be expressed in the thymic APC population that triggers 4.1-thymocyte deletion. Nevertheless, since thymocyte tolerance is a more sensitive response than peripheral T cell activation (52), the possibility that this peptide(s) is also expressed in peripheral APCs cannot be excluded.

Whatever the nature of the peptide ligand(s) involved, our data demonstrate that the ability of a given MHC molecule to restrict the negative selection of a TCR in the thymic medulla does not imply that the same MHC molecule should also be able to restrict this TCR’s positive selection in the thymic cortex. Assuming that positive selection of 4.1-thymocytes in 4.1-NOD mice is a peptide-dependent process, our data suggest that there are no peptides available in the thymic cortex to positively select 4.1-thymocytes in the context of I-Ab. In turn, this implies that this TCR cannot undergo positive selection via low affinity interactions with multiple I-Ab/peptide complexes totalling a positively selecting avidity threshold. Whether this unique ability of the 4.1-TCR to undergo sequential positive and negative selection on different MHC class II molecules is a peculiarity of certain autoreactive TCRs (i.e., those that are highly pathogenic and MHC promiscuous) or is also shared by some MHC-alloreactive TCRs and foreign Ag-specific TCRs that can cross-react with allo-MHC molecules remains to be determined. It appears, however, that most MHC-promiscuous TCRs do not behave like the 4.1-TCR; the ability of a given MHC molecule to restrict the deletion of a TCR in the thymic medulla is usually associated with the ability of this MHC molecule to restrict this TCR’s positive selection in the thymic cortex and its activation in the periphery. The K\(^\beta\)-restricted and L\(^d\)-alloreactive 2C TCR, for example, can undergo positive (but not negative) selection on K\(^\beta\) molecules and both positive and negative selection on L\(^d\) molecules (36). The conalbumin-specific D10-TCR undergoes weak positive selection in H-2\(^k\) mice and negative selection in H-2\(^d\) mice, but, unlike the 4.1-TCR, can also engage deleting MHC molecules (H-2\(^d\)) on peripheral APCs (35, 53). The foreign Ag-specific AND and DO11.10 TCRs (specific for pigeon cytochrome c/I-A\(^k\) and OVA/I-A\(^d\) complexes, respectively) can undergo positive selection on more than one MHC class II molecule (I-E\(^k\) and I-A\(^b\), or I-A\(^d\) and I-A\(^b\), respectively), but these MHC molecules do not trigger the negative selection of these TCRs in the thymic medulla (21, 22, 30, 31). On the basis of these differences, it is tempting to speculate that the selective promiscuity of the 4.1-TCR for antidiabetogenic MHC molecules in the thymic medulla is somehow associated with its exquisite diabetogenic potential, and that selection of this type of TCRs is promoted by the unique MHC class II molecule of the NOD mouse (I-A\(^g7\)).

Our findings also provide some insights into our understanding of the mechanisms underlying the MHC-associated susceptibility to autoimmunity. Previous studies have proposed that diabetogenic MHC class II molecules (which are poor peptide binders) provide susceptibility to diabetes by being able to reach expression levels on thymic APCs that are sufficient to induce the positive, but not.

**FIGURE 7.** Bone marrow-derived APCs expressing deleting MHC class II molecules bound to single peptides cannot trigger the deletion of 4.1-thymocytes. **A**, Thymocyte cytofluorometric profiles of radiation bone marrow chimeras. Bone marrow cells (7–12 \(\times\) 10\(^6\)) from 4.1-I-A\(^{g7}\)/b, 4.1-I-A\(^{g7}\)/g7, 4.1-H-2\(^M0\)/0, or 4.1-I-A\(^b\),l\(^{m0}\) were transfused into the tail vein of lethally irradiated NOD mice (H-2\(^g7\)). The chimeras were analyzed 6–8 wk after transplantation. The numbers of thymocytes in 4.1-H-2\(^M0\)/0–NOD, 4.1-H-2\(^g7\)/NOD, 4.1-I-A\(^{g7}\)/b, and 4.1-H-2\(^M0\)/0–NOD chimeras were 2 ± 1 \(\times\) 10\(^5\), 5 ± 1 \(\times\) 10\(^5\), 5 ± 1 \(\times\) 10\(^5\), and 6 ± 2 \(\times\) 10\(^6\), respectively. Results shown are average values from six to eight mice per group. **B**, Levels of I-A\(^b\) molecules on splenocytes from 4.1-I-A\(^{g7}\)/b, 4.1-I-A\(^{g7}\)/g7, and 4.1-H-2\(^M0\)/0 mice, as determined by staining with an anti-I-A\(^b\) mAb (AF6–120.1). **C**, Islet reactivity of splenic CD4\(^+\) T cells from bone marrow chimeras. Proliferation assays were performed as described in Fig. 6.
the negative, selection of autoreactive thymocytes (5, 27). This hypothesis implies that differences in the putative ability of dia-
abeticogenic and protective MHC class II molecules to delete patho-
genic T cells is the result of quantitative differences in the abilities of these molecules to form stable peptide/MHC complexes on thymic APCs and thus to present peptides at deleting doses on hemo-
poietic cells. Yet, pathogenic MHC molecules can positively select autoreactive T cells in the thymus, and can efficiently present autoantigenic peptides to mature T cells in the periphery. Two of our observations challenge the pure quantitative aspects of this theory, and provide one solution to this paradox. First, I-A\(g^7\) hemizygous 4.1-TCR transgenic mice selected 4.1-thymocytes as efficiently as I-A\(p^7\) homozygous mice, which are thought to express more I-A\(p^7\) molecules than the former. Second, protective I-A\(b\) molecules could efficiently delete, but could not positively select, 4.1-thymocytes, yet these molecules could not present the target molecules could not present this peptide or because the resulting complexes cannot be recognized by the 4.1-TCR.

The observation that this pathogenic TCR undergoes sequential positive and negative selection in diabetes-resistant H-2\(k^7\) mice by engaging different peptide/MHC complexes on two geographically distinct regions of the thymus not only has important implications for our understanding of the mechanisms through which protective MHC class II molecules afford autoimmune disease resistance, but also provides one solution of the so-called thymic paradox, i.e., how apparently similar interactions between individual TCRs and self peptide/MHC complexes on different thymic cell types can result in the formation of a T cell repertoire that can recognize foreign Ag in the context of self MHC yet be tolerant to self.

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