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Two Mechanisms for the Non-MHC-Linked Resistance to Spontaneous Autoimmunity

Joan Verdaguer, Abdelaziz Amrani, Brad Anderson, Dennis Schmidt, and Pere Santamaria

Genetic susceptibility and resistance to most autoimmune disorders are associated with highly polymorphic genes of the MHC and with non-MHC-linked polygenic modifiers. It is known that non-MHC-linked polymorphisms can override or enhance the susceptibility to an autoimmune disease provided by pathogenic MHC genes, but the mechanisms remain elusive. In this study, we have followed the fate of two highly diabetogenic β cell-specific T cell receptors (Kd and I-Aγ7 restricted, respectively) in NOR/Lt mice, which are resistant to autoimmune diabetes despite expressing two copies of the diabetogenic MHC haplotype H-2β7. We show that at least two mechanisms of non-MHC-linked control of pathogenic T cells operate in these mice. One segregates as a recessive trait and is associated with a reduction in the peripheral frequency of diabetogenic CD8+ (but not CD4+) T cells. The other segregates as a dominant trait and is mediated by IL-4- and TGF-β1-independent immune suppressive functions provided by lymphocytes that target diabetogenic CD4+ and CD8+ T cells, without causing their deletion, anergy, immune deviation, or ignorance. These results provide explanations as to how non-MHC-linked polymorphisms can override the susceptibility to an autoimmune disease provided by pathogenic MHC haplotypes, and demonstrate that protective non-MHC-linked genes may selectively target specific lymphoid cell types in cellularly complex autoimmune responses. The Journal of Immunology, 1999, 162: 4614–4626.
expressing diabeticogenic MHC haplotypes does not imply autoreactivity, and suggested that the diabetes resistance of these mice is controlled by non-MHC-linked genetic elements. The studies presented in this work were initiated to elucidate the mechanisms of action of non-MHC-linked antidiabeticogenic genes. This was done by following the fate of our two β cell-specific TCRs (4.1, I-A\(^{\beta}\)-restricted; and 8.3, K\(^{d}\)-restricted) in NOR and (NOD × NOR)F\(_1\) mice, both of which are resistant to islet inflammation (insulinitis) and diabetes, despite deriving ~88% of their genome from the NOD mouse, including two copies of the H-2\(^{k}\) haplotype (25, 26). These studies have resulted in the discovery of two mechanisms of non-MHC-linked control of diabeticogenic T cells. One of these mechanisms is recessive and induces a reduction in the peripheral frequency of diabeticogenic CD\(^{8}\), but not CD\(^{4}\), T cells. The other is dominant and is mediated by immune suppressive functions provided by endogenous lymphocytes that do not involve the deletion, anergy, or immune deviation of diabeticogenic T cells, nor their ignorance of β cells, and that target pathogenic TCRs regardless of their MHC restriction or fine antigenic specificity. These results provide an explanation as to how non-MHC-linked gene polymorphisms can override the susceptibility to an autoimmune disease provided by pathogenic MHC haplotypes and demonstrate that protective non-MHC-linked genes may selectively target specific cellular elements in cellularly complex pathogenic autoimmune responses.

Materials and Methods

**Mice**

8.3-NOD and 4.1-NOD mice, expressing K\(^{d}\), or I-A\(^{\beta}\)-restricted β cell-reactive TCR transgenes, derived from the CD\(^{8}\)- and CD\(^{4}\)-T cell clones NY8.3 and NY4.1, respectively, have been described (23, 24). NOR/Lt mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Diabetic 8.3-NOD or 4.1-NOD mice were outcrossed with NOR/Lt mice (H-2\(^{k}\) and ~88% genetically identical to NOD mice, including the diabetes susceptibility loci Idd2, Idd3, Iddb, Idd7, Idd8, and Idd10, but homozygous for diabetes-resistance alleles at Idd5, Idd9/11, and Idd13) (26) to generate 8.3-F\(_1\) and 4.1-F\(_1\) mice, respectively. NOR/Lt mice are also homozygous for Idd4, but this locus does not affect diabetes resistance in NOR mice (26). Diabetic 8.3-F\(_1\)- and 4.1-F\(_1\)-mice were then backcrossed with NOR/Lt mice for up to seven generations, to generate 8.3-NOR and 4.1-NOR mice. Mice of the N5 backcross were homozygous for NOR alleles at Idd5, Idd9/11, and Idd13 loci. RAG-2-NOR mice were generated by backcrossing the RAG-2 mutation of RAG-2\(^{-}\)C57BL/6J 129 mice (a gift from F. Alt, Boston Children’s Hospital, Boston, MA) onto the NOR background for 10 generations, followed by intercrossing N10 heterozygotes (typed as homozygous for NOR alleles at Idd5, Idd9/11, and Idd13) (the RAG-2 locus lies 13 cM away from the Idd13-containing region on chromosome 2). RAG-2 8.3-F\(_1\)- and 4.1-F\(_1\)-mice were generated by intercrossing RAG-2 8.3-NOR or RAG-2 4.1-NOR mice (24) with RAG-2 NOR or RAG-2\(^{-}\)NOR mice. 8.3-TCR\(^{b}\)-transgenic NOR mice were produced by backcrossing 8.3-TCR\(^{b}\)-transgenic NOR NOD mice (NOR × NOR)F\(_1\) mice, (27) with NOR mice for up to nine generations. Fas\(^{+/\text{NOD-lpr}}\) mice were generated by backcrossing the Fas\(^{+/\text{NOD-lpr}}\) gene of B6.MRL-Fas\(^{+/\text{NOD-lpr}}\) mice (from The Jackson Laboratory) onto the NOR background for up to seven generations. Fas\(^{+/\text{NOD-lpr}}\) mice were generated by intercrossing heterozygous mice of the N3 × N7 generation. Mice were screened for inheritance of the transgenes and mutated and wild-type RAG-2 or Fas alleles by PCR of tail DNA. NOR/NOD polymorphisms at Idd5, Idd9/11, and Idd13 loci were determined by PCR using primers for D1Mit46 (i5d), D4 Mit11 (Idd9/11), D2Mit144, and D2Mit490 (Idd13) (26), in the absence (for D2Mit490) or presence of I-A\(^{\beta}\)CTCP (for all other markers). D2 Mit490 lies between the Idd13 (3cM) and RAG-2 loci (10 cM). PCR products were resolved in 4% NuSieve agarose gels (D2Mit490) or in 7 M urea/6% acrylamide gels (other markers). All mice were housed in a specific pathogen-free facility.

**Diabetes**

Diabetes was assessed by measuring urine glucose levels with Diastix strips (Miles, Ontario, Canada) twice weekly. Animals were considered diabetic after two consecutive readouts ≥3\(^{+}\).
Target cells (L1210-Fas, L1210-Fas, and single NOD or NOR islet cells 5 x 10⁶) were labeled with ⁵¹Cr sodium chromate (DuAbelabs, Boston, MA) and seeded at 10⁴ cells per 100 μl/well. Some experiments (those in Fig. 5F) employed islet cells derived from islets that had been preincubated overnight with supernatants from 3-day-old islet-derived CD9 T cell lines, or with IL-1α (10⁴ U/L), to increase their susceptibility to Fas-mediated cytotoxicity (28). Effector cells (islet-infiltrating T cells, islet-derived serine esterase CD8 T cell lines, or islet-derived serine esterase CD8 T cell clones; 100 μl) were added to each well, in duplicate, at several E:T ratios. Plain medium or 1% Triton X-100 was added to sets of target cells for examination of spontaneous and total cell lysis, respectively. The plates were incubated at 37°C for 8 h, and the supernatants were collected at this point for determination of specific ⁵¹Cr release (24).

Cloning and sequencing of TCRα mRNAs

The TCRα-chain cDNA molecules of islet-stimulated splenic CD8⁺ T cells from 8.3-TCRβ-transgenic NOD and 8.3-TCRβ-transgenic NOR mice were amplified by anchor PCR, cloned, and sequenced, as previously described (27).

Cytokine RT-PCR

Total cellular RNA from islet-derived, CD4⁺ T cell-depleted T cell lines was reverse transcribed using oligo(dT)₁₂–₁₈ (Life Technologies) as a primer, and the resulting cDNAs were amplified by PCR (27).

In vitro and in vivo cytokine secretion

Spleenic CD4⁺ T cells (2 x 10⁶/well) were incubated with γ-irradiated NOD islet cells (10⁵/well) and splenocytes (10⁵/well) in 96-well plates for 48 h at 37°C in 5% CO₂. The supernatants (100 μl/well) were assayed for IL-2, IL-4, IFN-γ, and/or TGF-β content by ELISA using commercially available kits (Genzyme Diagnostics, Cambridge, MA). Determination of intraislet cytokine content was done as described in Cameron et al. (29), with modifications. Briefly, purified islets from nondenibetic mice were homogenized in PBS containing protease inhibitors (PMSF (1 mM), leupeptin (40 μM), pepstatin A (10 μM), sodium azide (0.2%), and EDTA (100 mM)), and centrifuged at 10,000 rpm to remove debris. The supernatants were adjusted at 200 μg of total protein/ml, and 100 μl of each sample was used in duplicate to measure the cytokine content by ELISA.

Histopathology and immunohistochemistry

Pancreata (one-half) were fixed in formalin, embedded in paraffin, sectioned at 4.5 μm, stained with hematoxylin and eosin, and examined for inflammation. The degree of insulitis was evaluated by scoring 15–30 islets/mouse using previously described criteria (24). The second half of each pancreas was snap frozen in liquid nitrogen, immersed in OCT, sectioned at 6–7 μm, fixed in cold acetone for 10 min, incubated with hydrogen peroxide to block endogenous peroxidase activity, and stained with anti-CD4 (GK1.5) and anti-CD8 (53-6.7) mAbs, followed by biotinylated anti-rat Ig Abs and horseradish peroxidase (HRP)-streptavidin conjugate (Dimension Laboratories, Mississauga, ON, Canada). The slides were developed with diaminobenzidine (DAB, Sigma).

To determine the percentages of granzyme B⁺ cells in islets, mice were euthanized and perfused with PBS and 4% paraformaldehyde in PBS. The pancreas of each mouse was then fixed overnight in 4% paraformaldehyde at 4°C, immersed in 20% sucrose overnight at 4°C, snap frozen in liquid nitrogen, and processed for immunopathology, as described above. Tissue sections were incubated with goat polyclonal anti-granzyme B IgG, biotinylated swine anti-goat IgG, and HRP-streptavidin; developed with DAB; and counterstained with hematoxylin.

In vivo bromodeoxyuridine (BrdU) labeling

Mice were given two i.v. injections of 200 μl of a 4 mg/ml solution of 5-bromo-2'-deoxyuridine (BrdU; Calbiochem, La Jolla, CA) 4 h apart. The pancreas and spleen of each mouse were collected 12 h later. Quantitation of the percentage of islet-infiltrating cells and splenocytes incorporating BrdU was done on frozen tissue using a BrdU staining kit (Calbiochem).

Statistical analyses

Statistical analyses were performed using Mann-Whitney U and χ² tests and by simple regression.

Results

IDDM resistance in 8.3-(NOD × NOR)F₁ and 8.3-NOR mice

We have shown that 8.3-CD8⁺ T cells are highly diabeticogenic in 8.3-NOD mice (24). Since these cells require the assistance of endogenous CD4⁺ T cells to accelerate diabetes (24), we reasoned that studies with the 8.3-TCR would be the most informative. To test the hypothesis that non-MHC-linked genes control the diabeticogenic activity of 8.3-CD8⁺ T cells, we first crossed 8.3-NOD mice with NOR/Lt mice to generate 8.3-(NOD × NOR)F₁ (8.3-F₁) mice. NOR/Lt mice are homozygous for the prodiabeticogenic H-²⁵ haplotype and are ~88% genetically identical to NOD/Lt mice, but unlike NOD/Lt mice and the 8.3-NOD mice used for these studies (homozygous for NOD alleles at the three Idd regions that provide diabetes resistance in NOR mice), are homozygous for antiabiabeticogenic alleles at Idd5, Idd9/11, and Idd13 loci. The incidence of diabetes in 8.3-F₁ mice was significantly lower than in 8.3-NOD mice, both in females and in males (p < 0.0002) (Fig. 1). These differences in diabetes incidence between 8.3-NOD and 8.3-F₁ mice were not due to genetic heterogeneity in the 8.3-NOD mouse population used as donor of the transgenes for this study, since the incidence of diabetes in the 8.3-TCR-transgenic littermates of the nontransgenic donors was similar to the current incidence of diabetes in our 8.3-NOD colony (78% vs 71% in females, and 60% vs 52% in males, respectively). As expected, none of the 20 female nontransgenic F₁ littermates that were followed developed diabetes (data not shown). We then backcrossed the 8.3-TCR of a diabetic 8.3-F₁ male mouse onto the NOR/Lt background for up to seven generations, to generate 8.3-NOR mice. Cohorts of mice from each backcross were followed for diabetes development. As expected, female 8.3-NOR mice of the N5-N7 backcrosses (homozygous for NOR alleles at Idd5, Idd9/11, and Idd13) also developed diabetes less frequently than female 8.3-NOD mice (p < 0.0002) (Fig. 1).

Surprisingly, however, 8.3-NOR males of the N5-N7 backcrosses displayed an increased incidence of diabetes when compared with 8.3-F₁ (but not 8.3-NOD) mice (p < 0.02) (Fig. 1). In both female and male 8.3-NOR mice (N5-N7 generations), there was a decrease in the average age at onset of the disease when compared with 8.3-NOD and/or 8.3-F₁ mice (37 ± 22 days vs 43 ± 26 days and 68 ± 31 days for females, p < 0.04; 30 ± 11 days vs 83 ± 35 and 41 ± 15 days for males, p < 0.0001). No obvious differences in disease incidence or age at diabetes onset were noted between N2, N3, or N4 mice and N5-N7 mice. In males, for example, the incidence and age at onset of diabetes for each generation were: N2, 2/4 mice at 39 ± 6 days; N3, 3/11 mice at 34 ± 6 days; N4, 2/5 mice at 35 ± 4 days. It therefore appears that NOR/Lt mice bear one or more chromosomal regions that, either

![FIGURE 1](http://www.jimmunol.org/)
alone or in combination, afford dominant protection from 8.3-CD8⁺ T cell-induced diabetes in 8.3-F₁ mice. The fact that this dominant protective effect is lost in male 8.3-NOR mice suggests that NOR/Lt mice also bear recessive prodiabetogenic genes that in males, but not females, can override the antidiabetogenic function of these dominant protective elements.

**Reduction in the peripheral frequency of 8.3-CD8⁺ T cells in 8.3-NOR mice, but not in 8.3-F₁ mice**

Three-color cytofluorometric studies of thymocytes revealed that the resistance of 8.3-F₁ and 8.3-NOR mice to 8.3-CD8⁺ T cell-induced diabetes was not a result of deletion or abnormal maturation of 8.3-CD8⁺ thymocytes. The thymi of these mice contained similar numbers of thymocytes and similar percentages of the four major thymocyte subsets as the thymi of 8.3-NOD mice (Fig. 2A). Likewise, no differences were found between the individual thymocyte subsets of all of these mice with respect to cell surface levels of the transgenic TCR (Fig. 2A) and several differentiation markers, including CD5, MHC class I (K<sup>d</sup>), CD24, CD44, and CD69 (data not shown).

The cytofluorometric profiles of the spleens of 8.3-F₁ mice were also remarkably similar to those of 8.3-NOD mice (Fig. 2B); however, the spleens (and, to a lesser extent, the lymph nodes) of 8.3-NOR mice contained significantly fewer CD8⁺ T cells than the spleens (or lymph nodes) of 8.3-NOD mice (Fig. 2B, and data not shown) (p < 0.0005). This reduction in the percentage of splenic CD8⁺ T cells in 8.3-NOR mice (defined as a splenic CD4⁺:CD8⁺ T cell ratio greater than the average plus 2 SDs of the 8.3-NOD values) segregated as a recessive trait: it occurred in none of 6 F₁ mice, in 4 of 10 N2 mice, and in 11 of 13 N4-N5 mice (8.3-F₁ vs 8.3-N2, p < 0.009; 8.3-N2 vs 8.3-N4/N5, p < 0.0001). This phenotype was not due to CD8 coreceptor down-regulation by 8.3-CD8⁺ T cells, since virtually all of the CD4⁺CD8⁺ splenic cells of these mice were Vβ8.1⁻ (data not shown), and could not be accounted for by disproportionate expansion of 8.3-CD8⁺ T cells in the spleens of 8.3-NOD vs 8.3-NOR mice, since the splenic CD8⁺ T cells of 8.3-NOD (and 8.3-NOR) mice did not express activation markers (24) and data not shown). Furthermore, the splenic periarteriolar lymphoid sheaths of 8.3-NOD mice contained very few actively proliferating cells, as determined by immunopathologic analyses of BrdU-injected mice, and the number of BrdU-incorporating cells in the spleens of 8.3-NOD and 8.3-NOR mice was similar (data not shown). Interestingly, this reduction in the percentage of splenic CD8⁺ T cells was accompanied by a reduction in the overall ability of the splenic CD8⁺ T cells of 8.3-NOR vs 8.3-NOD mice to proliferate in response to islet stimulation (Fig. 3A). Subsequent limiting dilution (Fig. 3B) and proliferation assays (Fig. 3C) using islet Ag or a plate-bound anti-Vβ8.1 mAb as stimuli, respectively, demonstrated that the lower proliferative responsiveness of 8.3-NOR-derived CD8⁺ T cells to islet stimulation was not due to anergy, but rather to a reduction in the peripheral frequency of β cell-reactive CD8⁺ T cells (~1/70–90 vs 1/17–30 CD8⁺ T cells in 8.3-NOR and 8.3-NOD mice, respectively). Paradoxically, however, 8.3-NOR mice developed an accelerated onset (but not an increased incidence) of diabetes. This suggested that this reduction in the peripheral frequency of β
cell-reactive CD8\(^+\) T cells in 8.3-NOR mice might increase with age, and that it would have to reach a certain threshold to be able to afford diabetes protection. Time course studies confirmed that deletion of 8.3-CD8\(^+\) T cells in 8.3-NOR mice was indeed an age-dependent phenomenon: contrary to what happened in 8.3-NOD mice, in which the percentage of splenic CD8\(^+\) T cells increased with age (\(r = 0.682, p < 0.0001\)), the percentage of splenic CD8\(^+\) T cells in 8.3-NOR mice did not increase as the mice grew older (\(r = 0.266\)). Studies with nontransgenic and TCR\(\beta\)-transgenic NOR mice confirmed that the deleting phenotype of 8.3-NOR mice was not mediated by an endogenous superantigen binding to the 8.3-TCR\(\alpha\)-chain; the percentages of V\(\beta\)8.1-2 splenic T cells in NOD (\(n = 3\)), NOR (\(n = 4\)), 8.3-TCR\(\beta\)-transgenic NOD (N8; \(n = 6\)), and 8.3-TCR\(\beta\)-transgenic NOR (N9; \(n = 2\)) mice. Endogenous VJ\(\alpha\) repertoire of islet-stimulated CD8\(^+\) T cells from 8.3-TCR\(\beta\)-transgenic mice. CD4\(^+\) T cell-depleted splenocytes from 8.3-TCR\(\beta\)-transgenic NOD (\(n = 3\)) and 8.3-TCR\(\beta\)-transgenic NOR mice (\(n = 4\)) were stimulated with irradiated NOD islets for 3 to 4 days, expanded with rIL-2, and restimulated twice with NOD islets and rIL-2. The TCR\(\alpha\) cDNAs of each line were amplified by anchor PCR, cloned, and sequenced. N is the ratio between each cDNA’s copy number/number of cDNAs sequenced. N-terminal residues homologous to those of the 8.3-TCR\(\alpha\)-chain are underlined.
focused on investigating this phenotype further. The dominant diabetes resistance observed in 8.3-F$_2$ mice, which does not involve T cell deletion, will be addressed further below.

**CD8$^+$ T cells expressing endogenously derived 8.3-TCR$\alpha$-chains are undetectable in 8.3-TCR$\beta$-transgenic NOR mice**

To confirm that the reduction in the peripheral frequency of 8.3-CD8$^+$ T cells in 8.3-NOR mice was not an artifact of the high peripheral frequency of clonotypic 8.3-CD8$^+$ T cells, we investigated whether this phenotype also occurred in single-chain 8.3-TCR$\beta$-transgenic NOR mice. We chose to use these mice to address this issue for several reasons: 1) unlike 8.3-TCR$\beta$-transgenic NOD mice, 8.3-TCR$\beta$-transgenic NOR mice do not develop diabetes (0% incidence, unpublished observations); 2) when compared with 8.3-NOD mice, 8.3-TCR$\beta$-transgenic NOD mice only have a minor increase in the peripheral frequency of $\beta$ cell-reactive CD8$^+$ CTL (27); and 3) the splenic CD8$^+$ T cells of 8.3-TCR$\beta$-transgenic NOD mice display a highly heterogeneous TCR$\alpha$ repertoire, yet most of the islet-associated CD8$^+$ T cells in acutely diabetic animals bear endogenously derived TCR$\alpha$-chains identical to the 8.3-TCR$\alpha$-chain, indicating that expression of the 8.3-TCR$\beta$ transgene fosters the maturation of some 8.3-CD8$^+$ T cells (27). To investigate whether 8.3-TCR$\beta$-transgenic NOR mice also have a reduction in the peripheral frequency of 8.3-CD8$^+$ T cells when compared with 8.3-TCR$\beta$-transgenic NOD mice, we compared the endogenous TCR$\alpha$ repertoire of $\beta$ cell-reactive CD8$^+$ T cells in 8.3-TCR$\beta$-transgenic NOR and 8.3-TCR$\beta$-transgenic NOD mice. To do this, we stimulated splenic CD8$^+$ T cells from several 8.3-TCR$\beta$-transgenic NOD and 8.3-TCR$\beta$-transgenic NOR mice with NOD islets in the presence of rIL-2 and sequenced multiple anchor PCR-generated TCR$\alpha$ cDNAs from each line. As shown in Fig. 3E, the 8.3-TCR$\alpha$ sequence was the predominant TCR$\alpha$ sequence within each of the three different 8.3-TCR$\beta$-transgenic NOD lines studied (46/60 cDNAs). In contrast, while some of the 49 TCR$\alpha$ cDNAs derived from four 8.3-TCR$\beta$-transgenic NOR lines encoded homologous junctional (CDR3) amino acid sequences, none of them encoded the 8.3-TCR$\alpha$-chain or the 8.3-TCR$\alpha$-CDR3 region sequence. These results therefore demonstrated that the reduction in the peripheral frequency of 8.3-TCR$\beta$-bearing CD8$^+$ T cells in 8.3-NOR mice was not an artifact of transgenesis. Furthermore, these data suggested that the putative CD8$^+$ T cell-deleting element(s) of NOR mice preferentially target(s) $\beta$ cell-reactive V$\beta$8.1$^+$CD8$^+$ T cells bearing the highly diabetogenic 8.3-TCR$\alpha$-chain, as opposed to all $\beta$ cell-reactive CD8$^+$ T cells regardless of TCR$\alpha$ usage.

**Diabetes resistance in 8.3-NOR mice is not associated with insulitis resistance or immune deviation**

To investigate whether the diabetes resistance of 8.3-NOR mice was associated with resistance to insulitis and/or local immune deviation, we determined whether nondiabetic 8.3-NOR mice (at least 1 SD older than the average age at onset of diabetes in these mice; <2.5% chances of ever becoming diabetic) developed insulitis, and whether the insulitis lesions of these mice were quantitatively and/or qualitatively similar to those of nondiabetic 8.3-NOD mice. Histopathologic and RT-PCR studies revealed that 8.3-NOR mice had severe insulitis (Fig. 4A), that the insulitis lesions of these mice had CD4$^+$:CD8$^+$ T cell ratios similar to those...
Cytofluorometric studies of 8.3-F1 mice indicated that their diabetes resistance or ignorance

seen in 8.3-NOD mice (Fig. 4B), and that the CD8+ T cells derived from these lesions displayed cytokine profiles similar to those derived from the insulitis lesions of 8.3-NOD mice (Fig. 4C). These cytokine profiles were confirmed by measuring the intraislet content of IL-2, IL-4, and IFN-γ in 8.3-NOD and 8.3-NOR mice: the insulitis lesions of both types of mice contained IFN-γ (120–1200 pg/mg), but undetectable levels of IL-4 and IL-2 (data not shown) (note that IL-2 was barely detectable by RT-PCR). These data demonstrated that the CD8+ T cells do not undergo immune deviation in 8.3-NOR mice, and that they accumulate in the islets of these mice quite efficiently.

Diabetes resistance in 8.3-F1 mice in the absence of T cell tolerance or ignorance

Cytofluorometric studies of 8.3-F1 mice indicated that their diabetes resistance could not be accounted for by deletion of 8.3-CD8+ T cells (Figs. 1 and 2). Our next set of experiments focused on attempting to elucidate the mechanism(s) underlying the genetically dominant diabetes resistance of these mice. Since the pancreatic islets of these mice were severely infiltrated and contained abundant CD8+ T cells (data not shown), we considered three possible mechanisms of diabetes resistance in these mice: 1) 8.3-CD8+ T cells undergo anergy in situ; 2) 8.3-CD8+ T cells fail to differentiate into β cell cytotoxic T cells (CTLs); and/or 3) the local 8.3-CD8+ CTLs are unable to kill NOR β cells because these cells do not express the target autoantigen, or are resistant to CTL-induced apoptosis. These questions were investigated by studying nondiabetic mice at ages beyond which their chances of developing diabetes were <5% (>10 wk).

To determine whether 8.3-CD8+ T cells of 8.3-F1 mice undergo anergy in situ, we compared the percentage of proliferating cells (incorporating exogenous BrdU) in islets of nondiabetic 8.3-F1 and 8.3-NOD mice. As shown in Fig. 5A, no significant differences were noted between these mice at any of three different age points. To determine whether these cells differentiated into CTLs in situ, we compared the percentages of insulitic cells from nondiabetic 8.3-F1 and 8.3-NOD mice that expressed granzyme B (a marker of cytoplasmic granule content), as detected by immunohistochemistry. As shown in Fig. 5B, no differences were observed in these studies. The ability of 8.3-NOR CD8+ T cells to differentiate into CTL in situ was confirmed by comparing the percentages of CD8+ T cell clones containing serine esterase activity that could be isolated from islets of these two types of mice. As shown in Fig. 5C, islet-derived CD8+ T cell clones from 8.3-F1 mice contained as many serine esterase+ clones as lines derived from 8.3-NOD mice. Differentiation of these islet-derived clones into CTL was not an artifact of in vitro stimulation, because none of 41 clones derived from splenic CD8+ T cells of 8.3-NOD mice using the same stimulation protocol expressed serine esterase activity (Fig. 5C). Subsequent cytotoxicity assays using serine esterase+ CD8+ CTL clones from 8.3-F1 and 8.3-NOD mice demonstrated that the diabetes resistance of 8.3-F1 mice could not be accounted for by an

![FIGURE 5](http://www.jimmunol.org/)}
FIGURE 6. Diabetogenesis and T cell development in 4.1-NOD, 4.1-NOR, and 4.1-F1 mice. A, Incidence of IDDM in female (4.1-NOD \( n = 57 \)), 4.1-F1 \( (n = 23) \), and 4.1-NOR \( (n = 26) \) and male (4.1-NOD \( n = 48 \), 4.1-F1 \( (n = 13) \), and 4.1-NOR \( (n = 20) \) mice. Since the incidence and age at onset of diabetes in 4.1-NOR N5-N7 mice were similar to those seen in N2-N4 mice (see text), the figure shows values for N2-N7 mice. The average age at onset of diabetes in 4.1-F1, 4.1-NOR, and 4.1-NOD females was: 60 ± 26 vs 76 ± 49 vs 47 ± 21 days. B, Flow-cytometry profiles of thymocytes and splenocytes from 4.1-NOD \( (n = 14; \text{age } 61 ± 25 \text{ days}) \) and 4.1-NOR mice \( (n = 6; \text{age } 62 ± 15 \text{ days}) \). Similar results were obtained with older mice (data not shown). C, Proliferative activity of splenic CD4\(^+\) T cells from 4.1-NOD and 4.1-NOR mice. CD4\(^+\) T cells \( (2 \times 10^5/well) \) were incubated, in triplicate, with \( 10^5 \) irradiated NOD islet cells and \( 10^5 \) irradiated NOD splenocytes for 3 days, pulsed with \(^{3}H\)thymidine, harvested, and counted. D, Peripheral frequency of \( \beta \) cell-reactive CD4\(^+\) T cells in nondiabetic NOD, 4.1-NOD, and 4.1-NOR mice, as determined by limiting dilution assay. The figure shows values for two of the four dilutions tested. E, Insulitis scores of nondiabetic 4.1-NOD and 4.1-NOR mice \( (n = 3–6 \text{ mice/group; } 7–12 \text{ wk old}) (15–30 \text{ islets/mouse}) \). Bars show the SD of the means. All mice had severe insulitis. F, Phenotype of insulitis T cells in 4.1-NOD and 4.1-NOR mice, as determined by immunopathology.
inability of 8.3-F1-derived 8.3-CD8+ T cells to kill β cells, or by an inherent resistance of NOR β cells to perforin-based cytotoxicity (Fig. 5D); NOR β cells were at least as, if not more, susceptible to CTL-induced lysis as NOD β cells.

Since only a small percentage of the insulitic T cells of 8.3-F1 and 8.3-NOD mice contained cytotoxic granules, we reasoned that the diabetes resistance of 8.3-F1 mice might result from the relative inability of the insulin CD8+ T cells of 8.3-F1 vs 8.3-NOD mouse to effect Fas-dependent cytotoxicity and/or from resistance of NOR β cells to Fas-induced apoptosis. To investigate this, we first determined whether 8.3-CD8+ T cells within islet cell suspensions from both types of mice could kill Fas+ targets. As shown in Fig. 5E, cells from both types of mice killed Fas+ (but not Fas−) fibroblasts with similar efficiency. We then tested the ability of 4–5-day-old serine esterase+ islet-derived T cell lines from 8.3-NOD mice to kill L1210-Fas- and L1210-Fas+ fibroblasts, and NOD, NOR, and NOD.lpr (Fas+) β cells, both in the absence and presence of cytokines that potentiate Fas-mediated cytotoxicity (28). As shown in Fig. 5F, these lines efficiently killed L1210-Fas+ cells, but not L1210-Fas− fibroblasts or Fas− NOD β cells; thus, 8.3-F1 CD8+ CTLs can kill Fas-expressing targets, and NOR and NOD β cells are equally susceptible to Fas-mediated cytotoxicity.

The results of these experiments suggested that the genetically dominant resistance of (NOD × NOR)F1 mice to diabetes was mediated by a novel mechanism. This prompted us to ask one fundamental question: does this mechanism selectively target 8.3-CD8+ T cells, or does it also target other highly diabetogenic T cells regardless of phenotype or antigenic specificity? To address this question, we followed the fate of another highly pathogenic, but I-β7-restricted, β cell-specific TCR (4.1-TCR) (23) in (NOD × NOR)F1 (4.1-F1) and NOD (4.1-NOR) mice derived from a diabetic 4.1-NOD mouse. Interestingly, both 4.1-F1 and 4.1-NOR mice (N2-N7 generations) displayed much lower incidences of diabetes than 4.1-NOD mice (pg < 0.0001 for both females and males) (Fig. 6A). No obvious differences in disease incidence were noted between 4.1-TCR-transgenic mice of the N2-N4 and N5-N7 backcrosses (the latter typed as homozygous for NOR alleles at Idd5, Idd9/11, and Idd13 loci) (females, 4/13 and 2/11, respectively; males, 1/10 and 0/7, respectively). The 4.1-F1 and 4.1-NOR mice that developed diabetes did so slightly later than 4.1-NOD mice (i.e., 60 ± 26 vs 76 ± 49 vs 47 ± 21 days, respectively). As expected, the incidence of diabetes in the nontransgenic F1 littermates that were followed was 0% at 32 wk (data not shown). Thus, like 8.3-F1 mice, 4.1-F1 mice also display dominant resistance to diabetes.

**Table I.** Cytokine profile of peripheral 4.1-CD4+ T cells in 4.1-NOD, 4.1-(NOD × NOR)F1, and 4.1-NOR mice

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Expt.</th>
<th>IL-2 (pg/ml)</th>
<th>IL-4 (pg/ml)</th>
<th>IFN-γ (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1-NOD</td>
<td>1</td>
<td>75</td>
<td>&lt;10</td>
<td>285</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>50</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>57</td>
<td>17</td>
<td>69</td>
</tr>
<tr>
<td>4.1-(NOD × NOR)F1</td>
<td>1</td>
<td>55</td>
<td>&lt;5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>70</td>
<td>&lt;5</td>
<td>73</td>
</tr>
<tr>
<td>4.1-NOR</td>
<td>1</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>NOD</td>
<td>1</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>

* Splenocytes from nondiabetic mice (>11 wk old) were depleted of CD8+ T cells using anti-CD8 mAb (53-6.7)-coated magnetic beads, adjusted to 2 × 10^6 cells/100 μl and cultured for 48 h in the presence of 1 × 10^5 NOD islet cells. The cytokine contents of the culture supernatants were determined in duplicate by ELISA.

**Table II.** Intra-islet content of cytokines in 4.1-NOD, 4.1-(NOD × NOR)F1, and 4.1-NOR mice

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Expt.</th>
<th>IL-2 (pg of cytokines/mg of protein)</th>
<th>IL-4 (pg of cytokines/mg of protein)</th>
<th>IFN-γ (pg of cytokines/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1-NOD</td>
<td>1</td>
<td>50</td>
<td>125</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>75</td>
<td>262</td>
</tr>
<tr>
<td>4.1-(NOD × NOR)F1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>4.1-NOR</td>
<td>1</td>
<td>25</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>NOD</td>
<td>1</td>
<td>0</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

* Purified islets from nondiabetic mice (>11 wk old) were homogenized in PBS containing protease inhibitors and centrifuged at 10,000 rpm to remove debris. The supernatants were adjusted at 200 μg of protein/ml, and 100 μl of each sample was used in duplicate to measure the cytokine content by ELISA.
progression in prediabetic NOD mice and disease recurrence in islet-grafted NOD mice (31), and diabetes does not develop in IL-4-treated mice (32), it was also possible that the genetic resistance of 4.1-F1 or 4.1-NOR mice was due to recruitment of IL-4-secreting cells to islets. To address these possibilities, we quantitated the IL-2, IL-4, and IFN-γ content of islet extracts from nondiabetic mice of all three mouse strains by ELISA. These experiments revealed that while the islets of nondiabetic 4.1-NOD mice contained significant levels of both IL-4 and IFN-γ, the islets of nondiabetic 4.1-F1 and 4.1-NOR mice (>10 wk of age; <4% chances of developing diabetes) contained IL-2 and/or IFN-γ, but undetectable levels of IL-4 (Table II). Islets from 4.1-F1 and 4.1-NOR mice contained lower levels of IFN-γ than islets from 4.1-NOD mice, but the significance of these differences is unclear (Table II). Next, since TGF-β1 has been shown to have suppressive effects on the action of Th1 and Tc1 cells in vitro and in vivo, and can suppress the development of experimental autoimmunity in mice (33–35), it was also important that we determined whether the diabetes resistance of 4.1-F1 mice was associated with recruitment of TGF-β1-producing cells to islets. The levels of TGF-β1 in islets of seven different 4.1-F1 and 4.1-NOR mice, however, were barely detectable (1.8±2 pg/mg) (data not shown). The mechanism that prevents diabetogenesis in F1 (and NOR) mice does not therefore promote local immune deviation and does not involve the recruitment of IL-4- or TGF-β1-producing cells to islets.

Spontaneous diabetes in monoclonal 8.3-F1 and 4.1-F1 mice

The remarkable similarity between the results of studies with 4.1-F1 and 8.3-F1 mice prompted us to investigate whether the diabetes resistance of these mice was due to a form of immune suppression involving suppressor lymphocytes other than Th2 (IL-4-producing) or Th3 (TGF-β1-producing) cells. Since the diabetogenic potential of 4.1-CD4+ and 8.3-CD8+ T cells in NOD mice is not negatively affected by the presence of endogenous (nontransgenic) lymphocytes able to compete with the transgenic T cells for Ag recognition (24), this possibility could be tested by comparing the natural history of diabetes in RAG-2−/− 8.3-F1, RAG-2−/− 8.3-NOD, RAG-2−/− 4.1-F1, and RAG-2−/− 4.1-NOR mice; these mice cannot rearrange Ig or endogenous TCR genes and thus express monoclonal TCR repertoires (24). Interestingly, RAG-2−/− 8.3-F1 and RAG-2−/− 4.1-F1 mice developed diabetes almost as frequently, and as early as RAG-2−/− 8.3-NOD and RAG-2−/− 4.1-NOD mice, respectively (Fig. 7). As expected, the incidence of diabetes in RAG-2−/− 4.1-F1 littermates was much lower: only 2 of 11 RAG-2−/− 4.1-F1 females (18%) and none of 8 RAG-2−/− 4.1-F1 males (0%) developed diabetes (p<0.015 vs RAG-2−/− 4.1-NOR females, and p<0.004 vs RAG-2−/− 4.1-NOD males) (Fig. 7). The same trend was noted in RAG-2−/− 8.3-F1; only 1 of 3 females, 33%, and none of 4 males, 0%, developed diabetes, incidences comparable with those seen in RAG-2−/− 8.3-F1 mice (Fig. 1). It should be pointed out that direct comparison of the incidences of diabetes in RAG-2−/− and RAG-2−/− 8.3-F1 mice is inappropriate in the context of this study, since the diabetogenic potential of 8.3-CD8+ T cells is dramatically reduced in the absence of CD4+ T cells bearing endogenous TCRs (24). Taken together, these findings demonstrated that the diabetes resistance of 8.3-F1 and 4.1-F1 mice is, at least in part, the result of a form of lymphocyte-mediated suppression not involving IL-4 or TGF-β1 that targets diabetogenic CD8+ and CD4+ T cells regardless of their fine antigenic specificity and MHC restriction.

Discussion

Genetic susceptibility and resistance to most spontaneous autoimmune disorders, including IDDM, are associated with highly polymorphic genes of the MHC and with multiple non-MHC-linked genes via unknown mechanisms. In this study, we have followed the fate of two highly diabetogenic TCRs in NOR and (NOD×NOR)f1 mice, which are genetically resistant to spontaneous IDDM despite being H-2k+ homozygous (25, 26). These studies have revealed the existence of at least two simultaneous, but independent, mechanisms of non-MHC-linked genetic control of diabetogenic T cells. One segregates as a recessive trait and is associated with a marked reduction in the peripheral frequency of diabetogenic CD8+ T cells. The second mechanism segregates as a dominant trait and is mediated by immunoregulatory functions provided by mature lymphocytes that interfere with the diabetogenicity of β cell-reactive CD4+ and CD8+ T cells, without causing their deletion, anergy, immune deviation, or ignorance, and without involving the recruitment of protective IL-4- or TGF-β1-producing cells to the pancreas. Although the existence of peripheral deletion and immune regulation as mechanisms of immune homeostasis is well established, our findings provide evidence for an association between a breakdown in these two specific forms of tolerance and the non-MHC-linked control of autoimmunity. They also demonstrate that protective non-MHC-linked genes may selectively target specific T cell types, rather than all autoreactive T cells, in cellularly complex autoimmune disorders.

The reduction in the peripheral frequency of 8.3-CD8+ T cells that was observed in 8.3-NOR, but not 8.3-F1, mice increased with age and was not mediated by endogenous superantigens binding to the transgenic TCRβ-chain. Studies of 8.3-TCRβ-transgenic NOR mice revealed that this phenotype was not an artifact of the high peripheral frequency of 8.3-CD8+ T cells in 8.3-TCR-transgenic mice when compared with nontransgenic animals, and that it preferentially targeted CD8+ T cells expressing TCRα-chains with CDR3 sequences identical to those of the CTL clone donating the 8.3-TCRβ transgene, rather than all autoreactive CD8+ T cells regardless of TCR usage. While the underlying mechanisms remain unclear, we suspect that this reduction in the peripheral frequency of 8.3-CD8+ T cells in 8.3-NOR mice is caused by deletion of 8.3-CD8+ T cells in the periphery. Since initiation of diabetogenesis in nontransgenic NOD mice requires CD8+ T cells...
(36–38), and the 8.3-TCR uses a TCRα-CDR3 sequence that is highly homologous to TCRα-CDR3 sequences used by many NOD islet-derived β cell-cytotoxic CD8+ T cells (27, 39), it is possible that some of the diabetes resistance of nontransgenic NOR mice results from their ability to delete the most pathogenic of all autoreactive CD8+ T cells, rather than all autoreactive CD8+ T cells regardless of their pathogenicity. The incomplete penetrance of 8.3-CD8+ T cell deletion and diabetes resistance in 8.3-NOR mice does not argue against this view; the high frequency of 8.3-CD8+ T cells in 8.3-NOR mice may overwhelm the mice’s deleting machinery, particularly since NOR mice also appear to bear recessive prodiabetic genes. The complete absence of 8.3-TCRα sequences in TCRα cDNA libraries generated from islet-reactive T cell lines of 8.3-TCRβ-transgenic NOR mice, which have a much lower frequency of β cell-reactive CD8+ T cells and do not develop diabetes, supports this interpretation of the data. Whatever the relative role of this phenomenon in the genetic resistance of NOR/Lt mice to spontaneous IDDM, these results demonstrate the existence of non-MHC-linked genetic elements other than endogenous superantigens that can control the fate of pathogenic autoreactive CD8+ T cells in the periphery. Importantly, these elements target diabetogenic CD8+ T cells while sparing diabetogenic CD4+ T cells.

While we do not yet know the nature of these genetic elements, interpretation of our findings vis-à-vis the results of previous genetic studies provides some clues. It has been shown that IDDM resistance in (NOD × NOR)F2, mice segregates with Idd5, Idd9, and Idd13 (26). Although Idd5', linked to cto4 and cld28 in chromosome 1, is associated with increased susceptibility of T cells to cyclophosphamide-induced apoptosis in vivo (40), two lines of evidence suggest that this reduction in the peripheral frequency of 8.3-CD8+ T cells in 8.3-NOR mice and the Idd5'-encoded susceptibility to cyclophosphamide-induced apoptosis are unrelated phenomena: 1) the putative gene within the Idd5' region of NOR mice that controls apoptosis susceptibility/resistance is of NOD origin (40); and 2) 8.3-CD8+ T cells from 8.3-NOR and 8.3-NOD mice show equal susceptibility to reactivation-induced apoptosis in response to several different stimuli, including islet cells and anti-TCR or anti-Fas mAbs (our unpublished observations). Although a contribution of Idd5 to the “deleting” phenotype of 8.3-NOR mice cannot be ruled out, the fact that deletion of diabetogenic T cells in NOR mice selectively targets MHC class I-restricted CD8+ T cells, but not MHC class II-restricted CD4+ T cells, makes Idd13 a more attractive candidate: Idd13 contains the dimorphic β2m (β2-microglobulin) locus; NOD and NOR mice express different β2m isofoms; and these isoforms account for conformational differences between the otherwise identical Kd and Dd MHC class I molecules of these mice (41–43). Since the Idd13'-controlled diabetes resistance of NOR mice resides in a radioresistant nonhemopoietic cell type, perhaps the β cell itself (44), and since NOR β cells display a somewhat greater susceptibility to 8.3-CTL-induced lysis than NOD β cells (see Fig. 5), it would be reasonable to speculate that the reduction in the peripheral frequency of 8.3-CD8+ T cells in 8.3-NOR mice results from engagement of target Kd/β2m+ complexes on β cells or APCs with an affinity/ avidity that surpasses a deleting threshold. This view is compatible with two observations: 1) “deletion” segregates as a recessive trait (i.e., two copies of the β2m molecule would increase the avidity of the TCR-MHC class I interaction and hence the chances of reaching the deleting threshold); and 2) in 8.3-TCRβ-transgenic NOR mice, “deletion” does not systematically target all β cell-reactive CD8+ T cells, but rather only those bearing the pathogenic 8.3-TCRα-CDR3 sequence. This interpretation would also provide an explanation for the paradoxical acceleration of IDDM in the few 8.3-NOR mice that developed IDDM: in young 8.3-NOR mice, in which the reduction in the peripheral frequency of 8.3-CD8+ T cells is minimal, such an increased affinity would actually promote IDDM. The reasons behind the increased incidence (and accelerated onset) of diabetes in 8.3-NOR vs 8.3-F1 male mice might also be due to this. However, we favor the alternative possibility that NOR/Lt mice also bear recessive prodiabetic genes that in males, but not females, can override the antidiabetic function of the dominant protective element(s). Ongoing studies of Idd13'-congenic 8.3-NOD mice should answer some of these questions.

Peripheral deletion of diabetogenic CD8+ T cells, however, is clearly not the only mechanism of diabetes resistance operating in NOR mice; 8.3-F1 male mice are diabetes resistant, but do not delete transgenic 8.3-CD8+ T cells. Experiments with another highly diabetogenic, but I-Aβ2'-restricted, β cell-specific TCR revealed that the diabetes resistance of F1 mice is primarily determined by one (or more) dominant protective element(s) that interfere(s) with the pathogenic activity of diabetogenic CD8+ and CD4+ T cells, regardless of their MHC restriction and antigenic specificity. This genetic element does not function by causing the deletion, anergy, or immune deviation of the transgenic T cells, but instead by promoting a form of immune suppression that is affected by mature B cells or endogenous T cells. The nature of the lymphocyte type and the specific mechanisms through which it prevents 4.1-CD4+ and 8.3-CD8+ -induced β cell death are not yet known, but there are several possibilities. B cells constitute a substantial fraction of islet-infiltrating lymphocytes in our TCR-transgenic mice (24), and thus may be able to mediate this local immunosuppressive effect. Although B lymphocytes have a greater capacity than other APC types to preferentially activate Th2 cytokine responses (45), the absence of local immune deviation in TCR-transgenic NOR mice suggests that this is not the mechanism by which these cells might function. Alternatively, since anti-idiotypic and anti-TCR-V region Abs can dampen T cell-induced autoimmune responses (46, 47), B cells might afford diabetes protection by mounting powerful local anti-idiotypic responses in NOR (but not NOD) mice (i.e., against the diabetogenic TCRs). The fact that B cell-deficient NOD mice are resistant to diabetes (48), however, argues against a role for B cells in this immunosuppressive response, as this would imply that B cells have qualitatively opposed functions in NOD vs NOR mice. Rather, we favor the alternative, but not exclusive, possibility that this protective function is affected by T cells; T cells capable of suppressing autoimmune diabetes have been described, and some of these cells have actually been isolated from NOD mice (49–54). Since disease suppression in TCR-transgenic F1 mice is dissociated from local production of IL-4 and TGF-β1, these suppressor T cells would more likely be related to a recently described type of antidiabetic CD4+ Th1 cells (54) or to anti-idiotypic T cells (55–57), than to Th2, Th3, or IL-4-producing TCR CD4+ CD8+ cells (58). It is noteworthy that this protective mechanism is not very efficient at suppressing CD8+ T cell-induced diabetes in male 8.3-NOR mice, suggesting that NOR/Lt mice also bear recessive genes that potentiate the diabetogenic activity of pathogenic CD8+ T cells in males.

Too little is known about the antidiabetic loci of NOR mice, to speculate as to whether this form of genetically dominant resistance to diabetes is encoded on one of these loci, or on a combination of them, or on other unknown loci. Nonetheless, since the NOR mouse derives some of its genetic material from C57BL/6 and DBA/2 mice (25, 26), it is likely that these elements are also present in other genetic backgrounds. This mechanism may account, in part, for the diabetes resistance of 4.1-(NOD × C57BL/6J-Aβ2-+)/F1 mice, which were completely resistant to spontaneous
IDDM, despite not being able to delete 4.1 thymocytes (23). It would also be reasonable to expect that less powerful forms of some protective elements also exist in mice that are susceptible to autoimmune diabetes. These variants, which would be able to suppress some autoreactive T cells, but not the most pathogenic ones (i.e., 8.3-CD8+ and 4.1-CD4+ T cells), might account for the clustering of multiple autoimmune disorders in affected individuals or their relatives (4, 59). Reductionist approaches such as the one used in this study should help define the mechanisms of action of specific non-MHC-linked chromosomal regions associated with spontaneous autoimmune diabetes.

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References


