β2-Microglobulin–Deficient NOD Mice Do Not Develop Insulitis or Diabetes

LINDA S. WICKER, EDWARD H. LEITER, JOHN A. TODD, ROBERT J. RENJILIAN, ERIN PETERSON, PAUL A. FISCHER, PATRICIA L. PODOLIN, MAARTEN ZIJLSTRA, RUDOLF JAENISCH, AND LAURENCE B. PETERSON

The role of CD8$^+$ T-cells in the development of diabetes in the nonobese diabetic (NOD) mouse remains controversial. Although it is widely agreed that class II–restricted CD4$^+$ T-cells are essential for the development of diabetes in the NOD model, some studies have suggested that CD8$^+$ T-cells are not required for β-cell destruction. To assess the contribution of CD8$^+$ T-cells to diabetes, we have developed a class of NOD mouse that lacks expression of β2-microglobulin (NOD-*B2m*^{null}). NOD-*B2m*^{null} mice, which lack both class I expression and CD8$^+$ T-cells in the periphery, not only failed to develop diabetes but were completely devoid of insulitis. These results demonstrate an essential role for CD8$^+$ T-cells in the initiation of the autoimmune response to β-cells in the NOD mouse. *Diabetes* 43:500–504, 1994

The NOD mouse is a spontaneous model of human autoimmune insulin-dependent diabetes mellitus. The T-cell dependency of the disease in NOD mice has been demonstrated by showing that athymic nude NOD-*nu/nu* mice do not develop insulitis or diabetes and that diabetes can be readily transferred to young irradiated NOD mice with T-cells obtained from diabetic animals (1,2). The roles of autoreactive CD4$^+$ and CD8$^+$ T-cells in the normal pathogenesis of diabetes in the NOD mouse are, however, controversial. On the one hand, cloned islet-reactive NOD CD4$^+$ T-cells have been shown to mediate rejection of islet cell grafts (3,4) and to transfer diabetes to NOD-*scid/scid* mice (5). In contrast, using freshly isolated uncloned T-cells, most groups report that both CD4$^+$ and CD8$^+$ T-cells are necessary to transfer diabetes and that CD4$^+$ T-cells alone transfer only insulitis to recipients (6–8). We (7) and Bendelac et al. (8) proposed an interaction between the two subsets of cells in which CD4$^+$ cells were involved in the homing and activation of CD8$^+$ islet-reactive cells, the physiologically relevant effectors of β-cell destruction. At the time, this scenario was a simple explanation for the requirement of both subsets to efficiently transfer diabetes.

The conflicting results for the role of CD8$^+$ T-cells in autoimmune diabetes can be explained by the existence of at least two pathways producing β-cell destruction: *1*) one in which class I–restricted CD8$^+$ T-cells destroy β-cells by direct contact (9,10) and *2*) one where class II–restricted CD4$^+$ T-cells initiate β-cell destruction by producing cytokines that recruit nonspecific effector cells into the islet (3,4). Although CD8$^+$ T-cells may normally be the most efficient effectors of β-cell destruction, given enough time and/or numbers of cells, CD4$^+$ T-cells are able to mediate disease.

Additional insight into the roles of CD4$^+$ and CD8$^+$ T-cells in diabetes has been obtained with the NOD-*scid/scid* mouse (11). In contrast to adoptive transfer protocols using normal NOD recipients, CD4$^+$ T-cells from diabetic NOD donors were able to transfer diabetes to NOD-*scid/scid* mice, albeit with less efficiency (58% diabetes and a mean onset of 71 days) than unseparated T-cells from diabetic NOD donors (100% incidence with a mean onset of 24 days). However, in the same studies, CD4$^+$ T-cells from prediabetic NOD donors were unable to transfer diabetes into NOD-*scid/scid* recipients, whereas unseparated T-cells from the same prediabetic

From the Departments of Autoimmune Diseases Research (L.S.W., P.L.P.), Cellular and Molecular Pharmacology (R.J.R., E.P., L.B.P.), and Immunology Research (P.A.F.), Merck Research Laboratories, Rahway, New Jersey; The Jackson Laboratory (E.H.L.), Bar Harbor, Maine; Nuffield Department of Surgery (J.A.T.), University of Oxford, John Radcliffe Hospital, Headington, Oxford, United Kingdom; Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology (M.Z., R.J.) Cambridge, Massachusetts.

Address correspondence and reprint requests to Dr. Linda Wicker or Dr. Larry Peterson, Merck Research Laboratories, Mail Code R80W-107, P.O. Box 2000, Rahway, NJ 07065–0900.

Received for publication 1 November 1993 and accepted in revised form 17 November 1993.

Type I diabetes, insulin-dependent diabetes mellitus; *B2m*, β2-microglobulin; PCR, polymerase chain reaction; bp, base pair.

donors induced disease (44% incidence with a mean onset of 85 days). The failure of $CD4^+$ T-cells from prediabetic donors to transfer disease suggests that $CD8^+$ T-cells are required to initiate the autoimmune response as well as play a role in the destruction of β-cells. Data supporting a role for $CD8^+$ T-cells in disease initiation have come from analysis of islet-associated leukocytes by flow cytometry (12). The first cells to appear in the islets of young prediabetic mice were $CD8^+$ T-cells and class II^+ monocytes, whereas only later in disease development did $CD4^+$ T-cells and β-cells appear in the islet infiltrate. Perhaps an interaction between the early infiltrating $CD8^+$ T-cells and the $CD4^+$ T-cells is required to initiate the autoimmune response to β-cells, thus explaining the failure of $CD4^+$ cells from prediabetic donors to transfer disease to NOD-*scid*/*scid* mice.

An experimental approach to understand the role of $CD8^+$ T-cells in the initiation and development of autoimmune diabetes in the NOD mouse is to prevent the expansion of class I–restricted $CD8^+$ T-cells. It has been shown recently that disruption of the β2-microglobulin gene (*B2m*), which encodes the light chain of class I molecules, results in a nearly complete lack of class I expression. By selective backcrossing, we have placed the disrupted *B2m* gene onto the NOD background (NOD-$B2m^{null}$). Class I expression is very low in NOD-$B2m^{null}$ mice and as a result, very few $CD8^+$ T-cells develop. The scarcity of $CD8^+$ cells and/or lack of class I expression results in a strain of NOD mouse that does not develop insulitis or insulin-dependent diabetes mellitus (type I diabetes). These data support the hypothesis that $CD8^+$ T-cells play a crucial role in the development of diabetes in the NOD mouse.

RESEARCH DESIGN AND METHODS

Development of the 129/Sv-*ter* strain with a disrupted *B2m* gene (129-$B2m^{null}$) has been described previously (13–15). NOD/MrkTacfBR (NOD) mice were obtained from Taconic Farms (Germantown, NY). 129-$B2m^{null}$ mice were outcrossed to NOD mice and subsequently backcrossed to NOD. At each backcross generation, DNA was isolated from peripheral blood leukocytes of potential breeders and tested for the presence of the disrupted *B2m* allele using the polymerase chain reaction (PCR). The oligonucleotide primer set 5′-ACGTCT GTCTTCCCCTGTGCCCTCAGAAAC-3′ and 5′-AGTTTA AGTCCAACACAGATGGAGCGTCCAGA-3′ produces a 400–base pair (bp) product with the wild type allele and a 1,600-bp product with the allele containing the *neo*^{r} insert-disrupted second exon of *B2m*. The PCR reaction with peripheral blood DNA was conducted for 40 cycles under the following conditions: a denaturation step at 94°C for 1 min, an annealing step at 60°C for 1 min, and an extension step at 72°C for 3 min. At the N7 generation, NOD-$B2m^{null/+}$ mice were intercrossed to produce homozygous NOD-$B2m^{null}$ mice. After establishment of the NOD-$B2m^{null}$ stock, flanking genetic regions on chromosome 2 were assessed with the following microsatellite markers: *D2Mit45*, *D2Mit43*, *D2Mit58*, *D2Mit17*, and *D2Mit47* (16).

Analysis of cell surface antigens. Detection of cell surface antigens by flow cytometry has been described previously (17). To quantitate expression of the class I K^d antigen, CD8, CD4, and B220 on splenic cells, monoclonal antibodies SF1-1.1, 53-6.7, RM-4-5, and RA3-6B2 were used. All antibodies, which were used under saturating conditions, were directly labeled with fluorescein or phycoerythrin and were purchased from PharMingen (San Diego, CA). Propidium iodide was added to eliminate dead cells from the flow cytometric analysis performed on a FACStar PLUS (Becton Dickinson, Mountain View, CA).

Assessment of diabetes and insulitis. Mice were monitored for the development of diabetes by testing for elevated levels of urinary glucose with Tes-Tape (Lilly, Indianapolis, IN). Animals were classified as diabetic after producing Tes-Tape values of ≥3. Diabetic mice also displayed polydipsia, polyuria, and weight loss. The presence of insulitis and sialitis was assessed in pancreas and submandibular glands, respectively, after fixation in buffered 10% formalin and paraffin sectioning. Tissue sections (5 μm) were stained with either hematoxylin and eosin or with aldehyde fuchsin and examined for the presence of mononuclear cell infiltration. Two noncontiguous sections of each tissue were examined. Classification of each animal was made using the most severe inflammatory lesion observed. Plasma glucose concentrations were determined using a hexokinase enzymatic assay kit with an ultraviolet endpoint (Sigma, St. Louis, MO).

Adoptive transfer of diabetes. Adoptive transfer of diabetes was performed as described previously (7). Single cell suspensions of spleen cells were prepared from recent onset diabetic female NOD mice. After erythrocyte lysis and washing, 40×10^6 cells were injected intravenously into female NOD (6-week-old) and NOD-$B2m^{null}$ (8-week-old) mice that had received 850 rad irradiation (^{137}Cs source, Gammacell 40, Atomic Energy of Canada, Ottawa, Ontario). Animals were monitored frequently for the onset of diabetes by measuring glycosuria.

RESULTS AND DISCUSSION

129-$B2m^{null}$ and 129-$B2m^{null/+}$ mice do not develop diabetes or insulitis. $B2m^{null}$ mice lack β2-microglobulin, resulting in little or no expression of class I molecules on cell surfaces (14,18). Because of this reduced class I expression, mature $CD8^+$ T-cells are not positively selected in the thymus and consequently are not found in the periphery of $B2m^{null}$ mice (14,18). It has been suggested by Faustman et al. (19) that reduced class I expression causes autoimmune diabetes. To address this hypothesis, we examined 18- to 19-month-old parental 129-$B2m^{null}$ male and female mice for hyperglycemia and pancreatic insulitis (Table 1). None of the 129-$B2m^{null}$ mice of either sex or their class I positive heterozygous littermates (129-$B2m^{null/+}$) developed hyperglycemia. Further, no histological changes were observed that were unique to the $B2m^{null}$ class. Instead, a variety of morphological alterations were noted in both

TABLE 1
Analysis of 129-*B2m*^{null} and 129-*B2m*$^{null/+}$ mice

Sex	*B2m* genotype	Insulitis	Plasma glucose (mg/dl)
F	null/null	0 of 3	104 ± 32
M	null/null	0 of 3	89 ± 12
F	null/+	0 of 7	116 ± 21
M	null/+	0 of 6	118 ± 9

Data are means ± SD for plasma glucose levels. Mice were between 18 and 19 months of age at the time of analysis.

genotypes (*B2m*^{null} and *B2m*$^{null/+}$). These histological phenomena also are commonly observed in pancreases of aging mice and included an increase in both the size and number of pancreatic islets, as well as focal regions of exocrine tissue replacement by fat. Aldehyde fuchsin staining for β-cell granulation showed the hypertrophied islets contain numerous granulated β-cells. In the larger islets, some of the β-cells were partially degranulated (possibly suggestive of insulin secretion). Small clusters of leukocytes and fibroblasts, primarily associated with pancreatic ducts and blood vessels, were present in both the *B2m*^{null} and *B2m*$^{null/+}$ classes. The perivascular or periductal infiltrates sometimes extended to an adjacent islet but were generally concentrated at only one pole and were rarely observed to penetrate the islet. The type of fulminant insulitis characteristic of NOD mice was never observed in any of the 129-*B2m*^{null} or *B2m*$^{null/+}$ pancreases observed.

These findings with ≥18-month-old, nonautoimmune 129-*B2m*^{null} and 129-*B2m*$^{null/+}$ mice are in marked contrast to those of Faustman et al. (19). All of our mice were normoglycemic (Table 1), whereas the *B2m*^{null} mice reported previously developed hyperglycemia (360 ± 50 mg/dl), and one class I positive control mouse became hyperglycemic at 20 months of age. Faustman et al. (19) also reported reduced body weights in the *B2m*^{null} mice (21.9 ± 3.8 g) and normal weights in the class I positive control mice (37.3 ± 5.6 g); in our study, class I positive and negative mice did not show any weight differences (all mice weighed between 40 and 45 g). Because the *B2m*^{null} mice in both our study (13,15) and the previous study (19) were derived from the same embryonic stem cell clone, genetic differences contributing to the two studies are limited. In the current study, inbred 129-*B2m*^{null} mice were examined, whereas in the other study, the 129-*B2m*^{null} strain had been outcrossed to the C57BL/6 strain. An outcross to C57BL/6 produces a change in genetic background, but this is unlikely to account for the abnormalities reported because one of us (E.H.L.) has not observed hyperglycemia, weight loss, or insulitis in a separate study of 129/Ola-*B2m*^{null} mice outcrossed to C57BL/6 (this issue, D.V. Serreze et al., p. 505–509). A likely explanation for the contrasting observations is environmental factors. The 129-*B2m*^{null} and 129-*B2m*$^{null/+}$ mice examined in this study were maintained in a sterile microisolater barrier facility, free of any known mouse pathogens, whereas the mice described previously (19) were not pathogen-free. The dramatic weight differences observed between the

TABLE 2
NOD-*B2m*^{null} mice do not develop diabetes or insulitis

Sex	*B2m* genotype	Diabetes (%)	Insulitis
F	null/null	0 of 38	0 of 29
M	null/null	0 of 40	0 of 31
F	null/+	9 of 15 (60)	4 of 4
M	null/+	4 of 16 (25)	9 of 9
F	+/+	10 of 15 (67)	3 of 4
M	+/+	3 of 5 (60)	1 of 1

NOD-*B2m*^{null} (N7F1–4 generations), NOD-*B2m*$^{null/+}$ (N7F1), and NOD-*B2m*$^{+/+}$ (N7F1) mice were observed for the development of diabetes until ≥7 months of age. Insulitis scores are reported for mice that did not develop diabetes by ≥7 months of age. In a contemporaneously performed study, the incidence of diabetes in the parental NOD strain was 70% in females (n = 50) and 42% in males (n = 50) at 7 months of age.

B2m^{null} and *B2m*$^{null/+}$ mice in Faustman et al. (19) could be attributable to an infectious process that was more pathogenic in the *B2m*^{null} class of mice lacking CD8$^+$ T-cells. In addition, the occurrence of hyperglycemia in one class I–expressing *B2m*$^{null/+}$ mouse suggests that factors other than class I expression influenced the previous results.

NOD-*B2m*^{null} mice do not develop insulitis or diabetes. After six backcrosses of (NOD × 129-*B2m*^{null})F1 mice to the NOD parental strain, with selection for the disrupted *B2m* allele at each generation, NOD-*B2m*$^{null/+}$ mice were intercrossed to produce homozygous NOD-*B2m*^{null} mice. Analysis of spleen cells from NOD-*B2m*^{null} mice showed the expected lack of both CD8$^+$ T-cells and the class I antigen, K^d. NOD-*B2m*$^{null/+}$ and NOD-*B2m*$^{+/+}$ mice had equivalent numbers of CD8$^+$ T-cells. Expression of K^d on NOD-*B2m*$^{null/+}$ spleen cells ranged from 50 to 90% of that on NOD-*B2m*$^{+/+}$ spleen cells. Mice of all three genotypes had comparable numbers of B220$^+$ and CD4$^+$ cells (data not shown).

NOD-*B2m*^{null} mice, as well as NOD-*B2m*$^{null/+}$ and NOD-*B2m*$^{+/+}$ mice, were observed for the development of insulitis and diabetes (Table 2). In both groups of class I–expressing mice, NOD-*B2m*$^{null/+}$ and NOD-*B2m*$^{+/+}$, the incidence of diabetes was similar to the parental NOD strain. This high incidence of disease suggests that the NOD-derived diabetogenic alleles were fixed at most, if not all, of the loci contributing to diabetes. In addition, homozygous expression of the NOD alleles were demonstrated for several diabetogenic loci including the major histocompatibility complex (*Idd-1*), *Idd-3*, *Idd-5*, *Idd-6*, *Idd-9*, and *Idd-10* (20) (data not shown). In addition to the parental incidence of diabetes in NOD-*B2m*$^{null/+}$ and NOD-*B2m*$^{+/+}$ mice, all but one of the class I–expressing N7F1 mice that did not develop diabetes had mild to severe insulitis (Table 2). The development of insulitis in nearly all animals also is a characteristic of the NOD parental strain.

In contrast to the high incidence of diabetes in NOD-*B2m*$^{null/+}$ and NOD-*B2m*$^{+/+}$ mice, NOD-*B2m*^{null} mice did not develop insulitis or diabetes (Table 2). No homozygous NOD-*B2m*^{null} mice (0 of 38 females and 0 of 40 males) exhibited any symptoms of diabetes at ≥7 months of age. In addition, the most severe histological

TABLE 3
Protection from diabetes is not associated with homozygosity for the 129/Sv-*ter* allele at loci linked to *B2m*

NOD-*B2m*^{null} (N7F2)	Genotype of diabetic animals					
	D2Mit45 (45 cM)	*D2Mit43* (45 cM)	*D2Mit58* (46 cM)	*D2Mit17* (51 cM)	*B2m* (54 cM)	*D2Mit47* (69 cM)
#11 (female)	129	129	129	129/NOD	null/+	NOD
#15 (female)	129	129	129	129/NOD	null/+	NOD
# 7 (female)	129/NOD	129/NOD	129	129/NOD	null/+	NOD

Locus position on chromosome 2 as listed in *Locus Map of the Mouse* issued 30 September, 1993 by The Jackson Laboratory, Bar Harbor, Maine. 129 and NOD indicate the presence of the 129/Sv-*ter* and the NOD allele, respectively, at the indicated locus.

lesion in the NOD-*B2m*^{null} mice was a mild peri-islet infiltration of mononuclear cells. These mild histological changes were seen in 13% of male (n = 31) and 48% of female (n = 29) NOD-*B2m*^{null} mice and were associated only with a small proportion (<10%) of the islets in the affected individuals. Mild to moderate perivascular and periductal infiltrates were noted in an additional 25 and 21% of male and female NOD-*B2m*^{null} mice, respectively. Additional evidence of autoimmunity was observed in NOD-*B2m*^{null} mice; mild to extensive perivascular and periductal submandibular gland infiltrates were seen in 8 of 8 female and 13 of 14 male NOD-*B2m*^{null} mice examined.

Protection from diabetes and insulitis is not associated with regions linked to the *B2m* locus. During the process of transferring the *B2m* locus from the 129-*B2m*^{null} mouse to the NOD genetic background, linked regions on chromosome 2 were selected along with the 129-*B2m*^{null}–derived *B2m* locus. Thus, it was possible that the protective effect observed in NOD-*B2m*^{null} mice was not from the *B2m* locus but from a linked recessive gene. We tested this hypothesis by examining microsatellite markers flanking the *B2m* locus (Table 3). We found that at the sixth backcross generation, non-NOD alleles were present at loci centromeric of *B2m*: *D2Mit45*, *D2Mit43*, *D2Mit58*, and *D2Mit17*. The closest telomeric marker analyzed, *D2Mit47*, was fixed for the NOD allele. Several diabetic animals, which were all typed as *null/+* at *B2m*, were homozygous for the 129-derived allele at *D2Mit45*, *D2Mit43*, and/or *D2Mit58*. In addition, we have developed a control strain that is homozygous for the 129-derived alleles at *D2Mit45*, *D2Mit43*, and *D2Mit58*, but homozygous for the NOD alleles at *B2m* and *D2Mit17*. These mice develop diabetes at a high frequency (data not shown). Because all mice homozygous for the 129-derived allele at *B2m* were protected from diabetes (Table 2), the locus responsible for disease protection is located in the 23-centiMorgan (cM) interval between (but not including) *D2Mit58* and *D2Mit47*. This 23-cM interval includes the *B2m* locus, suggesting strongly that the lack of class I expression is responsible for the protection from diabetes observed in the NOD-*B2m*^{null} strain.

Adoptive transfer of diabetes. One possible reason for the protection from diabetes in NOD-*B2m*^{null} mice is that islets lacking expression of class I are not susceptible to autoimmune destruction. To test this hypothesis, spleen cells from diabetic NOD mice were transferred to irradiated NOD-*B2m*^{null} recipients and monitored for diabetes. Control adoptive transfer of spleen cells from diabetic NOD donors into NOD recipients efficiently produced diabetes with 3 of 3 mice glycosuria positive 19 days post-transfer. For comparison, spleen cells from the same diabetic NOD donors were transferred into NOD-*B2m*^{null} recipients. Although delayed, 5 of 5 NOD-*B2m*^{null} recipients became glycosuria positive between 76 and 126 days post-transfer (105 ± 22 days). Pancreases from the five diabetic NOD-*B2m*^{null} recipients were examined. All five had massive insulitis in the few islets remaining and were indistinguishable from diabetic NOD mice. These results demonstrate that NOD-*B2m*^{null} islets can be targeted by islet-specific autoimmune T-cells. Although other potential interpretations exist, one possible reason for the delayed kinetics, compared with NOD recipients, is the inability of class I–restricted CD8$^+$ T-cells to effectively kill the class I negative β-cells, whereas the islet-specific class II–restricted CD4$^+$ T-cells remain fully functional. Adoptive transfer experiments using CD4$^+$- and CD8$^+$-depleted T-cell populations are in progress to address this hypothesis.

Concluding remarks. The total absence of diabetes in NOD mice lacking expression of β2-microglobulin supports the hypothesis that CD8$^+$ T-cells are essential for the development of autoimmune diabetes in the NOD mouse. The surprising finding that NOD-*B2m*^{null} mice do not develop CD4$^+$ T-cell–mediated insulitis suggests that at least one role of CD8$^+$ T-cells is at the initiation of the autoimmune response to the β-cell. With the absence of insulitis, other possible roles for β-cell–specific CD8$^+$ T-cells, perhaps in the effector phase of the autoimmune response, cannot be directly assessed in the NOD-*B2m*^{null} strain because the β-cell–specific response does not appear to be initiated. However, the NOD-*B2m*^{null} strain can provide the basis for experiments in which various combinations of the disease-related components such as pancreas, thymus, and bone marrow, can be class I expressing or class I deficient.

Although the results obtained in this study demonstrate that expression of class I is necessary for the initiation of β-cell–specific autoimmunity in the NOD mouse, the reason class I is required is unknown. Perhaps an early induction of cytotoxic, class I–restricted CD8$^+$ T-cells specific for β-cells is required to cause low-level islet destruction. The death of a few β-cells and subsequent release of β-cell components early in the autoimmune response could prime CD4$^+$ T-cells, which in turn aug-

ment and recruit other β-cell–recognizing T-cells. In support of this hypothesis is the observation that the first T-cells entering the NOD islets are $CD8^+$ (12).

It seems unlikely that the requirement for class I expression and $CD8^+$ T-cells for the initiation of autoimmune diabetes in the NOD mouse is attributable to nonspecific effects on the immune system. $CD4^+$ T-cells are functional in $B2m^{null}$ mice because these mice are able to generate anti-viral responses (21,22), skin graft rejection (15), and antibody responses (23,24). However, it is possible that the lack of $CD8^+$ T-cells could, in some fashion, lead to subtle changes in the $CD4^+$ T-cell population that reduces the autoimmune potential of these cells. In this context, note the unexpected results of Mozes et al. (24) where disruption of the β2-microglobulin gene produced resistance to an antibody-mediated disease, experimental systemic lupus erythematosus. The development of the NOD-$B2m^{null}$ strain should enable further characterization of the role of class I–restricted $CD8^+$ T-cells in type I diabetes.

ACKNOWLEDGMENTS

E.H.L. is supported by National Institutes of Health Grants DK-27722 and DK-36175.

This is dedicated to the memory of Dr. Maarten Zijlstra, a promising young investigator, who died during the course of these studies.

REFERENCES

1. Wicker LS, Miller BJ, Mullen Y: Transfer of autoimmune diabetes mellitus with splenocytes from nonobese diabetic (NOD) mice. *Diabetes* 35:855–60, 1986
2. Yagi H, Matsumoto M, Kunimoto K, Kawaguchi J, Makino S, Harada M: Analysis of the roles of $CD4^+$ and $CD8^+$ T-cells in autoimmune diabetes of NOD mice using transfer to NOD athymic nude mice. *Eur J Immunol* 22:2387–93, 1992
3. Wang Y, Pontesilli O, Gill RG, La Rosa FG, Lafferty KJ: The role of $CD4^+$ and $CD8^+$ T-cells in the destruction of islet grafts by spontaneously diabetic mice. *Proc Natl Acad Sci USA* 88:527–31, 1991
4. Bradley BJ, Haskins K, La Rosa FG, Lafferty KJ: CD8 T-cells are not required for islet destruction induced by a $CD4^+$ islet-specific T-cell clone. *Diabetes* 41:1603–608, 1992
5. Peterson JD, Pike B, Dallas A, Haskins K: Transfer of diabetes to NOD-F1 and NOD-*scid* mice by $CD4^+$ islet-specific T-cell clones (Abstract). *J Immunol* 150:53, 1993
6. Hanafusa T, Sugihara S, Fujino-Kurihara H, Miyagawa J-I, Asakawa H, Kono N, Fujiwara H, Hamaoka T, Tarui S: Induction of insulitis by adoptive transfer with $L3T4^+$ $Lyt2^-$ T-lymphcytes in T-lymphocytes–depleted NOD mice. *Diabetes* 37:204–208, 1988
7. Miller BJ, Appel MC, O'Neil JJ, Wicker LS: Both the $Lyt2^+$ and $L3T4^+$ T-cell subsets are required for the transfer of diabetes in nonobese diabetic mice. *J Immunol* 140:52–58, 1988
8. Bendelac A, Carnaud C, Boitard C, Bach J-F: Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates: requirement for both $L3T4^+$ and $Lyt2^+$ T-cells. *J Exp Med* 166:823–32, 1987
9. Hayakawa M, Yokono K, Nagata M, Hatamori N, Ogawa W, Miki A, Mizoguti H, Baba S: Morphological analysis of selective destruction of pancreatic β-cells by cytotoxic T-lymphocytes in NOD mice. *Diabetes* 40:1210–17, 1991
10. Nagata M, Yoon J-W: Studies on autoimmunity for T-cell–mediated β-cell destruction: distinct difference in β-cell destruction between $CD4^+$ and $CD8^+$ T-cell clones derived from lymphocytes infiltrating the islets of NOD mice. *Diabetes* 41:998-1008, 1992
11. Christianson SW, Shultz LD, Leiter EH: Adoptive transfer of diabetes into immunodeficient NOD-*scid*/*scid* mice: relative contributions of $CD4^+$ and $CD8^+$ T-cells from diabetic versus prediabetic NOD.NON-$Thy1^a$ donors. *Diabetes* 42:44–55, 1993
12. Jarpe AJ, Hickman MR, Anderson JT, Winter WE, Peck AB: Flow cytometric enumeration of mononuclear cell populations infiltrating the islets of Langerhans in prediabetic NOD mice: development of a model of autoimmune insulitis for type I diabetes. *Reg Immunol* 3:305–17, 1991
13. Zijlstra M, Li E, Sajjadi F, Subramani S, Jaenisch R: Germ-line transmission of a disrupted β2-microglobulin gene produced by homologous recombination in embryonic stem cells. *Nature* 342: 435–38, 1989
14. Zijlstra M, Bix M, Simister NE, Loring JM, Raulet DH, Jaenisch R: β2-microglobulin–deficient mice lack $CD4^-8^+$ cytolytic T-cells. *Nature* 344:742–46, 1990
15. Zijlstra M, Auchincloss H Jr, Loring JM, Chase CM, Russell PS, Jaenisch R: Skin graft rejection by β2-microglobulin–deficient mice. *J Exp Med* 175:885–93, 1992
16. Dietrich W, Miller J, Katz H, Joyce D, Steen R, Lincoln S, Daly M, Reeve MP, Weaver A, Goodman N, Dracopoli N, Lander ES: SSLP genetic map of the mouse (*Mus musculus*) $2N = 40$. In *Genetic Maps: Locus Maps of Complex Organisms*. S.J. O'Brien, Ed. New York, Cold Spring Harbor Laboratory Press, 1993
17. Wicker LS, Podolin PL, Fischer P, Sirotina A, Boltz RC, Peterson LB: Expression of intra-MHC transporter (*Ham*) genes and class I antigens in diabetes-susceptible NOD mice. *Science* 256:1828–30, 1992
18. Koller BH, Marrack P, Kappler JW, Smithies O: Normal development of mice deficient in β2M, MHC class I proteins, and $CD8^+$ T-cells. *Science* 248:1227–30, 1990
19. Faustman D, Li X, Lin HY, Fu Y, Eisenbarth G, Avruch J, Guo J: Linkage of faulty major histocompatibility complex class I to autoimmune diabetes. *Science* 254:1756–61, 1991
20. Ghosh S, Palmer SM, Rodrigues NR, Cordell HG, Hearne CM, Cornall RJ, Prins J-B, McShane P, Lathrop GM, Peterson LB, Wicker LS, Todd JA: Polygenic control of autoimmune diabetes in nonobese diabetic mice. *Nature Genet* 4:404–409, 1993
21. Hou S, Doherty PC, Zijlstra M, Jaenisch R, Katz JM: Delayed clearance of sendai virus in mice lacking class I MHC-restricted $CD8^+$ T-cells. *J Immunol* 149:1319–25, 1992
22. Muller D, Koller BH, Whitton JL, LaPan KE, Brigman KK, Frelinger JA: LCMV-specific, class II-restricted cytotoxic T-cells in β2-microglobulin–deficient mice. *Science* 255:1576–78, 1992
23. Spriggs MK, Koller BH, Sato T, Morrissey PJ, Fanslow WC, Smithies O, Voice RF, Widmer MB, Maliszewski CR: β2-microglobulin$^-$, $CD8^+$ T-cell–deficient mice survive inoculation with high doses of vaccinia virus and exhibit altered IgG responses. *Proc Natl Acad Sci USA* 89:6070–74, 1992
24. Mozes E, Kohn LD, Hakim F, Singer DS: Resistance of MHC class I–deficient mice to experimental systemic lupus erythematosus. *Science* 261:91–93, 1993