Genetic Dissection of T Cell Receptor Vβ Gene Requirements for Spontaneous Murine Diabetes

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Summary

It has been demonstrated, in certain autoimmune disease models, that pathogenic T cells express antigen receptors of limited diversity. It has been suggested that the T cells responsible for the pathogenesis of type I diabetes mellitus might similarly demonstrate restricted T cell receptor (TCR) usage. Recently, attempts have been made to identify the Vβ subset(s) that initiates and/or perpetuates the antiislet response in a mouse model of spontaneous autoimmune diabetes (non-obese diabetic [NOD] mice). In studies reported here, we have bred NOD mice to a mouse strain that congenitally lacks approximately one-half of the conventional TCR Vβ alleles. Included in this deletion are TCR Vβ gene products previously implicated as being involved in the pathogenesis of NOD disease. By studying second backcross-intercross animals, we were able to demonstrate that this deletion of TCR Vβ gene segments did not prevent the development of insulitis or diabetes.

Insulin-dependent diabetes mellitus (IDDM)[1] results from the destruction of the insulin-producing β cells of the pancreatic islets of Langerhans. The development of spontaneous IDDM in animal models, as well as in humans, has been shown to have an autoimmune pathology that arises through a complex pattern of genetic inheritance (1–6). One animal model of spontaneous diabetes, the non-obese diabetic (NOD) mouse, has demonstrated pathology analogous to the human disease, including islet-associated mononuclear cell infiltration (insulitis) and serologically detectable autoantibodies to a number of β cell–specific components (7–10). Both occur before the onset of overt hyperglycemia.

A pathogenic role for T lymphocytes in the destruction of islets has been demonstrated for NOD mice (8–10) and also implicated for human IDDM. There are now numerous examples, both in normal and pathologic immune responses, where there is preferential if not exclusive activation of T cells expressing particular TCR Vβ gene segments (11–13). Several recent studies have suggested that the inflammation and destruction of pancreatic islet cells in NOD mice may be mediated by T cells expressing TCR Vβ5 or Vβ8 gene segments (14–16).

In this report we have used a naturally occurring deletion in the TCR Vβ locus to test directly the requirement for these particular Vβ gene segments in the development of NOD diabetes. Most inbred mouse strains, including NOD, have the TCR $V\beta^b$ haplotype, encoding ~20–25 functional Vβ gene elements. However, several strains, such as SWR, carry the $V\beta^a$ haplotype, where there has been an extensive deletion of ~10 Vβ gene segments, including the Vβ5 and Vβ8 gene families (17). We crossed the $V\beta^a$ haplotype from SWR onto the NOD background, and examined the influence of TCR Vβ haplotype on the incidence and severity of insulitis and diabetes (hyperglycemia). Our results demonstrate that deletion of about half the wild-type complement of TCR Vβ gene segments, including those previously implicated in disease pathogenesis (14–16), still allowed the development of insulitis and diabetes.

Materials and Methods

Mice. NOD mice used in the breeding analysis were derived from stock breeders generously provided by Dr. Yoko Mullen, University of California at Los Angeles (Los Angeles, CA). SWR mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and female NOD mice (6 wk old) used in the adoptive transfer experiments were purchased from Taconic Farms (German Town, NY). All mice were housed in Nalgene microisolator cages (Rochester, NY) under specific pathogen-free (SPF) conditions, and allowed food and water ad libitum. Development of diabetes was screened for by visual inspection of cages for evidence of polyuria, and confirmed by measurement of plasma glucose values.

Antibodies. mAb directed against mouse T cell markers Vβ8 (mAb F23.1) (18, 19) and Vβ17a (mAb KJ23) (20, 21), and against

[1] *Abbreviations used in this paper:* BC2-F_1, second backcross-intercross; IDDM, insulin-dependent diabetes mellitus; NOD, non-obese diabetic.

the I-A^q MHC class II antigen (mAb BP107) (22), were purified from mouse ascites on Affi-Gel protein A columns (Bio-Rad Laboratories, Richmond, CA). The antibodies were conjugated to FITC or biotin using standard procedures (23). Allophycocyanin-labeled mAbs directed against the Ly-1 pan-T cell marker (24) were the generous gift of Dr. Alan Stall, Stanford University (Stanford, CA).

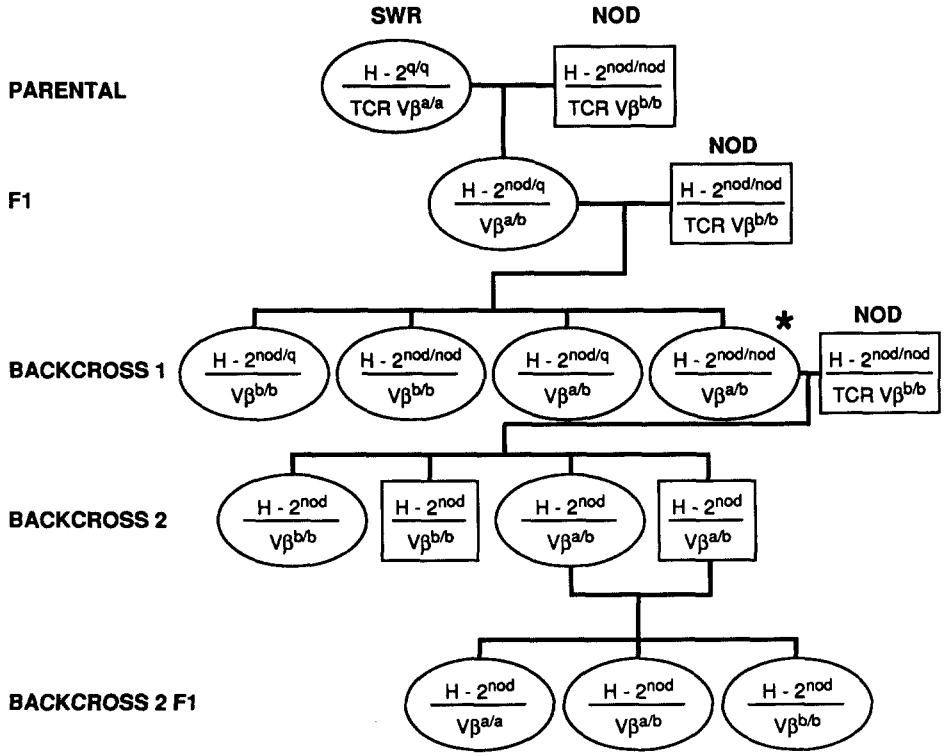
Fluorescence Staining. FACS® analysis of PBL from BC2-F_1 mice and control NOD, SWR, and (NOD × SWR)F_1 mice was carried out as previously described (25). Briefly, Ficoll-purified PBL were incubated with 25–50 μl titered, labeled antibodies at 4°C for 20 min. The cells were washed three times in ice cold PBS plus 2% FCS and 0.1% azide. The cells were resuspended in propidium iodide to label dead cells. Cells were analyzed on a modified dual laser FACS II® system (Becton Dickinson & Co., Mountain View, CA) equipped with logarithmic amplifiers. A primarily lymphocyte population was obtained for analysis by selective gating with forward and obtuse scatter.

Histology. Pancreata were dissected from freshly killed cadaver mice and prepared for histology by either: (*a*) quick freezing of tissue on OCT compound (Miles Inc., Elkhart, IN) over dry ice, or (*b*) overnight fixation in neutral buffered formalin and paraffin embedding. The resultant blocks were sectioned and stained with hematoxylin and eosin and viewed by light microscopy.

Adoptive Transfer. Splenocytes were transferred from BC2-F_1 mice and control NOD mice according to a modified protocol of Wicker et al. (26). Recipients for adoptive transfer were female NOD mice, 45–55 d old, that were whole body irradiated (775 rad) (250 kv, 16 milliamp; Philips, Hamburg, Germany) within 4 h of intravenous injection of donor splenocytes. Pancreata from splenocyte donor mice (NOD, $V\beta^{a/a}$, or $V\beta^{a/b}$ BC2-F_1) were screened for the presence of insulitis by hematoxylin and eosin staining of frozen pancreas sections before splenocyte preparation. Spleens from suitable donors were teased apart into single cell suspensions and washed twice in RPMI (Gibco Laboratories, Grand Island, NY) enriched with 2% FCS. 2×10^7 cells in 0.5 ml were injected into the tail vein of recipient NOD mice. Recipient mice were monitored for the presence of hyperglycemia by plasma glucose measurement (Beckman glucose analyze; Beckman Instruments, Palo Alto, CA) beginning 14 d after splenocyte transfer. Diabetes was defined by plasma glucose values >500 mg/dl.

Results

Breeding and Phenotyping of $V\beta^{a/a}$ Intercross Mice. To obtain progeny homozygous for the TCR Vβ deletion haplotype ($V\beta^a$) on the background of diabetogenic genes from the NOD strain, mice were bred according to the scheme shown in Fig. 1. NOD mice (H-2^{nod}, $V\beta^b$) were crossed with SWR mice (H-2^q, $V\beta^a$), and the F_1 progeny were crossed back to NOD. We previously reported that, in the first backcross generation, disease was observed at similar frequencies in both $V\beta^{a/b}$ and $V\beta^{b/b}$ mice homozygous for H-2^{nod} (25). To select for transmission of the various recessive alleles required for susceptibility to diabetes (5, 6), diabetic H-$2^{nod/nod}$ females typed as $V\beta^{a/b}$ were selected from this first backcross generation and crossed to NOD males. $V\beta^{a/b}$ progeny derived from one diabetic female from this second backcross (BC2) were intercrossed, and the resultant BC2-F_1 generation was typed by cytofluorometric analysis using two TCR Vβ-specific mAbs, F23.1 and KJ23. The an-



* Backcross 1 ♀ breeder had IDDM

Figure 1. Breeding protocol to derive mice homozygous for the TCR Vβ deletion ($V\beta^{a/a}$) on the NOD background. SWR mice (H-2^q, $V\beta^a$) were bred with NOD mice (H-2^{nod}, $V\beta^b$), and the F_1 progeny were backcrossed to NOD. From this first backcross generation, a diabetic female mouse that carried the H-2^{nod} haplotype and was TCR $V\beta^{a/b}$ was backcrossed to an NOD male to derive a second backcross generation that was H-$2^{nod/nod}$, carried the required genes for diabetes, and had TCR $V\beta^{a/b}$ progeny. The second backcross mice that carried the $V\beta^{a/b}$ TCR haplotype were intercrossed to derive a second backcross-intercross generation. The PBL of these progeny (BC2-F_1) were screened by cytofluorometric analysis; TCR haplotypes segregated in the expected distribution of 1:2:1 for the $V\beta^{a/a}/V\beta^{a/b}/V\beta^{b/b}$ haplotypes, respectively.

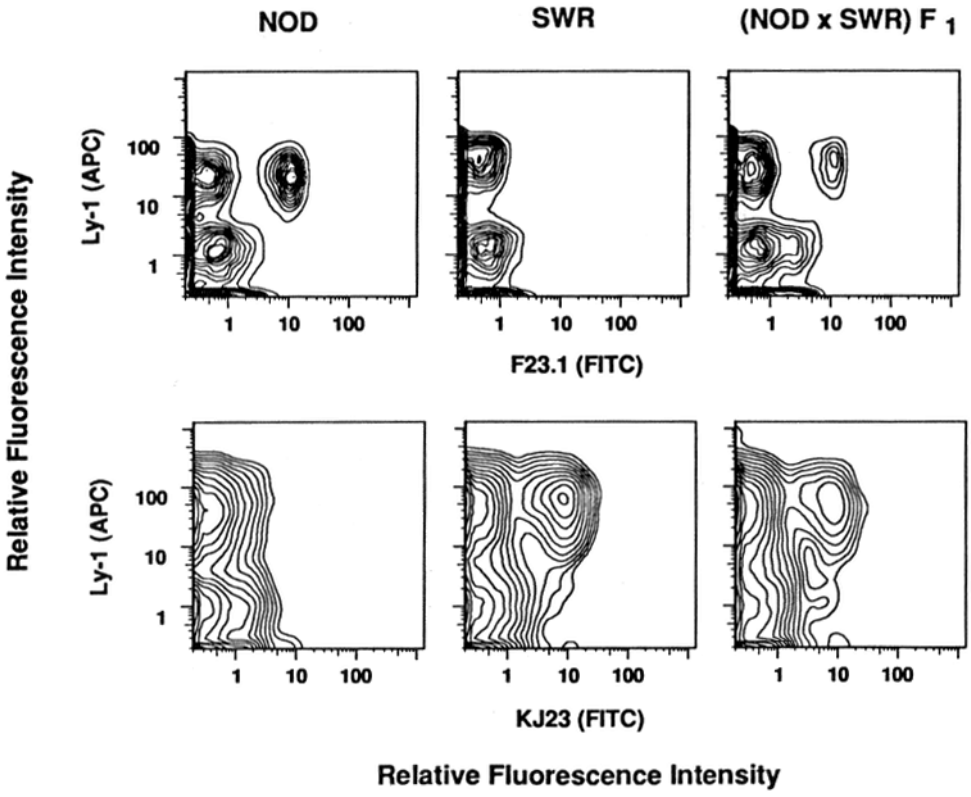


Figure 2. Determination of TCR haplotype for $V\beta^{a/a}$, $V\beta^{b/b}$ homozygotes, and $V\beta^{a/b}$ heterozygotes in (NOD × SWR) BC2-F_1 mice by cytofluorometric analysis. T lymphocytes from peripheral blood of BC2-F_1 mice were double labeled using an allophycocyanin-conjugated Ly-1 mAb in conjunction with either of two fluorescein-conjugated Vβ-specific mAbs, F23.1 or KJ23. mAb F23.1 binds to all three members of the Vβ8 family that are deleted in the $V\beta^a$ haplotype and therefore specifically labels $V\beta^b$ cells; thus, the antibody labels NOD ($V\beta^{b/b}$) and (NOD × SWR)F_1 ($V\beta^{a/b}$) but not SWR ($V\beta^{a/a}$) T cells. mAb KJ23 binds to the Vβ17a that is functionally expressed by $V\beta^a$ haplotype mice, but not $V\beta^b$; thus, the antibody labels SWR and (NOD × SWR)F_1 but not NOD T cells. Staining with F23.1 (*top*) is shown as a 5% probability contour plot, whereas staining with KJ23 (*bottom*) is expressed as a 50% logarithmic contour plot.

tibody F23.1 recognizes all three members of the Vβ8 gene family (18, 19); these gene segments are among those deleted in the $V\beta^a$ haplotype (17). KJ23 is specific for the Vβ17a gene product, encoded by the $V\beta^a$ haplotype (20, 21); the $V\beta^b$ haplotype has no functional Vβ17 gene (21). The BC2-F_1 generation could thus be typed as $V\beta^{a/a}$ ($F23.1^-$, $KJ23^+$), $V\beta^{a/b}$ ($F23.1^+$, $KJ23^+$), or $V\beta^{b/b}$ ($F23.1^+$, $KJ23^-$) (Fig. 2).

Effect of Vβ Haplotype on Insulitis. To determine the effect that TCR Vβ haplotype might have on the inductive phase of islet destruction, pancreata from BC2-F_1 mice were studied by histologic analysis for evidence of the lymphocytic infiltration (insulitis) into the islets. In our inbred NOD colony, insulitis begins at ~30–40 d of age with the accumulation of lymphocytes in the vasculature and ducts near the islets. Progressive inflammation of the islet parenchyma leads ultimately to islet β cell destruction. Loss of insulin secretory function and clinical diabetes occur at ~200 d. In the BC2-F_1 mice, severe insulitis was apparent in pancreata from mice expressing all three TCR phenotypes by 100 d of age. The morphology of these lesions was indistinguishable from those seen in inbred NOD mice. 8 of 10 $V\beta^{a/a}$ BC2-F_1 mice had widespread insulitic lesions, and the majority of these mice demonstrated severe intra-islet inflammation (Fig. 3). Most of the pancreata examined from $V\beta^{a/b}$ heterozygotes (9/11) and $V\beta^{b/b}$ homozygotes (4/5) also had extensive insulitis. Thus, T cells bearing receptors derived from the deletion haplotype $V\beta^a$, lacking both Vβ5 and Vβ8 families, could initiate the early stages of NOD disease.

Effect of Vβ Haplotype on Diabetes. Progression to clinical diabetes (hyperglycemia) in NOD mice is always preceded by insulitis. However, the mere presence of insulitis does not necessarily lead to overt disease (3). At least one non-MHC gene is thought to initiate inflammation, while the later pathogenic steps are controlled by other diabetogenic genes, including the NOD MHC (27). We therefore followed a cohort of BC2-F_1 mice for >200 d for evidence of overt diabetes. The criteria for diabetes was plasma glucose values in excess of 500 mg/dl (nl = <150). Diabetes incidence in our inbred NOD colony is 60–70% in females, and 10–20% in males by 200 d. As shown in Table 1, mice heterozygous or homozygous for the NOD-derived $V\beta^b$ locus had a disease incidence of ~30% by 210 d of age. Disease occurred in both male and females. In the $V\beta^{a/a}$ mice, the disease incidence was reduced compared to $V\beta^{b/a}$ or $V\beta^{b/b}$ litter mates; however, fulminant disease still occurred in 2 of 16 (12.5%) $V\beta^{a/a}$ mice. The incidence was not statistically different among the groups. The finding that the $V\beta^{a/a}$ mice could develop overt disease demonstrated that those TCR Vβ gene segments deleted in the $V\beta^a$ haplotype were not required for the development of diabetes.

$V\beta^a$ Splenocytes Transfer Disease. To establish whether lymphocytes from the BC2-F_1 mice that had not developed IDDM could mediate islet destruction in an environment containing all the background genes from NOD, we adoptively transferred lymphocytes from nondiabetic $V\beta^{a/a}$ and $V\beta^{a/b}$ BC2-F_1 mice into young NOD mice (26). Normoglycemic BC2-F_1 mice, >200 d old (all with documented insulitis) were used as lymphocyte donors. Lethally irradiated 7-wk-

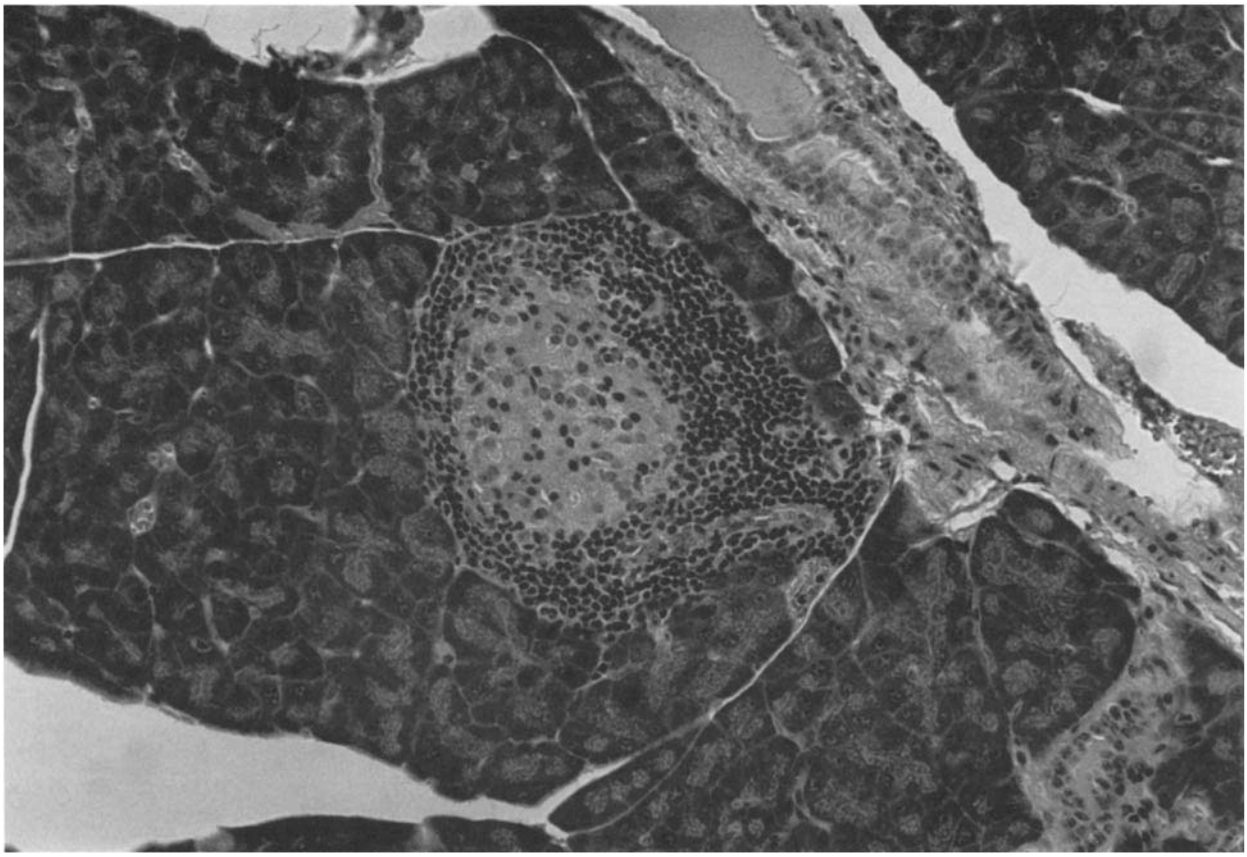


Figure 3. Inflamed pancreatic islet from a deletion haplotype ($V\beta^{a/a}$) mouse. A representative section from the pancreas of a >100-d-old $V\beta^{a/a}$ BC2-F_1 mouse showing massive accumulation and invasion of lymphocytes into the islet parenchyma. The section is visualized by light microscopy at ×400.

old female NOD mice were used as recipients. Without manipulation, these NOD recipients would not be expected to develop hyperglycemia for at least 100 d. The data presented in Table 2 demonstrate that spleen cells from either the Vβ deletion ($V\beta^{a/a}$) or heterozygous ($V\beta^{a/b}$) donors induced accelerated disease in recipients since overt hyperglycemia could be seen within 24 d after transfer in recipient mice. The incidence was as high or higher than that seen using cells transferred from inbred NOD mice.

Table 1. *Effect of TCR Vβ Haplotype on IDDM Incidence in BC2-F_1 Mice*

Vβ haplotype	IDDM incidence	Mean day of onset
$V\beta^{a/a}$	2/16 (12.5%)	160 ± 30
$V\beta^{a/b}$	10/35 (28.6%)	170 ± 30
$V\beta^{b/b}$	5/15 (33.3%)	165 ± 11

BC2-F_1 mice were followed by visual inspection of their cages for evidence of polyuria and periodically bled for blood glucose determination. The incidence and mean day of onset of overt hyperglycemia are presented. IDDM was defined as a blood glucose value in excess of 500 mg/dl (nl = <150 mg/dl).

Discussion

The results reported here demonstrate that TCR Vβ gene families 5, 8, 9, 11, 12, and 13, deleted in the $V\beta^a$ haplotype, are not required for development of insulitis or diabetes in this model of spontaneous diabetes. These findings differ significantly from previous reports in which the T cells

Table 2. *Disease Acceleration by Adoptive Transfer of BC2-F_1 Splenocytes*

TCR haplotype	Recipient with IDDM
NOD ($V\beta^{b/b}$)	0/3, 0/3, 3/4, 4/4
$V\beta^{a/b}$	0/2, 0/4, 2/4, 3/3, 3/3
$V\beta^{a/a}$	0/4, 3/3, 4/4, 4/4

Splenocytes from individual nondiabetic BC2-F_1 mice with the Vβ deletion ($V\beta^{a/a}$) were adoptively transferred into lethally irradiated (775 rad) young female NOD recipients (three to four recipients/donor spleen). Each fraction represents the number of diabetic recipients/transferred mice per donor spleen. Splenocytes from nondiabetic BC2-F_1 TCR heterozygotes ($V\beta^{a/b}$) and NOD mice were used as controls. Data are expressed as number of recipient mice with overt hyperglycemia 18–24 d after splenocyte transfer.

expressing receptors of the Vβ5 (14) and Vβ8 (15, 16) families were implicated as the major pathogenic T cell subsets in NOD diabetes. Those studies (14–16) utilized methods of T cell cloning or anti-Vβ-directed mAb therapy to address this issue. Here, by formal genetic analysis, we have demonstrated that diabetes can develop in the absence of T cells expressing gene segments from these families.

Our results may be interpreted in several ways. First, the antiislet T cell response may be polyclonal at its inception, therefore, the "inductive" pathologic T cells may not demonstrate predominant TCR Vβ gene usage. Such a polyclonal "inductive" response would contrast that seen in another murine autoimmune disorder, experimental allergic encephalomyelitis, where T cells of a single autoantigen specificity and conserved TCR Vβ usage have been shown to induce and perpetuate the autoimmune pathology (13, 28, 29). Second, initiation of the diabetogenic events in NOD mice may depend upon T cells utilizing a particular Vβ gene segment(s) that is encoded by the portion of chromosome 6 preserved in $V\beta^a$ mice, and thus shared by both $V\beta^a$ and $V\beta^b$ haplotypes. A third possibility is that in NOD mice, disease is indeed triggered by T cells using a TCR Vβ gene segment(s) that maps within the region deleted in the $V\beta^a$ haplotype (such as Vβ5 or Vβ8), but NOD $V\beta^a$ mice ($BC2\text{-}F_1$), in the absence of this gene segment, might compensate for the deletion(s) by shifting Vβ usage without affecting recognition of the autoantigen. We and others have recently shown that while T cells expressing particular TCR Vβ gene segments may predominate in response to a known antigen, excellent responses can be made by T cells expressing alternative TCR Vβ genes if T cells expressing the preferred genes are unavailable (30–34). Although we currently cannot choose among these three possibilities, the data presented in this manuscript demonstrate that the TCR Vβ genes deleted in the $V\beta^a$ haplotype are not required for the induction of IDDM in this model.

We thank Drs. Nora Sarvetnick and Irv Weissman for their comments on the manuscript, and Drs. Yoko Mullen and Masakazu Hattori for supplying the NOD mice from which our colony was derived. We also thank Paul Raju and Tim Knaak for support with the FACS® facility, and Robyn Kizer for her assistance with the preparation of the manuscript.

These studies were supported by the Juvenile Diabetes Foundation (J. A. Shizuru), and National Institutes of Health grant DK-39959.

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Received for publication 1 May 1991.

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