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GENETIC ANALYSIS OF DIABETES IN THE NONOBESE DIABETIC MOUSE

I. MHC and T Cell Receptor β Gene Expression[1]

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Backcross nonobese diabetic (NOD) ((NOD × SWR)F_1 × NOD) mice (108 females and 105 males) were typed for MHC, TCR Vβ, and monitored for 350 days for the onset of diabetes. The presence of "antipolar" antibodies in the sera and the occurrence of insulitis was examined in a proportion of these backcross mice. There was no difference in the incidence of diabetes in mice heterozygous for TCR $V\beta^{b/a}$ vs those homozygous for TCR $V\beta^{b/b}$. Among the 17 diabetics (all female) detected in this backcross, 14/17 were H-$2^{nod/nod}$ but 3/17 were H-$2^{nod/q}$. This supports a previous observation suggesting that the MHC-linked diabetogenic gene originally thought to be recessive may rather be dominant but have a low penetrance in the heterozygous state. Antipolar autoantibodies were found in both female and male backcross mice, and were similarly distributed in diabetic and nondiabetic mice. There appeared to be no correlation between the level of these autoantibodies and development of diabetes. The incidence and severity of insulitis was linked to MHC but no influence of TCR genes on insulitis nor an association between insulitis and antipolar antibodies could be demonstrated in this study. Further analyses of H-$2^{nod/nod}$ intercross mice homozygous for TCR $V\beta^a$ or TCR $V\beta^b$ are currently underway.

The NOD[4] mouse is studied as a model for human IDDM (1). Disease occurs principally in females, with onset after 12 wk of age. Outcross experiments have shown that at least three recessive genes from unlinked loci are required to produce diabetes (2–4). One of these maps to the MHC, and sequence analysis of MHC genes strongly suggests that the class II $A\beta^{nod}$ chain in mouse (and the HLA-DQβ chain in man) plays a central role in the induction of disease (5–7). Experiments from a number of groups have clearly shown that diabetes in NOD mice is the consequence of a T cell-mediated autoimmune response (8–15). One obvious possibility is that T cell expression of a particular TCR gene segment(s) is critically important for the induction of the disease. We approached this question by taking advantage of a major deletion within that segment of chromosome 6 carrying the TCR Vβ genes. The majority of inbred mouse strains have 20 to 30 TCR Vβ genes (16–20). A few strains, however, share a deletion involving at least 10 Vβ genes (21). Strains carrying this deletion have been given the haplotype $V\beta^a$, whereas strains with the full complement of Vβ gene segments have been designated $V\beta^b$. Binding experiments with TCR Vβ-specific mAb indicated that the NOD mouse carried the $V\beta^b$ haplotype (J. Danska and H. Acha-Orbea, personal communications). In this study, we looked at the incidence of diabetes and MHC association in $V\beta^{b/a}$ heterozygotes compared with $V\beta^{b/b}$ homozygotes in a large population of ((NOD × SWR)F_1 × NOD) backcross mice.

Inasmuch as these backcross mice were presumed to be segregating for at least three unlinked, recessive diabetogenic genes, and disease is expressed predominantly in female NOD mice, only a small proportion of the backcross was expected to become diabetic. We, therefore, also examined these mice for the appearance of insulitis and one characteristic autoantibody associated with disease, "antipolar" antibodies, to see whether any correlation was observed between TCR Vβ or MHC and these markers of disease in this outbred population. Insulitis is the major histopathologic lesion associated with diabetes and both incidence and severity of insulitis are linked to the MHC (4). We, therefore, examined these mice for the appearance of insulitis to confirm the MHC linkage in this outbred population. In addition, sera from a proportion of the backcross animals were tested for the presence of antipolar antibodies as it has been suggested (22) that these autoantibodies might act as a marker for the onset of type 1 diabetes. It has been previously demonstrated that sera from about 30% of new-onset type 1 diabetics and cytoplasmic islet cell Ag-positive relatives, as well as from 98% of NOD mice aged 1 wk to 6 mo, reacted in a polar fashion with tumor cells of the transplantable rat insulinoma RIN38. Control patients, normal mice, and (NOD × BALB/cJ)F_1 mice had no antipolar activity in their sera (22). The presence of antipolar antibodies was therefore determined for about 70% of the ((NOD × SWR)F_1 × NOD) backcross animals.

MATERIALS AND METHODS

Mice. NOD mice were maintained in a breeding colony in the conventional animal facility in the Department of Laboratory Animal

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[4] Abbreviations used in this paper: NOD, nonobese diabetic; IDDM, insulin-dependent diabetes mellitus; PFN, PBS plus 2% FCS and 0.1% sodium azide.

Medicine, Stanford University. SWR/J mice were purchased from The Jackson Laboratory, Bar Harbor, ME. (NOD × SWR)F_1 and [(NOD × SWR)F_1 × NOD) backcross mice were bred in the animal facility at Stanford.

Antibodies. BP107 (23) was purified from mouse ascites on a protein A column (Affi-Gel protein A maps II, Bio-Rad Laboratories, Richmond, CA) and conjugated to biotin by using standard procedures (24). FITC-conjugated KJ23 (25) and allophycocyanin-labeled anti Ly-1 (26) were generous gifts from Drs. C. Guidos and A. Stall, respectively.

Staining. A 0.5-ml blood sample was collected into 0.6 ml ice-cold Alsever's solution, mixed thoroughly, layered onto 1 ml Lympholyte M (Cedarlane Laboratories Ltd., Ontario, Canada), and centrifuged at 2000 rpm for 30 min at room temperature. Cells were harvested from the interface and washed once in ice cold PFN. All subsequent steps were carried out at 4°C. Any remaining E were lysed, and the cells washed twice more in PFN. They were then resuspended in 75 μl (or 125 μl for cells used as medium and second stage controls) HBSS plus 2% FCS, 0.1% sodium azide, and dispensed in 25-μl aliquots into round-bottomed microtiter wells for staining. MHC genotype was determined with biotin-labeled BP107. A 25-μl sample of antibody was added, and the cells were incubated for 15 min, washed twice with PFN, resuspended in 50 μl PFN containing a 1/800 dilution of avidin-Texas red, and incubated for 15 min. They were then washed three times and resuspended in 200 μl PFN plus propidium iodide. The percentage of $KJ23^+$ T cells was determined by double staining with FITC-KJ23 and allophycocyanin-anti-Ly-1. Then 25 μl of a mixture of these two antibodies was added to the cells, which were incubated in PFN for 15 min, washed three times in PFN, and resuspended in 200 μl PFN plus propidium iodide. Control wells (generally with NOD and SWR cells) received either medium alone, or medium followed by avidin-Texas red. Cells were analyzed on a FACScan (Becton Dickinson, Mountain View, CA).

Antipolar antibodies. Frozen sections of RIN38 tumor (5 μm) were fixed with acetone for 2 min at room temperature, then fixed with methanol for 1 min at room temperature. Sections were incubated with 30 μl of a 1/5 dilution of mouse sera in Tris buffer (pH 7.4) + 1% BSA, for 1 h at room temperature, washed with Tris buffer three times, and left in the third wash for 10 min. Sections were then incubated with 30 μl of 1/100 peroxidase conjugate protein A in Tris + 1% BSA at room temperature for 1 h with Tris buffer. The detecting color was developed by immersing the slides in 0.5 mg/ml diaminobenzidine tetrachloride dissolved in Tris buffer (pH 7.6) (0.5 M) containing 0.001% of 30% hydrogen peroxide (33 μl/100 ml) for 5 min. Slides were then washed for 5 min with tap water and sealed with AFT system (Behring Diagnostic, Los Angeles, CA).

Insulitis. Pancreata were obtained, fixed, and stained as previously described (4). Scores used to assess the degree of insulitis were: 0, no inflammatory cell observed; 1, periductal or perivascular inflammatory cells; 2, modest infiltrating cells in the pancreatic islets; and 3, severe inflammation of the islets (4).

Diabetes. Mice were bled after onset of polyuria. Plasma glucose was determined by a Beckman Glucose Analyzer II. They were considered diabetic when plasma glucose values exceeded 450 mg/dl.

RESULTS

[(NOD × SWR)F_1 × NOD) backcross animals were typed for MHC and TCR Vβ genotype by flow cytometry. $H\text{-}2^{nod/q}$ and $H\text{-}2^{nod/nod}$ mice were distinguished with the mAb BP107 (23), which binds to $I\text{-}A^q$ but not to $I\text{-}A^{nod}$ (5). $V\beta^{b/a}$ mice were distinguished from $V\beta^{b/b}$ mice by using the mAb KJ23, which binds to T cells expressing the Vβ17a gene product (25). The $V\beta^a$ haplotype has a functional copy of Vβ17a, whereas the homologous gene segment associated with the $V\beta^b$ haplotype contains a stop codon in the coding region, and is therefore inactive (27). SWR mice are $V\beta^a$ (25), whereas NOD mice appear, by typing with anti-TCR Vβ antibodies, to be $V\beta^b$ (J. Danska, personal communication). The use of KJ23 to distinguish $V\beta^a$ from $V\beta^b$ cells has one potential complication: Vβ17a cells show a very high frequency of reactivity to I-E molecules (25). To maintain self tolerance, mice carrying the $V\beta^a$ haplotype, but also expressing I-E, delete Vβ17a T cells intrathymically and have greatly reduced to undetectable levels of $KJ23^+$ peripheral T cells (28). However, the $H\text{-}2^q$ haplotype has defects in both Eα and Eβ expression (29, 30), whereas the NOD Eα gene has a well-characterized (31) 627-bp deletion (H. Acha-Orbea, personal communication). Thus the $H\text{-}2^q$ and $H\text{-}2^{nod}$ haplotypes have noncomplementing defects in I-E expression, so that parental, F_1 hybrid, and backcross mice are all I-E negative.

BP107 stained SWR and, at reduced levels, (NOD × SWR)F_1 peripheral blood cells, but not NOD cells (Fig. 1). Backcross animals were either negative (i.e., $H\text{-}2^{nod/nod}$) or positive at the reduced levels characteristic of $H\text{-}2^{nod/q}$ heterozygotes. As expected, T cells from NOD mice identified by high expression of Ly-1 (26) were $KJ23^-$, whereas SWR T cells were $KJ23^+$. Cells from (NOD × SWR)F_1 mice showed an unexpectedly low level (about 2%) of $KJ23^+$ T cells, compared with SWR (approximately 14%) (Table I). On the basis of allelic exclusion alone, these mice would be expected to have about 7% $KJ23^+$ T cells (32). The same low number of $KJ23^+$ T cells was seen in $H\text{-}2^{nod/q}$ ($BP107^+$) backcross animals (Table I), and $H\text{-}2^{nod/nod}$ ($BP107^-$), $KJ23^+$ backcross mice had even fewer $KJ23^+$ T cells, about half the number seen in the $H\text{-}2^{nod/q}$, $KJ23^+$ mice. Possible explanations for this are considered in the *Discussion.* Despite this low level of expression, there was no problem in distinguishing between $KJ23^+$ and $KJ23^-$ backcross mice.

Association of TCR Vβ genotype with IDDM. The results of BP107 and KJ23 typing assigned the backcross animals to one or other of the four expected genotypes: 1) $H\text{-}2^{nod/nod}$, $V\beta^{b/b}$; 2) $H\text{-}2^{nod/nod}$, $V\beta^{b/a}$; 3) $H\text{-}2^{nod/q}$, $V\beta^{b/b}$; and 4) $H\text{-}2^{nod/q}$, $V\beta^{b/a}$. One hundred eight female and 105 male mice were typed unambiguously. They were distributed fairly evenly among the four genotypes (Fig. 2), demonstrating the expected independent segregation of MHC (chromosome 17) and TCR Vβ (chromosome 6). Backcross mice were monitored for at least 350 days for the onset of diabetes. Only females (17/108) became diabetic (numbers shown in brackets in Fig. 2). Among the diabetic mice, 11/66 (16.7%) were $V\beta^{b/a}$ and 6/42 (14.3%) were $V\beta^{b/b}$. Thus, there was no association of diabetes with TCR Vβ haplotype.

MHC association of IDDM. The majority of diabetic females (14/17) were, as expected, $H\text{-}2^{nod/nod}$ homozygotes (2, 3), but 3/17 diabetics typed as $H\text{-}2^{nod/q}$. Retyping of these three mice, and of four of the $H\text{-}2^{nod/nod}$ homozygotes, confirmed the original MHC assignment. Unfortunately, all three $H\text{-}2^{nod/q}$ diabetic animals died, so we were unable to carry out a pedigree analysis. However, this result does support a previous study (4, 33) suggesting that the MHC-linked diabetogenic gene may in fact be dominant but with low penetrance in the heterozygous state, rather than recessive, as was originally reported (2, 3).

Lack of association of "antipolar" antibodies and IDDM. Sera from two-thirds of both female and male backcross mice were collected at approximately 60 days of age and were tested for the presence of antipolar antibodies (22). The results (Table II) show that levels of antipolar antibodies were distributed from negative to high within each of the four genotypes. The antibodies were found as frequently in males as in females, and in the same proportion in $H\text{-}2^{nod/nod}$ homozygotes and $H\text{-}2^{nod/q}$ heterozygous. Most strikingly, the female diabetics (numbers shown in parentheses in Table II) showed the entire range of antipolar antibody activity,

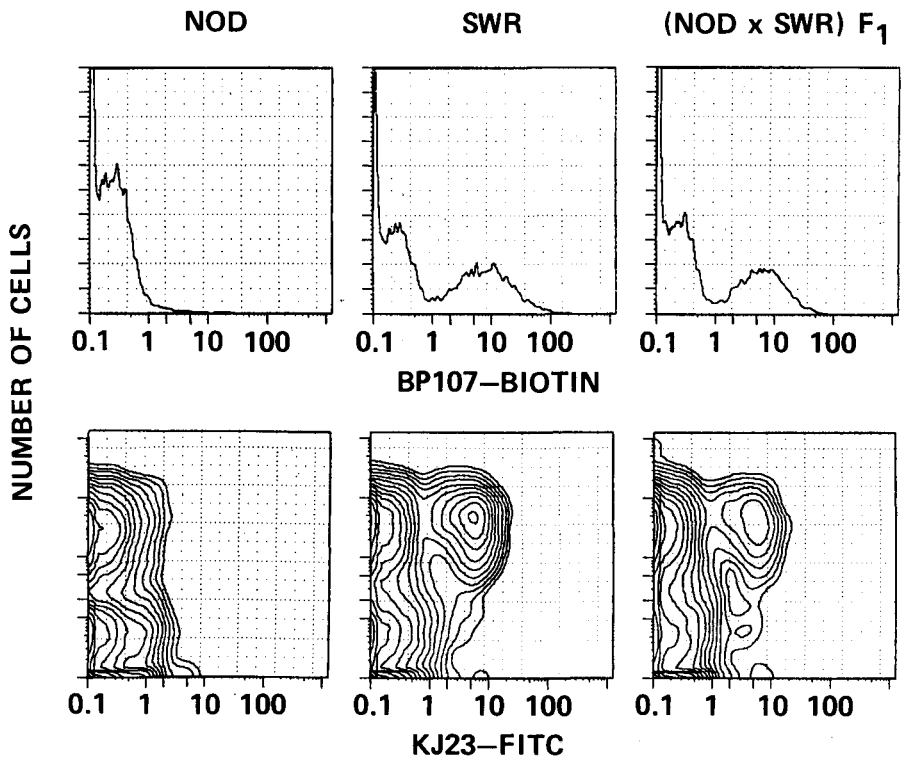

Figure 1. Flow microfluorimetry profile of PBL gated lymphocytes stained with anti IA^q (BP107) and anti-Vβ17a (KJ23) as described in *Materials and Methods.*

TABLE I

VB17a$^+$ T lymphocytes in (NOD × SWR) backcross mice[a]

Expt.	Controls		VB17a$^+$ Backcross	
	SWR	(NOD × SWR)F$_1$	H-2$^{nod/q}$	H-2$^{nod/nod}$
a	12.37% (1)	1.68% (1)	1.65% (7)	0.81% (4)
b	13.42% (1)	1.56% (1)	1.71% (5)	0.86% (3)
c	14.67% (1)	2.21% (1)	1.82% (5)	1.06% (4)
d	14.65% (1)	2.33% (1)	1.77% (3)	0.91% (5)

[a] Vβ17a$^+$ lymphocytes expressed as a percent of total T cells (see *Materials and Methods*) assessed by cytofluorimetry. Numbers in parenthesis are numbers of animals assayed per experiment.

from negative to extremely positive. Thus, from this study, there appeared to be no obvious correlation between the presence of antipolar antibodies and the development of diabetes.

MHC association of insulitis. Thirty-six female ((NOD × SWR)F$_1$ × NOD) backcross mice were sacrificed and their pancreata were examined by histopathologic methods to determine the association of insulitis with H-2 and TCR β gene families (Vβ^a vs Vβ^b). Previous studies (3, 4) had suggested that the MHC influenced both the incidence and severity of insulitis. Although MHC heterozygosity permitted low levels of inflammation, MHC homozygosity (H-2$^{nod/nod}$) correlated with fulminant insulitis (4, 34). Data presented in Figure 3 support this MHC association with insulitis levels but there was no evidence of any association between insulitis and TCR homozygosity for Vβ^b. Additionally, among those mice sacrificed for histopathology who had antipolar antibodies assays performed (31/36), no association was seen between insulitis and the autoantibody.

DISCUSSION

In this study, we monitored the incidence of diabetes in 108 female and 105 male ((NOD × SWR)F$_1$ × NOD) backcross mice typed for MHC and TCR Vβ genotypes. Among these animals, 72 females and 70 males were also tested for the presence in their sera of antipolar antibodies and 36 female mice were tested for the presence of insulitis. In our NOD colony, ≥80% of females and approximately 20% of males become diabetic within 250 days. In the backcross animals, diabetes was found

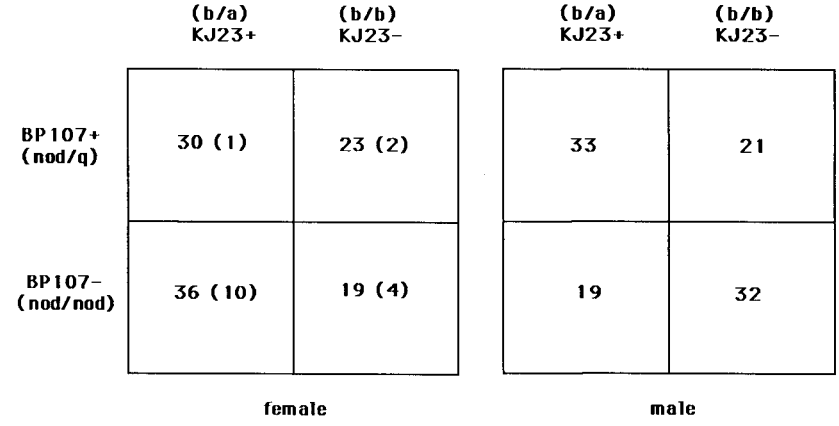

Figure 2. Numbers in each quadrant represent the number of mice (female or male) that are phenotyped by using described methods. Numbers in parentheses indicate the number of diabetic mice.

TABLE II

Distribution of polar antibody titers among ((NOD × SWR)F_1 × NOD) mice[a]

Polar Antibody	H-2$^{nod/nod}$ KJ23$^+$	H-2$^{nod/nod}$ KJ23$^-$	H-2$^{nod/q}$ KJ23$^+$	H-2$^{nod/q}$ KJ23$^-$
Males				
−	2	5	2	3
±	3	5	8	0
+	4	8	7	4
++	3	2	2	3
+++	2	5	1	1
Females				
−	3 (2)	0	2	2
±	4	4 (2)	5	5 (1)
+	5 (3)	3 (1)	6	8
++	4 (1)	3 (1)	7	1
+++	5 (1)	2	2	1

[a] Numbers in parenthesis indicate number of diabetic mice.

	$V\beta^{a/b}$		$V\beta^{b/b}$	
	# of animals	Insulitis score	# of animals	Insulitis score
H-2$^{nod/q}$	5/9	0	6/9	0
	2/9	1+	3/9	1+
	2/9	2+		
H-2$^{nod/nod}$	1/9	0	3/9	0
	2/9	1+	2/9	1+
	3/9	2+	0/9	2+
	3/9	3+	4/9	3+

Figure 3. Insulitis was determined by histopathological analysis of pancreata as described in *Materials and Methods*. Nine mice of each of the four phenotypes (H-2 and Vβ) were studied.

only in females, at a frequency (17/108) that agreed well with previous estimates from NOD outcrosses (2–4) of at least three independently segregating recessive diabetogenic genes.

The principal aim of this study was to determine whether any of the genes implicated in disease susceptibility mapped to that region of chromosome 6 present in the Vβ^b haplotype but deleted in the Vβ^a haplotype. We found that the incidence of disease in V$\beta^{b/a}$ heterozygotes was similar to that seen in the V$\beta^{b/b}$ homozygous mice. This result does not, of course, rule out the possibility that Vβ^b gene segments deleted in the Vβ^a haplotype play an essential role in the induction of diabetes. Models where T cells expressing a particular TCR gene segment precipitate disease generally work equally well whether the gene is present in the heterozygous or homozygous state. We are currently continuing to cross diabetic V$\beta^{b/a}$ females with NOD males in order to derive NOD congenic lines homozygous for Vβ^a and Vβ^b respectively.

This study also examined the relationship between the presence of antipolar antibodies (22) and insulitis and diabetes. Insulitis has been shown to be linked to the development of diabetes (4), and it has been suggested that antipolar antibodies might also be a useful marker for predisposition to disease. The original experiments on antipolar antibody expression (22) compared NOD mice, with a high incidence of diabetes, and (NOD × BALB/c)F_1 mice, with zero incidence. We took advantage of the predicted low frequency of diabetes in the backcross mice studied here to determine if there was any concordance between the presence of antipolar antibodies and disease. Analysis of two-thirds of the backcross population showed that these antibodies were present in both males and females, and in the same proportion in H-2$^{nod/nod}$ homozygotes and H-2$^{nod/q}$ heterozygotes (Table II). Those tested females that developed diabetes showed the entire spectrum of antipolar antibody activity, from negative to highly positive. Thus, in this study, there appeared to be no obvious correlation between the presence of serum antipolar antibodies and the development of disease.

Previous studies on the genetic control of insulitis in the NOD mouse used (NOD × C57BL/10) F_1, F_2, and (F_1 × NOD) backcross analyses (4). These studies suggested that a single non-MHC-linked gene controlled the development of insulitis but that the incidence and severity of insulitis were controlled by an MHC-linked gene (4). Since we have studied only backcross animals, we could not analyze the development of insulitis, but only the incidence and severity. Our studies confirm the previous demonstration that an MHC-linked gene(s) controls the incidence and severity of insulitis (4, 34) and further show that there is no association between insulitis and TCR V$\beta^{a/b}$ vs TCR V$\beta^{b/b}$ or with antipolar antibodies. Thus, among the H-2$^{nod/nod}$ mice, there was no difference in the expression of insulitis in TCR V$\beta^{a/b}$ compared with TCR V$\beta^{b/b}$ homozygote mice (Fig. 3). Furthermore, the entire spectrum of antipolar antibody activity was seen among the mice with insulitis irrespective of TCR Vβ genotype.

It was originally thought, from experiments looking at NOD outcross progeny (2, 3), that diabetes occurred only in mice homozygous for the NOD MHC. However, the experiments of Wicker et al. (4, 33) indicated that the MHC-linked diabetogenic gene was more likely dominant, but with low penetrance in heterozygotes. The results reported here support and extend this conclusion. We found that although 14/17 diabetic females were H-2$^{nod/nod}$, 3/17 were H-2$^{nod/q}$. One explanation for the occurrence of diabetes in animals apparently heterozygous for the NOD MHC is of course intra-MHC recombination, so that the mice in question would in fact be homozygous for the relevant MHC-linked gene. Wicker et al. decided from a pedigree analysis that this was not the explanation for their results (33). We were unable to set up pedigree analyses because the three H-2$^{nod/q}$ diabetic mice died. However, the fact that these mice were BP107$^+$ indicated that they were almost certainly I-A$\beta^{nod/q}$. BP107 binds to I-A^d and to I-A^q (23), but not to I-A^{nod} (5); as I-A^d and I-A^{nod} have identical Aα-chains (5), the inability of BP107 to bind to I-A^{nod} maps to the Aβ-chain. BP107$^+$ backcross mice therefore probably carried the Aβ^q gene. Sequence analyses have implicated Aβ^{nod} in the mouse, and HLA-DQβ in man, as the MHC-linked diabetogenic genes (5–7). If so, then it seems very likely that intra-MHC recombination can be discounted as an explanation for our results.

The frequency of disease in the first backcross MHC heterozygotes (3/17) in this study differs from the data of Wicker et al. (4, 33). In those studies, none of 29 female

first backcross H-2 heterozygote animals became diabetic by >7 mo (4). The most likely explanation for this difference lies in the strains selected for breeding. The SWR mice used in the present study were derived from the same Swiss outbred stock from which the NOD strain was derived (1, 35); whereas, Wicker et al. used C57BL/10 mice, which were derived from an entirely different founder stock (35). It is probable that the background of the Swiss mouse line may be more disease permissive and allows the higher level of expression of diabetes seen in our H-2 heterozygote mice.

In two additional reports studying NOD mice outcrossed with different mouse strains, diabetes was not seen in any of the mice heterozygous for H-2 (2, 3, 34). Why the difference between the results of these outcross experiments and those of Wicker et al. (4, 33) and ourselves? As discussed by Wicker and colleagues, the explanation again most probably lies in the selection of the strains used in the outcrosses. The first experiments used either C3H (2) or NON (3), both of which strains express a functional I-E molecule. The C57BL/10 strain used by Wicker et al and the SWR strain used in the present study are both I-E negative. Nishimoto et al. (36) showed that NOD mice carrying an Eα transgene (and therefore expressing a functional I-E molecule) failed to develop diabetes, and Reich et al. (37) have recently suggested an explanation for this. Several TCR Vβ gene segments preferentially recognize I-E, and so to maintain self tolerance, I-E$^+$ mice delete T cells expressing these particular Vβ genes intrathymically during T cell maturation (38–41). Reich et al. found that a number of T cell clones that could produce insulitis in NOD mice expressed Vβ5, one of the gene segments deleted in I-E$^+$ mice (40). They therefore proposed that I-E$^+$ mice were protected against disease by the intrathymic elimination of T cells that contributed to the development of diabetes. Because the Vβ5 gene family is among the gene segments deleted in the Vβ^a haplotype, we will be able to test his directly with the development of NOD-Vβ^a and NOD-Vβ^b strains, as described above.

Analysis of KJ23 expression in the backcross mice suggested that gene(s) derived from the NOD strain might cause the deletion of Vβ17a$^+$ T cells. The number of KJ23$^+$ T cells in (NOD × SWR)F_1 mice was surprisingly low compared with SWR mice. In our experiments, SWR mice had approximately 14% KJ23$^+$ T cells, a value in good agreement with that observed by others (25). However (NOD × SWR)F_1 mice had only about 2% KJ23$^+$ T cells, rather than 7%, as would be predicted simply by allelic exclusion of TCR β-chains (32). The H-2$^{nod/q}$, V$\beta^{b/a}$ backcross mice were very similar to the F_1 hybrids, while the number of KJ23$^+$ cells in the H-2$^{nod/nod}$, V$\beta^{b/a}$ backcross mice was reduced further still, to about 1% of peripheral T cells. The most likely explanation for this was that KJ23$^+$ cells were deleted during the induction of tolerance to NOD self Ag. The slightly higher level of KJ23$^+$ T cells in the H-2$^{nod/q}$, V$\beta^{b/a}$ mice compared with the H-2$^{nod/nod}$, V$\beta^{b/a}$ mice could be explained by the observation that I-A^q is particularly effective at positive selection of KJ23$^+$ T cells (42, 43), and in addition notably ineffective at deletion of particular TCR Vβ subsets via the recognition of Mls-like structure (44, 45). It could be argued that H-2^{nod} was particularly inefficient at the positive selection of KJ23$^+$ cells, but the very low level of KJ23$^+$ T cells in both H-2$^{nod/nod}$, V$\beta^{b/a}$ and H-2$^{nod/q}$, V$\beta^{b/a}$ mice was more consistent with a dominant deletional mechanism. A sizeable proportion of TCR Vβ gene segments confer reactivity to self Ag, and cells expressing these Vβ regions are deleted intrathymically to ensure self tolerance. TCR Vβ8.1 (44), Vβ6 (46, 47), Vβ3 (48, 49), Vβ9 (50) and Vβ7 (51) T cells are deleted in strains expressing Mls Ag, whereas Vβ5.1 (52), Vβ5.2 (41, 53), Vβ11 (38–40, 54), or Vβ17a (28) T cells are deleted in strains expressing a functional class II I-E molecule. It has been shown that deletion of Vβ5.2 (53), Vβ11$^+$ (40, 54) and Vβ17a$^+$ (28) T cells occur only in strains where particular non-MHC Ag, or cotolerogens, are expressed in addition to I-E. For instance, Vβ17a$^+$ T cells respond to a self Ag present on B cells, but absent from I-E$^+$ macrophages and from fibroblasts expressing transfected I-E molecules (55). However, this cannot explain the deletion of Vβ17a$^+$ T cells in the (NOD × SWR)F_1 and (NOD × SWR)F_1 × NOD backcross mice, because these animals, as discussed above, are I-E negative. We have recently obtained evidence that NOD mice may express Mls^c, because they have reduced levels of Vβ3 (J. Danska manuscript in preparation); moreover, it has been suggested that Mls^c can delete Vβ17a$^+$ T cells (M. McDuffie, manuscript submitted for publication). It is tempting to speculate that the reduced levels of Vβ17a$^+$ T cells in our F_1 and backcross animals are due to deletion of those cells reactive with an Mls-like structure. However, it has been suggested that deletion of particular T cell subsets may in fact protect against disease (37), and so this deletion mechanism may not be relevant to the development of diabetes in NOD mice. Resolution of the mechanism of Vβ17a$^+$ T cell deletion in the NOD × SWR cross will probably require the development of NOD × SWR H-2$^{nod/nod}$ and H-2$^{q/q}$ backcross or recombinant inbred strains.

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