Autoimmune Syndromes in Major Histocompatibility Complex (MHC) Congenic Strains of Nonobese Diabetic (NOD) Mice. The NOD MHC is Dominant for Insulitis and Cyclophosphamide-induced Diabetes

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Summary

The development of autoimmune diabetes in the nonobese diabetic (NOD) mouse is controlled by multiple genes. At least one diabetogenic gene is linked to the major histocompatibility complex (MHC) of the NOD and is most likely represented by the two genes encoding the α and β chains of the unique NOD class II molecule. Three other diabetogenic loci have recently been identified in the NOD mouse and are located on chromosomes 1, 3, and 11. In addition to the autoimmune diabetes which is caused by destruction of the insulin-producing β cells in the pancreas, other manifestations of autoimmunity are seen in the NOD mouse. These include mononuclear cell inflammation of the submandibular and lachrymal glands, as well as the presence of circulating autoantibodies. To determine the effect of the non-MHC diabetogenic genes on the development of autoimmunity, we constructed the NOD.B10.H-2b (NOD.H-2b) strain, which possesses the non-MHC diabetogenic genes from the NOD mouse, but derives its MHC from the C57BL/10 (B10) strain. The NOD.H-2b strain does not develop insulitis, cyclophosphamide-induced diabetes, or spontaneous diabetes. It does, however, develop extensive lymphocytic infiltrates in the pancreas and the submandibular glands that are primarily composed of Thy 1.2+ T cells and B220+ B cells. In addition, autoantibodies are present in NOD.H-2b mice which recognize the “polar antigen” on the insulin-secreting rat tumor line RINm38. These observations demonstrate that the non-MHC genes in the NOD strain, in the absence of the NOD MHC, significantly contribute to the development of autoimmunity. The contribution of a single dose of the NOD MHC to autoimmunity was assessed with a (NOD × NOD.H-2b)F1 cross. Although only ~3% of F1 females developed spontaneous diabetes, approximately 50% of both female and male F1 mice developed insulitis, and 25% of females and 17% of males became diabetic after treatment with cyclophosphamide. These data demonstrate that the MHC-linked diabetogenic genes of the NOD mouse are dominant with decreasing levels of penetrance for the following phenotypes: insulitis > cyclophosphamide-induced diabetes > spontaneous diabetes.

1 Abbreviation used in this paper: NOD, nonobese diabetic.
Materials and Methods

Animals. A breeding nucleus of inbred NOD mice was kindly provided by Dr. Yoshihiro Tochino (Aburabi Laboratories, Shionogi and Co., Osaka, Japan). After C57BL/10SnJ mice (Kβ, I-Aβ, Dd, and no expression of I-E) were crossed to C57BL/10SnJ mice (Kβ, I-Aβ, Dd, and no expression of I-E), we localized three diabetogenic genes, Idd-3, -4, and -5, on chromosomes 3, 11, and 1, respectively (2, 3). It is interesting that each of these diabetogenic genes did not function in a strictly recessive manner, but rather as dominant genes with reduced penetrance.

In other studies involving outcrosses of the NOD to the C3H and nonobese nondiabetic (NON) strains of mice, the MHC-linked diabetogenic gene of the NOD mouse appeared to function in an absolutely recessive manner since only NOD MHC (H-2b) homozygotes became diabetic (4). In contrast, in backcross generations of (NOD × B10) outcrosses to the NOD parental strain, a small percentage of H-2b mice became diabetic (1, 5). Analysis of the prevalence of diabetes in offspring produced from one diabetic MHC homozygote supported the hypothesis that the low incidence of diabetes in MHC homozygotes was not due to a rare recombination event, but was due to a low penetrance of a dominant MHC-linked diabetogenic gene in the heterozygous state (5). Insulitis was also observed in many MHC homozygotes in the backcross generations, suggesting that homozgyosity at the NOD MHC was not required for manifestations of subclinical autoimmunity. To assess the roles of the MHC-associated and the non-MHC diabetogenic genes in the autoimmune pathogenesis of the NOD mouse, we developed a MHC congenic strain on the NOD background, the NOD.H-2b, where the MHC has been derived from the normal B10 strain.

Results

Establishment of the NOD.H-2b Strain. After an outcross of the NOD strain to the B10 strain, repetitive backcrosses with NOD were performed with breeder selection based on the expression of I-Aβ as described in Materials and Methods. At the N6 generation, and later at the N12 generation, an intercross was performed to fix the H-2k haplotype on the NOD background. A representative MHC typing experiment (Fig. 1) demonstrates that the NOD.H-2b strain expresses Kk and I-Aβ, and is negative for NOD MHC class I and II antigens. Mice that were homozygous for the B10 MHC at the fifth (N6) and eleventh (N12) backcross intercross generations were used to establish the NOD.H-2k (N6) and NOD.H-2b (N12) strains, respectively, which were then maintained by brother-sister mating.

Prevalence of Diabetes in NOD.H-2b Mice. We have previously shown that by the N4 generation, the prevalence of diabetes in the NOD MHC homozygotes had reached frequencies observed in the NOD parental strain in our colony (5). These results imply that by the N4 generation, non-
Figure 1. Spleen cells from NOD, NOD.H-2\(b\), and B10 mice were tested for the presence of the K\(^b\) and K\(^d\) class I antigens and the I-A\(^b\) and I-A\(^d\) class II antigens as described in Materials and Methods. Each histogram includes the staining pattern obtained with the relevant counterstain (dashed lines).

MHC-linked recessive diabetogenic genes must have been fixed in the backcross population. Therefore, it is reasonable to assume that the non-MHC-linked recessive diabetogenic genes are also present in the NOD.H-2\(^b\) strains fixed at the N6 and N12 generations. Additional support for this hypothesis comes from the incidence of diabetes in the N6 and N12 intercross generations (Table 1). As anticipated, the frequencies of diabetes in NOD MHC homozygous females and males were not different from those observed in NOD females and males in our colony (80 and 50%, respectively). No spontaneous diabetes was observed in either the NOD.H-2\(^b\) or (NOD \times NOD.H-2\(^b\))\(F_1\) mice present in the N6 or N12 generations. In addition, no diabetes was observed among more than 100 female and 100 male NOD.H-2\(^b\) mice monitored for a minimum of 1 yr (data not shown). We previously reported (1, 5) a low incidence (~3%) of spontaneous diabetes in female (NOD \times NOD.H-2\(^b\))\(F_1\) mice, and presumably did not observe any diabetics in this population in the current study because of its relatively small size (Table 1, \(n = 23\)).

Pancreatic and Submandibular Gland Pathology in NOD.H-2\(^b\) Mice. Table 2 summarizes the pathology observed in the pancreata and submandibular glands of 22 female and 20 male NOD.H-2\(^b\) (N6) mice examined between 7 and 11 mo of age. None of the mice were diabetic. Three noncontiguous H and E stained sections of pancreas were examined by light microscopy for evidence of mononuclear cell inflammation. Histology scoring criteria: 0, no inflammatory cells present in the pancreas; 1, infiltrating cells observed in periductal and/or perivascular locations; 2, inflammatory cells observed at islet periphery; 3, mild inflammation of the islet in which <25% of the islet area contains infiltrating cells; and 4, moderate to severe inflammation of the islet. Classification of each animal was made using the most severe inflammatory lesion observed. For the examination of submandibular glands, three noncontiguous H and E stained sections were examined by light microscopy for evidence of mononuclear cell inflammation. Histology scoring criteria: 0, no inflammatory cells present; 1, mild inflammatory response in which only small accumulations of lymphoid cells are observed within the submandibular gland; 2, moderate inflammatory response in which multiple large periductal foci are observed; and 3, massive inflammatory response in which a majority of the submandibular gland is affected.

Table 1. Incidence of Diabetes in the NOD.H-2\(^b\) N6 and N12 Intercross Generations

| MHC        | N6 female | N6 male | N12 female | N12 male |
|------------|-----------|---------|------------|----------|
| NOD/NOD    | 7/9 (78%) | 1/6 (17%) | 5/5 (100%) | 5/10 (50%) |
| NOD/b      | 0/13      | 0/15    | 0/10       | 0/10     |
| b/b        | 0/7       | 0/6     | 0/6        | 0/1      |

MHC heterozygous mice from the fifth (N6) and eleventh (N12) backcross generations were intercrossed and the MHC type of the resulting progeny was determined. Intercross progeny were observed for the onset of diabetes for 8-12 mo.

Table 2. Pancreatic and Submandibular Gland Pathology in NOD.H-2\(^b\) Mice

|                | Pancreas score | Submandibular gland score |
|----------------|----------------|---------------------------|
|                | 0   | 1   | 2   | 3   | 4   | 0   | 1   | 2   | 3   |
| Females        |     |     |     |     |     |     |     |     |     |
|                | 2/22| 8/22| 11/22| 1/22| 0/22| 0/22| 1/22| 7/22| 14/22|
|                | (9%)| (36%)| (50%)| (5%)| (0%)| (0%)| (5%)| (32%)| (64%)|
| Males          |     |     |     |     |     |     |     |     |     |
|                | 3/20| 14/20| 3/20| 0/20| 0/20| 0/20| 2/20| 14/20| 4/20|
|                | (15%)| (70%)| (15%)| (0%)| (0%)| (0%)| (10%)| (70%)| (20%)|

NOD.H-2\(^b\) (N6) mice were examined between 7 and 11 mo of age. None of the mice were diabetic. Three noncontiguous H and E stained sections of pancreas were examined by light microscopy for evidence of mononuclear cell inflammation. Histology scoring criteria: 0, no inflammatory cells present in the pancreas; 1, infiltrating cells observed in periductal and/or perivascular locations; 2, inflammatory cells observed at islet periphery; 3, mild inflammation of the islet in which <25% of the islet area contains infiltrating cells; and 4, moderate to severe inflammation of the islet. Classification of each animal was made using the most severe inflammatory lesion observed. For the examination of submandibular glands, three noncontiguous H and E stained sections were examined by light microscopy for evidence of mononuclear cell inflammation. Histology scoring criteria: 0, no inflammatory cells present; 1, mild inflammatory response in which only small accumulations of lymphoid cells are observed within the submandibular gland; 2, moderate inflammatory response in which multiple large periductal foci are observed; and 3, massive inflammatory response in which a majority of the submandibular gland is affected.

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Autoimmunity in NOD.H-2b and (NOD × NOD.H-2b)F1 Mice
NOD.H-2\(^d\) (N6) mice between 7 and 11 mo of age. Peri-
ductal and perivascular lymphoid infiltrates (Fig. 2 A) were
frequently seen in the pancreas of both female and male mice
with females generally developing more severe inflammation.

Such inflammation was often extensive and in close prox-
imity to islets (Fig. 2 B), although insulitis, a lymphocytic
invasion of the islets present in almost all NOD mice, was
only occasionally observed (Fig. 2 C). When insulitis was
present, it was of a mild category, and usually only one islet was affected in an individual NOD.H-2b mouse.

The phenotypic composition of the pancreatic infiltrates was determined in selected mice with established lesions and was found to contain a mixture of Thy 1.2+ (Fig. 3) and B220+ (results not shown) lymphocytes. It is noteworthy that the lymphocytic infiltrates observed within the pancreata of NOD.H-2b mice were not seen in the parental B10 strain. The NOD.H-2b infiltrates do, however, resemble those in the NOD mouse except that in NOD mice the lymphocytes invade the islets and selectively destroy the β cells, whereas in NOD.H-2b mice, the infiltrates rarely penetrate the islets.

To evaluate the influence of age on the frequency and severity of insulitis in NOD.H-2b mice, pancreata from ten 16-mo-old female NOD.H-2b (N6) mice were examined. Only one islet from one of the ten mice examined displayed a mild form of insulitis. These data suggest that the frequency and severity of insulitis does not progressively intensify with age in NOD.H-2b mice.

Sialitis, loci of periductal lymphocytic infiltration in the submandibular glands, has been reported in NOD mice and could be transferred to syngeneic NOD neonates by splenic T cells (11, 12). Sialitis is also detected in most NOD mice from our colony by 6 mo of age and is not sex-restricted. We noted similar inflammatory responses in the submandibular glands of NOD.H-2b mice with lesions being more severe in females (Fig. 4) than in males. Multifocal lymphoid accumulations associated with large ductular elements were seen in the mixed serous-mucous glands of submandibular tissues. It is interesting that sialitis was not encountered in those portions of submandibular tissue comprised of either purely serous or mucous glandular components. Like the pancreatic inflammatory lesions, Thy-1.2+ and B220+ cells were the primary constituents of the submandibular gland infiltrates of the NOD.H-2b strain (results not shown). In contrast, although occasional small lymphoid accumulations were seen in the submandibular glands of B10 mice >7 mo of age, large infiltrates were never observed in B10 mice even up to 1 yr of age (unpublished observations).

Time-course of Pancreatic and Submandibular Gland Inflammation in NOD.H-2b Mice. Prospective analysis of the pancreatic and submandibular gland inflammation was carried out on male and female NOD.H-2b (N12) mice at 2, 4, 6, and 8 mo of age. The development and severity of the inflammatory responses in NOD.H-2b mice was found to be highly influenced by age. In mice that were 2 mo old, 2/5 females and 0/5 males displayed pancreatic infiltrates, while submandibular gland histology remained within normal limits in all mice. At 4 mo of age, 1/5 females and 1/5 males had pancreatic infiltrates, while submandibular infiltrates remained within normal limits in all mice. A 4 mo of age, 1/5 females and 1/5 males had pancreatic infiltrates while 4/5 females had developed sialitis.

Figure 4. Submandibular gland histology of a female NOD.H-2b mouse. Extensive mononuclear cell infiltrates are seen in the mixed serous-mucous component of the gland. Lymphatic vessels are distended by the resident lymphocytes contained within. Hematoxylin and eosin (x 50).
6 mo of age, a majority of male and female mice exhibited mild to moderate levels of inflammation in the pancreas and/or submandibular glands. 8-mo-old mice of both sexes nearly always displayed abnormal lymphoid accumulations in the pancreas and/or submandibular glands, and many of the mice had extensive areas of pathology (Table 2). A time-course for submandibular gland pathology, similar to that detailed above for the NOD.H-2b strain, was also seen in NOD mice from the current study (data not shown) and in a recent publication (12).

Detection of Autoantibodies Recognizing the Polar Antigen in NOD.H-2b Mice. In sera from 30% of new onset type 1 diabetic human patients and of prediabetics, but not of control subjects, autoantibodies have been detected which bind to the rat insulinoma line RINm38 in a polar fashion, i.e., cytoplasmic binding that is typically observed at the secretory pole of the insulinoma cells (13). In recent onset diabetics, the presence of antipolar antibodies was independent of the presence of cytoplasmic islet cell antibodies or of antiinsulin autoantibodies. The polar antigen-containing cells are usually at the periphery of islet tumor cell clusters, often adjacent to blood vessels or to fibrous connective tissue. The polar antigen is pronase sensitive, but acetone-, methanol-, and neuraminidase-resistant, suggesting it is a protein. Polar autoantibodies are also present in 100% of NOD mice >1 wk and <6 mo of age, but are not observed in normal inbred strains of mice including the B10 strain (13, and unpublished results).

NOD.H-2b mouse sera were evaluated for the presence of polar autoantibodies. Polar autoantibodies were found in 21/22 NOD.H-2b (N12) mice tested (Fig. 5). These results suggest that polar autoantibody production is not specific for mice with β cell destruction, and that polar antibodies do not mediate islet destruction. Even though the presence of the polar antibody is not diabetes specific, production of the polar autoantibodies in NOD.H-2b mice may be caused by one or more of the non-MHC–linked diabetogenic genes which, together with the NOD MHC, are responsible for the development of diabetes.

Time-course of Pancreatic and Submandibular Gland Inflammation in (NOD × NOD.H-2b)F1 Mice. As we reported earlier, (NOD × NOD.H-2b)F1, mice develop significant levels of insulitis and occasionally a small percentage spontaneously become diabetic (1, 5). Thus, one dose of the NOD MHC confers a dominant susceptibility for insulin and diabetes, however, there is only a low penetrance (~3%) for

Figure 5. Demonstration of polar antigen expression in a RINm38 cryostat section using indirect immunoperoxidase histochemistry. Dense intracellular inclusions are typically seen at the secretory pole of the insulinoma cells (×200).
Table 3. Prevalence of Insulitis in NOD and (NOD × NOD.H-2b)F1 Mice

| Age (wk) | Sex | NOD | (NOD × NOD.H-2b)F1 |
|----------|-----|-----|---------------------|
| 6        | F   | 2/3 | ND                  |
| 6        | M   | 2/3 | ND                  |
| 8        | F   | 3/3 | 0/10                |
| 8        | M   | 3/3 | 1/10                |
| 16       | F   | 3/3 | 0/10                |
| 16       | M   | 3/3 | 2/10                |
| 24       | F   | 3/3 | 5/10                |
| 24       | M   | 2/3 | 1/10                |
| 32       | F   | 2/3 | 6/10                |
| 32       | M   | 3/3 | 5/10                |

All mice were not diabetic at the time of the analysis. Each group of 10 male and female (NOD × NOD.H-2b)F1 mice was composed of five (NOD × NOD.H-2b)F1 and five (NOD.H-2b × NOD)F1 mice.

The time-course for the development of insulitis in (NOD × NOD.H-2b)F1 mice was compared with that of the NOD parental strain (Table 3). Although insulitis was observed as early as 8 wk of age in (NOD × NOD.H-2b)F1 mice, it was a rare event compared with the nearly complete incidence of insulitis in NOD mice at 6 and 8 wk. In addition, nearly all NOD mice display widespread insulitis between 2 and 4 mo of age, while (NOD × NOD.H-2b)F1 mice developed insulitis comparatively slowly, since only 3/40 (NOD × NOD.H-2b)F1 mice ≤4 mo of age exhibited insulitis. In addition, the total incidence of insulitis is only ~50% in (NOD × NOD.H-2b)F1 mice as compared with >95% in the NOD strain. Thus in (NOD × NOD.H-2b)F1 mice, islet pathology is very heterogeneous, ranging from no insulitis to widespread insulitis (Fig. 6). These observations suggest that the penetrance of a single dose of the NOD MHC varies in individual mice.

Cyclophosphamide Increases Diabetes in (NOD × NOD.H-2b)F1 but Not NOD.H-2b Mice. Cyclophosphamide increases the rapidity and incidence of diabetes in the NOD mouse. However, it does not cause normal strains of mice to develop insulitis or diabetes (14, 15). It is likely that cyclophosphamide disrupts the established regulatory balance.
of the immune system and allows the existing, although dormant, effectors of the autoimmune response to be reactivated. We therefore treated both NOD.H-2b and (NOD × NOD.H-2b)F1 mice with cyclophosphamide to determine if either insulitis or diabetes could be enhanced. Of the 17 NOD.H-2b female mice treated, insulitis was not observed, and none of the 17 developed diabetes (Table 4). This data suggests that despite the rare occurrence of mild insulitis in NOD.H-2b mice, this strain is not capable of generating β cell-specific effectors even after treatment with cyclophosphamide. In contrast, 23 and 17% of female and male (NOD × NOD.H-1b)F1 mice, respectively, developed diabetes when treated with cyclophosphamide. These results suggest that the insulitic infiltrates in F1 mice contain T cells that can become effectors of β cell destruction and that these cells are normally held in check in the (NOD × NOD.H-2b)F1 environment.

Table 4. Cyclophosphamide Induces Diabetes in NOD and (NOD × NOD.H-2b)F1 Mice but Not in NOD.H-2b Mice

| Strain                        | Sex    | No. diabetic/ total treated |
|-------------------------------|--------|----------------------------|
| (NOD × NOD.H-2b)F1           | Female | 13/56 (23%)                |
| (NOD × NOD.H-2b)F1           | Male   | 4/24 (17%)                 |
| NOD.H-2b                     | Female | 0/17                       |
| NOD.H-2b                     | Male   | ND                         |
| NOD                          | Female | 8/9 (89%)                  |
| NOD                          | Male   | 19/26 (73%)                |

Mice received 200 mg/kg of cyclophosphamide intraperitoneally on days 0 and 14. Mice were nondiabetic and 6-9 mo of age at the time of cyclophosphamide treatment. Diabetes was confirmed by histologic examination of the pancreas.

Discussion

An MHC-linked diabetogenic gene, Idd-1, was the first NOD chromosomal region identified as essential for the development of diabetes in the NOD mouse (1, 4, 16). It was also clear from these studies that regions outside the MHC contribute to the development of diabetes in the NOD mouse (1, 4, 16). In a backcross of (NOD × NON)F1 mice to NOD, a non-MHC-linked diabetogenic gene, Idd-2, was identified on chromosome 9 (4). More recently, in an outcross with the B10.H-2b strain followed by a backcross to the NOD parental strain, three additional diabetogenic genes, Idd-3, -4, and -5, were localized to chromosomes 3, 11, and 1, respectively (2, 3). Our long-term goal is to develop NOD strains that have B10-derived alleles at each of the Idd loci identified in the outcross with B10.H-2b. Such strains will be invaluable in deciphering the role each diabetogenic locus plays in the development of diabetes and other autoimmune manifestations in the NOD mouse.

The NOD.H-2b strain described in the current study could more accurately be termed the NOD.B10-Idd1 strain, since the number and nature of the MHC-linked diabetogenic genes ascribed to Idd-1 remains to be defined. Thus, this strain carries NOD alleles at all loci contributing to diabetes except Idd-1. We have demonstrated that this strain develops abnormalities not commonly associated with other strains of mice. In particular, lymphoid accumulations in the pancreas and the submandibular glands were seen in mice as young as 2 mo of age. These abnormalities increased in frequency and severity with age and nearly all NOD.H-2b mice showing such pathology by 8 mo of age.

The pathology seen in the NOD.H-2b pancreas was particularly striking. Large accumulations of lymphocytes were present and in some cases surrounded the islets. However, few, if any, lymphocytes actually penetrated into the islets making even mild insulitis a rare occurrence. These observations have led us to hypothesize that non-MHC genes on the NOD background cause a general failure in self-tolerance, and that one or more self-proteins within the pancreas and submandibular glands become the target(s) of an autoimmune response. Presumably these self-proteins are processed in antigen-presenting cells, and the resulting peptides bound and presented on MHC class II molecules to self-reactive T cells. In the NOD mouse, the class II gene product efficiently presents a β cell-derived peptide which stimulates a β cell-specific autoimmune response, while the class II product in the NOD.H-2b strain does not present an equivalent β cell-derived epitope. Alternatively, the non-MHC diabetogenic genes of the NOD may cause or allow lymphocytes to accumulate in the pancreas and submandibular glands, and only in the NOD mouse does a unique class II peptide complex stimulate a specific autoimmune response. Data supporting the first hypothesis include the description of autoantibodies specific for salivary ducts in the NOD mouse (12). Their presence suggests that the observed sialitis is at least partially focused on a specific antigen in this gland.

It is interesting that spontaneous infiltrates of both pancreatic and submandibular glands are observed in some strains of mice and under certain experimental procedures. In the autoimmune NZB strain, mononuclear cell infiltrates have been identified in the lung, liver, kidney, salivary glands, mesentery, and pancreas (17). A second autoimmune strain, MRL/lpr, also develops inflammatory cell infiltrates in the salivary glands (18). These lesions are primarily composed of CD4+ T cells, many of which express class II antigens. In addition, Hayashi et al. (19) found that aged C57BL/6 mice develop spontaneous organ-specific autoimmune lesions in the salivary glands, kidney, pancreas, lung, and liver. This pathology was first observed at 6 mo, and increased in incidence and severity as mice were monitored up to 2 yr of age. The infiltrating cells in the salivary glands were composed mainly of CD4+ T cells. These authors hypothesized an age-related disruption of regulatory T cells resulting in a breakdown of self-tolerance to certain organ-specific antigens. In a cyclosporin A-induced model of autoimmunity, neonatal mice develop thyroiditis, insulitis, adrenitis, sialitis, oophoritis or orchitis,
and gastritis (20). With this experimental system, genetic factors appeared to influence the tissues selected for immune attack. BALB/c mice developed mainly gastritis and oophoritis, whereas other organ-specific autoimmune diseases predominated in other strains. Therefore, a failure in self-tolerance as manifested by a variety of organ-specific autoimmune responses can result from a number of mechanisms: various genetic predispositions, aging, experimental alteration of proper thymic education, etc.

Thus, from the above discussion, although the development of sialitis and pancreatic infiltrates is not unique to the NOD.H-2b strain, it is likely that the relatively early, spontaneous appearance of such lymphocytic infiltrates in these tissues represent the activity of genes responsible for the autoimmune background in the NOD strain. This autoimmune background, in conjunction with the NOD MHC region, results in the selective targeting of the pancreatic $\beta$ cells for destruction.

The development of the NOD.H-2b strain has also enabled us to examine the effect of a single dose of the NOD MHC in the presence of NOD homozygosity at all of the non-MHC diabetogenic loci. Depending on the phenotype examined in the (NOD $\times$ NOD.H-2b)$F_1$, we observed that the NOD MHC can be considered dominant with high penetrance (insulitis), dominant with moderate penetrance (cyclophosphamide-induced diabetes), or dominant with low penetrance (spontaneous diabetes). Therefore, the NOD MHC clearly is active when present at a single dose suggesting that the $\beta$ cell-derived peptide mentioned above is able to associate with the NOD class II product in the (NOD $\times$ NOD.H-2b)$F_1$ strain. However, the islet-specific immune response is more protracted as evidenced by the delay in the onset of insulitis in those (NOD $\times$ NOD.H-2b)$F_1$ mice that eventually develop pathology (Table 3). Conceivably, reduced surface expression of pathogenic NOD class II-$\beta$ cell peptide complexes is achieved on antigen-presenting cells of (NOD $\times$ NOD.H-2b)$F_1$, as compared with NOD, resulting in a less vigorous autoimmune response. A recent report from Livingstone et al. (21) suggests that the NOD MHC has a higher penetrance for spontaneous diabetes in combination with the H-2$^v$ haplotype. In their study, 3/19 diabetic (NOD $\times$ SWR)$F_1 \times$ NOD first backcross mice were MHC heterozygotes. It is possible that the concentration of the pathogenic NOD class II-$\beta$ cell peptide complexes is higher in H-2$^v$/$\beta$ than in H-2$^b$/$\beta$ antigen-presenting cells.

It is intriguing that while spontaneous diabetes is rare in (NOD $\times$ NOD.H-2b)$F_1$ mice (1, 5), cyclophosphamide-induced diabetes occurs in $\sim$25% of the treated mice (Table 4). This suggests that cyclophosphamide modulates an immune regulatory event that normally slows or stops the progression of insulin in the (NOD $\times$ NOD.H-2b)$F_1$ strain. The observation that cyclophosphamide treatment of NOD.H-2b mice did not result in widespread insulitis or diabetes (Table 4) is consistent with the hypothesis that in the absence of at least one dose of the NOD MHC, $\beta$ cell-specific autoimmune T cells are not generated and thus cannot be upregulated by cyclophosphamide.

It is interesting to compare the NOD.H-2b congenic strain with the NOD.N-H-2K$^b$ strain described by Prochazka et al. (22). The MHC of this congenic strain was derived from the NON strain which expresses in I-E product. In this MHC congenic strain, only focal aggregates of leukocytes were detected and occasionally some of the aggregates were observed adjacent to an islet. This minimal pathology seen in 10-mo-old NOD.N-H-2K$^b$ mice contrasts sharply with the extensive accumulations of $T$ cells observed in older NOD.H-2b mice. Perhaps the presence of an I-E product in the NOD.N-H-2K$^b$ strain reduces the level of the non-$\beta$ cell-specific autoimmune response as compared with the NOD.H-2b strain which does not express an I-E product. A dramatic reduction in the incidence of insulitis and diabetes in NOD mice expressing an I-E transgene has been reported by several groups (23, 24).

The observation that NOD.H-2b mice produce polar autoantibodies emphasizes the potential utility of constructing congenic strains for each of the non-MHC diabetogenic genes involved in this complex disease. Since NOD.H-2b mice do not develop insulitis or diabetes but still produce polar autoantibodies, the presence of polar antibodies does not appear to reflect an ongoing $\beta$ cell-specific autoimmune response for expression. Thus, the polar antibody phenotype may be a subclinical or nonpathogenic manifestation of a genetic abnormality common to both NOD mice and human diabetics that contributes to overt disease by a currently undefined mechanism. If the NOD allele is replaced by the B10 allele at one of the non-MHC-linked diabetogenic loci, and production of polar antibodies is absent, this phenotype may act as a functional marker for that diabetogenic gene.

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