The Role of Lymphocyte Subsets in Accelerated
Diabetes in Nonobese Diabetic–Rat Insulin Promoter–B7-1
(NOD-RIP-B7-1) Mice

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Summary

B7-1 transgene expression on the pancreatic islets in nonobese diabetic (NOD) mice leads to accelerated diabetes, with >50% of animals developing diabetes before 12 wk of age. The expression of B7-1 directly on the pancreatic β cells, which do not normally express costimulator molecules, converts the cells into effective antigen-presenting cells leading to an intensified autoimmune attack. The pancreatic islet infiltrate in diabetic mice consists of CD8 T cells, CD4 T cells, and B cells, similar to diabetic nontransgenic NOD mice. To elucidate the relative importance of each of the subsets of cells, the NOD–rat insulin promoter (RIP)-B7-1 animals were crossed with NOD.β2microglobulin −/− mice which lack major histocompatibility complex class I molecules and are deficient in peripheral CD8 T cells, NOD.CD4 −/− mice which lack T cells expressing CD4, and NOD.μMT −/− mice which lack B220-positive B cells. These experiments showed that both CD4 and CD8 T cells were necessary for the accelerated onset of diabetes, but that B cells, which are needed for diabetes to occur in normal NOD mice, are not required. It is possible that B lymphocytes play an important role in the provision of costimulation in NOD mice which is unnecessary in the NOD-RIP-B7-1 transgenic mice.

Key words: nonobese diabetic mice • rat insulin promoter–B7-1 transgene • accelerated diabetes • T cells • B cells

1 Insulin-dependent diabetes in the nonobese diabetic (NOD) mouse occurs as a result of an autoimmune attack on pancreatic β cells. Although T cells play a very important role in the process, and both CD4 and CD8 T cells are required to adoptively transfer diabetes (1, 2), other cell types are clearly also important in the pathogenesis of disease.

Compelling evidence for the role of CD8 T cells in the initiation of diabetes comes from studies where the β2microglobulin (β2m) −/− mutation was bred onto the NOD background (NODβ2mnull), generating mice in which MHC class I expression is deficient (3, 4); consequently, these mice have very poor development of CD8 T cells. These NODβ2mnull mice do not develop either insulitis or diabetes (3–6). In addition, treatment of NOD mice with antibodies against CD8 T cells, when given before 5 wk of age, prevents both insulitis and diabetes (7). Further support for the role of CD8 T cells in initiation of disease has come from studies that demonstrate that T cells from young pre-diabetic NOD mice can transfer diabetes to MHC class I–positive NOD.SCID mice but not NOD.SCID mice with the β2mnull mutation lacking MHC class I (8). It is clear that CD8 T cells also play a role in the final effector phases of diabetes. This is shown by the fact that development of diabetes is delayed when diabetic spleen cells are adoptively transferred into NODβ2mnull mice, but there was no delay in disease transfer into NODβ2mnull mice bearing β2m on the rat insulin promoter (RIP), which express MHC class I exclusively on the pancreatic β cells (9).

CD4 T cells are also clearly important in the evolution of insulitis and for diabetes to occur, as it has also been shown that both insulitis and diabetes are prevented using antibodies against CD4 (10). Although adoptive transfer experiments have shown, for the most part, that both CD4 and CD8 T cells are required for diabetes to occur (1, 2), both CD4 T cells (11) and CD4 T cells (12) are able to transfer disease when given alone to NOD.SCID animals, which lack lymphocytes. T cell receptor transgenic mice that bear a single specificity of CD4 T cells, which are reactive to pancreatic β cells, having been crossed with recom-
binase activating gene (RAG)-2 -/- mice on the NOD background, develop accelerated diabetes (13). In addition, NOD mice crossed with mice that lack MHC class II molecules and are therefore deficient in CD4 T cells do not develop insulitis (5). However, this experiment is not easily interpreted, since these mice also have altered MHC class I molecules. This is because the MHC class II knockout mutation is made directly into I-A<sup>b</sup>, and therefore, when NOD mice are crossed with I-A<sup>b</sup> -/- mice, they will have the MHC class I molecules K<sup>b</sup> and D<sup>b</sup> (rather than K<sup>d</sup> and D<sup>b</sup> in NOD). CD4 T cells are clearly involved in the development of disease, as adoptive transfer of diabetic CD4 T cells alone into NOD.SCID animals will eventually cause disease, whereas isolated CD8 T cells do not (14). However, when cells from younger NOD donors are used, both CD4 and CD8 T cells are required.

B cells also play a role in the development of diabetes (15–17), the lack of B cells preventing development of diabetes. Although B cells are not required for adoptive transfer of diabetes (18), unable to cause disease when transferred alone to NOD.SCID mice (our unpublished observations), and serum does not transfer disease, B cells clearly influence the development of diabetes in a manner that probably relates to their antigen-presenting function. However, the precise role these cells play is still to be elucidated.

We have previously described NOD mice that express the costimulatory molecule B7-1 using the RIP on the pancreatic <b> cells, which develop clearly accelerated diabetes, with >50% of mice developing diabetes before the age of 12 wk when their nontransgenic littermates are just beginning to develop disease (19). This phenomenon is seen as early as the first backcross generation in mice that are homozygous for H-2<sup>b</sup> and B7-1 transgene positive. Diabetes in such mice occurs as early as 3 wk of age, and when examined histologically, the infiltrate is very similar to that seen in NOD mice, with large numbers of CD4 and CD8 T cells as well as a substantial number of B220-positive B cells (19). This study aimed at examining the role of CD4 and CD8 T cells, as well as B cells, in the accelerated model of diabetes of the NOD-RIP-B7-1 transgenic mice and comparing them to the role of these cells in the nontransgenic NOD mouse. We postulate that the B7-1-expressing β cells are a potent stimulus for CD8 T cells that are specific for β cell peptides presented by MHC class I molecules. When β cells are attacked by CD8 T cells, they release soluble antigens that are taken up by antigen-presenting cells which then present peptide to CD4 T cells in a similar manner to that found in the nontransgenic NOD mouse.

Materials and Methods

Mice. NOD-RIP-B7-1 transgenic mice were generated by crossing C57BL/6 mice transgenic for the human B7-1 molecule with NOD/Caj mice from our colony (19). Mice used in the experiments were all homozygous for H-2<sup>b</sup>, ascertained at the first backcross, and were used at the fourth backcross generation to NOD. NOD<sup>B2m<sup>m<sup>nu</sup>nu</sup> mice at the ninth backcross generation were provided by Linda Wicker (Merck, Rahway, NJ) and David Serreze (The Jackson Laboratory, Bar Harbor, ME). CD4 -/- mice on H-2<sup>b</sup> background (20) were bred with NOD/Caj mice and then backcrossed for four generations, μMT -/- mice lacking B cells (21) were obtained originally from Klaus Rajewsky (University of Cologne, Germany) and backcrossed for nine generations onto NOD/Caj mice. The mice were all housed in specific pathogen–free conditions. NOD.SCID animals were obtained originally from The Jackson Laboratory, and NOD.SCID-RIP-B7-1 mice were generated by crossing the NOD-RIP-B7-1 transgenic mice with NOD.SCID mice at The Jackson Laboratory (by David Serreze) and backcrossed to NOD.SCID mice. All the NOD.SCID-RIP-B7-1 mice used in the experiments were heterozygous for the RIP-B7-1 transgene. All the animal studies were performed under protocols approved by the Yale University Animal Care and Use Committee.

Breeding Scheme. The mice bearing the knockout mutations were bred initially onto the NOD-RIP-B7-1 transgenic mice. The F1 mice are all heterozygous for the knockout mutation and are either transgene positive (heterozygous) or transgene negative. F1 mice positive for the B7-1 transgene were then intercrossed with F1 mice which were B7-1 transgene negative, in order not to generate mice that were homozygous for the B7-1 transgene. The frequency of mice from this cross that were homozygous for the knockout mutation, were homozygous for H-2<sup>b</sup>, and had the B7-1 transgene was 2 out of 32.

Diabetes Screening. Animals were tested weekly for glycosuria using Diatix (Bayer Corp., Elkhart, IN), and if present, diabetes was confirmed by a blood glucose measurement using One Touch test strips (LifeScan, Inc., Milpitas, CA) of >250 mg/dl (13.9 mmol/liter).

Genotyping. The presence or absence of the hB7-1 transgene was determined by PCR on tail DNA using the following primers made in the Keck Facility (Yale University): 5′ TGA AGC CAT GGG CCA CAC and 5′ GAC ACT GTT ATA CAG GGC.

Typing for the various null mutations was carried out using PCR for neomycin to identify the presence of the mutation, and then staining of peripheral blood with mAbs to identify homozygous mice as follows. Heterozygous carriers of the β<sup>2m<sup>nu</sup>nu</sup> allele, CD4 -/- allele, and μMT -/- allele were identified using the following primers specific for the neomycin in the knockout mutation: 5′ GCC ACA ACA GAC AAT CGG CT and 5′ CCT GAT GCA CTT CGT CCA GA. Homozygosity for the β<sup>2m<sup>nu</sup>nu</sup> gene was tested for by staining peripheral blood lymphocytes with FITC-conjugated anti-CD8 (GIBCO BRL, Gaithersburg, MD). Homozygosity for the CD4 -/- mutation was tested for by staining with FITC-conjugated anti-CD4 (GIBCO BRL). Homozygosity for the μMT -/- mutation was tested for by staining of peripheral blood lymphocytes with FITC-conjugated anti-mouse Ig (Sigma Chemical Co., St. Louis, MO). The mice were screened for homozygosity for H-2<sup>b</sup> by testing for the absence of K<sup>b</sup> using the mAb Y-25 and FITC-conjugated anti-mouse IgG (Sigma Chemical Co.) followed by flow cytometric analysis as described previously (19).

Histology. Pancreatic tissue was fixed in formalin, paraffin embedded, and stained with hematoxylin and eosin. The sections were examined microscopically, and insulitis of individual islets was assessed by two independent observers according to the scale of 0 to 4 as follows: 0, no insulitis; 1, peri-insulitis; 2, perisinusitis with some insulitis; 3, >50% of the islet infiltrated; and 4, complete islet destruction. Additionally, some of the pancreata were fixed in periodate-lysine-paraformaldehyde, sucrose infused, and then frozen in Tissue-Tek OCT (Bayer Corp.). Sections (7 mm thick) were stained with biotinylated YT 4.3 antibody recognizing
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CD4, TIB 105 antibody recognizing CD8, and B220 antibody which stains the majority of B cells. The color was developed using diaminobenzidine tetrahydrochloride and nickel ammonium sulphate. The sections were then counterstained with hematoxylin.

Adoptive Transfer Studies. Spleens were removed from 6- and 12-wk-old female NOD mice that were nondiabetic (ascertained by blood glucose measurement of <250 mg/dl), and recently diabetic NOD mice (ascertained by a blood glucose measurement of >250 mg/dl).

Splenocytes (2 × 10⁷ cells) were adoptively transferred to female NOD.SCID and NOD.SCID-RIP-B7-1 mice, and the onset of diabetes was monitored, initially by checking for glycosuria and confirmed by measurement of blood glucose. At the onset of diabetes, the animals were killed, and the pancreas was removed for immunohistochemistry. In addition, 10⁷ CD8 NOD-derived islet-reactive cloned T cells (11) were also adoptively transferred to these groups of mice.

Results

NOD-RIP-B7-1/β₂mnull Mice Develop a Low Incidence of Accelerated Diabetes. NOD-β₂mnull mice have <1% peripheral CD8 T cells but do not develop diabetes, as reported previously (3, 4, 6). NOD-RIP-B7-1/β₂mnull also had

Figure 1. Mice were observed and screened for glycosuria. All mice which had glycosuria had a blood glucose measurement taken using One Touch test strips (LifeScan, Inc., Milpitas, CA), and diabetes was diagnosed if the blood glucose was >250 mg/dl. The graph shows the percentage of diabetes in mice that were RIP-B7-1 transgene positive and β₂m sufficient (filled circles), n = 41; RIP-B7-1 transgene positive and β₂m deficient (open circles), n = 16; RIP-B7-1 transgene negative and β₂m sufficient (filled triangles), n = 46; and RIP-B7-1 transgene negative and β₂m deficient (open triangles), n = 16. The numbers indicate the total population of both male and female mice.

Figure 2. Pancreatic section from an NOD-RIP-B7-1/β₂mnull mouse that was nondiabetic is shown (top), illustrating staining with anti-CD4, anti-CD8, and anti-B220 antibodies at 24 wk, and showing that when diabetes did not occur, there was no insulitis. (Middle) Staining from one of the two NOD-RIP-B7-1/β₂mnull mice that became diabetic, showing the presence of CD4 T cells and B cells. (Bottom) Staining of a pancreatic section from a diabetic NOD-RIP-B7-1/β₂m-sufficient mouse.
<1% CD8 T cells in peripheral blood, compared with 5–10% total lymphocytes in the β2m-sufficient mice (data not shown), and 14 out of 16 mice failed to develop diabetes (Fig. 1), even when followed until 24 wk of age. Histology showed that these mice also did not develop insulitis (Table 1, and Fig. 2). However, surprisingly, 2 out of 16 mice that were NOD-RIP-B7-1/β2mnull developed accelerated diabetes (Fig. 1). In the two animals that developed diabetes, the islet infiltrate was made up of CD4 T cells and B cells (Fig. 2), compared with NOD-RIP-B7-1 animals, which develop diabetes in which the infiltrate consists of CD8 and CD4 T cells as well as B cells.

NOD-RIP-B7-1/CD4−/− Mice Do Not Develop Accelerated Diabetes. NOD mice crossed with CD4−/− to the fourth backcross generation do not develop either insulitis or diabetes (Fig. 3). When NOD.CD4−/− mice were crossed with NOD-RIP-B7-1 mice, no diabetes occurred before the age of 12 wk. After 12 wk, some mice (mainly males) did develop diabetes (Fig. 3). In contrast to the NOD.CD4−/− mice that are RIP-B7-1 transgene negative, where no insulitis is seen, even in the NOD-RIP-B7-1/CD4−/− mice that did not develop diabetes, insulitis was seen, as shown in Fig. 4. The insulitis scores are shown in Table 1. Although these mice lack the CD4 coreceptor, it has been reported previously that there is an increased number of double-negative CD3+ T cells in CD4−/− mice that may play the role normally taken by CD4 T cells. In NOD-RIP-B7-1/CD4−/− mice, many of the islet-infiltrating CD3+ cells are stained with anti-CD8.

NOD-RIP-B7-1/μMT−/− Mice Develop Accelerated Diabetes. We have shown that NOD.μMT−/− mice at the ninth and tenth backcross generation to NOD, which are homozygous for all the NOD susceptibility markers described (15), do not develop diabetes (our unpublished observations). In contrast, when the mice are crossed with the NOD-RIP-B7-1 mice, they develop diabetes very rapidly, and absence of B cells in this model does not impair the development of the accelerated diabetes (Fig. 5). Histology shows that NOD.μMT−/− mice develop some insulitis by the age of 30 wk, although they do not develop diabetes (Fig. 6). In the NOD-RIP-B7-1 mice lacking B cells, histology at the time of diabetes shows that there is a large number of CD4 and CD8 T cells present. There are a few B220-positive cells that are not B cells but rather B220T cells, such as those seen in MRL/lpr/lpr mice, as shown by the absence of staining for Ig (Fig. 6). NOD.SCID-RIP-B7-1 Mice Develop Diabetes More Rapidly After Adoptive Transfer of Splenocytes and CD8 Cloned T Cells Than NOD.SCID Mice. Splenocytes from 6-wk-old prediabetic NOD mice are able to transfer diabetes to NOD.SCID mice in 6–10 wk. This time course is accelerated by ~3 wk, when the cells are transferred into NOD.SCID-RIP-B7-1 mice (Fig. 7a). A similar result is seen when just prediabetic or diabetic spleen cells are transferred into NOD.SCID mice compared with NOD.SCID-RIP-B7-1 mice, in that diabetes is accelerated in the latter recipients (Fig. 7b and c). When CD8 T cell clones (11), which respond to an undefined β cell antigen, are transferred, diabetes is also accelerated in the NOD.SCID-RIP-B7-1 mice, showing that these cells can be costimulated in vivo (Fig. 7d). Histology indicates that there is increased presence of CD8 T cells bearing Vβ6 in the infiltrate of the diabetic NOD.SCID-RIP-B7-1 mice (Fig. 8). Staining with anti-Vβ8 did not show any excess of these cells in either the diabetic NOD.SCID-RIP-B7-1 mice or the diabetic NOD.SCID mice (Fig. 8).

### Table 1. Insulitis Scores in NOD-RIP-B7-1/β2mnull and NOD-RIP-B7-1/CD4−/− Mice

| Insulitis score | Mice                                                        |
|-----------------|-------------------------------------------------------------|
|                 | NOD-RIP-B7-1/β2mnull                                      |
|                 | NOD-RIP-B7-1/CD4−/−                                       |
| 0               | 86                                                          |
| 1               | 6                                                           |
| 2               | 23                                                          |
| 3               | 13                                                          |
| 4               | 35                                                          |

Insulitis of individual islets was assessed according to the scale of 0 to 4 as follows: 0, no insulitis; 1, periinsulitis with some infiltrate; 2, periinsulitis; 3, >50% of the islet infiltrated; 4, complete islet destruction. Insulitis in NOD-RIP-B7-1/β2mnull mice was assessed in 86 islets from 10 nondiabetic mice. Insulitis in NOD-RIP-B7-1/CD4−/− mice was assessed in 77 islets from 9 nondiabetic mice.

### Figure 3. The graph shows the percentage of diabetes in mice that were RIP-B7-1 transgene positive and CD4 sufficient (filled circles), n = 71; RIP-B7-1 transgene positive and CD4 deficient (open circles) n = 23; RIP-B7-1 transgene negative and CD4 sufficient (filled triangles), n = 56; and RIP-B7-1 transgene negative and CD4 deficient (open triangles), n = 18. The numbers indicate the total population of both male and female mice.

### Discussion

We have shown that pancreatic islets of NOD-RIP-B7-1 mice are very potent stimulators of CD8 T cells, and the use of these islets has allowed us to clone CD8 T cells that are able to very rapidly transfer diabetes in the absence of CD4 T cells (11). We had noted that in the original NOD-
RIP-B7-1 mice, there was no difference in the incidence of diabetes in male and female animals, unlike the female preponderance seen in NOD mice (19). This lack of sex difference was also seen in the NOD-RIP-B7-1 mice that were homozygous for the CD4−/− and μMT−/− mutations. In the NOD-RIP-B7-1 accelerated model of diabetes, we postulate that on β cells, which normally do not express costimulatory molecules even when exposed to cytokines (reference 22, and our unpublished data), the provision of the B7-1 molecule has increased the capacity of CD8 T cells to initiate damage to the islets, accelerating the process that normally takes 12 wk to develop in the NOD mouse. Indeed, under normal circumstances, as β cells do not express costimulatory molecules, the initiation process would be expected to occur outside the islets, perhaps in the peripancreatic lymph nodes, with antigens shed from the islets and presented by dendritic cells (23). As cells are stimulated most optimally when the antigen is presented together with the costimulatory molecules on the same cell (24), the B7-1-expressing β cells are a potent stimulus for CD8 T cells that are specific for β cell peptides presented by MHC class I molecules. This may allow more T cells to become activated or provoke local clonal expansion of these autoreactive T cells, or alternatively become activated earlier than in the NOD mouse. There are several possible explanations for the fact that diabetes was seen in 2 out of 16 NOD-RIP-B7/β2mnull mice, but has not been found in NODβ2mnull mice. It has been documented that H-2Db is less dependent on β2m than other MHC class I molecules, and that although reduced, the Db molecule is expressed even in the absence of endogenous β2m (25).

Figure 4. (Top) Pancreatic sections from a non-RIP-B7-1 transgenic mouse that was CD4 deficient. All these mice were nondiabetic, and there was no insulitis seen, as illustrated by staining with anti-CD4, anti-CD8, and anti-B220 antibodies at 24 wk. The islets are intact, as shown by staining with insulin (red). (Middle) Staining from an NOD-RIP-B7-1/CD4−/− mouse that did not become diabetic. (Bottom) Staining of a pancreatic section from a diabetic NOD-RIP-B7-1/CD4-sufficient mouse.

Figure 5. The graph shows the percentage of diabetes in mice that were RIP-B7-1 transgene positive and B cell sufficient (filled circles), n = 35; RIP-B7-1 transgene positive and B cell deficient (open circles), n = 9; RIP-B7-1 transgene negative and B cell sufficient (filled triangles), n = 10; and RIP-B7-1 transgene negative and B cell deficient (open triangles), n = 13. The numbers indicate the total population of both male and female mice.
cells. In any event, the result does show that CD8 T cells do, indeed, play an important role in the accelerated diabetes of this model, as they do in diabetes in the NOD mouse.

It is clear from these results, and from those of other investigators, that CD4 T cells are required for the development of diabetes in the NOD mouse. The fact that accelerated diabetes does not occur in the absence of cells bearing the CD4 coreceptor indicates that these cells are also important for the development of the accelerated diabetes seen in the NOD-RIP-B7-1 transgenic mice. It has been reported that CD4\(^{-/-}\) mice have CD4\(^{-/-}\)CD8\(^{+}\) T cells that are able to perform the function of CD4 T cells (26). In the current experiments, clearly any CD4\(^{-/-}\)CD8\(^{+}\) T cells present do not function sufficiently to induce early diabetes in the NOD-RIP-B7-1 transgenic mice. However, the fact that the NOD-RIP-B7-1 transgenic mice lacking CD4 T cells can develop diabetes at a slower rate indicates either that the CD8 T cells which have been activated can kill the \(\beta\) cells alone, although less efficiently, or alternatively, that the CD4\(^{-/-}\)CD8\(^{+}\) T cells can help the process of \(\beta\) cell damage, but not unless more CD8 T cells have been activated to perform the final effector function. Certainly, these mice had massive infiltration of CD8 T cells, which suggests that the CD8 T cells can cause diabetes under the conditions where they can be maximally costimulated, but that the process is very much slower in the absence of CD4 T cells. However, the reduction of diabetes that occurs

**Figure 6.** (Top) Pancreatic sections from a nontransgenic mouse that was B cell deficient taken at 30 wk. Mild insulitis is seen, as illustrated by staining with anti-CD4, anti-CD8, and anti-B220 antibodies. (Middle) Staining from an NOD-RIP-B7-1/\(\mu\)MT\(^{-/-}\) mouse that became diabetic, showing intense insulitis. There are a few B220-positive cells seen, but these are negative for staining with anti-Ig antibody and are likely to be T cells. (Bottom) Staining of a pancreatic section from a diabetic NOD-RIP-B7-1, B cell-sufficient mouse.

**Figure 7.** Incidence of diabetes after adoptive transfer of 6-wk-old NOD spleen cells (a), 12-wk-old NOD spleen cells (b), diabetic spleen cells (c), and CD8 cloned T cells (d) into NOD.SCID-RIP-B7-1 mice (filled circles) and NOD.SCID mice (open circles).
without CD4 T cells implies that the process whereby both CD4 and CD8 T cells in the NOD mouse are required to develop diabetes also applies to this model, and that even in the presence of optimal stimulation of CD8 T cells, these alone cannot cause diabetes in an accelerated fashion, although diabetes does occur after many weeks. This suggests that soluble antigens released after damage of the islets are taken up by professional antigen-presenting cells, such as dendritic cells, which then present peptide to MHC class II–restricted CD4–CD8– T cells in a similar manner to that found in the nontransgenic NOD mouse. It would appear that CD4 T cells are ultimately required for the generation of accelerated diabetes, and any CD4–CD8– T cells that are present are not sufficient to perform this function. Ultimately, the role of CD4 T cells selected on MHC class II could be tested by crossing the NOD-RIP-B7-1 mice with class II transactivator (CIITA) −− mice (which lack MHC class II without changing MHC class I) (27) crossed onto the NOD background.

The presence of B cells within the islet in diabetic NOD-RIP-B7-1 mice suggests either that B cells are present because of bystander recruitment, or that B cells play a role in antigen presentation to the CD4 T cells in this accelerated model of diabetes, as in the native NOD mouse. However, the NOD-RIP-B7-1 mice that lack B cells are just as able to develop accelerated diabetes as those that are B cell sufficient. This suggests that the dendritic cells and macrophages are sufficient to present antigens to the CD4 T cells in this model, and that B cells are not required for this function. It perhaps also suggests that under normal circumstances, the B cells are required to maximally costimulate cells to develop diabetes. Alternatively, it is also possible that in addition to the costimulation of autoreactive cells, the loss of costimulation of regulatory cells also plays a role in the accelerated diabetes seen in this model. This suggests that in addition to the role that B cells play in antigen presentation to CD4 T cells, an important function of B cells in diabetes in NOD mice is to provide costimulation.

It has been demonstrated previously that diabetes could be adoptively transferred to NOD.SCID animals using young spleen cells (28). The current study shows that there is a delay of 3–6 wk when young spleen cells are used compared with diabetic spleen cells. There appears to be a proportional delay in the onset of diabetes, presumably related to the presence of cells that have already been primed to damage the β cells, taken from the older NOD animals. It is of interest that there is a predominant population of CD8 T cells expressing Vβ6 in the islets of adoptively transferred NOD.SCID-RIP-B7-1 mice. CD8 T cells expressing Vβ6 are also found in the diabetic nontransgenic NOD.SCID animals. In this study, T cells expressing Vβ8, postulated to be important in early initiating events (29), were not seen. We have previously isolated very potent CD8 T cell clones that also express Vβ6 from the islets of young, prediabetic NOD mice (11), which are maintained in culture on nontransgenic NOD islets. These are able to cause rapid diabetes in irradiated NOD mice and NOD.SCID mice. This suggests that these cells are present in the islets of NOD mice, but in the presence of the B7-1 transgene, this particular pathogenic population is increased and is therefore able to accelerate the onset of diabetes in the NOD.SCID mice bearing the RIP-B7-1 transgene.

In conclusion, the results presented here suggest that in the NOD-RIP-B7-1 mice, CD8 T cells are required for the accelerated diabetes seen, and in addition, CD4 T cells are also required, although diabetes can occur later in the absence of T cells bearing the CD4 coreceptor. Whether this result would be obtained in the absence of cells selected on MHC class II remains to be tested. However, unlike the nontransgenic NOD mouse, where B cells are required for the development of disease, these cells are not required in the RIP-B7-1 transgenic μMT −−− mouse. The mechanism by which the accelerated diabetes occurs is...
likely to be the increase in a potent population of CD8 T cells, which are able to initiate damage and destroy the islets, but this clearly requires the presence of CD4 T cells for the maximum effect to be seen. Although costimulatory molecules have not been shown to be expressed on the pancreatic islets under normal circumstances, this model has many features similar to that seen in NOD mice, and may allow us to elucidate the nature of the interaction between CD4 and CD8 T cells in the production of diabetes. In addition, as both CD4 and CD8 T cells appear to be required for this accelerated diabetes, the model will also be useful for testing preventative strategies.

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References

1. M iller, B.J., M.C. Appel, J.J. O’Neil, and L.S. W icker. 1988. Both the Lyt-2+ and L3T4+ T cell subsets are required for the transfer of diabetes in nonobese diabetic mice. J. Immunol. 140:52–58.

2. Bendelac, A., M. Carnaud, C. Boitard, and J.-F. Bach. 1987. Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates: requirement for both L3T4+ and Lyt2+ T cells. J. Exp. Med. 166:823–832.

3. Serreze, D.V., E.H. Leiter, G.J. Christianson, D. Greiner, and L.B. Peterson. 1994. Major histocompatibility complex class I-deficient NOD-B2mnull mice are diabetes resistant. J. Exp. Med. 180:505–509.

4. W icker, L.S., E.H. Leiter, J.A. Todd, R.J. Renjilian, E. Peterson, P.A. Fischer, P.L. Podolin, M. Zijlstra, R. Jaenisch, and D.C. Roopenian. 1994. Major histocompatibility complex class I molecules are required for the development of insulitis in nonobese diabetic mice. J. Immunol. 153:350–360.

5. Katz, J., C. Benoist, and D. Mathis. 1993. Major histocompatibility complex class I molecules are required for the development of insulin in nonobese diabetic mice. Eur. J. Immunol. 23:3358–3360.

6. Sumida, T., M. Furukawa, A. Sakamoto, T. Namakata, T. Maeda, M. Zijlstra, I. Iwamoto, T. Koike, S. Yoshida, H. Tomioka, et al. 1994. Prevention of insulin and diabetes in beta 2-microglobulin-deficient nonobese diabetic mice. Int. Immunol. 6:1445–1449.

7. W ang, B., A. Gonzales, C. Benoist, and D. Mathis. 1996. The role of CD8+ T cells in the initiation of insulin-dependent diabetes mellitus. Eur. J. Immunol. 26:1762–1769.

8. Serreze, D.V., H.D. Chapman, D.S. Varum, I. Gerling, E.H. Leiter, and L.D. Shultz. 1997. Initiation of autoimmune diabetes in NOD/Lt mice is MHC class I-dependent. J. Immunol. 158:3978–3986.

9. Kay, T.W., J.L. Parker, L.A. Stephens, H.E. Thomas, and J. Allison. 1996. RIP-beta 2-microglobulin transgene expression restores insulitis, but not diabetes, in beta 2-microglobu-
18. Bendelac, A., C. Boitard, P. Bedossa, H. Bazin, J.-F. Bach, and C. Carnaud. 1988. Adoptive T cell transfer of autoimmune nonobese diabetic mouse diabetes does not require recruitment of host B lymphocytes. J. Immunol. 141:2625–2628.

19. Wong, S., S. Guerder, I. Visintin, E.P. Reich, K.E. Swenson, R.A. Flavell, and C.J. Janeway. 1995. Expression of the costimulator molecule B7-1 in pancreatic beta-cells accelerates diabetes in the NOD mouse. Diabetes. 44:326–329.

20. Rahemtulla, A., L.W. Fung, M.W. Schilham, T.M. Kundig, S.R. Sambhara, A. Narendran, A. Arabian, A. Wakeham, C.J. Paige, R.M. Zinkernagel, et al. 1991. Normal development and function of CD8+ cells but markedly decreased helper cell activity in mice lacking CD4. Nature. 353:180–184.

21. Kitamura, D., J. Roes, R. Kuhn, and K. Rajewsky. 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin mu chain gene. Nature. 350:423–426.

22. Stephens, L.A., and T.W. Kay. 1995. Pancreatic expression of B7 co-stimulatory molecules in the non-obese diabetic mouse. Int. Immunol. 7:1885–1895.

23. Kurts, C., W.R. Heath, F.R. Carbone, J. Allison, J.F. Miller, and H. Kosaka. 1996. Constitutive class I-restricted exogenous presentation of self antigens in vivo. J. Exp. Med. 184:923–930.

24. Liu, Y., and C.J. Janeway. 1992. Cells that present both specific ligand and costimulatory activity are the most efficient inducers of clonal expansion of normal CD4 T cells. Proc Natl. Acad. Sci. USA. 89:3845–3849.

25. Zijlstra, M., M. Bix, N.E. Simister, J.M. Loring, D.H. Rautel, and R. Jaenisch. 1990. Beta 2-microglobulin deficient mice lack CD4-8+ cytolytic T cells. Nature. 344:742–746.

26. Locksley, R.M., S.L. Reiner, F. Hatam, D.R. Littman, and N. Killeen. 1993. Helper T cells without CD4: control of leishmaniasis in CD4-deficient mice. Science. 261:1448–1451.

27. Chang, C.H., S. Guerder, S.C. Hong, W. van Ewijk, and R.A. Flavell. 1996. Mice lacking the MHC class II transactivator (CIITA) show tissue-specific impairment of MHC class II expression. Immunity. 4:167–178.

28. Rohane, P.W., A. Shimada, D.T. Kim, C.T. Edwards, B. Charlton, L.D. Shultz, and C.G. Fathman. 1995. Islet-infiltrating lymphocytes from prediabetic NOD mice rapidly transfer diabetes to NOD-scid/scid mice. Diabetes. 44:550–554.

29. Yang, Y., B. Charlton, A. Shimada, C.R. Dal, and C.G. Fathman. 1996. Monoclonal T cells identified in early NOD islet infiltrates. Immunity. 4:189–194.