IFN-γ Action on Pancreatic Beta Cells Causes Class I MHC Upregulation but Not Diabetes

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Abstract

We have generated transgenic nonobese diabetic (NOD) mice expressing dominant negative mutant IFN-γ receptors on pancreatic beta cells to investigate whether the direct effects of IFN-γ on beta cells contribute to autoimmune diabetes. We have also quantitated by flow cytometry the rise in class I MHC on beta cells of NOD mice with increasing age and degree of islet inflammatory infiltrate. Class I MHC expression increases gradually with age in wild-type NOD mice; however, no such increase is observed in the transgenic mice. The transgenic mice develop diabetes at a similar rate to that of wild-type animals. This study associates class I MHC upregulation from progression to diabetes, shows that the rise in class I MHC is due to local IFN-γ action, and eliminates beta cells as the targets of IFN-γ in autoimmune diabetes. (J. Clin. Invest. 1998. 102:1249–1257.) Key words: nonobese diabetic mouse • cytokine • autoimmune disease • class I major histocompatibility antigen • transgenic mouse

Introduction

Insulin-dependent diabetes mellitus (IDDM) is characterized by a mixed lymphocytic infiltrate in the pancreatic islets of Langerhans (termed insulitis), followed by beta cell destruction leading to hyperglycaemia. The pleiotropic cytokine IFN-γ is detected in the islets in increasing amounts during progression to diabetes in the nonobese diabetic (NOD) mouse and biobreeding (BB) rat models of IDDM (1, 2). Recent data with IFN-γ receptor–deficient NOD mice, which do not develop diabetes and have a reduced level of insulitis, confirms that IFN-γ has an important pathogenic role in the disease (3).

However, it is unclear whether the cellular target of IFN-γ relevant to diabetes is the beta cell or other cells such as antigen-presenting cells. Treatment of NOD mice with anti-IFN-γ antibody or a nonimmunogenic soluble IFN-γ receptor prevents disease (4–6), and IFN-γ-deficient mice crossed with the lymphocytic choriomeningitis virus-glycoprotein (LCMV-GP) mouse model of diabetes are protected from disease after challenge with LCMV (7), which normally activates LCMV-GP–specific T cells (8, 9). Given the large amount of evidence suggesting involvement of IFN-γ, it is surprising that IFN-γ-deficient NOD mice develop insulitis and diabetes, albeit at a delayed and reduced rate (10).

Many direct effects of IFN-γ on beta cells have been demonstrated in vitro, including transcriptional regulation of genes implicated in beta cell dysfunction. The free radical nitric oxide (NO) is produced in beta cells by inducible NO synthase (iNOS), which is induced by IFN-γ in combination with IL-1 (for review see reference 11). Upregulation of iNOS by these cytokines leads to inhibition of glucose-stimulated insulin secretion by islets and DNA fragmentation in islet cells (12–14). Fas is a member of the TNF receptor family able to transduce an apoptotic signal (15). Its expression can be induced in vitro by combinations of the cytokines IL-1, IFN-γ, and TNF-α, and once induced, the islets are susceptible to lysis by anti-Fas antibody, demonstrating that Fas-mediated cell death is possible in mouse islets (16). Adhesion molecules such as ICAM-1 are also regulated by IFN-γ (17), and may aid in the trafficking of inflammatory mediators to the islets.

Class I major histocompatibility complex (MHC) expression is also upregulated by IFN-γ, and is induced on beta cells during destructive insulitis. Beta cell class I MHC overexpression is observed in humans (18–20), NOD mice (21, 22), and BB rats (23), and is temporally associated with initiation of insulitis in the LCMV-GP model (24). This upregulation has been proposed as a mechanism for beta cell–specific CD8+ T cell activation, either by increasing recognition of beta cells by CD8+ T cells, or indirectly by upregulating the antigen-processing pathway (25). Insulitis and diabetes do not occur in beta 2-microglobulin knockout NOD (NODβ2mnull) mice (26–29) that are deficient in cell surface class I MHC and CD8+ T cells (30). The absence of insulin in NODβ2mnull mice is consistent with a role for CD8+ T cells and class I MHC expression in the initiation of diabetes in NOD mice. When class I MHC was transgenically reconstituted on beta cells, insulitis returned, suggesting that initiation of autoimmune diabetes in the NOD mouse may involve a direct interaction between CD8+ T cells and peptide-class I MHC complexes on beta cells (31). There is, however, also evidence that CD8+ T cells can be activated by cross-presentation of antigens derived from non-antigen-presenting cells (APCs) in association with class I MHC on professional APCs (32).

The effects of IFN-γ on beta cells in vitro and the evidence for its role in vivo have led to the hypothesis that IFN-γ may...
promote the course of diabetes in the NOD mouse by acting directly on the beta cell. We have tested this possibility by expressing a dominant negative mutant IFN-γ receptor α chain in transgenic mice to generate beta cells that are unresponsive to IFN-γ. This mutant receptor has been used previously in transgenic mice under the control of T cell and macrophage-specific promoters to study the effects of IFN-γ on these lineages (33). The IFN-γ receptor α chain has been truncated intracellularly so that when expressed at high levels, the mutant receptor is able to dimerize with endogenous receptors and bind ligand; however, it is unable to transduce a signal in response to IFN-γ (34). We have used this system to study the direct effects of IFN-γ on the beta cell while maintaining both an intact immune system and the ability of other islet cells to respond to IFN-γ. We have found that while IFN-γ response is essential for upregulation of class I MHC on beta cells, it is not necessary for insulin or progression to diabetes in the NOD mouse.

Methods

Generation of transgenic mice. The rat insulin promoter (RIP) was inserted in place of the human lysozyme promoter in the plasmid BSSK-hL-P-myc-mgRΔIC-hGH (53). The transgene sequence was verified and then excised with KpnI and NotI. C57Bl/6 × SJL F2 embryos were microinjected with the transgene and transferred into pseudopregnant CBA × C57Bl/6 female fosters. Offspring were screened by Southern blot analysis of EcoRI-digested tail DNA using the RIP as a probe. Three founder lines obtained were crossed with BC2 generations. One line was intercrossed to generate mice homozygous for the transgene. All mice were housed in the animal facilities of BBSRC Harwell laboratories, Inc., Burlingame, CA) and additional blocking with 10% mouse serum. Incubations were for 30 min followed by a 10-min wash in PBS. Insulin was detected by incubation with guinea pig anti-insulin antibody followed by incubation with horseradish peroxidase–conjugated anti–guinea pig Ig (Dako Corp., Santa Barbara, CA). The ΔγR transgene was detected with biotinylated 9E10 followed by streptavidin–alkaline phosphatase, developed with CDP-Star substrate. The sequence used was the IFN-γ receptor (39), and GR20 rat mAb was used for detection of the murine IFN-γ receptor α chain (40). To stain class I MHC, 34-1-2s, a rat mAb directed against H-2Kd and Dd, which cross-reacts with H-2Kq and s, r, p, H-2 haplotypes (41), was used. A2B5, a monoclonal IgM antiangioglioside (42) obtained from American Type Culture Collection (Rockville, MD), was used in some instances for detecting islet endocrine cells by flow cytometry.

Flow cytometry. Islet cell suspensions were incubated for 30 min on ice with mAb diluted in balanced salt solution (BSS) containing 2% FCS. They were then washed and incubated for 15 min with a secondary Ab as necessary. Second antiserum used was as follows: biotinylated anti-rat Ig (PharMingen, San Diego, CA) followed by phycoerythrin-conjugated streptavidin (Caltag Labs, San Francisco, CA) and phycoerythrin-conjugated anti-mouse Ig (Silenus Labs Pty., Ltd., Hawthorn, Australia). Cells were finally washed and resuspended in BSS with 2% FCS and 1 μg/ml propidium iodide to stain dead cells. Analysis was performed on a FACScan (Becton Dickinson, Mountain View, CA). Beta cells were sorted on a FACStarplus (Becton Dickinson) on the basis of flavin adenine dinucleotide autofluorescence according the method described (43).

Immunohistochemistry. Pancreas and spleen were dissected, snap-frozen in OCT (Miles-Yeda Inc., Elkhart, IN) in a hexane/liquid nitrogen bath, and stored at −70°C. Serial 5-μm cryostat sections were cut, fixed in acetone, air-dried, and stored at −20°C. Immunohistochemical staining was performed using an avidin–biotin immunoperoxidase technique. Endogenous biotin present in pancreas exocrine tissue was blocked using an avidin/biotin blocking kit (Vector Laboratories, Inc., Burlingame, CA) and additional blocking with 10% mouse serum. Incubations were for 30 min followed by a 10-min wash in PBS. Insulin was detected by incubation with guinea pig anti-insulin Ab followed by incubation with horseradish peroxidase–conjugated anti–guinea pig Ig (Dako Corp., Santa Barbara, CA). The ΔγR transgene was detected with biotinylated 9E10 followed by streptavidin-conjugated horseradish peroxidase. Stains were developed for 4 min with 3,3’diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) at 1 mg/ml in PBS containing 0.02% hydrogen peroxide and counterstained with hematoxylin.

Electrophoretic mobility shift assay (EMSA). Nuclear extracts were prepared by a method adapted from Schreiber (44). Cells were lysed in a buffer containing 10 mM Heps, pH 7.9, 10 mM KCl, 1.5 mM MgCl2, protease inhibitors (0.5 mM PMSF, 10 μg/ml leupeptin), and 0.5 mM DTT. The lysate was vortexed with 25 μl 10% NP-40, and was centrifuged at 12,000 rpm for 5 min at 4°C. Nuclear extracts were stored at −70°C. EMSAs were carried out using oligonucleotides labeled with 32P-1 pmol of annealed double-stranded oligonucleotide was labeled by filling in the ends with Klenow polymerase and [32P]-dATP, and the labeled probe was purified on a spin column. The sequence used was the IFN-γ-activated sequence (GAS; from the FcyRI gene promoter [45]): 5′-GTACGAGATGTATTTCCCAGAAAA.

Histological scoring and diabetes incidence. Pancreases and spleen were dissected from mice at 50 and 90 d of age and placed into cold 10% formalin. 5-μm paraffin-embedded sections were cut and stained with hematoxylin and eosin. Insulitis was scored on three sections cut 40 μm apart on a 0–4 scale as described (46). Three mice were analyzed per group, with ~100 islets scored in each group.

For adoptive transfer, diabeticogenic spleen cells were purified from NOD mouse donors with blood glucose levels >15 mmol/liter.
Splenocytes were suspended at $1 \times 10^6$ cells/ml in RPMI, and 200 µl was injected intravenously into irradiated recipient mice. Eight male transgenic and eight nontransgenic mice aged 82 d were used as recipients. Blood glucose was measured every 5 d starting 2 wk after transfer. Mice were considered diabetic when blood glucose exceeded 15 mmol/liter.

Male mice (eight mice per group) > 100 d of age were used to assess the incidence of cyclophosphamide-accelerated diabetes. A single dose of 300 mg/kg body weight of cyclophosphamide (Cyclophosphamide; Farmitalia: Carloerba, Hawthorn, Australia) was injected intraperitoneally, and blood glucose levels were monitored weekly starting 2 wk after cyclophosphamide.

Female transgenic and nontransgenic mice (15–30 per group) were set aside to assess the spontaneous incidence of diabetes. After 120 d of age, the mice were monitored weekly by urine glucose measurement with a follow-up blood glucose determination if required. Mice were considered diabetic with a blood glucose over 15 mmol/liter.

Results

**Generation of dominant-negative IFN-γ receptor transgenic mice.** To generate mice with beta cells resistant to IFN-γ, we used a dominant negative form of the IFN-γ receptor α chain. The mutant IFN-γ receptor α chain (ΔγR) was truncated intracellularly so that it lacks the binding sites for Jak protein tyrosine kinases and Stat1, thus acting as a dominant negative receptor when expressed at high levels compared with endogenous receptors (34). Transgene expression was detected with an epitope from the myc proto-oncogene, recognized by the mAb 9E10. The gene was cloned under the control of the rat tyrosine kinase Stat1 normally binds to the IFN-γ receptor α chain where it is phosphorylated by members of the Janus (Jak) family of tyrosine kinases. It then forms homodimers and translocates to the nucleus where it binds to the specific DNA sequence termed the gamma-activated sequence (GAS). When bound to the GAS, Stat1 dimers are able to regulate IFN-γ-responsive genes transcriptionally (for review see reference 47). To analyze the activation of Stat1 in the RIP-ΔγR mice, beta cells were purified by FACS sorting of the A2B5-positive (endo-

The expression of IFN-γ receptors on the beta cells of RIP-ΔγR mice was also examined using the mAb GR20 that recognizes both the endogenous and transgenic murine IFN-γ receptors (40). Fig. 1C shows levels of expression in mice wildtype, heterozygous, and homozygous for the mutant receptor. The level of GR20 staining is greatly increased in the beta cells of mice homozygous for the RIP-ΔγR gene, possibly due to the lack of turnover of the mutant receptor within the cell.

*Stat1 is not activated in beta cells of RIP-ΔγR mice.* As a measure of IFN-γ responsiveness, we first looked at the ability of the transcription factor Stat1 to bind DNA in response to IFN-γ. Stat1 normally binds to the IFN-γ receptor α chain where it is phosphorylated by members of the Janus (Jak) family of tyrosine kinases. It then forms homodimers and translocates to the nucleus where it binds to the specific DNA sequence termed the gamma-activated sequence (GAS). When bound to the GAS, Stat1 dimers are able to regulate IFN-γ-responsive genes transcriptionally (for review see reference 47). To analyze the activation of Stat1 in the RIP-ΔγR mice, beta cells were purified by FACS sorting of the A2B5-positive (endo-

**Expression of RIP-ΔγR in beta cells.** The mAb 9E10 recognizes the myc epitope tag (39) was used to detect expression of the ΔγR mutant receptor in pancreas sections from transgenic mice by immunohistochemistry. Transgene expression was detected in the islets of transgenic mice, but not in the surrounding exocrine pancreas, nor in the islets of littermate control animals (Fig. 1A). The relative level of expression on the three lines of mice generated was compared by staining purified islet cells with 9E10, and by analysis by flow cytometry. The three lines showed similar levels of transgene expression (Fig. 1B).

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**Figure 1.** Expression of RIP-ΔγR transgene in islets. (A) Immunoperoxidase staining with the mAb 9E10 (recognizing the myc epitope tag) was performed on frozen sections of pancreas taken from transgenic and nontransgenic littermates. Insulin staining was used to visualize the islets in the sections. 200×; 340× (inset). (B) Islets were isolated from two mice of each of the three transgenic lines generated and nontransgenic littermates. Islet cell suspensions were stained with the biotinylated mAb 9E10 and streptavidin–phycoerythrin, and were analyzed by flow cytometry. (C) Islets isolated from nontransgenic NOD/Lt, heterogeneous, and homozygous RIP-ΔγR mice were dispersed and analyzed by flow cytometry with the rat anti-IFN-γ receptor mAb (GR20), and were then detected with biotinylated anti-rat Ig and streptavidin–phycoerythrin. Staining is shown on beta cells identified by autofluorescence.
Transgenic: – +
A2B5: – + –
IFN-γ: + + +

Figure 2. Stat1 is not activated in purified beta cells from RIP-ΔγR mice. Islet cells from transgenic and nontransgenic littermates were sorted by flow cytometry into endocrine (FITC-A2B5+) and nonendocrine (FITC-A2B5−) populations. Cells were treated with 100 U/ml IFN-γ for 15 min, and nuclear extracts prepared and EMSA carried out with radio-labeled GAS oligonucleotide probe.

crine) and A2B5-negative (nonendocrine) cells. The cells were treated with 100 U/ml IFN-γ for 15 min before nuclear extract preparation. Nuclear extracts were analyzed by EMSA for the presence of Stat1 DNA-binding activity. While Stat1 is activated by IFN-γ in the nontransgenic beta cells, the heterozygous transgenic mice have a greatly reduced level of Stat1 DNA binding (Fig. 2). The small amount of Stat1 DNA binding in the heterozygous mice could be due to insufficient levels of the transgene expression to achieve the dominant negative effect, or due to the use of a very high level of IFN-γ, causing a small response (see below).

Class I MHC is not upregulated on RIP-ΔγR beta cells. We next looked at the ability of IFN-γ to upregulate a responsive gene in the transgenic beta cells. It is well-documented that surface expression of class I MHC on beta cells increases in response to IFN-γ. We analyzed the expression of class I MHC on beta cells by flow cytometry after incubation of purified islets in vitro for 48 h with 100 U/ml IFN-γ. The nontransgenic beta cells showed a rise in cell surface class I MHC expression after incubation with IFN-γ, which was reduced in the mice homozygous for the transgene (Fig. 3A). There was a slight but reproducible increase in class I MHC levels in the transgenic mice after incubation with 100 U/ml IFN-γ that could be due to contaminating non-beta cells in the flow cytometry analysis or to the high concentration of IFN-γ during the 48-h in vitro culture. To address the issue of IFN-γ concentration, we looked at class I MHC expression after incubation with varying doses of IFN-γ, from 0.1 U/ml to 1,000 U/ml. Nontransgenic beta cells responded to as low as 1 U/ml IFN-γ with maximal upregulation of class I MHC after 10 U/ml. The RIP-ΔγR beta cells did not upregulate class I MHC in response to 10 U/ml IFN-γ; however, they showed a slight increase in class I MHC with 100 U/ml IFN-γ (Fig. 3A), which was not further increased with up to 1,000 U/ml IFN-γ (not shown). Therefore, it is likely that with in vivo concentrations of IFN-γ, the RIP-ΔγR beta cells are not able to respond to IFN-γ, even though with high levels of cytokine in vitro class I MHC is minimally upregulated (see results below also). Basal level of class I MHC on transgenic islets was not reduced as compared with the wild-type mice, demonstrating that IFN-γ responsiveness is not required for basal class I MHC expression in beta cells.

Quantitation of class I MHC expression increase with age on beta cells. Class I MHC surface protein levels were analyzed on A2B5-positive islet endocrine cells isolated from NOD mice ranging in age from 27 d at the start of insulitis to 120 d when mice are developing diabetes. Class I MHC levels continued to gradually increase from 50 d of age with no definite evidence of a plateau to this effect or step-wise change at the time that diabetes presents (Fig. 4A). No difference was seen in female and male mice at any age tested despite the well-recognized difference in risk of developing diabetes (Fig. 4B). Staining on three consecutive days of islet cells isolated at the same time showed that class I MHC levels were stable in vitro for at least 48 h (not shown).

NOD.scid mice do not develop insulitis or diabetes, but are congenic with NOD mice; that is, the genes controlling autoimmunity, including MHC genes, are the same as in NODs. D2.GD mice have the same class I MHC genes as NOD mice, but have different class II MHC and other genes, and are not prone to autoimmunity. We used these two strains of mice to ask whether the increase in class I MHC expression requires

Figure 3. Dose response of IFN-γ on RIP-ΔγR beta cells in vitro. Islets isolated from nontransgenic NOD/Lt mice or RIP-ΔγR(+/+·+) mice were cultured for 48 h in the absence or presence of IFN-γ at the indicated concentrations. Class I MHC was stained with the mAb 34-1-2s followed by phycoerythrin anti-mouse Ig, and is shown for the autofluorescent beta cell population of the islet cell suspension.
the presence of insulitis. No increase in class I MHC expression with age was seen in either NOD.scid or D2.GD islet cells (Fig. 5, A and B), and the levels seen were similar to those on islets from four to six mice of the same age have been pooled in each experiment. The NIT-1 insulinoma cell line (53) was included in each experiment to control for variability of class I MHC expression.

To determine if the rise in surface class I MHC expression seen in NOD mice of increasing age is due to the effect of local IFN-γ action on beta cells, we analyzed RIP-ΔγR mice at ages after the onset of insulitis and compared beta cell class I MHC expression with that of NOD mice of similar age. NOD.scid mice were used as an insulitis-free baseline class I MHC expression level. The class I MHC level of beta cells from RIP-ΔγR mice of ages 72–99 d remained unchanged at the level seen in NOD.scid beta cells, whereas the class I MHC level on beta cells of 116-d-old NODs was elevated (Fig. 6). These data suggest that the class I MHC increase in NODs is due to effects of local IFN-γ. This result further demonstrates that beta cells from the transgenic mice are not responsive to IFN-γ in vivo. While it is conceivable that other IFN-γ-responsive genes may be regulated in the transgenic beta cells via an alternative signaling pathway, we have found no evidence for this.

Diabetic spleen cells transfer disease into RIP-ΔγR recipients. We next looked at the ability of diabetogenic spleen cells to transfer disease into RIP-ΔγR recipients. After transfer of 2 × 10⁷ splenocytes from diabetic donors into irradiated RIP-ΔγR(+/-) or NOD mice, diabetes incidence was assessed by regular blood glucose measurements. The incidence of diabetes was the same in the transgenics and control NOD mice.
IFN-γ and other cytokines to the pathogenesis of diabetes (for reviews see references 48–50). While plausible circumstantial evidence for this link has existed, it has not previously been directly tested. These in vitro effects remain valid, but they do not explain the ability of IFN-γ to promote diabetes. If direct effects of IFN-γ do play a role, it must be minor because spontaneous disease and diabetes development in two accelerated models of diabetes were not affected by transgene expression. This finding is important because of the emphasis placed on in vitro effects of IFN-γ on beta cells in diabetes research, which we now believe to be of limited relevance.

Class I MHC expression on pancreatic beta cells and progression to autoimmune diabetes have been linked in humans, BB rats, and NOD mice. In the current study they have been dissociated for the first time because RIP-ΔγR mice developed diabetes without beta cell class I overexpression. This result suggests that basal levels of class I expression are sufficient for CD8⁺ T cell recognition of beta cells, known to be required for diabetes in the NOD mouse model (51).

Class I MHC overexpression on beta cells is closely associated with insulitis, and is upregulated very close to the time that insulitis begins. It is not found by immunohistology in islets without insulitis (21). In the current study, class I MHC overexpression on NOD beta cells has been quantitated for the first time by flow cytometry. Increased expression of class I MHC was first recognized at 50 d of age, and mean class I expression then rose gradually with age until 90–110 d when hy-

**Table I. Histological Scoring of Insulitis in Nondiabetic Wild-type NOD/Lt and RIP-ΔγR(+/-) Mice**

<table>
<thead>
<tr>
<th></th>
<th>No insulitis</th>
<th>Noninvasive insulitis</th>
<th>Destructive insulitis</th>
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<tbody>
<tr>
<td>NOD 50 d</td>
<td>86.7</td>
<td>12</td>
<td>1.3</td>
</tr>
<tr>
<td>RIP-ΔγR 50 d</td>
<td>88.6</td>
<td>10.1</td>
<td>1.3</td>
</tr>
<tr>
<td>NOD 90 d</td>
<td>37.2</td>
<td>33.3</td>
<td>29.5</td>
</tr>
<tr>
<td>RIP-ΔγR 90 d</td>
<td>39.1</td>
<td>38.3</td>
<td>22.6</td>
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Three mice were analyzed per group. The score is the percentage of islets in each category. At least 100 islets were scored per group.
perglycaemia began to occur. We did not observe increased class I MHC expression before insulitis, and therefore it is likely to be a consequence of rather than required for its development. This conclusion was supported by the absence of increased class I expression with age in mice without insulitis, and the development of insulitis in RIP-ΔγR mice in the absence of class I MHC overexpression. Similarly, increased class I expression is not likely to be due to the class I genes of the NOD since D2.GD mice with identical class I type do not show this phenotype. In contrast, total absence of class I MHC expression in the NODβ2mnull completely protects NOD mice from insulitis (for review see reference 51). We did not find any difference between class I MHC expression on beta cells from male and female NOD mice, making it also likely that class I overexpression is not sufficient for progression to diabetes since male mice in our colony rarely develop diabetes.

Our study shows that IFN-γ is the major factor associated with increased class I MHC expression on NOD beta cells. Other cytokines, endogenous or exogenous viruses, diet, or other environmental agents, although possibly contributing indirectly to this phenomenon, are unlikely to have a direct effect. This result is not surprising as, in vitro, IFN-γ is the only agent we have used that increases beta cell class I to the extent seen in vivo in NOD mice. An important implication of this result is that beta cell class I can be regarded as a local indicator of IFN-γ action. If this is the case, our data do not support a sudden shift to local IFN-γ activity, but rather a gradual rise with age.

The IFN-γ knockout mice develop diabetes at a modestly reduced and delayed rate (10). It is conceivable that global IFN-γ deficiency results in the development of compensatory mechanisms that preserve immune homeostasis, and also substitute for IFN-γ in the pathogenesis of diabetes. In contrast, IFN-γ receptor knockout NOD mice fail to develop diabetes, and the results indicate that this is likely to be due to the absence of IFN-γ-responsive macrophages or beta cells rather than T cells (3). The discrepancy between these results is unexplained, but both mice are known to have abnormal immune responses that are not always identical to each other in other disease states (52). It is also possible that an as yet unidentified second ligand for the IFN-γ receptor exists that substitutes for IFN-γ in the IFN-γ–deficient animals. Because of these considerations and because we wished to test the relevance to diabetes of in vitro effects of IFN-γ on beta cells, we chose an approach designed to produce IFN-γ–unresponsive beta cells, leaving the immune system intact. The approach of tissue-specific unresponsiveness to IFN-γ allows elucidation of which cell type is the target of IFN-γ relevant to the pathogenesis of IDDM. This cannot be achieved by transplantation of islets from knockout animals as there are many different cell types within the islet, including macrophages and endothelial cells, that are potential targets of IFN-γ action.

This study demonstrates the power of genetic manipulation of the NOD model to address clinically important questions directly. The use of class I–deficient mice back-crossed onto the NOD model dramatically changed our understanding of the role of class I MHC expression and CD8+ T cells in the NOD, and the current data clarify that the requirement for class I expression is at basal levels only and have confirmed that IFN-γ is likely to play at most a minor role by its action on beta cells. Clearly genetic manipulation of this sort is not applicable to IDDM in humans, in which virtually nothing definitive is known about pathogenesis beyond limited descriptive immunopathology. The challenge is to apply our understanding of diabetes pathogenesis in the NOD to human IDDM to enable rationally based treatment and prevention strategies.

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