Proinflammatory cytokines are believed to be important in pancreatic β-cell destruction in the development of type 1 diabetes. They act by upregulation of genes including Fas and inducible nitric oxide synthase (iNOS), which have both been shown to lead to β-cell death in vitro. We used mice deficient in the interleukin (IL)-1 receptor (IL-1R) to assess the contribution of IL-1 to different models of diabetes. IL-1R–deficient islets were protected from the damaging effects of tumor necrosis factor (TNF) and interferon (IFN)-γ in vitro, and β-cell expression of iNOS was reduced, suggesting that IL-1 mediates the induction of iNOS by TNF and IFN-γ. IL-1 action was not required for induction of class I major histocompatibility complex or Fas by TNF and IFN-γ. IL-1R–deficient nonobese diabetic (NOD) mice developed diabetes significantly slower than wild-type mice. IL-1R deficiency did not affect diabetes in 8.3 TCR transgenic NOD mice but prolonged the time to diabetes in BDC2.5 TCR transgenic NOD mice. We conclude that IL-1R deficiency slows progression to diabetes in NOD mice but on its own does not prevent diabetes. *Diabetes* 53:113–121, 2004

Interleukin (IL)-1 is produced by activated macrophages and is involved in acute-phase inflammatory responses (1,2). Functional IL-1 receptors (IL-1Rs) are present on pancreatic β-cells (3,4). In vitro, IL-1 is toxic to pancreatic β-cells, and it has been suggested that it may be involved in the pathogenesis of type 1 diabetes. IL-1, together with interferon (IFN)-γ, has been shown to inhibit the function of rodent and human pancreatic islets in vitro (5) as well as induce DNA damage and β-cell death (6,7). IL-1 and IFN-γ induce expression of inducible nitric oxide synthase (iNOS), which leads to production of the toxic molecule nitric oxide (NO). NO is believed to be largely responsible for islet cell damage in response to IL-1 and IFN-γ (8,9). Evidence suggests that IL-1 and IFN-γ are directly toxic to β-cells and that NO production by the β-cell itself induces damage. It is likely that NO produced by other cells within the islet, such as resident leukocytes, is at an insufficient local concentration to induce β-cell damage (10).

In addition to the effects of exogenous IL-1 on β-cells, there is also evidence that IL-1 is produced endogenously within islets by resident leukocytes (11). Inflammatory cytokines, such as tumor necrosis factor (TNF) and IFN-γ, and lipopolysaccharide (LPS) induce NO production within islets, but this is blocked by inhibition of IL-1 action with the IL-1R antagonist (IL-1Ra), suggesting that the cytotoxic action of these molecules depends on stimulation of IL-1 production within the islet (12,13).

Inflammatory cytokines including IL-1, TNF, and IFN-γ are all found within islets during the pathogenesis of diabetes (14–16). Because of the effects of these cytokines on islets in vitro, it is likely that they are playing some role in diabetes development in vivo. IFN-γ has been shown to be present in islets from pre-diabetic NOD mice at sufficient concentrations to upregulate class I major histocompatibility complex (MHC) gene expression on β-cells in vivo (17). Additionally, islet cell iNOS expression has been detected (16), presumably upregulated by the cytokines present in the inflammatory lesion in islets. Inhibition of IL-1 in vivo by injection of anti–IL-18 antisera had a modest effect on reducing the incidence of cyclophosphamide-induced diabetes (18), as did treatment with soluble IL-1R (19).

A further way that IL-1 and other cytokines may lead to β-cell destruction in vivo is via induction of the cell death receptor Fas. Whereas Fas does not contribute to IL-1–induced β-cell damage in vitro, it is a potential mechanism of β-cell death in vivo if cells expressing the Fas ligand are nearby. IL-1 upregulates Fas on β-cells, and this is enhanced by IFN-γ (20,21). TNF and IFN-γ also induce Fas expression and whether IL-1 is required for this, as it is for β-cell iNOS expression, is unknown.

We obtained IL-1R–deficient mice to examine whether IL-1 action plays a role in diabetes development in the NOD mouse and other models of diabetes. IL-1R–deficient mice develop normally; however, they are highly susceptible to *Listeria* infection and have reduced acute-phase and delayed-type hypersensitivity responses (1,2). The IL-1R is composed of two chains: IL-1R1 and IL-1R2 AcP. Both are required for all signaling in response to IL-1 (22). IL-1R1– and IL-1R2 AcP–deficient mice were used to analyze the effect of intraslet IL-1 on TNF- and IFN-γ–induced...
gene regulation. We also backcrossed both type I IL-1R– and IL-1R AcP–deficient mice on to the NOD genetic background to examine the incidence of diabetes in different models.

RESEARCH DESIGN AND METHODS

Mice and reagents. IL-1R–deficient and IL-1R AcP–deficient mice were obtained from Dr. M. Labow (Roche). Mice were backcrossed from a mixed 129/C57Bl/6 background onto the NOD genetic background for 10 generations and were then intercrossed to produce knockout and wild-type backcrossed mice. In addition to screening for the knockout locus, DNA samples from the established knockout strain were genotyped across the genome (~15 cm) genotyping polymorphic markers that also flank the previously described Id1 loci (23). The genome-wide screen was performed using fluorescently labeled primers at the Australian Genome Research Facility (Melbourne, Australia). PCR products were detected on a Perkin Elmer 377 and analyzed using Genescan and Genotyper software (Applied Biosystems) as described (24).

NOD8.3 mice expressing the TCRββ rearrangements of the H-2Kb-restricted β-cell–reactive CD8+ T-cell clone NYS.3 (25) were provided by P. Santamaria (University of California, San Francisco). NOD BDC2.5 TCR transgenic mice carrying a TCR transgene derived from an I-Abβ β-cell–reactive CD4+ T-cell clone were provided by D. Mathis and C. Benoist (Harvard University). NOD/Lt and 129/Sv mice were bred in specific pathogen-free conditions and housed under the auspices of the animal facility at the Walter and Eliza Hall Institute. All animal studies were carried out in accordance with accepted standards of humane animal care and were approved by the institutional animal ethics committee. Recombinant murine IFN-γ (used at 100 units/ml) and recombinant murine TNF-α (used at 1,000 units/ml) were obtained from Genentech (San Diego, CA). Recombinant human IL-1 (used at 10 units/ml) was obtained from Genzyme (Cambridge, MA), and IL-1Ra (5 μg/ml) was obtained from Amgen. Nω-nitroso-L-arginine (2 mM/ml) was purchased from Sigma (St. Louis, MO).

Islet isolation. Islets of Langerhans were isolated from mice as previously described (10,27). Purified islets were washed, hand picked, and cultured at 37°C in 5% CO2 in CMRL medium-1066 (Life Technologies, Gaithersburg, MD) containing antibiotics, glutamine, and 10% FCS.

Cell death assay. Quantitation of apoptosis was determined according to the method of Nicoli et al. (28). Islets were cultured for 4 days with cytokines, followed by analysis for DNA fragmentation on the flow cytometer (Becton Dickinson, Mountain View, CA) as previously described (21). Data from multiple experiments were pooled, as indicated in the figure legends.

Flow cytometry. Islet cells were analyzed for expression of Fas by flow cytometry as previously described (21). Islets were dispersed into single cells using trypsin (0.2% trypsin [Calbiochem, La Jolla, CA], 10 mM EDTA in Hank’s balanced salt solution) and stained using standard procedures. Antibodies were used for anti-Fas (Jo2; Pharmingen, San Diego, CA) and anti-IL-1R (clone 28-14-8; Pharmingen) followed by phycoerythrin-conjugated streptavidin. Cells were analyzed on a FACScan (Becton Dickinson, Franklin Lakes, NJ). β-Cells were identified based on their autofluorescence (21). Infiltrating inflammatory cells were excluded from analysis by gating out cells stained with anti-CD45 (anti-leukocyte common antigen) conjugated to PerCP-Cy5.5 (3F11; Pharmingen).

Western blotting. Islets were incubated for 48 h with cytokines, transferred to microcentrifuge tubes, and washed three times in PBS; they were then resuspended in 25 μl lysis buffer (10 mM HEPES, pH 7.4, 2 mM EDTA, 0.1% saponin, 5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 20 μg/ml leupeptin). Tubes were then subjected to three cycles of freezing and thawing, followed by centrifugation at 13,000 rpm for 15 min at 4°C. Lysed islets were kept at -70°C until required. Samples were boiled for 5 min in 2× sample buffer (125 mM Tris, pH 6.8, 4% SDS, 2% mercaptoethanol, 0.1% bromophenol blue, and 20% glycerol). Samples were separated by SDS-PAGE and transferred to nitrocellulose using standard procedures. Western blotting was performed with anti-NOS2 Ab (Santa Cruz Biotechnology, Santa Cruz, CA) followed by horseradish peroxidase (HRP)-conjugated anti-rabbit Ig (Silenus Laboratories, Hawthorn, Australia) and detection with Lumi-Light Western blotting substrate (Roche Diagnostics, Mannheim, Germany).

Immunostaining. Islets were cultured for 48 h with cytokines in complete CMRL medium-1066. For cytopsins, islets were dispersed into single cells with trypsin, resuspended in 4 × 104 cells per slide, and centrifuged onto glass slides. Slides were fixed for 30 min in 4% paraformaldehyde, air dried, and stored at -20°C until staining. Two-color immunofluorescence was performed using standard procedures. Blocking was with 10% FCS and 2% skim milk in PBS; antisera were incubated for 30 min with 5-min washes in PBS. Antisera used were anti-NOS2 Ab (Santa Cruz Biotechnology) with anti-rabbit Ig–Texas red (Jackson Immunoresearch, West Grove, PA), anti-insulin Ab (Dako, Carpenteria, CA) with anti–guinea pig Ig–fluorescein isothiocyanate (Becton Dickinson, Franklin Lakes, NJ), and anti-CD45 Ab (clone 30-F-11; Pharmingen) with anti-rabbit Ig–fluorescein isothiocyanate (Silenus). Staining was visualized using confocal microscopy.

Spontaneous diabetes and insulin. Backcrossed female wild-type and IL-1R–deficient mice were kept for 350 days to assess the spontaneous incidence of diabetes. Mice were visually monitored regularly, and blood glucose analyses were performed on any mice that appeared diabetic. Mice with a blood glucose >12 mmol/l were considered diabetic. For histological analysis, female wild-type and IL-1R–deficient mice were killed at 120 days of age, the pancreas was removed and placed in Bouin’s fixative, and 5-μm paraffin-embedded sections were cut at 40-μm intervals. Hematoxylin and eosin–stained sections were scored for insulitis using a 0–4 scoring system as previously described (29). The scores are expressed as percentage infiltration. Three mice were analyzed per group, with ~100 islets scored in each group.

Enzyme-linked immunosorbent assay. To measure IL-1Ra, two recipients were given a single injection of 300 μg/kg cyclophosphamide i.p. and monitored for onset of diabetes. The remaining mice were left until diabetes developed, at which time the graft was harvested. Grafts were placed in Bouin’s fixative and embedded in paraffin. The 5-μm sections were cut, and serial sections were stained with hematoxylin and eosin and with anti-insulin followed by anti–guinea pig HRP and anti-glucagon followed by anti-rabbit HRP (all antisera from Dako). Grafts were histologically scored using the following scale: 0 = no infiltrate; 1 = peri-islet infiltrate; 2 = intra-islet infiltrate <50% destruction; 3 = intra-islet infiltrate >50% destruction; and 4 = total β-cell loss, glucagon staining without insulin.

RESULTS

The contribution of intraislet IL-1 production to islet cell death induced by TNF plus IFN-γ. In islets, TNF plus IFN-γ plus LPS induces iNOS expression, NO production, and inhibition of glucose-stimulated insulin secretion (12). It is believed that intraislet IL-1 production by resident leukocytes, detectable by immunostaining, directly contributes to this toxicity of TNF plus IFN-γ plus LPS to islets. By inhibiting IL-1 action within islets, it is possible to assess the contribution of intraislet IL-1 production to islet cell death and iNOS expression induced by TNF plus IFN-γ in mouse islets. IL-1 action was inhibited in two ways. First, we coincubated islets with IL-1Ra, and second, we isolated islets...
from mice deficient in IL-1R. Islets were incubated in culture with cytokines, and nuclear fragmentation was analyzed after 4 days by flow cytometry. Whereas TNF alone had no effect on islet viability, the combination of TNF plus IFN-γ induced nuclear fragmentation of islet cells (Fig. 1). Inhibition of IL-1 action in islets with either IL-1Ra or IL-1R deficiency attenuated cell death of islets treated with TNF plus IFN-γ (Fig. 1). As expected, IL-1 plus IFN-γ did not induce death of islets from IL-1R−/− mice or of wild-type islets cocultured with IL-1Ra. IL-1 or IFN-γ alone were unable to kill mouse islets (10).

We also measured IL-1β production after culture of islets in vitro with cytokines. Because IL-1 is produced by resident leukocytes in islets and the number of these cells per islet is very low, IL-1β was not detected in the wild-type islets (untreated, 9.1 pg/ml IL-1β; TNF, 7.4 pg/ml IL-1β; TNF plus IFN-γ, 10.6 pg/ml IL-1β). However, we were able to detect very low levels of IL-1β secreted by IL-1R−/− islets (untreated, 9.3 pg/ml IL-1β; TNF, 18.4 pg/ml IL-1β; TNF plus IFN-γ, 23.8 pg/ml IL-1β). The inability to detect these low amounts of IL-1 in wild-type islets is most likely due to the receptor binding IL-1, thus preventing its detection by enzyme-linked immunosorbent assay. The low but detectable levels of IL-1β secreted by IL-1R−/− islets indicate that the reduced cell death in these islets treated with TNF plus IFN-γ is due to a lack of IL-1 action and not a lack of IL-1 production.

These data confirm that TNF plus IFN-γ induces intra-islet IL-1 production in mouse islets, and this IL-1 contributes to cell death induced by TNF plus IFN-γ. It remains possible that TNF plus IFN-γ act directly on islet cells to induce iNOS expression and NO production independent of IL-1 production. To address this, we looked at TNF plus IFN-γ-induced gene expression in IL-1R−/− deficient islets. TNF plus IFN-γ-induced gene expression in islet cells. TNF plus IFN-γ is known to induce expression of iNOS in islet cells, and NO production is believed to be at least one mechanism by which these cytokines damage islets in vitro. To investigate the islet cell types upon which TNF plus IFN-γ act, we used iNOS expression as a marker for cytokine action. Western blotting and immunofluorescence were performed to examine iNOS expression in cytokine-treated islets (Fig. 2). In wild-type islets, combinations of IL-1 plus IFN-γ and TNF plus IFN-γ induced iNOS expression (Fig. 2A). Inhibition of IL-1, with either IL-1Ra or IL-1R deficiency, reduced but did not prevent iNOS expression. Two-color immunofluorescence of cyto-}

![Graph showing cytokine-dependent killing of islet cells in vitro](image)

**FIG. 1.** Cytokine-dependent killing of islet cells in vitro depends on IL-1. Wild-type or IL-1R−/− deficient islets were cultured in vitro for 4 days with cytokines with or without IL-1Ra. After cytokine treatment, islets were dispersed into single cells and percent nuclear fragmentation was analyzed by flow cytometry as a measure of cell death. Means ± SE of data from four to five separate experiments are shown. *P < 0.001 by ANOVA, cytokine-treated groups vs. untreated. ND, not done; NMMA, Nω-monomethyl-L-arginine.

Spontaneous diabetes is delayed in IL-1R−/− deficient NOD mice. We examined the effects of IL-1R deficiency on spontaneous diabetes in the NOD mouse. IL-1R−/− deficient mice were backcrossed onto the NOD genetic background for 10 generations and then intercrossed to produce either IL-1R−/− deficient or backcrossed wild-type mice. Apart from the knockout locus, a genome-wide scan confirmed that all genetic markers, including those flanking previously described Idd loci, were homozygous for the NOD allele. Because this genotyping was performed after intercrossing at the tenth backcross generation, there is a statistical chance that small genomic intervals may yet retain donor alleles, but based on the genome-wide scan, it is unlikely that these intervals encompass loci previously linked to diabetes onset. In addition, the 129-derived interval encompassing the IL-1R knockout has been localized, at present, between D1Mit167 and D1Mit322 (not included). This interval is ~6.9 cM proximal to the previously described boundary for Idd5.1 and, to date, has not been implicated in disease susceptibility or resistance (32,33).

Female mice (IL-1R deficient and backcrossed wild type) were kept to 350 days of age to determine the incidence of spontaneous diabetes (Fig. 4A). Although we detected a slight but significant delay in diabetes incidence in the absence of IL-1 receptors, the overall incidence of diabetes remained substantial at 75%. There was no difference in diabetes between homozygous wild-type and het-
erozygous mice (not shown). Wild-type mice had an insulitis score of 46.5 ± 17% in infiltration, whereas IL-1R–deficient mice had a score of 41.8 ± 7% in infiltration. We also examined the incidence of diabetes in IL-1R–deficient mice after adoptive transfer of diabetogenic NOD splenocytes. Mice receiving 2 × 10⁷ splenocytes from newly diabetic NOD donors developed diabetes by 50 days posttransfer, even in the absence of IL-1R (Fig. 4B). We recently demonstrated that Fas is expressed on β-cells after adoptive transfer, although not during spontaneous diabetes (34). Fas expression after adoptive transfer was not affected by IL-1R deficiency (Fig. 4C).

Previous data suggest that cyclophosphamide-induced diabetes is reduced in NOD mice treated with either soluble IL-1R (19) or anti–IL-1β (18). We therefore tested the susceptibility of IL-1R–deficient NOD mice to diabetes induced by cyclophosphamide (Fig. 4D). Whereas IL-1R–deficient NOD mice developed cyclophosphamide-induced diabetes at a slightly reduced rate (66%) compared with wild-type mice (78%), the difference was not statistically significant (P > 0.1, log-rank test).

**IL-1R deficiency does not protect syngeneic grafts from destruction.** It has been reported that systemic administration of IL-1Ra prevents recurrence of disease after syngeneic transplantation of islets into diabetic NOD mice. This protection, however, only occurred for as long as IL-1Ra was administered (35). Using IL-1R–deficient islets, we are able to determine whether the protection afforded by IL-1Ra in this previous experiment is due to direct effects on β-cells or systemic effects on other cells such as macrophages, dendritic cells, or other immune cells. We therefore performed fetal pancreas grafts with wild-type and IL-1R–deficient donors into NOD scid recipients. Diabetes was then induced by adoptive transfer of splenocytes from newly diabetic NOD mice. Some mice were also treated daily with IL-1Ra or placebo.

Deficiency of IL-1R did not protect fetal pancreas grafts from autoimmune attack. Grafts were histologically scored as described in RESEARCH DESIGN AND METHODS, with a score of 0 being no infiltration and 4 being total β-cell destruction. Wild-type grafts had a score of 2.93 ± 0.1 compared with IL-1R–deficient grafts (2.77 ± 0.7). Both wild-type and IL-1R–deficient grafts had an infiltrate of mononuclear cells and destruction of insulin-producing β-cells (Fig. 5). This is consistent with results of adoptive transfer of diabetic cells into IL-1R–deficient NOD mice.
Mice treated with IL-1Ra had well-preserved grafts compared with placebo-treated mice, confirming that this treatment does help to prolong graft survival. However, there was no difference between wild-type (graft score 1.33 ± 0.36) and IL-1R–deficient grafts (graft score 1.12 ± 0.6). These results suggest that deficiency of IL-1R on β-cells alone does not prevent autoimmune destruction and that the effects of IL-1 in development of diabetes in this setting are likely to be systemic rather than direct effects on β-cells.

Adoptive transfer of diabetes by BDC2.5 cells is delayed in IL-1R–deficient NOD mice. BDC2.5 transgenic mice have diabetogenic CD4+ T-cells that rapidly kill β-cells by an as yet unknown mechanism that involves activation of the cells through the type I TNF receptor (36). After treatment of BDC2.5 NOD mice with cyclophosphamide, cytokines such as IL-1, TNF, and IFN-γ are present in the insulitis lesions and treatment of these mice with neutralizing antisera to IL-1 or TNF reduces the incidence of cyclophosphamide-induced diabetes, suggesting that inflammatory cytokines such as IL-1 do play a role in β-cell destruction by these CD4+ T-cells; however, other mechanisms can compensate for the lack of IL-1 action, although this takes a longer time.

Diabetes in NOD8.3 TCR transgenic mice. NOD8.3 TCR transgenic mice express a T-cell receptor from a CD8+ clone that was derived from infiltrating T-cells of an acutely diabetic mouse. The NOD8.3 TCR transgenic mice develop diabetes rapidly, with a mean age of diabetes of ~40 days. Perhaps, surprisingly, progression to diabetes occurs normally on a perforin-deficient background, and the T-cells from these mice appear to kill β-cells in a Fas-dependent manner (38). To test if IL-1 is required for this, for example, to upregulate Fas expression on β-cells, we produced NOD8.3 mice deficient in the IL-1R. These mice and their IL-1R wild-type littermates were studied for development of diabetes. No difference in frequency or age of onset of diabetes was observed (Fig. 7A). Furthermore, when islets from these mice were isolated before onset of diabetes, there was no evidence that Fas expression on β-cells was affected by IL-1R deficiency (Fig. 7B).
DISCUSSION
The main result from these studies is that IL-1 is not essential for progression to diabetes in several variations of the NOD mouse model of type 1 diabetes. Spontaneous diabetes occurred in ~75% of IL-1R-deficient NOD mice, which indicates a minor impact on diabetes frequency. This result is consistent with the lack of impact on diabetes frequency observed in caspase-1-deficient NOD mice (38a). We observed a delay in onset of diabetes in NOD mice and in NOD mice given diabetogenic BDC2.5 T-cells. This is the first time that the in vivo role of IL-1 in an animal model of diabetes has been directly tested with the use of genetic deficiency of IL-1 or IL-1 action.

IL-1 kills mouse β-cells in vitro primarily by iNOS.

**FIG. 4.** Diabetes frequency in IL-1R-deficient NOD mice. A: Diabetes frequency was studied in cohorts of 23 female wild-type (■) and 20 IL-1R-deficient (▲) mice. Diabetes was diagnosed by measuring blood glucose in mice with clinical features of diabetes. The groups are statistically significantly different from each other (\( P < 0.05 \), log-rank test). B: Diabetes after adoptive transfer of \( 2 \times 10^7 \) splenic T-cells from diabetic wild-type NOD mice into irradiated wild-type or IL-1R-deficient recipients (five mice per group). No difference was observed (NS). C: Fas expression on islet cells isolated from wild-type or IL-1R-deficient NOD recipients of splenic T-cells from diabetic wild-type NOD mice. Cells with high autofluorescence (predominantly β-cells) are circled. Islets were isolated 25 days after adoptive transfer and dispersed before staining. Leukocytes were excluded from analysis by staining with anti-CD45. The figure is representative of at least four individual mice. D: Diabetes frequency was studied after administration of cyclophosphamide. Cohorts of 14 wild-type and 21 IL-1R-deficient male mice were given a single dose of 300 mg/kg cyclophosphamide at 12–15 weeks of age. Mice were then monitored for diabetes. The groups are not statistically different (\( P > 0.1 \), log-rank test).

**FIG. 5.** IL-1R deficiency does not protect syngeneic grafts from destruction. Fetal pancreas grafts were harvested from NODscid mice after adoptive transfer of splenocytes from diabetic NOD mice. Recipient mice were diabetic treated with placebo (A and B) or non-diabetic treated with IL-1Ra (D and E). Serial sections were stained with insulin and glucagon to visualize islet structures. Representative wild-type and IL-1R-deficient grafts of the two groups stained with insulin are shown. C and F: Pancreas sections from each group stained with hematoxylin and eosin are also shown to demonstrate that insulitis caused by the adoptive transfer was unaffected by the treatment.
upregulation and production of NO, although some residual cell death is seen in IL-1–treated iNOS-deficient islets (39). This is also the main way that TNF kills primary mouse β-cells in combination with IFN-γ (40,41). TNF-stimulated β-cell iNOS expression is at least partly due to intraslet IL-1 production (11). In our studies, as expected, lack of IL-1 signaling eliminated β-cell iNOS induction due to TNF, IL-1, and IFN-γ. Immuneactive iNOS was still produced in IL-1R–deficient islet non–β-cells by TNF plus IFN-γ, indicating that regulation of iNOS differs between β-cells in which it is IL-1 dependent and other cell types in which it is not. The amount of iNOS induced in non–β-cells is insufficient to damage β-cells in vitro (10). Consequently, IL-1R–deficient islets are protected from TNF and IFN–γ as well as from IL-1 and IFN–γ in vitro. IL-1R–deficient islets were not protected in vivo, indicating that cytokine-induced iNOS induction is not the dominant pathway to β-cell death in vivo. The lack of substantial impact on diabetes frequency is consistent with the reported lack of impact of iNOS deficiency on diabetes in NOD mice.

Redundancy with TNF that has very similar effects to IL-1 may be an explanation for the unchanged frequency of diabetes. Both activate the nuclear factor–κB and mitogen-activated protein kinase pathways and induce an overlapping set of genes involved in inflammation (42). If TNF effects on β-cells are mediated by intraslet production of IL-1, then TNF effects should be blocked in IL-1R–deficient β-cells. Our data using NOD IL-1R–deficient islets or wild-type islets in the presence of IL-1Ra showed that this is true for iNOS expression but not for Fas or class I MHC upregulation by TNF. These do not require local IL-1 expression, indicating substantial variation in how genes are regulated by TNF plus IFN-γ. Therefore, if both IL-1 and TNF are being produced in the islets of NOD mice, perhaps removing IL-1 would have effects limited to genes regulated like iNOS that require IL-1. Other IL-1–inducible genes regulated like class I MHC, which can be upregulated by TNF alone or Fas and can be upregulated by TNF and IFN-γ independently of IL-1, would still be expressed on β-cells of IL-1R–deficient NOD mice as long as TNF is present.

Blockade of IL-1 would also not be effective if IL-1 is not significantly expressed in NOD islets. There are reports that IL-1 has been detected in NOD islets (43,44), but this may be at too low levels or, alternatively, inhibitors of IL-1 such as IL-1Ra may be concomitantly expressed (43). Fas expression on β-cells is one way of tracking local expression of IL-1 and TNF. Our data suggest that Fas cannot be detected on β-cells during spontaneous diabetes in NOD mice (21,34). This suggests that there is not sufficient local IL-1 (or TNF) action to induce Fas by itself or with IFN-γ. In contrast, in accelerated models of diabetes, β-cell expression of Fas can be detected, indicating that sufficient IL-1 or TNF may be present. Fas expression in these settings is not affected by IL-1 signaling deficiency and may be due to TNF, although other inducers of Fas on β-cells have also been studied in vitro (45).

Another possible reason why IL-1R deficiency did not protect more fully from diabetes is that it may not block the effects of perforin and granzymes. These factors are secreted by the cytotoxic granule of the CD8+ T-cell and are important mediators of β-cell destruction in NOD diabetes (46). If an intervention to block β-cell death does not block perforin-dependent killing either by blocking the interaction between β-cells and CTL (e.g., by downregulation of class I MHC) or by blocking the function of perforin/granzymes, then it may not significantly reduce diabetes in NOD mice. There is no direct way that IL-1

![Graph](image-url)

**FIG. 6.** Diabetes is delayed after adoptive transfer of BDC2.5 T-cells into IL-1R–deficient NOD mice. BDC2.5 cells (2 × 105) obtained from diabetic BDC2.5scid mice were transferred into irradiated wild-type or IL-1R–deficient NOD recipients, and the mice were followed for diabetes. Five mice per group were used (*P* < 0.05, log-rank test).

![Graph](image-url)

**FIG. 7.** Diabetes in NOD8.3 mice is not affected by IL-1R deficiency. A: Frequency of diabetes was studied in 5 wild-type and 10 IL-1R–deficient NOD8.3 mice. No difference in diabetes frequency or onset was seen (*P* > 0.05). B: Fas expression on islet cells from NOD8.3 mice or IL-1R–deficient NOD8.3 mice. Islets were isolated at 35 days of age. Cells with high autofluorescence (predominantly β-cells) are circled. Representative data from four individual mice are shown.
receptor deficiency would block perforin/granzyme function in β-cells. It is likely, however, that multiple pathways (not only perforin) can lead to β-cell death. In perforin-deficient NOD mice, there is residual delayed diabetes (~17% of mice become hyperglycemic) (46). We hypothesized that in settings where β-cell destruction is thought to be perforin independent, particularly those mediated by cytokines, an effect of IL-1R deficiency might be more apparent. Therefore, the IL-1R–deficient mice were bred with NOD TCR transgenic mice BDC2.5, a CD4+ TCR transgenic and 8.3, a CD8+ TCR transgenic in which β-cell killing is believed to be perforin independent (38). In both cases, the frequency of diabetes was unaffected by IL-1R deficiency, although an increase in the time to diabetes was observed in BDC2.5 mice.

We performed fetal pancreas graft experiments to determine whether the small effects of IL-1R deficiency were due to action of IL-1 on β-cells or on effector cells of the immune system. Systemic treatment with IL-1Ra improved both wild-type and IL-1R–deficient graft survival. This treatment is equivalent to the total absence of IL-1 responses in the IL-1R–deficient mice and had similar effects to spontaneous diabetes (i.e., a slight reduction in diabetes incidence). However, IL-1R–deficient grafts survived as poorly as wild-type grafts in placebo-treated mice. This treatment is equivalent to the adoptive transfer of wild-type splenocytes into IL-1R–deficient mice (Fig. 4B), where no change in diabetes incidence was observed. In this experiment, the transferred cells are responsive to IL-1, whereas the recipient mice remain IL-1R deficient. These data suggest that IL-1 action on the effector cells may be more important than that on the β-cells. The exact effect that IL-1 has on these cells remains to be elucidated.

That IL-1 has relatively small effects on diabetes in NOD mice does not rule out a more important role in other rodent models of autoimmune diabetes or in human type 1 diabetes. There are subtle differences between mouse, rat, and human islet responses to cytokines, which may make IL-1 action more important in human diabetes. These differences include regulation of the iNOS gene and its less important role in cytokine-induced death of human β-cells (47,48). Also, Fas appears to be expressed in islets from biopsies of diabetic patients (49), whereas we are unable to detect Fas expression on β-cells of spontaneously diabetic NOD mice (34).

The reduction observed in diabetes frequency in the NOD mouse may reflect more the difficulty of achieving large reductions in diabetes by blockade of a single pathway than the lack of involvement of IL-1. Therefore, it remains to be tested whether blockade of other cytokine pathways together with IL-1R deficiency will have a greater impact on diabetes. These results, however, must make it less of a priority to test neutralization of IL-1 or protection from its effects as monotherapy in human diabetes than if the current experiments had completely blocked diabetes in NOD mice. It also focuses attention on the effector mechanisms more likely to be dominant, including perforin, Fas, and possibly TNF, although TNF has been claimed to affect T-cell activation more than it does β-cell death (36,50). IL-1 has been a major focus of efforts to understand and prevent β-cell death over many years, and we believe our data clarify its importance in the NOD mouse.

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