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# IFN-γ receptor deficiency prevents diabetes induction by diabetogenic CD4+ T cells but not CD8+ T cells[1]

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## Summary

IFN-γ is generally believed to be important in the autoimmune pathogenesis of type 1 diabetes (T1D). However, the development of spontaneous β cell autoimmunity is unaffected in NOD mice lacking expression of IFN-γ or the IFN-γ receptor (IFNγR), bringing into question the role IFN-γ has in T1D. In the current study an adoptive transfer model was employed to define the contribution of IFN-γ in $CD4^+$ versus $CD8^+$ T cell-mediated β cell autoimmunity. NOD.*scid* mice lacking expression of the IFNγR β chain (NOD.*scid*.IFNγRB$^{null}$) developed diabetes following transfer of β cell-specific $CD8^+$ T cells alone. In contrast, β cell-specific $CD4^+$ T cells alone failed to induce diabetes despite significant infiltration of the islets in NOD.*scid*.IFNγRB$^{null}$ recipients. The lack of pathogenicity of $CD4^+$ T cell effectors was due to the resistance of IFNγR-deficient β cells to inflammatory cytokine-induced cell death. On the other hand, $CD4^+$ T cells indirectly promoted β cell destruction by providing help to $CD8^+$ T cells in NOD.*scid*.IFNγRB$^{null}$ recipients. These results demonstrate that IFN-γR may play a key role in $CD4^+$ T cell-mediated β cell destruction.

### Keywords

IFN-γ; NOD mice; Type 1 diabetes

## Introduction

The development of spontaneous type 1 diabetes (T1D) is the result of autoimmune destruction of the insulin-producing β cells. The primary immune effectors of β cell destruction are $CD8^+$ and $CD4^+$ T cells that exhibit a type 1 phenotype [1]. $CD8^+$ T effector cells kill β cells directly through granzyme B-mediated cytotoxicity upon recognition of MHC-peptide complexes presented on the surface of β cells. Both $CD4^+$ and $CD8^+$ T effector cells also mediate β cell destruction indirectly through secretion of proinflammatory cytokines. IFN-γ has long been believed to play a key role in driving the autoimmune

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pathogenesis of T1D [2, 3]. How IFN-γ contributes to β cell autoimmunity and T1D development remains unclear, however.

Studies have shown that blocking IFN-γ function in NOD mice with either IFN-γ-specific Abs [4, 5] or soluble IFN-γ receptors (IFNγR) [6] reduces the incidence of spontaneous diabetes and prevents diabetes transfer by splenocytes from diabetic NOD donor mice [7]. Furthermore, transgenic expression of IFN-γ by β cells induces autoimmunity resulting in overt diabetes in otherwise diabetes-resistant mice [8]. *In vitro* studies have also shown that IFN-γ has cytotoxic effects on β cells. Exposure of human and murine islets to IFN-γ with either IL-1β or TNF-α induces β-cell death *in vitro* [9-14]. IFN-γ alone, however, has no effect on β cell-death. Notably, β cells deficient of STAT-1, a key signaling molecule activated in β cells by IFN-γ, are resistant to apoptosis induced *in vitro* by IFN-γ with either IL-1β or TNF-α [12, 15, 16].

Nevertheless, despite IFN-γ being the signature cytokine of type 1 effector T cells, the differentiation of type 1 effector T cells and the development of spontaneous diabetes are not significantly affected in NOD mice genetically lacking expression of IFN-γ [17] or IFNγR [18, 19]. T cells from IFNγR β chain (IFNγRB)-deficient NOD mice (NOD.IFNγRB$^{null}$) transfer diabetes as efficiently as wild-type NOD T cells [20]. Although NOD mice lacking IFNγR α chain expression were reported to remain diabetes-free, it was later found that disease protection was independent of the IFNγR deficiency, and due to linked genes derived from the 129 mouse strain genotype [18, 21]. Furthermore, NOD mice in which the IFN-γ-mediated signaling in β cells is selectively disrupted by expression of dominant negative mutants of IFNγR develop spontaneous diabetes comparable to wild-type NOD mice [22]. The fact that the lack of IFNγR expression has little or no effect on the development of spontaneous diabetes in NOD mice is perplexing in view of studies showing an important role for IFN-γ in autoimmune destruction of β cells *in vitro* and *in vivo* [2, 3].

In the current study, we employed an adoptive transfer model to determine how IFNγR deficiency influences the development of diabetes mediated by diabetogenic CD4$^+$ versus CD8$^+$ T cells. Our results demonstrate that IFNγR deficiency has a distinct effect on the development of diabetes mediated by CD4$^+$ T cells versus CD8$^+$ T cells, which was previously unrecognized in IFNγR-deficient NOD mice. These findings provide new insight into the mechanisms by which IFN-γ contributes to the pathogenesis of T1D.

## Results

### Diabetogenic CD8$^+$ T cells induce diabetes in IFNγR-deficient NOD.*scid* mice

Previous studies have demonstrated that systemic deficiency of either the α or β chain of IFNγR has a minimal effect on the development of spontaneous diabetes in NOD mice [18, 19]. These model systems are limited, however, since the possibility that IFNγR deficiency has differential effects on CD4$^+$ and CD8$^+$ T cell-mediated β-cell autoimmunity cannot be addressed. Accordingly, an adoptive transfer model was employed to individually assess the impact of IFNγR deficiency on the pathogenicity of diabetogenic CD4$^+$ versus CD8$^+$ T effectors. NOD.*scid* mice lacking expression of the IFNγR β chain (NOD.*scid*.IFNγRB$^{null}$) were used as recipients in this study. Initially, splenocytes from wild-type NOD mice were adoptively transferred into NOD.*scid* and NOD.*scid*.IFNγRB$^{null}$ mice, and diabetes monitored. All of the NOD.*scid*.IFNγRB$^{null}$ and NOD.*scid* recipients developed diabetes although diabetes onset was delayed in NOD.*scid*.IFNγRB$^{null}$ mice (Fig. 1A). These results are consistent with previous observations that NOD mice lacking IFNγR expression continue to develop spontaneous diabetes [18, 19].

To determine whether IFNγR deficiency selectively affected $CD8^+$ T cell-mediated β cell destruction, TCR transgenic CL4 $CD8^+$ T cells specific for *influenza* hemagglutinin (HA) were adoptively transferred into NOD.*scid*.InsHA or NOD.*scid*.InsHA.IFNγ$RB^{null}$ mice. All recipients of CL4 $CD8^+$ T cells developed diabetes regardless of genotype (Fig. 1B), although the onset of diabetes was delayed in some of the NOD.*scid*.InsHA.IFNγ$RB^{null}$ recipients. These results demonstrate that $CD8^+$ T cells continue to mediate β cell destruction despite the lack of IFNγR expression in recipient animals.

## Diabetogenic $CD4^+$ T cells induce insulitis but fail to transfer diabetes in IFNγR-deficient NOD.*scid* recipients

Next, the effect of IFNγR deficiency on $CD4^+$ T cell-mediated diabetes was investigated. BDC2.5 $CD4^+$ T cells were isolated from the spleen of BDC2.5.NOD.C$\alpha^{null}$ mice, which lack $CD8^+$ T cells, and then injected into NOD.*scid*.IFNγ$RB^{null}$ and NOD.*scid* mice. As expected, diabetes was induced in all NOD.*scid* recipients 9 wks post-transfer. Strikingly, none of the NOD.*scid*.IFNγ$RB^{null}$ recipients of BDC2.5 $CD4^+$ T cells developed diabetes over 20 wks post-transfer (Fig. 1C). Histological analysis of the pancreases showed that NOD.*scid* mice receiving BDC2.5 $CD4^+$ T cells developed severe insulitis by 5 wks post-transfer (Fig. 2). In contrast, insulitis was significantly reduced in NOD.*scid*.IFNγ$RB^{null}$ recipients with the majority of the islets remaining free of infiltration. By 15 wks post-transfer, however, NOD.*scid*.IFNγ$RB^{null}$ recipients exhibited significant insulitis (Fig. 2).

The delayed onset and progression of insulitis in NOD.*scid*.IFNγ$RB^{null}$ recipients could be attributed to inefficient priming of the transferred BDC2.5 $CD4^+$ T cells. To test this possibility, CFSE-labeled BDC2.5 $CD4^+$ T cells were transferred into NOD.*scid* and NOD.*scid*.IFNγ$RB^{null}$ mice, and T cell activation and proliferation assessed in the spleen, mesenteric lymph nodes (MLN), draining pancreatic lymph nodes (PLN) and islets. As reported previously [23], early proliferation of BDC2.5 $CD4^+$ T cells was detected in the PLN but not the spleen and MLN of NOD.*scid*.IFNγ$RB^{null}$ and NOD.*scid* recipients 3 to 4 days post-transfer (data not shown), and few cells were found in the islets at this time. At day 6 post-transfer, over 50% of BDC2.5 $CD4^+$ T cells in the PLN of both NOD.*scid*.IFNγ$RB^{null}$ and NOD.*scid* recipients had proliferated and undergone more than seven rounds of division as measured by CFSE dilution (Fig. 3A). The proliferation of BDC2.5 $CD4^+$ T cells in the PLN was antigen-specific because there were few proliferating BDC2.5 $CD4^+$ T cells (less than 10%) in the spleen and MLN of both groups of recipients (data not shown). Proliferating T cells in both groups of animals exhibited up-regulation of CD44 and down-regulation of CD62L (Fig. 3A). Thus, the lack of IFNγR expression by recipient animals had no effect on priming of the transferred BDC2.5 $CD4^+$ T cells in the draining PLN.

On the other hand BDC2.5 $CD4^+$ T cells displayed a markedly different profile in the islets of NOD.*scid*.IFNγ$RB^{null}$ versus NOD.*scid* recipients. Significant numbers of activated and proliferating BDC2.5 $CD4^+$ T cells were detected in the islets of NOD.*scid* recipients (Fig. 3B). In contrast, only few BDC2.5 $CD4^+$ T cells were found in the islets of NOD.*scid*.IFNγ$RB^{null}$ recipients, which in turn exhibited minimal proliferation and activation (Fig. 3B). These results indicate that trafficking of the transferred BDC2.5 $CD4^+$ T cells to the islets is delayed in NOD.*scid*.IFNγ$RB^{null}$ recipients, thereby explaining the later onset and progression of insulitis in these animals (Fig. 2).

Albeit delayed, NOD.*scid*.IFNγ$RB^{null}$ recipients of BDC2.5 $CD4^+$ T cells developed severe insulitis (Fig. 2) suggesting that IFNγR-deficient β cells were resistant to ongoing inflammation. To better assess the functional status of the β cell mass in these recipients, an intraperitoneal glucose tolerance test (IPGTT) was performed [24]. Twenty weeks post-transfer of BDC2.5 $CD4^+$ T cells, NOD.*scid*.IFNγ$RB^{null}$ recipients were injected i.p. with

glucose and blood glucose levels measured. As expected, recently diabetic NOD mice in which the majority of β cells have been destroyed, failed to control hyperglycemia after glucose injection (Fig. 4A). Pre-diabetic BDC2.5.NOD.Cα$^{null}$ mice that are known to develop destructive insulitis exhibited elevated blood glucose levels that persisted after glucose administration, reflecting impaired β cell function. Despite severe insulitis, blood glucose was similarly controlled in NOD.*scid*.IFNγRB$^{null}$ recipients of BDC2.5 CD4$^{+}$ T cells and untreated NOD.*scid* mice that have normal β cell mass and function (Fig. 4A). Together these results demonstrate that IFNγR-deficient β cells are resistant to CD4$^{+}$ T cell-mediated destruction.

## Lack of pathogenicity of CD4$^{+}$ T cells in IFNγR-deficient mice is due to the resistance of β cells to IFN-γ-induced death

To further assess the extent to which IFNγR-deficient β cells were resistant to CD4$^{+}$ T cell-mediated killing, NOD.*scid*.IFNγRB$^{null}$ mice were treated with cyclophosphamide (CY) after the transfer of BDC2.5 CD4$^{+}$ T cells. Treatment with CY accelerates diabetes development in NOD mice [4, 5] by in part enhancing inflammatory cytokine and chemokine production by islet infiltrating cells [25]. NOD.*scid*.IFNγRB$^{null}$ recipients were injected with CY 20 wks after BDC2.5 CD4$^{+}$ T cell transfer, and monitored for diabetes. As controls, NOD.*scid* recipients of BDC2.5 CD4$^{+}$ T cells prior to diabetes onset and pre-diabetic BDC2.5.NOD.Cα$^{null}$ mice with established insulitis were also treated with CY. All NOD.*scid* recipients of BDC2.5 CD4$^{+}$ T cells and pre-diabetic BDC2.5.NOD.Cα$^{null}$ mice developed diabetes within 2 wks after one injection of CY (Fig. 4B). In contrast, the majority of CY treated NOD.*scid*.IFNγRB$^{null}$ recipients (12/14) remained diabetes-free, even after a second injection of CY (Fig. 4B). This result demonstrates that IFNγR-deficient β cells are highly resistant to BDC2.5 CD4$^{+}$ T cell-mediated destruction, even when the proinflammatory *milieu* of the islets is enhanced.

To determine whether the failure of BDC2.5 CD4$^{+}$ T cells to transfer diabetes in NOD.*scid*.IFNγRB$^{null}$ mice was β cell-intrinsic, two sets of experiments were carried out. In the first, bone-marrow chimera were established in which β cells lacked IFNγR expression. NOD.*scid*.IFNγRB$^{null}$ and NOD.*scid* mice were irradiated and reconstituted with bone marrow from NOD.*scid* mice to restore IFNγR expression by all hematopoietically-derived APC [26]. Eight wks after bone marrow reconstitution, animals were adoptively transferred with BDC2.5 CD4$^{+}$ T cells and monitored for diabetes. All control chimeric NOD.*scid* recipients of BDC2.5 CD4$^{+}$ T cells (10/10) became diabetic by 78 days post-transfer (Fig. 5), whereas the majority (6/8) of chimeric NOD.*scid*.IFNγRB$^{null}$ mice receiving BDC2.5 CD4$^{+}$ T cells remained diabetes-free when monitored for over 20 wks (Fig. 5).

Secondly, IFNγR-deficient β cells were tested *in vitro* for sensitivity to cytokine-induced death. Wild-type and IFNγR-deficient β cells were incubated with IFN-γ plus IL-1β or TNF-α, and β cell death measured. As shown in Fig. 6, significant death of wild-type β cells was readily induced by exposure to IFN-γ plus either IL-1β or TNF-α. In contrast, the same treatment had a minimal effect on the viability of IFNγR-deficient β cells, similar to β cells exposed to individual cytokines. Studies have also shown that the combination of TNF-α and IL-1β is cytotoxic to β cells [27]. Interestingly, the death of IFNγR-deficient β cells treated with TNF-α and IL-1β was significantly reduced compared to wild-type β cells (Fig. 6). Together these results suggest that the lack of diabetes in NOD.*scid*.IFNγRB$^{null}$ mice following BDC2.5 CD4$^{+}$ T cell transfer is due to resistance of β cells to proinflammatory cytokine-induced cell death.

### CD4+ T cells accelerate the onset of diabetes induced by CD8+ T cells in NOD.*scid*.IFNγRB$^{null}$ mice

Despite the inability of CD4$^+$ T cells to kill β cells lacking IFNγRB expression (Fig. 1C), NOD.IFNγR$^{null}$ mice have been shown to develop spontaneous diabetes. This suggests a scenario in which CD4$^+$ T cells in NOD.IFNγR$^{null}$ mice provide help to CD8$^+$ T cells that directly destroy β cells. To test this hypothesis, 8.3 CD8$^+$ T cells which express a transgenic TCR specific for glucose-6-phosphatase catalytic subunit-related protein (IGRP) were transferred alone or with CD4$^+$ T cells from NOD mice into NOD.*scid*.IFNγRB$^{null}$ or control NOD.*scid* mice. 8.3 CD8$^+$ T cells were used based on previous work showing that CD4$^+$ T cells are required for IGRP-specific 8.3 CD8$^+$ T cells to efficiently mediate diabetes [28]. As expected, diabetes onset was accelerated in NOD.*scid* recipients injected with both 8.3 CD8$^+$ T cells and NOD CD4$^+$ T cells relative to diabetes onset in NOD.*scid* recipients of only 8.3 CD8$^+$ T cells (Fig. 7). Diabetes onset in NOD.*scid*.IFNγRB$^{null}$ recipients of 8.3 CD8$^+$ T cells alone was delayed compared to that in NOD.*scid* recipients. In contrast, co-transfer of NOD CD4$^+$ T cells and 8.3 CD8$^+$ T cells significantly accelerated the onset of diabetes in the NOD.*scid*.IFNγRB$^{null}$ recipients (Fig. 7). These results indicate that CD4$^+$ T cells nevertheless, contribute to β cell destruction in NOD.*scid*.IFNγRB$^{null}$ recipients by providing help to 8.3 CD8$^+$ T cells.

## Discussion

Despite much effort the role(s) of IFN-γ in T1D remains ill-defined. In this study we employed an adoptive transfer model to dissect the relative contribution of IFN-γ in CD4$^+$ versus CD8$^+$ T cell-mediated β-cell autoimmunity, and in turn better define the function of this proinflammatory cytokine in the development of T1D. We found that IFNγR deficiency prevents diabetes induction by β cell-specific CD4$^+$ but not CD8$^+$ T cells. Furthermore, the resistance of IFNγR-deficient animals to CD4$^+$ T cell-mediated diabetes was primarily due to the lack of IFNγR expression by β cells.

Consistent with the previous finding reported by Chervonsky and colleagues [20], diabetes was induced in IFNγR-deficient animals by diabetogenic CD8$^+$ T cells (Figs. 1 & 7). The minimal impact IFNγR deficiency had on the development of diabetes was not surprising since CD8$^+$ T cells are known to primarily destroy β cells by a perforin/granzyme B mechanism. In contrast, β cell-specific BDC2.5 CD4$^+$ T cells failed to transfer diabetes in NOD.*scid*.IFNγRB$^{null}$ recipients (Fig. 1). Our findings differ from results obtained in an earlier study in which the development of overt diabetes was unaffected in IFNγR-deficient NOD mice expressing the IA$^{g7}$-restricted 4.1 transgenic TCR specific for β cells [29]. The different results seen between the two studies could be due to the animal models used. NOD4.1 mice possess significant numbers of CD8$^+$ T cells that express the transgenic TCR β chain paired with endogenous TCR α chains [30]. Some of these CD8$^+$ T cells in IFNγR-deficient NOD4.1 mice are expected to be β cell-specific, and in turn drive β cell destruction. Since NOD.BDC2.5.Cα$^{null}$ mice were used as donors in our study, the transferred BDC2.5 CD4$^+$ T cells were monoclonal, and devoid of CD8$^+$ T cells.

The lack of diabetogenicity by transferred BDC2.5 CD4$^+$ T cells in NOD.*scid*.IFNγRB$^{null}$ recipients was not due to aberrant priming. BDC2.5 CD4$^+$ T cells proliferated similarly in the PLN of NOD.*scid* and NOD.*scid*.IFNγRB$^{null}$ mice (Fig. 3). Interestingly, the progression of insulitis by transferred BDC2.5 CD4$^+$ T cells was delayed in NOD.*scid*.IFNγRB$^{null}$ recipients. Impaired homing of insulin B chain specific CD8$^+$ T cells to the islets of IFNγR-deficient recipients has also been reported [20]. The delayed onset and progression of islet infiltration by primed BDC2.5 CD4$^+$ T cells is likely due to altered expression of adhesion molecules in NOD.*scid*.IFNγRB$^{null}$ recipients. For instance, IFN-γ is known to control T cell trafficking by regulating several adhesion molecules expressed by

endothelial cells [31]. Nevertheless, it is very unlikely that the observed delay in insulitis accounts for the complete lack of β cell destruction by BDC2.5 $CD4^+$ T cells. Indeed, CY treatment of NOD.*scid*.IFNγ$RB^{null}$ mice at a time when the islets were significantly infiltrated had no effect on diabetes incidence (Fig. 4). This was in marked contrast to NOD.*scid* recipients injected with CY, in which the pathogenicity of transferred BDC2.5 $CD4^+$ T cells was enhanced. CY has recently been shown to induce proinflammatory cytokine secretion by islet infiltrating cells and drive β cell autoimmunity [25]; our results suggest that IFN-γ is a key effector cytokine in this model.

Evidence is provided demonstrating that the resistance of IFNγR-deficient animals to BDC2.5 $CD4^+$ T cell-induced diabetes is β cell-intrinsic. β cells lacking IFNγR expression were no longer sensitive to cytokine-induced cell death *in vivo* and *in vitro*. For instance, BDC2.5 $CD4^+$ T cells failed to transfer diabetes in NOD.*scid*.IFNγ$RB^{null}$ mice reconstituted with “wild-type” NOD.*scid* bone marrow in which hematopoietic-derived cells expressed IFNγR but not β cells (Fig. 5). Although non-hematopoietic cells, such as endothelial cells, also lacked IFNγR expression in NOD.*scid*.IFNγ$RB^{null}$ bone marrow recipients, our *in vitro* experiments argue against a role for these cells affecting the diabetogenicity of transferred BDC2.5 $CD4^+$ T cells. Pakala and colleagues provided data indicating that IFN-γ has no role in $CD4^+$ T cell-mediated destruction of β cells. In this study IFNγR-deficient islet allografts were implanted into NOD.*scid* mice receiving BDC2.5 $CD4^+$ T cells [32]. The observed destruction of the IFNγR-deficient islets may reflect allograft-rejection mediated by natural killer (NK) cells. Numerous studies have shown the importance of NK cells in acute allograft rejection [33, 34]. Our results clearly demonstrate that *in vitro* cytokine-mediated β cell death required IFNγR expression by β cells (Fig. 6), consistent with other studies [11, 29]. β cell death has been shown to be readily induced through the synergistic effects of IFN-γ with IL-1β or TNF-α [12, 15, 35]. It has also been reported that β cells are susceptible to apoptosis mediated by the combination of TNF-α and IL-1β, presumably independent of IFN-γ [27]. Therefore it was somewhat surprising that IFNγR-deficient β cells exhibited reduced sensitivity to the cytotoxic effects of TNF-α and IL-1β (Fig. 6), indicating a central role for IFN-γ in cytokine-induced β cell death. How IFN-γ-mediated signaling in β cells governs the susceptibility to TNF-α and IL-1β-mediated death is currently unclear. However, IFN-γ sensitizes β cells to apoptosis by triggering activation of STAT-1, and inducing expression of several genes directly or indirectly associated with β cell death, including IFN-γ regulatory factor-1, caspase-1 and -11 [12, 25]. Furthermore, β cells pretreated *in vitro* with IFN-γ are more susceptible to apoptosis when subsequently exposed to TNF-α and IL-1β [12, 36]. This scenario may explain the resistance of IFNγR-deficient β cells to TNF-α and IL-1β-induced cell death *in vitro* and $CD4^+$ T cell-mediated destruction *in vivo*.

NOD mice deficient of $CD4^+$ T cells fail to develop insulitis and diabetes [37-39]. Why then do NOD mice lacking IFNγR or selectively expressing a dominant negative IFNγR by β cells continue to develop diabetes [18, 19, 22], if our results indicate that $CD4^+$ T cell-mediated β-cell destruction should be blocked? Co-transfer experiments carried out in this study demonstrate that IFNγR-deficiency does not impair the helper function of $CD4^+$ T cells. For example, the onset of $CD8^+$ T cell-induced diabetes was accelerated in NOD.*scid*.IFNγ$RB^{null}$ recipients when $CD4^+$ T cells were co-transferred (Fig. 7). These results suggest that $CD4^+$ T cells indirectly contribute to the development of spontaneous diabetes in IFNγR-deficient NOD mice by providing help necessary for β cell-specific $CD8^+$ T cells to initiate β-cell autoimmunity and destroy β cells via cytotoxic activity.

Taken together, our study demonstrates that IFNγR-deficiency has distinct effects on $CD4^+$ versus $CD8^+$ T cell-mediated diabetes, and that IFN-γ may play a critical role in $CD4^+$ T cell-mediated destruction of β cells. These results may also provide an explanation for the

"unexpected" development of spontaneous diabetes in NOD.IFNγR$^{null}$ mice [18, 19] and in NOD mice selectively expressing a dominate negative IFN-γ receptor by β cells [22].

## Materials and Methods

### Mice

NOD/Lt and NOD.*scid* mice were bred and maintained under specific pathogen-free conditions. NOD.IFNγRB$^{null}$ mice deficient in IFNγR β chain expression have been characterized [19] and were kindly provided by Dr. D. Serreze (The Jackson Laboratories Bar Harbor, ME). NOD.IFNγRB$^{null}$ mice were bred with NOD.*scid* mice to generate NOD.*scid*.IFNγRB$^{null}$ mice. NOD.BDC2.5 mice expressing an IA$^{g7}$-restricted transgenic TCR have been described [40] and were crossed to NOD.Cα$^{null}$ mice to generate NOD.BDC2.5.Cα$^{null}$ mice. NOD.CL4 mice expressing an H2K$^{d}$-restricted clone-4 TCR [41] were bred with NOD.*scid* mice to generate NOD.*scid*.CL4 mice. NOD.*scid*.InsHA.IFNγRB$^{null}$ mice were established by breeding NOD.*scid*.IFNγRB$^{null}$ mice with NOD.*scid*.InsHA mice [42]. NOD.8.3 mice that express an H2K$^{d}$-restricted TCR specific for IGRP [43] have been previously described [28]. All animal experiments were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committees.

### Adoptive transfer of diabetes

CL4 CD8$^{+}$ T cells were purified from the spleen of nondiabetic NOD.*scid*.CL4 mice by negative selection according to the manufacturer's instructions (Miltenyi Biotec), and adoptively transferred into 6 to 8 wk-old NOD.*scid*.InsHA and NOD.*scid*.InsHA.IFNγRB$^{null}$ female mice ($2\times10^{6}$ cells/mouse). BDC2.5 CD4$^{+}$ T cells isolated from the spleen of 2-3 wk-old nondiabetic BDC2.5.NOD.Cα$^{null}$ mice were purified as described above and injected i.p. ($2\times10^{6}$ cells/mouse) into 6 to 8 wk-old NOD.*scid*.IFNγRB$^{null}$ and NOD.*scid* female mice.

In some experiments 8.3 CD8$^{+}$ T cells were co-transferred with CD4$^{+}$ T cells. Here, 8.3 CD8$^{+}$ and CD4$^{+}$ T cells were isolated from the spleen of 5 wk-old 8.3-NOD and 12 wk-old NOD female mice, respectively. NOD.*scid* and NOD.*scid*.IFNγRB$^{null}$ mice 6-8 wks of age received either 8.3 CD8$^{+}$ T cells ($2\times10^{6}$ cells/mouse) alone or both 8.3 CD8$^{+}$ T cells and NOD CD4$^{+}$ T cells ($2\times10^{6}$ cells/mouse).

### Insulitis

Pancreases were removed, fixed in 10% formalin buffer (Fisher Scientific), and embedded in paraffin. Serial sections were stained with H&E and non-overlapping sections evaluated for insulitis as previously described [44]. Islets were scored as intact (no infiltration), peri-insulitis (infiltrates surrounding the islet) and intra-insulitis (infiltrates within islets) which was further scored as <50% and >50% infiltration.

### Blood glucose tolerance test

IPGTT was performed as described [45, 46]. Briefly, mice were fasted for 12 hr and tested for baseline of blood glucose levels prior to glucose administration. Mice were then injected i.p. with a 10% glucose solution in PBS (3g/kg body weight) and blood glucose levels measured (Abbott Diabetes Care Inc).

### Bone marrow chimera

Bone marrow reconstitution experiments were carried out according to the method of Tatekawa [26]. Briefly, bone marrow was prepared from femurs and tibias of 6 wk-old NOD.*scid* or NOD.GFP mice and depleted of red blood cells. NOD.*scid* and

NOD.*scid*.IFNγRB$^{null}$ mice 6 wks of age were irradiated with 200 cGy using a gamma irradiator and injected i.v. with $3\times10^7$ bone marrow cells 24 hr after irradiation. Eight wks post-transplantation, bone marrow reconstitution was confirmed by examining GFP expression by B cells and T cells. Chimeric NOD.*scid* and NOD.*scid*.IFNγRB$^{null}$ mice were then adoptively transferred with $2\times10^6$ BDC2.5 CD4$^+$ T cells purified from BDC2.5.NOD.Cα$^{null}$ mice and monitored for diabetes.

### *In vitro* β cell death

Islets were isolated and cytokine-induced β cell death was performed as described [47, 48]. Briefly, hand-picked islets were cultured for 4 days at 37°C in complete CMRL-1066 medium with recombinant cytokines (IFN-γ, 100 units/ml; IL-1β, 10 units/ml; and TNF-α, 1000 units/ml) (Peprotech). Islets were dispersed into single cells with 0.2% trypsin and 10 mM EDTA in Hank's balanced salt solution, and cell death evaluated by flow cytometry after staining with propidium iodide (Invitrogen).

### *In vivo* T cell proliferation

CFSE-labeled BDC2.5 CD4$^+$ T cells ($2\times10^6$ cells/mouse) were injected i.v. into NOD.*scid* and NOD.*scid*.IFNγRB$^{null}$ mice. Cells were harvested from the spleen, PLN, MLN and islets 4 and 6 days post-transfer and stained with mAbs (eBioscience, San Diego, CA) specific for Vβ4, CD4, CD44 and CD62L. Proliferation and activation of BDC2.5 CD4$^+$ T cells were analyzed by flow cytometry (DakoCytomation).

### Statistical analysis

Diabetes incidence was assessed with a Kaplan–Meier Log Rank Test (Prism,GraphPad, San Diego, CA). The statistical analysis of β-cell death induced by cytokines was performed by using the log-rank (Mantel–Cox) test (Prism,GraphPad, San Diego, CA).

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## Abbreviations

| | |
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| **PLN** | pancreatic lymph node |
| **T1D** | type 1 diabetes |
| **IFNγRB** | IFN-γ receptor β chain |

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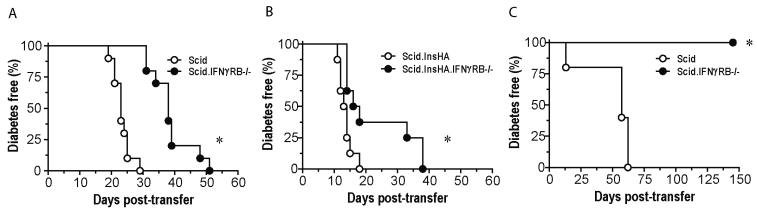


**Figure 1. IFNγR deficiency in NOD.*scid* recipients prevents diabetes induction by BDC2.5 CD4$^+$ T cells**

A. Diabetes incidence in NOD.*scid* (Scid, n=10) and NOD.*scid*.IFNγRB$^{null}$ (Scid.IFNγRB−/−, n=10) mice adoptively transferred with splenocytes ($1\times10^7$ cells/mouse) from diabetic NOD mice; *P<0.0001. B. Diabetes incidence in NOD.*scid*.InsHA (Scid.InsHA, n=8) and NOD.*scid*.InsHA.IFNγRB$^{null}$ (Scid.InsHA.IFNγRB−/−, n=8) recipients of purified CL4 CD8$^+$ T cells ($2\times10^6$ cells/mouse) from the spleen of NOD.*scid*.CL4 mice; *P<0.0144. C. Diabetes incidence in NOD.*scid* (Scid, n=5) and NOD.*scid*.IFNγRB$^{null}$ (Scid.IFNγRB−/−, n=6) recipients of purified BDC2.5 CD4$^+$ T cells ($2\times10^6$ cells/mouse) from the spleen of NOD.BDC2.5.Cα$^{null}$ mice; *P<0.0011.

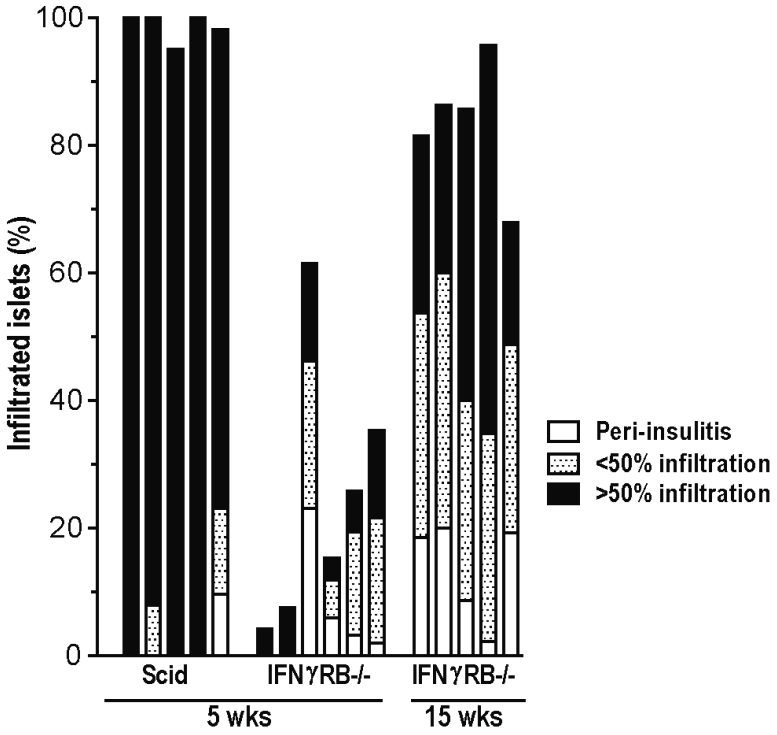


**Figure 2. NOD.*scid*.IFNγRB$^{null}$ recipients of BDC2.5 CD4$^{+}$ T cells exhibit delayed onset and progression of insulitis**

Pancreases were harvested from NOD.*scid*.IFNγRB$^{null}$ (IFNγRB−/−) recipients of BDC2.5 CD4$^{+}$ T cells and evaluated for insulitis 5 and 15 wks post-transfer. Insulitis in NOD.*scid* (Scid) recipients of BDC2.5 CD4$^{+}$ T cells was examined 5 wks post-transfer. The data presented in the figure shows the percentage of infiltrated islets of total islets examined in individual animals. At least 50 islets per mouse were examined and bars represent individual animals.

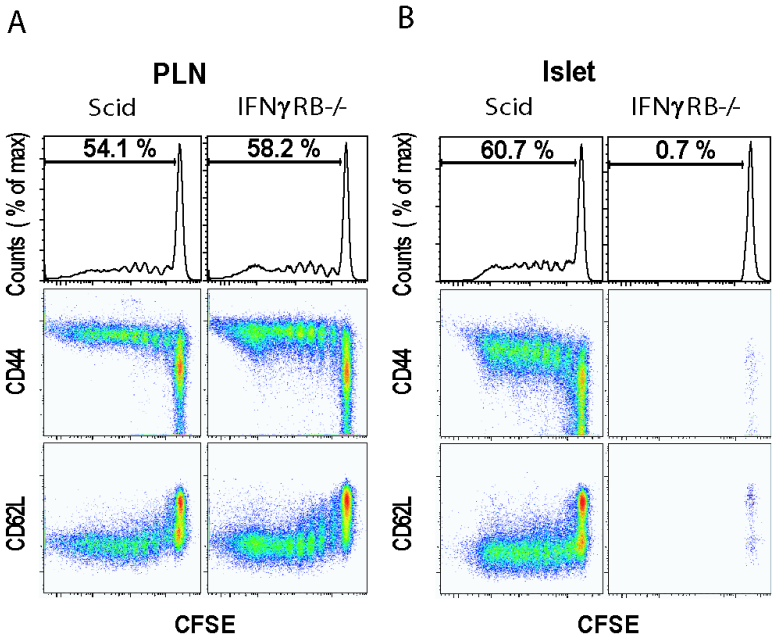


**Figure 3. IFNγR deficiency has no impact on the priming of BDC2.5 CD4$^+$ T cells in the pancreatic lymph nodes of NOD.*scid*.IFNγRB$^{null}$ recipients**

CFSE-labeled BDC2.5 CD4$^+$ T cells were adoptively transferred into NOD.*scid* (Scid) and NOD.*scid*.IFNγRB$^{null}$ (IFNγRB−/−) mice. Cells were harvested from the PLN and islets 6 days after T cell transfer and stained with Abs specific for Vβ4, CD44, and CD62L. Proliferation and activation of BDC2.5 CD4$^+$ T cells in the PLN (A) and the islets (B) were measured by CFSE dilution and the expression of CD44 and CD62L. Data shown are representative of 3 experiments.

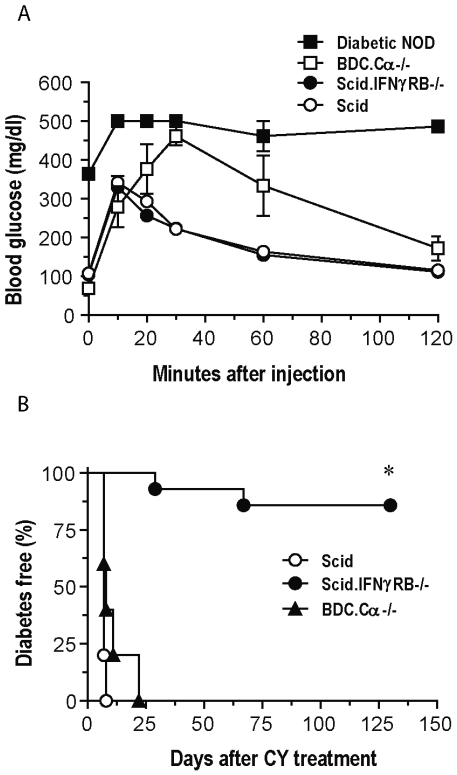


**Figure 4. β cells in NOD.*scid*.IFNγRB$^{null}$ recipients of BDC2.5 CD4$^{+}$ T cells exhibit normal function, and are resistant to diabetes induction by CY-treatment**

(A) NOD.*scid*.IFNγRB$^{null}$ (Scid.IFNγRB−/−) recipients of BDC2.5 CD4$^{+}$ T cells (n=6) were injected (i.p.) with glucose solution (3 g/kg body weight) 20 wks post-transfer. NOD.*scid* (Scid) mice (n=4) with no T cell transfer, NOD (Diabetic NOD) mice (n=2) with recent onset of diabetes; and 10 wk-old pre-diabetic BDC2.5.NOD.Cα$^{null}$ (BDC.Cα) mice (n=5) were injected with glucose and served as controls. Blood glucose levels (mg/dl) were measured after glucose injection. (B) NOD.*scid*.IFNγRB$^{null}$ (Scid.IFNγRB−/−, n=14) recipients of BDC2.5 CD4$^{+}$ T cells were injected with CY (200ng/kg body weight) 20 wks post-transfer. Pre-diabetic NOD.*scid* (Scid, n=4) recipients were injected with CY 5 wks after T cell transfer. Pre-diabetic BDC2.5.NOD.Cα$^{null}$ (BDC.Cα, n=5) mice receiving the same treatment were used as controls. Data show the percentage of diabetes free animals. *P<0.0001 for NOD.*scid*.IFNγRB$^{null}$ recipients versus NOD.*scid* recipients or BDC2.5.NOD.Cα$^{null}$ mice. Data are representative of 3 experiments.

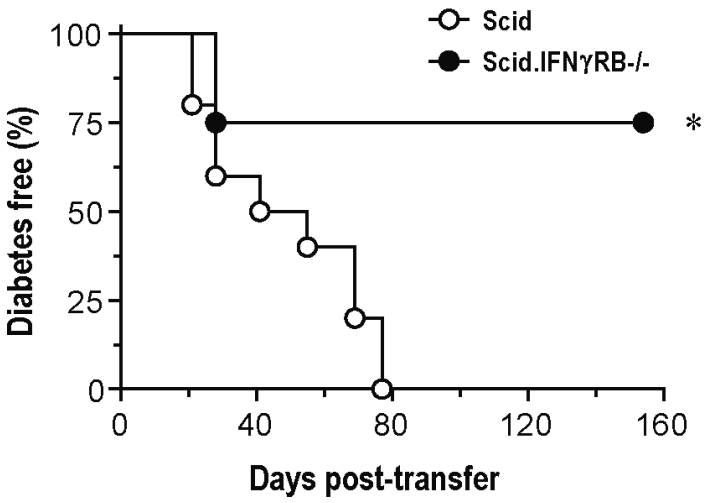


**Figure 5. Chimeric NOD.*scid*.IFNγRB$^{null}$ mice are resistant to diabetes induced by BDC2.5 CD4$^+$ T cells**

NOD.*scid*.IFNγRB$^{null}$ and NOD.*scid* mice were irradiated and reconstituted with bone marrow from NOD.*scid* mice. Eight weeks after bone marrow reconstitution, the chimeric NOD.*scid*.IFNγRB$^{null}$ (Scid.IFNγRB−/−, n=8) and NOD.*scid* (Scid, n=10) mice were adoptively transferred with purified BDC2.5 CD4$^+$ T cells ($2\times10^6$ cells/mouse) and followed for the development of diabetes. The percentage of diabetes-free animals is shown. *P<0.0034. Data are representative of 2 experiments.

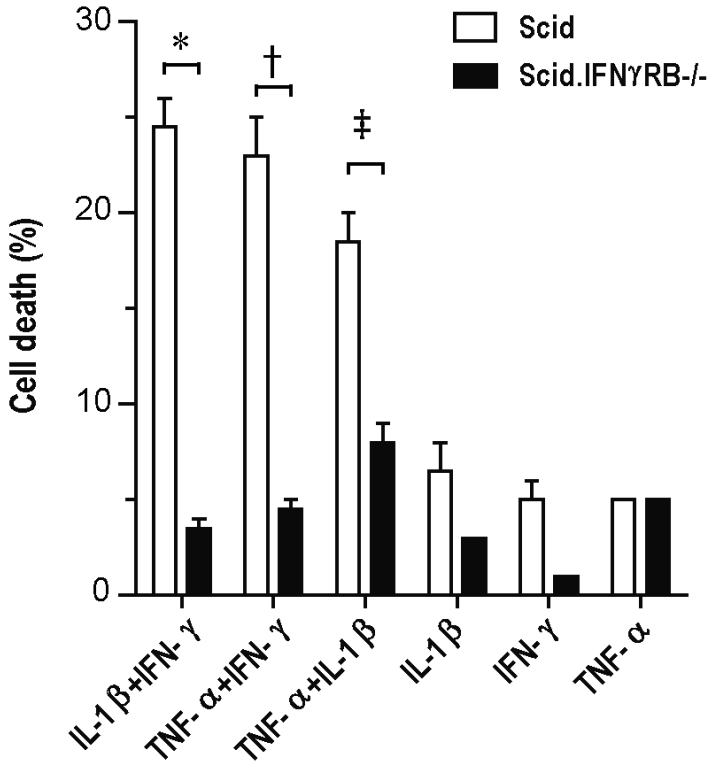


**Figure 6. IFNγR-deficient β cells are resistant to proinflammatory cytokine-induced cell death *in vitro***

Fresh islets isolated from NOD.*scid* (white bar) or NOD.*scid*.IFNγRB$^{null}$ (black bar) mice were cultured for 4 days in the presence of cytokines indicated. Cytokine-induced death of β cells was determined by FACS after staining with propidium iodide. *P=0.0056; **P=0.0122; ***P=0.0282. Data are representative of 3 experiments.

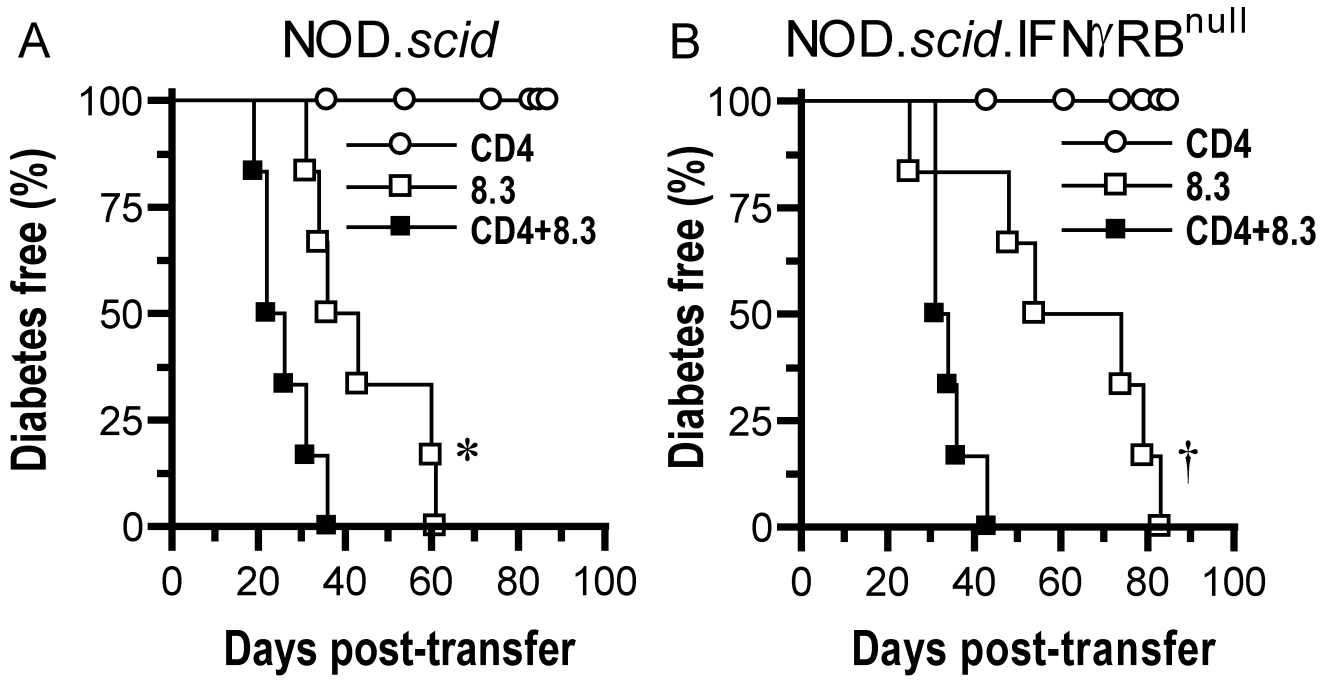


**Figure 7. CD4$^+$ T cells accelerate the onset of diabetes induced by CD8$^+$ T cells in NOD.*scid*.IFNγRB$^{null}$ mice**

NOD.*scid* (A) and NOD.*scid*.IFNγRB$^{null}$ (B) mice (6 to 8 mice per group) were adoptively transferred (i.p.) with 8.3 CD8$^+$ T cells (8.3) alone ($2\times10^6$ cells/mouse;) or purified CD4$^+$ T cells (CD4) ($2\times10^6$ cells/mouse) from 12-wk-old pre-diabetic NOD mice or co-transferred with both CD4$^+$ and 8.3 CD8$^+$ T cells (CD4+8.3). The data show the percentage of diabetes-free animals. *P=0.0121 for NOD.*scid* recipients of 8.3 versus CD4+8.3. †P=0.0109 for NOD.*scid*.IFNγRB$^{null}$ recipients of 8.3 versus CD4+8.3. Data shown are representative of two experiments.