Dual role of interferon- γ signalling pathway in sensitivity of pancreatic beta cells to immune destruction

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Abstract

Aims/hypothesis. Disruption of the interferon-gamma (IFN- γ) signalling pathway at the level of interferon regulatory factor-1 (IRF-1) protects islets against cytokine-induced nitric oxide production and cell death in vitro. The aim of this study was to investigate the effects of a global disruption of IFN- γ signalling, or a selective disruption of IRF-1, on beta-cell sensitivity to in vivo immune destruction.

Methods. In a first set of experiments, IFN- γ receptor knockout mice (IFN- γ R^{-/-}) and interferon regulatory factor-1 knockout mice (IRF-1^{-/-}) were rendered diabetic by injections of 50 mg streptozotocin i.p. on 5 consecutive days (MLDSTZ).

Results. Whereas no difference in sensitivity to MLDSTZ-induced diabetes could be observed between IFN- γ R^{-/-} mice and their 129/Sv/Ev controls (50% vs 55%, NS), there was an increased incidence of diabetes in IRF-1^{-/-} mice (100% vs 67% in C57Bl/6 mice, $p < 0.05$). A similar increased sensitivity to

immune destruction of IRF-1^{-/-} islets was observed when these islets were used as allografts. Islet graft survival rate of IFN- γ R^{-/-} and 129/Sv/Ev islets, when transplanted in alloxan-diabetic BALB/c recipients, was comparable (12.0 ± 1.9 days vs 12.9 ± 2.3 days, NS). Allograft rejection, however, of IRF-1^{-/-} islets by BALB/c recipients occurred more rapidly than following transplantation to their C57Bl/6 controls (9.1 ± 2.0 days vs 13.1 ± 1.5 days, $p < 0.003$).

Conclusions/interpretation. These data indicate that IFN- γ signal transduction at the beta-cell level is not essential for immune beta-cell destruction in vivo. Moreover, disruption of the IRF-1 gene in pancreatic islets increases susceptibility to beta-cell killing, suggesting that IRF-1 might be necessary for the expression of putative beta-cell “defence and/or repair” genes. [Diabetologia (2001) 44: 567–574]

Keywords Type I diabetes, cytokines, interferon regulatory factor-1, interferon- γ , signal transduction, islet destruction.

The ability of pancreatic islets to endure assaults of the immune system could be relevant in the development of Type I (insulin-dependent) diabetes mellitus. We and other investigators have suggested that inter-

leukin-1 β (IL-1 β) and other cytokines, especially interferon-gamma (IFN- γ) and tumor necrosis factor- α (TNF- α) contribute to beta-cell dysfunction and death during early Type I diabetes [1–3]. Combinations of cytokines can contribute to beta-cell dysfunction in rodents by inducing inducible nitric oxide synthase (iNOS) expression, leading to the production of the radical nitric oxide [4–6]. Nitric oxide has been shown to cause Krebs cycle dysfunction and directly damage nuclear DNA in islet cells [7–10]. The presence of IL-1 β is sufficient to induce iNOS transcription and nitric oxide formation in rodent islets, while a mixture of IL-1 β , IFN- γ and TNF- α is required for iNOS mRNA expression in human islet cells [2].

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Abbreviations: iNOS, Inducible nitric oxide synthase; IFN- γ , interferon-gamma; IRF-1, interferon regulatory factor-1; IL, interleukin; MLDSTZ, multiple low dose streptozotocin; STAT, signal transducer and activator of transcription; STZ, streptozotocin.

These data suggest that IFN- γ can play an important part in the development of diabetes. Indeed, islet-infiltrating lymphocytes produce IFN- γ during insulinitis in animal models of Type I diabetes and administration of anti-IFN- γ antibodies attenuates the development of the disease [11, 12].

Interferon-gamma activates a family of transcription factors known as the signal transducers and activators of transcription (STATs). Phosphorylation of STAT-1 α by the receptor-associated Janus kinase 1 and 2 (JAKs) in response to IFN- γ results in STAT-1 α homodimerization and nuclear translocation. Activated STAT-1 α complexes stimulate transcription of new genes by binding to gamma-activated site (GAS) elements in their promoter regions [13, 14]. One of these genes encodes interferon regulatory factor-1 (IRF-1) [15], a transcription factor involved in the induction of iNOS expression in pancreatic islet cells and known as a key player in the regulation of cell growth, apoptosis and function of a variety of immune effector cells [16–20].

It has been recently shown that IRF-1 mRNA expression is induced by IFN- γ , and to a minor degree by IL-1 β , and precedes iNOS expression and nitric oxide production in islets of rodent and human origin [16]. Moreover, by using the IRF-1 knockout mice (IRF-1 $^{-/-}$) (a mouse with a targeted disruption of the IRF-1 gene) [21], our group has recently shown that in vitro exposure of whole IRF-1 $^{-/-}$ islets to a mixture of cytokines led to a significant decrease in iNOS expression, nitric oxide formation and islet cell death [22]. On the other hand, FACS-purified IRF-1 $^{-/-}$ beta cells were not protected against these deleterious effects of cytokines [22]. These in vitro findings suggest a relevant role for IRF-1 gene expression in islet non-beta cells for cytokine-mediated destruction of beta cells in whole islets.

To further evaluate the role of IFN- γ , and especially IRF-1, as possible mediators of in vivo immune-mediated beta-cell destruction, we chose two genetically modified mouse models, namely the IFN- γ receptor knockout mice (IFN- γ R $^{-/-}$) (a mouse deficient in the IFN- γ receptor) and the IRF-1 $^{-/-}$ (a mouse with a targeted disruption of the IRF-1 gene) mouse. We tested in these mice the susceptibility of beta cells towards immune destruction in multiple low dose streptozotocin (MLDSTZ)-induced diabetes and when transplanted in an allogeneic host.

Materials and methods

Animals. Breeding pairs of IRF-1 $^{-/-}$ (3-times backcrossed into a C57Bl/6 mice) were kindly provided by Dr. T. Mak of the Ontario Cancer Institute (Ontario, Canada). As wild-type controls, C57Bl/6 mice were used (Harlan CPB, Zeist, the Netherlands). IFN- γ R $^{-/-}$ and their controls (129/Sv/Ev mice) were kindly provided by Dr. P. Matthys. All animals were kept under semi-barrier conditions in the animal house of the Rega-

Institute (Leuven, Belgium). As recipients for islet grafting, we used 8 to 10 week-old BALB/c mice (Harlan CPB). All experimental designs were conducted with the approval of the Animal Ethics Committee of the Katholieke Universiteit Leuven.

MLDSTZ-induced diabetes. Streptozotocin (STZ) (Sigma Chemical, St. Louis, Mo., USA) was dissolved in 25 mmol/l citrate buffer (pH 4.5). Injections were made intraperitoneally (50 mg/kg) within 15 min of preparation. Male recipients (IRF-1 $^{-/-}$, IFN- γ R $^{-/-}$ and the respective controls) were treated with 5 consecutive daily injections [23] and development of glucosuria was monitored with Clinistix (Bayer Diagnostics, Tarrytown, N. Y., USA). Blood glucose was measured (Glucocard, Menarini, Firenze, Italy) in mice having glucosuria. A mouse was considered diabetic when measurements of blood glucose were higher than 11.0 mmol/l on two consecutive days. Mice were followed up until 45 days after the last injection of STZ. At that time point the animals were killed, and the pancreas removed for histology and insulin content measurement.

Islet allograft model. Islets from IRF-1 $^{-/-}$, IFN- γ R $^{-/-}$ or of wild-type mice of each respective strain were isolated as described previously [24]. Briefly, pancreases of young mice were digested with collagenase in cold Hank's balanced salt solution by vigorous shaking. Islets were handpicked under a stereomicroscope. Islet recipients used were 8 to 10-week-old BALB/c mice. One day before islet grafting, BALB/c mice were rendered diabetic by a single intravenous injection of 90 mg/kg alloxan (Fluka Chemika, Bornem, Belgium). Mice were anaesthetised by injection of avertin intraperitoneally (0.02 ml/mg BW) (Fluka Chemika). The left kidney was exposed through a lumbar incision and recipient mice were transplanted with 500 fresh islets. Allograft function was followed by daily blood glucose measurements (Glucocard, Menarini, Firenze, Italy). Graft rejection was defined as measurements of blood glucose higher than 11.0 mmol/l on two consecutive days. Recipient mice were killed on the day of graft rejection or in a separate experiment for cytokine analysis in the graft on day 2 post transplantation. At that moment, the graft was removed for PCR analysis of infiltrating cytokines.

Histology and insulin content determination. In the MLDSTZ-model, pancreata were removed at the end of the experiment (45 days after STZ injection). One half of each pancreas was snap-frozen in liquid nitrogen and stored at -80°C until processing. Serial cryostat sections (5 μm thickness) were cut and stained with haematoxylin and eosin to assess pancreatic islet integrity [25]. Immunostaining was done with a polyclonal rabbit-anti-insulin antibody as previously described [26]. The beta-cell fraction in the islets from 5 pancreata per group (10 sections per pancreas) was analysed in a semi-quantitative blinded fashion. The insulin-positive surface of each individual islet was graded as percentage of the total islet surface (> 80 , 60–80, 40–60, 20–40, or $< 20\%$ of insulin-positivity per islet). The remaining half of each pancreas was used for insulin content determination as described [25].

Total RNA extraction, cDNA synthesis and real time RT-PCR analysis. In the allogeneic islet transplant model, total RNA was extracted from both islet graft and control kidney tissue (background level) using TRIzol reagent (Life Technologies, Gaithersburg, Md., USA) as described by the manufacturer. Target RNA (5 μg) was reverse transcribed using 100 U Superscript II RT (Life Technologies) at 42°C for 80 min in the presence of random primers. For each reaction set, one RNA sam-

Fig. 1 A, B. Multiple low-dose streptozotocin (MLDSTZ)-induced diabetes in (A) IFN- γ R^{-/-} ($n = 10$, ●) and 129/Sv/Ev ($n = 11$, ▲) mice; (B) IRF-1^{-/-} ($n = 13$, ■) and C57Bl/6 ($n = 15$, ▼) mice. MLDSTZ-diabetes was induced in male mice as described under materials and methods. * $p < 0.05$ vs STZ-treated C57Bl/6 control mice

ple was carried out without Superscript II RT (RT⁻ reaction) to provide a negative control in the subsequent PCR reactions.

For IL-1 β , TNF- α , IFN- γ , IL-6, iNOS and the “house-keeping gene” β -actin, real time RT-PCR analysis of the islet grafts was done in the ABI prism 7700 Sequence detector (Perkin Elmer, Foster City, Calif., USA) as described previously [27]. Briefly, the PCR reactions contained in 25 μ l reaction solution: 1 μ l cDNA, 100 to 200 nmol/l of forward and reverse primers, 200 μ mol/l dATP, dCTP, dGTP and 400 μ mol/l dUTP, 2.5 μ l TaqMan Buffer A, 3 to 9 MgCl₂, 0.25 U AmpErase Uracil N-Glycosylase and 0.625 units AmpliTaq Gold DNA polymerase (Perkin Elmer). Each reaction also contained 100 nmol/l of the corresponding probe and was done in triplicate wells, using the following conditions: 2 min at 50°C and 10 min at 94°C, followed by a total of 40 to 45 two-temperature cycles (15 s at 94°C and 1 min at 60°C).

To enable comparisons between the amounts of target cDNA present in the different samples, we used external standards for each target cDNA, as well as for β -actin. To prepare these standards, plasmid clones containing a partial cDNA sequence of each target were constructed as described previously [27]. Serial dilutions of the resulting clones were used as standard curves each containing a known amount of input copy number. Cytokine mRNA expression was defined as a mean of cytokine copies/ β actin copies.

Statistical analysis. Data are presented as means $\pm$ standard deviation and statistical analysis was done using the chi-squared test for the incidence of MLDSTZ-induced diabetes, the log-rank test for graft survival, the Student t test for metabolic evaluation and the analysis of variance (ANOVA) for cytokine analysis. Statistical significance was defined as a p value of less than 0.05.

Results

MLDSTZ-induced diabetes. All experimental mice were sensitive to the hyperglycaemic action of MLDSTZ treatment. Diabetes incidence in full IFN- γ R^{-/-} mice ($n = 10$) was not different from that of the 129/Sv/Ev control mice ($n = 11$) (50% vs 55%, NS) (Fig. 1). Interferon- γ receptor knockout mice as well as 129/Sv/Ev control mice became diabetic from 8 to 29 days after MLDSTZ injection. Surprisingly, all IRF-1^{-/-} mice ($n = 13$) exposed to MLDSTZ injection became diabetic (100%) compared to only 10 out of 15 of STZ-treated C57Bl/6 control mice (67%, $p < 0.05$). Timing of diabetes onset was not different between IRF-1^{-/-} and C57Bl/6 control mice (5 to 36 days after treatment with MLDSTZ).

Histological analysis of pancreata removed from IFN- γ R^{-/-} or 129/Sv/Ev control mice at the end of the experiment showed comparable depletion of insulin positive beta cells in the few islets present. At this time point, little evidence remained of lymphocytes infiltrating into the residual insulin-depleted islets of both strains (data not shown). Pancreata from MLDSTZ-treated IRF-1^{-/-} mice clearly contained fewer islets, which were reduced in size and number of insulin positive beta cells, compared with MLDSTZ-treated C57Bl/6 mice at day 45 after STZ injection (Fig. 2A–D). At this late time point hardly any lymphocyte infiltrates remained in either group. Semi-quantitative analysis of the pancreatic sections showed that in IFN- γ R^{-/-}, 129/Sv/Ev and C57Bl/6 mice, respectively 23, 34, 24% of the counted islets had an insulin-positive area of more than 50% of the islet, whereas in IRF-1^{-/-} mice only 3% of the islets maintained insulin-positivity in more than 50% of their surface ($p < 0.01$ vs STZ-treated control C57Bl/6 mice) (Table 1).

At the end of the experiment, insulin content determinations in pancreata from diabetic mice with a full IFN- γ receptor knockout or from 129/Sv/Ev control mice showed no differences (Table 1). On the

Fig. 2A–D. Histopathology of pancreata from STZ-treated mice. Animals were killed at day 45 after STZ injection. (**A**, **C**) MLDSTZ-injected IRF-1^{-/-}, (**B**, **D**) MLDSTZ-injected wild type C57Bl/6 control mice. Haematoxylin and eosin (**A**, **B**), insulin staining (**C**, **D**). Magnification: $\times 50$ (**A–B**), $\times 100$ (**C–D**). Note the severe depletion of insulin positive beta cells (in the few small residual islets) of the pancreata of STZ-treated IRF-1^{-/-} mice as compared to their respective C57Bl/6 controls

other hand, insulin content of pancreata from diabetic IRF-1^{-/-} mice was significantly decreased compared with C57Bl/6 control mice ($p < 0.05$), indicating a more important destruction of insulin-producing beta cells in IRF-1^{-/-} mice (Table 1).

Islet allograft survival. To evaluate the effect of a global (IFN- γ R^{-/-}), or selective (IRF-1^{-/-}), disruption of IFN- γ signalling on sensitivity of the islets to allograft rejection, we transplanted either IFN- γ R^{-/-} or IRF-1^{-/-} islets and their respective control islets in alloxan-diabetic BALB/c mice. In the absence of the IFN- γ receptor ($n = 8$), islet allograft survival was comparable to that observed in islets from 129/Sv/Ev

control donors ($n = 9$) (12.0 ± 1.9 days vs 12.9 ± 2.3 days, NS) (Fig. 3). On the other hand, islets from IRF-1^{-/-} donors transplanted to alloxan-diabetic BALB/c mice ($n = 9$) had a significantly reduced survival time than the C57Bl/6 control islets ($n = 9$) (9.1 ± 2.0 days vs 13.1 ± 1.5 days, $p < 0.003$) (Fig. 3), indicating an increased susceptibility of the IRF-1^{-/-} islets to immune-mediated destruction.

To investigate whether the more rapid rejection of IRF-1^{-/-} islets was not due to a higher expression of inflammatory cytokines induced in BALB/c mice by these islets, we analysed the patterns of mRNA expression of IL-1 β , TNF- α , IFN- γ , and iNOS in the islet grafts of IFN- γ R^{-/-}, IRF-1^{-/-} and respective control mice at day 2 post transplantation ($n = 4$ per group) and at the time of graft rejection ($n = 8–9$ per group). No differences were observed between the cytokine profiles present in the IRF-1^{-/-} and their control C57Bl/6 islet grafts. As expected, no significant differences were observed between the inflammatory cytokine patterns or iNOS mRNA expression in islet grafts of IFN- γ R^{-/-} and corresponding 129/Sv/Ev control mice (Fig. 4A,B). Similar results were obtained for IL-6 mRNA expression in islet grafts (data not

Fig. 3 A, B. Survival of allogeneic islets of (A) IFN- γ R^{-/-} (*n* = 8, ●) and 129/Sv/Ev (*n* = 9, ▲) mice; (B) IRF-1^{-/-} (*n* = 9, ■) and C57Bl/6 (*n* = 9, ▼) mice in alloxan-diabetic BALB/c mice. Rejection was defined as a return to hyperglycaemia (> 11.0 mmol/l) after a period of normoglycaemia. * *p* < 0.003 vs C57Bl/6 mice. Note the faster destruction of IRF-1^{-/-} islet grafts compared to C57Bl/6 control islets in alloxan-diabetic BALB/c mice

shown). These observations show that it is not likely that the faster destruction of IRF-1^{-/-} islets is caused by a more vigorous inflammatory response.

Discussion

There is increasing evidence that cytokines, such as IL-1 β , TNF- α and IFN- γ , secreted by infiltrating immune cells and free radicals are effector molecules involved in beta-cell dysfunction and damage in Type I diabetes [3]. Many of the cytokine-induced effects involved in inhibition of beta-cell function and beta-cell destruction are regulated at the transcriptional level. We have examined the effects of a global disruption of IFN- γ signalling or a selective disruption of the IRF-1 pathway, on beta-cell sensitivity to immune destruction in vivo. In vitro, full IFN- γ receptor knockout islets are resistant to cytokine-induced damage, as suggested by a near complete reduction in iNOS

and MHC-I mRNA expression after a 24 h exposure to IL-1 β + IFN- γ , as compared with wild-type control islets (Eizirik, unpublished data). Our in vivo data, however, show that absence of the IFN- γ receptor at the beta-cell level does not reduce the rate of MLDSTZ-diabetes development. Moreover, our islet transplantation data illustrate that β -cell-IFN- γ receptor deficiency has no protective effect on the outcome of islet allograft survival. Together, these data suggest that IFN- γ signal transduction at the beta-cell level does not play a major role in immune-mediated beta-cell destruction in vivo. These data are consistent with a previous report showing that transgenic NOD mice with a mutant IFN- γ receptor on their pancreatic islet cells have a similar rate of diabetes progression as wild-type controls [28]. These observations suggest that, in the absence of IFN- γ signal transduction, the immune system utilizes other mechanisms to damage mouse beta-cells. Of note, IFN- γ , in combination with IL-1 β , is required to induce death of human islet cells [9], whereas IL-1 β alone is sufficient to kill rat islet cells [1, 5]. Thus, it is conceivable that IFN- γ plays a more relevant role for human than for mouse beta-cell death in autoimmune diabetes.

We have previously observed a reduction in iNOS expression, nitric oxide formation and islet cell death in whole islet cells from mice with a selective disruption of the IRF-1 gene [22]. On the other hand, lack of IRF-1 gene expression did not protect FACS-purified beta cells against cytokine-induced cell death [22]. These data suggest that IRF-1 expression, probably in the non-beta-cells present in whole islets, is involved in cytokine-induced islet cell damage. Surprisingly, we observed that the absence of the IRF-1 gene in pancreatic islets in vivo promotes MLDSTZ-induced diabetes and accelerates destruction of beta cells in an allogeneic setting. These conflicting data cannot be attributed to more vigorous inflammatory responses elicited in vivo by the lack of beta-cell IRF-1 expression, since there were no differences in pro-inflammatory cytokine expression patterns and

Table 1. Evaluation of pancreatic insulin content in multiple low-dose streptozotocin-(MLDSTZ)-treated IFN- γ R^{-/-}, IRF-1^{-/-} and respective control mice (immunohistochemistry and ELISA)

Strain	Number of islets with respective beta-cell fraction (%)					Pancreatic insulin content (pmol/mg)
	> 80 (% insulin-positivity of islet surface)	60–80	40–60	20–40	< 20	
IFN- γ R ^{-/-}	0	5	18	5	72	1.024 $\pm$ 0.307
129/Sv/Ev	0	11	23	6	60	1.656 $\pm$ 0.608
IRF-1 ^{-/-}	0	0	3	11	86 ^a	0.566 $\pm$ 0.191 ^b
C57Bl/6	2	9	13	14	62	1.118 $\pm$ 0.882

MLDSTZ-diabetes was induced in male mice as described in materials and methods. Histological examination of pancreata from IFN- γ R^{-/-} and 129/Sv/Ev mice showed a similar decrease in islet beta-cell fraction, while IRF-1^{-/-} pancreata had a higher depletion of their beta-cell fraction 45 days post STZ treatment compared with STZ-treated control C57Bl/6 mice. ^a *p* < 0.01 vs STZ-treated C57Bl/6 control mice.

Insulin content was also determined by ELISA. Pancreatic insulin content in control mice (not treated with STZ) is in the range of 14.383 $\pm$ 6.969 pmol insulin/mg tissue. ^b *p* < 0.05 vs STZ-treated C57Bl/6 control mice

Fig. 4A, B. Real time RT-PCR analysis of cytokine mRNA patterns in islet transplants into alloxan-treated mice at day 2 post transplantation (**A**) and at the time of graft rejection (**B**). Islets were isolated from IFN- γ R^{-/-}; IRF-1^{-/-} and respective control mice, and transplanted into alloxan-diabetic BALB/c mice. No differences in pro-inflammatory cytokine expression or iNOS mRNA were noted between the different experimental groups. Of note, however, is the much lower global expression pattern at day 2 post transplantation than at day of rejection (2–100 times lower)

iNOS mRNA levels between knockout and respective wild type control islet grafts. These data suggest that disruption of the IRF-1 gene in pancreatic islets increases susceptibility to in vivo immune beta-cell destruction.

Although inflammatory cytokines, such as TNF- α and IFN- γ , are clear mediators of beta-cell destruc-

tion in vitro, their role in beta-cell killing in in vivo models of Type I diabetes is less clear. Accelerated models of diabetes generated by transgenic expression of cytokines by islet beta cells have provided evidence that IFN- γ is not sufficient to cause complete destruction of pancreatic beta cells in vivo because transgenic mice, expressing IFN- γ under the control of the glucagon promoter do not have insulinitis nor diabetes [29]. On the other hand, TNF- α has been more strongly implicated in the susceptibility to Type I diabetes, since mice with a transgenic expression of TNF- α in their beta cells are prone to the development of autoimmune diabetes. Indeed, TNF- α -NOD mice develop an accelerated form of diabetes through a more vigorous infiltration of the islets by immune cells [30].

These findings, together with our data emphasise that caution is required when extrapolating observations of effects of cytokines on beta cells observed in

vitro to a possible role in the pathogenesis of Type I diabetes. Most in vitro studies focus on the destructive properties of the cytokines as mediators of beta-cell damage, but recent data suggest that, under specific conditions, cytokines can also protect beta cells against a subsequent assault [31]. Whether the cytokines produced by islet-infiltrating macrophages and T cells are "directly" destructive to beta cells in vivo remains to be elucidated. In vivo, other destructive processes such as direct killing of beta cells by CD8 + cytotoxic cells, might also play an important role [32]. Moreover, during the in vivo immune response there is a mixture of endocrine and immune cells, some with membrane-bound cytokines, in close proximity. This can lead to a different outcome than the one observed in vitro, where pure beta cells are exposed to soluble recombinant cytokines.

Our data point towards a dual role for the IFN- γ signalling pathway in immune-mediated beta-cell destruction, with possibly a protective role for IRF-1. Interferon- γ -induced STAT-1 α interacts directly and indirectly, through other transcription factors such as IRF-1, with IFN- γ -inducible genes such as iNOS [17, 33]. Because in IRF-1^{-/-} mice the IRF-1 arm is knocked out, IFN- γ -induced STAT-1 α will only induce iNOS expression by direct GAS binding. The present observation that the absence of IRF-1, but not of IFN- γ receptor expression leads to a more severe and faster immune-mediated beta-cell destruction, suggests that IFN- γ induces beta-cell damaging (through STAT-1 α) and beta-cell protective (through IRF-1) mechanisms. In this context, it is of interest to identify these anti-survival and pro-survival genes, regulated by the transcription factors STAT-1 α and IRF-1.

We conclude that in contrast to the in vitro situation full or selective destruction of the IFN- γ signalling pathways in pancreatic islet cells cannot protect beta cells from immune destruction in vivo. Our data indicate that IFN- γ by itself is redundant for immune-mediated beta-cell killing and even generates dual effects since IRF-1 gene disruption in islets of Langerhans increases sensitivity to immune-mediated beta-cell destruction in vivo.

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