Original Articles

Early Graft Failure of Xenogeneic Islets in NOD Mice Is Accompanied by High Levels of Interleukin-1 and Low Levels of Transforming Growth Factor-β mRNA in the Grafts

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Early graft failure, graft rejection, and autoimmune recurrence remain unresolved issues in islet xenotransplantation in type 1 diabetes. The first aim of this study was to examine the existence of early graft failure in spontaneously diabetic autoimmune NOD mice after rat islet transplantation under technically controlled circumstances. The second aim was to examine the mediators of this early xenograft dysfunction. First, we demonstrated a higher percentage of early xenograft failure (48%) in spontaneously diabetic NOD mice as compared with chemically diabetic old NOD (13%, $P < 0.05$) and C57Bl/6 (7%, $P < 0.01$) mice. In addition, in spontaneously diabetic NOD mice, xenogeneic islets displayed early graft failure more frequently than allogeneic (23%, $P \leq 0.05$) or isogeneic islets (7%, $P < 0.01$). No early graft failure was observed in allotransplantation or isotransplantation in chemically diabetic mice. Reverse transcriptase–polymerase chain reaction analysis of cytokine mRNA in islet xenografts 8 h after transplantation showed higher levels of interleukin (IL)-1 mRNA in autoimmune diabetic mice compared with chemically diabetic old NOD mice (1.40 ± 0.32 vs. 0.90 ± 0.14 IL-1 copies/β-actin copies, $P < 0.05$). In contrast, mRNA levels of transforming growth factor (TGF)-β were lower in spontaneously diabetic NOD mice than in chemically diabetic old NOD mice (0.67 ± 0.16 vs. 1.36 ± 0.50 TGF-β copies/β-actin copies, $P < 0.05$). No differences in tumor necrosis factor-α, IL-6, and inducible nitric oxide synthase were seen between autoimmune and nonautoimmune diabetic mice. T-cell cytokines (IL-2, IL-4, IL-10, and γ-interferon) were absent in all mice until 48 h after transplantation. These data suggest that early islet xenograft failure is more common in spontaneously diabetic NOD mice and could be due to a nonspecific inflammatory reaction locally in the grafts. *Diabetes* 49:1992–1997, 2000

Whole-pancreas transplantation remains the most reliable surgical option for restoring normal glucose homeostasis in type 1 diabetes, but transplantation of isolated pancreatic islets holds great promise. A central problem in islet allotransplantation is the lack of suitable human organs for widespread islet replacement. Animals are a more likely tissue source. Xenotransplantion of islets in animal models of experimental diabetes, however, remains associated with many unresolved problems (1).

The NOD mouse that spontaneously develops autoimmune diabetes serves as an animal model for human type 1 diabetes. These spontaneously diabetic autoimmune mice undergo, in addition to islet graft rejection, a recurrence of the original autoimmune disease. Indeed, even when rejection of the graft is prevented or when an isogeneic transplantation is performed, islets will be destroyed by an immune attack, which resembles the one causing the earlier diabetic process (2,3). Autoimmune T-cells carry the memory responsible for this autoimmune recurrence (AIR).

Early graft failure, defined as the immediate destruction of tissues or grafted cells such as islets, is a process occurring before immunological graft rejection. The inability of both allogeneic and xenogeneic islets to achieve normoglycemia in chemically and spontaneously diabetic recipients after transplantation has already been reported (4–7). Until recently, it was believed that early graft failure was solely a technical problem that was related to one or more of the following: *1*) grafting of insufficient islet mass, *2*) the purity of the islet grafts, or *3*) the site of implantation (4), although immune-mediated mechanisms might be involved as well (5–9).

In fact, little is known about the involvement of AIR in this immediate failure of islets after grafting. Because spontaneously

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AIR, autoimmune recurrence; IFN-γ, γ-interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; NK, natural killer; RT-PCR, reverse transcriptase–polymerase chain reaction; TGF, transforming growth factor; TNF, tumor necrosis factor.

diabetic autoimmune NOD mice already have an immune system primed to anticipate an attack on pancreatic β-cells, it has been postulated that a certain percentage of immediate posttransplant losses of grafted islets may be due to AIR.

On the other hand, data concerning the impact of the xenogeneic nature of the islets on this phenomenon are very limited. Early graft failure is reported after both allogeneic and xenogeneic islet transplantation, but the mechanisms underlying both allograft and xenograft immune reactions are quite different. Allogeneic islet rejection is predominantly mediated through T-cells, whereas macrophages and natural killer (NK) cells are more involved in xenogeneic islet rejection (10,11).

Although the exact mechanisms involved in this early graft failure are still unexplained, several investigators have suggested a role for inflammatory processes mediated by macrophages (12,13). Indeed, macrophages are the first cells to infiltrate the pancreatic islets, even before T-cells, and they can secrete proinflammatory cytokines, which allow for the recruitment and activation of other invasive immune cells. In addition, inflammatory cytokines, especially interleukin (IL)-1, have been shown in vitro to inhibit insulin secretion and to be cytotoxic for pancreatic β-cells (14,15).

The first aim of this study was to examine the existence of early graft failure in spontaneously diabetic autoimmune NOD mice after rat islet transplantation under controlled technical circumstances. The second aim was to examine the mediators of this early xenograft dysfunction.

In the present study, we confirmed the high prevalence of early graft failure in spontaneously diabetic autoimmune NOD mice after xenogeneic islet transplantation (8). We demonstrated that both the autoimmune background of the recipient and the xenogeneic origin of the islets play a role in this early graft failure. Moreover, this high susceptibility of spontaneously diabetic autoimmune NOD mice to develop early xenograft failure correlates with the presence of high levels of inflammatory cytokines, in particular IL-1, and the absence of high levels of transforming growth factor (TGF)-β. No arguments for a T-cell–mediated AIR could be found.

RESEARCH DESIGN AND METHODS

Animals and induction of diabetes. NOD mice were provided by Professor C.Y. Wu (Beijing, China) and have been inbred in our animal facility since 1989. At the time of the experiments, this colony had a diabetes incidence of 72% in female and 23% in male mice. Housing of NOD mice occurred under semibarrier conditions (16). Spontaneously diabetic NOD mice, characterized by glucosuria (Clinistix; Bayer Diagnostics, Tarrytown, NY) and hyperglycemia (blood glucose >200 mg/dl) (Glucocard; Menarini, Florence, Italy) on two consecutive days, were used as autoimmune recipients for grafting.

As chemically induced models of experimental diabetes, old (>180 days of age) NOD mice that escaped development of spontaneous diabetes and C57Bl/6 mice purchased from Charles River (Wiga, Sulzfeld, Germany) were used. Diabetes was induced in these mice by an intravenous injection of 90 mg/kg alloxan (Fluka Chemika, Bornem, Belgium) 24 h before transplantation.

Xenogeneic donors were piebald virol glaxo (PVG) C6-sufficient (C+) rats (8–12 days old), obtained from the breeding colony at the Rega Institute (Leuven, Belgium). Allogeneic donors were young BALB/c mice (Harlan CPB, Zeist, the Netherlands) and isogeneic donors were either young NOD, NOD/ltSz-scid/scid (NOD-scid), or C57Bl/6 mice (8–21 days old).

Islet isolation and transplantation. Islets of rats or mice were isolated by previously published isolation procedures (17,18). Briefly, after aseptic removal, the pancreatic glands were digested with collagenase in cold Hanks' balanced salt solution during vigorous shaking. To avoid possible interference of endotoxin contamination of the collagenase, we used one and the same batch of collagenase for all islet isolation procedures, and all islet transplantations were performed at random in all experimental groups. In addition, the collagenase used (Serva, Heidelberg, Germany) had low endotoxin activity (2,750 EU/ml) compared with collagenase from other manufactures (19). Rat islets were hand picked under a stereomicroscope after dextran gradient centrifugation for removal of the endocrine tissue, whereas mouse islets were picked without prior centrifugation.

Both male and female recipient mice (age- and sex-matched per group) were anesthetized by intraperitoneal injection of avertin (0.02 ml/mg body wt) (Fluka Chemika). The left kidney was exposed through a lumbar incision and recipient mice were given 300 fresh islets.

Follow-up of mice. Nonfasting blood glucose levels of each recipient mouse were measured to monitor the rate of early graft failure and graft survival after transplantation. The measurement of glycemia after tail vein puncture was performed every day during the first week and then every other day until recurrence of hyperglycemia (>200 mg/dl blood glucose) (Glucocard). Early graft failure was defined as blood glucose levels never falling below 200 mg/dl for at least 3 consecutive days after islet transplantation, whereas islet graft rejection was defined as a return to hyperglycemia (>200 mg/dl blood glucose) after a period of normoglycemia.

Real-time reverse transcriptase–polymerase chain reaction of the grafts. A separate experiment of islet grafting for all experimental groups was performed for reverse transcriptase–polymerase chain reaction (RT-PCR) analysis of the transplants. Mice were sacrificed 8, 16, or 24 h after transplantation, when islet grafts were removed. Real-time RT-PCR was performed for IL-1, IL-2, IL-4, IL-6, IL-10, IL-12, tumor necrosis factor (TNF)-α, γ-interferon (IFN-γ), TGF-β, inducible nitric oxide synthase (iNOS), and β-actin as the housekeeping gene, as described previously (20). Briefly, total RNA was extracted from both graft and control kidney tissue (as background level) using TRIzol reagent (Life Technologies, Gaithersburg, MD) as described. Then, 5 µg target RNA was reverse transcribed using Superscript II RT (Life Technologies) at 42°C for 80 min in the presence of random primers. PCRs were performed in the ABI prism 7700 Sequence detector (PerkinElmer, Foster City, CA). The assay requires specifically designed hybridization probes that are dual-labeled with a fluorescent reporter and quencher dye and also specifically designed PCR primers. The 5′ nuclease activity of the *Taq* polymerase cleaves the fluorogenic probe during the extension phase of the PCR reaction, liberating the reporter dye and causing an increase in fluorescence emission. The ABI prism continuously measures this increase in fluorescence, providing real-time detection of the PCR product accumulation. For each sample, we performed a correction for RT variability by normalization to β-actin. PCR amplifications were performed in duplicate wells and repeated at least three times. The average cytokine mRNA values in the islet grafts were expressed as mean cytokine copy numbers (copies) related to mean β-actin copy numbers (copies), e.g., IL-1 copies/β-actin copies.

Statistical analysis. The following statistical tests were used as appropriate: the Kaplan-Meier survival technique, the log-rank test, the χ^2 test, the analysis of variance, and Fisher's least significant differences test were used. Significance was defined at the 0.05 level. Data are expressed as means ± SD.

RESULTS

Early graft failure after xenogeneic, allogeneic, or isogeneic islet grafting in autoimmune diabetic versus nonautoimmune diabetic mice. Transplantation of xenogeneic PVG (C+) rat islets under the kidney capsule of spontaneously diabetic autoimmune NOD mice resulted in early graft failure in 13 of 27 mice (48%) (Table 1). This early graft failure was defined as an inability of the graft to achieve normoglycemia within the first 3 days after transplantation. Early failure of graft function was less frequent in chemically diabetic nonautoimmune old NOD mice (2 of 16 mice [13%], $P < 0.05$) or in chemically diabetic C57Bl/6 mice (1 of 14 mice [7%], $P < 0.01$). Moreover, in recipients without early graft failure, a significantly shorter functional islet xenograft survival time (5.4 ± 2.1 days) was shown in spontaneously diabetic autoimmune NOD mice than in chemically diabetic nonautoimmune old NOD mice (9.4 ± 2.4 days, $P < 0.0005$) or chemically diabetic C57Bl/6 mice (10.1 ± 2.4 days, $P < 0.0005$).

After allotransplantation using BALB/c donors, we observed in 7 of 30 (23%) spontaneously diabetic autoimmune NOD mice early islet graft failure, whereas no early graft failure occurred in chemically diabetic recipients. Again, allogeneic islets had a significantly shorter graft survival time in spontaneously diabetic autoimmune NOD

TABLE 1
Outcome of islet xenogeneic, allogeneic, and isogeneic transplantation in spontaneously diabetic autoimmune NOD mice (autoimmune NOD), chemically diabetic nonautoimmune old NOD (Allox-NOD) mice, and chemically diabetic nonautoimmune C57Bl/6 mice (Allox-C57Bl/6)

Recipient	Donor	*n*	Early graft failure (%)	Survival time (days) of functioning grafts
Autoimmune NOD	PVG (C+)	27	48	5.4 ± 2.1 (3–9)
Allox-NOD	PVG (C+)	16	13*	9.4 ± 2.4 (5–14)‡
Allox-C57Bl/6	PVG (C+)	14	7†	10.1 ± 2.4 (8–16)‡
Autoimmune NOD	BALB/c	30	23*	9.2 ± 4.8 (3–17)*
Allox-NOD	BALB/c	18	0‡‖	15.0 ± 3.0 (11–20)§**
Allox-C57Bl/6	BALB/c	18	0‡‖	9.8 ± 1.9 (8–13)§
Autoimmune NOD	NOD	15	7†	9.9 ± 2.2 (8–15)‡
Autoimmune NOD	NOD-scid	12	25	18.3 ± 16.6 (8–54)‡
Allox-NOD	NOD	10	0†	>60 (13–60)¶‡‡§§
Allox-C57Bl/6	C57Bl/6	5	0*	>60 (>60)§††

Data are median (range) unless otherwise indicated. *$P \leq 0.05$ vs. xeno-autoimmune NOD, †$P < 0.01$ vs. xeno-autoimmune NOD, ‡$P < 0.0005$ vs. xeno-autoimmune NOD, §$P < 0.0001$ vs. xeno-autoimmune NOD, ¶$P < 0.00001$ vs. xeno-autoimmune NOD, ‖$P < 0.05$ vs. allo-autoimmune NOD, **$P < 0.005$ vs. allo-autoimmune NOD, ††$P < 0.0001$ vs. iso autoimmune NOD, ‡‡$P < 0.00001$ vs. iso-autoimmune NOD; §§except for one animal with AIR at day 13 posttransplantation.

mice than in chemically diabetic nonautoimmune old NOD mice (Table 1).

By performing isogeneic islet transplantations or transplantations using islets from NOD-scid donors into the spontaneously diabetic autoimmune NOD mice, we determined the impact of autoimmunity on both early graft failure and islet graft rejection. Early graft dysfunction was observed in 7% of isografts and 25% of NOD-scid grafts in spontaneously diabetic autoimmune NOD mice, but was absent in isografts transplanted in chemically diabetic recipients. Moreover, recurrence of autoimmune disease caused destruction of isogeneic grafts in all autoimmune diabetic NOD mice after 9.9 ± 2.2 days, whereas except for one animal, all chemically diabetic nonautoimmune old NOD mice had long-term acceptance of their isografts without any signs of AIR (Table 1). As expected, all chemically diabetic nonautoimmune C57Bl/6 mice maintained perfect isograft function.

Early (8 h) cytokine mRNA analysis of islet xenografts of autoimmune diabetic versus nonautoimmune diabetic NOD mice. Our results indicate that spontaneously diabetic autoimmune NOD mice had a significantly stronger early intragraft expression of IL-1 (1.40 ± 0.32 IL-1 copies/β-actin copies) 8 h after transplantation than did chemically diabetic nonautoimmune old NOD recipients (0.90 ± 0.14 IL-1 copies/β-actin copies, $P < 0.05$) (Fig. 1). In the rat islet grafts, cytokine gene expression for other macrophage products such as TNF-α, IL-6, and iNOS revealed no significant differences between spontaneously diabetic autoimmune NOD mice and chemically diabetic nonautoimmune animals (data not shown). Interestingly, all T-cell cytokines examined (IL-2, IL-4, IL-10, and IFN-γ) were absent in both recipients at the earliest timepoint checked. We measured significantly higher levels of TGF-β intragraft mRNA expression in chemically diabetic nonautoimmune old NOD mice (1.36 ± 0.50 TGF-β copies/β-actin copies) relative to the expression level in the islet xenografts of spontaneously diabetic autoimmune NOD mice (0.67 ± 0.16 TGF-β copies/β-actin copies, $P < 0.05$) (Fig. 1).

Late (16 and 24 h) cytokine mRNA expression in islet xenograft of autoimmune diabetic versus nonautoimmune diabetic NOD mice. When studying the time course of cytokine mRNA expression in the rat islet grafts of both types of NOD recipients, we observed a progressive decline (from 1.40 ± 0.32 IL-1 copies/β-actin copies [8 h] to 0.89 ± 0.34 IL-1 copies/β-actin copies [24 h], $P < 0.05$) in gene expression for IL-1 in xenogeneic islet grafts of spontaneously diabetic autoimmune NOD mice to levels comparable with those of chemically diabetic nonautoimmune old NOD mice (0.82 ± 0.09 IL-1 copies/β-actin copies [24 h]) (Fig. 2*A*). Consistent with these decreasing levels of IL-1 gene expression in the autoimmune situation, TGF-β mRNA concentrations showed a similar decline from 8 until 24 h in the rat islet grafts of spontaneously diabetic autoimmune NOD mice. In contrast, TGF-β expression levels within the islet xenografts of chem-

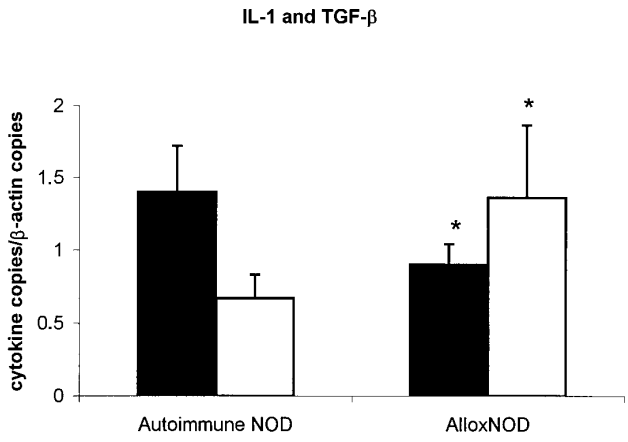

FIG. 1. Real-time RT-PCR analysis of islet xenografts 8 h after transplantation in spontaneously diabetic autoimmune NOD mice (autoimmune NOD, *n* = 7) compared with chemically diabetic nonautoimmune old NOD mice (Allox-NOD, *n* = 6). Note the high IL-1 (■) and low TGF-β (□) mRNA expression in islet xenografts of spontaneously diabetic autoimmune NOD mice compared with chemically diabetic nonautoimmune old NOD mice. *$P < 0.05$ vs. autoimmune NOD.

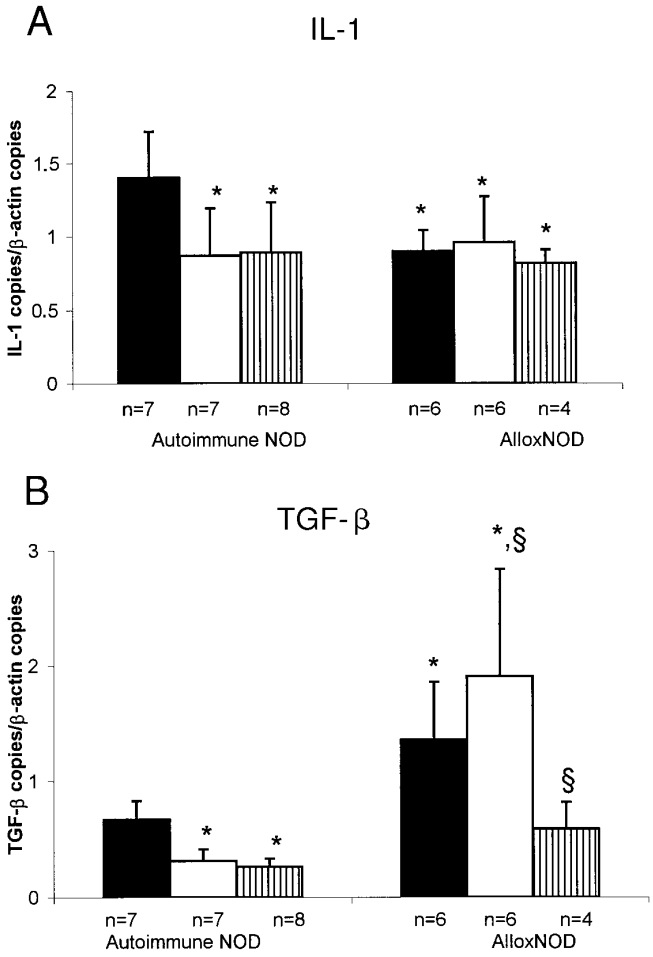

FIG. 2. Time course of real-time RT-PCR analysis of islet xenografts 8 h (■), 16 h (□), and 24 h (▥) after transplantation in spontaneously diabetic autoimmune NOD mice compared with chemically diabetic nonautoimmune old NOD mice. Note the gradual decrease of IL-1 mRNA expression in islet xenografts of spontaneously diabetic autoimmune NOD mice to levels comparable with the nonautoimmune situation. In contrast, gene expression level of TGF-β, which was significantly higher in nonautoimmune mice than in autoimmune mice, remained upregulated until 16 h after transplantation. *P < 0.05 vs. autoimmune NOD 8 h; §P < 0.05 vs. allox-NOD 8 h.

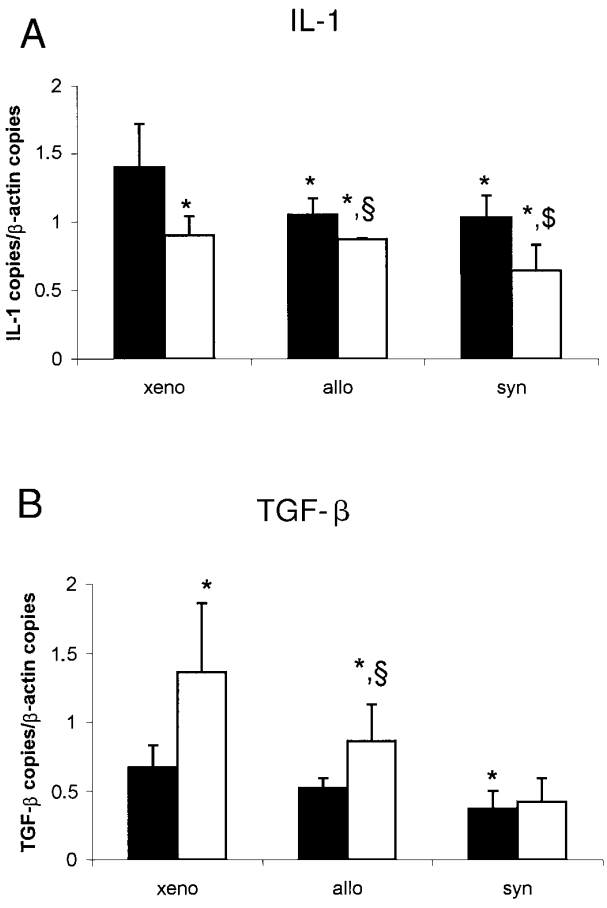

FIG. 3. Real-time RT-PCR analysis of xenogeneic, allogeneic, and isogeneic islet grafts 8 h after transplantation in spontaneously diabetic autoimmune NOD mice (autoimmune NOD [■], n = 4 per transplantation setting) and chemically diabetic nonautoimmune old NOD mice (Allox-NOD [□], n = 4 per transplantation setting). Note the lower IL-1 mRNA expression in allogeneic and isogeneic islet grafts of spontaneously diabetic autoimmune NOD mice compared with xenogeneic islet grafts in these recipients. TGF-β mRNA expression was low in all circumstances in spontaneously diabetic autoimmune NOD mice. *P < 0.05 vs. xeno-autoimmune NOD; §P < 0.05 vs. allo-autoimmune NOD; $$P$ < 0.05 vs. iso-autoimmune NOD.

ically diabetic nonautoimmune recipients remained significantly upregulated from 8 until 16 h after operation as compared with the autoimmune situation, but there was a relative decline in expression at 24 h after transplantation (0.59 ± 0.23 TGF-β copies/β-actin copies, P < 0.05 vs. 8 and 16 h) (Fig. 2*B*). No T-cell cytokines (IL-2, IL-4, IL-10, and IFN-γ) were detectable until 48 h after transplantation. Only after 48 h posttransplantation were similar signals for IL-2 and IFN-γ gene expression detected in islet xenografts of both diabetic NOD recipients (data not shown).

Cytokine mRNA analysis of islet allografts and isografts of autoimmune diabetic versus nonautoimmune diabetic NOD mice. Because islet xenografts possess a higher susceptibility to islet graft failure than do allogeneic and isogeneic islet grafts, we hypothesized that the high prevalence of early xenograft failure could be due to the xenogeneic nature of the islets, driving aspecific immune attack mediators such as macrophages and inflammatory cytokines. Interestingly, islet allografts and isografts of spontaneously diabetic autoimmune NOD mice showed lower IL-1 expression than did islet xenografts (1.05 ± 0.12 and 1.03 ± 0.16, P < 0.05 vs. 1.40 ± 0.32 IL-1 copies/β-actin copies) (Fig. 3*A* and *B*), suggesting indeed a "xeno-specific" element in the observed early graft failure. Interestingly, IL-1 gene expression in islet allografts and isografts in spontaneously diabetic autoimmune NOD mice still remained higher than in the nonautoimmune mice, suggesting a role for the autoimmune setting itself in early graft failure.

TGF-β mRNA expression in islet grafts in the autoimmune situation was low in all circumstances, with lowest levels in isogeneic islet grafts (Fig. 3*B*). A striking difference again was noted between the autoimmune and the nonautoimmune situation in xenogeneic islet grafts (0.67 ± 0.16 vs. 1.36 ± 0.50 TGF-β copies/β-actin copies, P < 0.05) and allogeneic islet grafts (0.52 ± 0.07 vs. 0.86 ± 0.27 TGF-β copies/β-actin copies, P < 0.05), but not in the isogeneic islet grafts (0.37 ± 0.13 vs. 0.42 ± 0.17 TGF-β copies/β-actin copies, NS).

DISCUSSION

Islet xenotransplantation in experimental models of type 1 diabetes is still characterized by many unresolved problems that limit the use of this treatment as a possible cure for human type 1 diabetes (1). Indeed, many transplants function poorly or not at all, and the cause of this failure remains unclear. One cause limiting the success of experimental xenotransplantation—early graft failure, a tendency of islet grafts to fail to function after a technically perfect transplantation—has been subject of many investigations. According to recent reports, the underlying basis for this inability to function and maintain normoglycemia after islet grafting is the action of local inflammatory agents (6,12,13). However, the exact triggers of this phenomenon remain unknown.

In the first set of experiments, we studied the impact of autoimmunity itself on the rate of early graft injury after islet xenotransplantation in spontaneous autoimmune type 1 diabetes. We observed a greater incidence in islet xenograft failure and a significantly shorter islet xenograft survival in spontaneously diabetic autoimmune NOD mice than in both chemically diabetic models. These data suggest that spontaneously diabetic autoimmune mice display a more aggressive or hyperactive immune system toward the grafted islets than do chemically diabetic mice. Indeed, in these spontaneously diabetic autoimmune NOD mice, it is known that the immune system is the major mediator of the β-cell assault and is already primed to destroy the newly transplanted β-cells (2,3), whereas nonautoimmune diabetic animals do not possess this recurrent and accelerated immune response against the islet grafts and are therefore under a milder immunological attack.

To unravel the mediators of early graft failure, we performed real-time RT-PCR mRNA analysis of islet xenografts for local cytokines (20). First of all, we could prove that the typical autoimmune memory, carried by T-cells, was not involved in this early graft destruction, because T-cell cytokines could not be detected locally in the grafts earlier than 48 h after transplantation. Furthermore, we demonstrated that spontaneously diabetic autoimmune NOD mice have an early and strongly upregulated intragraft expression of the inflammatory cytokine IL-1 compared with that of nonautoimmune diabetic recipients. It has been shown by others that IL-1 production originates from nonspecific inflammation carried by macrophages in response to the trauma of grafting and may produce direct cytotoxicity to the grafted β-cells (14,15). Moreover, hypersecretion of IL-1 by NOD macrophages has been described as one of the central immune abnormalities contributing to the onset of autoimmune diabetes in the NOD mouse (21). So, macrophages and their byproducts seem to be particularly important in mediating early dysfunction of islet xenografts in both spontaneously diabetic and chemically diabetic animals. It is intriguing that these macrophages invade the grafts very early after transplantation and seem to exert their damaging effects in an aspecific manner, without prior activation by other cell types such as NK cells, because NOD mice are NK-cell deficient (22). Previously, it has also been established that depletion of host macrophages with clodronate-loaded liposomes or silica reduces early graft failure of xenogeneic islets (23,24). Moreover, Fox et al. (25) demonstrated that macrophages are the first effectors of graft destruction and promote T-cell infiltration of islet xenografts. Indeed, the absence of all T-cell cytokines tested in the islet xenografts of all recipients until 48 h after transplantation suggests that the role of (autoreactive) T-cells is of a secondary nature and that these cells are probably recruited after macrophage activation and production of their cytotoxic byproducts. In this context again, IL-1 in particular has been described as being a central mediator of β-cell damage (26).

Another cytokine of interest is TGF-β. TGF-β can be produced by every leukocyte lineage (27). Besides its roles in development, in epithelial cell growth and differentiation, and in the process of carcinogenesis, this molecule has profound effects on immune cells, including all classes of lymphocytes, macrophages, and dendritic cells. Convincing evidence exists that TGF-β has anti-inflammatory properties, inhibits production of proinflammatory cytokines from macrophages, T-cells and B-lymphocytes, and may also prevent autoaggressive Th1-type responses (28–30). Indeed, transgene expression of TGF-β in the pancreatic islets of NOD mice prevents the development of spontaneous diabetes (31). Moreover, in vitro incubation of islets with TGF-β can reduce the susceptibility of β-cells to autoimmune destruction by diabetogenic spleen cells and to cytokine-mediated lysis of islet cells (32). Here we confirm that TGF-β might have protective capacities against early xenogeneic β-cell injury in the nonautoimmune diabetic mice, possibly by downregulation or suppression of the early β-cell–destructive agents like IL-1. Indeed, imbalance between high IL-1 and low TGF-β levels in spontaneously diabetic autoimmune NOD animals might be the triggering factor in early graft failure.

We conclude that early graft failure occurs very frequently when xenogeneic rat islet cells are transplanted into spontaneously diabetic autoimmune NOD mice. Autoimmune background, but also specific xenogeneic triggers, might underlie this phenomenon. Mediators in early graft failure are high intragraft IL-1 levels in the presence of low TGF-β levels, pointing toward abnormal macrophage function in spontaneously diabetic autoimmune NOD mice as the basis for early graft failure.

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