

# Recurrent Autoimmunity Accelerates Destruction of Minor and Major Histoincompatible Islet Grafts in Nonobese Diabetic (NOD) Mice

Teru Okitsu<sup>a</sup>, Stephen T. Bartlett<sup>a,\*</sup>,  
Gregg A. Hadley<sup>a</sup>, Cinthia B. Drachenberg<sup>b</sup> and  
Alan C. Farney<sup>a</sup>

<sup>a</sup> Division of Transplantation and <sup>b</sup> Department of Pathology, University of Maryland School of Medicine, Baltimore Maryland, USA

\* Corresponding author: Stephen T. Bartlett, sbartlett@smail.umaryland.edu

**Recurrent autoimmunity destroys nonobese diabetic (NOD) islet isografts, but whether recurrent autoimmunity contributes to islet graft destruction in immunocompetent allogeneic recipients is unknown. In the NOD, a single dose of streptozocin prevents or delays primary autoimmunity, allowing the detection of alloimmunity alone in chemically diabetic hosts (streptozocin-NOD) to be compared to the combined effects of autoimmunity and alloimmunity in spontaneously diabetic NODs (autoimmune-NOD). Islets were isolated from prediabetic NOD (H-2K<sup>d</sup>D<sup>b</sup>), nonobese resistant (NOR) (H-2K<sup>d</sup>D<sup>b</sup>), Balb/cByJ (H-2<sup>d</sup>) and B10.BR (H-2<sup>k</sup>) donors and transplanted to either the renal subcapsule or the intraportal site in autoimmune-NODs or streptozocin-NODs. MHC-matched NOR islets had indefinite graft survival in streptozocin-NODs. However, NOR islets showed graft loss at  $12.6 \pm 3.2$  days in renal subcapsule and at  $6.8 \pm 0.1$  days in intraportal site of autoimmune-NODs. Partially MHC-matched Balb/cByJ islet grafts failed significantly sooner in autoimmune-NODs than in streptozocin-NODs ( $p < 0.005$ ). Fully MHC-mismatched B10.BR islet grafts also failed sooner in autoimmune-NODs, but the difference did not reach significance ( $p < 0.06$ ). Although the streptozocin-NOD was functionally tolerant of MHC-matched NOR islets, NOR islets transplanted into autoimmune-NODs failed sooner than NOD islets in both renal subcapsule ( $12.6 \pm 3.2$  days vs.  $26.4 \pm 10.5$  days,  $p = 0.009$ ) and intraportal sites ( $6.8 \pm 0.1$  days vs.  $11.5 \pm 1.7$  days,  $p = 0.014$ ). In the autoimmune-NODs, the intraportal site consistently showed shorter graft survival than the renal subcapsule site (NOD:  $p = 0.009$ , NOR:  $p = 0.014$ , Balb/cByJ:  $p = 0.008$ , B10.BR:  $p = 0.032$ ). In conclusion, autoimmune processes facilitate the alloimmune response to minor and major histocompatibility antigens and accelerate graft destruction. The same autoimmune processes are more pronounced in the intraportal site.**

**Key words:** Autoimmunity, islets of Langerhans, major histocompatibility complex, NOD mouse, rejection

Received 21 December 2000, revised and accepted for publication 22 March 2001

## Introduction

Type I diabetes mellitus results from autoimmune destruction of the insulin-producing beta cells of the islets of Langerhans (1,2). If isologous beta cells are re-introduced by transplantation, they are susceptible to recurrent autoimmune disease. In humans, Sutherland et al. (3) found recurrence of autoimmune disease following whole organ pancreas transplantation between identical twins. Likewise, recurrent autoimmune disease destroys isologous islet or whole-organ pancreas grafts in the diabetes-prone BB rat (4,5) and the nonobese diabetic (NOD) mouse (6,7).

Whether autoimmune disease recurrence occurs following clinical allogeneic transplantation is less clear. In humans, disease recurrence after whole-organ pancreas transplantation in immunosuppressed hosts is rare and reportable (8). However, recurrent autoimmunity may play a significant role in allogeneic human islet transplantation. The Newsletter of the International Islet Transplantation Registry (9) shows that islet transplantation in type I diabetic patients has a markedly lower success rate than the success rate in patients rendered diabetic by pancreatectomy.

Animal models of type I diabetes provide some evidence suggesting a role for autoimmune destruction of allogeneic islet grafts. Both Wang et al. (10) and Markmann et al. (11) observed that immunomodulated islet grafts could be destroyed in the diabetic NOD. In addition, there are numerous strategies that prolong allogeneic islet survival in autoimmune-free, chemically induced diabetic murine recipients. However, similar strategies are not effective in autoimmune NOD mice (12–14). These investigations demonstrated that the recurrent autoimmune process could destroy alloislet grafts in the absence of alloimmunity.

This study addresses the question: what happens to allogeneic islet grafts when the alloimmune system is fully intact in the presence of autoimmunity? A model was developed to test the contribution of autoimmunity to alloislet destruction by observing the fate of islet grafts in the presence of both autoimmunity and alloimmunity vs. alloimmunity alone.

Administered in the prediabetic state, streptozocin prevents

or significantly delays primary autoimmunity in the NOD mouse (12,15). Prevention of early primary autoimmunity clearly obviates recurrent autoimmunity; however, the mechanism of this is incompletely understood (12,15). We took advantage of the ability of streptozocin to prevent recurrent autoimmunity in the NOD mouse.

Islet donor strains were selected based on major histocompatibility complex (MHC) match with the NOD mouse. Non-obese resistant (NOR) mice are MHC identical and differ from NODs only in minor antigens (16). The NOR strain was first identified in the Jackson NOD colony after inadvertent contamination of the NOD colony with C57BL/KsJ mice housed in the same facility. Genetically, the NOR mice have approximately 11.6% of the NOD genome replaced with that from the C57BL/KsJ strain. Segments of the C57BL/KsJ genome have been identified on chromosomes 1, 2, 4, 5, 7, 11, 12, and 18 of the NOR mice. The C57BL/KsJ strain, itself, is a recombinant congenic strain resulting from genetic contamination of C57BL/6J with DBA/2J. Studies by Serreze et al. show that the diabetes resistance of the NOR strain is most likely conferred by a protective diabetes resistance locus, *Idd13<sup>r</sup>* on chromosome 2. The presence of homozygosity at the *Idd13<sup>r</sup>* allele confers diabetes resistance even in the presence of other diabetes susceptibility (*Idd*) genes (17). The NOD alloimmune response to NOR is weak, as NOR skin grafts survive 5–6 weeks in NOD recipients (16). Because of the genetic similarity between NOR and NOD, autoimmunity might predominate in NOR islet graft failure in the NOD. In contrast, the B10.BR strain is fully MHC mismatched with the NOD mouse; whether autoimmunity contributes to allogeneic destruction of fully MHC-mismatched grafts is unknown. The Balb/c strain was selected as an islet donor because it has a single MHC class I disparity with the NOD.

Islet graft survival was compared between streptozocin-treated NODs (no recurrent autoimmunity) and autoimmune diabetic NODs (potentially both recurrent autoimmunity and alloimmunity). The isolated effect of alloimmunity was compared to the combined effects of alloimmunity and autoimmunity for both renal subcapsule and intraportal sites in the NOD mouse.

## Materials and Methods

### Animals

Female NOD (H-2K<sup>d</sup> I-E<sup>-</sup> D<sup>b</sup>) mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and were maintained under pathogen-free conditions at our facility. NOR (H-2K<sup>d</sup> I-E<sup>-</sup> D<sup>b</sup>), Balb/cByJ (H-2<sup>d</sup>) and B10.BR (H-2<sup>k</sup>) mice were also purchased from Jackson Laboratory. NOR, Balb/cByJ, B10.BR and prediabetic (6–7-week-old) NOD mice served as islet donors. Spontaneous autoimmune diabetic NOD mice (12–25 weeks old) and young NOD mice (6–7 weeks old) with chemically induced diabetes served as islet recipients. Chemical diabetes was induced in the recipients by a single (220 mg/kg) intraperitoneal injection of streptozocin (Sigma, St Louis, MO, USA). Blood glucose levels were measured with the Accu-Chek Easy blood glucose monitoring system (Boehringer-Mannheim Biochemicals, Indianapolis, IN, USA). Spontaneous autoimmune and

streptozocin-induced diabetes were diagnosed by blood glucose levels greater than 400 mg/dL on 2 consecutive days. NOD mice typically became diabetic within 5 days after streptozocin injection. Spontaneous autoimmune NOD mice (auNOD) and streptozocin-induced NOD mice (stzNOD) received islet grafts within 2 weeks of the diagnosis of diabetes.

### Islet isolation

Islets were prepared as described by Gotoh et al. (18). For islet isolation, 2 mL of Hanks' balanced salt solution (HBSS) (Life Technologies, Rockville, MD, USA) containing 2 mg/mL collagenase (Sigma type V) was injected into the common bile duct of mice. The distended pancreas was removed and incubated at 37 °C for 16 min. The islets were purified by centrifugation on Ficoll gradients comprising four different densities (25, 23, 21.5, 11%). The tissue fragments at the interface of the 21.5% and 23% layers were collected, washed and resuspended in HBSS. Individual islets, free of attached acinar, vascular, and ductal tissue were selected and removed with a Pasteur pipette under a dissecting microscope, yielding highly purified islets for transplantation.

### Islet transplantation

Four hundred and fifty to five hundred and fifty freshly isolated islets (approximately 500 islet equivalents) were picked up with an infusion set and transplanted into the renal subcapsular space of the left kidney or into the hepatic portal system. Intraportal islet transplantation was selectively directed into the right hepatic lobe. Just before islet graft injection, the left branch of the portal vein was clamped using a Kleinert-Kutz microclip with angled blade (Pilling Weck, Fort Washington, PA, USA). Using a 27-gauge needle infusion set, the injection was made into the ileo-colic vein. After the removal of the needle from the vein, the clamp was also removed. The total clamping time was less than 5 min. Hemostasis was easily achieved with gentle pressure using a cotton tip.

### Assessment of graft function

Islet graft function was monitored daily by measuring nonfasting blood glucose. All recipient mice became normoglycemic within 24 h after transplantation. Graft loss was determined when the nonfasting blood glucose exceeded 200 mg/dL for 2 consecutive days.

### Histology

The left kidneys or the right lobes of the liver containing islet grafts were removed within 48 h after the determination of graft loss. The tissues were fixed in 10% formalin solution, embedded in paraffin, and stained with hematoxylin and eosin. Paraffin-embedded sections were stained for insulin and glucagon (DAKO, Carpinteria, CA, USA) using standard immunoperoxidase techniques, and evaluated blindly by a pathologist.

### Statistics

Differences in the duration of graft survival between groups were evaluated with the Kaplan-Meier log-rank test. A p-value of <0.05 was considered statistically significant.

## Results

### NOD islets in stzNODs and auNODs

StzNOD recipients of isologous NOD islets demonstrated more than 150 days' graft survival in both renal subcapsule and intraportal sites. Some of the grafts failed starting at Day 153 in the intraportal site and Day 183 in the renal subcapsule site. In contrast to the result in stzNODs, isologous NOD islets transplanted into auNODs failed after  $26.4 \pm 10.5$  days ( $p < 0.011$ ) in the renal subcapsule site and after  $11.5 \pm 1.7$  days ( $p < 0.01$ ) in the intraportal site (Table 1). Graft

**Table 1:** Survival of intraportal and renal capsular isogeneic prediabetic NOD donor islets in chemically (stzNOD) and spontaneously (auNOD) NOD recipients

Donor/recipient	Graft site	Graft survival time (d)	Mean $\pm$ SD
NOD/stzNOD	Renal subcapsule site	183, 202, >300, >300	>180
NOD/stzNOD	Portal site	153, 186, >300, >300	>150
NOD/auNOD	Renal subcapsule site	19, 20, 21, 28, 44	26.4 $\pm$ 10.5
NOD/auNOD	Portal site	10, 11, 11, 12, 14	11.5 $\pm$ 1.7

failure in auNOD recipients could only result from autoimmune recurrence since these grafts were isologous.

#### ***Allo- and autoimmunity contribute to the failure of NOR and Balb/c, B10.BR, and B10.D2 islets in auNODs***

In the stzNOD (alloimmunity only), NOR islets functioned for more than 100 days in the renal subcapsule ( $n=4$ ) and for more than 60 days ( $n=2$ ) in the intraportal site. In auNOD mice (alloimmunity and recurrent autoimmunity), NOR islet grafts failed after  $12.6 \pm 3.2$  days in the renal subcapsule site ( $p < 0.005$  compared to stzNOD, Figure 1A-1) and  $6.8 \pm 1.0$  days in the intraportal site ( $p < 0.05$  compared to stzNOD, Figure 1A-2). Note that NOR and NOD functioned indefinitely in stzNOD recipients; however, NOR islets failed much more quickly than NOD islets in auNOD recipients (Figure 2), strongly suggesting that the minor histocompatibility antigens of the NOR strain become immunogenic only in the presence of an active autoimmune process.

In another experiment, 500 islet equivalents from allogeneic MHC-mismatched Balb/c mice were transplanted into the renal subcapsule or the portal system of stzNODs and auNODs. Although their genetic backgrounds are different, Balb/c (H-2D<sup>d</sup>) mice differ from NOD (H-2D<sup>b</sup>) at only one MHC class I locus. In the stzNOD (alloimmunity only), Balb/c islets survived  $19.8 \pm 7.5$  days in the renal subcapsule ( $n=5$ ) and  $13.8 \pm 5.4$  days ( $n=5$ ) in the intraportal site. In auNOD mice (alloimmunity and recurrent autoimmunity), Balb/c islet grafts failed after  $10.5 \pm 1.5$  days ( $n=5$ ) in the renal capsule site ( $p=0.002$  compared to stzNOD, Figure 1B-1) and  $7.8 \pm 0.8$  days ( $n=5$ ) in the intraportal site ( $p=0.002$  compared to stzNOD, Figure 1B-2).

Next, a fully MHC-mismatched donor strain was selected. Five hundred islet equivalents from B10.BR mice (H-2<sup>k</sup>) were transplanted into the renal subcapsule or the portal system of stzNODs and auNODs. In the stzNOD (alloimmunity only), B10.BR islets survived  $11.8 \pm 0.8$  days in the renal subcapsule ( $n=5$ ) and  $12.0 \pm 4.2$  days ( $n=5$ ) in the intraportal site. In auNOD mice (alloimmunity and recurrent autoimmunity), B10.BR islet grafts failed after  $10.2 \pm 1.3$  days ( $n=5$ ) in the renal capsule site ( $p=0.074$  compared to stzNOD, Figure 1C-1) and  $7.8 \pm 1.3$  days ( $n=4$ ) in the intraportal site ( $p=0.067$  compared to stzNOD, Figure 1C-2).

Islet grafts from the strain B10.D2, whose MHC genotype is the same as Balb/c (H-2<sup>d</sup>), but whose genetic background is similar to B10.BR, were transplanted to the renal subcapsule

of stzNODs and auNODs. B10.D2 islets failed sooner in the auNOD ( $n=4$ ,  $8.3 \pm 1.0$  days) than in the stzNOD ( $n=4$ ,  $18.0 \pm 10$  days,  $p=0.017$ ).

#### ***Renal subcapsule vs. intraportal site for islet engraftment in the auNOD***

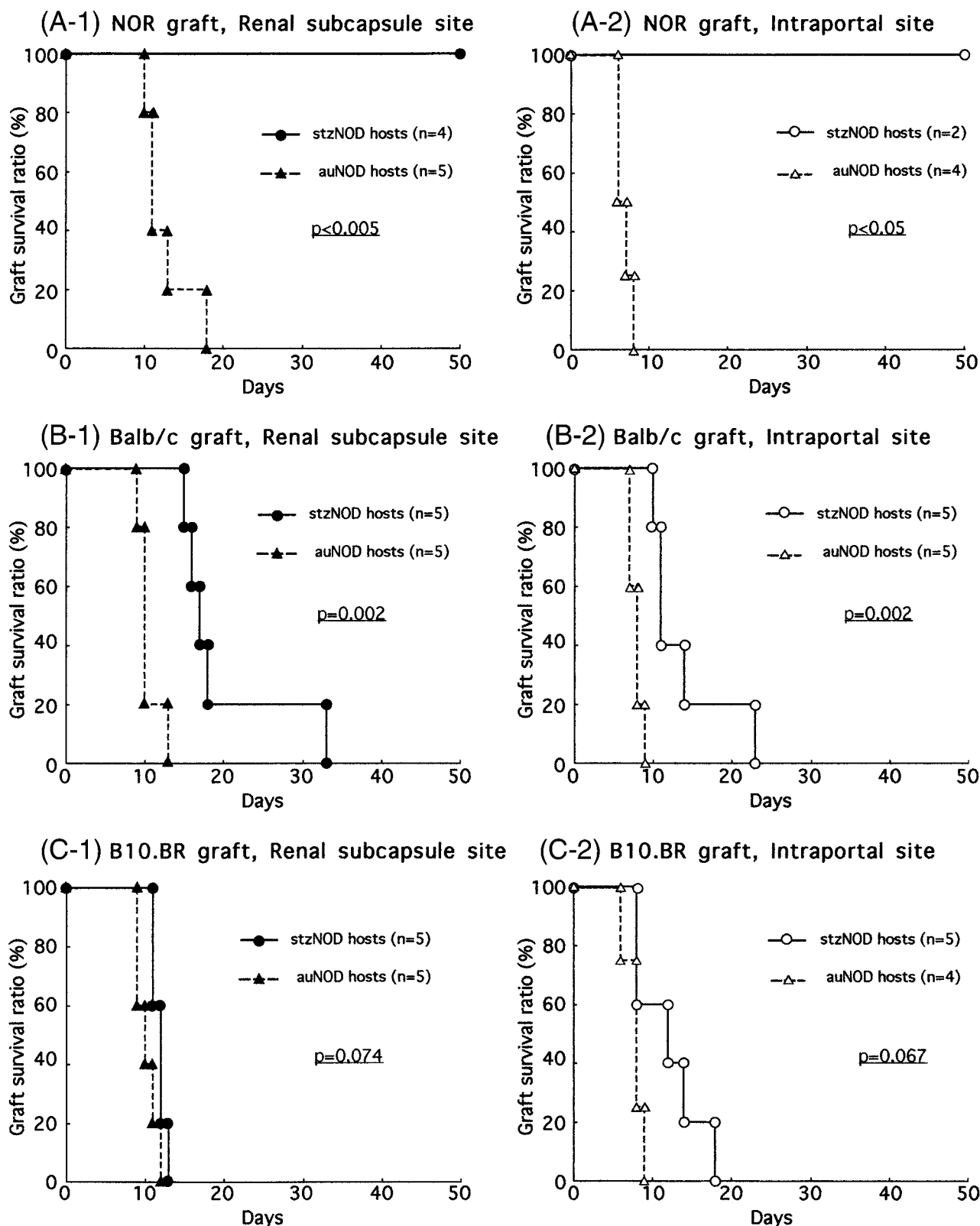
From the previous experiments, it was observed that islet grafts in the auNOD failed sooner in the intraportal site than in the renal subcapsule. For NOD, NOR, Balb/c and B10.BR donor islets, failure in the renal subcapsule site occurred at  $26.4 \pm 10.5$ ,  $12.6 \pm 3.2$ ,  $10.5 \pm 1.5$  and  $10.5 \pm 1.3$  days compared to  $11.5 \pm 1.7$ ,  $6.8 \pm 0.1$ ,  $7.8 \pm 0.8$  and  $7.8 \pm 1.3$  days, respectively (all  $p < 0.05$ ).

#### ***The effect of autoimmunity on the immune response to minor histocompatibility antigens***

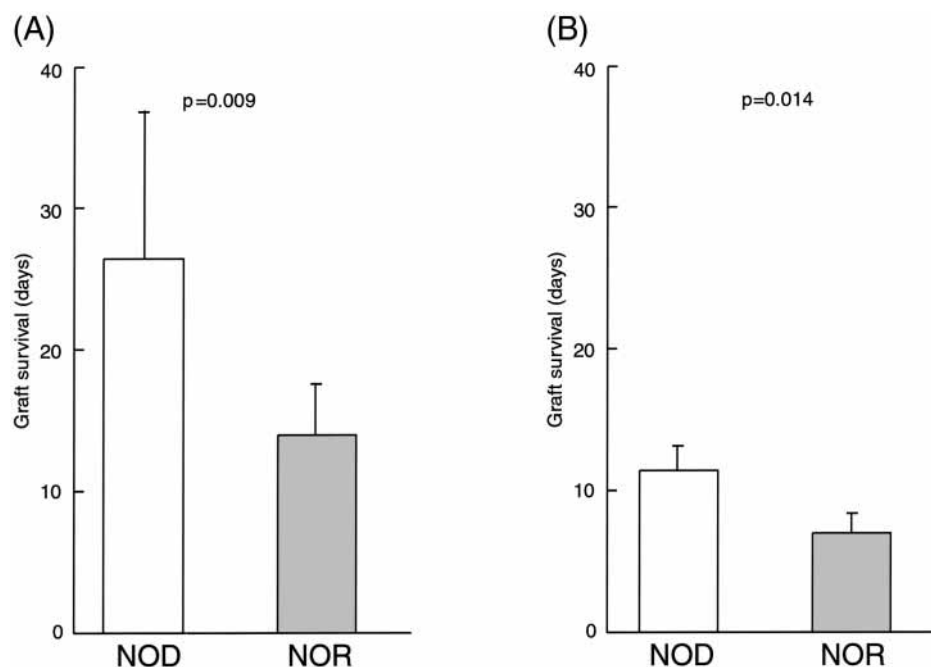
There are two possible explanations for the difference in the rate of islet graft loss between NOD and NOR islets in auNODs. These are: (i) that NOR islets are more susceptible to recurrent autoimmunity or (ii) that autoimmunity augments what would otherwise be a negligible alloimmune response to NOR minor antigens. To help differentiate between those possibilities, NOR and NOD islet grafts in auNODs were evaluated by insulin and glucagon staining. If NOR islets are destroyed by a pure autoimmune process, then alpha cells should persist following autoimmune beta cell destruction. Destruction of alpha cells would suggest the presence of alloimmunity or both processes together.

Histologic analysis of NOD islet isografts procured at the time of graft failure in auNODs revealed persistence of glucagon-staining cells. In the renal subcapsule grafts, some islets contained beta cells, but these islets were heavily infiltrated with lymphocytes. However, some 'islets' contained only aggregates of alpha cells, suggesting alpha cell persistence (Figure 3B,C). In contrast, NOR islet allografts often stained negatively for insulin and glucagon, indicating beta and alpha cell destruction. Aggregates of NOR alpha cells were not demonstrated, and the few remaining isolated alpha cells were associated with heavily infiltrated beta cell islet remnants (Figure 3E,F).

NOD and NOR islet transplants into the intraportal site of the auNOD showed a pattern of alpha cell staining similar to that seen with the renal subcapsular grafts. NOD islet isografts revealed persistence of alpha cells despite the total loss of beta cells (Figure 4B,C). In contrast, the few alpha cells that remained in NOR allografts were associated with residual



**Figure 1: Allogeneic islet graft survival in NOD mice.** Islet grafts from NOR (A), Balb/c (B), B10.BR (C) were transplanted into spontaneously autoimmune NOD recipients (auNOD) and streptozocin-treated NOD recipients (stzNOD) using two different sites: renal subcapsule (1) and intraportal (2).



**Figure 2: Comparison of the graft survival between isogenic NOD and MHC-compatible allogeneic NOR islet grafts in spontaneously autoimmune diabetic NODs transplanted into two sites: (A) renal subcapsule and (B) intraportal.**

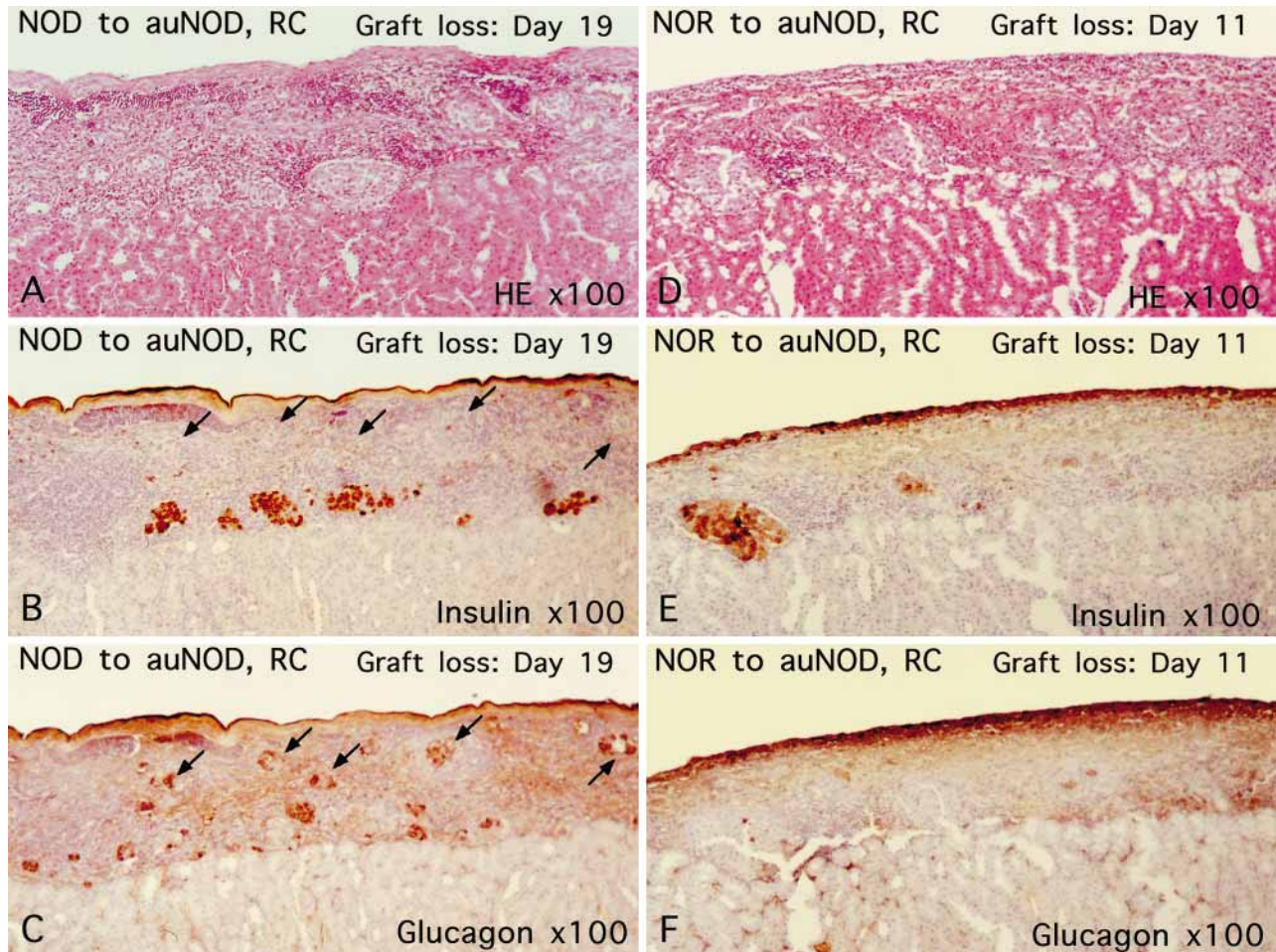
beta cells, and occasional apoptotic alpha cells were present (Figure 4E,F).

## Discussion

Streptozocin is commonly used to induce diabetes in rodents and other animal models of diabetes. In the prediabetic NOD mouse, a single dose of streptozocin prevents or significantly delays recurrent autoimmune destruction of beta cells in isologous islet transplants (12,15). In our experiments, NOD islet grafts functioned for more than 150 days both in the renal subcapsular and in the intraportal site of stzNODs. Graft failure was seen in some of the NOD-to-stzNOD transplants, starting at Day 153 in the intraportal site and at Day 183 in the renal subcapsule site (Table 1). This might be due to 'primary autoimmunity' or unspecific graft failure. Why streptozocin prevents or delays recurrent autoimmune disease is unclear, but the necessary timing of the injection, before the development of autoimmune diabetes, suggests that loss of autoantigen before the development of primary disease interferes with the later development of autoimmune mechanisms. Thus, maneuvers to eliminate the autoantigen before the onset of autoimmune diabetes abrogate the mechanisms leading to recurrent autoimmunity following isologous islet transplant. For example, Itoh et al. (19) found that pancreatectomized prediabetic NODs do not destroy NOD islet isografts. Because of the effect of streptozocin on recurrent autoimmunity in the NOD mouse, a long autoimmune disease-free window is created, during which it is possible to observe the isolated effects of alloimmunity on islet graft function

(stzNOD). Comparison between islet recipients in which there is alloimmunity only (stzNOD) and recipients which potentially have both alloimmunity and autoimmunity (auNOD) allows direct observation of an interaction between autoimmune and alloimmune processes.

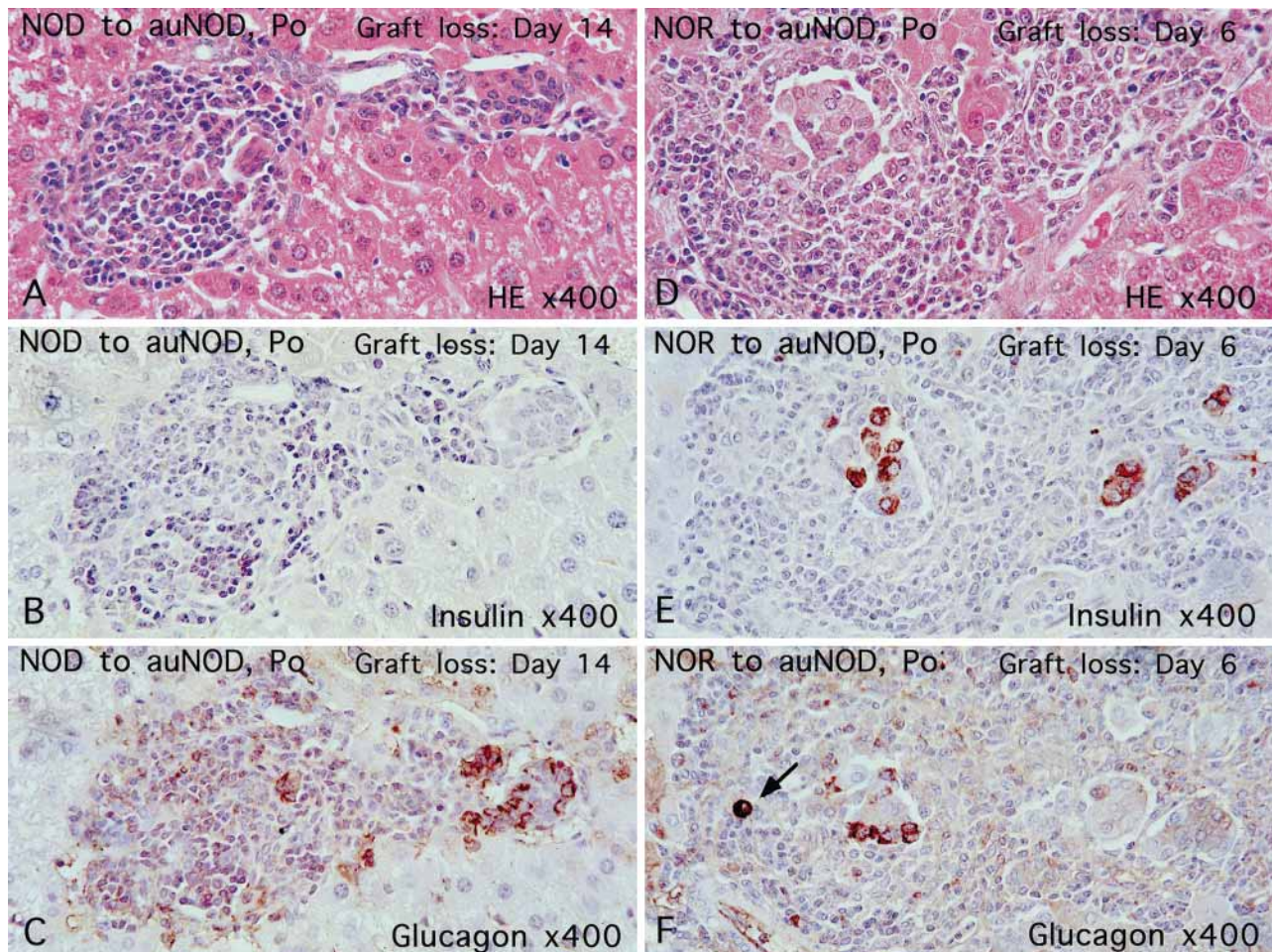
Allogeneic islet donor strains were picked for their differing degree of MHC compatibility with the NOD mouse. Fully MHC-compatible NOR and partially MHC-compatible BALB/c islets transplanted to the renal capsule and portal vein were destroyed more rapidly in the auNOD than in the stzNOD, suggesting that the combined effects of alloimmunity and autoimmunity contributed to alloislet graft destruction. In contrast, little difference in islet graft survival was seen for fully MHC-incompatible B10.BR islets in the auNOD and stzNOD. The important role of MHC in the contribution of recurrent autoimmunity was confirmed by the study using B10.D2 donors, which have the same MHC as Balb/c and the same background as B10.BR. In contrast to B10.BR, the B10.D2 islet grafts showed the same pattern of accelerated graft loss in the auNOD as Balb/c islet grafts. Although these experiments were not designed to determine whether recurrent autoimmunity has MHC-restricted pathways, the effect of recurrent autoimmunity on alloislet graft destruction was more clearly observed when the MHC was better matched. It is possible that MHC-compatible islet grafts are more susceptible to recurrent autoimmunity. Alternatively, autoimmunity may be operative in the ultimate failure of poorly MHC-matched islet grafts, but graft failure was so rapid that the relative contributions could not be determined with our model.



**Figure 3: NOD (A, B, C) and NOR (D, E, F) islet grafts in the renal subcapsular site (RC) of a spontaneously autoimmune NOD recipient.** A, D: Grafts from NOD and NOR. Both demonstrate that residual islets are visible but are surrounded and infiltrated by mononuclear cells. B: Some residual NOD islets showed complete absence of insulin-containing beta cells (arrows). C: Immunological staining of an adjacent section for glucagon reveals aggregates of alpha cells (arrows) in the insulin-negative residual islets of section B. E, F: Although some alpha cells are visible in islet grafts from NOR, they are always accompanied by beta cells.

Another interesting finding of this investigation is that recurrent autoimmunity appears to augment alloimmunity. This was suggested by the significantly more rapid graft loss of NOR islets than isogeneic NOD islets, and by the observation that NOR islet grafts were accepted by NODs unless autoimmunity was present. Although the immunopathologic pattern of recurrent autoimmune destruction of isolated islet grafts is not established, it was hypothesized that recurrent autoimmunity might spare nonbeta cells. If so, then an immunopathologic approach could be used to determine if autoimmunity was the sole mechanism of NOR islet graft destruction. Selective nonbeta cell (alpha and PP cells) persistence has been demonstrated in primary disease in NOD mice (20) and humans (21). However, it is not known whether this same phenomenon would be seen with transplanted islet grafts that fail from recurrent autoimmunity. The histologic pattern of primary autoimmune isletitis in the native pancreas and recurrent autoimmune destruction of transplanted islet grafts might be different. For example, a primed autoimmune re-

sponse might nonspecifically injure 'innocent bystanders' such as the alpha cell. Kaufman et al. (22) suggested that the contribution of nonspecific islet destruction, initiated by macrophages and macrophage-derived cytokines, significantly affects islet transplant outcome. Both *in vitro* (23,24) and *in vivo* (25) studies show that various inflammatory cytokines may damage isolated or transplanted islets. Some *in vitro* studies using isolated islets (26) and beta cell lines (27) show no difference in the sensitivity of beta cells and alpha cells to cytokine-induced destruction. These reports suggest that alpha cells as well as beta cells of isolated islet grafts might be destroyed by recurrent autoimmunity alone. However, the immunohistochemical analysis in our study did not show alpha cell loss. At the point of graft loss, the isogeneic islet grafts in auNODs (only recurrent autoimmunity can occur) showed aggregates of alpha cells in the renal subcapsule consistent with the previous observations of Markees et al. (13). Similarly, we found high concentrations of alpha cells within NOD islet remnants in the intraportal site of auNODs.



**Figure 4: NOD (A, B, C) and NOR (D, E, F) islet grafts in the intraportal site (Po) of spontaneously autoimmune diabetic NOD recipients.** A, B, C: Adjacent sections of a typical section containing residual NOD islets showing persistence of alpha cells with complete absence of beta cells. The residual NOD alpha cells lack degenerative changes. D, E, F: Residual NOR islet grafts always contain both beta cells and alpha cells. Some residual alpha cells show apoptosis (arrow).

These findings are evidence that recurrent autoimmunity, just like the primary disease, results in selective beta cell destruction and alpha cell persistence. The histological pattern of NOR islet graft destruction differed from that of isogeneic NOD islet grafts. NOR islet grafts lacked evidence of selective alpha cell persistence. The main point is that evidence of alloimmune destruction of NOR islets was found only in autoimmune-competent NODs. Thus, autoimmunity can enhance the alloimmune response to minor antigen differences.

The interaction and relative contribution of alloimmunity and autoimmunity to alloislet graft destruction is the next subject to pursue. However, our current experiments clearly pointed out that the combination of alloimmunity and autoimmunity is more aggressive than alloimmunity or autoimmunity alone.

Two possible mechanisms may underlie the rapid islet graft failure seen with alloimmunity and recurrent autoimmunity. First, the destruction of target cells by one immune pathway, alloimmunity or autoimmunity, could increase the availability

of antigen for presentation to T cells reactive in the other pathway. Second, common cytokine cascades, such as INF- $\gamma$  (28), utilized by both alloimmunity and autoimmunity could enhance the T cell activation for both of these pathways.

Finally, the study found differences in graft survival between the intraportal and renal subcapsule. In the human, the intraportal site is currently the standard (29). However, these experiments demonstrate that recurrent autoimmunity leads to rapid graft failure in the intraportal site. Since there do appear to be site differences in the NOD, and because the intraportal site has become the standard in humans, intraportal islet transplantation in NODs may be a more relevant experimental model for type I diabetes.

In summary, the findings in this study indicate that autoimmunity contributes to allogeneic islet transplant destruction in the NOD mouse. Furthermore, autoimmune processes may facilitate or augment alloimmune responses to minor or major histocompatibility differences. The findings underscore

the barriers to intraportal islet transplantation in autoimmune type I diabetics without the benefit of immunosuppression. Strategies to induce 'tolerance' to islet grafts in type I diabetic patients will need to address these two important and interacting mechanisms of islet graft destruction.

## Acknowledgment

The study was supported by an unrestricted grant from the Fujisawa Corporation.

## References

- Eisenbarth GS. Type I diabetes mellitus. A chronic autoimmune disease. *N Engl J Med* 1986; 314: 1360–1368.
- Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med* 1994; 33: 1428–1436.
- Sutherland DE, Goetz FC, Sibley RK. Recurrence of disease in pancreas transplants. *Diabetes* 1989; 38 (Suppl. 1): 85–87.
- Bartlett ST, Chin T, Dirden B, Quereshi A, Hadley G. Inclusion of peripancreatic lymph node cells prevents recurrent autoimmune destruction of islet transplants: evidence of donor chimerism. *Surgery* 1995; 118: 392–398.
- Bartlett ST, Killewich LA. Recurrent diabetes in pancreas transplants in the BB rat: evidence for a defect extrinsic to the beta cell. *Transplant Proc* 1991; 23 (Part 1): 689–690.
- Wang T, Singh B, Warnock GL, Rajotte RV. Prevention of recurrence of IDDM in islet-transplanted diabetic NOD mice by adjuvant immunotherapy. *Diabetes* 1992; 41: 114–117.
- Mottram PL, Murray-Segal LJ, Han W, Maguire J, Stein-Oakley A, Mandel TE. Long-term survival of segmental pancreas isografts in NOD/Lt mice treated with anti-CD4 and anti-CD8 monoclonal antibodies. *Diabetes* 1998; 47: 1399–1405.
- Tyden G, Reinholt FP, Sundkvist G, Bolinder J. Recurrence of autoimmune diabetes mellitus in recipients of cadaveric pancreatic grafts. *N Engl J Med* 1996; 335: 860–863.
- International Islet Transplantation Registry. Newsletter No. 7 1996; 6.
- Wang Y, Pontesilli O, Gill RG, La Rosa FG, Lafferty KJ. The role of CD4+ and CD8+ T cells in the destruction of islet grafts by spontaneously diabetic mice. *Proc Natl Acad Sci USA* 1991; 88: 527–531.
- Markmann JF, Bassiri H, Desai NM et al. Indefinite survival of MHC class I-deficient murine pancreatic islet allografts. *Transplantation* 1992; 54: 1085–1089.
- Stegall MD, Loberman Z, Ostrowska A, Coulombe M, Gill RG. Autoimmune destruction of islet grafts in the NOD mouse is resistant to 15-deoxyspergualin but sensitive to anti-CD4 antibody. *J Surg Res* 1996; 64: 156–160.
- Markees TG, Serreze DV, Phillips NE et al. NOD mice have a generalized defect in their response to transplantation tolerance induction. *Diabetes* 1999; 48: 967–974.
- Guo Z, Mital D, Shen J et al. Immunosuppression preventing concordant xenogeneic islet graft rejection is not sufficient to prevent recurrence of autoimmune diabetes in nonobese diabetic mice. *Transplantation* 1998; 65: 1310–1314.
- Takayama Y, Ichikawa T, Maki T. Effect of STZ administration on islet isograft and allograft survival in NOD mice. *Diabetes* 1993; 42: 324–329.
- Prochazka M, Serreze DV, Frankel WN, Leiter EH. NOR/Lt mice: MHC-matched diabetes-resistant control strain for NOD mice. *Diabetes* 1992; 41: 98–106.
- Serreze DV, Prochazka M, Reifsnnyder PC, Bridgett MM, Leiter EH. Use of recombinant congenic and congenic strains of NOD mice to identify a new insulin-dependent diabetes resistance gene. *J Exp Med* 1994; 180: 1553–1558.
- Gotoh M, Maki T, Kiyozumi T, Satomi S, Monaco AP. An improved method for isolation of mouse pancreatic islets. *Transplantation* 1985; 40: 437–438.
- Itoh A, Maki T. Protection of nonobese diabetic mice from autoimmune diabetes by reduction of islet mass before insulinitis. *Proc Natl Acad Sci USA* 1996; 93: 11053–11056.
- Gomez Dumm CL, Console GM, Luna GC, Dardenne M, Goya RG. Quantitative immunohistochemical changes in the endocrine pancreas of nonobese diabetic (NOD) mice. *Pancreas* 1995; 11: 396–401.
- Foulis AK, Liddle CN, Farquharson MA, Richmond JA, Weir RS. The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. *Diabetologia* 1986; 29: 267–274.
- Kaufman DB, Platt JL, Rabe FL, Dunn DL, Bach FH, Sutherland DE. Differential roles of Mac-1+ cells, and CD4+ and CD8+ T lymphocytes in primary nonfunction and classic rejection of islet allografts. *J Exp Med* 1990; 172: 291–302.
- Mandrup-Poulsen T, Spinas GA et al. Islet cytotoxicity of interleukin 1. Influence of culture conditions and islet donor characteristics. *Diabetes* 1987; 36: 641–647.
- Pukel C, Baquerizo H, Rabinovitch A. Destruction of rat islet cell monolayers by cytokines. Synergistic interactions of interferon-gamma, tumor necrosis factor, lymphotoxin, and interleukin 1. *Diabetes* 1988; 37: 133–136.
- Nagata M, Mullen Y, Herrera M, Matsuo S. Non-specific vs. autoimmune destruction of islet grafts in mice. *Horm Metab Res* 1990; 25: 153–156.
- Ling Z, In't Veld PA, Pipeleers DG. Interaction of interleukin-1 with islet beta-cells. Distinction between indirect, aspecific cytotoxicity and direct, specific functional suppression. *Diabetes* 1993; 42: 56–65.
- Hamaguchi K, Leiter EH. Comparison of cytokine effects on mouse pancreatic alpha-cell and beta-cell lines. Viability, secretory function, and MHC antigen expression. *Diabetes* 1990; 39: 415–425.
- Suarez-Pinzon W, Rajotte RV, Mosmann TR, Rabinovitch A. Both CD4+ and CD8+ T-cells in syngeneic islet grafts in NOD mice produce interferon-gamma during beta-cell destruction. *Diabetes* 1996; 45: 1350–1357.
- Shapiro AM, Lakey JR, Ryan EA et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; 343: 230–238.