Effect of STZ Administration on Islet Isograft and Allograft Survival in NOD Mice

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We examined the effect of a single STZ administration on subsequent islet isograft and allograft survival in NOD mice. Young prediabetic NOD mice were rendered diabetic by STZ administration and transplanted with islet isografts 8–11 days later. The earliest loss of islet function occurred on postgraft day 49. In sharp contrast, 15 of 16 isografts in spontaneously diabetic mice were destroyed within 17 days postgrafting. A comparison of the age of islet isograft destruction in STZ-induced diabetic NOD mice with the course of diabetes development in the NOD mouse colony clearly showed that STZ administration at the prediabetic stage led to a significant delay of diabetes onset in isografts. When STZ was given to overtly diabetic NOD mice, both islet isografts and allografts survived significantly longer than those in untreated, spontaneously diabetic NOD mice. However, the degree of prolongation induced by STZ was much smaller compared with that induced by ALS, a potent immunosuppressive reagent. In vitro mixed lymphocyte culture experiments showed that spleen cells of mice given STZ exhibited time-dependent reduction of their alloantigen reactivities. These results demonstrate that STZ, which is commonly used to induce diabetes in various experimental animals, also possesses an immunosuppressive property, although it is relatively weak compared with ALS. *Diabetes* 42:324–29, 1993

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STZ, streptozocin; ALS, anti-mouse lymphocyte serum; type I diabetes, insulin-dependent diabetes mellitus; H-2, mouse major histocompatibility complex; FITC, fluorescein isothiocyanate; PE, phycoerythrin; MoAb, monoclonal antibody; MLC, mixed lymphocyte culture; MST, mean survival time; cpm, counts/min.

STZ, a naturally occurring nitrosourea produced by *Streptomyces achromogenes* (1), has been widely used to induce diabetes mellitus in various experimental animals. STZ alkylates DNA and leads to the cessation of cellular function and eventual cell death. Its affinity for pancreatic β-cells is apparently mediated through the glucose moiety in its structure (2,3). Although repeated administration of subdiabetogenic doses of STZ could induce autoimmune-type diabetes (4), diabetes induced by a single diabetogenic dose of STZ is attributable to its direct cytotoxic effect on pancreatic β-cells. Thus, a single injection of 200–250 mg/kg STZ in a mouse results in the elevation of blood glucose levels >17 mM (300 mg/dl), usually within 4–7 days. The absence of autoimmunity in these diabetic mice is evidenced by subsequent indefinite acceptance of islet isografts.

In contrast, diabetes mellitus that spontaneously develops in the NOD mouse is caused by autoimmune processes affecting the pancreatic β-cells. Progressive lymphocytic infiltration into the islets begins around 5 wk of age and is followed by β-cell destruction and loss of insulin secretion, resulting in polyuria, glucosuria, hyperglycemia, loss of body weight, and fatal ketoacidosis (5). Once a NOD mouse becomes overtly diabetic, it rapidly destroys subsequent islet isografts by autoimmune mechanisms (6,7). Similarly, recurrence of the disease in the form of insulitis and β-cell destruction occurs in type I diabetes patients receiving pancreatic transplants from identical twins unless recipient immunosuppression is instituted (8,9). The development of autoimmune diabetes in the NOD mouse can be prevented by treatment at an early age before the insulitis stage with immunosuppressive agents such as anti-thymocyte serum or anti-Thy 1.2 antibody (10), cyclosporine (11), and monoclonal anti-L3T4 antibody (12). In addition, we recently showed that treatment of overtly diabetic NOD mice with ALS

induces long-term abrogation of autoimmunity, enabling subsequent islet isografts to survive indefinitely (7).

In this study, we investigated the autoimmune destruction of islet isografts transplanted into prediabetic NOD mice that were rendered diabetic by STZ administration (STZ-diabetic NOD mice). We also examined the effect of STZ administration in overtly diabetic mice, which are considered to have few viable β-cells left in the pancreas. The results described herein show that the development of spontaneous diabetes was significantly delayed when the NOD mice were given STZ and received islet isografting at the prediabetic age, and that STZ appears to have an immunosuppressive property in addition to its cytotoxicity to pancreatic β-cells.

RESEARCH DESIGN AND METHODS

Breeding of NOD/Jos//Tm mice was initiated in 1988 from two sibling pairs (F43-NOD/Shi//Jos), which were kindly provided by Dr. Masakazu Hattori of the Joslin Diabetes Center (Boston, MA). The breeding colony is currently in its 54th generation and is maintained under pathogen-free conditions at the Cancer Research Institute, New England Deaconess Hospital (Boston, MA). After 8 wk of age, NOD mice are tested once a week for urinary glucose levels with test strips (Tes-Tape, Lilly, Indianapolis, IN). Once urinary glucose becomes positive, mice are also tested for morning nonfasting blood glucose concentrations weekly by using test strips and a blood glucose monitor (Chemstrip and Accu-Chek II, Boehringer Mannheim, Indianapolis, IN). Mice with >17 mM (300 mg/dl) blood glucose levels are treated with 0.3–0.5 U of insulin (1:1 mixture of regular and NPH insulin, Lilly) twice a day adjusted daily by their urinary glucose levels. According to the incidence of spontaneous diabetes, our NOD mouse colony has been segregated into two strains, a high-incidence strain (line II) and a low-incidence strain (line I). The overall incidence of diabetes at 37 wk of age is 88.8% in females and 73.1% in males for the high-incidence strain and 63.6% in females and 24.0% in males for the low-incidence strain. Unless indicated otherwise, the line II NOD mice (high-incidence strain) were used as islet graft recipients in all experiments. Diabetes-resistant inbred strains of C3H/He, C57BL/6, and DBA/2 mice were purchased from Jackson Laboratory (Bar Harbor, ME).

Reagents. STZ was purchased from Upjohn (Kalamazoo, MI) as Zanosar containing 0.22 g of citric acid per 1 g of STZ and was injected intraperitoneally into mice immediately after it was dissolved in saline. The diabetogenic dose of STZ was 246 mg/kg (or 300 mg/kg Zanosar) for NOD mice and 204 mg/kg (or 250 mg/kg Zanosar) for C57BL/6 mice. ALS was made by immunizing rabbits with a pool of lymph node cells prepared from NOD, C3H/He, DBA/2, and (C57BL/6xA)F_1 mice according to the method described previously (13).

Islet isolation and transplantation. Islet isografts were prepared from NOD mice at 8–12 wk of age. To suppress lymphocyte infiltration into the islets, NOD islet donors were treated with two doses of 0.4 ml ALS intraperitoneally at 5 wk of age. Islet allografts were prepared from C3H/He mice, which are fully mismatched to NOD mice at H-2, at 10–14 wk of age. Islets were isolated by a collagenase digestion and Ficoll gradient separation method (14), further hand-picked under a dissection microscope, and transplanted into the renal subcapsular space. Although our previous study showed that isografts and allografts consisting of 200 islets consistently restore normoglycemia in STZ-induced diabetic (C57BL/6xA)F_1 mice (15), it was found that consistent reversal of hyperglycemia in diabetic NOD mice requires at least 500 islets (data not shown). Therefore, all transplantation experiments used grafts composed of 500 islets. Postoperatively, blood glucose levels were determined 3 times/wk for the first 4 wk, 2 times/wk for the next 10 wk, and 1 time/wk thereafter. Loss of islet function and onset of diabetes were defined when blood glucose concentrations were >17 mM for two consecutive determinations.

Flow cytometric analysis. Spleen cells were doubly stained with FITC conjugates of MoAb 53–6.7 (anti-CD8)/PE conjugate of MoAb 30.H12 (anti-Thy 1.2) and PE conjugate of MoAb GK1.5 (anti-CD4)/FITC conjugates of MoAb 30.H12. Two-color analysis was performed with a single-beam flow cytometer, FACScan (Becton Dickinson, Mountain View, CA) and the FACSCAN Research Software (Becton Dickinson). MoAbs were obtained from Becton Dickinson.

MLC. Splenocyte suspension was prepared either from C57BL/6 mice (H-2^b) at various time points after STZ injection or from untreated or STZ-administered prediabetic and diabetic NOD mice. These cells (7.5×10^5 cells) were mixed with irradiated 5×10^5 DBA/2 splenocytes (H-2^d) or C3H/He splenocytes (H-2^k) in RPMI-1640 medium supplemented with 5% fetal bovine serum, 5×10^{-5} 2-mercaptoethanol, penicillin (100 U/ml), and streptomycin (100 μg/ml) in a total volume of 200 μl in 96-well U-bottom plates. The proliferative response was determined on day 3 of incubation after an 8-h pulse with 1 μCi [^{3}H]thymidine/well. The generation of cytotoxicity was assessed after a 5-day incubation with an identical set of cultures. Cells in each well were thoroughly mixed and then 100 μl of the mixtures was transferred to a V-bottomed microtiter well that contained 1×10^4 ^{51}Cr-labeled P815 mastocytoma cells (H-2^d) or C3H MT1820 cells (H-2^k). Percentage of specific lysis was calculated as described previously (16). Results are means ± SE.

Statistical analysis. The analysis of graft survival and the incidence of diabetes was calculated by the Kaplan-Meier estimate using Mac SYSTAT 5.1 and SURVIVAL programs (Systat, Evanston, IL). The significance level (*P* value) was obtained by Mantel's log-rank test in the SURVIVAL program as well as by Student's *t* test.

RESULTS

Islet isograft and allograft survival in STZ-induced diabetic NOD mice. Nine female and nine male nondiabetic NOD mice were rendered diabetic by STZ injection between 52 and 57 days of age. These mice were transplanted with islets prepared from nondiabetic NOD mice 8–11 days later. Isograft survival in STZ-induced

TABLE 1
Islet isograft survival in STZ-induced diabetic and spontaneously diabetic NOD mice

Cause of diabetes	Sex of recipient	Age at grafting (days)	*n*	Graft survival (days postgrafting)
STZ	F	61–70	9	49, 54, 68, >104, 123, 143*, 167, >228, >269
STZ	M	62–65	9	52, 97†, 103†, 207†, 255†, 255†, 256†, 256†, 256†
Spontaneous	F	109–167	12	2, 3, 3, 5, 5, 8, 13, 15, 15, 17, 17, 98*
Spontaneous	M	142, 143	4	3, 3, 4, 4

Islet isografting in spontaneously diabetic mice was conducted 20–41 days after diabetes onset. STZ injection in nondiabetic NOD mice was done 8–11 days before isografting.
*Death of mouse with functioning islets.
†Removal of functioning graft.

diabetic NOD mice was compared with isograft survival in spontaneously diabetic NOD mice. As shown in Table 1, the earliest loss of islet function in STZ-induced diabetic NOD mice occurred on day 49 postgrafting, whereas virtually all isografts (15/16) were destroyed within 17 days postgrafting in spontaneously diabetic mice. Because destruction of islet isografts in NOD mice must be exerted by autoimmune processes, the loss of graft function can be considered the onset of overt diabetes. Therefore, by redefining the age of diabetes onset in these STZ-induced diabetic NOD mice as [age at the time of isografting plus graft survival days], the development of spontaneous diabetes in these mice was compared with that of the high-incidence strain littermates (Fig. 1). Figure 1*A* clearly demonstrates that the overall onset of diabetes in isografted, STZ-induced diabetic female NOD mice was significantly delayed compared with that in the females of our mouse colony ($P = 0.016$). Of isografted STZ-induced diabetic NOD females, 35% were free of diabetes at 300 days (42 wk) of age compared with 8% diabetes-free females in the control colony. The difference in the incidence of diabetes was more evident between isografted STZ-induced diabetic NOD males and nontreated spontaneously diabetic males (Fig. 1*B*).

To determine whether the operative procedure of islet isografting influences the development of diabetes, we gave a group of female NOD mice between 61 and 63 days of age NOD islet isografts without STZ administration. Another group of litter-matched females was observed for the development of diabetes without any treatment. As shown in Table 2, no significant difference was found in the onset of diabetes between the two groups.

C3H islet allograft survival in young STZ-induced diabetic NOD mice was also compared with that in spontaneously diabetic NOD recipient mice. As shown in Table 3, allografts were rapidly destroyed in spontaneously diabetic mice within 10 days postgrafting, whereas they survived significantly longer ($P = 0.005$ for females and $P = 0.000$ for males) in STZ-induced diabetic NOD mice.

Effect of STZ and ALS administration on isograft survival in overtly diabetic NOD mice. Islet isograft survival in overtly diabetic NOD mice administered STZ was compared with that in untreated diabetic NOD mice. The overall duration of diabetes at the time of grafting was not significantly different between the two groups: 36 ± 16 days (range 21–73 days) for NOD mice given STZ 8–11 days before grafting and 30 ± 10 days (17–43 days) for the untreated group. An additional group of diabetic NOD mice (duration of 44 ± 16 or 20–78 days) was given ALS on days 0 and 2 relative to isografting on day 0. Because no significant difference was observed in graft survival between male and female mice as well as between line I and line II mice once they became overtly diabetic, results of the experiments with both male and female as well as line I and line II mice were combined. As illustrated in Fig. 2, STZ administration induced a significant prolongation of islet isografts ($P = 0.023$) with MST of 41 days, whereas most isografts in untreated

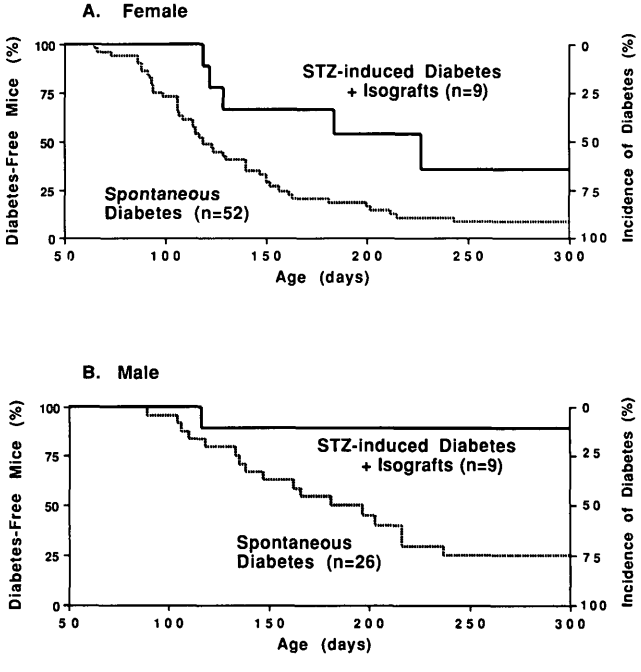

FIG. 1. Development of spontaneous diabetes in isografted STZ-administered young mice and their control colony mice. *A*: Of 9 female mice given isografts, 5 mice became diabetic 49, 54, 68, 123, and 167 days postgrafting, 1 mouse died with a functioning graft on day 143 postgrafting, and 3 mice were subjected to graft removal 104, 228, and 269 days postgrafting. *B*: Of 9 male mice given isografts, 1 mouse became diabetic on day 52 postgrafting, and the remaining 8 mice were subjected to graft removal on days 97, 103, 207, 255, 255, 256, 256, and 256 postgrafting. In both groups, removal of grafts by nephrectomy resulted in a rapid rise in blood glucose levels >17 mM. Age of spontaneous diabetes onset in these isografted STZ-induced diabetic NOD mice was defined as age at isografting plus graft survival days.

TABLE 2
Onset of diabetes in mice given islet isografts without STZ administration

Treatment	*n*	Age of diabetes onset (days)*
Islet isografting	7	97, 106, 151, 175, 187, 219, >234
No treatment	7	129, 142, 185, >223, >226, >234, >242

*Islet isografting was done between 61 and 63 days of age. Incidence of diabetes between above two groups, is not significantly different with $P = 0.158$ by Kaplan-Meier estimation (log-rank test).

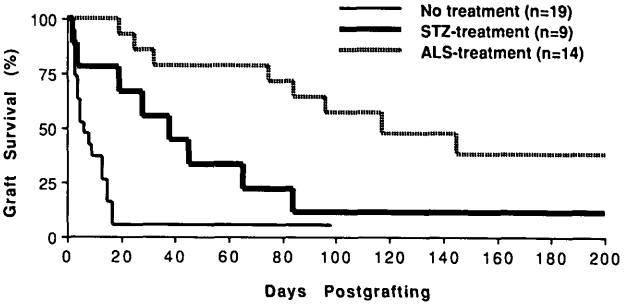

FIG. 2. Islet isograft survival in spontaneously diabetic NOD mice. Three groups of overtly diabetic mice were isografted on day 0 either without any treatment (16 line II and 3 line I mice) or after administration of STZ (9 line II mice) or ALS (13 line II and 1 line I mouse). Isografts were destroyed on days 2, 3, 3, 3, 3, 4, 4, 5, 5, 6 (line I), 8, 9 (line I), 13, 13 (line I), 15, 15, 17, and 17 for untreated mice; days 2, 4, 19, 28, 38, 45, 65, and 84 for STZ-administered mice; and days 19, 25, 32 (line I), 75, 84, 96, 117, and 145 for ALS administered mice. Remaining mice either died with functioning grafts on day 98 (no treatment), 246 (STZ), or 109 and 180 (ALS) or were subjected to graft removal on days 106, 177, 179, or 221 (ALS).

diabetic NOD mice were destroyed rapidly (MST 8 days). ALS administration at grafting induced marked prolongation of isograft survival, with 8 of 14 mice maintaining normoglycemia for >100 days (MST 102 days).

Effect of STZ and ALS administration on allograft survival in overtly diabetic NOD mice. Similarly, C3H islet allografts were transplanted into three groups (untreated, STZ administered, and ALS administered) of overtly diabetic NOD mice (Fig. 3). As seen with islet isografts, longer allograft survival was observed in STZ-administered mice than in untreated recipients, with MST of 12 and 6 days, respectively ($P = 0.007$). ALS administration induced maximal prolongation of allografts, with MST of 35 days.

Effect of STZ administration on in vitro MLC reactivity. To examine the effect of STZ on immune reactivity, STZ was administered to C57BL/6 mice. Splenocytes were prepared at various time points after STZ administration and used as responder cells in MLC against DBA/2 stimulator cells. Table 4 shows that alloantigen reactivities (both proliferative responses and generation of cytotoxicity) progressively decreased until days 14 and 28, when they were at their lowest point. The reactivity was recovered on day 56 after STZ administration. In a different series of experiments, splenocytes were prepared from prediabetic and overtly diabetic NOD mice that were given STZ 14 days before and used in flow cytometric analysis for Thy-1.2$^+$, CD4$^+$, and CD8$^+$ cell distribution and in determination of their ability to generate cytotoxicity against fully allogeneic C3H/He stimulator cells. Untreated prediabetic and diabetic littermates served as the controls. Table 5 shows that STZ administration induced a significant reduction of cytotoxic alloreactivities in both prediabetic and overtly diabetic groups. The suppression of the reactivity was not attributable to the shifting of time kinetics or the changes in dose responses (data not shown). No statistically significant changes were seen among four groups in total cell yields, percentage of Thy 1.2$^+$ cells, or CD4$^+$-CD8$^+$ cell composition of the splenocytes, although the tendency seemed to be towards lower cell yields and decreased CD8$^+$ cells. It is interesting to note that splenocytes of overtly diabetic mice responded to alloantigens as well as those of prediabetic mice.

DISCUSSION

We demonstrated that islet isografts transplanted into young STZ-induced diabetic NOD mice survived for prolonged periods, in sharp contrast to the rapid destruction of all isografts in untreated spontaneously diabetic NOD mice. The prolongation of isograft survival was particularly evident in male mice, with most grafts functioning for >100 days. Weak autoimmune activity in the young nondiabetic NOD mouse, as suggested previously (17), could explain longer isograft survival in these mice. However, weak autoimmunity at isografting alone fails to explain why the subsequent destruction of islets (i.e., onset of diabetes) in these STZ-induced diabetic mice was significantly delayed compared with the course of diabetes development in the littermates. In the presence of isogeneic islets transplanted shortly after STZ injection, anti-islet autoimmunity should have fully developed and destroyed the isografts about the same time it would have destroyed the islets in the untreated hosts.

Several possible mechanisms underlie the delayed

TABLE 3
C3H/He islet allograft survival in STZ-induced diabetic and spontaneously diabetic NOD mice

Cause of diabetes	Sex of recipient	Age at grafting	*n*	Graft survival (days postgrafting)
STZ	F	63–69	3	12, 12, 22
STZ	M	67–81	9	12, 13, 16, 19, 22, 27, 31, 64, 82
Spontaneous	F	134–231	17	2, 2, 3, 3, 3, 4, 4, 5, 6, 7, 7, 9, 9, 9, 10, 10, 10
Spontaneous	M	139–221	3	2, 10, 10

Islet allografting in spontaneously diabetic NOD mice was done between 21 and 105 days after diabetes onset.

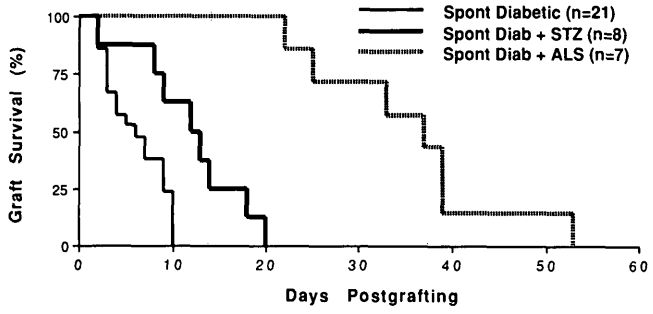

FIG. 3. Islet allograft survival in diabetic NOD mice. Three groups of spontaneously diabetic mice were grafted with C3H islet allografts either without any treatment (20 line II and 1 line I mice), or after administration of STZ (8 line II mice) or ALS (6 line II and 1 line I mouse). Allografts were destroyed on days 2, 2, 2, 3, 3, 3, 3 (line I), 4, 4, 5, 6, 7, 7, 9, 9, 9, 10, 10, 10, 10, and 10 for untreated mice, days 2, 8, 9, 12, 13, 14, 18, and 20 for STZ-administered mice, and days 22, 25, 33 (line I), 37, 39, 39, and 53 for ALS-administered mice.

onset of diabetes in isografted STZ-induced diabetic NOD mice. First, it could be argued that the shift of diabetes onset may have been caused by the operative procedure of islet grafting rather than by STZ itself. This is unlikely, however, because the untreated prediabetic NOD mice given islet isografts without STZ administration showed a similar pattern of diabetes incidence to the untreated nongrafted control littermates. Second, the STZ-induced sudden destruction of β-cells at an early stage of insulitis could have shifted the balance between the autoreactive effector cells and the suppressor cells within the infiltrating lymphocytes toward a suppressor cell–dominant condition. Previous studies have indicated that lymphoid cells infiltrating the NOD islets contain autoreactive suppressor cells capable of inhibiting the development of diabetes (18). Thus, isografts enjoyed prolonged survival until the autoreactive effector cells became dominant again at a later time. The most likely explanation, however, is that STZ has an immunosuppressive potential in addition to its diabetogenic property, and that suppression of weak autoimmunity at the prediabetic stage prevented the timely destruction of isograft β-cells and delayed the development of diabetes in isograft-bearing mice. The immunosuppressive activity of STZ was illustrated more clearly by the finding that administration of STZ to overtly diabetic NOD mice led to significantly longer survival of both islet isografts and allografts than that in age-matched, untreated diabetic NOD mice. Because few β-cells in islets remain viable in overtly diabetic mice, the diabetogenic activity of STZ itself should have little influence on the isograft and allograft survival. The prolonged graft survival in these mice must be induced by the suppression of autoimmunity, which affects both islet isografts and allografts. The immunosuppressive property of STZ was also demonstrated by in vitro allogeneic MLC experiments. Injection of STZ in normal C57BL/6 mice induced gradual reduction in both proliferative and cytotoxic activities of their splenocytes. The maximum changes in alloreactivity were observed at 2–4 wk after STZ injection, followed by full recovery at 8 wk. STZ administration to both prediabetic and overtly diabetic NOD mice also caused reduction in generation of the allogeneic cytotoxic reactivities of their splenocytes. Whether the reduced alloreactivity could be attributable to changes in T-cell subpopulations, especially reduced $CD8^+$ T-cells, after STZ administration is not clear at present.

The immunosuppressive property of STZ probably stems from its DNA-alkylating activity. Although STZ mediates its strong toxic effect in pancreatic β-cells through reduction of nicotinamide adenine dinucleotide (19) and other complex mechanisms (reviewed in 20), the alkylating activity of STZ in other types of cells is generally extremely weak compared with other nitrosoureas (21). Indeed, this study indicates that the immunosuppressive activity of STZ at a diabetogenic dose is weak, especially compared with that of ALS, a widely used immunosuppressive reagent. STZ achieved marked prolongation of islet isograft survival in spontaneously diabetic mice, with the MST being 33 days longer than that of the untreated controls. When STZ administration to the diabetic mice was followed by islet allografting, however, the prolongation of graft survival in these mice was significant but much smaller, with the MST being only 6 days longer than the untreated controls. Because destruction of allografts in overtly diabetic NOD mice is probably mediated by both autoimmune processes and alloantigen responses, this finding may suggest inability of STZ to suppress allogeneic rejection responses. In contrast, ALS mediates much stronger immune suppression. We have shown previously that the treatment of overtly diabetic NOD mice with ALS led to

TABLE 4
Effect of STZ administration on MLC reactivity

	[^{3}H]Thymidine uptake		Cytotoxicity	
Days after STZ	(cpm)	*P*	(% specific lysis)	*P*
0 (Untreated)	15,654 ± 4056	—	51.1 ± 13.0	—
Day 7	11,663 ± 3060	0.002	40.5 ± 10.8	0.012
Day 14	7283 ± 3285	0.000	32.5 ± 13.8	0.002
Day 28	10,012 ± 4159	0.002	20.3 ± 7.6	0.000
Day 56	15,474 ± 2742	0.906	52.8 ± 6.6	0.724

Splenocytes were prepared from untreated and STZ-administered C57BL/6 mice at the indicated time after treatment. At least 3 mice were used in each group. Splenocytes from each mouse were individually sensitized in vitro against DBA/2 stimulator cells for 4 days (see METHODS). Results were pooled within the same group and expressed as mean cpm/culture and mean percentage of specific lysis/culture ± SE. Levels of significance (*P* values) were calculated against the untreated group with Student's *t* test.

TABLE 5
Effect of STZ administration of prediabetic and diabetic NOD mice on splenic T-cell populations and their MLC reactivity

NOD mouse	STZ	Splenocyte yield ($\times 10^{-6}$)/mouse	Splenic T-cells (%)			Cytotoxicity (% specific lysis)
			Thy 1.2$^+$	CD4$^+$	CD8$^+$	
Nondiabetic	−	101–139	55.7	65.3	27.5	58.0 ± 2.5
Nondiabetic	+	54–106	45.6	73.5	24.4	32.4 ± 2.0*
Diabetic	−	108–152	53.0	71.7	26.3	51.8 ± 1.8
Diabetic	+	86–147	48.3	72.0	25.0	19.1 ± 3.2*

Prediabetic and overtly diabetic NOD mice were treated with diabetogenic dose of STZ. Fourteen days later, splenocytes were prepared individually from these mice as well as untreated prediabetic and diabetic littermates and used in flow cytometric analysis and in vitro MLC. At least 3 mice were used in each group. CD4$^+$ and CD8$^+$ cells were expressed as percentage of Thy-1.2$^+$ cells. Generation of anti-C3H/He cytotoxicity was determined separately for each mouse. Results were pooled within the same group and expessed as mean percentage of specific lysis/culture ± SE.
*$P < 0.01$, vs. untreated controls.

abrogation of autoimmunity, with subsequent isografts surviving indefinitely, or induced marked prolongation of isografts when ALS was given at the time of islet grafting, with 8 of 13 grafts surviving for >100 days (7). ALS treatment of diabetic NOD mice also induced a 23–29 day prolongation of islet allograft survival compared with the untreated controls. The weaker immunosuppressive activity of STZ was also demonstrated by in vitro MLC experiments; STZ administration to normal mice resulted in only a 50% reduction of their splenocyte alloreactivity, whereas ALS treatment induces complete abrogation of in vitro alloantigen responsiveness (16).

In summary, we demonstrated the immunosuppressive property of STZ with an islet isograft and allograft model in NOD mice. Because STZ is widely used in various experimental animals as a diabetogenic agent, its immunosuppressive activity may need to be considered in analyzing the experimental results.

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REFERENCES

1. Wiggans RG, Woolley PV, MacDonald JS, Smythe T, Ueno W, Schein PS: Phase II trial of streptozotocin, mitomycin-c and 5-fluorouracil (SMF) in treatment of advanced pancreatic cancer. *Cancer* 41:387–91, 1958
2. LeDoux SP, Wilson GL: Effects of streptozotocin on a clonal isolate of rat insulinoma cells. *Biochim Biophys Acta* 804:387–92, 1984
3. Wilson GL, Hartig PC, Patton NJ, LeDoux SP: Mechanisms of nitrosourea-induced β cell damage: activation of poly (ADP-ribose) synthetase and cellular distribution. *Diabetes* 37:213–16, 1988
4. Like AA, Rossini AA: Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. *Science* 193:415–17, 1976
5. Makino S, Harada M, Kishimoto Y, Hayashi Y: Absence of insulitis and overt diabetes in athymic nude mice with NOD genetic background. *Exp Anim* 35:495–98, 1986
6. Nomikos IN, Prowse SJ, Carotenuto P, Lafferty KJ: Combined treatment with nicotinamide and desferrioxamine prevents islet allograft destruction in NOD mice. *Diabetes* 35:1302–304, 1986
7. Maki T, Ichikawa T, Blanco R, Porter J: Long-term abrogation of autoimmunity in spontaneously diabetic NOD mice by immunotherapy with anti-lymphocyte serum. *Proc Natl Acad Sci USA* 89:3434–38, 1992
8. Sutherland DE, Sibley R, Xu Xz, Chinn P, Micheal A, Srikanta AM, Taub F, Nigerian J, Getz SC: Twin-to-twin pancreas transplantation: reversal and reenactment of the pathogenesis of type I diabetes. *Clin Res* 32:551A, 1984
9. Sutherland DER, Goetz FC, Sibley RK: Recurrence of disease in pancreatic transplants. *Diabetes* 38 (Suppl. 1):85–87, 1989
10. Harada M, Makino S: Suppression of overt diabetes in NOD mice by anti-thymocyte serum or anti-Thy 1.2 antibody. *Exp Anim* 35, 501–504, 1986
11. Mori Y, Suko M, Okudaira H, Matsuba I, Tsuruoka A, Sasaki A, Yokoyama H, Tanase T, Shiba T, Nishimura M, Terada E, Ikeda Y: Preventive effects of cyclosporin on diabetes in NOD mice. *Diabetologia* 29:244–47, 1986
12. Koike T, Itoh Y, Ishii T, Takabayashi K, Maruyama N, Tomioka H, Yosida S: Preventive effect of monoclonal anti-L3T4 antibody on development of diabetes in NOD mice. *Diabetes* 36:539–41, 1987
13. Monaco AP, Wood ML, Gray JG, Russel PS: Studies on heterologous antilymphocyte serum in mice: I. In vitro and in vivo properties. *J Immunol* 96:217–28, 1966
14. Gotoh M, Maki T, Kiyoizumi T, Satomi S, Monaco AP: An improved method for isolation of mouse pancreatic islets. *Transplantation* 40:437–38, 1985
15. Gotoh M, Porter J, Kanai T, Monaco AP, Maki T: Multiple donor allotransplantation: a new approach to pancreatic islet transplantation. *Transplantation* 45:1008–12, 1988
16. Maki T, Gottschalk R, Wood ML, Monaco AP: Specific unresponsiveness to skin allografts in antilymphocyte serum treated, marrow injected mice: participation of donor marrow derived suppressor T-cells. *J Immunol* 127:1433–38, 1981
17. Wang Y, McDuffie M, Nomikos IN, Hao L, Lafferty KJ: Effect of cyclosporine on immunologically mediated diabetes in nonobese diabetic mice. *Transplantation* 46:101–106S, 1988
18. Reich E-P, Scaringe D, Yagi J, Sherwin RS, Janeway CA Jr: Prevention of diabetes in NOD mice by injection of autoreactive T lymphocytes. *Diabetes* 38:1647–51, 1989
19. Yamamoto H, Uchigata Y, Okamoto H: Streptozotocin and alloxan induce DNA strand breaks and poly(ADP-ribose) synthetase in pancreatic islets. *Nature* (Lond) 294:284–86, 1981
20. Wilson GL, Leiter EH: Streptozotocin interactions with pancreatic β cells and the induction of insulin-dependent diabetes. *Curr Top Microbiol Immunol* 156:27–54, 1990
21. Wheeler GP: A review of studies on the mechanism of action of nitrosourea. In *Cancer Chemotherapy.* Vol. 30. Sartorelli AC, Ed. Washington, DC, American Chemical Society, 1976, p. 87–119