Testicular Sertoli Cells Protect Islet β-Cells From Autoimmune Destruction in NOD Mice by a Transforming Growth Factor-β1–Dependent Mechanism

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Testicular Sertoli cells protect pancreatic islet grafts from allo- and autoimmune destruction; however, the mechanism(s) of protection is unclear. The aim of this study was to determine whether Fas ligand (FasL) and/or transforming growth factor (TGF)-β1, immunoregulatory proteins produced by Sertoli cells, might mediate the protective effects of these cells against autoimmune destruction of islet β-cells. Sertoli cells were purified from testes of NOD mice and implanted under the right renal capsule of diabetic NOD mice, whereas NOD islets were implanted under the left renal capsule. Of the mice that received islet and Sertoli cells grafts, 64% (9 of 14) remained normoglycemic at 60 days posttransplantation compared with 0% (0 of 6) of the mice that received islet grafts alone. Immunohistochemical examination of Sertoli cell grafts in normoglycemic mice revealed that TGF-β1 expression by Sertoli cells remained high, whereas FasL expression by Sertoli cells decreased progressively posttransplantation. Also, plasma levels of TGF-β1 were significantly elevated in mice that received Sertoli cells and islet grafts, and anti–TGF-β1 antibody administration completely abrogated the protective effect of Sertoli cells on islet graft survival, whereas anti-FasL antibody did not. Islet graft destruction in anti–TGF-β1–treated mice was associated with increases in interferon (IFN)-γ–producing cells and decreases in interleukin (IL)-4–producing cells in the islet grafts. We conclude that 1) Sertoli cell production of TGF-β1, not FasL, protects islet β-cells from autoimmune destruction and 2) TGF-β1 diverts islet-infiltrating cells from a β-cell–destructive (IFN-γ+) phenotype to a non-destructive (IL-4+) phenotype. Diabetes 49:1810–1818, 2000

Certain tissues such as brain, anterior chamber of the eye, and testis have been termed immunologically privileged because immune responses to “non-self” antigens are reduced at these sites (1). For example, allogeneic (2) and xenogeneic (3) pancreatic islets survive transplantation in the testis with little immunosuppression. Sertoli cells have been identified as the component in testicular tissue that confers immune privilege to tissue allografts (4), including islet allografts transplanted into chemically diabetic rats (5–8) and islet isografts transplanted into NOD mice with autoimmune diabetes (9). The mechanism(s) of Sertoli cell–induced protection of β-cells from immunological destruction, however, have not been fully elucidated.

Sertoli cells produce a wide variety of proteins, including immunoregulatory factors such as Fas ligand (FasL) and transforming growth factor (TGF)-β1 (10,11). FasL expression by testicular tissue has been reported to protect mouse testicular (12) and islet (13) allografts from rejection. In other studies, however, FasL expression by mouse β-cells, achieved transgenically (14,15) or by gene transfection (15,16), failed to protect islet allografts, or even isografts, from destruction. TGF-β1 is a potent suppressor of inflammatory and immune responses, including autoimmune diabetes (17–22); however, this cytokine has not been examined as a possible mediator of the protective effects of Sertoli cells against immunological destruction of islet β-cells.

Therefore, the aim of the present study was to determine whether FasL and/or TGF-β1 might mediate the protective effect of Sertoli cells against autoimmune β-cell destruction. To address this question, we used the model of syngeneic islet transplantation in NOD mice. We implanted syngeneic Sertoli cells into diabetic NOD mice at the time of syngeneic islet transplantation; then we studied FasL and TGF-β1 expression by the implanted Sertoli cells and the effects of anti-FasL and anti–TGF-β1 monoclonal antibody administration on islet graft survival. Our findings demonstrate that TGF-β1, not FasL, is a mediator of the protective effect of Sertoli cells against autoimmune destruction of islet β-cells.

RESEARCH DESIGN AND METHODS

Animals. Male and female NOD mice (Taconic, Germantown, NY) aged 4 weeks were used as islet donors, and male NOD mice aged 9–12 days were used as recipients. Sertoli cells were purified from testes of NOD mice aged 11–12 weeks by the method of Rabinovitch et al. (10). Purified Sertoli cells were cultured and then washed three times in phosphate-buffered saline (PBS) before being injected into recipient mice. Islets were purified as described by Korbutt et al. (13). Briefly, islets were isolated from the pancreas of NOD mice aged 6–8 weeks and cultured overnight in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 10% FCS, and 1 ng/ml bovine insulin. The islets were then washed in PBS and placed into culture dishes in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS without insulin. After 24 h, viable islets were used for implantation. Sertoli cells were cultured in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS.

Animals were housed at the Animal Services Facility at the University of Alberta. All animals were housed in an environment with controlled temperature and humidity and with a 12-h light–dark cycle. The Animal Services Facility is accredited by the Canadian Council on Animal Care and operates in accordance with guidelines of the Canadian Council on Animal Care. Animals were sacrificed by isoflurane overdose or by CO2 asphyxiation as permitted by the University of Alberta’s Animal Care Committee.

The recipient mice were aged 4–6 weeks and were rendered diabetic by streptozotocin injection of 50 mg/kg body weight i.p. The mice were allowed to recover for 1 week and received either 100 or 200 μg islets. Sertoli cells were purified from testes of NOD mice aged 11–12 weeks by the method of Rabinovitch et al. (10). Purified Sertoli cells were cultured and then washed three times in PBS before being injected into recipient mice. Islets were purified as described by Korbutt et al. (13). Briefly, islets were isolated from the pancreas of NOD mice aged 6–8 weeks and cultured overnight in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 10% FCS, and 1 ng/ml bovine insulin. The islets were then washed in PBS and placed into culture dishes in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS without insulin. After 24 h, viable islets were used for implantation. Sertoli cells were cultured in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS.

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ABP, androgen-binding protein; BSA, bovine serum albumin; FasL, Fas ligand; FSHR, follicle-stimulating hormone receptor; HBSS, Hanks’ balanced salt solution; IL, interleukin; IFN, interferon; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PBS-saponin, PBS with 3% saponin; TGF, transforming growth factor; Th, T helper.
used as testicular Sertoli cell donors. Diabetic female NOD mice aged 17–22 weeks were used as recipients of islet and Sertoli cell grafts. Diabetic mice (diabetes duration 3–5 weeks) were transplanted into non-tissue culture-coated Petri dishes, and incubated for 3 days at 37°C (5% 
vein blood glucose was monitored daily after transplantation by using an Accu-Check Ii glucometer (Roche, Mannheim, Germany). Grafts were washed twice in RPMI 1640 medium (Life Technologies) supplemented with 10% heat-inactivated fetal calf serum (Life Technologies, Burlington, ON, Canada) and incubated at 37°C with trypsin (25 µg/ml; Boehringer Mannheim, Laval, PQ, Canada) and DNase (4 µg/ml; Boehringer Mannheim) in HBSS/EDTA, and washed four times in HBSS. The final cell pellet was resuspended in IM9 medium (Life Technologies, Burlington, ON, Canada) supplemented with 10% heat-inactivated horse serum, passed through a 500-µm mesh, placed in 150–15× mm nontissue culture-coated Petri dishes, and incubated for 3 days at 37°C (5% CO2) before transplantation to allow the testicular cells to reaggregate (8). There was a preferential survival of Sertoli cells during the 3-day culture period. Survival of testicular cell viability was evaluated by immunohistochemical staining for markers that are specific to Sertoli cells in testicular cell preparations: follicle-stimulating hormone receptor (FSHr) (24) and androgen binding protein (ABP) (25). Thus, fresh preparations of testicular cells from 9- to 12-day-old NOD mice were composed of 52 ± 4% FSHr+ cells and 60 ± 6% ABP+ cells, and the Sertoli cell purity increased to 83 ± 4% FSHr+ cells and 94 ± 3% ABP+ cells after 3 days of culture (means ± SE, n = 4 preparations). Each preparation of Sertoli cells was obtained from a pool of testes (10 NOD mice per culture). -1 × 106 Sertoli cells were recovered from each graft. Before transplantation, an aliquot (1 ml) of a suspension of the 3-day cultured Sertoli cell aggregates (35 ml for cells prepared from 20 testes) was dissociated into single cells by incubation at room temperature for 10 min in enzyme-free Hank’s-based cell dissociation buffer (Life Technologies), followed by trypan blue (0.4% in HBSS) to obtain a cell count and estimate the number of Sertoli cells in the cell aggregates transplanted. Sertoli cell aggregates were washed twice in RPMI 1640 medium (Life Technologies) supplemented with 1% NOD mouse serum before transplantation. Transplantations. Diabetic NOD mice were anesthetized with Halothane and then transplanted with 500 freshly isolated islets alone or with 500 islets and 4 × 106 Sertoli cells. Transplantations of islets and Sertoli cells were carried out as follows. A total of 500 islets were aspirated into polyethylene tubing (PE-50), pelleted by centrifugation for 2 min, then implanted under the left renal capsule on the lateral aspect of the kidney. Other diabetic NOD mice were similarly transplanted with 500 islets under the left renal capsule and, in addition, aggregates of 4 × 106 Sertoli cells were transplanted under the right renal capsule using the PE-50 tubing and pelleting technique used for islet transplants. Transplantation was considered successful if the nonfasting blood glucose returned to normal (<7.0 mmol/l) and remained normal for the first 4 days posttransplantation. Tail vein blood glucose was monitored daily after transplantation by using an Accu-Check Ii glucometer (Boehringer Mannheim). Graft rejection was diagnosed by return of hyperglycemia (blood glucose >11 mmol/l) accompanied by glycosuria and ketonuria on two consecutive days. Experimental design. In the first study, we examined the effects of Sertoli cells implanted under the right renal capsule on survival of syngeneic islets implanted under the left renal capsule in diabetic NOD mice. The transplanted mice were monitored daily by blood glucose measurements, and the day of diabetes recurrence after transplantation defined the duration of graft survival. The mice were killed at the time of diabetes recurrence or at 60 days posttransplantation if still normoglycemic. Islet and Sertoli cell grafts from diabetic and normoglycemic NOD mice were examined histologically. In the second study, we examined the cell compositions of islet and Sertoli cell grafts by immunohistochemistry at 8 h posttransplantation (day 1), at 12–18 days posttransplantation in mice in which diabetes recurred (within the previous 24 h), and at 12–18 and 30 days posttransplantation in mice that were still normoglycemic. In the third study, we measured plasma levels of TGF-β1 in nontransplanted diabetic and normoglycemic NOD mice transplanted with either islets or islets plus Sertoli cells. In the fourth study, we examined the effects of treating the mice transplanted with both islets and Sertoli cells with the following: I) a mouse anti-mouse FasL neutralizing monoclonal antibody (mAb) (Kaiy; PharMingen, San Diego, CA) (26), 250 µg i.p. three times a week from day 0–28 posttransplantation, or the same dose of an isotype control mouse IgG2b mAb (PharMingen); or II) a mouse anti-human TGF-β1 neutralizing mAb (MAB240, R&D Systems, Minneapolis, MN) that cross-reacts to mouse TGF-β1 (27), 250 µg i.p. three times a week starting from either day 0 or day 20 posttransplantation until diabetes recurrence, or the same dose of an iso
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cells by immunohistochemical staining (31). Cells to be stained for TGF-β1 were first permeabilized by incubation in PBS with 3% saponin to detect intracellular cytokine (29,30). TGF-β1 antigen was stained by incubating the cells at 25°C for 2 h with a rabbit antibody specific for TGF-β1 (sc-146; Santa Cruz Biotechnology) or IgG control antibody, 1 µg/ml diluted in PBS with 3% saponin. All subsequent cell incubations and washes were carried out in PBS for cells stained with anti-Fasl or control antibody and in PBS with 3% saponin (PBS-saponin) for cells stained with anti–TGF-β1 or control antibody. After staining with antibodies to Fasl or TGF-β1, the cells were washed in PBS or PBS-saponin for 30 min and then incubated for 30 min at room temperature with a secondary antibody, biotinylated goat anti-rabbit IgG (Zymed Laboratories), diluted 1:1 in PBS or PBS-saponin. Cells were then washed in PBS or PBS-saponin for 30 min and incubated for 15 min at room temperature in PBS or PBS-saponin diluted 1:1 with streptavidin-peroxidase conjugate (Histostain-SP kit; Zymed Laboratories). After a brief washing in PBS or PBS-saponin, cells were incubated with the substrate chromogen 3-amino, 9-ethylcarbazole for 3–5 min until staining (red) of Fasl, or TGF-β1 was at its maximum. By this method, Fasl- and TGF-β1+- cells were stained red intracellularly, and Sertoli cells expressing Fasl (FShr+ FasL+) or TGF-β1 (FShr+ TGF-β1+) were stained blue on the surface (FShr+) and red intracellularly (Fasl- or TGF-β1-).

**Determination of plasma TGF-β1 Levels.** To minimize the activation of platelets and subsequent release of endogenous TGF-β1, a platelet-poor plasma was obtained as follows. Whole blood was mixed with a 1.5% EDTA solution, mixed thoroughly, and put on ice immediately after blood collection. The blood/EDTA mixture was then layered gently on 20% sucrose and centrifuged for 2 h with a rabbit antibody specific for TGF-β1 (sc-146; Santa Cruz Biotechnology) or IgG control antibody. After staining with antibodies to TGF-β1, the cells were washed in PBS or PBS-saponin diluted 1:1 with streptavidin-peroxidase conjugate (Histostain-SP kit; Zymed Laboratories) or IgG control antibody, 1 µg/ml diluted in PBS with 3% saponin. All subsequent antibody, biotinylated goat anti-rabbit IgG (Zymed Laboratories), diluted 1:1 in PBS or PBS-saponin. Cells were then washed in PBS or PBS-saponin for 30 min and incubated for 15 min at room temperature in PBS or PBS-saponin diluted 1:1 with streptavidin-peroxidase conjugate (Histostain-SP kit; Zymed Laboratories). After a brief washing in PBS or PBS-saponin, cells were incubated with the substrate chromogen 3-amino, 9-ethylcarbazole for 3–5 min until staining (red) of Fasl, or TGF-β1 was at its maximum. By this method, Fasl- and TGF-β1+- cells were stained red intracellularly, and Sertoli cells expressing Fasl (FShr+ FasL+) or TGF-β1 (FShr+ TGF-β1+) were stained blue on the surface (FShr+) and red intracellularly (Fasl- or TGF-β1-).

**RESULTS**

**Effects of Sertoli cells on islet graft survival.** Syngeneic islet transplantation restored normoglycemia (blood glucose <7.0 mmol/l) in diabetic NOD mice 8–12 h posttransplantation; however, diabetes recurred (blood glucose >11.1 mmol/l) between day 8 and day 14 posttransplantation. In contrast, when syngeneic Sertoli cells were transplanted under the capsule of the right kidney, the time that islets were transplanted under the capsule of the left kidney. 64% (9 of 14) of mice remained normoglycemic at 60 days posttransplantation (Fig. 1). Median survival was 9 days for islets transplanted alone and >60 days for islet grafts in mice that also received Sertoli cell grafts. Histological examination of the grafts revealed that islet-only grafts were heavily infiltrated by leukocytes and few insulin-staining β-cells remained, whereas abundant β-cells were surrounded but not destroyed by leukocytes in islet grafts of mice that also received Sertoli cell grafts (Fig. 2).

**Cell compositions of islet and Sertoli cell grafts.** Cellular compositions of islet and Sertoli cell grafts that prevented diabetes recurrence were compared with compositions of grafts that failed to maintain normoglycemia (Fig. 3). At 12–18 days posttransplantation, β-cells were significantly decreased, and leukocytes were significantly increased in islet grafts of diabetic mice compared with values in islet grafts initially transplanted. In contrast, β-cell numbers were maintained, and leukocytes were significantly less numerous in islet grafts of normoglycemic mice than diabetic mice at 12–18 days and at 30 days posttransplantation. Regarding Sertoli cell grafts, leukocytes infiltrated the grafts in similar numbers in diabetic and normoglycemic mice. Sertoli cells in the grafts were significantly decreased in diabetic mice and to a lesser extent in normoglycemic mice at 12–18 days but not at 30 days posttransplantation. Notably, Fasl expression by Sertoli cells (percent of Sertoli cells that expressed Fasl) decreased significantly from 97 ± 2% at the time of transplantation to 44 ± 3% in diabetic mice and 45 ± 3% in normoglycemic mice at 12–18 days posttransplantation. At 30 days posttransplantation, FasL expression was very low in normoglycemic mice (26 ± 2%) despite total Sertoli cell numbers remaining unchanged since the time of transplantation. In contrast to Fasl, TGF-β1 expression by Sertoli cells (percent of Sertoli cells that expressed TGF-β1) remained high (>80%) after transplantation in all groups of mice (Fig. 3). Total numbers of TGF-β1+Sertoli cells, however, were decreased in diabetic mice (1.9 ± 0.1×10⁶ cells) and to a lesser extent in normoglycemic mice (2.9 ± 0.2×10⁶ cells) at 12–18 days posttransplantation but were maintained in normoglycemic mice at 30 days posttransplantation (3.7 ± 0.2×10⁶ cells) compared with the number of TGF-β1+Sertoli cells initially transplanted (3.8 ± 0.2×10⁶ cells). Some of the mice that were normoglycemic at 12–18 days posttransplantation later developed diabetes (Fig. 1); therefore, these prediabetic mice could account for the lower mean values for Sertoli cell numbers and percent of TGF-β1+Sertoli cells in normoglycemic mice at 12–18 days than at 30 days posttransplantation (Fig. 3).

**Effects of anti-Fasl antibody.** Anti-Fasl mAb treatment of NOD mice transplanted with islets and Sertoli cells did not abrogate Sertoli cell–induced prolongation of syngeneic islet graft survival in NOD mice; rather, islet graft survival was...
FIG. 2. Photomicrographs of islet grafts (A, B, and D, stained with anti-insulin antibody and hematoxylin) and a Sertoli cell graft (C, stained with hematoxylin and eosin) in NOD mice. At 8 h after islet transplantation, abundant β-cells (stained brown with anti-insulin antibody) were detected in an islet graft placed under the left renal capsule and overlying the kidney (A). At 10 days after transplantation, the islet graft was heavily infiltrated by leukocytes (small cells with blue-stained nuclei), few β-cells (stained brown) remained, and diabetes had recurred (B). In other mice, islets were implanted under the left renal capsule, and Sertoli cells were implanted under the right renal capsule: at 60 days after transplantation, Sertoli cells were arranged in clusters (c) and tubules (t) in the Sertoli cell graft (C), and abundant β-cells (stained brown) were surrounded but not destroyed by leukocytes (stained blue) in the islet graft (D).

FIG. 3. Cell composition of islet and Sertoli cell grafts in NOD mice. A total of 500 islets were transplanted under the left renal capsule, and 4 × 10⁶ Sertoli cells were transplanted under the right renal capsule in diabetic NOD mice. The grafts were examined at 8 h posttransplantation (day 1, n = 6), at 12–18 days posttransplantation in mice in which diabetes had recurred within the previous 24 h (day 12–18, diabetic, n = 4) and in mice that were normoglycemic (day 12–18, normoglycemic, n = 7), and at 30 days posttransplantation in mice that remained normoglycemic for this period (day 30, normoglycemic, n = 4). Total β-cells and leukocytes in islet grafts (A), total Sertoli cells and leukocytes in Sertoli cell grafts (B), and percent of Sertoli cells expressing FasL (FasL⁺ FSHr⁺ cells) and TGF-β1 (TGF-β1⁺ FSHr⁺) in Sertoli cell grafts (C) were determined by immunohistochemistry using antibodies to the cell types indicated. Values are means ± SE. *P < 0.05, **P < 0.01 vs. day 1 posttransplantation; †P < 0.05, ‡P < 0.01 vs. day 12–18, diabetic. Significance was determined by analysis of variance followed by Tukey-Kramer’s test. □, Day 1; ■, day 12–18, diabetic; ⊙, day 12–18, normoglycemic; □, day 30, normoglycemic.
slightly improved (Fig. 4). Thus, 75% (9 of 12) of mice treated with anti-FasL mAb were normoglycemic at 30 days after receiving islet and Sertoli cell grafts compared with 67% (4 of 6) of mice treated with a control mAb (P = 0.059, not significant by the Mann-Whitney rank sum test).

**Plasma TGF-β1 levels.** Plasma TGF-β1 levels were measured to determine whether TGF-β1 expressed by Sertoli cells implanted in the right kidney led to increased circulating levels of the cytokine. The plasma TGF-β1 level in mice that received both islet and Sertoli cell grafts and remained normoglycemic was significantly increased (3.72 ± 0.31 ng/ml) compared with the plasma TGF-β1 level in normoglycemic mice that received islets alone (1.97 ± 0.22 ng/ml), and the latter value was similar to that in normoglycemic nontransplanted NOD mice (2.06 ± 0.30 ng/ml) (Fig. 5). Also, plasma TGF-β1 levels were 2- to 4-fold higher in normoglycemic mice than in diabetic mice in each group. All TGF-β1 values reported here represent the latent form of TGF-β1 (detected as active TGF-β1 after acidification). Active TGF-β1 was not detected (<4 pg/ml) in the plasma samples before acidification.

**Effects of anti-TGF-β1 antibody.** Anti-TGF-β1 mAb was administered to NOD mice to determine whether Sertoli cell expression of TGF-β1 and the consequent increase in plasma level of TGF-β1 was responsible for prolonged islet graft survival in mice implanted with Sertoli cells in addition to islets. Anti-TGF-β1 mAb treatment of NOD mice transplanted with islets and Sertoli cells completely abrogated Sertoli cell–induced prolongation of syngeneic islet graft survival in NOD mice. Thus, when anti-TGF-β1 mAb was administered to mice from the time of islet and Sertoli cell transplantation, islet grafts were rejected at the same time after transplantation (7–17 days), as in mice that received islet grafts alone, whereas Sertoli cell–induced prolongation of islet graft survival persisted in mice treated with a control mAb (Fig. 6A). Similarly, administration of antianti–TGF-β1 mAb, starting at 20 days after islet and Sertoli cell transplantation, completely abrogated the protective effect of Sertoli cells on islet graft survival within 10 days of anti–TGF-β1 administration (Fig. 6B). Immunohistochemical analysis of the cell composition of the islet grafts revealed that anti–TGF-β1 mAb treatment of mice transplanted with islets and Sertoli cells significantly decreased β-cells and increased infiltrating leukocytes in islet grafts compared with values in islet grafts of mice transplanted with islets and Sertoli cells and treated with a control mAb (Fig. 7). The percent of total leukocytes (CD45+ cells) present in each leukocyte subset (CD4+ and CD8+ T-cells, B-cells, and macrophages) was similar in islet grafts of mice treated with anti–TGF-β1 and control mAbs. IFN-γ–expressing cells, however, were significantly increased, and IL-4–expressing cells were significantly decreased as percentages of total leukocytes in islet grafts of mice treated with anti–TGF-β1 mAb compared with values in mice treated with a control mAb (Fig. 7).
These cells against autoimmune destruction of islet TGF-β-immunoregulatory products of Sertoli cells, FasL and cells mediated this protection. We investigated two result suggested that circulating factors produced by Sertoli cells implanted separately from syngeneic islet grafts in diabetic NOD mice; however, the mechanism of pro-
together) with syngeneic islets prolonged survival of islet 64% of mice for 60 days posttransplantation. In a previous study (9), we found that Sertoli cells cotransplanted (mixed geneic islet transplantation significantly prolongs survival of Sertoli cells was associated with protection of islet grafts from autoimmune destruction. Thus, immunohistochemical analysis of Sertoli cell grafts revealed that TGF-β1 was highly expressed by Sertoli cells initially implanted in diabetic NOD mice (96 ± 2% of Sertoli cells were TGF-β1+); however, this decreased progressively after transplantation. At 30 days posttransplantation, whereas the total number of Sertoli cells in the grafts remained unchanged, only 26 ± 3% of Sertoli cells expressed FasL. Nevertheless, despite this loss of FasL expression by the Sertoli cells, the mice were normoglycemic. At present, we do not have an explanation for the loss of FasL expression by the transplanted Sertoli cells; however, we have found that this reduction also occurs after culture of Sertoli cells in vitro for 2–3 weeks, despite continued expression of other major Sertoli cell products, such as ABP and FSHr (data not shown).

Because FasL expression by Sertoli cells in the grafts decreased over several weeks and was low but not absent at 30 days posttransplantation (26 ± 3% of Sertoli cells were FasL+), FasL expression by Sertoli cells might still be a mechanism of islet β-cell protection from autoimmune destruction. To examine this possibility, we treated NOD mice transplanted with islets and Sertoli cells with a neutralizing monoclonal antibody specific to mouse FasL. The failure of anti-FasL antibody to abrogate the protective effect of Sertoli cells on islet graft survival clearly excluded FasL as a mediator of Sertoli cell protection of islet grafts. Rather, it appeared that FasL may have contributed to islet graft destruction because anti-FasL antibody slightly improved Sertoli cell–induced protection of islet grafts. These findings are consistent with reports that FasL expression by islet β-cells achieved transgenically (14,15) or by gene transfection (15,16) failed to protect mouse islet allografts (or even isografts) from rejection; rather, FasL was proinflammatory and induced an intense neutrophilic infiltration into the islets and graft destruction (14–16). In contrast, other studies have reported that FasL expression by testicular cells delays rejection of islet allografts (12,13). These studies differ from the present study, however, in that islet allografts were transplanted into mice with chemically induced (streptozotocin) diabetes (12,13), whereas we studied the recurrent autoimmune response to syngeneic islets. It remains to be determined whether FasL might play a protective or destructive role in the response to islet allografts transplanted into NOD mice with autoimmune diabetes.

In contrast to FasL, we found that TGF-β1 expression by Sertoli cells was associated with protection of islet grafts from autoimmune destruction. Thus, immunohistochemical analysis of Sertoli cell grafts revealed that TGF-β1 was highly expressed by Sertoli cells initially implanted in diabetic NOD mice (96 ± 2% of Sertoli cells were TGF-β1+), and this expression remained high in normoglycemic mice at 30 days posttransplantation (95 ± 4%). Also, the total number of TGF-β1+ Sertoli cells in grafts of normoglycemic mice remained unchanged at 30 days posttransplantation, whereas TGF-β1+ Sertoli cells were significantly decreased in grafts of diabetic mice. Similarly, plasma levels of TGF-β1 were significantly increased in normoglycemic mice but not in diabetic mice that received Sertoli cell grafts in addition to islet grafts. Finally, anti–TGF-β1 monoclonal antibody administration rapidly and completely abrogated the protective effect of Sertoli cells on islet graft survival and

DISCUSSION
This study demonstrates that implantation of syngeneic testicular Sertoli cells in diabetic NOD mice at the time of syngeneic islet transplantation significantly prolongs survival of β-cells in the islet grafts and maintains normoglycemia in 64% of mice for 60 days posttransplantation. In a previous study (9), we found that Sertoli cells cotransplanted (mixed together) with syngeneic islets prolonged survival of islet grafts in diabetic NOD mice; however, the mechanism of protection was not identified. In the present study, we found that Sertoli cells implanted separately from syngeneic islets (Sertoli cells in the right kidney and islets in the left kidney) protected islet β-cells from autoimmune destruction. This result suggested that circulating factors produced by Sertoli cells mediated this protection. We investigated two immunoregulatory products of Sertoli cells, FasL and TGF-β, as possible mediators of the protective effects of these cells against autoimmune destruction of islet β-cells.

Our findings indicate that FasL does not qualify as a mediator of the protective effects of Sertoli cells against autoimmune β-cell destruction. Thus, immunohistochemical analysis of Sertoli cell grafts revealed that FasL was highly expressed by Sertoli cells initially implanted in diabetic NOD mice (97 ± 2% of Sertoli cells were FasL+); however, this decreased progressively after transplantation. At 30 days posttransplantation, whereas the total number of Sertoli cells in the grafts remained unchanged, only 26 ± 3% of Sertoli cells expressed FasL. Nevertheless, despite this loss of FasL expression by the Sertoli cells, the mice were normoglycemic. At present, we do not have an explanation for the loss of FasL expression by the transplanted Sertoli cells; however, we have found that this reduction also occurs after culture of Sertoli cells in vitro for 2–3 weeks, despite continued expression of other major Sertoli cell products, such as ABP and FSHr (data not shown).

Because FasL expression by Sertoli cells in the grafts decreased over several weeks and was low but not absent at 30 days posttransplantation (26 ± 3% of Sertoli cells were FasL+), FasL expression by Sertoli cells might still be a mechanism of islet β-cell protection from autoimmune destruction. To examine this possibility, we treated NOD mice transplanted with islets and Sertoli cells with a neutralizing monoclonal antibody specific to mouse FasL. The failure of anti-FasL antibody to abrogate the protective effect of Sertoli cells on islet graft survival clearly excluded FasL as a mediator of Sertoli cell protection of islet grafts. Rather, it appeared that FasL may have contributed to islet graft destruction because anti-FasL antibody slightly improved Sertoli cell–induced protection of islet grafts. These findings are consistent with reports that FasL expression by islet β-cells achieved transgenically (14,15) or by gene transfection (15,16) failed to protect mouse islet allografts (or even isografts) from rejection; rather, FasL was proinflammatory and induced an intense neutrophilic infiltration into the islets and graft destruction (14–16). In contrast, other studies have reported that FasL expression by testicular cells delays rejection of islet allografts (12,13). These studies differ from the present study, however, in that islet allografts were transplanted into mice with chemically induced (streptozotocin) diabetes (12,13), whereas we studied the recurrent autoimmune response to syngeneic islets. It remains to be determined whether FasL might play a protective or destructive role in the response to islet allografts transplanted into NOD mice with autoimmune diabetes.

In contrast to FasL, we found that TGF-β1 expression by Sertoli cells was associated with protection of islet grafts from autoimmune destruction. Thus, immunohistochemical analysis of Sertoli cell grafts revealed that TGF-β1 was highly expressed by Sertoli cells initially implanted in diabetic NOD mice (96 ± 2% of Sertoli cells were TGF-β1+), and this expression remained high in normoglycemic mice at 30 days posttransplantation (95 ± 4%). Also, the total number of TGF-β1+ Sertoli cells in grafts of normoglycemic mice remained unchanged at 30 days posttransplantation, whereas TGF-β1+ Sertoli cells were significantly decreased in grafts of diabetic mice. Similarly, plasma levels of TGF-β1 were significantly increased in normoglycemic mice but not in diabetic mice that received Sertoli cell grafts in addition to islet grafts. Finally, anti–TGF-β1 monoclonal antibody administration rapidly and completely abrogated the protective effect of Sertoli cells on islet graft survival and.
diabetes recurred. These findings clearly implicate TGF-β1 as a mediator of the protective effect of Sertoli cells against autoimmune destruction of islet β-cells.

Although Sertoli cells prevented destruction of β-cells in islet grafts and diabetes recurrence (in 64% of mice at 60 days posttransplantation), leukocytic infiltration of islet grafts was significantly reduced but not prevented in these mice. Administration of anti–TGF-β1 antibody to mice that received Sertoli cell and islet grafts revealed significant differences between the types of islet graft–infiltrating cells in normoglycemic and diabetic mice. Thus, islet β-cell destruction and diabetes recurrence in mice treated with anti–TGF-β1 antibody was associated with increases in IFN-γ–producing cells and decreases in IL-4–producing cells in the islet grafts. Differences in frequencies of cytokine-producing cells occurred without any changes in distribution of leukocyte subsets (CD4+ T-cells, CD8+ T-cells, B-cells, and macrophages). These findings are consistent with many other reports that the β-cell–destructive insulitis lesion in autoimmune diabetes is characterized by a dominance of Th1 helper (Th)-1-type infiltrating leukocytes (IFN-γ-producing) over Th2-type cells (IL-4-producing) (33).

Our finding that anti–TGF-β1 administration increased IFN-γ–producing cells and decreased IL-4–producing cells in islet grafts is consistent with recent studies (34,35) that demonstrate that TGF-β (β1 or β2) can modulate macrophage activity in a way that favors Th2 over Th1 cell differentiation. For example, TGF-β1, expressed transgenically in pancreatic islet β-cells, protected NOD mice from diabetes, and the protective action of TGF-β1 was related to diverting islet antigen responses toward a Th2 phenotype (20). Similarly, TGF-β1 somatic gene therapy decreased insulitis and diabetes in cyclophosphamide-accelerated and natural course autoimmune diabetes in NOD mice, and pancreatic IL-12 and IFN-γ mRNA expression was decreased, as was the ratio of IFN-γ to IL-4 mRNA (22). In other studies, transgenic expression of TGF-β1 in pancreatic islet α-cells protected NOD mice from diabetes, and the protective action of TGF-β1 was related to blocking the cytotoxic effects of diabetogenic effector lymphocytes (21). Therefore, Sertoli cell–derived TGF-β1 could have protected β-cells in islet grafts from autoimmune destruction by diverting islet-infiltrating cells from a Th1 to a Th2 phenotype (20,22; the present study) and, in addition, possibly by blocking diabetogenic effector lymphocytes (21). Our findings are consistent with other studies in which in vivo administration of TGF-β1 neutralizing antibody has highlighted the key role of TGF-β1 in autoimmune regulation, suppressing autoimmune T-cells in both diseased and nondiseased states (36–38).

The plasma level of TGF-β1 achieved by implanting Sertoli cells and islets into diabetic NOD mice was similar (3.72 ng/ml at 30 days posttransplantation) to levels achieved by treating NOD mice with a plasmid DNA expression vector encoding mouse latent TGF-β1 (4–5 ng/ml at 3–14 days after intramuscular injection of the TGF-β1 DNA construct) (22), and both approaches protected NOD mice from autoimmune β-cell destruction and diabetes. Only the latent form of TGF-β1 was detected in plasma, both in the present study using Sertoli cells and in the study using latent TGF-β1 somatic gene therapy (22). Cells secrete TGF-β1 as a latent complex formed by noncovalent interaction of the mature biologically active TGF-β1 homodimer with a dimer of the gly-
cosylated and phosphorylated NH2-terminal precursor segments. Activation of this latent complex into the biologically active form of TGF-β1 is observed after treatment with acid or proteases such as plasmain. Therefore, systemic administration of the latent form of TGF-β1 is probably an advantageous feature, since it may become biologically active only at an inflammatory site, such as the insulitis lesion, through the action of macrophages, low pH, or other factors (39).

In conclusion, we have demonstrated that testicular Sertoli cells protect syngeneic islet grafts from recurrent autoimmune destruction after transplantation into diabetic NOD mice. The protective effect was dependent on TGF-β1 and not FasL expression by the Sertoli cells. TGF-β1 prevented islet β-cell destruction by diverting the islet-infiltrating cells from a Th1 phenotype (IFN-γ–producing) to a Th2 phenotype (IL-4–producing). Although Sertoli cells can protect rat islet allografts from rejection after transplantation into rats with chemically induced diabetes (8), it remains to be determined whether Sertoli cells can protect islet allografts from destruction after transplantation into NOD mice with autoimmune diabetes.

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