

Long-Term Survival of Segmental Pancreas Isografts in NOD/Lt Mice Treated With Anti-CD4 and Anti-CD8 Monoclonal Antibodies

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Spontaneously diabetic nonobese diabetic (NOD/Lt) mice were treated with anti-T-cell monoclonal antibodies (mAbs) at the time of grafting with vascularized segmental pancreas isografts. Recipients were either untreated or given anti-CD4 and/or anti-CD8 mAbs (0.5 mg/20-g mouse on each of 4 consecutive days), which reduced target cell levels to <5% of normal. Graft function was monitored by measuring blood glucose (BG) levels. Transplants were removed for histological examination when BG returned to >20 mmol/l for two consecutive readings. Isografts from 3- to 4-week-old prediabetic mice placed in untreated diabetic NOD mice ceased functioning in 9–13 days with a mean survival time (MST) \pm SD of 10 ± 2 . Treatment with anti-CD4 prolonged survival significantly (MST = 61 ± 35 days, $P < 0.05$ compared with untreated control mice). Anti-CD8 treatment was less effective, but it still significantly improved graft survival (MST = 24 ± 9 days, $P < 0.05$ compared with untreated control mice). Anti-CD8 plus anti-CD4 treatment was highly effective in inhibiting autoimmune destruction of the grafts (MST = 97 ± 8 days). This clearly demonstrates that transient inactivation of most T-cells with anti-CD4 plus anti-CD8 mAbs effectively controls autoimmune disease in the isograft, despite recovery of CD4 and CD8 T-cells to normal levels. Although insulinitis developed in the long-term grafts, insulinitis scores did not increase between 33 and 100 days, and none of the mice progressed to IDDM in 100 days. Histology showed a predominantly perislet T-cell and macrophage infiltrate with ductal expression of the cytokines interleukin (IL)-4, IL-2, and interferon- γ . There was little infiltrate or expression of cytokines within the islets. Thus, mAb treatment at the time of grafting allowed isograft survival and prevented progression from insulinitis to β -cell destruction. *Diabetes* 47:1399–1405, 1998

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BG, blood glucose; CsA, cyclosporine A; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate; GAF, Gomori's aldehyde fuchsin; IFN, interferon; Ig, immunoglobulin; IL, interleukin; mAb, monoclonal antibody; MST, mean survival time.

Vascularized pancreas transplantation is at present the only clinically applicable cure for IDDM. The 1-year survival rate for simultaneous pancreas and kidney transplants is 93%, equivalent to survival rates for heart, liver, and single kidney transplants. However, results for single pancreas transplants have not improved in recent years, and 50% of pancreas-only transplants fail within 6 months, with the 4-year graft survival rate for single pancreas transplants being only 41% (1). These results are comparable with 1994 Pancreas Registry data showing a 49% 1-year survival rate of single pancreas transplants (2). Current evidence suggests that many of these grafts fail for immunological reasons (1), with recurrent disease and rejection being hard to distinguish in pancreas allografts. Thus, there is considerable room for improvement in the survival rate of pancreas-only transplants, which ideally should be done before kidney failure or the development of host vascular disease. Our use of the NOD mouse isograft model for pancreas-only transplantation, performed in recipients with functioning kidneys (i.e., without the immunomodulatory effects of uremia) in which rejection does not occur, is therefore ideal for studies of new immunosuppressive treatments.

IDDM is T-cell-mediated, and the transplants are destroyed by T-cells causing both rejection and recurrent autoimmune disease (1,3). The autoimmune attack is directed toward antigens expressed on islet β -cells in individuals genetically susceptible to diabetes, is organ-specific, and may be major histocompatibility complex-restricted (4). Both CD4 and CD8 T-cells play a role, and antibodies directed against these cells can delay or prevent the occurrence of disease in NOD mice treated before the appearance of increased blood glucose (BG) levels, but they have failed to cure IDDM when the disease was well established (5–8). Anti-T-cell monoclonal antibodies (mAbs) also delay the autoimmune attack on islets placed under the kidney capsule (9). Because the immune response to primarily vascularized grafts can be quite different from the response to neovascularized grafts (10) and because the survival of vascularized pancreas isografts in mice treated with anti-T-cell antibodies has not been previously reported, this study was undertaken to determine the effect of anti-T-cell mAbs on graft survival in NOD mice in which T-cell-mediated autoimmune disease was present and isograft failure at >10 days was invariably due to recurrent autoimmune disease.

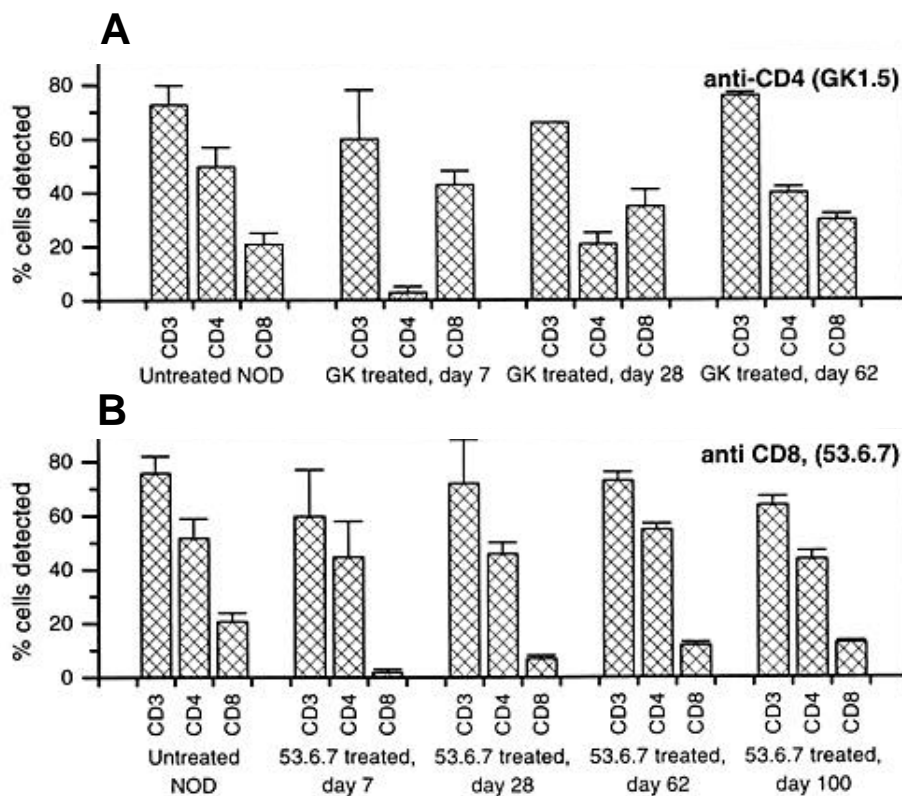


FIG. 1. FACS analysis of lymph node cells from untreated NOD mice or animals treated with either anti-CD4 (GK1.5; **A**) or anti-CD8 (53.6.7; **B**) at 0.5 mg/20-g mouse on 4 consecutive days (total dose 2 mg). Cells were harvested 7, 28, 60, or 100 days after treatment commenced, and freshly isolated cells were incubated with FITC-coupled anti-CD3, anti-CD4, or anti-CD8 mAb. Mean percent positive cells \pm SD in the gated small lymphocyte populations are shown. There were three to five animals per group.

RESEARCH DESIGN AND METHODS

Mice. NOD/Lt ($K^{dIA^{g7D}b}$) female mice were bred and maintained in the Department of Surgery at the University of Melbourne in Australia and were kept in accordance with the animal ethics guidelines of the National Health and Medical Research Council of Australia.

Pancreas transplantation. NOD/Lt mice >100 days old, weighing 20–30 g, and with a >20 mmol/l BG level received heterotopic segmental pancreas isografts from female NOD/Lt mice, aged <28 days old (pre-insulinitis, weighing 10–15 g). Pancreas transplantation was carried out as previously described (11). The donor segment of pancreas perfused by the splenic vessels was harvested along with the splenic vein, a cuff of portal vein, and a cuff of aorta, including the outlet for the splenic artery. The spleen and pancreatic lymph nodes were removed, and the pancreatic duct was cauterized. The donor pancreas was attached to the recipient's aorta and vena cava below the left kidney with the donor aorta attached to the recipient aorta and the donor portal vein to the recipient inferior vena cava by end-to-side anastomoses. Graft function was measured by checking urine glucose and BG levels at regular intervals. Mice were killed and samples collected for histology when BG levels exceeded 20 mmol/l or at >100 days.

mAbs. Rat anti-mouse CD4, GK1.5 (immunoglobulin [Ig]G2b) (12) and CD8, 53.6.7 (IgG2a) (13) mAbs were isolated from ascites collected from pristane-treated nude mice and purified on a protein-G sepharose column. These mAbs have been previously shown to prolong pancreas allograft survival in NOD mice (14). Recipients were treated intraperitoneally with the doses listed in RESULTS on the day before grafting and days 0, 1, and 2 after transplantation. T-Cell subpopulations in single-cell suspensions of lymph nodes from treated animals were detected with fluorescein isothiocyanate (FITC)-conjugated antibodies purchased from Boehringer Mannheim (Mannheim, Germany). Fluorescent cells in the small lymphocyte population were detected using a Becton Dickinson (Sunnyvale, CA) FACScan. The antibodies used for fluorescence-activated cell sorting (FACS) were 53.6.7 (CD8), H129.19 (CD4), and 145-2c11 (CD3). Binding of H129.19-FITC would have been blocked by any cell-surface-bound GK1.5, since they bind to the same epitope (15), but T-cell depletion was detected by a decrease in the percentage of CD3 cells. The mean percent \pm SD for each lymphocyte subset was calculated (>2 mice per group).

Histology and immunohistology. Pancreas samples were removed and placed in Bouin's fixative for 2 h before transfer to 70% ethanol and then embedded in paraffin. This tissue was stained using Gomori's aldehyde fuchsin (GAF) to identify insulin-containing β -cells. Glucagon was detected as previously described by a three-layer immunoperoxidase technique using a rabbit anti-human polyclonal antibody that cross-reacts with mouse glucagon (Dako, Carpinteria, CA) (16). For other immunohistology, tissue was embedded in optimal cutting temperature (Tissue-Tek;

Miles, Elkhart, IN) medium and frozen in liquid nitrogen, and cryostat sections (4–6 μ m) fixed in 4% paraformaldehyde were stained using four-layer immunoperoxidase techniques as previously described (17,18). Anti-mouse mAbs used to detect cell infiltrates and cytokines were either affinity-purified from ascites from nude rats in the Department of Surgery at Royal Melbourne Hospital (anti-CD3, KT3, anti-CD4, GK1.5, and anti-CD8, 53.6.7) or used as supernatants from cell cultures obtained from the American Type Culture Collection and grown at the Walter and Eliza Hall Institute for Medical Research or Monash University in Australia (11B11, anti-interleukin [IL]-4; S4B6, anti-IL-2; XMG1-2, anti-interferon [IFN]- γ ; B220, anti-B-cell; and F4/80, anti-macrophage). Control mice, in which there was use of an irrelevant antibody and omission of primary antibody, showed some background staining, but this was clearly less than that seen with specific antibodies.

Given the difficulty of staining cytokines in tissue sections, with nonspecific and false-positive staining frequently seen, we tested our cytokine-specific antibodies on tissues from knockout mice (IL-4, IL-10, IFN- γ) and on cytokine-expressing cell lines (IL-4 and IL-2), as described previously (17). In other studies, we have used semiquantitative polymerase chain reaction to verify the presence of cytokines detected with these antibodies in heart allografts (19). Results from two anti-IL-10 mAbs and one anti-IFN- γ mAb were not acceptable because they bound to many lymphoid cells in the IL-10 and IFN- γ knockout mice.

Graft survival and degree of infiltration. In grafts stained with GAF, islets were assessed by a semiquantitative score of 0–4, with 0 representing an islet with no stained β -cells. Scores of 2 or 3 were for islets with increasing peri-islet infiltration, and a score of 4 represented a well-preserved islet with the majority of cells stained purple. Infiltrates were also scored as 0 (no infiltrate), 1 (focal peri-islet or peri-ductal infiltrate), 2 (peri-islet infiltrate with 25–75% of the islet affected), 3 (100% peri-islet infiltrate and some intra-islet invasion and disruption of β -cells), or 4 (extensive infiltrate throughout the islet). All individual islet scores for each treatment or harvest group were summed and presented as means \pm SD. Scores were compared using Student's *t* test (20).

RESULTS

T-Cell depletion in NOD mice treated with CD4 or CD8 mAbs. GK1.5 treatment was effective in reducing NOD lymph node CD4 cells to <4% (Fig. 1A). Depletion of CD4 T-cells in the NOD mice was not as effective as in CBA mice, since twice the dose of the same batch of antibody was required to deplete CD4 T-cells to this level (21). Nevertheless, the level of CD4 T-cell depletion seen here was sufficient to

TABLE 1
NOD to NOD pancreas isograft survival times

Treatment	Dose	Survival (days)	Mean \pm SD	<i>P</i> values
None	—	9, 9, 10, 13	10 \pm 1.9	—
Anti-CD4	2 mg	21, 26, 44, 92, 100	61 \pm 35	0.016*
Anti-CD8	2 mg	13, 14, 20, 23, 24, 34, 38	24 \pm 9.4	0.01*
Anti-CD4 plus CD8	2 mg each	62, 76, 100, 100, 100, 100, 100, 100, 100, 100, 100	97 \pm 10	0.001*

Recipients were treated with mAb, 0.5 mg i.p./20-g mouse on days -1, 0, 1, and 2 with transplantation on day 0 (total dose 2 mg). *Significantly different from untreated control mice.

allow long-term allograft survival in this and many other strains of mice (14,15,22). The rate of recovery of CD4 cells was also similar to that seen in other strains (21), with cells at 50% of normal by 28 days and back within the normal range by 60 days (day 62 levels not significantly different from untreated NOD levels, $P > 0.05$).

The anti-CD8 mAb 53.6.7 (Fig. 1B) was also not as effective in depleting CD8 T-cells in NOD mice as it was in CBA mice. A 2-mg total dose was required in NOD mice to achieve equivalent depletion to that seen with a 0.5-mg total dose in CBA mice. The 2-mg dose reduced detectable CD8⁺ cells from 21 \pm 3 to 2 \pm 1% at day 7. Recovery was slower than for GK1.5-treated CD4 cells, since the lymph node percentage of CD8 cells did not return to within the normal range until 100 days after treatment.

Isograft survival times. Graft function was assessed by measuring BG levels on the day of transplant and at weekly intervals thereafter. Transplantation caused a return to normal BG levels within hours. A return to diabetic BG levels was recorded as the time of rejection and β -cell destruction was confirmed by graft histology.

Treatment with a short course of anti-CD4 (2-mg total dose) significantly improved graft survival time from a mean survival time (MST) of 10 \pm 2 days in untreated control mice

to 61 days ($P < 0.05$ compared with untreated control mice) (Table 1). There was a wide range of survival times from 21 to >100 days, with four of five grafts failing at <100 days. Anti-CD8 treatment was less effective, and although survival times (MST = 24 \pm 9 days) were significantly better than in untreated control mice ($P < 0.05$), all grafts failed by 38 days (range 13–38 days). In contrast, in the group that received a combination of anti-CD4 plus anti-CD8 (GK1.5 and 53.6.7; 2 mg total of each mAb), 8 of 10 grafts survived >100 days. This was significantly better ($P < 0.01$) than the group treated with anti-CD8 (53.6.7) alone, although not different from the anti-CD4 (GK1.5)-treated group ($P > 0.05$), which is due to the wide range of survival times in the latter group.

Figure 2 shows BG levels before and after transplantation for three representative mice from each group: untreated control mice (A), mice treated with GK1.5 (B), mice treated with 53.6.7 (C), and mice treated with a combination of GK1.5 and 53.6.7 (D). The BG measurements showed rapid graft failure in untreated recipients from 7 days postgraft. The BG readings in the anti-CD4-treated group showed various times for graft survival, ranging from 21 to >100 days. In the anti-CD8-treated group, no grafts survived >38 days. Graft survival in animals treated with both anti-CD4 and anti-CD8 was

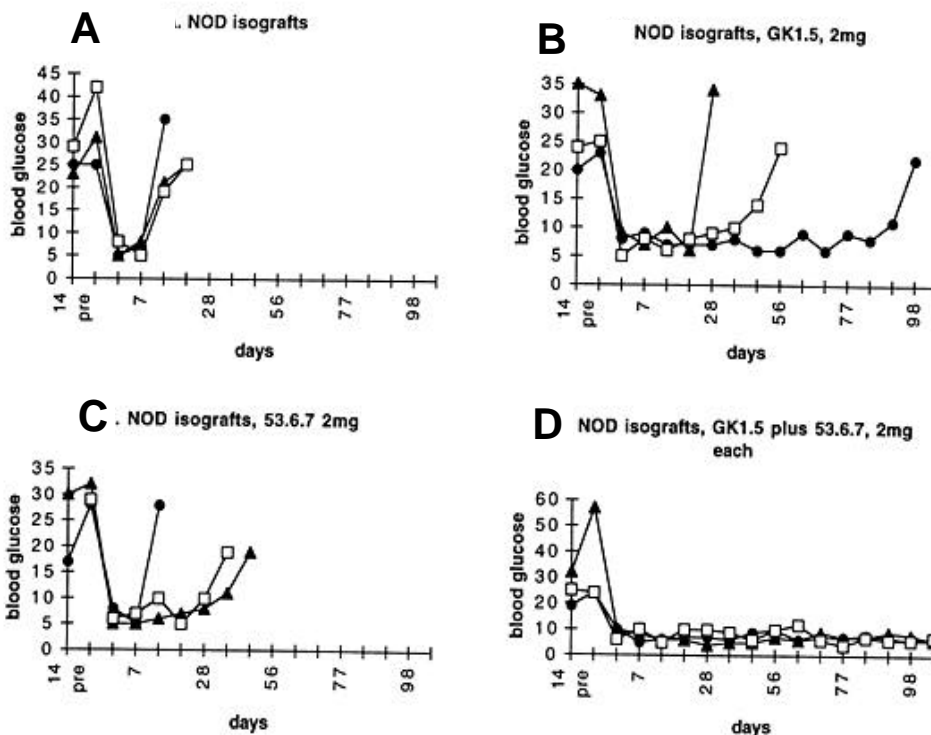


FIG. 2. Weekly BG (mmol/l) readings from three representative isografted mice (\blacktriangle , \square , \bullet) from each treatment group are shown for 14 days before and up to 100 days after transplantation. Mice were classified as diabetic at BG >20 mmol/l and euglycemic at BG <20 mmol/l. Treatment groups were as follows: A, untreated; B, given anti-CD4 (GK1.5); C, given anti-CD8 (53.6.7); or D, given both anti-CD4 and anti-CD8 at total doses of 2 mg i.p./20-g mouse, i.e., a 0.5-mg dose of each antibody on days -1, 0, 1, and 2 with transplantation on day 0.

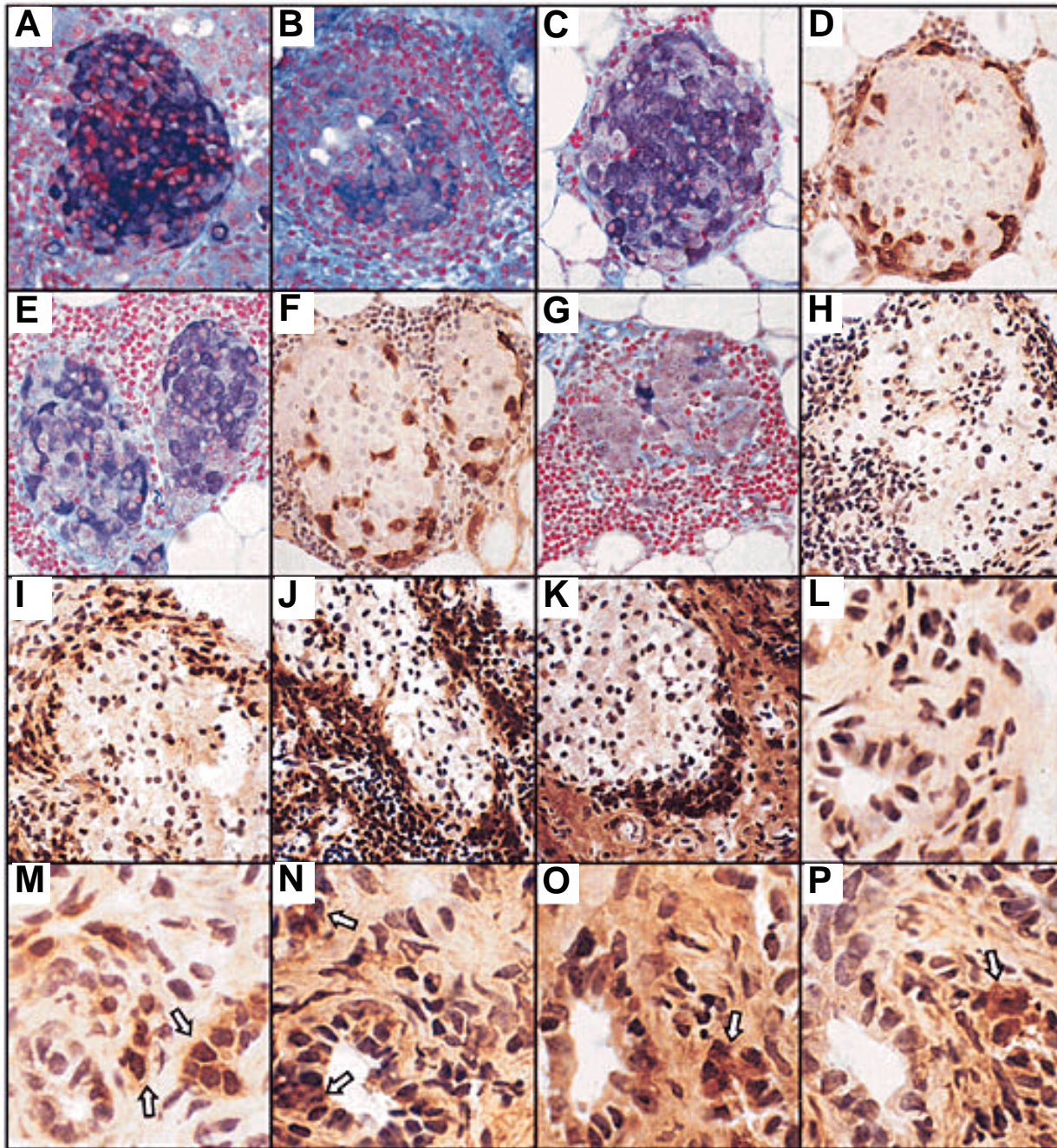


FIG. 3. *A, B, C, E, and G*: Representative GAF-stained sections (4–6 μ) of pancreas isografts to reveal insulin-producing β -cells (purple staining). *D* and *F*: Serial sections of *C* and *E*, respectively, stained to show glucagon-producing α -cells. Untreated isografts were harvested at 4 days (*A*) and 7 days (*B*). Long-surviving allografts from recipients treated with anti-CD4 plus anti-CD8 were harvested at >100 days (*C–P*). Immunoperoxidase-stained frozen tissue from a single islet-rich area of a representative long-surviving graft are shown (*H–P*). These show serial or closely associated sections of islets (*H–K*) or neighboring ducts (*L–P*). These sections were stained with no primary antibody (*H*), anti-CD3 (*J*), anti-macrophage (*K*), rat immunoglobulin (*L*), anti-CD3 (*M*), anti-IL-4 (*N*), anti-IFN- γ (*O*), or anti-IL-2 (*P*). The cell-surface markers showed peri-islet infiltration of CD3 T-cells and macrophages (*I–K*) with a few cells inside the islets. B-cells were seen in clusters near some islets only (*K*). The level of background staining of duct tissue seen here was typical of this antibody. Cytokine staining was not seen in the peri-islet regions, with sections looking like *H*. Clusters of cytokine-positive cells, some associated with CD3 cells (arrows in *N–P*) were, however, seen in the duct tissue. Background staining was higher in the sections stained for secreted cytokines (*N–P*) compared with the cell-surface marker stain (CD3, *M*).

remarkable in the maintenance of normal BG levels from the time of grafting to >100 days in 8 out of 10 animals. This indicated that transient reduction of both CD4 and CD8 T-cells could successfully inhibit the autoimmune response and usually provide indefinite survival of the isografts.

Histology of NOD native pancreas and isografts. In sections stained with GAF to show both β -cells and infiltrating leukocytes, rapid destruction of β -cells in untreated isografts was seen, with insulinitis visible on day 4 postgraft (Fig. 3*A*). The β -cells within the graft were severely depleted on day 7

TABLE 2
Isograft function and insulinitis scores in mice with normal BG levels or recurrence of disease

Days after graft	Islets scored	Grafts examined	Graft assessment	Mean \pm SD
33–40*	77	3	Islet score	2.25 \pm 0.49
			Insulinitis score	1.65 \pm 0.49
>100*	52	4	Islet score	2.12 \pm 0.75
			Insulinitis score	2.03 \pm 0.68
9–13†	31	3	Islet score	0.03 \pm 0.02
			Insulinitis score	3.97 \pm 0.34

*Recipients treated with 0.5 mg i.p./20-g mouse with both anti-CD4 and anti-CD8 mAb on days –1, 0, 1, and 2 with transplantation on day 0 (total dose 2 mg of each mAb). These animals had normal blood glucose levels. †Untreated recipients with recurrent disease.

(Fig. 3B), with a marked increase in infiltrating cells around and within the islets. In contrast, long-surviving grafts (>100 days) from animals treated with anti-CD4 plus anti-CD8 mAb showed many islets with prominently stained β -cells, although the number of β -cells in each islet varied from the majority (Fig. 3C and E) to very few cells (Fig. 3G). Immunostaining for glucagon in serial sections showed that α -cells were also present in the long-surviving islets (Fig. 3C compared with 3D and 3E compared with 3F). The lack of acinar tissue in the long-term isografts is typical of transplants in which the drainage of the exocrine pancreas is occluded at the time of grafting. All islets in the long-surviving grafts were surrounded by a peri-islet infiltrate. Native pancreases from long-term survivors (>100 days) showed severe islet atrophy, no GAF-positive β -cells, and no surrounding insulinitis as reported previously (20).

Table 2 shows the condition of islets within long-surviving grafts from anti-CD4-treated plus anti-CD8-treated recipients at >33 days and >100 days postgraft. Scores were not significantly different at the two time points, and they were significantly different from the untreated isografts. In untreated isografts at 10 days, islets had no or few GAF-positive cells (islet score 0), and the total islet area was invaded by infiltrating mononuclear cells (infiltrate score 4).

Immunoperoxidase staining of frozen sections revealed many T-cells in the peri-islet infiltrate, with CD3 cells present (Fig. 3J) in all four long-term grafts (>100 days) examined. Macrophages in the peri-islet area were shown by the F4/80 antibody (Fig. 3J), and there were isolated areas of B-cells, seen with the B220 antibody (Fig. 3K). The negative control (Fig. 3H) showed no staining of islets with the primary antibody deleted. Cytokine staining showed no clearly positive cells in the peri-islet areas, with sections not different from the negative control (Fig. 3H). Expression of all three cytokines examined was seen in ductal tissue, as compared with negative controls (Fig. 3L, stained with rat IgG first antibody). There was expression of IL-4 (Fig. 3N), IFN- γ (Fig. 3O), and IL-2 (Fig. 3P) with individual cells in the ductal tissue staining for these cytokines. Cytokine-positive cells were associated with CD3-positive (Fig. 3M) and F4/80-positive cells (not shown) seen in sequential sections, suggesting that these cells were responsible for cytokine expression. The presence of positive cells showed clearly that cytokines could be detected in these grafts but were not found in the

peri-islet infiltrate. This reflected the nonaggressive state of the infiltrate.

DISCUSSION

We have shown that transient depletion of both CD4 and CD8 cells allows long-term survival of vascularized pancreas isografts in spontaneously diabetic NOD mice by inhibiting the recurrence of autoimmune disease. Normal BG readings in the long-surviving isografts show that there was β -cell function in these grafts. The long-surviving islets within the isografts had varying degrees of insulinitis, with a T-cell and cytokine profile that suggested that the progress of autoimmune disease had been arrested at the insulinitis stage.

The short course of treatment used here, combining anti-CD4 with anti-CD8 mAb, was able to maintain isograft function for >100 days in most grafts, despite a major regeneration of T-cells. However, transient depletion of T-cells did not prevent the reappearance of insulinitis. Although there was sufficient β -cell function in these grafts to maintain euglycemia, the histology revealed an immune response within the graft similar to the insulinitis seen in prediabetic NOD mice. Short-term CD4 plus CD8 mAb treatment was clearly effective in preventing the progression of disease in the graft, since islet structure and insulinitis scores were not significantly different on day 33 and day 100 after transplantation.

Vascularized pancreas transplantation gives the advantage of a return to normal BG levels within hours of receiving the graft. In adult islet grafts, vascularization starts at 3 days, with normal BG levels seen by day 4 (23). The vascularized grafts also use only one donor per recipient and consistently cure diabetes, comparable to clinical pancreas transplantation, in contrast to the multiple donors required and low success rate for clinical islet transplantation (24). Vascularized grafts are, however, as susceptible to disease recurrence as islet grafts in NOD mice, with both islets and pancreas grafts destroyed by autoimmune disease within 10 days (9,20), and they are an appropriate model for observing the effects of immunosuppressive treatment.

Both CD4 and CD8 T-cells are required for the development of IDDM in NOD mice and are seen in insulinitis in both prediabetic young mice and in older euglycemic males and females (25). The insulinitis lesions contain T-cells that can destroy islets (26), and anti-T-cell treatment of prediabetic NOD mice can prevent progression to IDDM. This treatment cleared insulinitis, and the withdrawal of therapy did not lead to IDDM, although insulinitis recurred (6,8). Insulinitis lesions may also include "regulator" T-cells involved in preventing the progression to disease, as demonstrated by rapid disease induction with cyclophosphamide treatment of prediabetic NOD mice (27). "Protective insulinitis" was also seen in older nondiabetic NOD mice (28). Thus, disease can be prevented by changing the balance of cells within the insulinitis lesion to favor those protecting the β -cells from autoimmune attack. Transient depletion of CD4 and CD8 T-cells has been effective in doing this in islet isografts in NOD mice (29). Our data demonstrate that this treatment is even more effective for prolonging the survival of vascularized pancreas transplants.

Although many treatments can prevent or delay the progress of disease in neonatal or prediabetic NOD mice, treatment of older mice and mice with established diabetes was less effective (8). Pancreas isografts placed under the kidney capsule were destroyed by autoimmune disease in 6–10

days (9). Attempts to prevent this with conventional immunosuppression were not successful. Immunosuppressive doses of cyclosporine A (CsA) failed to prevent disease recurrence in diabetic NOD mice given neovascularized newborn isografts (30). Short-term treatment with anti-CD4 ± CsA did not prolong organ-cultured fetal pancreas isografts, and insulinitis in the grafts was the same as that in the host pancreas at 56 days (31). Thus, transient treatment to remove only CD4 cells was not effective in maintaining neovascularized isografts in diabetic NOD mice (32).

Continuous treatment with anti-CD4 and anti-CD8 mAbs for >50 days postgraft effectively maintained normal BG levels in NOD mice receiving islet isografts, and mild or no insulinitis was seen (9). Long-term immunosuppression with mAbs would not be applicable to clinical transplantation, given the profound immunosuppression and the toxic side effects seen with agents such as these (33). Anti-CD4 treatment for 4 weeks postgraft also prevented destruction of islet isografts but not the reappearance of insulinitis (34). IDDM prevention was seen when prediabetic NOD mice were given treatments, such as a fatty acid-deficient diet (35), oral insulin (32), or adjuvant therapy (36), that increased IL-4 and/or IL-10 and decreased IFN- γ expression. Successful prevention of diabetes by treating neonates with IL-4 has been reported, but treatment of diabetic NOD mice with neovascularized isografts and IL-4 and IL-10 delayed, but did not prevent, β -cell destruction within most of the grafts (50% failure by 30 days) (37).

Increases in IFN- γ expression are clearly associated with the development of disease, while decreases prevent disease progression (38); however, the role of the other cytokines in IDDM is not as clear. Insulinitis lesions showed progressive increases in the mRNA levels of the inflammatory cytokines IL-1 β , IL-2, IL-4, IL-10, and IFN- γ from 5 weeks to diabetes onset, with only IFN- γ showing a significant increase in diabetes-prone mice compared with low-incidence adjuvant-treated mice (38). In islet allografts, β -cell destruction was associated with increased levels of IL-2, IL-10, and IFN- γ within the graft, while IL-4-treated and IL-10-treated grafts showed decreased levels of IFN- γ , tumor necrosis factor- α , IL-2, and IL-10. IL-4 levels in the grafts remained low in all groups (38). In our study, the T-cell infiltrate and cytokine pattern observed in the long-term isografts was very similar to that reported by Rabinovitch et al. (38) for insulinitis of young diabetes-prone NOD mice, which expressed low levels of IL-2, IL-4, IFN- γ , and IL-10 in the peri-islet insulinitis lesions, suggesting that the anti-T-cell treatments used here have restricted the autoimmune response to the insulinitis phase of the disease. In our study, the levels of cytokines seen in the peri-islet regions of long-surviving islets were very low, reflecting the noninflammatory nature of this infiltrate. In conclusion, we have shown that a short peritransplant course of anti-CD4 plus anti-CD8 mAb treatment allows isografts in NOD mice to function for >100 days by preventing progression of the disease from the insulinitis phase to overt disease.

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