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CD4⁺ T CELLS ARE SUFFICIENT TO ELICIT ALLOGRAFT REJECTION AND MHC CLASS I MOLECULE IS REQUIRED TO INDUCE RECURRENT AUTOIMMUNE DIABETES AFTER PANCREAS TRANSPLANTATION IN MICE

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Abstract

Background—We characterized the role of T cell subsets and MHC molecules in allograft rejection and recurrence of autoimmune diabetes.

Methods—Adoptive cell transfer and vascularized segmental pancreas transplantation were performed in mice.

Results—In an alloimmune response model, transfer of non-diabetic CD4⁺, but not CD8⁺ T cells, elicited pancreas allograft rejection in streptozotocin (STZ)-induced diabetic NOD/scid mice. Pancreas allografts were acutely rejected in STZ-induced diabetic NOD/β2m^{−/−} mice (confirmed the absence of MHC class I and CD8⁺ T cells) and permanently accepted in NOD/CIIT^{−/−} mice (confirmed the absence of MHC class II and CD4⁺ T cells). The results suggest that rejection of pancreas allograft is CD4 dependent and MHC class I independent. In the autoimmune diabetes model, whole spleen cells obtained from diabetic NOD mice induced autoimmune diabetes in NOD/scid and NOD/CIIT^{−/−} mice, but the onset of diabetes was delayed in NOD/β2m^{−/−} mice. However, the purified diabetic T cells failed to elicit autoimmune diabetes in NOD/β2m^{−/−} mice. NOD/scid and NOD/CIIT^{−/−} pancreas grafts were acutely destroyed while four of six NOD/β2m^{−/−} pancreas grafts were permanently accepted in autoimmune diabetic NOD mice.

Conclusion—CD4⁺ T cells are sufficient for the induction of allograft rejection and MHC class I molecule is required to induce recurrent autoimmune diabetes after pancreas transplantation in mice.

Keywords

Pancreas transplantation; mice; MHC; T cells; allograft rejection; autoimmune response

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INTRODUCTION

Exogenous insulin prevents the acute complications of diabetes and death from ketosis; however, the underlying absence of islet beta-cells is not substantially influenced by this therapy. Pancreas or islets allograft transplantation represents a currently preferred approach for replacing destroyed islet beta-cells and curing Type 1 diabetes (T1D).

Although islet transplantation offers the advantage of being less invasive with fewer complications, current clinical and experimental studies show that multiple pancreas donors are required to achieve and maintain insulin-independence in the intermediate term, and long-term function remains a problem even with multiple transplants (1–3). Vascularized pancreas allograft transplantation remains one of the major approaches for the treatment of T1D; however, much of the immunology has been with islet but not pancreas transplantation because of a technical challenge of the pancreas transplantation mouse model. Previous studies with cell reconstitution and cell depletion experiments have shown that the rejection of the allograft and recurrence of autoimmune disease after pancreas transplantation were induced by T cell-mediated, especially CD4⁺ T cell-mediated, immune response (4–6). CD4⁺ T cells themselves can develop into cytolytic T lymphocytes that mediate the destruction of allografts (7,8). However, another report has indicated that both CD4⁺ and CD8⁺ T cell subsets are necessary for the successful transfer of autoimmune diabetes (9). Whether either CD4⁺ or CD8⁺ T cells alone contributes to the recurrence of autoimmune diabetes after pancreas transplantation remains to be investigated. This is of special importance in clinical pancreas transplantation for the treatment of autoimmune diabetes because the autoimmune response is already established and the autoimmune CD4⁺ and CD8⁺ T cells have already activated or primed at the time of transplantation.

Previous studies in an islet transplantation mouse model suggest that major histocompatibility complex (MHC) class I molecule plays a very important role in the pathogenesis of diabetes recurrence. The islet grafts from MHC class I deficient NOD mice (NOD/β2m^{-/-}) or MHC class I and class II deficient NOD mice survived indefinitely when transplanted under the kidney capsule of diabetic NOD recipients (10,11). Conversely, MHC class II expression is not a necessary component of autoimmune destruction. Thus, MHC class I-restricted cytotoxic T-lymphocytes (CTLs) play a dominant role in disease recurrence in the islet transplantation model (10–14). Whether MHC class I is required for the recurrence of autoimmune diabetes following vascularized pancreas transplantation remains unknown.

The aim of the present study is to design strategies of adoptive transfer of T cell subsets and vascularized pancreas transplantation and to determine the role of CD4⁺ and CD8⁺ T cells and MHC class I and class II molecules in allograft rejection as well as in the recurrence of autoimmune diabetes. The current study demonstrates that diabetic CD4⁺ T cells can induce allograft rejection and MHC class I molecules are required to initiate tissue-specific destruction of syngeneic pancreas grafts in a pancreas transplantation mouse model.

MATERIALS AND METHODS

Mice and vascularized segmental pancreas transplantation

C57BL/6 (H-2b), NOD/LtJ (I-Ag7), NOD/β2m^{-/-} (beta 2-microglobulin deficient), NOD/CIIT^{-/-} (class II transactivator deficient), NOD/scid (severe combined immune deficiency spontaneous mutation), and NOR mice (15) were purchased from Jackson Laboratory (Bar Harbor, ME) and the National Cancer Institute (NIH, Frederick, MD). All mice were housed in pathogen-free conditions in the Animal Resources Center at the University of Chicago and the Division of Animal Care at Vanderbilt University Medical Center, according to the National Institutes of Health guidelines. A mouse with blood glucose values over 300 mg/dl for more

than two consecutive days was defined as diabetic. The rejection or recurrence was defined as the day when blood glucose value was >200mg/dl, measured by a blood glucose meter (SureStep, LifeScan, Milpitas, CA), following a period of normal blood glucose level.

Heterotopic vascularized segmental pancreas transplantation was performed using microsurgical techniques as described previously (16,17). In the donor operation, the pancreas was isolated by sequential ligatures, with care taken to ligate the common bile duct and pancreatic duct. A long segment of the donor aorta with celiac artery and superior mesenteric artery was prepared. The portal vein was isolated and transected. Before harvest of the pancreas graft, 100 units of heparin was injected through the inferior vena cava (IVC) and the pancreas was perfused with 0.4ml of saline (4°C) through the aorta below the left renal artery. In the recipient operation, a median laparotomy was performed. The donor aorta was sutured to the recipient aorta from end to side using 10-0 sutures (Davis & Geck) and the portal vein to the IVC in the same manner. About 60% to 70% of the segmental pancreas was transplanted into the recipients.

Immunosuppressive reagents

Anti-CD4 (GK1.5) and anti-CD8 (2.4.3) mAbs were purified from protein-free culture supernatants, precipitated by 45% ammonium sulfate, and dialyzed in PBS (18). All of the hybridoma clones were from American Type Culture Collection (ATCC, Manassas, VA). The protein concentrations were determined by spectrophotometry (OD280) and compared with a standard curve of BSA. The purity of the mAb preparation was determined by SDS-PAGE analysis to be >90% pure. 10mg/kg of anti-CD4 mAb and anti-CD8 mAb were administered (i.v.) at days -1, 0, +1, and +2 relative to transplantation.

Lymphocyte subsets transfer

Spleens were removed from 8 to 12-week-old mice. A single-cell suspension was obtained by passing the splenocytes through a 70-µm cell strainer (Becton Dickinson Labware, Franklin Lakes, NJ) and the viable washed cells were counted using Trypan-blue exclusion (9,19,20). Red blood cells were lysed with ACK lysis buffer (Sigma). Afterwards, T cells, CD4⁺ and CD8⁺ T subsets were isolated using T cell isolation kit (R&D Systems, Minneapolis, MN) and CD4⁺ or CD8⁺ T cell isolation Kit (Miltenyi Biotec Inc., Auburn, CA) according to manufacturer's protocol.

Histological Examination

All rejected and long-term surviving mice, engrafted with pancreas grafts, were sacrificed for histopathological examinations. The grafts were frozen rapidly in liquid nitrogen. Four to six µm sections were stained with H&E for the assessment of infiltration. For detecting expression of CD4⁺, CD8⁺, and IgM/IgG in the pancreas grafts, immunohistochemistry was performed with a standard avidin-biotin peroxidase complex method (21,22). Cryosections were stained with the following primary mAbs: CD4 (GK1.5) and CD8 (Ly-2, PharMingen, San Diego, CA). Biotinylated goat-anti-rat IgG (Jackson Immunochem. Labs, West Grove, PA) and horseradish peroxidase-streptavidin (Zymed, South San Francisco, CA) were then stained at room temperature. Immunostaining was developed by DAB and counterstained with Mayer's hematoxylin.

Statistics

The diabetes-free or pancreas graft survival days were presented as mean survival time ± standard deviation (MST ± SD) and evaluated for statistical significance by ANOVA analysis (Abacus Concepts, Berkeley, CA). *P*-value of < 0.05 was considered significant.

RESULTS

CD4⁺ T cells are sufficient to mediate pancreas allograft rejection

To identify the role of T cell subsets in allograft rejection, C57BL/6 pancreas allografts were transplanted into NOD/scid mice, and T cell subsets from NOD males that have a very low incidence of developing spontaneous diabetes (23) were transferred into recipients on the day of transplantation. The purity of T cell subsets, determined by flow cytometry, was >95% (data not shown). Adoptive transfer of whole spleen cells (20×10^6) from NOD males elicited rejection of C57BL/6 pancreas allografts in 24.3 days in STZ-induced diabetic NOD/scid mice. CD4⁺ T cells (10×10^6) resulted in pancreas allograft rejection in 26.8 days. In CD8⁺ (10×10^6) cell-transferred mice, 50% of pancreas allografts were rejected in 80 days while the other 50% pancreata survived for over 100 days (MST $\geq$ 92.0 days, Fig. 1).

Next, NOD males were made diabetic with STZ and C57BL/6 mice pancreas allografts were transplanted into diabetic NOD males. The results in Figure 2A show that pancreas allograft was rejected in a mean time of 13.6 days (MST $\pm$ SD = 13.6 ± 6.43 days, $n = 5$). Histopathological examinations showed massive TCR⁺/CD4⁺ T cell infiltration in the untreated pancreas allografts on day 5 and massive CD8⁺ T cells deposition on day 9 after transplantation (Figure 2B). Survival of pancreas allografts was modestly improved to 21.7 days (MST $\pm$ SD = 21.7 ± 2.89 , $n = 4$) in mice treated with anti-CD8 mAb. Survival of pancreas allografts was significantly prolonged (MST $\pm$ SD = 62.6 ± 43.9 , $n = 8$) but not permanently accepted.

Finally, MHC class I (NOD/ $\beta 2m^{-/-}$) and class II (NOD/CIIT^{-/-}) deficient mice, which have significantly reduced numbers of CD8⁺ and CD4⁺ cells, respectively, and no autoimmune diabetes occurs in the lifetime, were used as recipients of pancreas allografts. After development of diabetes induced by STZ, C57BL/6 pancreas allografts were transplanted into STZ-induced diabetic NOD/ $\beta 2m^{-/-}$ and NOD/CIIT^{-/-} mice. Pancreas allografts were acutely rejected by STZ-induced diabetic NOD/ $\beta 2m^{-/-}$ in 11 days (MST $\pm$ SD = 9.75 ± 2.36 days, $n = 4$) but no allograft rejection occurred in C57BL/6 to NOD/CIIT^{-/-} combination (MST >100 days, $n = 4$, Fig. 2A). The results indicate that CD4⁺ T cells are sufficient to initiate allograft rejection and MHC class I is not required in pancreas allograft rejection.

MHC class I molecules are required for the autoimmune destruction of pancreas grafts

To investigate the role of autoimmune response in the destruction of pancreas allografts, NOR mice were used as donors of pancreas grafts that were transplanted into STZ-induced diabetic NOD males or autoimmune females. NOR is a recombinant congenic strain in which limited regions of the NOD genome have been replaced by genome from the C57BLKS/J strain. NOR mice share the same MHC with NOD mice but are insulinitis resistant and diabetes free (15). The results in Figure 3 show that NOR pancreas grafts survived for more than 100 days in STZ-induced diabetic NOD males and for 74.2 days (MST $\pm$ SD = 74.2 ± 23.63 days) in STZ-induced diabetic NOD females in the absence of immunosuppression. NOR pancreata were destroyed in spontaneous diabetic NOD females (MST $\pm$ SD = 11.8 ± 1.89) and anti-CD4 treatment marginally prolonged survival of NOR pancreas grafts in autoimmune diabetic NOD mice (MST $\pm$ SD = 18.0 ± 5.29 days). In addition, NOR cardiac grafts were transplanted into non-diabetic NOD females to test tissue-specific response. All NOR heart grafts ($n = 5$) were permanently accepted by NOD females without any immunosuppressant. When 3 of 5 NOD mice developed autoimmune diabetes at 60 days post-transplantation, the heart grafts continued to function normally and were not rejected (data not shown). The results suggest a tissue specific role of recurrent autoimmune response in the destruction of pancreas grafts.

The deficiency of either MHC class I or class II abrogates the development of autoimmune diabetes in NOD mice; however, it is unclear whether MHC class I and class II molecules also contribute to the recurrence of autoimmune diabetes. First, un-purified diabetic or primed spleen cells (20×10^6) from diabetic NOD mice were transferred into NOD/ $\beta 2m^{-/-}$ and NOD/CIIT $^{-/-}$ mice. The results in Figure 4A show that diabetic or primed spleen cells induced autoimmune diabetes in a mean time (MT) of 72 days in MHC class I deficient NOD/ $\beta 2m^{-/-}$ mice (MT $\pm$ SD = 72.25 ± 10.53 d), 44 days in class II deficient (NOD/CIIT $^{-/-}$) mice (MT $\pm$ SD = 44.0 ± 8.49 d), and 28.8 days in NOD/scid mice (MT $\pm$ SD = 28.8 ± 6.06 d, $n = 5$). It is possible that unpurified diabetic spleen cells containing non-T cells that express MHC class I molecules or the autoreactive T cells themselves may mediate autoimmune response (24, 25). Next, T cells were isolated from diabetic NOD mice using T cell isolation kit (R&D Systems) and the purity of T cells, determined by flow cytometry, was $>90\%$ (data not shown). The purified T cells (10×10^6) were transferred into NOD/ $\beta 2m^{-/-}$ mice. The results show that all mice survived for more than 100 days with normoglycemia in NOD/ $\beta 2m^{-/-}$ mice after adoptive T cell transfer (Fig. 4A). The data indicate that purified T cells fail to induce autoimmune diabetes in the absence of MHC class I molecules.

To ask whether the deficiency of MHC class I molecule expression on the pancreas graft can prevent recurrent autoimmune diabetes, NOD/scid, NOD/ $\beta 2m^{-/-}$, and NOD/CIIT $^{-/-}$ pancreas grafts were transplanted into autoimmune diabetic NOD mice. The results in Figure 4B show that NOD/scid and NOD/CIIT $^{-/-}$ pancreas grafts were acutely destroyed in 9.4 days (MST $\pm$ SD = 9.4 ± 1.7 days, $n = 3$) and 6.6 days (MST $\pm$ SD = 6.6 ± 0.5 days, $n = 3$) respectively. Four of six NOD/ $\beta 2m^{-/-}$ pancreas grafts survived for over 120 days in diabetic NOD mice with a mean survival time of 72.3 days (MST $\pm$ SD = 72.2 ± 61.0 days, versus NOD/scid and NOD/CIIT $^{-/-}$ pancreas grafts, $p < 0.01$). The remaining two NOD/ $\beta 2m^{-/-}$ pancreata were destroyed within 14 days and 18 days, respectively. The results further show an important role of MHC class I molecule expression by the pancreas graft for destruction by recurrent autoimmune diabetes.

CD4⁺ and CD8⁺ T cells mediate the destruction of allogeneic pancreas grafts in diabetic NOD mice

In the allo- and auto-immune response model, C57BL/6 pancreas allografts were transplanted into autoimmune diabetic NOD mice. Without any treatment, C57BL/6 pancreas allografts were rejected in 11.4 days in autoimmune diabetic NOD mice. CD4⁺ cell infiltration in the pancreas transplant was observed at days 3 and 5 and CD8⁺ cell infiltration was noticed at days 4 and 7 after transplantation, which is consistent with the results in pancreas allograft rejection (Fig. 1B). Because of an important role of T cells in the destruction of pancreas allografts, we used anti-CD4 mAb (GK1.5, 10mg/kg $\times$ 4) and anti-CD8 mAb (2.43, 10mg/kg $\times$ 4) to prevent allograft rejection and recurrence of autoimmune diabetes. Our unpublished data and a previous report indicate that more than 95% of CD4⁺ cells are depleted after three times of treatment (5mg/kg)(26). The current results indicate that anti-CD4 modestly prolonged survival of pancreas allografts in autoimmune diabetic NOD recipients (MST $\pm$ SD = 27.2 ± 10.33 days, $n = 5$). Anti-CD8 alone was unable to prevent rejection of pancreas allografts (MST = 13.0 days, $n = 3$). Anti-CD4, in combination with anti-CD8, significantly prolonged survival of pancreas allografts in NOD mice (MST $\pm$ SD = 49.33 ± 8.69 days, $n = 6$, Fig. 5).

To determine if diabetic CD4⁺ T cells can accelerate the destruction of pancreas allografts, STZ-induced diabetic NOD/scid mice were transplanted with syngeneic (NOD/scid) or allogeneic (C57BL/6) pancreas grafts. The recipients were transferred with CD4⁺ cells (10×10^6 CD4⁺ spleen cell), obtained from non-diabetic NOD males, non-diabetic NOD females, and autoimmune diabetic NOD females. CD4⁺ T cells were isolated by T cell isolation kit (Miltenyi Biotec Inc.) and the purity of T cell subsets, determined by flow cytometry, was

>95% (data not shown). The results in Figure 6 show that non-diabetic CD4⁺ cells induced C57BL/6 allograft rejection in a mean time of 40.3 days (MST $\pm$ SD = 40.3 $\pm$ 2.52 days), diabetic CD4⁺ T cells elicited NOD/scid autoimmune diabetes in 30 days (MST $\pm$ SD = 29.8 $\pm$ 3.63 days), and diabetic CD4⁺ T cells induced destruction of pancreas allografts in 19 days (MST $\pm$ SD = 19.3 $\pm$ 10.97 days). The results indicate that diabetic CD4⁺ T cells induce an accelerated destruction of pancreas allografts.

In addition, we observed that diabetic CD8⁺ T cells (10×10^6) also induced destruction of C57BL/6 pancreas allografts in a mean time of 21.4 days in NOD/scid mice (MST $\pm$ SD = 21.4 $\pm$ 2.96 days, n = 5, Fig. 6); whereas, the same number of NOD male CD8⁺ T cells failed to elicit allograft rejection (Fig. 2) suggesting that once activated or primed, CD8⁺ T cells alone have the ability to induce autoimmune diabetes.

DISCUSSION

The current results suggest that CD4⁺ cells are necessary and sufficient to induce allograft rejection, which is evidenced by the following results: (i) pre-transplant anti-CD4 mAb but not anti-CD8 significantly prolonged survival of allogeneic pancreas grafts in STZ-induced diabetic NOD males; (ii) adoptive transfer of CD4⁺ but not CD8⁺ T cells elicits allograft rejection, and (iii) pancreas allografts were permanently accepted by STZ-induced NOD/CIIT^{-/-} males that are CD4⁺ T cell deficient. However, MHC class I molecule is not required for allograft rejection evidenced by the fact that pancreas allografts are rejected in MHC class I deficient NOD/ $\beta 2m^{-/-}$ mice. These results suggest that CD4⁺ T cells play a critical role in the induction of allograft rejection independent of MHC class I molecule in a pancreas transplantation mouse model.

Autoimmune response in the deterioration of pancreas allografts following pancreas transplantation has been documented in clinic and research (5,27,28). However, experimental studies indicate that NOD congenic mice are almost completely protected from autoimmune diabetes and costimulatory blockade fails to prolong skin allograft survival in NOD congenic mice; whereas, the same regimen induces prolonged survival of skin grafts in non-NOD background mice. They conclude that the NOD strain is genetically resistant to tolerance induction (29,30). In our study, NOR pancreas grafts survive for more than 100 days in STZ-induced diabetic NOD males but are acutely rejected in autoimmune diabetic NOD mice (MST = 11.8 days). When NOR pancreas grafts are transplanted into STZ-induced NOD females, 50% pancreas grafts destroy within 60 days when autoimmune diabetes occurs suggesting that autoimmune response mediates destruction of pancreas grafts. However, NOR heart grafts are permanently accepted indicating a tissue specific effect of autoimmune response. Our data collectively demonstrate that recurrent autoimmune response indeed occurs in autoimmune diabetic NOD mice after pancreas allograft transplantation.

Conflicting observations exist regarding the interplay between autoimmunity and alloimmune response. Previous results showed that islet grafts survived the worst if the donor and recipient shared MHC class II antigens (31). Another study suggested that MHC mismatching did not protect islet allografts from disease recurrence and MHC mismatched islet allografts remained vulnerable to autoimmune response (27). Our data show the following results: non-diabetic CD4⁺ cells induced allograft rejection within 40 days in STZ-induced diabetic NOD/scid males (MHC mismatch); diabetic CD4⁺ cells (obtained from diabetic NOD mice) elicited autoimmune diabetes within 30 days (MHC match); and diabetic CD4⁺ cells resulted in destruction of allogeneic pancreas grafts within 20 days (MHC mismatch). The current results suggest that MHC matching is required for long-term survival of pancreas allografts in alloimmune response, and it is likely that MHC class II mismatching elicits alloreactivity and

may enhance the effect of autoreactivity (27). Simultaneous presence of both allo- and auto-immune responses accelerates destruction of pancreas allografts.

CD4⁺ cells recognize allo-antigen through antigen presentation by MHC class II and CD8⁺ cells via MHC class I molecule. Both MHC class I and class II play an important role in the development of autoimmune diabetes. NOD mice do not develop diabetes in the absence of either MHC class I or class II molecule (10,31–33). Specific MHC class II alleles are associated with the disease (I-Ag⁷ in NOD mice)(34). Mutation of I-Ab is able to prevent development of autoimmune diabetes (35) and the deficiency of MHC class II in NOD mice does not develop into autoimmune diabetes (34). However, after onset of autoimmune diabetes, absence of MHC class II could not prevent recurrence of autoimmune diabetes in the adoptive transfer or islet transplantation model (10,11,34). In contrast, MHC class I deficient islet grafts survived in diabetic NOD mice indefinitely (10,11). Our studies, using a mouse pancreas transplantation model, show that four of six syngeneic pancreas grafts are permanently accepted by autoimmune diabetic NOD mice. The results suggest that autoimmune destruction of pancreas grafts depends on the expression of MHC class I by the pancreas graft.

In an adoptive spleen cell transfer study, whole spleen cells, obtained from diabetic NOD mice, induced autoimmune diabetes in NOD/scid and NOD/β2m^{-/-} mice. However, adoptive transfer of purified T cells (in the absence of donor APCs) results in autoimmune diabetes in NOD/scid and MHC class II deficient mice but not in MHC class I deficient NOD/β2m^{-/-} mice implicating a necessity of MHC Class I expression by donor-derived APCs. Although either primed CD4⁺ or CD8⁺ T cells have the ability to elicit autoimmune diabetes, no diabetes occurs when transfer of diabetic T cells (including both CD4 and CD8). Thus, MHC Class I molecule is required for the pancreas graft to be destroyed by autoimmune T cells.

In conclusion, the current results indicate that CD4⁺ T cells are sufficient to elicit allograft rejection and MHC class I molecules are required for recurrence of autoimmune diabetes after vascularized pancreas transplantation in NOD mice. In the clinical setting of pancreas transplantation for T1D patients, elimination of MHC class I molecule and control of CD4⁺ T cells are necessary (see Table 1).

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Figure 1. CD4⁺ spleen cells induce allograft rejection

Whole spleen cells, CD4⁺ and CD8⁺ T cells from NOD males were isolated (Miltenyi Biotec Inc.) and transferred into STZ-induced diabetic NOD/scid mice that were transplanted with C57BL/6 pancreas allografts on the day of cell transfer. Whole spleen cells (20×10^6) induce pancreas allograft rejection in a mean survival time (MST) of 24.3 days (MST = 24.3d, $n = 3$), CD4⁺ T cells in 43 days (10×10^6 , MST = 26.8d, $n = 5$), and CD8⁺ T cells in ≥ 92.0 days (10×10^6 , MST ≥ 92.0 d, $n = 6$, versus whole spleen cells and CD4⁺ T cells, $p < 0.05$).

Figure 2.

Figure 2A Survival of pancreas allografts (C57BL/6) in STZ-induced diabetic NOD males, NOD/ $\beta 2m^{-/-}$ and NOD/CIIT $^{-/-}$ mice.

NOD males, NOD/ $\beta 2m^{-/-}$, and NOD/CIIT $^{-/-}$ mice were made diabetic with streptozocin (STZ, 170mg/kg, i.p.) and transplanted with pancreas allografts. In the control group (from C57BL/6 to NOD males), pancreas allografts were acutely rejected (MST $\pm$ SD = 13.6 $\pm$ 6.43d, n = 5). Anti-CD4 mAb (GK1.5, 10mg/kg $\times$ 4, MST $\pm$ SD = 62.6 $\pm$ 43.9d, n = 8) but not anti-CD8 mAb (2.43, 10mg/kg $\times$ 4, MST $\pm$ SD = 21.7 $\pm$ 2.89d, n = 4) significantly prolongs survival

of pancreas allografts ($p < 0.01$ compared to the control). Pancreas allografts were accepted in STZ-induced diabetic MHC class II deficient NOD/CIIT^{-/-} mice (MST > 100d, n = 4) but acutely rejected in MHC class I deficient NOD/β2m^{-/-} mice (MST ± SD = 9.75 ± 2.36d, n = 4).

Figure 2B *CD4⁺ and CD8⁺ T cells infiltration in the pancreas allografts.*

Histopathological examinations showed a predominant CD4⁺ T cell infiltration in the untreated pancreas allografts (C57BL/6), engrafted in autoimmune diabetic NOD recipient, on day 5 and a massive of CD8⁺ T cells deposition in the rejected pancreas allografts on day 9 after transplantation.

Figure 3. NOR pancreas were rejected in spontaneous diabetic NOD females but permanently accepted in STZ-induced diabetic NOD males

NOR pancreas grafts were rejected acutely in spontaneous diabetic NOD mice (NOR-NOD Sp, $MST \pm SD = 11.8 \pm 1.89d$, $n = 4$) and anti-CD4 mAb delayed rejection slightly (NOR-NOD Sp + anti-CD4, $MST \pm SD = 18.0 \pm 5.29d$, $n = 6$). However, all pancreas grafts survived for more than 100 days in STZ-induced diabetic NOD males (NOR-NOD STZ male, $MST > 100d$, $n = 4$) and NOR pancreas grafts were rejected in a mean time of 74.2 days in STZ-induced diabetic NOD females (NOR-NOD STZ female, $MST = 74.2 \pm 23.63d$, $n = 6$).

Figure 4.

Figure 4A Purified diabetic T cells fail to induce autoimmune diabetes in NOD/ $\beta 2m^{-/-}$ mice.

Whole spleen cells (WSC) were collected from autoimmune diabetic NOD mice and T cells were purified by T cell isolation kit (R&D Systems). Purity of isolated cells for this experiment was 90 to 95% (data not shown). Unpurified diabetic WSC (20×10^6) elicit autoimmune diabetes in a mean time (MT) of 72.25 days (MT $\pm$ SD = $72.25 \pm 10.53d$, $n = 6$, versus WSC to NOD/scid, $p = 0.012$) in MHC class I deficient NOD/ $\beta 2m^{-/-}$ mice, 44.5 days in class II deficient NOD/CIIT^{-/-} mice (MT $\pm$ SD = $41.0 \pm 7.48d$, $n = 4$, versus WSC to NOD/scid $p > 0.05$), and 28.8 days in NOD/scid mice (MT $\pm$ SD = $31.4 \pm 7.54d$, $n = 5$). However, the purified T cells (10×10^6) induced autoimmune diabetes in NOD/scid mice (MT $\pm$ SD = $48.5 \pm 5.74d$) and NOD/CIIT^{-/-} mice (MT $\pm$ SD = $34.3 \pm 10.05d$) but not in NOD/ $\beta 2m^{-/-}$ mice (MT $> 100d$, $n = 4$) suggesting that donor-derived MHC class I molecules mediate autoimmune response.

Figure 4B Four of six MHC class I deficient pancreas grafts survived indefinitely in autoimmune diabetic NOD mice.

MHC class I deficient (NOD/ $\beta 2m^{-/-}$), class II deficient (NOD/CIIT^{-/-}), and NOD/scid pancreas grafts were transplanted into autoimmune diabetic NOD mice. Four of six NOD/ $\beta 2m^{-/-}$ pancreas grafts survived for over 100 days without any immunosuppressant (MST $\pm$ SD $\geq 58.17 \pm 45.90d$, $n = 6$), compared to NOD/CIIT^{-/-} and NOD/scid pancreas grafts (MST $\pm$ SD = $8.2 \pm 1.10d$ and $9.0 \pm 2.31d$ respectively).

Figure 5. Anti-CD4 plus anti-CD8 mAbs delay allograft rejection and recurrence of autoimmune diabetes in autoimmune diabetic NOD mice

C57BL/6 pancreas allografts were rejected in a mean time of 11.4 days in autoimmune diabetic NOD mice (control, $\text{MST} \pm \text{SD} = 11.4 \pm 1.14\text{d}$, $n = 5$). Anti-CD4 mAb ($\text{MST} \pm \text{SD} = 27.2 \pm 10.33\text{d}$, $n = 5$, versus control, $p < 0.05$) but not anti-CD8 mAb ($\text{MST} \pm \text{SD} = 13.0 \pm 2.5\text{d}$, $n = 5$) prolonged survival of pancreas allografts. Anti-CD4 mAb plus anti-CD8 mAb ($\text{MST} \pm \text{SD} = 49.33 \pm 8.69\text{d}$, $n = 6$, versus control, $p < 0.05$) significantly prolonged survival of pancreas allografts.

Figure 6. Autoimmune response accelerates allograft rejection

Syngeneic non-diabetic (NOD males) and diabetic (diabetic NOD females) T cell subsets (10×10^6) were transferred into STZ-induced diabetic NOD/scid mice. The results show that non-diabetic CD4⁺ cells, obtained from non-diabetic NOD males, elicited rejection of C57BL/6 allografts in a mean time of 40.3 days (MST $\pm$ SD = 40.3 ± 2.52 d, n = 4), diabetic CD4⁺ cells, obtained from diabetic females, induced autoimmune diabetes in NOD/scid mice engrafted with NOD male pancreas grafts in a mean time of 29.8 days (MST $\pm$ SD = 29.8 ± 3.63 d, n = 5), and diabetic CD4⁺ cells accelerated pancreas allograft rejection in a mean time of 19.3 days (MST $\pm$ SD = 19.3 ± 10.97 d, n = 5). In addition, diabetic CD8⁺ cells induced pancreas allograft destruction in a mean time of 21.4 days (MST $\pm$ SD = 21.4 ± 2.96 d, n = 5). The results suggest that autoimmune response is able to accelerate allograft rejection and activated CD8⁺ cells alone have the ability to induce autoimmune response.

Table 1

Summarize the critical roles of T cell subsets and MHC molecules in allograft rejection and recurrent autoimmunity following vascularized pancreas transplantation

Subsets	Allo-rejection	Auto-immunity
CD4 ⁺ T	Required and sufficient	Required and sufficient
CD8 ⁺ T	Help CD4 to mediate rejection	Activated CD8 can elicit autoimmunity
Class I	Not required	Required
Class II	Required	Not required