The Role of Autoimmunity in Islet Allograft Destruction

Major Histocompatibility Complex Class II Matching Is Necessary for Autoimmune Destruction of Allogeneic Islet Transplants After T-Cell Costimulatory Blockade

Leila Makhlouf,1 Koji Kishimoto,1 Rex N. Smith,2 Reza Abdi,1 Maria Koulmanda,3 Henry J. Winn,3 Hugh Auchincloss, Jr.,3 and Mohamed H. Sayegh1,4

Although it has often been assumed that transplanted allogeneic islets can be destroyed by recurrent autoimmunity in recipients with type 1 diabetes, definitive evidence is lacking and the settings in which this may occur have not been defined. To address these issues, we compared the survival of islet transplants (subject to tissue-specific autoimmunity) with cardiac transplants (not subject to tissue-specific autoimmunity) from various major histocompatibility complex (MHC)-matched and -mismatched donors transplanted into autoimmune NOD recipients. We found that when recipients were treated with combined B7 and CD154 T-cell costimulatory blockade, hearts survived best with better MHC matching, whereas islets survived worst when the donor and recipient shared MHC class II antigens. In the absence of full or MHC class II matching, there was no difference in the survival of islet and cardiac allografts. We also found that the tendency of NOD mice to resist tolerance induction by costimulation blockade is mediated by both CD4+ and CD8+ T-cells, not directly linked to the presence of autoimmunity, and conferred by non-MHC background genes. These findings have clinical importance because they suggest that under some circumstances, avoiding MHC class II sharing may provide better islet allograft survival in recipients with autoimmune diabetes, since mismatched allogeneic islets may be resistant to recurrent autoimmunity. Our results may have implications for the design of future clinical trials in islet transplantation. Diabetes 51: 3202–3210, 2002

Islet transplants for patients with type 1 diabetes potentially face two distinct types of immune destruction: one generated by the allogeneic response to foreign tissues and the other generated by the recurrence of the tissue-specific autoimmune process that caused the disease in the first place. Indeed, previous reports showed that human islets from genetically identical twins (1) or cadaver donors (2) were subject to recurrent autoimmunity. Several other findings have suggested that recurrent autoimmunity might be responsible for destruction of xenogeneic (3) and allogeneic (3–6) islets. In interesting studies by Woehrle et al. (7) and Markmann et al. (8) in BB rats, precultured major histocompatibility complex (MHC)-mismatched islets were not subject to recurrent autoimmunity, and the survival was significantly better for MHC-mismatched grafts than for MHC-matched grafts. Furthermore, until recently, human islet transplants have generally been notably unsuccessful compared with other types of transplants in patients with type 1 diabetes (9). In addition, several investigators have found that strategies that induce long-term survival of allogeneic islets in ordinary mice have been less successful when used in NOD mice that have developed spontaneous autoimmune diabetes (5,10,11). In these cases, the assumption has been that the existence of the autoimmune process in NOD mice was responsible for the failure of the tolerance-induction strategies. On the other hand, other evidence has not supported the idea that recurrent autoimmunity plays a role in the destruction of islets transplanted from genetically disparate donors (12). First, although the results of clinical islet transplantation have often been poor, survival rates for whole-organ pancreas transplants are similar to those of kidneys (13), and even islet transplantation has been very successful recently when performed with newer immunosuppressive protocols (14). Second, it has been shown that xenogeneic islets survive for longer periods than syngeneic islets in diabetic NOD mice when they have been treated with a variety of immunomodulating therapies (15). Finally, Markees et al. (16) recently reported that the resistance of NOD mice to tolerance induction applies to tissues other than islets, suggesting that this resistance may not necessarily reflect recurrent tissue-specific auto-

From the 1Laboratory of Immunogenetics and Transplantation, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts; the 2Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; the 3Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; and the 4Nephrology Division, Children’s Hospital, Harvard Medical School, Boston, Massachusetts.

Address correspondence and reprint requests to Mohamed H. Sayegh, MD, Laboratory Immunogenetics and Transplantation, Renal Division, Department of Medicine, Brigham and Women’s Hospital, Boston, MA 02115. E-mail: msayegh@rics.bwh.harvard.edu.

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MHC, major histocompatibility complex; MST, mean survival time.

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immunity. Thus, it is not clear at this point what role recurrent autoimmunity plays in islet transplant destruction and, if it does, under what circumstances. This is of obvious clinical relevance to islet transplantation in humans with type 1 diabetes who are potentially subject to recurrent autoimmunity in the transplanted islets.

To address these questions, we conducted a series of transplant experiments in NOD mice, one of the best available animal models for the study of type 1 diabetes (17), using two types of tissues: hearts, which would only be subject to allogeneic destruction, and islets, which would potentially be subject to recurrent autoimmunity in addition to alloreactivity. We also used donors that expressed a range of genetic disparities to determine what degree of antigen matching might allow for the expression of recurrent autoimmunity. Our results indicate that when B7 and CD154 T-cell costimulatory blockade is used to prolong islet transplant survival, recurrent autoimmunity is only evident when the MHC class II antigens are matched between the donor and recipient. Our data also show that the resistance of NOD mice to tolerance by costimulation blockade is mediated by both CD4+ and CD8+ T-cells, not absolutely linked to the presence of an autoimmune response, and conferred by non-MHC background genes.

### TABLE 1

MHC class I and class II haplotypes in different mouse strains

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>K</th>
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<th>E</th>
<th>D</th>
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<td>d</td>
<td>g7</td>
<td>--</td>
<td>b</td>
</tr>
<tr>
<td>C57BL/6 (partial class I)*</td>
<td>b</td>
<td>b</td>
<td>--</td>
<td>b</td>
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<tr>
<td>BALB/c (partial class I)*</td>
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<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>B10.BR (none)*</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
</tr>
<tr>
<td>B6.NOD (full MHC)*</td>
<td>d</td>
<td>g7</td>
<td>--</td>
<td>b</td>
</tr>
<tr>
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<td>g7</td>
<td>--</td>
<td>q1</td>
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<tr>
<td>B10.ITG (class I only)*</td>
<td>d</td>
<td>d</td>
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*Indicates degree of MHC matching with NOD mice

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**RESEARCH DESIGN AND METHODS**

**Mice.** Diabetic female NOD/Lt (H-2 g7) mice (referred to as NOD) were used as recipients. BALB/c (H-2 b), C57BL/6 (H-2 b), NOD/LtSz-Pkdcs/cld/Prkdcscid (referred to as NOD-scid), B10.BR (H-2 k), and B6.NOD (H-2 g7) mice, aged 8–12 weeks, were obtained from The Jackson Laboratory (Bar Harbor, ME) and used as donors. BIOZZI ABH/RijHsdH (IA-g7) mice, referred to as BIOZZI mice, were purchased from Harlan (England, U.K.), and B10.IPTG (H-2 g) were a kind gift of Dr. Hugh McDevitt (Stanford University).

**Murine model for islet transplantation.** Once NOD mice spontaneously developed diabetes, 700 islets were transplanted under the renal capsule. In nonautoimmune recipients, diabetes was chemically induced with streptozotocin (225 mg/kg i.p.) and 400 islets were transplanted under the renal capsule. Diabetes was defined as blood glucose >250 mg/dl on at least 2 consecutive days. Reversal of diabetes was defined as blood glucose <200 mg/dl for at least 2 consecutive days. Graft rejection was defined as blood glucose >250 mg/dl for at least 2 consecutive days and confirmed histologically. Islet graft function was assessed by blood glucose measurements (ACCU-Check Advantage; Boehringer Mannheim) twice a week.

All experiments were performed in compliance with institutional guidelines regarding animal care.

**Isolation of pancreatic islets.** Pancreatic islets were isolated using collagenase digestion followed by histopaque 1077 (Sigma 1077; Sigma, St. Louis, MO) density gradient separation and then handpicking as described elsewhere (18).

**Cardiac transplants.** Cardiac transplants were performed in the same mouse strain combinations as islets. Cardiac graft survival was assessed by graft palpation (19). Rejection was confirmed histologically.

**Histology.** Grafts (three to six per group) were harvested soon after rejection and fixed in 2% buffered formalin. Paraffin-embedded sections (4 μm) were stained with hematoxylin and eosin. Immunohistochemical staining was performed on both paraffin-embedded tissue sections using guinea pig anti-insulin (1/10 dilution; Dako) or rabbit anti-glucagon (1/10 dilution; Dako). Second antibodies were goat anti–guinea pig or goat anti-rabbit at 1:200 (Vector Laboratories). Slides were blocked using an avidin-biotin blocking kit (Vector Laboratories, Burlingame, CA) and normal rabbit sera at 1/200 before staining with primary antibodies. After staining, slides were developed with avidin-conjugated horseradish peroxidase using the vectastain ABC Kit (Vector Laboratories). Biotinylated isotype-matched antibodies were used as negative controls for all staining experiments. Photographs were taken with a RT Color Spot camera (Diagnostic Instruments) and a Zeiss Axioskop microscope. All magnifications are 400x. Composite pictures were created with Adobe Photoshop.

**Agents, antibodies, and in vivo T-cell depletion.** MR1 is a hamster monoclonal antibody that is specific for murine CD154 (the hybridoma is a kind gift from Dr. Randy Noelle (Dartmouth College of Medicine, Lebanon, NH), and the antibody was manufactured commercially by Bioexpress, West

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**FIG. 1.** Effect of combined CTLA4Ig and anti-CD154 on islet graft survival. Combined CTLA4Ig and anti-CD154 (MR1) were given to recipients of islet transplants. A: Combined costimulation blockade induced indefinite islet allograft survival in BALB/c recipients of C57BL/6 islets (treated group MST >115 days, n = 7 vs. control nontreated group MST 13 ± 4 days, n = 8; P < 0.001). B: Combined costimulation blockade had no effect on recurrent autoimmunity in NOD-scid isografts; all recipients lost their grafts as rapidly as nontreated recipients (treated group MST 8.3 ± 2.2 days, n = 6 vs. nontreated group MST 13.6 ± 4 days, n = 5; P < 0.017). C: Combined costimulation blockade induced prolonged allograft survival in NOD mice recipients of B10.BR islets (treated group MST 30 ± 15 days, n = 6 vs. nontreated control group MST 8 ± 0.5 days, n = 50; P < 0.001). D: NOD-scid islets survived indefinitely in nonautoimmune C57BL/6 recipients treated with combined costimulation blockade (treated group MST >100 days, n = 4 vs. nontreated group MST 16 ± 1 days, n = 3; P < 0.001). A, no treatment; B, CTLA4Ig and anti-CD154.
Lebanon, NH). CTLA4Ig is a kind gift of Dr. Robert Peach (Bristol-Meyers Squibb, Princeton, NJ). The agents were administered intraperitoneally (250 μg) at days 0, 2, 4, and 6 posttransplantation, as previously reported (20). Anti-CD8 ascites (116-13.1; anti-Lyt2.1 IgG2a) and anti-CD4 ascites (GK1.5; anti-L3T4 rat IgG2b) were obtained from the American Type Culture Collection (Rockville, MD). Anti-CD8– and anti-CD4–treated mice received 0.2 ml i.p. of unpurified ascites of the appropriate antibody (roughly equivalent to 200 mg of purified antibody) on day −1 and day 0 and then twice a week after transplantation until rejection or day 60 posttransplantation in animals with surviving grafts (21). This resulted in >98% depletion of CD4+ or CD8+ T-cells throughout the follow-up period.

**Statistics.** Survival data were plotted by using the Kaplan-Meier method. Significance of difference between groups was tested by comparing group means and medians (mean survival time [MST]) by either the two-tailed t test or the Wilcoxon’s signed-rank test, as appropriate. Statistical significance was defined as a P value <0.05.

**RESULTS**

The effect of costimulatory blockade on islet transplant survival in normal and NOD mice. We performed our studies using a regimen of T-cell costimulatory blockade that has been found to be very effective in promoting long-term allograft survival in several transplant models, including the stringent skin allograft model (20). Combined treatment with CTLA4Ig and anti-CD154 mAb caused long-term islet allograft survival when C57BL/6

**FIG. 2.** Photomicrographs of islet tissue transplanted in different strain combination evaluated by immunohistology. A: NOD-scid islets were transplanted into syngeneic diabetic NOD recipients treated with costimulatory blockade. Islets were destructed within weeks after transplantation. Islets show marked peri-islet inflammation with no staining for insulin but focal staining for glucagon, indicating selective autoimmune β-cell destruction. B: Allogeneic C57BL/6 islets were transplanted into diabetic NOD mice treated with costimulatory blockade. Mice rejected their grafts ~8 weeks after transplantation. Grafts show intense inflammation without detectable islets and without insulin or glucagon staining. C: Allogeneic C57BL/6 islets transplanted into chemically diabetic BALB/c recipients treated with costimulation blockade. Recipients were euglycemic, and long-term surviving grafts (>100 days) showed islets without inflammation as well as strong insulin and glucagon staining. D: Allogeneic NOD-scid islets were transplanted into chemically diabetic C57BL/6 recipients treated with costimulation blockade. Islets harvested at >100 days after transplantation were without inflammation and strong insulin and glucagon staining. H & E, hematoxylin and eosin.
islets were transplanted into BALB/c mice that were made chemically diabetic and did not have autoimmune diabetes (Fig. 1A). We then tested the same treatment regimen in NOD mice that had developed spontaneous diabetes. Combined costimulatory blockade did not prevent the recurrent autoimmunity responsible for the destruction of NOD-scid islet isografts in these recipients (Fig. 1B). The syngeneic islets were destroyed within 2–3 weeks. Next, we tested the effect of the same treatment regimen on the rejection of allogeneic islets by NOD mice with spontaneous diabetes. B10.BR (fully MHC disparate) islets transplanted into diabetic NOD recipients showed somewhat prolonged survival (Fig. 1C), but unlike the nonautoimmune BALB/c recipients, all NOD recipients rejected their transplants within 8 weeks. No long-term survival was achieved. To assure that NOD-scid islets were not unusually susceptible to destruction, we transplanted them into nonautoimmune C57BL/6 recipients along with costimulatory blockade. NOD-scid islets survived indefinitely in C57BL/6 recipients (Fig. 1D).

Qualitative immunohistological analyses of islet transplants described above were performed (Fig. 2). Staining of NOD-scid islets transplanted into syngeneic recipients treated with combined costimulatory blockade after loss of islet function (MST 8.3 ± 2.2 days, n = 6) showed positive staining for glucagon but negative staining for insulin on all harvested and analyzed grafts (n = 6), which confirms the selective autoimmune β-cell destruction similar to that found in the pancreas of an established diabetic NOD mouse (Fig. 2A). On the other hand, islets from C57BL/6 donors transplanted into diabetic NOD recipients showed no staining for either insulin or glucagon (n = 4) associated with the complete loss of islet cells morphologically, indicating alloimmune destruction of islet cells, including α- and β-cells (Fig. 2B). Long-term surviving grafts (for >100 days) transplanted from C57BL/6 mice

FIG. 2. Continued.
into BALB/c recipients showed positive staining for both glucagon and insulin (n = 3) (Fig. 2C). Also, NOD-scid islets transplanted into C57BL/6 recipients treated with costimulation blockade survived indefinitely, as well as stained positive for both glucagon and insulin (n = 3) (Fig. 2D). Absence of cellular infiltrate was observed in all long-term surviving islets. The pattern of insulin and glucagon staining found in the islets in Fig. 2C and D is similar to that found in the islets of a normal pancreas.

These results confirm the general findings reported by others: that immunomodulatory therapies that achieve long-term survival for allogeneic transplants are not necessarily effective in preventing autoimmune destruction of syngeneic islets in NOD mice, and that these therapies are generally less effective for allogeneic islet transplants in recipients with spontaneous diabetes. However, it was not clear from these data whether the destruction of the syngeneic islets in the autoimmune recipients was due to a recurrence of autoimmunity.

Is tissue-specific autoimmunity responsible for the destruction of islet allografts? To examine the role of autoimmunity in the destruction of islets by NOD mice, we transplanted either islet or cardiac allografts into NOD mice that were treated with combined T-cell costimulatory blockade as above. First, unlike islets (Fig. 1B), NOD-scid cardial allografts were not rejected by NOD mice (graft survival >150 days, n = 4). Therefore, we concluded that cardiac grafts were not subject to tissue-specific autoimmunity. We then performed islet or cardiac transplants using a number of donor and recipient combinations that were designed to match some, or all, of the transplantation antigens of the donor and recipient (Table 1), assuming that different mouse strain islets have similar inherent sensitivity to immune injury in the NOD mouse recipient. Figure 3 shows the results of these experiments. The survival of cardiac allografts was nearly identical to that found in the syngeneic islets (subject to autoimmune destruction), but early destruction of islet allografts in NOD mice does not depend on the presence of autoimmunity.

Why are NOD mice resistant to tolerance induction? The difficulty in achieving long-term survival of allogeneic transplants in NOD mice has been observed by others (16). We further examined the mechanisms of this resistance to tolerance in several ways. First, we depleted either CD4+ or CD8+ T-cells vigorously in NOD recipients and then transplanted either allogeneic or syngeneic islets. Chronic CD4+ or CD8+ T-cell depletion led to long-term survival of the syngeneic islets (subject to autoimmune destruction only), but allogeneic islets were still rejected within 3 months (Fig. 4A and B). These data provide further support to the notion that the difficulty in achieving long-term islet allograft survival in NOD mice does not depend on the presence of autoimmunity.

CD8+ T-cells have been shown to be resistant to combined costimulation blockade in some stringent transplant models (22,23). However, the addition of CD8+ T-cell depletion to our regimen of costimulatory blockade did not further prolong the survival of syngeneic islets compared with combined costimulatory blockade alone (Fig. 4B). Thus, the resistance of NOD mice to long-term allograft survival is not due to costimulation blockade–resistant alloreactive CD8+ T-cells.

Finally, we used the same regimen of costimulatory blockade in recipient B6.NOD (NOD MHC genes on the
C57BL/6 background genes) mice to determine the importance of non-MHC genes in the resistance to long-term allograft survival. Long-term survival of allogeneic islets was achieved in this congenic strain (Fig. 4C). Thus, it is not just the unusual MHC haplotype of NOD mice, which makes these animals susceptible to autoimmunity, that is responsible for the difficulty in achieving long-term allograft survival—background non-MHC genes also appear to play a role.

**DISCUSSION**

The difficulty in achieving long-term survival of allogeneic islets in autoimmune NOD mice has been cited in the past as evidence that recurrent autoimmunity can contribute to the destruction of even MHC-disparate islet transplants. Recognizing, however, that NOD mice have a general resistance to immunomodulating therapies, which is not necessarily related to their autoimmune condition, we used a novel experimental system in which we systemat-
icantly compared the survival in NOD recipients of allografts that might be subject to recurrent autoimmunity (islets) with that of grafts that would not be (hearts). Our findings indicate that when using B7 plus CD154 T-cell costimulatory blockade for therapy, recurrent autoimmunity appears to contribute to the destruction of allogeneic islets only when there is MHC class II antigen matching between the donor and recipient.

It is of course possible that recurrent autoimmunity is contributing to the destruction of the class II–mismatched islet allografts or the autoimmune process happens to coincide in timing with the alloreactive process that causes cardiac graft rejection. There is no formal way of excluding this possibility as long as islet destruction occurs. However, the rapid destruction of MHC class II–matched islets, like that of the NOD-scid islets, strongly suggests that recurrent autoimmunity would be evident much sooner than the late destruction that is seen when using class II-mismatched donors. In addition, the nearly identical timing of the islet rejection with that of the cardiac grafts, when there is no MHC class II antigen matching, suggests that there is no need to invoke a tissue-specific autoimmune component as an explanation for the destruction of MHC-disparate islet allografts. It is possible that autoreactive T-cells contribute to the recognition of mismatched donor strain islets; however, the findings in Fig. 4, which show that vigorous CD4+ or CD8+ depletion completely prevented recurrent autoimmunity in NOD-scid islets transplanted into NOD recipients but was not as effective in promoting long-term survival of MHC-mismatched islet allografts in NOD recipients, provide strong support to our conclusion that it is predominantly the alloimmune response rather than the autoimmune response that is causing islets to be destroyed in NOD recipients of mismatched islets.

Strictly speaking, our conclusions apply primarily to the setting in which costimulatory blockade is used for treatment. It is possible that this particular immunomodulating regimen eliminates some, but not all, of the components of autoimmunity, leaving other components that can cause destruction of syngeneic and MHC class II–mismatched allogeneic islets. This is unlikely since both CTLA4Ig (24) and MR1 (25) are relatively ineffective in reversing established autoimmunity. Still, other treatments might not eliminate a component of the autoimmune response that could cause destruction of MHC-disparate allografts. Thus, it is important to be cautious about generalizing from our experiments to conclude that recurrent autoimmunity will only be a problem when there is MHC class II matching with all immunomodulatory strategies or in all clinical settings. It is possible that our observations do apply to other immunomodulatory strategies. Indeed, there is one supportive study in BB (autoimmune-prone) rats by Markmann et al. (8) showing that precultured (an immunomodulatory strategy that has been shown to prolong islet graft survival) MHC-matched islet allografts do worse than precultured MHC-mismatched islets. While these studies did not compare islet and heart graft survival and did not dissect class I versus class II matching effects, the data are consistent with our observations in NOD recipients.

It is interesting to note that although it appears that autoimmunity played a role in both cases, the rejection of B6.NOD islets did not take place quite as quickly as the destruction of NOD-scid islets in our experiments. One possible explanation for this observation is that some of the non-MHC genes responsible for the development of diabetes may do so by causing the islets in NOD mice to be more susceptible to immune destruction. In addition, the destruction of more than just the β-cells in the class II–matched allogeneic islet transplants indicates that more than just recurrent autoimmunity was involved. Thus, the process of allogeneic islet destruction, even when autoimmunity is involved, is not identical to the process of autoimmune destruction of syngeneic islets. Apparently, the recurrence of the autoimmune response helps to generate an allogeneic response that would otherwise be delayed by costimulatory blockade. One implication of this finding is that it is not possible to determine the absence of an autoimmune component in islet rejection by the presence of generalized islet destruction.

The difficulty in defining the contribution of recurrent autoimmunity to the destruction of allogeneic islets stems from the general resistance of NOD mice to immunomodulating therapies for all types of allografts (16). It is not clear, however, whether the features of the immune system of these mice that cause them to develop spontaneous autoimmunity are necessarily responsible for this general resistance. Our data showing long-term survival of allogeneic islet grafts in B6.NOD (Fig. 4C) indicate that the resistance to tolerance in NOD mice is not directly linked to the presence of their autoimmune disease and is conferred by non-MHC genes. However, it is not clear whether humans with type 1 diabetes will show this general resistance. Interestingly, the results of clinical kidney transplantation using standard immunosuppressive drugs have been at least as good for patients with type 1 diabetes as for patients without autoimmune diabetes (26). It remains to be determined, however, how diabetic patients receiving islet transplantation will respond to various types of selective immunomodulating therapies in the future.

It is difficult to draw definitive conclusions about the clinical implications of our findings for patients undergoing islet transplantation. If we consider the NOD mouse as one of the best nonhuman models to study autoimmune diabetes (17), then on the one hand, our data suggest that when using certain types of immunosuppression, islet transplants from MHC class II–mismatched donors may be resistant to recurrent autoimmunity. On the other hand, they suggest that unlike most solid organ transplants in nonautoimmune recipients, class II–matched islet grafts for patients with diabetes may be at a disadvantage because they will be susceptible to recurrent autoimmunity. Therefore, the possibility that MHC class II matching may lead to poorer clinical outcomes should be carefully examined in all future trials of islet transplantation. Finally, our observations may have important implications for the design of future immunomodulatory/tolerogenic therapies in islet transplantation based on the degree of MHC sharing between donor and recipients with autoimmune diabetes.
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