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Prevention of rejection of murine islet allografts by pretreatment with anti-dendritic cell antibody

(dendritic cells/transplantation/diabetes/islets of Langerhans/passenger lymphocytes)

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ABSTRACT **Previously we have demonstrated that islets of Langerhans treated with donor-specific anti-Ia serum and complement survive when transplanted across the major histocompatibility complex of the mouse. In this study, using immunofluorescence, we demonstrate two morphologically distinct populations of Ia-positive cells scattered within the Ia-negative islet tissue. A large irregularly shaped Ia-positive subset of cells were identified as dendritic cells by using the 33D1 antibody specific for a mouse dendritic cell antigen. The other small, round Ia-positive subset was 33D1 negative. Islets pretreated with anti-dendritic cell antibody and complement prior to transplantation survived in their histoincompatible recipients for >200 days. Rejection of stable islet allografts promptly occurred when transplant recipients were challenged with 1×10^5 donor dendritic cells 60 days after transplantation. These results demonstrate an important *in vivo* role for donor dendritic cells in the stimulation of allograft rejection.**

Rejection of mouse islet allografts is prevented when donor histoincompatible islets are treated with anti-Ia antiserum and complement prior to transplantation (1). Because the islet cells are Ia^- (2), the antiserum treatment presumably eliminates Ia^+ donor lymphoid cells. As proposed previously, these passenger lymphocytes may be the donor cells responsible for initiating islet graft rejection (1, 3).

Previous studies have suggested that the principal type of passenger leukocyte is the dendritic cell (4–6). Dendritic cells are bone marrow-derived (7) Ia^+ cells (8, 9) that express a specific antigen identified with a monoclonal antibody 33D1 (10) but lack the characteristic markers of other leukocytes such as F4/80 and Mac-1 (macrophages), surface Ig (B cells), and thy-1, Lyt-1,2 (T cells) (9–11). Dendritic cells are active stimulators of lymphocyte responses in culture, particularly the primary mixed lymphocyte response, where they are 100 times more potent than unfractionated spleen cell suspensions (4, 5). Small numbers of rat lymph dendritic cells can induce the rejection of rat kidney allografts (6). In all of these studies, dendritic cells have been derived from lymphoid organs or lymph. Dendritic cells have not been identified previously in graft tissue using a cell-specific antibody.

In this study, we demonstrate by immunofluorescence that dendritic cells are present in freshly isolated mouse islets of Langerhans and that these cells can be eliminated with a monoclonal antibody to dendritic cells and complement. Pretreatment of donor mouse islets with anti-dendritic cell antibody plus complement prior to transplantation prevented rejection of mouse islets transplanted across a major histocompatibility barrier. These findings indicate that dendritic cells are sufficient for initiating the rejection of mouse islet allografts.

MATERIALS AND METHODS

Mice. B10.BR/SgSnJ (B10.BR; $H\text{-}2^k$) and C57BL/6J (B6; $H\text{-}2^b$) male mice (age, ≈5–6 weeks) were obtained from The Jackson Laboratory.

B6 transplant recipients were made diabetic by the intravenous injection of streptozotocin (160 mg/kg of body weight). Nonfasting plasma glucose levels were determined three times a week on blood obtained from the orbital sinus of B6 diabetic mice, and only mice with plasma glucose levels of >400 mg/dl for three consecutive bleedings were used as recipients. Rejection was defined as a nonfasting serum glucose of >250 mg/dl for two consecutive bleedings.

Islet Isolation. Islets were isolated by the collagenase technique as described (1). The isolated islets were separated on a Ficoll gradient (Pharmacia) and hand picked with the aid of a dissecting microscope (12).

Antibodies. The following antibodies were used for immunofluorescence assays: (*i*) A.TH anti-A.TL, a mouse alloantiserum that is directed against I^k determinants; (*ii*) A.TL anti-A.TH, a mouse alloantiserum directed against I^s determinants; (*iii*) 10-2.16, a mouse monoclonal antibody that is directed against murine $I\text{-}A^k$ (13); and (*iv*) 33D1, a rat monoclonal antibody which is specific for mouse dendritic cells (5, 10). The 33D1 antibody also was used for the treatment of allogeneic islets prior to transplantation. The A.TH anti-A.TL and A.TL anti-A.TH antisera and the 10-2.16 reagent were produced in the Department of Genetics, Washington University School of Medicine. Cells for production of 10-2.16 were obtained from the Salk Institute (San Diego, CA). The monoclonal reagents were used in the form of culture supernatant.

Fluoresceinated goat anti-mouse IgG (Fl-anti-mouse IgG) was purchased from Tago (Burlingame, CA) and used to localize A.TH anti-A.TL, A.TL anti-A.TH, and 10-2.16 antisera. Biotin-labeled mouse anti-rat immunoglobulin (B-anti-rat Ig) and fluorescein-avidin (Fl-avidin) (Vector Laboratories, Burlingame, CA) were used for staining with the 33D1 reagent (10).

Rabbit sera (GIBCO) were screened by the microcytotoxicity test (14) in order to select one that lacked toxicity to islets while maintaining a high degree of specific complement activity.

Immunofluorescence. After isolation, islets either were processed immediately or were maintained in tissue culture medium (CMRL 1066) at 37°C in 95% air for 12 hr. The islets

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Abbreviations: B6, C57BL/6J; B10.BR, B10.BR/SgSnJ; Fl-anti-mouse IgG, fluoresceinated goat anti-mouse IgG; B-anti-rat Ig, biotin-labeled mouse anti-rat immunoglobulin; Fl-avidin, fluorescein-avidin.

were placed in glass Kimble tubes (100–150 islets per tube) and washed three times in Hanks' solution supplemented with 0.02% sodium azide. The islets were then incubated 45 min at room temperature with a 1:20 dilution of A.TH anti-A.TL, a 1:2 dilution of 33D1 culture supernatant, or with Hanks' solution and then were washed three times and incubated with rabbit complement (1:3 dilution) in Hanks' solution at 37°C for 30 min. The islets were treated for 15 min with DNase (1:10 dilution, 200 μg per tube) at 37°C and then washed by centrifugation three times in order to remove lysed cells. The islets were then treated with A.TH anti-A.TL (1:20 dilution), 10-2.16 (1:2 dilution), 33D1 (1:2 dilution), or Hanks' solution for 45 min at room temperature. After this second incubation, the islets were resuspended in a 1:20 dilution of Fl-anti-mouse IgG or a 1:40 dilution of B-anti-rat Ig (30 min at 4°C). The islets were washed three times and pelleted, and the islets treated with B-anti-rat Ig were subsequently treated with a 1:5 dilution of Fl-avidin (30 min at 4°C). The treated and washed islets were resuspended in 20 μl of Hanks' solution and examined with a Leitz Orthoplan fluorescence microscope equipped with an epi-illuminator. The islets were scored for the number of fluorescent-labeled cells with dendritic cell morphology and those cells resembling small lymphocytes. Fifty to 75 islets were counted in each experiment, and the mean number of fluorescent cells per islet was recorded. The treatment protocols were repeated at least twice with islets from different isolations. Similar numbers of Ia^+ cells were observed in islets tested either immediately after isolation or after 12 hr of incubation in tissue culture medium; thus, the data were pooled in Fig. 1.

Islet Transplantation. Freshly isolated B10.BR islets were incubated with monoclonal anti-dendritic cell reagent for 45 min at room temperature in Hanks' balanced salt solution containing 0.5% bovine serum albumin. The treated islets were washed twice by centrifugation and incubated for 30 min at 37°C with rabbit complement. Control islets were transplanted immediately after isolation or were treated with only rabbit complement. Each B6 ($H\text{-}2^b$) diabetic recipient received 550–880 treated or untreated B10.BR ($H\text{-}2^k$) islets by embolizing the islets into the terminal sinusoids of the liver by injection into the portal vein (15). We have demonstrated previously that islets transplanted as isografts via the portal vein will rapidly reverse the diabetic state to normal and maintain normoglycemia in the recipients for >300 days (1).

Dendritic Cells for Induction of Rejection. Donor B10.BR dendritic cells were prepared from spleen cell suspensions as described (16). Sixty-five days after transplantation, normoglycemic B6 mice with stable B10.BR islet transplants were given a single injection of 1×10^5 viable cells enriched for dendritic cells in 0.5 ml of medium via the tail vein.

RESULTS

Identification of Ia^+ Lymphoid and Dendritic Cells in Isolated Islets. Immunofluorescence studies using alloantiserum recognizing murine Ia determinants revealed two morphologically distinct populations of Ia^+ cells within B10.BR mouse islets (Fig. 1, group 1). One population was composed of large cells with finger-like protrusions, spaced between the Ia^- islet cells (Fig. 2) that were similar to the dendritic cells described in lymphoid organs (8–11, 16). These cells were present within the parenchyma of the islets and were rarely observed on the islet surface. A second Ia^+ population was composed of small, round cells with cytoplasmic projections that resembled small lymphocytes. The number of dendritic-like cells ranged from 0 to 5 cells per islet, and the number of lymphoid cells ranged from 0 to 3 cells per islet, with the larger islets having more Ia^+ cells than smaller islets. The ratio of dendritic cells to lymphocytes remained constant (3:1) even though the size of the islets varied.

A monoclonal anti-$I\text{-}A^k$ antibody also detected both lymphoid-like and dendritic-like populations of Ia^+ cells within B10.BR islets (Fig. 1, group 2), although the intensity of staining and the total number of Ia^+ cells per islet was decreased in comparison to that observed with the alloantiserum. This may reflect the lower density of determinants recognized by a monoclonal reagent or the qualitative or quantitative expression of Ia specificities on the lymphoid

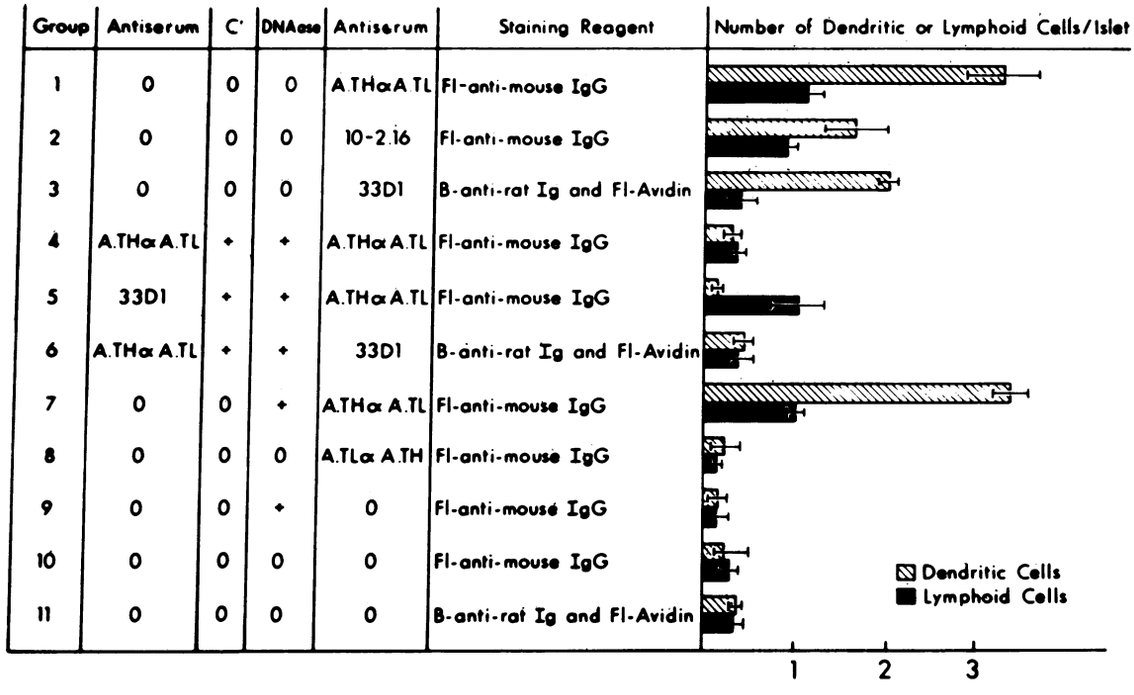

FIG. 1. Treatment of islets with allo-anti-Ia (A.TH anti-A.TL), monoclonal anti-Ia (10-2.16), or monoclonal anti-dendritic cell (33D1) reagents in the presence or absence of complement.

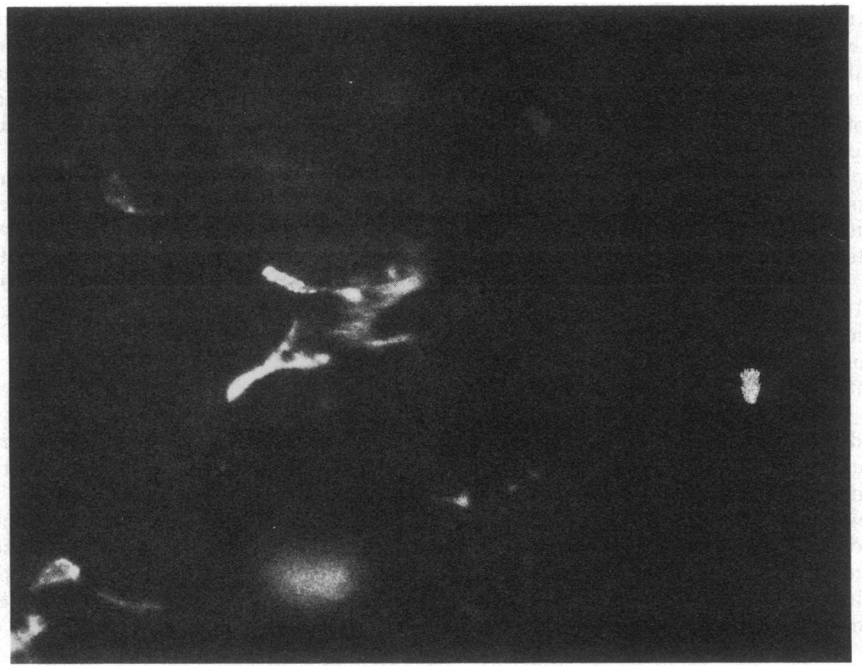

FIG. 2. Immunofluorescence appearance of a dendritic cell in a B10.BR islet. The islet was treated with allo-anti-Ia serum and Fl-anti-mouse IgG.

cells in the islets. Control B10.BR islets treated with an anti-Ia alloantiserum to an irrelevant haplotype (I^s) demonstrated background staining only (Fig. 1, group 8).

Treatment of islets with A.TH anti-A.TL antiserum, complement, and DNase reduced the number of dendritic and lymphocytic cells detected by immunofluorescence to background levels (Fig. 1, group 4).

A monoclonal antibody to mouse dendritic cells (33D1) was used to determine whether the large Ia^+ cells with elongated processes in the islets were dendritic cells. It is known that the number of determinants detected by 33D1 is very small (about 14,000 antibody binding sites per cell) compared to >200,000 anti-I-A binding sites (9, 10). Therefore, the more sensitive biotin/avidin modification of indirect immunofluorescence was used with 33D1 reagent. This technique produced readily detectable staining of large dendritic cells (Fig. 1, group 3) but did not stain the round cells which resembled small lymphocytes. The average number of $33D1^+$ dendritic cells per islet (2.0) was less than the number of Ia^+ cells with dendritic-like morphology (3.5). This difference was attributed to less intense staining with 33D1 and not to a population of Ia^+, $33D1^-$ dendritic cells because treatment of islets with 33D1 antibody and complement eliminated all of the Ia^+ dendritic cells (Fig. 1, group 5). As expected, removal of Ia^+ cells eliminated $33D1^+$ cells (Fig. 1, group 6). The Ia^+ lymphocyte-like cells lacked 33D1 determinants both by immunofluorescence (Fig. 1, group 3) and by antibody-mediated cytotoxicity (Fig. 1, group 5). These results indicate that most of the large Ia^+ cells in islets are related to the $33D1^+$ dendritic cells previously isolated from lymphoid organs.

Effects of Anti-Dendritic Cell Antibody on Islet Allograft Survival. The majority (83%) of B6 recipients receiving untreated B10.BR islets rejected the islets by 12 days after transplantation (Fig. 3, group 1). These results are comparable to our previous studies (1) and those of Morrow *et al.* (17). The small number that failed to reject the allografts may represent animals that received islets with insufficient numbers of passenger cells to induce rejection. Islet allografts treated with rabbit complement alone prior to transplantation were also acutely rejected (Fig. 3, group 2).

Transplants of B10.BR islets treated with monoclonal anti-mouse dendritic cell reagent (33D1) and complement resulted in allograft survival in eight of the nine recipients for at least 65 days after transplantation (Fig. 3, group 3). One islet graft was rejected at 12 days. As shown in Fig. 4*A*, the recipients remained normoglycemic beyond 200 days.

Initiation of Rejection of Established Islet Allografts with Donor Dendritic Cells. Previous studies have demonstrated that the intravenous injection of donor lymphoid cells would induce the acute rejection of established islet allografts in rats (3) and mice (1). For example, at 100 days after transplantation, established mouse islet allografts are rejected by two injections of 1×10^7 donor splenocytes.

We studied four mice with established allografts 65 days after transplantation of islets pretreated with anti-dendritic

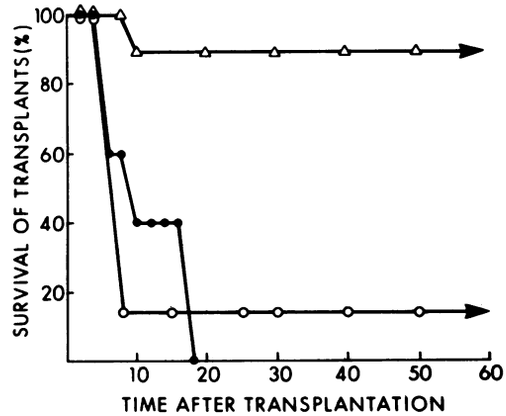

FIG. 3. Survival of fresh allogeneic islets (B10.BR) transplanted into nonimmunosuppressed diabetic recipients (B6). Group 1 (○) recipients were transplanted with fresh untreated islets (n = 6). Group 2 (●) recipients were transplanted with islets treated with rabbit complement (n = 5). Group 3 (△) recipients were transplanted with islets treated with anti-dendritic cell antibody (33D1) and rabbit complement (n = 9).

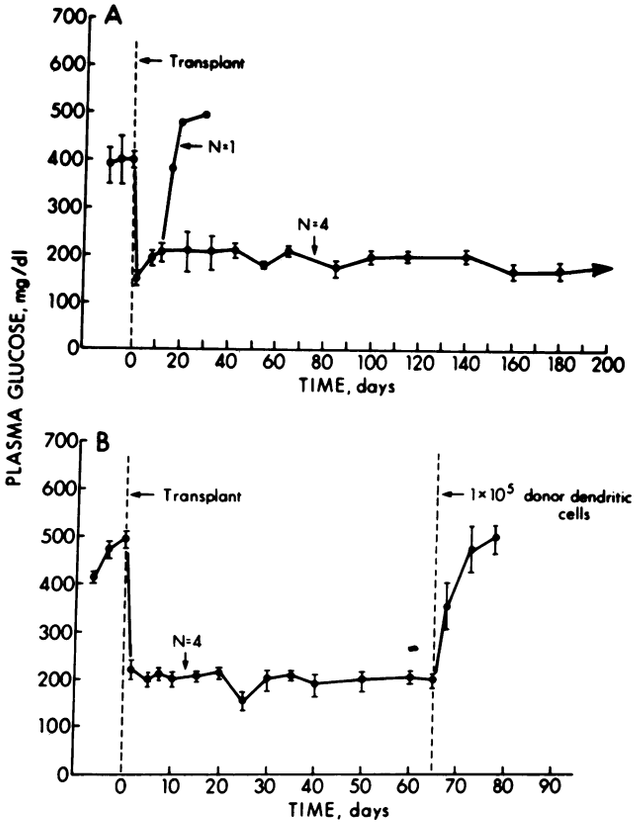

FIG. 4. Plasma glucose levels of diabetic B6 mice after transplantation with allogeneic islets. The allogeneic islets were pretreated with anti-dendritic cell antibody and rabbit complement prior to transplantation. (*A*) Group A: plasma glucose levels remained normal after an islet allograft transplant for >200 days. (*B*) Group B: four stable islet allograft recipients received 1×10^5 donor cells enriched for dendritic cells. Islet allograft rejection was promptly induced.

cell antiserum and complement. The mice were challenged intravenously with 1×10^5 donor cells enriched for dendritic cells. Hyperglycemia occurred acutely in all four recipients within 6 days (Fig. 4*B*), indicating that this low dose of donor dendritic cells was capable of initiating rejection.

DISCUSSION

Marked prolongation of allograft survival is achieved by a variety of procedures directed toward altering or eliminating donor passenger lymphocytes prior to transplantation. These procedures have been applied successfully to islets of Langerhans and include islet culture at low temperature (24°C) (18) or in the presence of 95% oxygen (19) and elimination of Ia^+ cells from the islets by specific antisera and complement (1). Rejection of long-term established islet transplants can be induced with donor lymphoid cells (1, 20, 21). However, cell-specific monoclonal antibodies have seen little use in defining the contribution of passenger lymphocytes in transplantation.

Morphological studies have demonstrated the presence of Ia^+ cells within rat islets (22) and within canine islets (23). The present immunofluorescence study demonstrates two structurally distinct populations of Ia^+ cells within isolated mouse islets. One population of Ia^+ cells possessed finger-like processes and reacted with the 33D1 anti-dendritic cell antibody, whereas the other Ia^+ population was $33D1^-$ and resembled lymphocytes. Only the dendritic-like cells were eliminated by the specific anti-dendritic cell antibody, whereas anti-Ia antiserum and complement eliminated both lymphoid and dendritic-like cells. It is likely that the $33D1^+$ cells within the islet cells are related to the Ia^+ cells described by Hart and Fabre in most nonlymphoid organs (22).

Dendritic cells *in vitro* express large quantities of I region determinants and are potent stimulators of mixed lymphocyte reactions (4, 5, 9, 16). A similar situation must exist *in situ*. Treatment of donor islets with anti-dendritic cell antibody and complement prior to transplantation prevented the rejection of islet allografts in 89% of the histoincompatible recipients for >65 days. Four transplant recipients were followed for >200 days, and all four animals remained normoglycemic. Successfully engrafted mice were challenged 65 days after transplantation with 1×10^5 donor cells enriched for dendritic cells and rejection was acutely induced in all four animals. The dose of enriched dendritic cells sufficient to induce rejection (1×10^5 per mouse) is notable because previous studies demonstrated that the rejection of established mouse islet allografts required $>1 \times 10^7$ donor cells if splenocytes were used 100 days after transplantation (1). Therefore, results of the present study demonstrate the potency of the dendritic cell preparations in eliciting graft rejections.

These *in vitro* and *in vivo* findings indicate that dendritic cells are a potentially important component of transplantation rejection reactions, and that the presence of dendritic cells alone within islets may be sufficient for the initiation of mouse islet allograft rejection.

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