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Prolongation of murine islet allograft survival by pretreatment of islets with antibody directed to Ia determinants

(diabetes/passenger leukocytes/transplantation/tissue culture)

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ABSTRACT **Islets of Langerhans treated with donor-specific anti-Ia serum and complement were transplanted across a major histocompatibility barrier into nonimmunosuppressed diabetic mice. The allografts survived in all recipients for at least 200 days after transplantation. Rejection of an established allograft could be induced by intravenous injection of donor splenocytes. This demonstrates that allografts can serve as targets for immune rejection and supports the possible role of Ia-positive passenger lymphoid cells in initiation of immune rejection. The results show that immunosuppression of the recipient is not a prerequisite for successful transplantation.**

The survival of islet of Langerhans allografts and xenografts can be prolonged by *in vitro* culture prior to transplantation (1–4). It has been suggested that organ culture removes passenger lymphoid cells and that these cells are required for immune rejection. Support for this idea comes from the observation that islets successfully transplanted into allogeneic recipients after culture are promptly rejected following injection of donor lymphoid cells (5). Similar prolongation of allografts by culture has been described for ovary and thyroid.

It has been demonstrated *in vitro* that stimulation of cell-mediated immunity depends on Ia-bearing macrophages or dendritic cells (6, 7). Furthermore, Ia antisera have been shown to enhance skin and probably kidney grafts (8–10). In view of our previous demonstration that beta cells lack Ia determinants (11), we have investigated the influence of Ia antisera on islet transplantation. Here we show that brief treatment of islets with donor-specific Ia antisera and complement *in vitro* permits transplantation across major histocompatibility barriers without immunosuppression of the recipient. This suggests that the passenger cell that is removed by culture bears Ia determinants.

MATERIALS AND METHODS

Mice. Recipients were C57BL/6J (H-2^b; B6) male mice made diabetic by the intravenous injection of streptozotocin (160 mg/kg). Islets for allografts came from male B10.BR/SgSnJ (H-2^k; B10.BR) mice; islets for isografts were from B6 male mice. All mice were from The Jackson Laboratory, Bar Harbor, ME.

Islet Isolation. Islets were isolated by the collagenase technique (12). The method was modified for mouse islets by directly injecting the pancreas at numerous sites with Hanks' balanced salt solution. Twenty mice were used for each isolation, with a yield of ≈50 islets per mouse. Isolated islets were separated on a Ficoll (Pharmacia) gradient (13). To distinguish islets from lymph nodes and other contaminating structures, the reflected green light technique (14) was used and the islets were hand picked with a dissecting microscope. Islets were transplanted either immediately or after exposure to antiserum and complement.

Islet Culture and Transplantation. The hand-picked clean islets that were to be incubated with antiserum and complement were placed in 6 × 50 mm glass tubes. These islets were cultured for 90 min at room temperature with anti-I^k or anti-I^s serum diluted 1:10 in Hanks' balanced salt solution/0.5% bovine serum albumin. Islets were washed twice and incubated for 30 min at 37°C with selected guinea pig serum diluted 1:3 (pooled, fresh frozen, GIBCO). The incubated islets were repicked before transplantation.

Treated and untreated islets were transplanted by the portal vein technique (15). Hand-picked islets (550–750) were suspended in tissue culture medium without fetal calf serum and injected into the portal vein of the diabetic recipients.

Antiserum. Anti-I^k serum (A.TH anti-A.TL) and anti-I^s serum (A.TL anti-A.TH) were produced as described (16). Both sera were tested against a panel of 35 independent and recombinant haplotypes to determine specificities of antibodies (Miroslav Hauptfeld, personal communication). Anti-I^k serum has a titer of 1:1024 and anti-I^s serum has a titer of 1:64–1:128 when assayed against donor splenocytes.

Criteria for Rejection. Nonfasting plasma glucose levels were determined three times a week on blood obtained from the orbital sinus of B6 diabetic animals and only mice with plasma glucose levels >400 mg % for three consecutive bleedings were used as recipients. Plasma glucose levels and body weights were determined three times a week before and after transplantation. Rejection was defined as a nonfasting serum glucose of >250 mg/dl for three consecutive bleedings.

Splenocytes for Induction of Rejection. Splenocytes (B10.BR) for intravenous injection into normoglycemic B6 mice with B10.BR transplants were prepared by mincing a spleen with scissors on an 80 mesh stainless steel screen, gently pressing the fragments through the screen into Hanks' balanced salt solution, and centrifuging the mixture. After centrifugation, erythrocytes were lysed by the addition of Tris-buffered isotonic ammonium chloride, and the suspension was washed twice in Hanks' balanced salt solution. Viability of the cells was determined by trypan blue exclusion, and 1–5 × 10^7 viable cells in 0.5 ml were injected into the tail vein.

RESULTS

Islet Isografts Survive and Restore Blood Glucose Levels to Normal. Isografts (B6 →B6) were performed to determine whether collagenase-isolated islets of Langerhans could be successfully transplanted into the portal vein of diabetic mice and restore the plasma glucose levels to normal. Diabetes was induced in recipient mice by intravenous injection of streptozotozin, a drug that permanently destroys the insulin-secreting

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Abbreviations: B6, C57BL/6J; B10.BR, B10,BR/SgSnJ.

beta cells of the pancreas. Nonfasting plasma glucose levels and body weight were the physiologic parameters chosen to monitor the diabetic condition.

Transplantation of 550–650 fresh isologous islets into the portal vein of diabetic mice resulted in return of the plasma glucose to normal levels within 7 days (Fig. 1, group 1). Histological sections of the liver showed that the transplanted islets lodged in the terminal branches of the portal vein. Islets transplanted in this fashion maintained normoglycemia for >200 days in all recipients (Table 1). The transplanted animals showed weight gains comparable with those of nondiabetic control siblings (data not shown).

Most Untreated Allografts Are Acutely Rejected. By using the transplant procedures used for isografts, untreated B10.BR islets were injected into the portal vein of diabetic B6 mice. Sixty percent of the recipients acutely rejected their islet allografts within 20 days (Fig. 1, group 2). Unexpectedly, two untreated allograft recipients remained normoglycemic for >200 days (Table 1). This is in marked contrast to untreated rat islet allografts, which are always rejected by day 9 (2). The occasional lack of rejection is possibly a result of selecting only islets free of acinar and ductal debris and using the green light technique, a procedure that allows identification and removal of lymphoid tissue (9). These procedures result in islets with fewer contaminating structures, which implies that clean islets may not be fully immunogenic. Histological sections of the liver of these normoglycemic mice showed granulated islets. In contrast, the livers of animals after acute rejection failed to show islets in the liver.

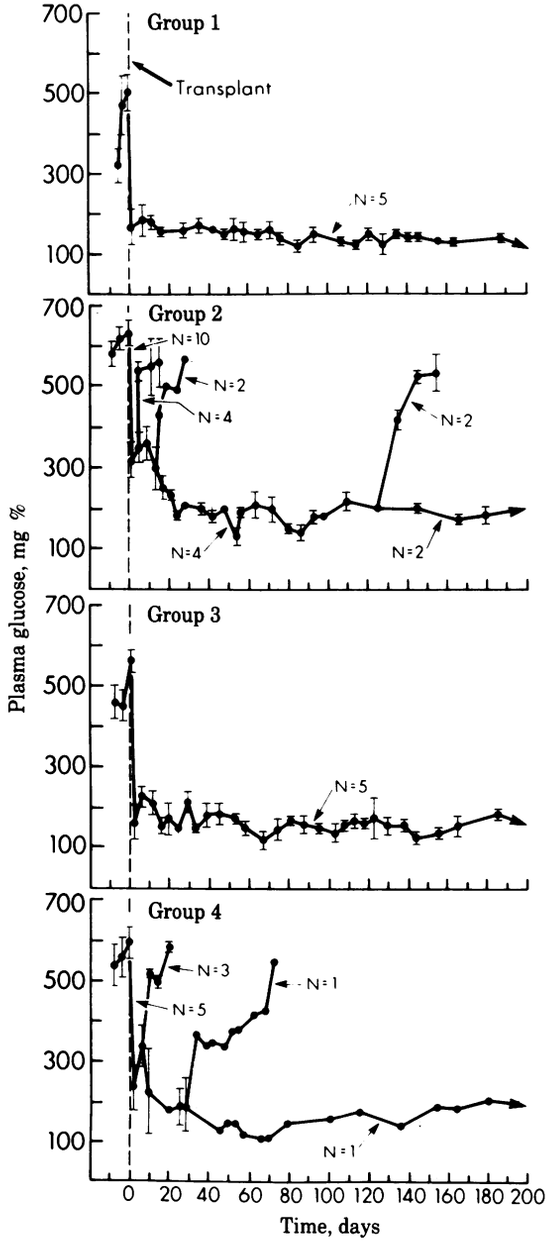

FIG. 1. Plasma glucose levels of diabetic B6 (H-2^b) mouse recipients after transplantation on day 0. Group 1, islet isografts (B6→B6); group 2, untreated fresh B10.BR (H-2^k) islets transplanted into B6 recipients; group 3, anti-I^k antiserum and complement-treated B10.BR (H-2^k) islets transplanted into B6 recipients; group 4, anti-I^s antiserum and complement-treated B10.BR (H-2^k) islets transplanted into B6 recipients.

Effect of Ia Antisera on Allograft Survival. Transplantation of B10.BR (H-2^k) islets treated with anti-I^k serum and complement resulted in allograft survival in all animals of >200 days (Table 1). The use of another anti-I^k serum produced in different strain combinations also resulted in allograft survival similar to that reported for A.TH anti-A.TL antiserum (data not shown). The nonimmunosuppressed recipients became normoglycemic 10 days after the transplant (Fig. 1, group 3) and gained weight comparable with that of isograft controls (data not shown). On the other hand, treatment of B10.BR islets with anti-I^s antiserum and complement did not alter the rapid rejection of 60% of the allografts (Fig. 1, group 4). Table 1 shows that there is no difference between those groups that received no treatment and those that were treated with anti-I^s antiserum and complement.

The effectiveness of donor-specific Ia antiserum in facilitating transplantation of allografts could be because an Ia-bearing passenger cell is required to stimulate allograft rejection or because the beta cells themselves are altered by this treatment. The latter alternative is less likely because of our demonstration that beta cells lack detectable Ia determinants. Two animals with established allografts were given intravenous injections of lymphoid cells (Fig. 2). Both exhibited prompt rejection of the islets after two injections of cells. Therefore, the islets, although incapable of stimulating allograft rejection by themselves, can serve as targets of an ongoing response.

DISCUSSION

Our results indicate that pretreatment of allogeneic mouse islets with donor-specific Ia antiserum and complement results in the prevention of their immune rejection in nonimmunosuppressed mice for at least 200 days after transplantation.

The exact cellular target of the Ia antiserum and complement is unknown but Ia-bearing cells have long been thought to be central to regulation of the immune response, particularly with regard to antigen presentation. I region-controlled T cell-proliferation responses (17) and T cell-dependent *in vitro* antibody responses (18) can be inhibited by Ia antisera but not by H-2K or H-2D antisera. Furthermore, recent studies have shown that antigen-induced proliferation of T cells can be inhibited by monoclonal antibody directed against Ia determinants on antigen-presenting cells (19). These experiments provide further support for the hypothesis that the Ia antigens on antigen-presenting cells are products of the *Ir* gene.

In 1957, Snell suggested that passenger leukocytes present in allografts might play a significant role in the initiation of immune rejection of the transplanted organ (20). The survival of islet of Langerhans and thyroid allografts can be prolonged by *in vitro* culture prior to transplantation (2, 4). It has been suggested that organ culture removes passenger lymphoid cells and that these cells are required for immune rejection. According

Table 1: Effect on murine islet allograft survival of pretreatment of islets with antibody directed to Ia determinants

Group	Type	Islet treatment	Individual transplant survival, days*	Survival after transplantation, % 10 days	20 days	30 days	40 days	200 days	Transplant survival*
1	B6→B6	None	>200, >200, >200, >200, >200	100	100	100	100	100	>200
2	B10.BR→B6	None	5, 5, 6, 6, 8, 12, 135, 139, >200, >200	100	40	40	20	20	>72 ± 27
3	B10.BR→B6	αI^k + C	>200, >200, >200, >200, >200	100	100	100	100	100	>200
4	B10.BR→B6	αI^s + C	6, 8, 10, 28, >200	100	40	20	20	20	>50 ± 38

Transplant survival was determined by plasma glucose levels. Rejection of transplanted islets was considered to have occurred when individual serum glucose levels were >250 mg % for three consecutive bleedings. C, complement.
* Results are mean ± SEM.

to Sollinger and Bach, two populations of T lymphocytes are generated *in vitro* in response to an alloantigeneic stimulus (21). One population, composed of proliferating T helper cells, responds primarily to Ia antigens, whereas cytotoxic T lymphocytes are activated by H-2K or H-2D antigens. Lafferty also proposed a two-signal hypothesis to explain T-cell responses to alloantigen (22) but, according to his model, induction occurs when the responsive T cell binds antigen (H-2K or H-2D) to its surface receptor and simultaneously receives an inductive signal from an Ia-bearing lymphoreticular cell. Delivery of both signals to the same cell results in T-cell activation. Both theories suggest that, if Ia differences are not present in the graft, the generation of cytotoxic T lymphocytes will not occur.

Because mouse β-cells lack Ia antigens (11), our results suggest that Ia antiserum and complement treatment of islets removes an Ia-bearing passenger cell that is necessary for cytotoxic T-lymphocyte generation. Recently, in preliminary *in vivo* and *in vitro* experiments using successfully transplanted rat islets (1-week culture of donor islets and a single injection of antilymphocyte antiserum), we found evidence of donor haplotype-specific tolerance, probably attributable to active suppression of rejection (23). Similarly, Greene *et al.* (24) found that adherent cells from UV-irradiated mice, which no longer were efficient stimulators of delayed hypersensitivity responses, stimulated antigen-specific suppressor T cells instead. UV irradiation is known to preferentially affect antigen-presenting cells (25). These and other studies show that inappropriate presentation of antigen may lead to suppression.

Finally, the prevention of immune rejection of islet allografts by brief pretreatment of the graft with antisera is an attractive method potentially applicable to a variety of organs. In addition, the elimination of the need for immunosuppression of the host

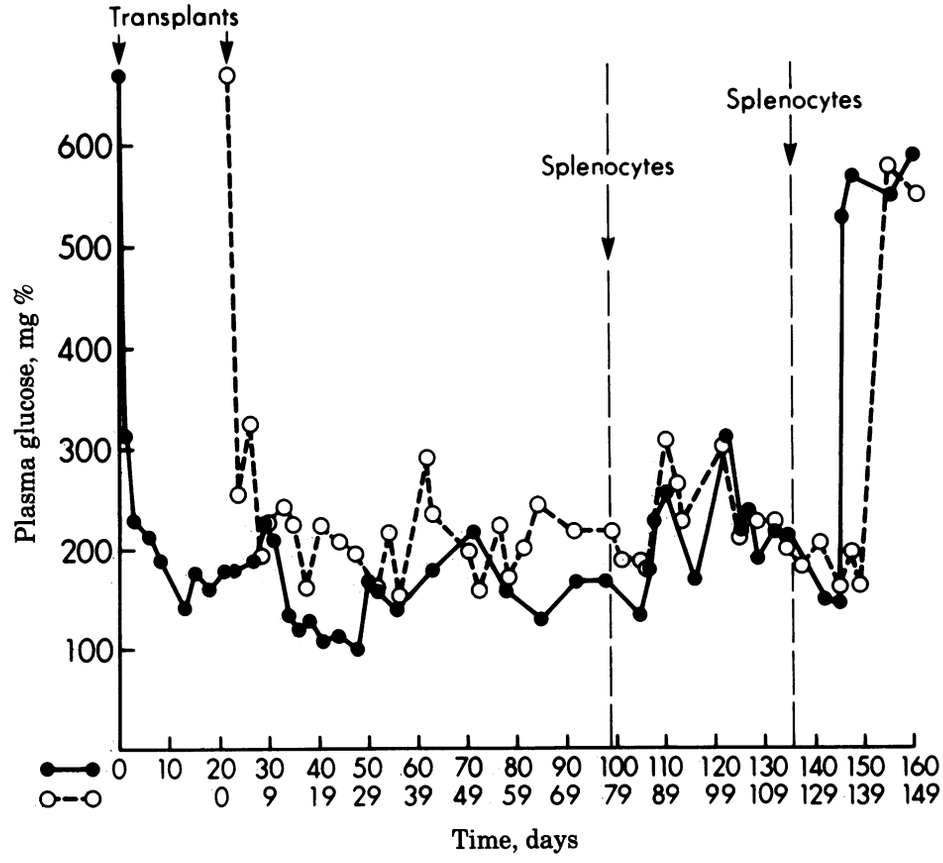

FIG. 2. Induction of islet rejection with B10.BR donor splenocytes. Two animals received splenocyte injections, a group 2 animal (○) that had received fresh untreated islets 78 days earlier and a group 3 animal (●) that had received Ia antiserum and complement-treated islets 99 days earlier. Each animal received two splenocyte injections, one of 1×10^7 and one of 5×10^7 cells.

is an important advance, particularly with regard to the eventual application of islet transplantation to the treatment of diabetes in man.

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