Cross-Reactivity of Organs in Allograft Rejection

Comparison of Effect of Thyroid Allografts on Established Islet Allografts

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SUMMARY

The effect of allotransplantation of thyroid or islet allografts into rats with established islet allografts was studied to determine the cross-reactivity of the thyroid and islets in allograft rejection. Islets obtained from cultured neonatal rat (F344) pancreas explants were transplanted bilaterally underneath the kidney capsule of Wistar-Furth rats. After 21 days these allografts did not exhibit signs of rejection. Thyroid (half lobe) from either F344 or Brown Norway rats was transplanted underneath the capsule of the remaining kidney. Transplant of the thyroid from F344 rats resulted in immediate rejection of the islet transplant, whereas transplant of the thyroid from Brown Norway rats was without effect on the islet allograft. This indicates that the thyroid contains immunocompetent cells (cells that present antigen or induce recognition of antigen) that are capable of initiating rejection of established islet allografts. The cytotoxic T-lymphocytes that result are specific for the organ bearing the immunocompetent cells at time of transplantation. *Diabetes* 36:1268–70, 1987

Recent developments in the understanding of the mechanism of allograft rejection and in the ability to render significant quantities of isolated islets nonimmunogenic have resulted in renewed interest in islet transplantation as a cure for diabetes. Theoretically, it will be possible to transplant sufficient quantities of islets to cure the diabetes without the need for immunosuppression to prevent rejection (1–4). Allograft rejection is apparently avoided by removing immunocompetent cells (dendritic cells or passenger leukocytes) from the isolated islets. These lymphoreticular cells may be responsible for presentation of antigen and/or production of a costimulator necessary for processing of antigen by host lymphocytes. Long-term survival of islet and thyroid allografts devoid of these dendritic cells has been reported (1–6).

Because islet transplantation in humans may be accompanied by renal transplantation, interaction of different organs in allograft rejection is important (7–9). Maneuvers to produce islet allografts that are nonimmunogenic may be futile if dendritic cells from other organs are capable of nonspecific initiation of allograft rejection (10–20). Observations that donor spleen cells accelerate rejection of allografts and that sensitized spleen cells initiate allograft rejection do not completely answer the question of organ specificity in the initiation of rejection. Antigen recognition or presentation is required for sensitization and may have specificity not observed in sensitized lymphocytes. The response of the host to the antigen-presenting cells might also be modified if these cells are contained in the whole organ rather than in a dispersed state. Although the cross-reactivity of different organism rejection in a sensitized host has been studied extensively, the interaction of different organs in the initiation of allograft rejection has not been well studied. We use combinations of thyroid and islet allografts that are incompatible at the major histocompatibility complex (MHC) locus to study the effect of thyroid allografts on the initiation of rejection of established islet allografts.

MATERIALS AND METHODS

Islet isolation. Islets were isolated from the neonatal rat pancreas by the method of Hegre et al. (17). Pancreases from a litter of newborn rats were placed in a concave dish with a small amount of sterile Hanks' balanced salt solution and minced with surgical blades, but not so vigorously as to decrease the yield of islets. The minced tissue was distributed to 35-mm plastic dishes and cultured in 95% air/5%

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CO_2 at 37°C in Ham's F12 medium with 5% newborn calf serum. After 7 days in culture, islets separated from the tissue pieces. Islets were then handpicked (with a siliconized capillary suction pipette) from harvested tissue pieces and subcultured in fresh media for 24 h. The free cells and non-islet debris attached to the dish surface. After 24 h, the medium was swirled to bring the free-floating islets to the center of the dish, where they were handpicked with the aid of an inverted microscope. This islet preparation was purified further by handpicking and subculturing in a 60-mm petri dish containing Ham's F12 medium with 25% fetal calf serum. In this medium, the islets remain free floating with minimal attachment. After 10 days of subculturing, the islets were handpicked before transplantation.

Islet and thyroid transplantation. Rats were anesthetized, and the kidney was exteriorized through a lateral lumbar incision (18). With a dissecting microscope, a small slit was made in the kidney capsule. Islets were drawn into a small siliconized pipette. The pipettes were formed by drawing out the end of a Pasteur pipette into a small-bore capillary tube with a 90° bend at the junction of the capillary portion and the body of the Pasteur pipette. The tip of the capillary tube was inserted under the kidney capsule, a pocket was formed at the tip by gentle manipulation, and fluid and islets were expelled into the pocket. In nondiabetic rats, 40–50 islets form a sufficient mass to determine if rejection has occurred.

The lobes of the thyroid were identified through a midline ventral neck incision and dissected free of connective tissue. The lobe was excised and placed in cold (4°C) culture medium. One-half of each lobe was inserted underneath the capsule of the kidney through a small incision in the capsule.

In these experiments, syngeneic (control) and allogenic transplants of Fischer (F344, $RT1^l$) rat islets were performed bilaterally under the kidney capsule of nondiabetic female F344 or Wistar-Furth ($RT1^u$; for allogenic transplants) recipients. The islets were allowed to establish for 20–30 days (to rule out indolent rejection) and a unilateral nephrectomy was performed to assess the viability and status of the islets. A section of the kidney containing the graft was prepared for sectioning. This tissue was sectioned serially, and every fifth section (5 mm) was stained with hematoxylin and eosin, aldehyde fuchsin, or immunoperoxidase for insulin. At the time of nephrectomy, a syngeneic or allogenic transplant of thyroid [F344 for allogenic transplants and Brown Norway ($RT1^n$) for third-party transplants] was performed in the remaining kidney. The recipient rats were killed at 4 or 10 days, and the transplanted thyroid and islets were examined for evidence of rejection. Rejection was classified by two unbiased observers according to the morphology of sections stained with hematoxylin and eosin or aldehyde fuchsin (aldehyde fuchsin staining was confirmed by immunoperoxidase staining for insulin on selected sections).

RESULTS

The comparisons made in these experiments are given in Table 1. The scale was: 0, no lymphocytes in allograft and viable allograft (aldehyde fuchsin positive); 1, peripheral lymphocyte accumulation surrounding the viable allograft (aldehyde fuchsin positive); 2, lymphocyte infiltration of allograft but viable allograft remaining (aldehyde fuchsin positive); 3, complete infiltration of allograft by lymphocytes and no viable allograft remaining evident (aldehyde fuchsin negative); and 4, complete sclerosis. The mean rating was used to compare different grafts. The islet and thyroid isografts all had ratings of 0 at 21 and 10–14 days after transplantation, respectively. Islet isografts were not affected by subsequent syngeneic thyroid transplants. Placement of the thyroid allograft at the same site of the islet isograft did not result in infiltration of the islet isograft by lymphocytes, even though complete rejection of the thyroid allograft had taken place.

Islet allografts examined 4 days after thyroid allotransplantation had a mean rating of 1, whereas the thyroid allograft had a mean rating of 2.8. Islet allografts examined 10 days after thyroid had a mean rating of 2.5, whereas the thyroid allografts had a mean rating of 3. With third-party thyroid allografts, one of six islet allografts exhibited signs

TABLE 1
Effect of thyroid allografts on established islet allografts

Donor/recipient	*n*		Days	Rating
F344/F344	6	Islet isograft before thyroid isograft	21	0, 0, 0, 0, 0, 0
		Islet isograft after thyroid (F344) isograft	10†	0, 0, 0, 0, 0, 0
		Thyroid isograft	10	0, 0, 0, 0, 0, 0
F344/F344	4	Islet isograft before thyroid allograft	21	0, 0, 0, 0
		Islet isograft after thyroid allograft (WF/F344)*	14†	0, 0, 0, 0
		Thyroid allograft (WF/F344)	14	4, 4, 4, 4
F344/WF	6	Islet allograft before thyroid allograft	7	0, 0, 0, 0, 0, 0
		Islet allograft after thyroid allograft (F344/WF)	4†	0, 0, 0, 2, 3, 1
		Thyroid allograft (F344/WF)	4	3, 3, 2, 3, 3, 3
F344/WF	6	Islet allograft before thyroid allograft	21	0, 0, 0, 0, 0, 0
		Islet allograft after thyroid allograft (F344/WF)	10†	3, 2, 3, 2, 3, 3
		Thyroid allograft (F344/WF)	10	3, 3, 3, 3, 3, 3
F344/WF	6	Islet allograft before third-party thyroid allograft	21	0, 0, 0, 0, 0, 0
		Islet allograft after third-party thyroid allograft (BN/WF)	10†	0, 0, 0, 3, 0, 0
		Third-party thyroid allograft (BN/WF)	10	3, 3, 3, 3, 2, 2

F344, Fischer rats; WF, Wistar-Furth rats; BN, Brown Norway rats.
*Thyroid allograft placed next to islet isograft.
†Time after thyroid allograft; total time of engraftment includes time before thyroid transplantation.

of rejection. In the instance of rejection, lymphocytes were present around the islet allograft before the thyroid allotransplantation, suggesting an indolent rejection in this islet allograft.

DISCUSSION

Classically, class I antigens and Lyt2$^+$ cells (cytotoxic subset) are involved in rejection of islets and thyroid allografts (21). This contrasts with kidney and skin, in which class II antigens are more important in allografts. By considering the T-lymphocyte subset responsible for allograft rejection, cross-reactivity among allotransplant rejections might be predicted, but these predictions have not been tested experimentally. The data are sufficiently controversial so that predictions without experimental verification might be misleading (8,21,22). Rejection of established allografts can be accomplished by adoptive transfer of sensitized lymphocytes, but this approach may ignore the role of dendritic cells in the rejection process (23,24).

Because of the renewed interest in islet transplantation as a cure for diabetes and the elucidation of the role of dendritic cells in initiating allograft rejection, we have developed a study to explore the cross-reactivity of different organs in allograft rejection. Neonatal islet allografts were accepted without evidence of rejection. When these were followed with thyroid allografts that were syngeneic to the islet allograft, rejection of the islet allografts occurred but required 10–14 days to complete. Thyroid allografts (third party) that were different from either the islet donor or recipient at the MHC in most instances did not initiate rejection of the established islet allograft within 10 days after thyroid transplantation. This has obvious implications in the choice of donors for multiple organ transplants into a single individual.

The research suggests that the immunocompetent cells of one organ are capable of initiating rejection of another organ. Whether this has any relation to the class of antigen expressed on the parenchymal cells remains to be determined. As a minimum, the organs or immunocompetent cells of the organs must have the same MHC for the cross-reactivity in allograft rejection to occur. If the same cross-reactivity occurs between renal and islet allografts, maneuvers to remove the immunocompetent cells from islets before transplantation may not be helpful if a simultaneous or subsequent renal transplant that is syngeneic to the islets takes place. On the other hand, if the recipient has an established renal allograft, it may be imperative to remove the immunocompetent cells from the islets to prevent initiation of an established renal allograft. The lack of cross-reactivity between organs from MHC-incompatible animals suggests that this might also be used to improve the success of multiple organ transplants into a single individual.

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