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20. Complementary DNA from *mbm2* mRNA was cloned into the plasmid pCD as previously described (*11*). For selection purposes, the dihydrofolate reductase (DHFR) transcription unit, derived from pFR400 (*28*), was also inserted into this vector. This construct was cotransfected with pSV2neo into F-MEL cells by electroporation, and transformants were selected by sequential growth in media containing geneticin and methotrexate. Individual transfected clones were obtained by limiting dilution.
21. pSst$^-$ was generated by partial digestion of pMbm2 with Sst I, removal of the protruding ends with T4 polymerase, and subsequent blunt-end re-ligation. Absence of the Sst I restriction site was verified by digestion with Sst I, Eco RI, and Cla I. The plasmid was transfected into F-MEL cells as described (*20*).
22. Mean values for percent of differentiated cells (benzidine-positive) after transfection with Eco RI–linearized pMbm2. Individual clones were treated with DMSO and compared to wild-type F-MEL cells; 0% DMSO, 3% benzidine-positive ($P = 0.55$); 0.5% DMSO, 21% benzidine-positive ($P = 0.79$); 1.5% DMSO, 35% benzidine-positive ($P = 0.78$).
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Induction of Donor-Specific Unresponsiveness by Intrathymic Islet Transplantation

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The application of isolated pancreatic islet transplantation for treatment of diabetes mellitus has been hampered by the vulnerability of islet allografts to immunologic rejection. Rat islet allografts that were transplanted into the thymus of recipients treated with a single injection of anti-lymphocyte serum survived indefinitely. A state of donor-specific unresponsiveness was achieved that permitted survival of a second donor strain islet allograft transplanted to an extrathymic site. Maturation of T cell precursors in a thymic microenvironment that is harboring foreign alloantigen may induce the selective unresponsiveness. This model provides an approach for pancreatic islet transplantation and a potential strategy for specific modification of the peripheral immune repertoire.

TRANSPLANTATION OF ISOLATED pancreatic islets is the most specific therapy for insulin-dependent diabetes mellitus (*1*). However, despite immunosuppression, no human islet allografts have escaped rejection, possibly because cellular allografts such as bone marrow or islets are more vulnerable to rejection than vascularized organ allografts such as kidney, liver, or whole pancreas (*2*). Greater success has been achieved in experimental animals by chronic administration of potent immunosuppressants or by reducing the immunogenicity of the graft before transplantation (*3, 4*). The latter can be achieved by prolonged tissue culture, ultraviolet irradiation, or treatment of islets with specific antibodies to delete or inactivate intra-islet antigen presenting cells (APCs) (*1, 3, 5*). An alternative strategy would be transplantation to an immunologically privileged site. Thus far only the brain and the testicle have shown promise in this regard (*6, 7*). Our data support the thymus as a new immunologically privileged site for islet transplantation. The prolonged residence of allogeneic islets in the thymus also induced unresponsiveness to donor islets transplanted extrathymically.

Islets were isolated from Lewis (RT1^l) or DA(RT1^a) donors and transplanted to major histocompatibility complex (MHC) incompatible Wistar Furth (WF,RT1^u) recipients (8 to 10 weeks old) in which diabetes (blood glucose > 300 mg/dl) had been induced 2 to 3 weeks earlier with intravenous streptozotocin (65 mg per kilogram of body weight). Islets (1000 to 1200) were inoculated into conventional islet transplant sites (beneath the renal capsule or into the liver, via the portal vein) (*8*), into a known privileged site (the testicle), or into the thymus by direct injection of 500 to 600 islets into each lobe. In some instances freshly isolated islets were transplanted, whereas in others islets were maintained in tissue culture for 14 days before transplantation (to delete intra-islet APCs). Where noted, recipients received a single intraperitoneal injection (1 cm^3) of rabbit antiserum to rat lymphocytes (ALS) at the time of islet transplantation. Islet grafts were considered technically successful if the nonfasting blood glucose returned to normal (<200 mg/dl) within 2 to 3 days. Rejection was diagnosed by subsequent recurrence of hyperglycemia (>200 mg/dl) and confirmed by histological examination of the graft.

All transplants of freshly isolated (non-APC–depleted) Lewis islets were rejected promptly by nonimmunosuppressed WF rats if transplanted to the liver or renal subcapsule [median survival times (MST), 8 and 9.5 days respectively; Table 1]. Survival of freshly isolated islets in the thymus was prolonged (MST, 17 days), and one of seven such islet allografts survived permanently.

Administration of ALS (1 cm^3) at the time of transplantation delayed rejection of freshly isolated islets transplanted to the liver (MST, 29 days) or renal subcapsule (MST, 47 days). Although rejection was further postponed by transplantation to a clas-

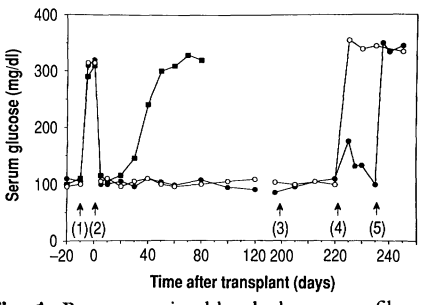

Fig. 1. Representative blood glucose profiles of WF rats transplanted with freshly isolated pancreatic islets in various sites. (1) Streptozotocin administration. (2) Transplantation of fresh Lewis islets (■–■, intraportal; ●–●, intrathymic, animal #1; ○–○, intrathymic, animal #2). All animals received ALS (1 cm^3) at time of transplantation. (3) Second renal subcapsular transplants of either Lewis (●–●) or DA (○–○) islets. (4) Thymectomy. (5) Removal of islet bearing kidneys.

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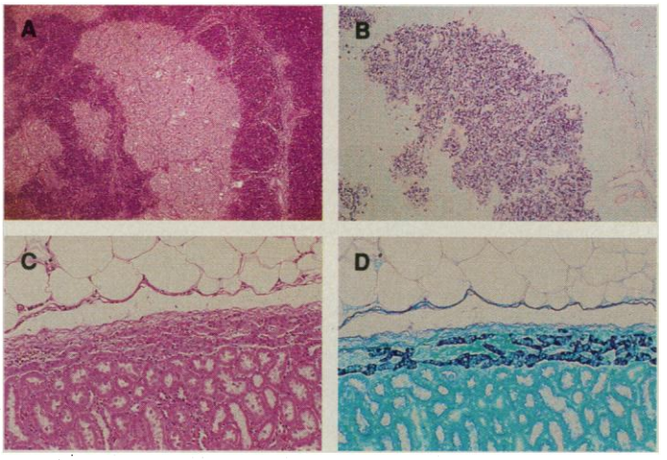

Fig. 2. Photomicrograph of thymus and kidney from WF recipients of Lewis islet allografts. (**A**) Section from a thymectomy specimen of a WF recipient 200 days after intrathymic Lewis islet transplantation. Several islands of endocrine tissue without evidence of intra-islet mononuclear infiltration are scattered throughout the thymic stroma (H & E, original magnification, ×20). (**B**) Insulin-specific staining of thymus from the same recipient showing abundant insulin granules (Scott stain, ×25). (**C**) Section of kidney from the WF animal in (A) that received a second renal subcapsular fresh Lewis islet allograft (H & E, ×50). Nephrectomy was performed 6 weeks after the second islet transplant and 2 weeks after thymectomy. Islet beta cells in subrenal capsule are verified by staining for insulin granules (**D**) (Scott, ×50).

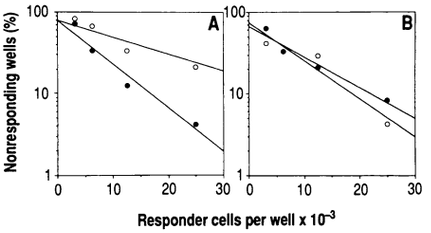

Fig. 3. Effect of intrathymic islet transplantation on CTLp frequencies. Limiting numbers of WF lymph node cells (24 wells per group) were cocultured with irradiated (2000 R) donor islet strain Lewis or "third party" DA lymph node stimulators (5×10^5 cells per well) in the presence of 15% α-methyl mannoside–treated supernatant from concanavalin A (Con A)–stimulated rat spleens. After 7 days of culture, specific cytotoxic activity was assessed in a 4.5-hour ^{51}Cr release assay with Con A spleen cell blast targets (1×10^4 per well). Spontaneous release was determined by addition of target cells to wells containing stimulators only. Wells were considered positive if ^{51}Cr release values were >3 SD above mean spontaneous release. Estimates of CTLp frequencies were derived by linear regression analysis (*24*). A representative experiment of three performed is shown. (**A**) Anti-Lewis CTLp of control WF (●–●, $f = 1/6240$) and WF recipients of intrathymic Lewis islets (○–○, $f = 1/16{,}000$). (**B**) Anti-DA CTLp of control WF (●–●, $f = 1/6930$) and WF recipients of intrathymic Lewis islets (○–○, $f = 1/6500$). Average CTLp frequencies ± SD of the three experiments were: Anti-Lewis control WF (6020 ± 2332) and WF recipients of intrathymic Lewis islets (13467 ± 2170). Anti-DA control WF (5690 ± 1098) and WF recipients of intrathymic Lewis islets (6400 ± 374). Coefficients of determination (r^2) ranged from 0.92 to 0.99.

sic immunologically privileged site (testicle), only two of six such allografts survived for 200 days, while the others were rejected after 50 to 110 days. Intrathymic allografts were superior, with 10 of 13 such grafts (including the last 10) surviving permanently.

Prolonged exposure to an allogeneic graft maintained by immunosuppression will sometimes diminish the host response to subsequent unprotected donor strain allografts (*1, 3, 9–12*). To determine whether a similar unresponsive state had developed in our model, four WF rats with long-term (>200 days) intrathymic Lewis islets received freshly isolated Lewis islet allografts under the renal capsule. No immunosuppression was given. In no instance were either the established or the new islet allografts rejected. Graft survival was confirmed by removing the thymus 21 days after the second transplant (which did not cause hyperglycemia), followed by removal of the islet-bearing kidney 2 weeks later (which did provoke hyperglycemia) (Fig. 1). Histologic examination of both the excised thymus and kidney revealed the presence of healthy islets containing abundant, well-granulated beta cells (Fig. 2). In three other rats harboring long-term intrathymic Lewis islets, "third party" DA renal subcapsular islet transplants were not accepted. This was demonstrated by histology and by performing a thymectomy 21 days after grafting, which resulted in hyperglycemia. The protection afforded to Lewis renal subcapsular islets by the intrathymic graft was unusual, because in all previous experiments by us and reports by others, recipients induced to accept allogeneic islets have failed to accept subsequent transplants of non-APC–depleted donor islets unless immunosuppression was continued after the second graft (*11, 12*). Allografts implanted successfully in privileged sites are usually rejected if second grafts of donor strain tissue are transplanted to a nonprivileged site in the same host (*13*).

We determined whether the development of peripheral unresponsiveness in animals bearing intrathymic islets was due to the unique immunological characteristics of the transplant site. The testicle was first selected as a site that should support permanent survival of fresh islets because of its established status as a privileged site (*13*). However, this was an unsatisfactory control for the thymus because only two of six WF rats retained their intratesticular fresh Lewis islet grafts for >200 days. In these two animals, second fresh Lewis islet allografts transplanted under the kidney capsule provoked rejection of both the intratesticular and renal subcapsular grafts in 10 and 15 days.

Identification of a proper control was difficult, because (except for implantation in the thymus) no method allows permanent survival of fresh islet allografts in nonimmunosuppressed rats. However, pretransplant in vitro culture of islets at 24°C permits their long-term (>200 days) survival under the kidney capsule of rats treated with a single injection (1 cm^3) of ALS (*1, 8*). Therefore, this model was used as an additional control for intrathymic islets in order to assess the effect of long-term residence of allogeneic islets on peripheral immune responsiveness. In three WF recipients that had maintained successful cultured Lewis islet allografts under the kidney capsule for more than 200 days, noncultured Lewis islets were transplanted to the opposite kidney. Although all rats remained normoglycemic after this procedure, removal 30 days later of the kidney that bore cultured islets resulted in hyperglycemia, demonstrating rejection of the second islet graft.

To examine mechanisms by which in-

Table 1. Survival of fresh Lewis islet allografts in WF recipients.

Site of islet transplantation	Individual graft survival (days)	
	Without ALS	1 ml of ALS*
Liver (intraportal)	5, 8, 8, 9 (8)†	6, 22, 29, 35, 36 (29)
Renal subcapsule	9, 9, 10, 13 (9.5)	27, 33, 38, 47, 61, >200 × 2 (47)
Thymus	13, 13, 16, 17, 17, 18, >200 (17)	28, 33, 57, >200 × 10 (>200)‡
Testicle		50, 50, 76, 110, >200 × 2 (76)

*Accurate Chemical & Scientific Co., Westbury, New York. †Median survival times. ‡Three recipients of intrathymic islets that were not used for further transplantation experiments remain normoglycemic after 17 months.

trathymic islet allografts modified responsiveness to donor alloantigens, we performed several in vitro functional and phenotypic analyses of T lymphocytes. Fluorescence-activated cell sorter (FACS) analysis of lymphocyte populations revealed no differences between thymic allograft recipients and normal controls or hosts of long-standing intratesticular or renal subcapsular allografts (*14*). Similarly, mixed lymphocyte culture responses of T lymphocytes from islet allograft acceptors to donor strain stimulator cells were indistinguishable from responses to "third party" stimulators (*15*). However, limiting dilution analysis of lymph node cells revealed a significantly reduced (40 to 60%) precursor frequency of cytotoxic T lymphocytes (CTLp) to donor strain alloantigen (Lewis) in recipients of intrathymic grafts as compared to untransplanted controls (Fig. 3), suggesting that deletion or functional inactivation of class I–restricted RT1[l] reactive T lymphocytes had occurred. In the same recipients the CTLp frequency for DA alloantigens was unchanged.

Our finding that the thymus is a privileged transplant site was unexpected because, unlike privileged sites such as the brain and the hamster cheek pouch, the thymus does not lack efferent lymphatics (*13, 16*). However, thymic cortical vessels contain numerous tight junctions between endothelial cells that do not permit extravasation of particulate dyes or radiolabeled circulating cells (*17*). In addition, mature T cells rarely return to the thymus, thus making it relatively protected from immune surveillance (*18*). Though these anatomic and physiological features might account for survival of allografts implanted in the thymus, they would not explain the favorable influence on allografts subsequently implanted in extrathymic sites. We hypothesize that this influence may be attributable to the role of the thymus in the induction of self tolerance. Allogeneic thymus transplants are capable of inducing tolerance, as can intrathymic inoculation of allogeneic thymus stem cells in lethally irradiated mice that are reconstituted with syngeneic bone marrow (*19–21*). Our data indicate that allogeneic cells of neither thymic nor lymphoid origin, if present in the thymus, can induce unresponsiveness, and that this may depend on deletion, inactivation, or both.

ALS depletes the peripheral circulation of T cells and accelerates the kinetics of stem cell traffic into the thymus (*22, 23*). In rats injected once with ALS, the peripheral T cell population is transiently (14 days) depleted to 10% of normal levels as assessed by FACS analysis (*14*). Subsequent reconstitution of the peripheral T lymphocyte pool in these animals presumably requires that prothymocytes migrate to and mature in the thymus. In our experiments, prothymocytes were forced to mature in a thymus containing cells expressing foreign MHC antigens.

These studies demonstrate that pancreatic islet allografts transplanted into the thymus survive indefinitely and are capable of inducing donor-specific unresponsiveness. This approach offers a novel strategy for successful pancreatic islet transplantation and may be relevant to our understanding of the mechanisms involved in the development of tolerance.

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LFA-3, CD44, and CD45: Physiologic Triggers of Human Monocyte TNF and IL-1 Release

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The monocyte-derived cytokines, tumor necrosis factor–α (TNF-α) and interleukin-1β (IL-1β), are central regulators of the immune response, but the physiologic stimuli for their release remain largely undefined. Engagement of three monocyte glycoproteins, LFA-3, CD44, and CD45, by specific monoclonal antibodies immobilized on plastic induced TNF-α and IL-1β release. In addition, TNF-α was released when monocyte LFA-3 bound immobilized, purified CD2, which is its physiologic receptor. Thus, a receptor-ligand interaction that mediates cell-cell adhesion can transmit the necessary signals for the release of monokines.

MONOCYTES PRODUCE A VARIETY of soluble mediators (monokines) that can influence surrounding cells. Among such mediators are TNF-α and IL-1β, both of which are important in the initiation and regulation of the immune response (*1, 2*). Although monocytes release TNF-α and IL-1β after stimulation with endotoxin, phorbol myristate acetate (PMA), or Gram-positive bacterial components (*2, 3*), other more physiologic mechanisms for induction of monokine release must also exist, because monokine production can be induced under conditions that exclude these factors. One such physiologic mechanism may be via cell-cell contact. For example, TNF-α is elicited when monocytes contact tumor cells (*4*), and IL-1β, which facilitates T cell proliferation, is released during antigen presentation to T cells (*2*). We therefore attempted to define the mechanisms by which monocyte interaction with other cells might induce monokine release.

Receptor-ligand interactions between surface molecules on monocytes and other cells are likely to trigger this release of monokines. The binding of a monocyte surface receptor with specific monoclonal antibody (MAb) immobilized on plastic might mimic the effects of receptor engagement with its

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