

LONG-TERM SURVIVAL OF DONOR-SPECIFIC PANCREATIC ISLET XENOGRAFTS IN FULLY XENOGENEIC CHIMERAS (WF RAT → B10 MOUSE)^{1,2}

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We recently reported that reconstitution of lethally irradiated B10 mouse recipients with 40×10^6 untreated WF rat bone marrow cells resulted in stable fully xenogeneic chimerism (WF rat → B10 mouse). In these animals, the tolerance induced for skin xenografts was highly MHC specific in that donor-specific WF rat skin grafts were significantly prolonged while MHC-disparate third-party xenografts were rapidly rejected (median survival time [MST] = 9 days). We have now examined whether islet cell xenografts placed under the renal capsule of chimeras rendered diabetic with streptozotocin would be accepted and remain functional to maintain euglycemia. Animals were prepared, typed for chimerism at 6 weeks, and diabetes induced with streptozotocin. Donor-specific WF (Rt1A^a) islet cell xenografts were significantly prolonged (MST >180 days) in WF → B10 chimeras, while MHC-disparate third-party F344 rat (Rt1A^b) grafts were rejected with a time course similar to unmanipulated B10 mice (MST=8 days). The transplanted donor-specific islet cells were functional to maintain euglycemia, since removal of the grafts at from 100 to 180 days in selected individual chimeras uniformly resulted in return of the diabetic state. These data suggest that donor-specific islet cell xenografts are accepted and remain functional in mice rendered tolerant to rat xenoantigens following bone marrow transplantation.

Great advances in the field of transplantation have occurred in the last two decades. The availability of nonspecific immunosuppressive agents has allowed pancreatic transplantation to become an accepted clinical modality for treatment of diabetes. The demand for grafts already exceeds the supply: as of December 1990, 477 patients were awaiting a pancreatic graft (1). However, a number of technical limitations are associated with transplantation of the whole pancreas, resulting in side effects including abscess formation, anastomotic leaks, and rejection. In recent years, the development of procedures to isolate large numbers of purified human pancreatic islets has made it possible to initiate a new phase of clinical trials in pancreatic islet cell transplantation (2-7). Transplantation of pancreatic islet cells is now the most specific replacement therapy for treatment

of type 1 insulin-dependent diabetes. Because only 1% of total pancreatic tissue is required to cure diabetes (the islet cells), transplantation of purified, pancreatic islet cells overcomes the associated limitations encountered with transplantation of the whole pancreas.

It is well recognized that both the whole pancreas and pancreatic islet cells are highly antigenic (2-14). Although nonspecific immunosuppressive agents have been instrumental in control of alloreactivity to transplanted islet cells, rejection often still occurs (8-10). Attempts to decrease antigenicity of islet cell grafts using in vitro pretreatment of grafts with monoclonal antibodies (11-14), in vitro culture protocols (5, 15-17), ultraviolet irradiation (18-20), microencapsulation (21-24), and isolation of hand-picked pure islet cells (25, 26) have resulted in some improvement in graft survival but have not reliably prevented rejection. Two limitations therefore exist: the supply of grafts does not meet the demand, and rejection remains a problem.

The induction of donor-specific transplantation across a species barrier, using bone marrow stem cells to produce chimerism, has been suggested as a potential approach to prevent rejection of transplanted pancreatic islet xenografts and overcome the shortage of solid organ grafts available for transplantation. The association between bone marrow chimerism and donor-specific transplantation tolerance has been recognized for forty years (27-42). The first association between bone marrow chimerism and tolerance was reported by Billingham, Brent, and Medawar in 1953 when they demonstrated the induction of permanent donor-specific transplantation tolerance for skin grafts by transplantation of bone marrow cells into newborn mouse recipients (27). Subsequently, numerous methods to induce similar tolerance in adult recipients using bone marrow transplantation have been reported (28-38). Monaco et al. demonstrated prolongation of skin allograft survival in mice treated with antilymphocyte serum followed by a critically timed transfusion of donor bone marrow stem cells (30, 31). Similar tolerance for alloantigens has now been achieved in a number of other species, including the dog (37) and monkey (38).

Recently, Ildstad et al. developed and characterized a model to induce similar donor-specific transplantation tolerance across a species barrier through preparation of fully xenogeneic chimeras (rat → mouse) (43, 44). Engraftment of rat bone marrow stem cells in mouse recipients was stable, as evidenced by the presence of rat-derived lymphocytes, myeloid cells, platelets, and red blood cells up to 12 months after reconstitution with untreated rat bone marrow cells. Survival was excellent (>80% at 180 days), and there was no evidence for graft-versus-

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host disease. Fully xenogeneic chimeras specifically accepted donor-strain rat skin grafts but were competent to reject MHC-disparate third-party mouse and rat skin grafts (43, 44).

The aim of the present studies was to determine whether long-term acceptance and function of pancreatic islet cell xenografts could be achieved in fully xenogeneic chimeras without requirement for chronic nonspecific immunosuppressive agents. We now report the long-term survival and function of donor-specific xenogeneic pancreatic islet cells in fully xenogeneic chimeras.

MATERIALS AND METHODS

Animals. Six to eight-week-old male C57BL/10SnJ (B10), B10.BR/Sgn (B10.BR) mice were purchased from the Jackson Laboratory, Bar Harbor, ME. Four to eight-week-old male Fischer 344 (F344, Rt1A⁺), and Wistar-Furth (WF, Rt1A⁺) male rats were purchased from the Harlan Sprague Dawley Company. Animals were housed in a specific pathogen-free facility at the Pittsburgh Cancer Institute and in a conventional animal facility after islet cell transplantation.

Fully xenogeneic reconstitution (non-TCD WF → B10). Fully xenogeneically reconstituted animals were prepared as previously described (43, 44). Briefly, inbred B10 male mice recipients were lethally irradiated with a single dose of 950R from a cesium source (Nordion). Using sterile technique, bone marrow was flushed, with Media 199 (Gibco, Grand Island, NY) containing 50 µl/ml Gentamicin (MEM) from the long bones of donors. The marrow was mechanically resuspended in MEM by gentle aspiration through an 18-gauge needle, and the suspension filtered through sterile nylon mesh. The cells were then pelleted at 1000 RPM for 10 min, resuspended, and counted. Recipient animals were reconstituted within 4–6 hr following lethal irradiation via the lateral tail veins using a 27-gauge needle and received 40×10⁶ non-TCD Wistar-Furth (WF → B10) rat bone marrow cells.

Characterization of chimeras by flow cytometry. After 28 days, animals were typed to document chimerism using flow cytometry with monoclonal antibody staining (43, 44). Briefly, peripheral blood was collected into heparized plastic serum vials. After thorough mixing, the suspension was layered over 1 ml of cold Ficoll-Hypaque (Bionetics, Kensington, MD), centrifuged at 4°C, and counterstained with sandwich when required. The anti-WF and anti-H-2^b class I monoclonal antibodies were determined to be noncrossreactive on the irrelevant species. Arbitrary levels on log scale were selected based on the inflection point where straining of the control negative population was minimized while retaining maximal numbers of positive cells.

Diabetes. Six weeks after reconstitution, chimeras were made diabetic by intravenous injection of streptozotocin (165 mg/kg) via the lateral tail vein. Nonfasting plasma glucose levels were determined on blood removed from the orbital sinus of the mice. Only mice with plasma glucose concentrations exceeding 300 mg/dl for at least 1 week were used as islet cell recipients.

Rat islet isolation and transplantation. Fischer and Wistar-Furth rat islet cells were separated by collagenase digestion using a modification of the automated method for human pancreatic islet isolation (3, 5). Briefly, the pancreatic duct of the rat was cannulated, and 7 ml of Hanks solution containing 1 mg/ml collagenase (Boehringer-Mannheim, type P, lot 09) was injected. The distended pancreata were then placed in a digestion chamber and subjected to 10–15 min of enzymatic digestion. The separated islets were purified with Euro-Collins Ficoll (Sigma, St. Louis, MO) (densities were 1.108, 1.096, 1.069, and 1.037). The purified islets were cultured overnight in medium (RPMI 1640: GIBCO, Grand Island, NY; 10% FCS: Hyclone Laboratories, Inc. + 1% penicillin [100 U/ml] Sigma Chemical Company, St. Louis, MO, + streptomycin [100 U/ml]) at 37°C, 5% CO₂. The following morning, the islet cells were transplanted beneath the left renal capsule. Two groups were prepared: 800 WF donor-specific rat pancreatic islets (group 1, n=8), or 800 MHC-disparate Fischer (F344) rat islets, were placed under the renal capsule. Six B10 mice that did not receive bone marrow were transplanted with 800 Wistar rat islets as a control.

TABLE 1. PBL typing of fully xenogeneic chimeras (WF rat → B10 mouse)^a

Animal	% Rat	% Mouse	Islet cell donor
1	86.1	14.8	WF
2	94.0	5.9	WF
3	60.2	40.5	WF
4	88.2	12.8	WF
5	58.6	40.6	WF
6	60.9	39.1	WF
7	67.3	32.8	WF
8	89.2	12.3	WF
9	94.5	5.5	F344
10	86.0	14.0	F344
11	68.2	32.5	F344
12	95.0	5.2	F344
13	61.5	40.0	F344
14	78.4	24.0	F344
Normal B10	0.1	99.6	NA ^b
Normal WF	99.8	0.2	NA ^b

^a Typing was performed by PBL at 6 weeks using flow cytometry. Anti-class I WF-FITC and anti-H2^b-FITC were utilized for staining.

^b NA: not applicable.

The nonfasting plasma glucose levels were determined in all of the animals prior to and daily after transplantation. Rejection of the islet xenografts was considered to have occurred when the plasma glucose concentrations exceeded 200 mg/dl on 3 consecutive days. In animals in which that occurred, grafts were removed and examined histologically.

Histological studies. Histology of the xenografts in the kidney and native pancreas was performed after rejection had occurred or after planned nephrectomy and pancreatectomy in normoglycemic recipients. The histological sections were stained with aldehyde fuchsin and hematoxylin and eosin to demonstrate beta cells and immunoperoxidase to detect insulin.

RESULTS

Typing of fully xenogeneic chimeras (WF rat → B10 mouse). Fully xenogeneic chimeras were prepared in the standard fashion using untreated WF rat bone marrow cells. Prior to the induction of diabetes, the recipients were typed for rat lymphoid chimerism 30 days following reconstitution. All mice tested exhibited xenogeneic rat chimerism when typed for the presence of rat peripheral blood lymphocytes (Table 1). As in our previous experience, chimerism ranged from 60.2% to 95%.

Long-term survival of rat islet xenografts in fully xenogeneic chimeras. After typing for chimerism, diabetes was induced in the fully xenogeneic chimeras using streptozotocin. Normal B10 mice were also prepared as controls. Daily blood-glucose monitoring was performed. All mice developed diabetes as evidenced by serum glucose levels over 300 mg/dl for a minimum of 7 days. Although the chimeras remained healthy, they exhibited a decrease in body weight compatible with the diabetic state.

Mice that remained hyperglycemic for over 7 days were subsequently transplanted with 800 WF donor-specific islet cells placed under the renal capsule. Plasma glucose was monitored daily as an assessment of islet cell function. Survival of the donor-specific islet cell xenografts was significantly prolonged (median survival time [MST]* >180 days) (Fig. 1). Recipients became normoglycemic within 2 days following placement of the islet cell xenografts. Two animals became

* Abbreviation: MST, median survival time.

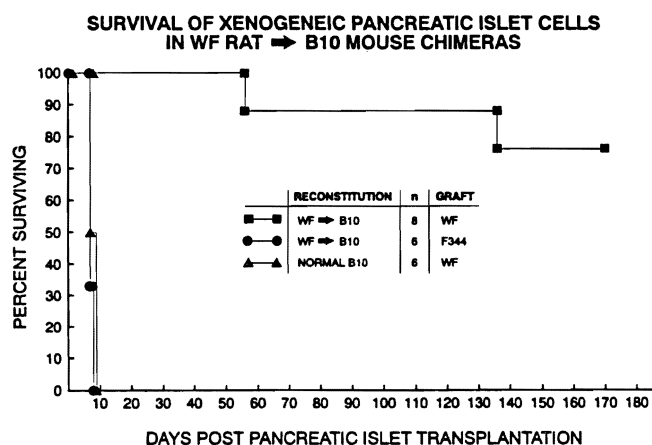


FIGURE 1. Life table survival of donor-specific (WF rat) and MHC-disparate third-party (F344 rat) pancreatic islet xenografts in fully xenogeneic chimeras (WF rat → B10 mouse). Recipients were followed for a minimum of 180 days.

hyperglycemic 59 and 120 days following transplantation of the donor-specific rat pancreatic islets. In these animals, an ipsilateral nephrectomy was performed to allow histological examination of the islet cell xenografts. Subcapsular fibrosis was present at the site of transplantation beneath the renal capsule, and mononuclear lymphoid cellular infiltrates were identified. No rat islet cells remained. These findings are compatible with chronic rejection. The remaining grafts have been functional for up to >180 days.

Specificity of tolerance to rat pancreatic islet in fully xenogeneic chimeras (Non-TCD WF → B10). To examine the specificity of tolerance to pancreatic islet xenografts in fully xenogeneic chimeras, MHC-disparate third-party F344 rat islets were transplanted beneath the left renal capsule of the fully xenogeneic chimeras. These xenografts were promptly rejected with a time course similar to normal B10 mice (MST=8 days), suggesting that the specificity of tolerance to rat islet xenografts was highly donor-strain specific. Blood sugar levels in the recipients initially normalized, suggesting engraftment of the islet cells. This was followed by a return of hyperglycemia (Fig. 2) and histological evidence for acute rejection (data not shown).

To determine that the euglycemic state was supported by the transplanted xenogeneic islet cells and not secondary to a return of function of the native pancreas, ipsilateral nephrectomy was performed at 100, 120, and 156 days in 3 animals to remove the islet cell xenografts. Following removal of the xenografts, daily blood sugar monitoring was performed. In all animals examined, a return of hyperglycemia occurred from 12 to 24 hr following removal of the donor-specific xenogeneic islet grafts. Figure 3 shows blood glucose measurements for a representative animal prior to and following removal of the transplanted islets at 100 days.

Histological examination of xenogeneic islet cells. After nephrectomy, immunohistochemical staining was performed on the islet cell xenografts to examine for the production of insulin and glucagon as an indication of function. In all tissue sections examined, the islet cells appeared healthy, and there was no evidence of chronic inflammation. Insulin production was present in all tissue sections examined, suggesting that the islet cells were functional and able to produce these hormones.

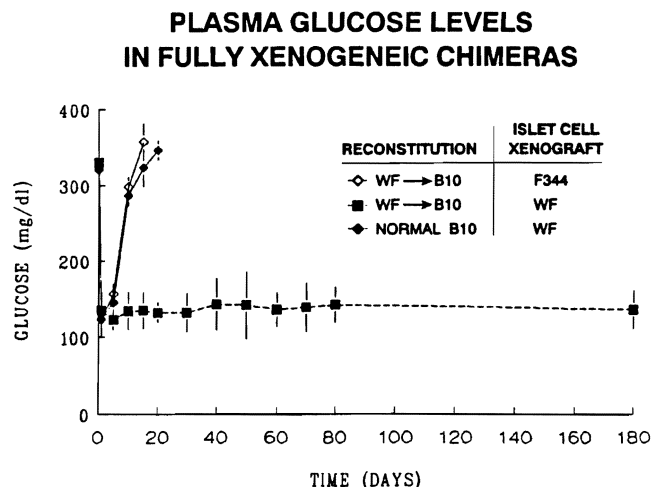


FIGURE 2. Mean plasma blood glucose levels (\pm SEM) in recipients of xenogeneic islets. Diabetes was induced by streptozotocin, and after ≥ 7 days of hyperglycemia (BS>300 mg/dl) donor-specific (WF) or third-party (F344) islet cell xenografts were placed. Two animals became hyperglycemic at 59 and 120 days following placement of WF islet cell grafts. Their blood sugar calculations were excluded from these calculations at those time points.

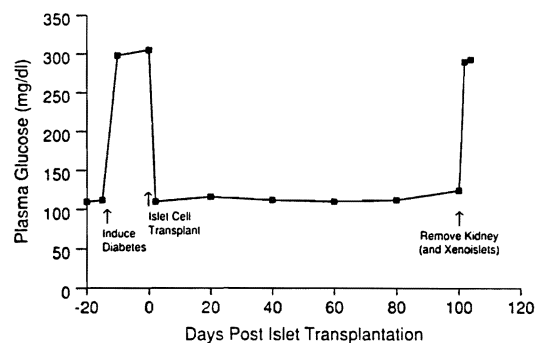


FIGURE 3. Kinetics of plasma glucose concentration for a representative chimera. Diabetes was induced using i.v. streptozotocin treatment at 6 weeks following reconstitution. After documentation of blood sugars >300 for 7 days, islet cell xenografts were then placed (day 0), and serum glucose determination followed daily. In this animal, the islet xenograft was removed on day 110 to demonstrate that the euglycemic state was supported by the xenogeneic islets and not the native pancreas.

Further examination of the native pancreas from the transplanted animals demonstrated the presence of islet cells, but absence of insulin production as assessed by immunoperoxidase, indicating efficacy of the streptozotocin treatment.

DISCUSSION

Significant progress has been made in pancreatic transplantation for treatment of diabetes. The use of multimodal non-specific immunosuppressive agents has allowed relatively good control of allograft rejection (5-7). More recently, the transplantation of isolated pancreatic islet cells has emerged as a more-focused approach for treatment of diabetes (2-7, 45, 46). Using this approach, anastomoses are avoided, and only the insulin-producing islet cells are administered. However, rejection and a shortage of organs persist as two major limitations. Although nonspecific immunosuppressive agents have revolutionized the field of transplantation, rejection may occur in

spite of their use. This is true for cellular grafts as well as whole pancreas allografts. A significantly increased rate of infection and malignancy is associated with the use of nonspecific immunosuppressive agents.

A renewed interest in xenotransplantation has emerged as a result of the critical shortage of solid organs available for transplantation. Transplantation of solid organs or even cellular grafts obtained from another species, such as the pig, has been suggested as a possible solution to the current shortage of solid organs. It is well recognized that rejection of xenografts is usually more vigorous than that for allografts and not controlled by the conventional immunosuppressive agents utilized to control alloreactivity (47). In fact, the mechanisms responsible for graft rejection for xenogeneic disparities are not well understood and may, in fact, differ from that for alloantigens (47, 48). Auchincloss et al. recently reported that skin xenograft rejection was mediated, for the most part, by CD4⁺ T lymphocytes (47, 48). The induction of donor-specific transplantation tolerance using bone marrow cells has therefore been suggested as one potential approach to overcome these limitations.

The association between bone marrow chimerism and donor-specific transplantation tolerance has been recognized for the past 40 years (27). Chimeras can be prepared using both lethal and nonlethal conditioning protocols, including lethal total-body irradiation (28), total lymphoid irradiation, (49), ablative chemotherapy (50), antilymphocyte serum (36–42). In all these models, the induction of systemic donor-specific transplantation tolerance was achieved as assessed by permanent acceptance of donor skin grafts and the presence of specific hyporeactivity to donor alloantigens *in vitro* (51–53). We recently reported the induction of similar chimerism and donor-specific transplantation tolerance across a species barrier in a model for fully xenogeneic chimerism (rat → mouse) (43, 44). Reconstitution of B10 mouse recipients with 40×10^6 untreated rat bone marrow cells resulted in long-term engraftment of rat pluripotent stem cells and maturation of rat T lymphocytes in the xenogeneic thymic stromal environment of normal irradiated B10 mouse recipients. Using this model, we have now determined that pancreatic islet xenografts are permanently accepted and remain functional to maintain euglycemia in a xenogeneic environment.

Long-term acceptance of xenogeneic pancreatic islet cells resulted when non-hand-picked purified islet cells were transplanted into fully xenogeneic chimeras (WF rat → mouse) rendered diabetic by streptozotocin treatment. Euglycemia resulted within 48 hr following the placement of the cellular xenografts under the renal capsule. In order to determine that the euglycemic state present in the chimeras was supported by the islet cell xenografts and not due to return of function of the native pancreas, we performed serial ipsilateral nephrectomy on selected chimeras to remove the transplanted islet cell xenografts. In all animals examined from 3½ to 6 months following placement of the xenografts, the diabetic state returned within 24 hr after removal of the graft, further confirming that the islet cell xenografts were in fact functional and responsible for the euglycemic state. Histologically, grafts appeared healthy, and there was evidence for production of both insulin and glucagon, suggesting normal function. Most importantly, there was no evidence for chronic rejection.

The tolerance induced by fully xenogeneic (rat → mouse) reconstitution for islet xenografts was highly MHC specific, as evidenced by rapid rejection of MHC-disparate third-party

(F344) grafts. Rat was not seen as “generic” rat but instead as specific strain of rat for the transplanted pancreatic islets. Similar results were present for skin grafts, where donor-specific skin xenografts were accepted while third-party rat skin grafts were promptly rejected (43, 44). The MHC-disparate islet cell grafts engrafted as evidenced by the presence of euglycemia for 4–8 days in individual recipients. However, hyperglycemia reappeared as the third-party grafts were rejected. Rejection was also confirmed histologically by the presence of inflammatory mononuclear cell infiltrates.

In 2 of the 8 animals, hyperglycemia returned at 59 and 120 days, respectively. After 3 days of persistent hyperglycemia, the grafts were removed and examined histologically. There was evidence for chronic fibrosis as well as mononuclear cell infiltrates, suggesting chronic rejection. In addition, no islet cells could be identified. Despite this graft loss, there was no change in the level of xenogeneic chimerism. We speculate that this effect may be due to islet-specific antigens expressed on islet cells but not on the rat bone marrow elements to which the mice were rendered tolerant. Skin-specific antigens present on skin, but not on lymphoid cells, have been well characterized using bone marrow chimeras (54, 55). In these studies, chronic, gradual skin graft rejection occurred without change in the level of donor bone marrow chimerism (54, 55). We recently reported evidence for similar skin-specific antigens across a species barrier in mixed xenogeneic chimeras (mouse + rat → mouse) (56), and we speculate that similar antigens may be present in the islet cell tissue. However, it is of note that in the majority of our recipients we do not have evidence for chronic rejection. Studies are in progress to further characterize the mechanism responsible for this late rejection and to further examine whether it is, in fact, due to islet-specific antigens recognized across a species barrier.

The islet cell preparation used for these studies was not hand-picked and therefore most closely approximated the cellular grafts currently utilized in human clinical trials at the present time. This form of islet cell preparation is well recognized to be of a heterogeneous, highly antigenic composition (2–7), and therefore it served as a test of the presence of tolerance in fully xenogeneic chimeras.

In summary, we report long-term survival and function of donor-specific pancreatic islet xenografts in fully xenogeneic (rat → mouse) chimeras. The tolerance induced was highly MHC specific, since third-party rat islet grafts were rejected with the same time course as that for unmanipulated B10 controls. Further studies are in progress to examine the putative role of islet-specific antigens and to evaluate whether other cellular endocrine xenografts will be similarly accepted and remain functional.

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ORAL DISCUSSION

DR. NAJI (Philadelphia, Pennsylvania): I have two specific questions. Was there any evidence of graft-versus-host disease in your recipients, and, secondly, could you tell us the composition of the rat T cells and whether there was any T cell maturation in the thymus of these mice?

DR. ILDSTAD: We have documented normal T cell matu-

ration in our chimeras. The rat-derived T cells enter the thymus and exhibit an immature profile: CD8 negative, CD4 negative without the alpha-beta TCR and CD3. They then go through the expected maturational steps and emerge with a mature phenotype. Lymphocytes from the chimeras are also functional, and the chimeras are functionally tolerant to both the donor strains of rat, yet respond in CTL and MLR responses to third party. Similarly, they are also specifically tolerant to the recipient strain of mouse. The tolerance we see is a very MHC-specific tolerance across a species barrier.

DR. NAJI: Isn't it surprising that the rat cells mature in the murine thymus—this is the first time that I think this has been demonstrated—in view of the fact that you don't really see that when you repopulate the SCID mouse with the human PBL?

DR. ILDSTAD: This is the first demonstration that the mouse stromal environment in the thymus is adequate to support development of mature T cells from another species. We found it surprising as well.

DR. BRAYMAN (Minneapolis, Minnesota): Is the Wistar-Furth islet graft a xenograft or an allograft in an animal that has been reconstituted with xenogeneic bone marrow? How are we considering this if you have a fully xenogeneic chimera where the immune system presumably is now of rat origin?

DR. ZENG: Your question is an important one, and we are in the process of evaluating whether it is the rat, mouse, or both lymphoid cells that respond to recognize MHC-disparate third-party rat.

DR. HARDY (New York, New York): In relation to this question of nomenclature, could you comment on the degree of chimerism? Was this a complete 100%?

DR. ILDSTAD: The chimerism is never 100%. In the fully xenogeneic situation presented today, we have rat chimerism for multiple lineages, including red cells, platelets, T cells, B cells, and macrophages that ranges from 65% to 99% for individual animals. The remaining cells of recipient origin seem to be radio-resistant cells that are predominantly CD4 positive and alpha-beta TCR negative. Other lineages are not present. We don't find mouse-derived red cells, platelets, or other lineages that would be produced by the mouse stem cell.

In mixed chimeras, when we administer a mixture of mouse plus rat bone marrow, we have observed lineage production from both the mouse-derived stem cell and the rat-derived stem cell.

DR. BRAYMAN: Is a fully xenogeneic chimera really an allograft response for third-party rat?

DR. ILDSTAD: In a sense that is correct. For fully xenogeneic chimeras, it would be the rat cells responding to the third-party rat graft as an allograft in a xenogeneic mouse environment.

DR. AUCHINCLOSS (Boston, Massachusetts): I doubt that you have had the opportunity to create this kind of chimera followed by an islet transplant in an NOD mouse or a spontaneous diabetic mouse. The reason I ask is that you could then see whether or not the xenoislets escape whatever the autoimmune disease is that causes diabetes. This possibility has been suggested as one of the attractive aspects of xenogeneic pancreatic islet transplantation.

DR. ILDSTAD: Yes, it is possible that one could overcome the autoimmune disease associated with type 1 diabetes with xenotransplantation. It is of note that we do not see graft-versus-host disease in our chimeras even when we give a very large dose of spleen cells. Since autoimmune diseases share

many similarities, it is possible that xenochimerism would also be protective against GVHD.

DR. HARDY: Have you tried to do this with any other type of rodent, for example, guinea pig to the mouse?

DR. ILDSTAD: Not yet. We plan to begin those studies in the near future. We attempted to achieve engraftment of human marrow in our mice but were unsuccessful.

DR. HARDY: This is a little too radical, but the guinea pig is a very good model of xenotransplantation. The rat-to-mouse has been prolonged indefinitely in terms of islets by various types of protocols, including that of my group, by modifying the islets themselves. I think the greatest test would be to go on to guinea pig-to-mouse; that might clarify Dr. Brayman's question.

DR. RICORDI: Compared with previous models of prolongation of islet xenograft survival, this experiment was different because it was possible to prolong xenograft survival despite the partial purity of the preparations used for transplantation. In fact, the islets were collected from the Ficoll gradients without hand-picking. The quality of the preparations was therefore similar to what we use in human islet transplants. I am not aware of any previous study showing prolongation of islet xenograft survival using islets that were not perfectly clean or hand-picked.

DR. HARDY: Dr. Ricordi, your preparation is so good that I am sure when it is scaled down to the rat, it's pretty pure.

DR. RICORDI: But if you perform an analysis of all the components, you will find that all of the constituents of the pancreas are still present in the purified islet preparation, even if their relative percentage is changed. That 10, 20% of contaminating nonislet component can be very immunogenic.

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