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CHAPTER 17

BONE MARROW CHIMERISM AND PANCREATIC ISLET GRAFTS

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Transplantation has significantly advanced in the past 30 years. The availability of T-cell-directed immunosuppressive agents (FK506, Rapamycin, Cyclosporine, Azathioprine) has allowed pancreatic transplantation to become an accepted therapeutic approach for treatment of diabetes. In recent years, the development of procedures to isolate large numbers of pancreatic islets has made it possible to initiate clinical trials for cellular replacement of pancreatic endocrine function.[1-6] Transplantation of pancreatic islets is the most focused approach to treat diabetes and may offer a number of advantages over transplantation of the whole pancreas as a primarily vascularized graft. In addition to the potential for cryopreservation and islet "banking", in vitro pretreatment of free cellular grafts is more feasible than with solid organs. Finally, one can eliminate the requirement for vascular and ductal anastomoses which must accompany the transplantation of solid pancreas grafts.

It is well recognized that all pancreas grafts are highly antigenic.[1-8] Although one might predict that free cellular grafts would be *less* antigenic than a primarily vascularized solid organ graft, the opposite has proven to be true.[1-8] Cellular grafts are even more antigenic than the whole pancreas if one uses rejection-free survival and function as the criteria for judging graft immunogenicity. Approaches to reduce immunogenicity of the islet grafts, including graft pretreatment with monoclonal antibody,[7,5,9-11] in vitro culture techniques,[4,10-13] ultraviolet irradiation of the grafts,[14-16] microencapsulation,[17-20] and isolation of hand-picked ß-cells[21,22] have resulted in prolonged graft survival, but these approaches have not reliably prevented graft rejection. The induction of donor-specific transplantation tolerance across MHC disparities, or even species barriers, has been suggested as a potential approach to overcome the limitation of graft rejection. To date, the only state of true systemic donor-specific transplantation tolerance has been that associated with chimerism, the engraftment of bone marrow stem cells in a conditioned recipient. The association of chimerism and tolerance will be the focus of this chapter.

The recognition that bone marrow stem cells possess a unique property to induce systemic and permanent transplantation tolerance to donor histocompatibility antigens was made in the late 1940s and early 1950s. Owen detected mixed red blood cell

chimerism in fraternal twins which shared a common placenta (freemartin cattle) and concluded that this was possible only if hematopoietic stem cells had been exchanged in utero.[23] This effect persisted in these animals throughout their lifespan. The implications of this phenomenon for transplantation immunology would prove to be profound: if donor bone marrow cells could be engrafted in normal recipients, they would not be rejected, but instead would persist in a mutual state of cotolerance.

Contemporaneously, Billingham, Brent and Medawar became aware of Owen's observation when they were asked by the Agricultural Research Council to develop a model to distinguish monozygotic from dizygotic cattle twins. It was important to the cattle industry because "freemartin" (chimeric) cattle were virtually always sterile and therefore not economical to raise. Billingham, Brent and Medawar had developed a method to use skin grafting to perform histocompatibility typing. They had demonstrated skin graft rejection in the presence of histocompatibility differences. However, to their surprise they found that dizygotic freemartin cattle accepted skin grafts from their sibling just as readily as did monozygotic (identical) twins. The presence of donor chimerism had rendered the recipients tolerant. They immediately attempted to transfer bone marrow cells into newborn mice to determine whether donor-specific skin graft survival could be achieved. This led to the report of "actively acquired tolerance of foreign cells" in which transplantation of Major Histocompatibility Complex (MHC)—disparate bone marrow stem cells into neonatal recipient mice induced specific and systemic tolerance to the donor with preservation of immunocompetence to reject genetically different third party grafts from other donors.[24] Moreover, the tolerance was stable and persisted into the adult life of the recipient.

The fetus and newborn possess a privileged state in which no conditioning is required to achieve engraftment of bone marrow cells in the form of chimerism. In the mouse, this stem cell engraftment can be achieved until 72 hours after birth. In the human fetus, this occurs until 16 weeks of gestation. After that, approaches to condition the recipient or "make space" to allow bone marrow stem cells to engraft must be utilized *(conditioning)*.

After the pioneering work of Billingham et al in neonatal mice, approaches to achieve similar chimerism in adult recipients were reported. Conditioning approaches to allow engraftment of donor stem cells included total body irradiation,[25,26] total lymphoid irradiation,[27] and pharmacologic cytoreductive approaches, i.e., cyclophosphamide.[28] With each of these approaches, donor-specific transplantation tolerance to subsequent solid organ or tissue grafts of donor-type was achieved. The tolerance induced was stable, systemic, and specific for the donor. It did not require the use of chronic nonspecific immunosuppressive agents. Hence the recognition that chimerism was associated with tolerance for tissue and solid organ grafts.

It is now well accepted that full replacement of the immune system of the recipient with that of donor *(fully allogeneic chimerism)* results in systemic donor-specific transplantation tolerance. However, this state is complicated by a relative state of recipient *immunoincompetence*.[29-31] The donor T-lymphocytes which develop in the recipient are restricted to interacting with antigen-presenting cells (APC) of the host. With full replacement of all bone-marrow-derived cells, only donor APCs are present. As a result, primary immune responses which rely on APCs, including antibody production,[32] antiviral responses,[30] and survival are significantly impaired, resulting in a state of relative recipient immunoincompetence.[33] Therefore, tolerance and immunocomperence are two independent variables.

The presence of syngeneic bone marrow cells in coexistence with allogeneic bone marrow *(mixed chimerism)* results in similar systemic donor-specific transplantation tolerance for solid organ grafts with the advantage that recipient immunocompetence is preserved. The appropriate host-derived APCs are present and a state of mutual cotolerance is induced. The donor and host bone marrow stem cells co-engraft and function to produce multilineage mixed chimerism. A mixture of donor and recipient red blood cells, platelets, B-cells, T-cells, NK cells, macrophages and other APCs, granulocytes and monocytes can be detected (manuscript submitted). In striking contrast to fully allogeneic chimeras, mixed allogeneic chimeras exhibit superior immunocomperence for antibody production, antiviral

responses, and survival.[31-33] Most importantly, a relative resistance to graft versus host (GVH) disease is present in mixed allogeneic chimeras due to mechanisms which have not yet been elucidated.[25,26,31,32] Similar resistance to GVH disease has been reported in human recipients of allogeneic bone marrow who by chance reconstituted as mixed chimeras.[34]

Mixed chimerism can be achieved by coadministration of syngeneic plus allogeneic bone marrow following conditioning with total body irradiation (TBI),[32] transplantation of untreated allogeneic bone marrow following total lymphoid irradiation (TLI),[27] or following administration of untreated (T-replete) bone marrow in conjunction with low-dose irradiation plus cyclophosphamide[28] or monoclonal antibody.[35] In each of these widely different approaches, the induction of donor-specific transplantation tolerance *plus* resistance to GVH disease was observed, reinforcing the strong association of tolerance with chimerism. Most importantly, the presence of chimerism, no matter how low the level, conferred stable systemic donor-specific transplantation tolerance. The tolerance induced was not incremental, but rather an *all-or-none* effect.

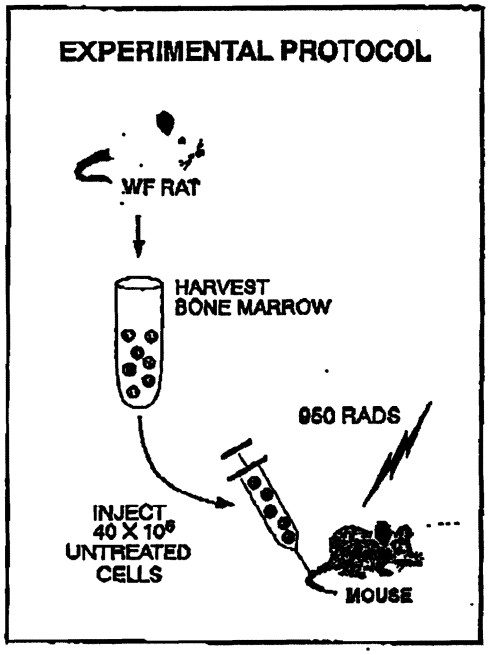

Figure 1

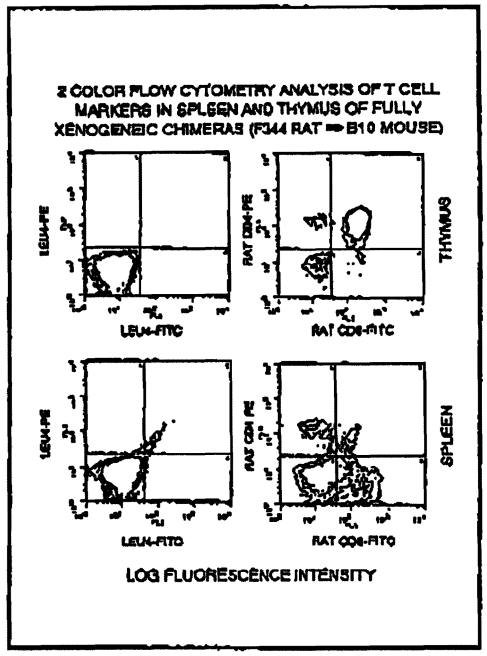

Figure 2

The transplantation of bone marrow cells across xenogeneic barriers has been demonstrated to produce bone marrow rescue following lethal irradiation.[36-39] As in allogeneic chimeras, the presence of xenogeneic chimerism is associated with the induction of donor-specific transplantation tolerance to solid organ or tissue grafts.[39-41] Until recently, xenogeneic chimerism was limited by transient engraftment of the donor bone marrow cells and inferior recipient survival.[39-41] It has now been reported that the administration of 40×10^6 untreated rat bone marrow cells into B10 mouse recipients conditioned with 950 rads of TBI (rat → mouse) (Fig. 1) resulted in stable fully xenogeneic chimerism, excellent survival, resistance to GVH disease, and the induction of stable donor-specific transplantation tolerance.[41] Rat T-cell maturation proceeded in a phenotypically normal fashion in the chimeric mouse recipients, as evidenced by an immature-staining profile by flow cytometry in the thymus and mature profile in the periphery (Fig. 2). Most importantly, the rat-derived lymphocytes were *functionally* tolerant to both recipient mouse and donor rat antigens, yet fully-reactive to MHC-disparate third party mouse and rat antigens indicating that mouse and rat are not seen as generic species but instead in an MHC-strain-

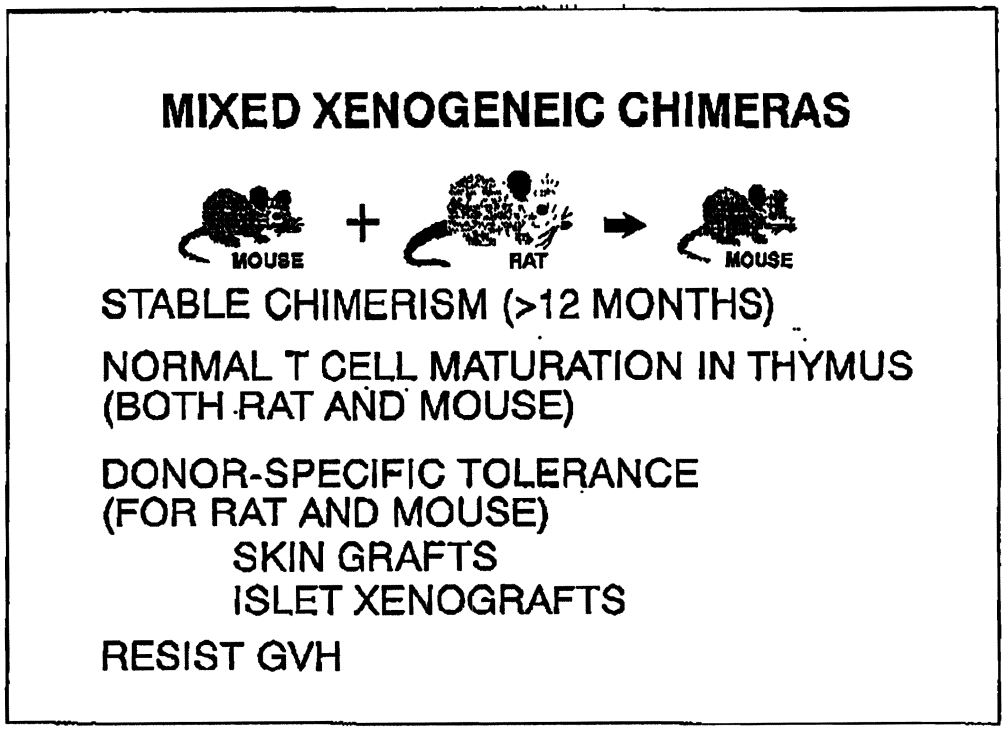

Figure 3

specific fashion when tolerance is induced across a species barrier[42] (manuscript in preparation).

Mixed xenogeneic chimeras (mouse + rat → mouse) have been prepared by coadministering T-cell-depleted syngeneic mouse plus untreated rat bone marrow to mouse recipients conditioned by TBI (Fig. 3).[43] Stable xenogeneic multilineage chimerism ranging from 1% to 56% rat for individual recipients has been demonstrated. As in mixed allogeneic chimeras, any level of detectable xenogeneic chimerism was associated with stable, systemic donor-specific transplantation tolerance in vivo and in vitro. Most importantly, mixed xenogeneic chimeras exhibited superior immunocompetence, probably for reasons similar to those observed in mixed allogeneic chimeras. Although untreated rat bone marrow cells are administered, there is no evidence for GVH, suggesting that there is a resistance to GVH across a species barrier. Mixed xenogeneic chimeras accept donor-specific skin grafts, but reject MHC-disparate third party mouse and rat skin grafts with a time course similar to unmanipulated mice.[43]

Although significant progress has occurred in transplantation in the past 30 years, two major limitations exist: (1) there is a critical shortage of allogeneic donors; and (2) rejection occurs in spite of conventional multimodal immunosuppression. These limitations are especially true for cellular grafts. The induction of donor-specific transplantation tolerance across species disparities has been suggested as a potential approach to overcome these limitations.

Recently, fully xenogeneic chimerism was applied to induce tolerance for pancreatic islet xenografts. Permanent, rejection-free graft survival (> 9 months) was demonstrated when nonhandpicked pancreatic islet xenografts were transplanted in fully xenogeneic (rat → mouse) chimeras.[42] In this experiment, chimeras were prepared and typed for chimerism at 6 weeks (Fig. 4). Diabetes was then induced using a single dose of intravenous streptozotocin (165 mg/kg). After 5 to 7 days of documented hyperglycemia (blood glucose > 300 mg/dl), either a donor-specific or MHC-disparate third party islet xenograft placed under the renal capsule. MHC-disparate third party grafts were rapidly rejected (median survival time = 9 days) in a time course similar to unmanipulated B10 mice while donor-specific islet grafts were permanently ac-

cepted (Fig. 5). To document that glucose homeostasis was maintained by the islet xenografts and was not due to regeneration of function in the native pancreatic endocrine tissue, transplant nephrectomy was performed at selected time points (Fig. 6). In all recipients analyzed from 90-270 days (n=8), hyperglycemia recurred, thereby confirming functional integrity of the islet xenografts (Fig. 7). The islet grafts and native pancreas were examined immunohistochemically using immunoperoxidase stains to detect insulin production. Normal appearing pancreatic islet tissue positive for insulin production was present, supporting functional integrity of the xenografts.

No evidence for mononuclear cell infiltrates could be detected. As expected, the native pancreas had no evidence for insulin production, confirming that glucose homeostasis was indeed maintained by the islet xenografts. Similar long-term rejection-free graft survival and normal function of donor-specific islet xenografts has been achieved in mixed xenogeneic chimeras (mouse + rat → mouse).[44] Hence, this approach for tolerance induction has proven effective to allow permanent survival and function of islet grafts.

It has become apparent that although T-cell-directed antirejection therapy has made a significant improvement in graft survival, permanent rejection-free graft survival has not yet been achieved. Agents directed at other mediators in the complex rejection pathway, i.e., cytokines, antigen-presenting cells, or B-lymphocytes, in combination with T-cell directed immunosuppressive agents (FK506, cyclosporine A, Rapamycin) may offer the optimal combination to achieve permanent rejection-free graft survival. If not, the use of one cellular graft (bone marrow) to induce tolerance to strain and even species disparities may provide an approach to achieve permanent, rejection-free survival of other cellular grafts, i.e., pancreatic islets.

The transplantation of bone marrow cells to treat malignancy, stem cell failure, and genetic defects represents the first successful clinical application of cellular transplantation.[47] After the demonstration that rescue from lethal irradiation could be achieved using the intravenous injection of bone marrow cells, evidence accumulated that the "barrier" to transplantation previously described by Alexis Carrel, may in fact not be totally impenetrable. The neonatal chimerism

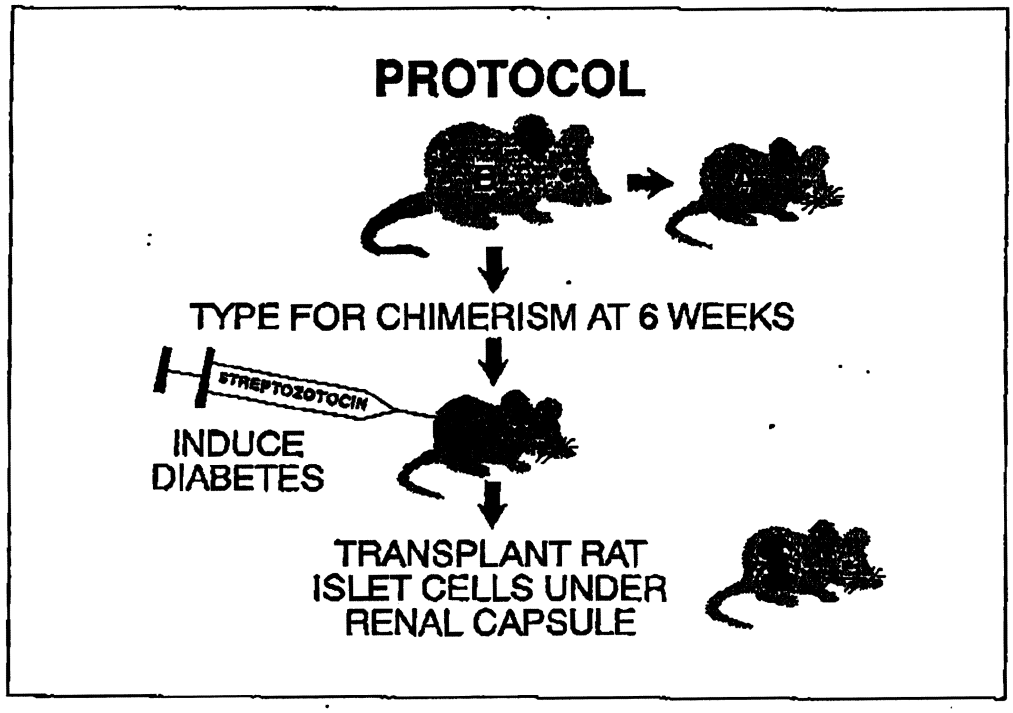

Figure 4

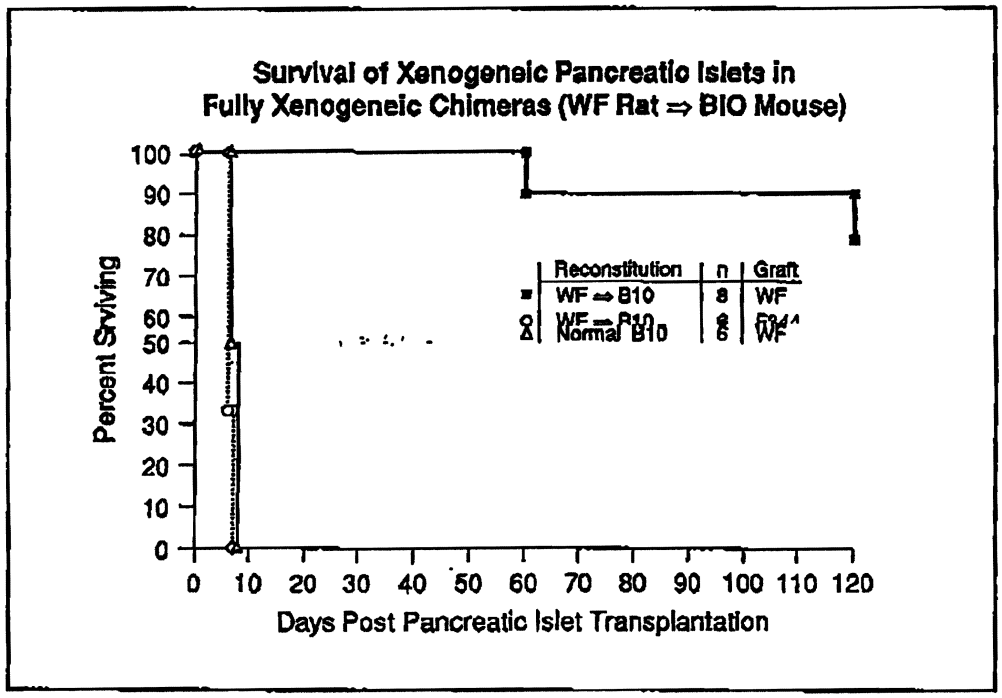

Figure 5

documented in Medawar's experiments gave further support to the paradigm that the histocompatibility barrier to transplantation was not totally insurmountable. The pioneering work of Dr. E. Donnall Thomas and colleagues applied these observations to the transplantation of cellular bone marrow grafts for treatment of hematologic malignancy and the field of bone marrow transplantation became a clinical reality.[46]

Although bone marrow transplantation developed in parallel and sometimes divergent pathway from solid organ transplantation, a recent mutual recognition has led to a merging of these two areas so that the lessons of each can be shared by both. In this way, it is quite appropriate to speculate that the use of one cellular graft, i.e. bone marrow, might be applied to achieve permanent survival of a second cellular graft, i.e., pancreatic islets.

The clinical application of bone marrow to prolong survival of solid organ allografts has begun. In a recent report, two patients who received HLA-matched living-related donor bone marrow grafts for hematologic malignancy developed renal failure as a late complication of their chemotherapy.[45] Both were given a renal

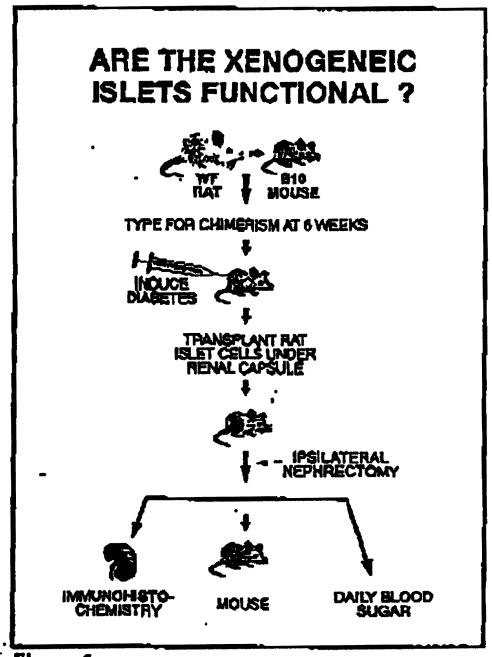

Figure 6

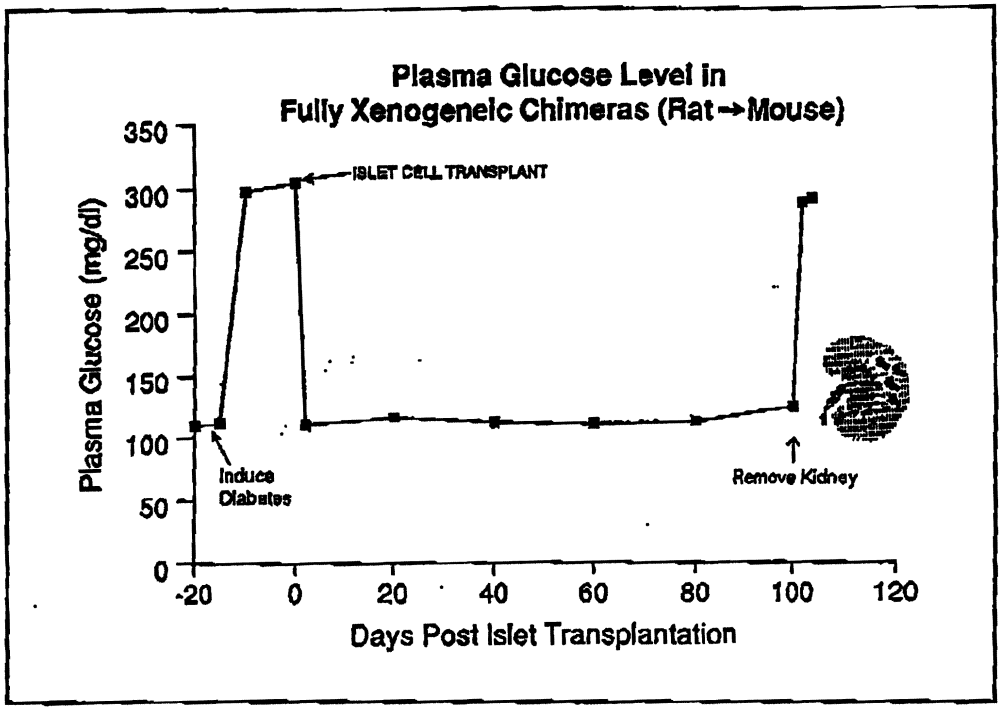

Figure 7

allograft from the same bone marrow donor and have experienced rejection-free graft survival without immunosuppression. In pioneering studies, Barber et al reported prolongation of renal allograft survival when cadaver donor bone marrow was administered 10 days following placement of the renal graft in conjunction with low dose cyclophosphamide.[46] A significant improvement in graft survival was observed and most importantly, no significant morbidity, i.e., GVH, was observed when T-cell replete donor bone marrow cells were administered.

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