Induction of Pancreatic Islet Graft Acceptance: The Role of Antigen Presenting Cells

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Diabetes mellitus is the most common endocrine disease. It is the fourth leading cause of death by disease in western countries and it is a worldwide public health problem.[1-3]

Prolongation of life is achieved by insulin therapy, but an increasing number of diabetic patients are treated for the complications associated with the disease, including blindness and end-stage renal failure. Fifty percent of all patients with diabetes develop renal failure in their lifetime.[1-5]

In patients with Type I diabetes mellitus, insulin production progressively declines and finally disappears as the beta cells within the islets are destroyed by an autoimmune process which results from a complex interplay between genetic and unknown environmental factors.[6-7] Replacement therapy with exogenous insulin has prevented acute death but is imperfect and has been ineffective in preventing the chronic complications of the disease. Thus, alternative methods for total endocrine replacement have been explored, including transplantation of isolated islets as a free graft.[8]

The idea of transplanting pancreatic tissue to reverse diabetes is a century old[9-10] and recent reviews on the subject are available.[11-15] Procedures for islet isolation[16-17] have improved significantly during the last decade[18-30] and the use of more powerful immunosuppressive agents such as cyclosporine[26,27,31-33] or FK 506[34-36] have resulted in prolonged human islet allograft survival. Insulin independence[31,33-37] was obtained in some patients indicating that it is possible to replace the endocrine function of the pancreas by an islet transplant in humans.

Despite these encouraging results, rejection remains the major factor limiting clinical trials of islet transplantation in Type I diabetes mellitus.[31,32,34-37] The solution to islet rejection cannot be provided by an increase in immunosuppressive protocols, since islet transplantation does not constitute a life-saving procedure. In contrast to other organ transplants such as heart or liver allografts, islet administration to Type I diabetic patients should be considered as prophylaxis to prevent the development of the chronic complications of the disease. Therefore, the risks associated with powerful immunosuppressive treatments cannot be justified at the present time. In the absence of any major breakthrough in the development of new and more benign immunosuppressive agents, it will be necessary to develop alternative procedures to prevent islet rejection.

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Several experimental approaches have been developed to reduce the immunogenicity of islet preparations by elimination or metabolic inactivation of the donor antigen presenting cells (APCs) within the islet grafts. Other approaches to prevent islet rejection that are currently under investigation include microencapsulation/macroencapsulation, bioartificial pancreas and treatment with antibodies to major histocompatibility complex (MHC) determinants. These approaches are not the subject of the present review. Instead this review will address the question of the role of APCs in islet rejection and the method to develop islet graft acceptance that could require a participating or determining effect of APCs.

The idea of treating tissues before transplantation to reduce immunogenicity is not new.[38,39] In 1934, Stone suggested a clinical benefit of in vitro culture of parathyroid tissue before transplantation in patients with hypoparathyroidism.[38] The hypothesis was that culture of tissue in the presence of recipient serum could result in graft "adaptation" to the new host. Lafferty[40] postulated that the facilitating effect of organ culture could be explained by the destruction or metabolic inactivation of bone marrow-derived donor antigen presenting cells (APCs). After two weeks of culture in an atmosphere of 95% O_2, significant prolongation of thyroid allograft survival was obtained (> 200 days).[40,41] Inactivation or destruction of the so-called "passenger leukocytes"[42,43] became the focus of many investigators who significantly contributed to developing procedures to prolong survival of endocrine tissues [44,45] and pancreatic islet [46-65] grafts.

Faustman et al[50] achieved prolongation of islet allograft survival following anti-Ia serum and complement treatment of the donor islets prior to transplantation, demonstrating a correlation between islet immunogenicity and the presence of Class II-positive cells in the transplanted islets. However, it has been shown that islet allograft rejection occurs despite Class II identity between the donor and the recipient,[66] indicating that rejection can occur for Class I disparities alone.[67-69]

Steinman and his associates described a specific type of interdigitating APC which he called the *dendritic cell*. He demonstrated that this Class II+ cell was a potent simulator of immune reaction in vitro.[70] He prepared a monoclonal antibody (mAb) to mouse dendritic cells and demonstrated that treatment with this antibody could eliminate these cells in vitro. In collaborative studies with Steinman, Faustman et al, demonstrated dendritic cells in mouse islets by immunochemical techniques and found that these cells could be eliminated by in vitro treatment of the islets with the mAb and complement. Pretreatment of BlO-BR (H-2k) donor mouse islets with the anti-dendritic cell mAb plus complement prevented rejection of the treated islets when transplanted into MHC-disparate diabetic C57 BL/6J (H-2b) recipients.[52] These findings indicated that in the mouse the

dendritic cell plays a major role in the initiation of rejection of islet allografts, since other Ia+ cells which remained in the graft after elimination of the dendritic cell did not initiate rejection.

Subsequent attempts to prevent rejection of rat islet allografts using antibodies that were reactive with Ia antigens in these species were not successful. In fact, either treatment of donor islets with a single antibody or with a mixture of anti-Ia antibodies and complement did not prevent rejection of rat islet allografts. Lacy and his associates demonstrated that in the rat, anti-Ia antibody and complement treatment decreased the number and/or quality of APCs in the islets but did not completely eliminate them. The inability to prevent rejection of rat islet allografts by treatment of the donor islets with anti-Ia antibodies was probably due to the larger size and more compact arrangement of rat islets as compared to mouse islets, thus making it more difficult for the antibody and complement to diffuse into the islets. Ia+ lymphoid cells can be demonstrated in freshly isolated rat islets using immunohistochemical techniques; however, relatively few Ia+ cells can be found after overnight culture. If the cultured islets are partially disrupted by a mechanical means or by a low calcium content in the medium, then Ia+ cells can be demonstrated in such islet preparations. These findings indicated that Ia+ cells were still present in the islets after overnight culture; however, the antibody was unable to penetrate the tightly compact islets to reveal their presence. The size and compactness of human islets is similar to the rat, and it may be difficult to obtain complete penetration of human islets with specific anti-Ia antibodies and complement. Thus, the rat model could be of assistance to test approaches that may be applicable to human islet transplants.[71]

Evidence is growing that APCs in different tissues are part of a bone marrow-derived system connected by movement and homing.[72] Coupled with this migratory ability is the capacity to capture antigens in an immunogenic form in situ. There is evidence to suggest that donor APCs from solid organ grafts, i.e., heart, migrate to splenic and other lymphoid tissues of the host and that allograft rejection is in fact initiated at a site distinct from the graft itself.[119] The progenitor for the putative dendritic cell lineage has not been isolated. Dendritic cells in spleen and lymph originate from a proliferating pool of precursors and undergo rapid turnover,[73-75] but the site for proliferation (3H-thymidine uptake) is not known. A bone marrow precursor exists but conditions have not been identified that direct its growth in culture.[73,74,76,77]

A recent study by Setum et al compared the potency of an enriched rat donor-strain dendritic cell population with fractionated spleen in relative ability to initiate an immune response in vivo.[78] While 103-104 dendritic cells were capable of stimulating graft rejection, or at least a severe immunologic response, 105 spleen cells were required to produce a similar effect, indicating that dendritic cells are powerful APCs. These findings extended the in vitro evidence that dendritic cells are potent stimulator cells and supported the hypothesis that APCs may be one of the most important inducers of allograft rejection.

Increasing interest has been focused on the thymus as a unique site for the induction of tolerance to both the endogenous (self) and transplantation antigens.[79-95] In radiation bone marrow chimeras it is now accepted that bone marrow-derived thymic stromal APCs played an essential role in the deletion of potentially autoreactive T-lymphocytes during T cell maturation. A renewed interest in the thymus as a "privileged" site for tolerance induction has therefore occurred. The induction of systemic donor-specific transplantation tolerance for islets but *not* skin was reported when Antilymphocyte Serum (ALS) was administered intraperitoneally (IP) and a simultaneous MHC-disparate islet allograft was placed intrathymically (IT).[96] A subsequent donor-specific islet graft was accepted at a distant site (renal subcapsular), but third-party islet grafts were rejected. Recipients were systematically hyporeactive to donor alloantigens in mixed lymphocyte culture proliferative assays (MLR). The thymus was critical for tolerance induction since placement of the first graft at the renal subcapsular (RSC) location did *not* induce tolerance. One might speculate that presence of bone marrow-derived APCs accompanying the islet graft could have resulted in the induction of tolerance. This is especially important since quiescent mature T-cells cannot re-enter the thymus, while activated T-lymphocytes *can*.[97]

Therefore, rejection has remained a limitation to survival of pancreatic islet allografts. The induction of donor-specific transplantation tolerance using bone marrow stem cells to produce chimerism, has been suggested as a potential approach to prevent rejection of transplanted pancreatic islets. The association between bone marrow chimerism and donor-specific transplantation has been recognized for 40 years.[79-94, 98-115] The first association between bone marrow chimeras 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.[98] Subsequently, numerous methods to induce similar tolerance in adult recipients using bone marrow transplantation have been reported.[101-111] Monaco et al demonstrated prolongation of skin allograft survival in mice treated with ALS followed by a critically timed transfusion of donor bone marrow stem cells.[103,104] Similar tolerance for alloantigens has now been achieved in a number of other species, including the dog[110] and monkey.[111]

Recently, Ildstad et al developed and characterized a model to induce donor-specific transplantation tolerance across a species barrier through preparation of fully xenogeneic chimeras (rat = >mouse).[116,117] 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 of graft-versus-host (GVH) 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.[116,117] We have recently demonstrated that long-term acceptance and function of donor-specific pancreatic islet xenografts could be achieved in fully xenogeneic chimeras without requirement of chronic nonspecific immunosuppressive therapy.[99,100] Euglycemia resulted within 48 hours following the placement of the cellular xenografts under the renal capsule. The pancreatic islet grafts were permanently accepted and remained functional for over eight months following transplantation.

To determine that the euglycemic state present in the chimeras was supported by the islet grafts and not due to return of function of the native pancreas, we performed serial nephrectomies of the kidneys bearing the grafts in selected chimeras. Following nephrectomy the animals returned to the diabetic state within 24 hours, further demonstrating that the islet xenografts were responsible for maintenance of the euglycemic state. Histologically, the grafts appeared healthy, and there was evidence for insulin positive cells (immunoperoxidase stains). Most importantly, there was no evidence for chronic rejection. These islets were not hand picked and therefore closely approximate the cellular grafts currently utilized in human trials. We have recently

observed that bone marrow-derived APCs are completely replaced in fully xenogeneic chimeras with those of the bone marrow donor, suggesting a potential role of donor APCs in the tolerance state that is associated with chimerism following bone marrow transplantation (manuscript submitted).

In conclusion, it is apparent that antigen-presenting cells exert a central role in islet allograft and xenograft rejection and/or tolerance induction. Methods to induce tolerance to islets as well as to other organ and tissue grafts using APCs as the target of immunoalteration procedures are currently the object of intense research. In the past APCs have been the target of procedures to eliminate and/or metabolically inactivate these cells to prolong islet graft survival. Today research evidence supports that APCs may play an active role in graft acceptance as well. Further studies will unmask the multifaceted role of this critical cellular component of tissue and organ grafts.

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