
Studies of Immune Mechanisms of Diabetes and Treatment with Isolated Pancreatic Islets

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four decades are the basis of a successful ongoing human islet transplant program.

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Introduction

In the early 1970's Dr. Clyde Barker at the University of Pennsylvania and the late Paul Lacy at the Washington University in St. Louis were the pioneers in exploring the concept of islet transplantation as a means to cure diabetes. In 1972, Ballinger and Lacy reported amelioration of diabetes in islet recipient rats.¹ In 1973, Barker and Reckard were the first to show that islet transplantation could completely and permanently restore normoglycemia in rodent models of chemically induced diabetes.² They found that islets, rather than being immunologically privileged tissue and thus partially exempt from rejection as others had assumed, are unusually vulnerable to destruction by immune mechanisms which could be avoided only with potent immunosuppression based on anti T cell antibodies.³ Transplanted islets also sensitized their hosts, inducing accelerated rejection of subsequent donor strain grafts of islets or other tissues. Barker's basic early findings heralded the challenges still being encountered in human trials of islet transplantation.

Barker and Naji next investigated the fate of islet transplantation in BB rats, the only animal model at that time of spontaneous genetically determined diabetes and closely reminiscent of human type I diabetes.⁴ Their experiments yielded two remarkable findings. First, BB rats rendered immunologically tolerant by neonatal inoculation of allogeneic bone marrow from normal rats were usually protected from

Abstract

Reviewed here are studies in animal models of type I diabetes that have led to better understanding of the disease and progress to its cure by of pancreatic islet transplantation and/or manipulations of the immune system. These studies include: 1) the first complete and permanent cure of chemically induced diabetes in rodents; 2) demonstration of autoimmune recurrence of diabetes in islet recipients; 3) induction of intrathymic tolerance to islets; 4) prevention of autoimmune diabetes by thymic manipulation; and 5) demonstration of the importance of B lymphocytes in autoimmune diabetes and islet rejection. These experimental studies over the last

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developing diabetes. The disease prevention afforded was shown to be attributable to the generation of hematopoietic cellular chimerism and restoration of self tolerance to beta cell autoantigens.⁵ This finding was the first conclusive evidence that diabetes was an autoimmune disease, at a time when different etiologies such as viral infections were being strongly considered. It also represented the first immunotherapy for the prevention of autoimmune diabetes. Second, those immunologically tolerant BB rats that did develop diabetes were found to permanently accept skin allografts yet mount a vigorous response to islet transplants. That this recurrent anti-beta cell autoimmunity destroyed transplanted islets but could be prevented by immunosuppression were crucial findings that several years later were confirmed in human pancreas transplants between identical twins.⁶ The biologic threat of recurrent autoimmune damage of transplanted islets is probably highly relevant to current clinical outcomes in human type I diabetic patients.

In view of their finding that autoimmune diabetes is a consequence of the failure in self-tolerance to beta cell antigen(s), Barker and Naji explored the impact of intrathymic inoculation of islets on the restoration of central immune tolerance. When a small number of islets were transplanted into the thymus of newborn BB rats, it was found that this exposure to putative islet autoantigens completely prevented the development of diabetes, indicating the restoration of tolerance to these antigens by 'educating' developing thymocytes during T cell ontogeny.⁷

In addition, these experiments showed the thymus to be a previously unrecognized privileged site, as evidenced by the permanent survival of allogeneic islets transplanted into the thymus.⁸ Akin to the impact on autoimmunity, exposure of developing thymocytes to alloantigens of islets or other cells implanted to the thymus led to a state of donor specific immunological tolerance *via* deletion or inactivation of T lymphocyte clones.⁹ This prevented

rejection of donor strain grafts transplanted outside of the thymus. Though intrathymic islet transplantation has yet to be utilized in patients because of biologic problems related to atrophy of the adult human thymus, these studies provided important proof of concept for induced central tolerance.

By the early 1980s, it was evident that type I diabetes is the result of the selective destruction of insulin-producing islet beta cells by autoreactive T lymphocytes. The presence of islet-specific autoantibodies in the serum of human subjects however suggested that the loss of B lymphocyte tolerance to islet autoantigens may also be involved in the destruction of beta cells and the development of diabetes. This concept was tested in a series of studies by Naji and Barker to assess the role of B lymphocytes in the pathogenesis of autoimmune diabetes.¹⁰⁻¹² With Hooman Noorchashm, they reported that depletion of B lymphocytes prevents insulinitis and the onset of diabetes in non-obese diabetic (NOD) mice. Insights gained from these basic studies have led to a current clinical trial of B cell depletion by rituximab (i.e., anti-CD20) for the reversal of recent onset type I diabetes.

Subsequently, they reported that the dysregulation of B cell tolerance is linked to a failure to eliminate from the developing B cell repertoire autoreactive B lymphocytes with beta cell autoantigen specificity.¹³ Physiologically, B cell tolerance occurs via two dominant mechanisms, central deletion of autoreactive B cell clones in the bone marrow or functional inactivation in the peripheral lymphoid system. The stringency of selection at the latter tolerance checkpoint is dominantly regulated by the TNF-related B cell survival factor, B lymphocyte stimulator (BLyS) which is the limiting survival factor required for successful B cell maturation. Naji is currently focusing on the processes that govern the tolerance checkpoint of immature transitional B cells in the peripheral immune system and the effect of modulating this checkpoint on the development of diabetes.

In this regard, Najj recently demonstrated in NOD mice that neutralization of BLYS leads to marked depletion of follicular and marginal zone B lymphocytes in the spleen, abrogating production of insulin autoantibodies, and offering protection from progression to spontaneous diabetes.¹⁴ As such, BLYS inactivation may be a logical and novel target of immunotherapy for the prevention of islet beta cell destruction of type I diabetic patients. Dr. Najj's work also provided a mechanistic rationale for testing the efficacy of the anti-BLYS antibody belimumab as a novel immunotherapeutic agent for the prevention or reversal of type I diabetes in the clinical setting.

Based on their findings on the requisite role of B lymphocytes in autoimmune diabetes, Barker and Najj have investigated the efficacy of B cell depletion therapy in the induction of immunological tolerance to islet allografts in non-human primates. These studies revealed that B cell depletion at the time of islet transplantation promotes prolonged islet allograft survival without the need for chronic immunosuppression.¹⁵ An immunosuppressive regimen including the anti B cell agent rituximab allows survival of islet allografts transplanted to diabetic cynomolgus monkeys for as long as 4 years.

Convinced of the progress they made over two decades of research to develop effective immune intervention strategies to prevent the rejection of islet allografts, Barker and Najj developed a clinical islet transplantation program at the University of Pennsylvania. The program, established in 1999 includes a state of the art cGMP islet isolation facility that produces high-quality human islets both for clinical transplantation and basic research. The facility has been selected as an Islet Cell Resource Center in the United States, not only for transplants in their own program but also for distributing high-quality human islets to other centers for transplantation and basic research in islet biology. The outcome of the

initial series of 18 human islet transplants performed at the University of Pennsylvania between 2001 and 2005 was similar to that of the widely reported simultaneous series at Edmonton.^{16,17} All patients who completed the protocol of 2-3 islet infusions became normoglycemic without requirement for insulin. But over the next 4-5 years, there was a progressive loss of islet allograft function and recurrence of diabetes indicating vulnerability of islets grafts to recurrent autoimmunity or rejection just as predicted by the earlier experiments of Barker and Najj in BB rats.

Encouraging results from the pre-clinical studies on the efficacy of B cell depletion therapy to promote tolerance to islet transplants was one factor leading to the selection by NIH of the University of Pennsylvania's human islet transplantation program as one of only three U.S. centers to be a member of the Clinical Islet Transplant Consortium. These consortium centers are charged with the mission to improve islet transplant outcome and avoid late failures by exploiting several new immunosuppressive protocols. A central aim of Najj and Barker's program at the University of Pennsylvania is implementing novel immunotherapies including the one based on Najj's primate studies. It targets B lymphocytes with the aim of achieving transplant tolerance. The first two islet transplants performed under this NIH supported trial were performed by Najj at the University of Pennsylvania in 2008. To date, the subjects show outstanding degrees of glycemic control and have remained insulin independent for more than a year.

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