Saving islets from allograft rejection

Terry B. Strom*

Harvard Medical School and Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine-1, Room 026, 77 Avenue Louis Pasteur, Boston, MA 02115

In a recent issue of PNAS, Lewis *et al.* (1) demonstrated that treatment with human α1-antitrypsin, the major serum serine-protease inhibitor whose substrates include many proinflammatory and prothrombotic molecules, serves to protect murine insulin-producing pancreatic islet transplants placed into insulin-deficient recipients grossly mismatched for major histocompatibility complex (MHC) genes from early rejection. This observation is interesting, very relevant to troublesome clinical practice issues, and somewhat counterintuitive. Although rejection of islet allografts is a T cell-dependent immune response (reviewed in ref. 2), α1-antitrypsin, an apparently safe agent approved by the Federal Drug Administration for use in other circumstances, is known as an anti-inflammatory agent, not as an immunosuppressive agent. Nonetheless, until mouse anti-human α1-antitrypsin antibodies are generated, the islet allografts are protected from rejection. Although it is not certain whether α1-antitrypsin can completely protect or (more likely) delay graft rejection, the respite from T cell-dependent rejection afforded by α1-antitrypsin is a delightful surprise.

Islet Allografts Are Very Vulnerable to Inflammation and Ischemia

The marked susceptibility of islets to injurious effects of activated macrophages (3) and proinflammatory cytokines (4), cytokines that are often expressed as the product of activated macrophages, are well known. Moreover, islet transplants are subjected to ischemia and anoxia as a consequence of the transplant procedures. During the harvesting of donor islets, the islets are literally stripped from their blood vessels. After transplantation, perfusion of the graft is drastically compromised for days until neovascularization of the islet allograft is established (2). Once blood flow is established, the islets obviously become an inviting target for ischemia–reperfusion type injury. In addition to the inherent ischemia and anoxia during the period of compromised blood flow, coagulation and thrombosis also contribute to the multiple insults suffered by the islet allografts during the peritransplant and early posttransplant period (5). Ischemia–reperfusion and coagulation–thrombosis lead to inflammation, and the islets, as noted above, are very sensitive to inflammation (3, 4). Therefore, islet allografts are inherently fragile and susceptible to inflammatory, ischemic, coagulative, and anoxic processes.

α1-Antitrypsin Protects Islet Allografts from Early Rejection

In this context, we can appreciate the potential role of antiinflammatory agents for aiding the engraftment of a critical mass of healthy and robust islets. It is well established that α1-antitrypsin inhibits the enzymatic activity of numerous proinflammatory and prothrombotic molecules (6), and α1-antitrypsin is shown by Lewis *et al.* (1) to exert potent antiinflammatory effects in several newly described settings. α1-Antitrypsin inhibits expression of proinflammatory genes and the infiltration of mononuclear leukocytes into inflamed areas (1). Insofar as proinflammatory cytokines induce expression of class II MHC molecules upon the surface of many cell types that, in the noninflamed state, lack expression on MHC class II, it is not surprising that α1-antitrypsin treatment served to restrain the expression of highly immunogenic MHC class II cell surface molecules upon the islets. In short, α1-antitrypsin exercises a wide spectrum of islet-protective effects (1). The expression of proinflammatory cytokines is reduced (1). The serine-protease inhibitor-related effects of α1-antitrypsin on destroying the enzymatic function of other proinflammatory and prothrombotic molecules act to further reduce damage. Expression of donor MHC class II molecules is a potent stimulus for activation of host anti-donor $CD4^+$ T cells. Therefore, the immune-stimulating potential for inflammation-induced expression of MHC class II molecules is reduced (1). Finally, α1-antitrypsin also confers a state of resilience or cytoprotection to the islets so that they are less vulnerable to a variety of insults (1). The cytoprotective property is α1-antitrypsin, demonstrated through resistance of islets to the detrimental effects of the beta cell toxic drug stretozotocin *in vivo* and to proinflammatory cytokines such as IL-1β and INF-γ *in vitro* (1). It is notable that cytoprotective agents often elicit anti-inflammatory effects. Indeed, these properties may be inextricably linked (reviewed in ref. 7).

α1-Antitrypsin exercises a wide spectrum of islet-protective effects.

By what means do the antiinflammatory, antithrombotic, and cytoprotective properties of α1-antitrypsin treatment protect islet allografts from rejection? Clearly, rejection of islet allografts is a T cell-dependent process that is orchestrated by a subpopulation of host T cells bearing specific T cell receptors for donor alloantigens (reviewed in ref. 2). T cell-deficient hosts do not reject allografts. It does not seem reasonable that the ability of α1-antitrypsin treatment to diminish MHC class II expression and, hence, the immunogenicity of the graft provides a sufficient explanation for prevention of early acute rejection. This change might weaken, but not eliminate, the vigor of rejection mediated by the T cell-dependent allograft response. By convention, the participation of inflammatory mechanisms in allograft rejection and other expressions of cytodestructive T helper 1 type immunity have been mapped as a downstream consequence of the T helper 1 type anti-donor-directed transplant immunity that creates rejection. In this scenario, $CD4^+$ T cell-dependent activation of inflammatory cells participates in the rejection process. Although the role of T cell-dependent processes in rejection is unquestioned, the ability of α1-antitrypsin, an antiinflammatory agent, to protect the graft from early rejection suggests a more proximal role for inflammation in the events of rejection than the scenario portrayed above. Indeed, the role of inflammation to provoke vigorous graft rejection and other forms of cytodestructive T cell-dependent immunity has become evident (8, 9). Aggressive, cytodestructive forms of T cell-dependent immunity are fostered when T cells recognize antigen within an inflamed mi-

See companion article on page 12153 in issue 34 of volume 102.

*E-mail: tstrom@bidmc.harvard.edu.

lieu (8, 9). In the context of organ or cell transplantation, inflammation occurs as a consequence of anoxic and ischemia–reperfusion type injury, and kidney transplants that express a high abundance of TNF-α and other proinflammatory cytokines are prone to rejection (10). Evidence of the fragility of human islet transplants is evident in the requirement for transplantation of islets harvested from more than one pancreas to render the patient free from insulin therapy (11). Although some circumstances allow success with use of islets derived from a single donor (12), serial islet transplants are typically performed with use of a different donor for each transplant. In contrast, a single donor is sufficient to relieve hyperglycemia when whole-organ pancreas transplantation is used for the treatment of type 1 diabetes mellitus.

There has been a great and recent revival of interest in clinical islet transplants as modern harvesting and purification procedures (reviewed in ref. 2), and the use of islets extracted from multiple pancreases along with deployment of immunosuppressive drug regimens that minimize use of agents that cause beta cell toxicity and insulin resistance have led to an excellent rate of engraftment in experienced centers at 1 year (11). Insofar as islet transplants, unlike whole pancreas, do not require invasive surgery, islet transplantation is an alluring alternative to whole-pancreas transplantation for insulin-deficient individuals with type 1 diabetes mellitus. Nonetheless, recent observations on the longer-term follow-up data of islet transplant recipients have partially dampened the reborn enthusiasm for islet transplantation because most recipients who are rendered free from insulin therapy at 1 year after transplantation need to reinstitute insulin therapy within 5 years after transplantation (13). The fragility of the islet preparations in the peritransplant and immediate posttransplant period, as evidenced by the usual requirement for transplantation with islets derived from multiple donors (11), is paralleled by a disturbing incidence of graft dysfunction evident by 5 years after transplantation (13).

In this context, the data provided by Lewis *et al.* (1) are indeed exciting. Inflammation provokes especially potent rejection, and inflammation is almost certainly involved in an inability to successfully engraft, under routine circumstances, one recipient with islets derived from a single donor (11). Perhaps the long-term viability of islet transplants is also compromised by consequences of inflammation occurring in the peritransplant period (13). It seems reasonable to pursue antiinflammatory as well as immunosuppressive or immune tolerizing approaches in parallel as a means to further improve clinical outcomes with patients receiving conventional immunosuppressive therapy. Can the use of islet cytoprotective agents, a list of drugs that includes but is not limited to α1-antitrypsin, in the early posttransplant period aid short- and long-term engraftment of islets? With some caution, I am optimistic.

Can the use of islet cytoprotective agents in the early posttransplant period aid engraftment of islets?

There are other important opportunities for the use of α1-antitrypsin and other cytoprotective, antiinflammatory, and antithrombotic agents to aid engraftment of allogeneic islet transplants. At present, islet transplantation is used as a means to slow the progression of diabetes-related disabilities in patients with important complications of the type 1 diabetic state such as life-endangering silent hypoglycemia or renal, retinal, or cardiovascular disease. Islet transplantation is not often recommended as a means to prevent complications in those with recent-onset diabetes (2). Why is islet transplantation recommended for patients with complications of the type 1 diabetic state but not to new-onset type 1 diabetics to prevent these complications? It is feared that the long-term complications of the daily antirejection regimens will lead to worse outcomes than long-term insulin therapy. In addition to the inherent problems associated with long-term drug-induced immunodeficiency, the immunosuppressive drugs produce adverse effects on a variety of nonimmune system tissues. For islet transplantation to become widely applicable, we will need to develop successful and safe strategies for inducing tolerance to islet transplants, thereby relieving the recipient from the untoward effects of long-term immunosuppression. Clearly, the induction of islet transplant tolerance in humans will prove challenging, but this goal seems altogether impossible until a means to control intragraft inflammation and its inherent impact upon catalyzing aggressive antidonor immunity is achieved. In this context, the successful α1-antitrypsin and/or other agents that alleviate intraislet inflammation and impart cytoprotection are of great importance. It will not be easy to induce tolerance to short-lived, inflamed, highly immunogenic, multidonor islet allografts. I believe that the development of immune-tolerizing strategies and cytoprotective agents should be pursued as part of an integrated strategy to provide the best opportunity for a breakthrough in the clinic. The work of Lewis *et al.* affords considerable hope that an effective antiinflammatory strategy can modify the vigor and tempo of rejection.

1. Lewis, E. C., Shapiro, L., Bowers, O. J. & Dinarello, C. A. (2005) *Proc. Natl. Acad. Sci. USA* **102,** 12153–12158.
2. Ricordi, C. & Strom, T. B. (2004) *Nat. Rev. Immunol.* **4,** 259–268.
3. Kaufman, D. B., Platt, J. L., Rabe, F. L., Dunn, D. L., Bach, F. H. & Sutherland, D. E. (1990) *J. Exp. Med.* **172,** 291–302.
4. Bendtzen, K., Mandrup-Poulsen, T., Nerup, J., Nielsen, J. H., Dinarello, C. A. & Svenson, M. (1986) *Science* **232,** 1545–1547.
5. Robson, S. C, Wu, Y., Sun, X., Knosalla, C., Dwyer, K. & Enjyoji, K. (2005) *Semin. Thromb. Hemostasis* **31,** 217–233.
6. Breit, S. N., Wakefield, D., Robinson, J. P., Luckhurst, E., Clark, P. & Penny, R. (1985) *Clin. Immunol. Immunopathol.* **35,** 363–380.
7. Otterbein, L. E., Soares, M. P., Yamashita, K. & Bach, F. H. (2003) *Trends Immunol.* **24,** 449–455.
8. Matzinger, P. (2002) *Science* **296,** 301–305.
9. Medzhitov, R. & Janeway, C. A., Jr. (2002) *Science* **296,** 298–300.
10. Avihingsanon, Y., Ma, N., Pavlakis, M., Chon, W. Y., Uknis, M. E., Monaco, A. P., Ferran, C., Stillman, I., Schachter, A. D., Mottley, C., *et al.* (2005) *J. Am. Soc. Nephrol.* **16,** 1542–1548.
11. Shapiro, A. M. J., Lakey, J. R. T., Ryan, E. A., Korbutt, G. S., Toth, E., Warnock, G. L., Kneteman, N. M. & Rajotte, R. V. (2000) *N. Engl. J. Med.* **4,** 230–238.
12. Hering, B. J., Kandaswamy, R., Ansite, J. D., Eckman, P. M., Nakano, M., Sawada, T., Matsumoto, I., Ihm, S. H., Zhang, H. J., Parkey, J., *et al.* (2005) *J. Am. Med. Assoc.* **293,** 830–835.
13. Ryan, E. A., Paty, B. W., Senior, P. A., Bigam, D., Alfadhli, E., Kneteman, N. M., Lakey, J. R. & Shapiro, A. M. (2005) *Diabetes* **54,** 2060–2069.