Pretransplant Immune Parameters Associate With Islet Allograft Outcome

Implications for Transplant Strategy?

Norma S. Kenyon and Camillo Ricordi

Extensive and informative immunological assessments of islet transplant recipients have been limited due, in part, to the smaller numbers of patients transplanted compared with solid organ trials. Limitations related to blood volume and the logistical challenges of collecting multiple posttransplant samples for drug levels as well as clinical and metabolic parameters also pose challenges. Several variables affect islet allograft outcome, including allo- and autoimmune responses to transplanted islets, β-cell mass and islet quality, efficacy of the immunosuppressive regimen and its effect on islet function, as well as glycemic control. In fact, much debate has centered on whether or not islet attrition is due to immune-mediated loss, death of overworked cells caused by insufficient mass, and/or diabetogenic effect of various immune intervention agents. In addition, many of these parameters may have differing effects based on the transplanted mass, with marginal mass transplants being more susceptible to immune-mediated or stress-induced loss.

Recently, several studies have demonstrated immunological alterations in islet allograft patients. Posttransplant changes in lymphocytes and lymphocyte subsets were reported to be variable depending on the immune suppression used but have not yet been associated with graft status or specific clinical outcomes (1–4). Higher posttransplant cytotoxic T-cell precursor frequency against donor antigens was associated with poorer outcome at 6 months in recipients treated with sirolimus-based maintenance therapy but not tacrolimus–mycophenolate mofetil (5). In one study, cytokine production in allogeneic mixed lymphocyte reactions (MLRs) was skewed toward a Th2 profile in recipients who achieved insulin independence, but no differences were noted between groups prior to transplant (6). Hyporesponsive posttransplant MLRs have been observed in islet recipients treated with steroid-free immune suppression (3) and in islet/stem cell recipients (2), with increases above baseline occurring subsequent to graft rejection. All of these findings are associated with eventual graft outcome, as opposed to demonstration of predictive changes in the posttransplant period, and provide indications to the immunological alterations that have occurred in successfully transplanted recipients. Elevation of cytotoxic lymphocyte gene expression has been shown to predict impending islet allograft rejection in nonhuman primates (7) and clinical islet transplant recipients (2,3,8). Alloantibodies are generally not observed until a patient has already lost significant islet function and immune suppression is being tapered or has been discontinued (3,9), and autoantibody status has not been proven to impact islet allograft outcome (2,3,10). In the absence of a clinical assay that is associated with rejection, such as creatinine for renal and amylase for pancreatic allografts, the lack of a validated marker of islet rejection remains a challenge to be overcome and limits the ability to interfere at an early stage of graft loss.

In this issue of *Diabetes*, Hilbrands et al. (4) demonstrate that higher baseline total and B lymphocyte cell counts, as well as T-cell autoreactivity to islet antigens, are associated with poorer islet transplant outcome. The analyses of immune and graft outcome parameters included 30 nonuremic, C-peptide–negative type 1 diabetic patients who received an intraportal islet cell graft under the cover of anti-thymocyte globulin induction and maintenance with tacrolimus and mycophenolate mofetil. Absolute leukocyte, lymphocyte, and lymphocyte subset (T-, B-, and natural killer cell) counts, autoantibodies, and T-cell autoreactivity were compared with achievement of insulin independence, plasma C-peptide, and glycemic variability at 6 months posttransplant. Fifteen patients were insulin independent at 6 months and were compared with those who were not. Observed correlations from univariate analysis were further examined in a multivariate model. At baseline, patients who would ultimately become insulin independent had significantly lower total lymphocyte, as well as CD19+ B- and CD3+ T-cell counts, with a lower count of CD3/8 T-cells contributing to the difference in CD3 cells. There was no association between baseline autoantibody positivity and graft outcome. However, T-cell autoreactivity against IA-2 and/or GAD was lower at baseline in the group that became insulin independent; this effect was specific for recipients of suboptimal β-cell mass (4,11). The authors conclude that prospective studies are needed to assess whether control of these characteristics can help increase the function of islet cell grafts during the first posttransplant year.

As previously mentioned, the logistics of posttransplant sample collection are challenging, and the blood volume required for many assays necessitates that relatively few samples are collected over time, with quarterly monitoring being fairly standard. This background, together with the constantly changing immunosuppressive landscape, makes it difficult to convincingly demonstrate the utility/

From the Department of Surgery and Diabetes Research Institute, Miller School of Medicine, University of Miami, Miami, Florida.
Corresponding author: Camillo Ricordi, cricordi@med.miami.edu.
DOI: 10.2337/db09-1147

See accompanying original article, p. 2267.

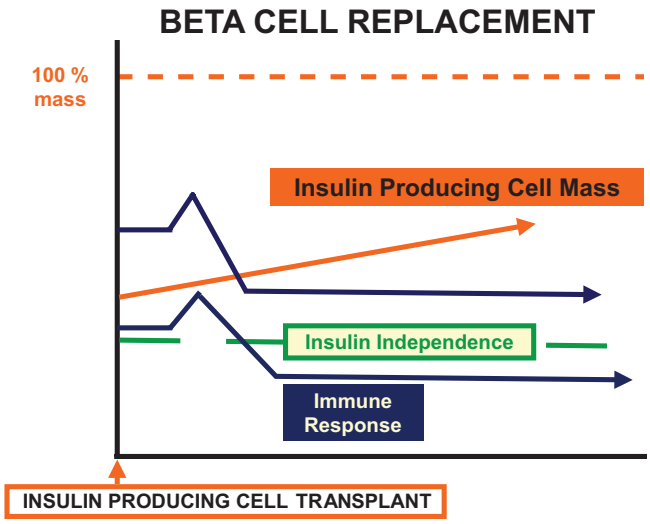

FIG. 1. Individuals with type 1 diabetes are generally diagnosed after a dramatic reduction in β-cell mass. It has been postulated that a person can lose up to 80% of his or her islet mass prior to showing clinical evidence of diabetes. The dashed orange line represents 100% mass, and the solid green line represents the mass required to maintain insulin independence. The two solid blue lines represent the immune status of an individual to both auto- and allo-antigens; the slight posttransplant increase represents the inflammatory response to the islet infusion, followed by decreased reactivity due to immune intervention. A lower pre-transplant immune profile (lower blue line) would result in a level of immunity that allows for maintenance of insulin independence, while a higher profile (upper blue line) would be suppressed but not enough to prevent gradual islet loss due to rejection and/or recurrent autoimmunity. If immunity is adequately suppressed, the transplanted islet mass can slowly increase over time (solid orange line).

validity of various assays in monitoring islet allograft outcome. The results in the article by Hilbrands et al. offer potentially very useful and logistically simple assessments that can be done prior to transplant to determine which patients are most likely to become insulin independent. The studies can be done while the patient is on the waiting list and can be relatively easily scheduled for a blood draw. Analyses of total lymphocyte as well as CD3/8 T- and B-cell counts in addition to T-cell–directed autoreactivity to IA-2 and GAD could provide a useful tool for tailoring transplant strategy. As an example, if the β-cell mass available for transplant is low, one would not want to transplant a patient with baseline T-cell autoreactivity. It will be of interest to undertake similar analyses for islet transplant patients being treated with immune intervention protocols that are not thymoglobulin–mycophenolate mofetil based. If baseline assessment of cell counts and T-cell autoreactivity can predict outcome, as suggested by the results and schematically represented in Fig. 1, it would be important to add an additional immune intervention agent for patients who have higher baseline immune parameters. Agents that target β-cells and memory T-cell populations are good candidates for additional therapeutics, as are blockers of costimulation. Especially in light of the limited numbers of donors available for pancreatic islet transplant trials, selection of patients who have a better prognostic profile would allow for optimal resource allocation.

ACKNOWLEDGMENTS

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Toso C, Edgar R, Pawlick R, Emamaullee J, Merani S, Dinyari P, Mueller TF, Shapiro AM, Anderson CC. Effect of different induction strategies on effector, regulatory and memory lymphocyte sub-populations in clinical islet transplantation. Transpl Int 2009;22:182–191
2. Mineo D, Ricordi C, Xu X, Pileggi A, Garcia-Morales R, Khan A, Baidal DA, Han D, Monroy K, Miller J, Pugliese A, Froud T, Inverardi L, Kenyon NS, Alejandro R. Combined islet and hematopoietic stem cell allotransplantation: a clinical pilot trial to induce chimerism and graft tolerance. Am J Transplant 2008;8:1262–1274
3. Froud T, Ricordi C, Baidal DA, Hafiz MM, Ponte G, Cure P, Pileggi A, Poggioli R, Ichii H, Khan A, Ferreira JV, Pugliese A, Esquenazi VV, Kenyon NS, Alejandro R. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant 2005;5:2037–2046
4. Hilbrands R, Huurman VAL, Gillard P, Velthuis JHL, De Waele M, Mathieu C, Kaufman L, Pipeleers-Marichal M, Ling Z, Movahedi B, Jacobs-Tulleneers-Thevissen D, Monbaliu D, Ysebaert D, Gorus FK, Roep BO, Pipeleers DG, Keymeulen B. Differences in baseline lymphocyte counts and autoreactivity are associated with differences in outcome of islet cell transplantation in type 1 diabetic patients. Diabetes 2009;58:2267–2276
5. Roelen DL, Huurman VA, Hilbrands R, Gillard P, Duinkerken G, van der Meer-Prins PW, Versteeg-van der Voort Maarschalk MF, Mathieu C, Keymeulen B, Pipeleers DG, Roep BO, Claas FH. Relevance of cytotoxic alloreactivity under different immunosuppressive regimens in clinical islet cell transplantation. Clin Exp Immunol 2009;156:141–148
6. Huurman VA, Velthuis JH, Hilbrands R, Tree TI, Gillard P, van der Meer-Prins PM, Duinkerken G, Pinkse GG, Keymeulen B, Roelen DL, Claas FH, Pipeleers DG, Roep BO. Allograft-specific cytokine profiles associate with clinical outcome after islet cell transplantation. Am J Transplant 2009;9:382–388
7. Han D, Xu X, Pastori RL, Ricordi C, Kenyon NS. Elevation of cytotoxic lymphocyte gene expression is predictive of islet allograft rejection in nonhuman primates. Diabetes 2002;51:562–566
8. Han D, Xu X, Baidal D, Leith J, Ricordi C, Alejandro R, Kenyon NS. Assessment of cytotoxic lymphocyte gene expression in the peripheral blood of human islet allograft recipients: elevation precedes clinical evidence of rejection. Diabetes 2004;53:2281–2290
9. Cardani R, Pileggi A, Ricordi C, Gomez C, Baidal DA, Ponte GG, Mineo D, Faradji RN, Froud T, Ciancio G, Esquenazi V, Burke GW 3rd, Selvaggi G, Miller J, Kenyon NS, Alejandro R. Allosensitization of islet allograft recipients. Transplantation 2007;84:1413–1427
10. Vantyghem MC, Kerr-Conte J, Arnalsteen L, Sergent G, Defrance F, Gmyr V, Declerck N, Raverdy V, Vandewalle B, Pigny P, Noel C, Pattou F. Primary graft function, metabolic control, and graft survival after islet transplantation. Diabetes Care 2009;32:1473–1478
11. Huurman VA, Hilbrands R, Pinkse GG, Gillard P, Duinkerken G, van de Linde P, van der Meer-Prins PM, Versteeg-van der Voort Maarschalk MF, Verbeeck K, Alizadeh BZ, Mathieu C, Gorus FK, Roelen DL, Claas FH, Keymeulen B, Pipeleers DG, Roep BO. Cellular islet autoimmunity associates with clinical outcome of islet cell transplantation. PLoS One 2008;3:e2435