Targeting Purinergic Receptors in Islet Transplantation

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Transplanted islets are exposed to a distinctively hostile immunological environment. Grafted islets typically encounter a combination of innate, allogeneic and anti-islet immune responses—the relative contributions of which are not fully understood. The relatively low success rate of islet transplantation compared with other organs suggests that current immunomodulatory protocols are inadequate to provide long-term islet graft protection. As the negative consequences of long-term immunosuppression can arguably outweigh the benefits of islet transplantation, there is intense focus on the development of strategies to induce immune tolerance toward grafts, thereby avoiding the requirement of chronic immunosuppression. In this issue of *Diabetes*, Vergani et al. (1) provide evidence that targeting purinergic receptor signaling is a viable strategy for islet allograft protection.

Extracellular ATP modulates many aspects of the immune system. For example, ATP signaling is able to promote the differentiation of proinflammatory Th17 cells (2), which may play a role in islet rejection and recurrent autoimmunity. ATP signaling can also suppress the differentiation and function of regulatory T cells (Tregs) (3), which are immunosuppressive T cells that play a role in immune tolerance (4). Thus, ATP signaling acts to shift the immune response toward a more inflammatory, less tolerant state (Fig. 1).

Extracellular ATP can be recognized by different cell surface receptors. The P2X family receptors are nonselective Ca^{2+} channels activated by ATP. Seven P2X receptors have been identified and mediate many immune cell responses. For example, T-cell activation requires autocrine activation of P2X1, P2X4, and P2X7 to amplify intracellular calcium levels to those required for downstream signaling and interleukin (*IL*)-*2* transcription (5). P2X7 is also expressed on Tregs and acts to suppress the function of these immunosuppressor cells (3). The fact that P2X7/ATP signaling promotes inflammatory T-cell differentiation and activation, while at the same time inhibits the generation and function of immunosuppressive Tregs, makes P2X7 an attractive target for therapies aimed at immunosuppression and tolerance induction.

Vergani et al. tested the role of purinergic signaling in a mouse model of islet allograft rejection using the irreversible purinergic inhibitor oxidized ATP (oATP). In a model where 100% of control islet allografts were rejected by 14 days, 30% of the transplanted mice treated with oATP during the first 14 days postsurgery demonstrated long-term graft function (>100 days). oATP-treated mice had significantly less activated T cells and Th17 cells. While there was no effect of oATP on Treg numbers, histological examination of the grafts demonstrated an enrichment of Tregs in the grafts of oATP-treated mice. Therefore, oATP shifted the immune response toward a more tolerant state (Fig. 1), resulting in long-term graft tolerance in 30% of mice.

To examine the specific role of P2X7 in islet allograft rejection, Vergani et al. performed islet allotransplants into *P2X7* null mice. The absence of P2X7 resulted in a marginal increase in graft survival of only 5 days, suggesting that *1*) other receptors are responsible for the protective effect of oATP, or *2*) a compensatory mechanism has developed in the *P2X7* null mice. The authors provide evidence supporting both possibilities. First, they demonstrate that oATP still has a protective effect in *P2X7* null animals, clearly showing a biological effect of this compound in the absence of P2X7 and confirming the promiscuous activity of this compound (6,7). Second, the authors found a compensatory change in expression of other P2X proteins in the absence of P2X7. Thus, the therapeutic target of oATP in this model remains unclear.

Rapamycin (sirolimus) is a clinically used immunosuppressant drug with tolerance-inducing properties (8). In the present work, Vergani et al. found that short-term treatment with a combination of oATP and rapamycin resulted in indefinite (>100 days) survival of grafts in 5 of 7 mice, demonstrating a synergistic effect of rapamycin and oATP on islet engraftment.

To gain insight into the potential relevance of P2X targeting in human islet transplantation, Vergani et al. examined the expression of P2X7 on immune cells of islet transplant recipients. The authors observed an expansion of P2X7 expressing memory T cells in long-term islet transplant recipients compared with either recently transplanted or healthy individuals. Expansion of memory T cells has previously been demonstrated in long-term islet transplant recipients receiving a standard immunosuppressive regimen (9) and may contribute to the limited long-term success of islet transplantation. Thus, the present findings suggest that P2X7 may be a viable target to modulate memory cell function.

An exciting finding in the current study is that short-term oATP treatment can induce graft tolerance, a property of P2X7 inhibition previously suggested in other contexts (10). Equally notable is the synergy between rapamycin and oATP in the induction of graft tolerance, which could arise from differential targeting of signals controlling T-cell activation, where purinergic receptor inhibition inhibits T-cell receptor–dependent signaling and IL-2 expression, while rapamycin decreases IL-2 signaling. These findings suggest that although rapamycin can negatively impact insulin action (11), its short-term use as part of a tolerance-inducing strategy could prove beneficial.

Many immunosuppressant agents negatively impact islet function. For example, tacrolimus (FK506) blocks calcium-mediated T-cell activation by targeting calcineurin, which is also required for islet function (12,13). oATP also affects human islet function, and this effect was suggested to be due to targeting of P2X3, not P2X7 (14). While it remains

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DOI: 10.2337/db13-0211

See accompanying original article, p. 1665.

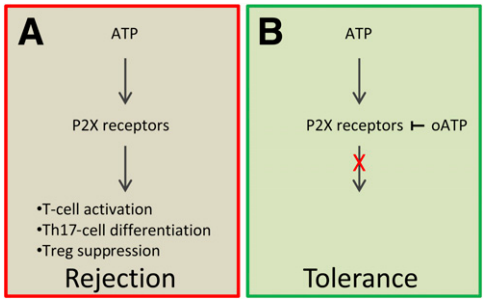

FIG. 1. Schematic summary of the proposed mechanism of action of ATP during islet allograft rejection. *A*: Extracellular ATP activates P2X receptors and promotes T-cell activation and Th17-cell differentiation, while suppressing Treg function. *B*: oATP treatment blocks P2X signaling, promoting a less inflammatory, more tolerant state.

unclear precisely which receptor is targeted by oATP in the study by Vergani et al. (1), the fact that P2X3 is not expressed on most immune cell types and that a P2X3 inhibitor did not afford protection in an in vitro alloimmune assay suggests it may be possible to target immune-related P2X signaling without affecting islet-specific P2X function. Regardless, a short-term impairment of islet function would be an acceptable compromise for long-term graft tolerance.

The present work offers new and exciting possibilities for immunosuppression in islet transplantation. Mechanistic studies identifying the particular receptors and target cell types of oATP are warranted and could prove valuable in optimizing tolerance-inducing therapies. In addition, the fact that purinergic inhibitors are already available for human use provides the opportunity for these findings to have a rapid clinical impact.

ACKNOWLEDGMENTS

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

The author thanks Quim Madrenas, McGill University, and Andrew Slack, Health Canada, for helpful comments and suggestions.

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