Tolerance and pancreatic islet transplantation

Luca Inverardi and Camillo Ricordi*

*Diabetes Research Institute, Cell Transplant Center, University of Miami School of Medicine,
1450 NW 10th Avenue, Miami, FL 33136, USA*

Islet transplantation holds renewed promise as a cure for type I diabetes mellitus. Results of recent clinical trials have shown remarkable success, and have reignited universal optimism for this procedure. In spite of this success, the need for life-long immunosuppression of the recipient still limits islet transplantation to patients with poorly controlled diabetes or to those requiring kidney transplantation. It is obvious that the achievement of immunological tolerance would broaden the indication for islet transplantation to a much larger cohort of patients with type I diabetes mellitus, most likely preventing long-term complications and contributing to a much improved quality of life. Increased understanding of the basic mechanisms of tolerance induction has resulted in the implementation of numerous experimental approaches to achieve long-term survival of islet grafts in the absence of chronic immunosuppression. In this brief review we will attempt to summarize the current status of research and knowledge.

Keywords: type I diabetes; islets of Langerhans; transplantation; immunological tolerance; islet transplantation

1. ISLET TRANSPLANTATION

Biological replacement of insulin-producing tissue represents a logical approach to the treatment of type I diabetes mellitus. Transplantation of a vascularized pancreas is now often successful in the long term but has relative disadvantages, since surgical complications and morbidity are quite often observed. In contrast, islet transplantation is a safe procedure, performed under local anaesthesia with extremely rare occurrence of complications. The success rate of islet transplantation, on the other hand, has historically been much lower than that of pancreas transplantation, substantially diminishing the overall enthusiasm for this procedure (International Islet Transplantation Registry, <http://www.med.uni-giessen.de/itr/>).

Recent data from at least three islet transplant centres have provided clear evidence of a much improved success rate in type I diabetic patients transplanted with allogeneic islets of Langerhans. At the University of Giessen, Germany, a first series of data was published (Bretzel *et al.* 1999), reporting graft success rates substantially superior to those observed previously. Patients received allogeneic islets under the umbrella of conventional immunosuppression, but were managed, in the peritransplant period, in a novel fashion, with administration of drugs, including pentoxifylline, and were kept under the strictest metabolic control for the first weeks following transplantation (total parenteral nutrition, intravenous insulin administration). The reported success rate, in terms of insulin independence, was 75–100% at three months.

More recently, data were reported by another European group (from Milan) that also showed much improved results in terms of insulin independence. Thirty-nine per cent of allogeneic islet recipients achieved insulin independence following intrahepatic islet transplantation with conventional immunosuppression (Maffi *et al.* 2000).

A group of researchers from Edmonton, Canada, has perhaps the most exciting data ever communicated in the field of clinical islet transplantation. Seven patients out of seven that were transplanted achieved insulin independence, with a mean follow-up approaching one year. Many novel features characterize the procedure followed by the Canadian group, including the use of rapamycin and low dose FK-506 as immunosuppressive therapy, after a brief induction that includes the administration of an anti-IL-2 receptor antibody (Shapiro *et al.* 2000).

It is therefore obvious to foresee that islet transplantation will soon become an accepted alternative to conventional insulin treatment in a much larger cohort of patients. The problems that remain to be solved are twofold: first, the increased demand for human islets for transplantation will make the existing discrepancy between organ availability and potential recipients even more acute than it is now; second, the need for chronic immunosuppression will still limit indications for islet transplantation to patients that are poorly controlled or that require a simultaneous kidney transplant for end-stage renal failure (Hering & Ricordi 1999).

The need for alternative sources of insulin-producing tissue can be addressed by increasing the pool of human donors, by generating alternative beta cells or by using non-human sources of islets. All of these approaches to the problem are being actively pursued (Groth *et al.* 1999;

*Author for correspondence (ricordi@miami.edu).

Newgard *et al.* 1997), and will hopefully contribute to a solution to tissue availability in the immediate future.

The search for effective protocols of tolerance induction is the obvious answer to the second problem, since it would broaden the indication of islet transplantation to virtually all type I diabetic patients, and especially children.

2. THE IMMUNE SYSTEM IN ISLET TRANSPLANTATION

The immune system learns to discriminate between self and non-self through an intricate series of mechanisms. The molecular basis of any individual's identity is represented by the combination of polymorphic antigens that differ amongst individuals. The most important system of polymorphic loci in humans is the human leucocyte antigen (HLA) complex. Minor histocompatibility antigens also play a critical role in allogeneic responsiveness. When a transplant is performed between two non-identical individuals, the differences that exist in the HLA and minor antigen profiles lead to recognition and rejection of the implanted tissue, unless effective immunosuppression is administered to the recipient. Self–non-self discrimination is an acquired characteristic of the immune system based on the positive selection of T-cell clones that react against non-self, and the elimination or silencing of autoreactive clones.

Experimental approaches aimed at the induction of tolerance to alloantigens have the common goal of educating the immune system into accepting selected allogeneic antigens as self.

Induction of tolerance to allogeneic antigens might not suffice in the case of islet transplantation for diabetes, since the pathogenesis of the underlying disease is, in most instances, an autoimmune phenomenon. There is the need, therefore, for tolerization to B-cell autoantigens as well, to promote survival of the grafted tissue and avoiding recurrence of autoimmunity that invariably leads to destruction of insulin-producing B cells in the transplanted islet. While targets of allorecognition and rejection are well defined, targets of autoimmunity in type I diabetes mellitus are only partly characterized in humans, making antigen-specific tolerance induction approaches quite cumbersome (Roep *et al.* 1999).

3. TOLERANCE INDUCTION IN SMALL ANIMALS

Rodents have been widely used to study fundamental questions in the immunobiology of transplantation rejection and tolerance induction. Islet transplantation has also been largely exploited because of the existence of well-defined animal models, the availability of inbred strains and the relative simplicity of the procedure. Also, the existence of the major histocompatibility complex (MHC) class I, MHC class II and minor histocompatibility mismatched congenic strains has made our comprehension of the mechanisms of islet allorecognition quite substantial. Additionally, numerous transgenic and knockout strains allow the precise pinpointing of cellular and molecular mechanisms of allorecognition and rejection. Of utmost value, both a mouse (NOD) and a rat (BB) model system of autoimmune diabetes exist (Wong & Janeway 1999), making it possible to analyse the role of

autoimmunity after islet transplantation, a potentially critical component of the overall fate of islet transplantation in the clinical setting in type I diabetic patients.

(a) *Allogeneic islet transplantation*

After islet isolation the islets are implanted usually in the portal system or under the kidney capsule. The first site of implantation is less often used today in tolerance models, but is potentially more relevant for extrapolation to the clinical setting since it is the preferred one for human islet transplantation. Rodents that receive an islet transplantation across a major or minor histocompatibility barrier invariably reject their grafts, unless immunosuppression is administered, making these animal models relevant for the study of tolerance induction. Numerous approaches to the achievement of tolerance induction have been used in rodents, including the induction of haemolymphopoietic chimerism, the intrathymic administration of alloantigens and administration of antibodies or other compounds capable of blockade of selected cellular and molecular pathways of allograft recognition.

(i) *Haemolymphopoietic chimerism*

The fundamental observations that the establishment of haemolymphopoietic chimerism leads to tolerance of donor-derived tissues and organs is reviewed in detail elsewhere in this issue (Sykes & Sachs). Achievement of mixed chimerism (the coexistence of haemolymphopoietic elements of donor origin with elements derived from the recipient) is characterized by general advantages in terms of overall immune competence and health of the recipient, when compared with full allogeneic chimerism (presence of donor-derived haemolymphopoietic elements only).

Induction of mixed chimerism has been generally achieved with preconditioning regimens based on quite harsh myeloablative treatments, often requiring total body irradiation (lethal or sublethal). The quest for milder induction regimens with a more acceptable rate of morbidity and occurrence of side-effects has indeed led to the development of alternative, efficient approaches.

Induction of stable mixed chimerism in mice and rats results in indefinite survival of donor-specific islet transplantation with reversal of chemically induced diabetes mellitus.

One of the first descriptions of this approach to tolerance induction to allogeneic islet transplantation was published by Britt *et al.* (1982). Total lymphoid irradiation followed by donor-specific bone marrow administration led to long-term islet allograft survival in rats. Chemically induced diabetes was reversed long term, demonstrating the efficacy of this approach for diabetes treatment (Britt *et al.* 1982).

Chimerism induction was also obtained by either lethal or non-lethal conditioning in mice by Li *et al.* (1995b), and led to donor-specific tolerance, long-term islet graft survival and reversal of chemically induced diabetes mellitus. Induction regimens were always based on the administration of total body irradiation. Reconstitution occurred with administration of a mixture of donor-derived and recipient-derived bone marrow cells. This represents a clear demonstration that establishment of mixed allogeneic chimerism results in acceptance of an

islet graft without the need for chronic administration of immunosuppressive drugs. Graft-versus-host disease (GVHD) represents a feared possible complication of chimerism-inducing regimens, especially based on irradiation preconditioning. T-cell depletion of the bone marrow inoculum is effective in preventing GVHD occurrence (Li *et al.* 1995b), but might result in reduced engraftment rate or efficacy (Neipp *et al.* 1999). An alternative approach has been proposed by Jin *et al.* (1992), based on the pretreatment of donor bone marrow with ultraviolet B (UVB) irradiation. The described approach leads to the establishment of long-lasting chimerism without GVHD in rats (Jin *et al.* 1992). Donor-specific islet grafts (as well as heart grafts) survived indefinitely.

A drawback to the described approach is the need for a radiation-based conditioning regimen that might be perceived as unacceptable for clinical trials, especially for the treatment of a non-life-threatening condition such as diabetes mellitus.

A non-myeloablative, non-radiation-based regimen leading to long-term survival of islet allografts in rats has been described by our group where transient administration of FK-506 together with donor-specific bone marrow led to long-lasting reversal of chemically induced diabetes mellitus (Ricordi *et al.* 1996).

Novel non-radiation-based preparative regimens for the successful achievement of long-lasting chimerism, based on co-stimulatory blockade or on the use of rapamycin and anti-lymphocyte globulin, have also been described but no published data are yet available on donor-specific islet graft survival in these models (Durham *et al.* 2000; Wekerle *et al.* 2000; Hale *et al.* 2000).

Microchimerism (chimerism at levels that require molecular biology techniques for detection) might be a much easier goal to achieve in large animals and humans, but there is still no definitive evidence that microchimerism might lead to tolerance induction, even if clear evidence of coexistence of the two phenomena has been convincingly presented (Starzl *et al.* 1993).

(ii) Intrathymic administration of donor antigens

Intrathymic inoculation of alloantigens is another well defined approach to tolerance induction in rodents. An early description of a model of islet transplantation in the thymus of rodents by Posselt *et al.* (1990) gave rise to a considerable body of work using this model. Intrathymic inoculation of islets resulted in long-term acceptance of the graft. Observations on the administration of alloantigens in different forms soon followed, demonstrating that donor cells, including lymphocytes and spleen cells, could similarly promote tolerance induction to donor-specific islet transplants (James *et al.* 1993; Oluwole *et al.* 1993, 1994a; Ohajekwe *et al.* 1995). Also, donor-derived soluble antigens (such as spleen cell membranes) were shown to induce tolerance to islet grafts when administered to young rats (Qian *et al.* 1993). Tolerance could be induced even to multiple donor strains by administration of spleen cell membrane preparations (Qian *et al.* 1995). More recently, evidence has been presented suggesting that donor-derived MHC peptides suffice in inducing tolerance to subsequent islet grafts (Chowdhury *et al.* 1998). Intrathymic inoculation of recipient-derived, donor

MHC class I peptide-pulsed dendritic cells has been described as another effective means of inducing tolerance to islet transplantation (Ali *et al.* 2000). Studies on the mechanisms underlying tolerance achieved via the intrathymic route have suggested the engagement of T-cell mediated regulatory circuits (Odorico *et al.* 1993; Perico *et al.* 1993; Oluwole *et al.* 1994b, 1995, 1999; Remuzzi *et al.* 1995; Turvey *et al.* 1999). While these observations have contributed to elucidating a fascinating area of transplantation immunobiology and tolerance induction, some concerns about its potential usefulness in the clinical arena are legitimate, including anatomical considerations in adult patients.

(iii) Co-stimulation blockade

T lymphocytes require the engagement of the T-cell receptor and a series of co-receptors (accessory signals and/or co-stimulatory signals) to undergo full-blown activation. Blockade of co-stimulation leads to incomplete activation and might result in the induction of antigen-specific unresponsiveness and operational tolerance. Several pathways of co-stimulation have been elucidated, and their manipulation explored as a potentially therapeutic means of tolerance induction. LFA-1, CD154 and CD28 have received keen attention because of their fundamental roles in T-cell activation.

Treatment of mice receiving an allogeneic islet graft with a short course of an anti-LFA-1 antibody resulted in long-term graft acceptance and donor-specific tolerance (Nishihara *et al.* 1997; Nicolls *et al.* 2000).

Similarly, an islet transplantation performed across a fully mismatched histocompatibility barrier was accepted permanently after a transient treatment aimed at blockade of the CD28–CD80/86 interaction (Steurer *et al.* 1995; Tran *et al.* 1997) alone or in combination with blockade of CD40–CD154 interaction (Rastellini *et al.* 1999). Blockade was obtained by the administration of a soluble chimeric molecule (cytotoxic T-lymphocyte antigen 4 immunoglobulin, CTLA-4-Ig) that prevents CD28 engagement and of an anti-CD154 monoclonal antibody.

CD154 blockade has also been explored as part of a strategy comprising the administration of donor-specific spleen cell subpopulations. Convincing data have been presented that transient blockade of co-stimulation, together with the administration of donor-specific cell subsets, can lead to permanent acceptance of allogeneic islet grafts (Parker *et al.* 1995). Mechanisms proposed to explain this effect include deletion of alloreactive CD8⁺ T cells (Iwakoshi *et al.* 2000), and require the engagement of the CTLA-4 signalling pathway (Zheng *et al.* 1999).

(iv) Other approaches

Efficient engagement of the T-cell receptor requires not only the participation of numerous co-stimulatory signals, but also the activity of additional components of the cell signal transduction machinery. Amongst these, the CD45 molecule plays a critical role in early events of T-cell mediated signalling. Isoform-specific antibodies (anti-CD45 RB) have been used as a means of modulating the T-cell signalling in a model of allogeneic islet transplantation in mice. A short course administration of anti-CD45 RB antibodies led to long-term survival of implanted allogeneic islets in fully MHC disparate

recipient mice. Chemically induced diabetes was reversed (Auersvald *et al.* 1997). Mechanistic analysis of the effects of anti-CD45 RB treatment suggests that cell surface isoform switch, and upregulation of CTLA-4, might be involved in the observed efficacy in tolerance induction (Basadonna *et al.* 1998).

Administration of an antibody specific for the common gamma chain of interleukin (IL) 2, IL-4, IL-7, IL-9 and IL-15 cytokine receptor has resulted in long-term survival of allogeneic islet grafted in chemically induced diabetic mice (Li *et al.* 2000).

Targeting of CD4 T lymphocytes with either a depleting (Alters *et al.* 1991; Song *et al.* 1993) or a non-depleting antibody (Lehmann *et al.* 1997) has also been demonstrated to result in permanent islet allograft survival in both mice and rats.

(b) *Xenogeneic islet transplantation*

The use of islets derived from a different species represents a highly desirable goal, since availability of human islets for transplantation is a major obstacle to large-scale application of biological replacement therapeutic strategies for the treatment of type I diabetes mellitus. Tolerance to concordant islets has been obtained in several instances in rodents with approaches similar to the ones described for allograft tolerance induction.

On the other hand, successful transfer of effective protocols from the allogeneic to the xenogeneic setting does not represent a general rule, and failures have also been reported.

(i) *Haemolymphopoietic chimerism*

Haemolymphopoietic chimerism induction has been successful in promoting long-term islet survival in chemically induced diabetic recipients. Rat islets were implanted in either full or mixed xenogeneic chimeras (rat to mouse) after chimerism induction, and indefinitely reversed diabetes (Zeng *et al.* 1992; Ricordi *et al.* 1992; Li *et al.* 1994, 1995a). Both lethal and non-lethal radiation approaches have been described.

In the xenogeneic setting, the development of non-lethal, non-radiation-based induction regimens to achieve tolerance via chimerism will certainly contribute to making this approach potentially applicable in clinical practice.

(ii) *Intrathymic administration of donor antigens*

The intrathymic route of tolerance induction has been explored in xenogeneic species combinations, to extend the promising results that had been observed in the allogeneic setting. Some controversy exists on the effectiveness of this approach, since both positive and negative results have been reported (Goss *et al.* 1994; Dellagiacoma *et al.* 1994; Zeng *et al.* 1993; Tran *et al.* 1999). Substantial differences in the experimental approaches (i.e. source of antigen) and the species combinations used could explain these apparent discrepancies.

(iii) *Co-stimulatory blockade*

Long-term survival of human islets in mice was described by Lenschow in 1992. Administration of CTLA-4-Ig induced donor-specific tolerance without the need for additional immunosuppression (Lenschow *et al.* 1992). Human islets were also used to explore the role of

ICAM-1 blockade on survival and function in a xenogeneic host (mouse). Pretreatment of the islets with an anti-ICAM-1 antibody led to long-term survival of the grafted tissue in 40% of the recipients, with reversal of chemically induced diabetes (Zeng *et al.* 1994).

Treatment of recipients with an anti-donor ICAM-1 antibody together with an anti-recipient LFA-1 antibody was described as an effective technique to induce tolerance to islets in a rat-to-mouse species combination (Grochowicki *et al.* 2000). Interestingly, pretreatment of islets with mitomycin C represented a prerequisite for the achievement of long-term graft survival. More recently, administration of CTLA-4-Ig together with an anti-CD154 antibody has been reported as capable of tolerance induction in a rat-to-mouse concordant xenogeneic combination (Lehnert *et al.* 2000). Tolerance appeared to be species specific but not strain specific. Also, mechanisms underlying the achievement of tolerance did not appear to involve immune deviation (Lehnert *et al.* 2000).

(iv) *Other approaches*

Administration of anti-lymphocyte serum resulted in long-term survival of xenogeneic islets. An early report by Serie *et al.* (1993) described the induction of tolerance to rat neonatal islet in mice by the administration of anti-lymphocyte serum together with the transplant (Serie *et al.* 1993). Tolerance appeared strain specific, since third party rat islets were promptly rejected by the recipient mice.

Masking of MHC class I molecules on islets prior to transplant with a Fab fragment of a specific antibody was reported as a successful strategy by Faustman in a human-to-mouse experimental combination (Faustman & Coe 1991). The efficacy of this approach was not limited to islets since hepatic grafts also showed indefinite survival.

Treatment with anti-CD4 antibodies has also shown promise in tolerance induction protocols in xenogeneic combinations. A non-depleting antibody was effective in a hamster-to-mouse species combination, while a depleting one was not (Lu *et al.* 1998). In fact, persistence of CD4⁺ T lymphocyte seemed a prerequisite for tolerance induction, since their elimination by the depleting antibody reversed the tolerant state obtained with the non-depleting antibody (Lu & Borel 1998).

Pig pro-islets, on the other hand, were accepted permanently in recipient mice treated with a depleting anti-CD4 antibody, suggesting intrinsic differences in the mechanisms underlying these two models of xenotransplantation (Morris *et al.* 1995).

Natural killer (NK) T cells have been postulated recently to play a fundamental role in permitting acceptance of islet xenografts in a concordant species combination (Ikehara *et al.* 2000), and this important observation might contribute to the design of efficient protocols of tolerance induction to xenogeneic islets.

(c) *Transplantation in the autoimmune background*

While tolerance to islet grafts in allogeneic and xenogeneic combinations clearly represents an exciting achievement, it can be argued that tolerance to B cells in the transplanted islets in an autoimmune background represents the ultimate goal of protocols of potential

clinical value for the treatment of type I diabetes. The NOD mouse and the BB rat are invaluable experimental tools in this respect. Interestingly, there is a substantial quantitative discrepancy between the amount of data available in purely allo- and xenogeneic combinations and those available in the transplant models in the auto-immune diabetic models.

Tolerance to islet allotransplants was obtained after induction of full allogeneic chimerism with lethal, radiation-based preconditioning by Li *et al.* (1995a) in the NOD mouse. Attempts to obtain stable mixed chimerism were unsuccessful when approaches successful in non-autoimmune strains were used (H. Li, personal communication). More recently, a sublethal conditioning that included administration of an anti-CD154 antibody resulted similarly in achievement of long-term allogeneic islet graft survival (Seung *et al.* 2000). Although sublethal, the latter approach still requires heavy radiation-based preconditioning of the recipient; refinement of the induction regimens and diminution of risk would be required to justify their clinical application.

The intrathymic approach has not proven effective in BB rats, as reported by the Rossini group (Battan *et al.* 1994). Similarly, a protocol of tolerance induction based on co-stimulatory blockade (via anti-CD154 administration), together with donor-specific transfusion, did not lead to islet graft prolonged survival in NOD mice (Markees *et al.* 1999). The same protocol had resulted in reproducible tolerance induction to islet allografts in a non-autoimmune background (Zheng *et al.* 1999).

4. TOLERANCE INDUCTION IN LARGE ANIMALS

While the use of rodents represents a precious experimental tool for the relatively easy screening of numerous protocols of tolerance induction, extrapolation of the results to the clinical setting is not immediate. The use of large animal model systems, and in particular the use of non-human primates, arguably represents a necessary and highly meaningful step before embarking on clinical trials.

Encouraging data have been reported in a concordant xenogeneic system where primates received intrahepatic islet transplantation together with a brief course of immunosuppression. Immunosuppression was based on the administration of an anti-CD3 antibody-toxin conjugate (CD3 immunotoxin), cyclosporin A and steroids. All recipient primates were rapidly and permanently restored to good endocrine function with excellent metabolic control. Immunosuppression needed to be administered only in the first four days peritransplant (Thomas *et al.* 1999a; Contreras *et al.* 2000). In a parallel series of experiments performed by the same group, primates that permanently accepted kidney grafts after a similar induction treatment were shown to be tolerant, as demonstrated by acceptance of second set of donor-specific grafts without additional immunosuppression (Thomas *et al.* 1999b, 2000).

5. CONCLUDING REMARKS

Tolerance induction represents the Holy Grail of transplantation immunobiology. Graft acceptance

without the need for chronic immunosuppression would broaden the indications of islet transplantation to insulin-dependent diabetic patients before the onset of secondary complications. A necessary prerequisite for acceptance of this paradigm is the reasonable safety of tolerance-inducing protocols. This is our next challenge before taking this exciting prospect to the clinical arena.

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