Diabetes mellitus comprises a series of clinically and genetically heterogeneous disorders associated by disturbances of carbohydrate, fat, and protein metabolism, the most common sign of which is HYPERGLYCAEMIA. The prevalence of diabetes increases with age, from less than 1% of western populations affected before age 20 to more than 13% above age 75. Approximately 90% of adult patients with diabetes have the ‘type 2’ variant, previously referred to as non-insulin dependent, which is generally associated with hyperglycaemia and insulin resistance, eventually leading to defects in insulin secretion. The most severe form of diabetes is the ‘type 1’ variant, which results from autoimmune destruction of the insulin-secreting cells (β-cells) contained in the pancreatic islets. In type 1 diabetes, daily treatment with exogenous insulin is required to support life. The metabolic abnormalities associated with diabetes can lead to a significant percentage of patients developing a series of chronic and degenerative complications, including retinopathy, nephropathy, neuropathy, atherosclerosis and lipid disorders. Experimental models and recent clinical trials have shown that islet transplantation could be an alternative to exogenous insulin treatment, as it results in normalization of metabolic control, which has been almost impossible to obtain with administration of exogenous insulin. The results of clinical islet transplantation have greatly improved owing to the introduction of more efficient methods for the separation of islets and more effective immunosuppressive strategies. The marked improvement in clinical results has been paralleled by a renewed enthusiasm and interest in this approach, but major challenges remain to be addressed, including the need for life-long recipient immunosuppression, which severely limits the current indications for islet transplantation. This article outlines the history of and recent progress in the field, as well as the present immunological challenges and possible strategies for tolerance induction that are crucial to make clinical islet transplantation more widely available.

The history of islet transplantation

The first attempts. In 1893, the English surgeon Watson Williams attempted to transplant sheep pancreatic fragments into a 15-year-old boy with end-stage diabetes. This first ‘islet xenograft’ was carried out at a time of intense debate as to whether the pancreas was producing a sugar-destroying substance. Minkowsky

Type 1 diabetes mellitus results from autoimmune destruction of the insulin-secreting cells in the pancreas. Daily treatment with exogenous insulin is required, but because of difficulties in achieving physiological control of blood-glucose concentrations, chronic and degenerative complications still occur in a marked fraction of patients. Islet transplantation can normalize metabolic control in a way that has been virtually impossible to achieve with exogenous insulin, but long-term immunosuppression of the recipients is required, limiting the procedure to the most severe forms of diabetes. This article outlines the history of and recent progress in the field, as well as the present immunological challenges and possible strategies for tolerance induction that are crucial to make clinical islet transplantation more widely available.
had reported in 1892 that removal of the pancreas was associated with diabetes in dogs, and that subcutaneous placement of a portion of the pancreas (the uncinate process), without any communication to the gastrointestinal tract, prevented the mortality associated with total pancreatectomy. This implied that a pancreatic substance that was not secreted into the gastrointestinal tract could be responsible for the regulation of glucose levels. However, after Williams had transplanted the pancreatic fragments from a freshly slaughtered sheep, the boy died a few days later. In those days, nothing was known about immune rejection of xenogeneic tissue transplants and Williams suggested that his inability to cure diabetes by transplanting pancreatic fragments should caution against attributing too much importance to the pancreas in the pathogenesis of the disease. This attempt preceded, by almost three decades, the discovery of insulin, which changed the fate of millions of patients affected by this condition.

However, the painful lesson of the twentieth century was that treatment with exogenous insulin alone could not prevent the chronic complications that are frequently associated with diabetes. The importance of maintaining strict metabolic control through frequent insulin injections was clearly shown in numerous clinical trials, as improved metabolic control was associated with a decreased incidence and/or progression of diabetic complications. However, intensive insulin treatment could be successfully applied to fewer than 10% of patients in the study because of an increased risk of severe episodes of hypoglycemia, compared with control patients who received traditional insulin treatments.

**Proof of concept in experimental models.** In 1967, Paul E. Lacy proposed that the transplantation of pancreatic islets (islets of Langerhans) might be a better solution than exogenous insulin treatment, as they contain the insulin-secreting cells that are specifically destroyed in the autoimmune process associated with the most severe form of the disease (type 1 diabetes). In 1972, he was the first to reverse diabetes by this method in a rodent model system in which diabetes was chemically induced (see later). These initial results showed the potential for pancreatic islet-cell transplants to restore metabolic control and prevent the development of chronic complications of diabetes. However, it proved difficult to reproduce the results that were obtained in small animal models in larger animal models, or even in rodent models of autoimmune diabetes, such as the NON-OBESI DIABETIC (NOD) MICE, owing to immune destruction of the transplanted insulin-producing cells by graft rejection or autoimmune processes.

Two basic mouse models have been used to study the transplantation of MHC-mismatched allogeneic islets into insulin-deficient hosts. One model uses non-autoimmune hosts that have been rendered insulin deficient by administration of streptozotocin—a toxin for insulin-producing pancreatic β-cells of the islets of Langerhans. In this model, untreated islet-allograft recipients become diabetic as a consequence of a T-cell-dependent alloimmune response. In the second model, autoimmune NOD mice with spontaneous diabetes are transplanted with MHC-mismatched islets. Rapid and vigorous T-cell-dependent loss of the islets results in a failure to restore a euglycaemic state in treated hosts from both autoimmune and alloimmune mechanisms.

**The first success in clinical trials.** In the clinical setting, in addition to the immunological challenges imposed by allo- and autoimmunity, a formidable obstacle emerged in the 1970s and 1980s in terms of the inability to extract and purify sufficient numbers of islets from the human pancreas. This problem was solved by the introduction of an automated method for islet isolation in 1986, which enabled for the first time the collection of sufficient numbers of human islets (BOX 1).
Preparation and testing of human islet cells for transplantation

**a Human islet-cell isolation**

The technologies currently used in human islet-cell processing are based on an automated method that was first introduced in 1986 and that rapidly replaced all previously tested procedures. The concept introduced by this method was to progressively disassemble the pancreas after injection of an enzyme blend through the pancreatic duct. This process allowed gradual digestion of the organ into fragments of decreasing size, until cell clusters of the volume range of islets are released. A constant flow through the digestion chamber allows the released islets to pass through a screen and to be collected in separate compartments, in which further enzymatic action is blocked by cooling and dilution. A final purification step is carried out by density-gradient separation of the islets, now carried out using a COBE2991 cell processor.

Several qualitative and quantitative tests are used to verify the quality of the final human islet-cell product. Pre-transplant criteria that must be met include determination of the total islet-cell number (>5,000 islet equivalents/kg recipient body weight), total pellet volume of the final preparation (<7 ml of tissue) and islet-cell purity (>30% islets). Product release criteria also include negativity of a Gram stain (to detect the presence of contaminating bacteria) and >70% viability as assessed by fluorescent inclusion/exclusion dyes. However, the best predictive test of post-transplant functional competence is the reversal of diabetes after transplantation of an aliquot of islets into an immunodeficient (athymic) mouse. At present, research is underway to develop alternative technologies for the assessment of final islet-cell preparations, including assessment of apoptosis, ATP and oxygen consumption, and mitochondrial membrane potentials, towards definition of improved prospective product release tests for the prediction of post-transplant islet-cell function. Images are redrawn with permission from Landes Bioscience from REF. 80.

**b Purification of islets by density-gradient separation**

Using this method, in 1989, Lacy and collaborators at Washington University in St Louis showed that it was possible to reverse diabetes and obtain insulin independence after transplantation of human islets. However, the islet-cell transplant failed a few days after insulin independence was achieved, most probably because of inadequate recipient immunosuppression.

In 1990, the first successful series of human islet allografts was reported by the Pittsburgh group: prolonged insulin independence was achieved with a steroid-free immunosuppressive regimen based on the then recently introduced agent FK506. This was the first unequivocal evidence of long-term reversal of diabetes after human islet allotransplantation, with insulin independence lasting up to five years. These unprecedented results led to great enthusiasm in the field, and several centres, including those in Milan, Miami, Edmonton, St Louis and Minneapolis, began or resumed testing of clinical islet allotransplantation protocols. During the same period, the survival of intrahepatic human islets after transplantation was confirmed in liver biopsies, indicating that allogeneic human islets can successfully engraft in the hepatic microenvironment.

The prevention of allogeneic human islet rejection and/or the recurrence of autoimmunity when the islets were transplanted to recipients with type 1 diabetes turned out to be a major challenge in the 1990s: only about one-third of islet transplants continued to function after one year and the rates of insulin independence were ~10% (REF. 71). More than 50% of the islet transplants failed within the first two months, indicating the susceptibility of islet cell transplants to early graft loss (BOX 2). However, the relatively few islet allografts that continued to function long-term in patients with diabetes clearly showed that islet transplantation allowed for normalization of metabolic control in the absence of severe episodes of hypoglycaemia.

**Improvement of clinical outcome**

Since the initial success of the Pittsburgh trial, the results of clinical islet allotransplantation have continued to improve, owing to improvements in islet preparation and the use of more effective immunosuppressive regimens. Initially, long-term success was limited by immunosuppressive protocols that included diabetogenic drugs, such as combinations of steroids and calcineurin inhibitors. These agents can severely impair insulin secretion and action, even when administered at low, maintenance doses. Recent observations in non-human primates clearly showed that when diabetogenic drugs are avoided, islet allografts maintain long-term insulin independence and also provide improved insulin secretory function over time. Particularly notable is the success recently achieved in clinical trials by the Edmonton group, which introduced the use of Rapamycin in islet transplantation, allowing for clinical implementation of a steroid-free protocol. This improved immunosuppression, in combination with multiple islet infusions from different donors, resulted in insulin independence in 7/7 islet-cell transplant recipients in an initial series of patients. A high rate of
Box 2 Early loss of transplanted islets

Given the disparity between the availability of islets for transplantation and the number of potential recipients, it will be crucial to maximize the number of healthy islets that can be engrafted from a single donor. Prevention of early islet loss, which is estimated to occur for up to 50% of the transplanted islet mass, would also contribute significantly towards the definition of tolerance protocols that can result in insulin independence. Islets exposed to allogeneic blood are subject to an immediate blood-mediated inflammatory reaction that involves coagulation and complement activation\(^\text{11-13}\). Interestingly, human islets express tissue factor, an integral component in the coagulation cascade; studies in an in vitro system have shown that heat-inactivated factor VIII or antibody specific for tissue factor could inhibit this process\(^\text{11,12}\). Moreover, increased levels of thrombin–thrombin-specific antibody complex are detected in islet-transplant recipients in the immediate post-transplant period\(^\text{15}\). In addition to complement activation and coagulation, a key role for macrophages and their pro-inflammatory products, such as interleukin-1 and tumour-necrosis factor, in islet survival and function has been shown\(^\text{13-17}\). Clearly, attempts to control intra-graft inflammation, complement activation and coagulation in the early post-transplant period as a means to prevent unnecessary early graft loss will be important. Moreover, the link between intense inflammation and the stimulation of immune responses indicates that immunological, as well as non-immunological, graft loss might be reduced by these measures.

FK506
The initial name given to tacrolimus, a metabolite of the fungus Streptomyces tsukubaensis. It is a potent immunosuppressive agent that inhibits the formation of important growth-promoting cytokines, including interleukin-2.

RAPAMYCIN
A new immunosuppressive drug that is structurally related to tacrolimus. It is produced by the organism Streptomyces hygroscopicus. It prevents the translation of mRNAs encoding cell-cycle regulators and controls progression from the G1 to S phase of the cell cycle.

CENTRAL TOLERANCE
This form of tolerance refers to the lack of self-reactivity found as lymphoid cells develop, and is associated with the deletion of autoreactive clones. For T cells, this occurs in the thymus. Many facets of classical central tolerance are evident in mixed donor and recipient haematopoietic chimaeras that are rendered tolerant to transplanted donor tissue allografts.

The introduction of the more effective, but also more powerful, rapamycin-based immunosuppressive protocols has led to an increased risk of complications, including mouth ulcers, hyperlipidaemia and hypertension\(^\text{15,16}\), which limits the applicability of islet transplantation to only the most severe cases of type 1 diabetes, in which patients fail to recognize hypoglycaemia (hypoglycaemia unawareness) or are unable to achieve acceptable metabolic control, despite attempts to implement intensive insulin regimens.

At present, islet transplantation is mainly used as a means to slow the progression of disease-related disabilities in patients with advanced renal disease, retinopathy and neuropathy, but it is not widely recommended as a means to prevent complications in those with recent-onset diabetes. Most clinicians reason that long-term use of maintenance immunosuppressive drugs will lead to worse outcomes than long-term insulin therapy. For islet transplantation to become widely applicable, we will eventually need to develop successful strategies for tolerance induction to the transplanted insulin-producing cells in the absence of continuous recipient immunosuppression. These strategies, to be clinically relevant, cannot introduce marked risks for the recipients, such as in the case of lethal or sub-lethal radiation strategies. For islet transplantation to become a widely applicable treatment for diabetes, we need to develop better immunomodulatory strategies and ultimately donor-specific tolerance induction.

We believe that the induction of tolerance in humans to allogeneic islets will prove to be challenging, and that many of the preclinical models are not precisely relevant. Why is this situation unusually challenging? First, the T-cell response to MHC-mismatched allografts recruits a large number of T-cell clones\(^\text{17}\). The number of T-cell clones that participate in the allograft response is exponentially larger than the T-cell response mounted against nominal- or auto-antigens. Second, tolerance must be achieved in hosts that have pre-activated autoreactive lymphocytes.

**Strategies for donor-specific transplant tolerance**

Clonal deletion of donor-reactive T cells. Donor-specific transplant tolerance has been obtained through treatments that mimic central tolerance, leading to near total, or total and permanent, elimination of donor-specific T-cell clones in the recipient. Selective long-term deletion of host donor-specific T-cell clones and so donor-specific transplant tolerance has been achieved in preclinical rodent models by creating mixed donor and recipient multi-lineage haematopoietic chimaeras\(^\text{18}\). Donor bone marrow or haematopoietic stem cells are transplanted into prospective tissue-transplant recipients to establish a mixed chimerism before the tissue transplant. In hosts with established robust multi-lineage haematopoietic mixed chimerism, host donor-specific T cells are destroyed in peripheral immune tissues and new thymic emigrants are depleted of donor-reactive T cells in the thymus\(^\text{18}\). In small animal preclinical models, immunosuppression is required to achieve initial engraftment of donor haematopoietic cells, but immunosuppressive treatment is not required to maintain tolerance in allograft recipients with established chimerism. The highly satisfactory outcome of this approach is the creation of donor-specific tolerance with recovery and preservation of other immune responses. Moreover, humans with bone-marrow or haematopoietic malignancies who have been successfully engrafted with haematopoietic stem cells from HLA-identical siblings and who have subsequently developed renal failure, have been successfully transplanted with a kidney from the haematopoietic-cell donor in the absence of further immunosuppressive therapy\(^\text{19}\).

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insulin independence continues to be reported by Shapiro and collaborators in Edmonton\(^\text{25}\) and by other centres that have used rapamycin-based immunosuppression since these initial promising results\(^\text{15,19}\). Marked improvement after islet transplantation was observed in terms of metabolic indicators and the frequency and severity of hypoglycaemic episodes, which virtually disappeared, even after the first islet infusion\(^\text{15,19,25,29}\).

Follow-up observations in the initial islet-cell transplant recipients in Edmonton and elsewhere indicated that some islet-cell transplants can fail to maintain insulin independence long-term, with about one quarter of the recipients requiring an additional islet infusion during the second or third year post-transplant\(^\text{25}\). The reasons for the chronic failure of a portion of the islet transplants are currently under investigation, but could be associated with immune rejection, recurrence of autoimmune or chronic exposure to diabetogenic immunosuppressive agents. Nevertheless, the results of clinical trials in the past three years have been clearly superior to those achieved in the preceding two decades, at least in patients with autoimmune, type 1 diabetes\(^\text{25}\).

**Current indications and limitations**

The introduction of the more effective, but also more powerful, rapamycin-based immunosuppressive protocols has led to an increased risk of complications, including mouth ulcers, hyperlipidaemia and hypertension\(^\text{15,25}\), which limits the applicability of islet transplantation to only the most severe cases of type 1 diabetes, in which patients fail to recognize hypoglycaemia (hypoglycaemia unawareness) or are unable to achieve acceptable metabolic control, despite attempts to implement intensive insulin regimens.

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Why is this promising approach not presently being tested in the clinic for patients in need of an islet transplant? First, the treatments that are most often used to achieve long-lasting, robust mixed chimerism are aggressive and use short courses of depleting pan-lymphocyte-specific or pan-T-cell-specific antibodies, irradiation, cytotoxic drugs and conventional immunosuppression. This treatment often results in marked, albeit temporary, lymphodepletion and immunoincompetence. Second, even transient mixed chimerism is not readily achieved across the broad donor and recipient HLA barriers that are usually encountered with non-related transplant donors. Indeed, long-lasting and robust multi-lineage mixed haematopoietic chimerism has not been achieved in the clinic. Third, the depletion of host donor-specific alloreactive T cells in the mixed chimeric host does not guarantee that the host autoimmune clones have been destroyed or that the balance of islet-reactive cytotoxic and regulatory clones now favours regulation and cessation of overt autoimmunity. In this setting, it is feared that pre-existing autoimmunity, not allograft rejection, would destroy the newly engrafted islet allograft in patients with type 1 diabetes. Nonetheless, a new protocol that incorporates mixed haematopoietic chimerism and co-stimulation blockade has succeeded in producing successful engraftment of allogeneic islets and abolition of diabetic autoimmunity in overtly diabetic NOD mice (M. Sykes, personal communication). If similar attempts prove successful in primates, this approach would receive great attention as a candidate for clinical use.

A novel strategy was recently reported to increase the level of chimerism after donor bone-marrow-cell infusions, while avoiding the risks that are associated with conditioning of the recipient with external radiation. This was made possible by the use of targeted delivery of radiation to the bone marrow using a bone-seeking compound (a diphosphonate derivative, Lexidronam), coupled with a short-lived radioactive isotope (Samarium). Administration of Samarium-Lexidronam (Quadramet®) as a single infusion 7–14 days before allogeneic bone-marrow transplantation promoted engraftment and the achievement of long-term mixed chimerism in mice. Engraftment was dependent on the administration of transient co-stimulatory blockade (CD 154-specific antibody). Chimerism resulted in donor-specific hyporesponsiveness to skin and islet allografts and was paralleled by a depletion of alloreactive T cells in the recipients. This targeted radioisotopic strategy is now being tested in large animal and preclinical models.

**Immunoregulation.** As an alternative to clonal deletion, peripheral tolerance might be achieved by treatments that shift the balance from pathogenic donor-reactive effector T cells to graft-protecting regulatory T cells in favour of immunoregulation, thereby allowing cessation of immunosuppressive treatment. After transplantation, most alloactivated host CD4+ T cells in untreated recipients strongly express the T helper 1 (Th1)-type cytokines interleukin-2 (IL-2) and interferon-γ (IFN-γ), but not Th2-type cytokines such as IL-4, IL-5, IL-10 and IL-13 (REFS 32–35). By contrast, most tolerizing regimens, such as co-stimulation blockade, that are known to create peripheral tolerance foster an early phase (within the first few weeks) of the allograft response that is characterized by high levels of expression of Th2, rather than Th1-type cytokines (Th1 to Th2 immune deviation) 32–35. In a successfully treated host, alloactivated CD4+ T cells express IL-4, IL-5, IL-10 and IL-13, but not IL-2 and IFN-γ during the induction of allograft tolerance 32–35.

A list of regimens that can produce induced Th1 to Th2 immune deviation and peripheral allograft tolerance includes, but is not limited to, CD3-specific antibodies, certain non-depleting CD4-specific antibodies and co-stimulation blockade with donor-specific whole blood transfusion and CD154-specific antibody and/or cytotoxic T lymphocyte antigen 4 (CTLA4)-immunoglobulin fusion protein.

Is Th1 to Th2 immune deviation the direct cause of peripheral transplant tolerance? Rejection of MHC-mismatched allografts can be achieved in both Th1- and Th2-polarized conditions. Short-term treatment with IL-12-specific monoclonal antibody — a treatment that directly deviates the immune response from a Th1 to Th2 pattern — can produce transplant tolerance in non-autoimmune hosts transplanted with MHC-matched islet allografts, but this regimen does not produce tolerance in more stringent, MHC-mismatched models. Nonetheless, Th1 to Th2 immune deviation seems necessary, but not entirely sufficient, to produce peripheral tolerance to MHC-mismatched allografts. We suspect that Th1 to Th2 immune deviation, a correlate to the creation of tolerance in MHC-matched hosts, aids but does not cause tolerance in this situation. By contrast, Th1 to Th2 immune deviation is not a prerequisite for the induction of tolerance to MHC-compatible allografts. It would be interesting to examine the susceptibility of IL-4 and IL-10 double-knockout mice to various tolerizing regimens. In short, Th1 to Th2 immune deviation does not cause tolerance to MHC-mismatched allografts.

It seems reasonable to conclude that these tolerizing treatments must produce Th1 to Th2 immune deviation as well as other beneficial effects. It is notable that CD3-specific antibodies, some non-depleting CD4-specific monoclonal antibodies and co-stimulation blockade with CD154-specific antibody and/or CTLA4-immunoglobulin cause Th1 to Th2 immune deviation and antigen-specific unresponsiveness (anergy), enhanced activation-induced cell death (AICD) and strengthened CD4+CD25+ T-cell-dependent, antigen-specific immunoregulatory networks. Indeed, the acquisition of tolerance with these treatment regimens seems to be totally dependent on the integrity of these immunoregulatory T-cell networks.
REVIEWS

a Cytolytic IL-2-immunoglobulin fusion protein

- AICD of effector T cells
- Survival of regulatory T cells

IL-2

CH2 CH3

Mouse IgG2a Fc region

Hinge

Increased serum half life

- Fc-receptor binding
- Complement (C1q) binding

Cell lysis

b Cytolytic mutant IL-15-immunoglobulin fusion protein

- Blockade of anti-apoptotic signals to T cells

Mutant IL-15

Hinge

Mouse IgG2a Fc region

Mutation

Increased serum half life

- Fc-receptor binding
- Complement (C1q) binding

Cell lysis

Figure 1 | T-cell-growth-factor-related immunoglobulin fusion proteins. a Through the use of a gene-fusion strategy, wild-type interleukin-2 (IL-2) sequences have been fused to the Fc region of mouse IgG2a (REF. 40). The resulting dimeric protein contains both the biological properties of IL-2 and those of Fcγ2a, which retains the ability to activate complement and phagocytes expressing Fc receptors. b An identical strategy has been used to produce a dimeric fusion protein that is comprised of a mutant, antagonist-type IL-15-related Fcγ2a fusion protein13. AICD, activation-induced cell death.

continuum of tolerizing therapeutic outcomes, is likely to be fallacious40. Clonal depletion might never be complete and the functional supremacy of immunoregulatory over pathogenic T cells might often require the depletion of pathogenic T cells in individuals who receive MHC-mismatched allografts39. To use non-T-cell-depleting regimens to produce transplant tolerance in allograft models, adjunctive measures, including those that enhance the apoptotic depletion of antigen-activated T cells, are required for success44-46. By contrast, treatments or abnormalities in the host allograft response that compromise IL-2-triggered AICD or PASSIVE CELL DEATH of pathogenic T cells inhibit tolerance induction through co-stimulation-based strategies42,44,46. In short, the unusually large pool size of pathogenic T cells that participate in the allograft response demands that the number of alloaggressive T cells must be markedly reduced if donor-specific tolerance, even peripheral, immunoregulatory dominant tolerance, is to be achieved44-46,49.

Preventing autoimmune destruction of islets

It is notable that co-stimulation blockade with CD154-specific antibody/CTLA4–immunoglobulin — a treatment that is designed to promote T<sub>reg</sub> immune deviation — can, in many models, produce transplant tolerance in non-autoimmune hosts transplanted with MHC-matched allografts, but these regimens do not produce tolerance in many stringent MHC-mismatched models. Moreover, this regimen does not enable long-term engraftment of allogeneic islets into overtly diabetic NOD mice49. Therefore, we suspect that in patients with type 1 diabetes, total deletion or marked depletion of donor-reactive pathogenic T cells, together with T<sub>T<sub>reg</sub></sub>, immune deviation of alloreactive clones will probably prove necessary, but not sufficient, to produce tolerance to the islet allograft, as these autoimmune patients also have pre-activated autoreactive, islet-specific pathogenic T-cell clones. Some strategies, such as blockade of the co-stimulatory CD28 and CD154 pathways, that are well suited for creating tolerance among naive T cells (for example, most alloreactive clones) experiencing their first encounter with (allo-) antigen, are not equally well suited to create tolerance among activated, autoimmune T cells reactivated by (auto-) antigen. Activation and expansion of naive alloreactive T-cell populations requires vigorous antigen stimulation, co-stimulation and denovo expression of receptors for T-cell growth factors, but the signals required for reactivation of previously activated T cells are less stringent than those for naive T cells. So, it is probable that strategies that can produce T-cell tolerance in human islet-transplant recipients that have only naive alloreactive T cells might not necessarily prove effective in hosts that have both pre-activated, autoimmune T cells and alloreactive T cells. For example, both direct targeting of T cells, or major subsets thereof, with depleting antibodies and co-stimulation-blockade-based strategies have been pursued to create tolerance to islet allografts in naive non-autoimmune mice, but these strategies have not been reported to restore a euglycaemic state in recent-onset NOD mice or to enable long-term engraftment of allogeneic islets in overtly diabetic NOD mice50.

Promising new strategies

CD3-specific antibody treatment. Until recently, only CD3-specific monoclonal antibody treatment, which skews the response of antigen-activated T cells from a T<sub>eff</sub>1 programme to T<sub>eff</sub>2 cells51 and regulatory T-cell activation, had proven successful in restoring a euglycaemic state to overtly diabetic NOD mice52. In mice, this approach removes autoreactive T cells and restores T-cell tolerance and the proper balance of regulatory to pathogenic T cells in the diabetic host51,53. Interestingly, the mechanism by which treatment with CD3-specific antibody restores self-tolerance in NOD mice apparently depends on the expression of transforming growth factor-β (TGF-β) by CD4<sup>+</sup> regulatory T cells55. CD3-specific monoclonal antibody treatment of humans with recent-onset diabetes slows the progression to permanent diabetes56. By contrast, CD3-specific monoclonal antibody treatment of recent-onset overtly diabetic NOD mice has
not been reported to enable long-term engraftment of allogeneic islets in NOD hosts. So, we suspect that CD3-specific monoclonal antibodies might prove useful as a component, but not as a monotherapy, in regimens that are designed to tolerize humans with type 1 diabetes to islet allograft donors.

**Selective targeting of pathogenic T cells.** The therapeutic application of pan-T-cell-specific polyclonal or monoclonal antibodies can lead to broad, marked and long-lived T-cell depletion. A treatment regimen that only targets recently activated cytopathic donor-reactive T cells and spares regulatory T cells might prove to be a potent and selective means of achieving long-term engraftment and tolerance. CD25 (IL-2 receptor-α, IL-2Rα)-specific antibodies or cytoxic toxins (IL-2) directed against high-affinity tri-molecular IL-2Rs, which are expressed by activated, but not resting, T cells have been used as immunosuppressive agents. However, these therapies might not readily discriminate between IL-2Rα+ activated pathogenic T cells and regulatory T cells.

AICD and passive cell death are routine downstream consequences of T-cell activation by antigen together with co-stimulation, and, as noted earlier, the integrity of these apoptotic pathways is required for the induction of peripheral transplant tolerance across MHC barriers with treatments that do not directly kill T cells. We have adopted a new strategy that is aimed at producing marked apoptosis of recently activated pathogenic, but not regulatory, T cells in the absence of T-cell proliferation. The strategy is based on the knowledge that the fate of activated effector T cells, which unlike resting T cells express high-affinity receptors for T-cell growth factors, is dictated by the nature of their response to T-cell growth factors. IL-2 triggers AICD, whereas IL-15 counteracts this signal and protects activated effector T cells from passive cell death, and rapamycin blocks the proliferative, but not pro-apoptotic, effects of IL-2. With this as a framework, we have shown that the combined administration of rapamycin, agonist IL-2–immunoglobulin fusion protein and a mutant, antagonist-type IL-15-related cytokolytic immunoglobulin fusion protein (FIG. 1) enables long-term engraftment/tolerance in exception-ally stringent allotransplantation models, including transplantation of allogeneic islets into overtly diabetic NOD mice. This is achieved by limiting the early clonal expansion of activated T cells, preserving and even exaggerating their subsequent apoptotic clearance, and further amplifying the depletion of these activated T cells by antibody-dependent mechanisms (FIG. 2), while preserving CD4+CD25+ T-cell-dependent immunoregulatory networks. The IL-2- and IL-15-related fusion proteins are genetically linked to immunoglobulin tails, which provide longevity and can be engineered to activate

**Figure 2** | **Selective destruction of activated effector T cells.** As activated effector, but not resting, T cells express tri-molecular, high-affinity receptors for interleukin-2 (IL-2) and IL-15, the IL-2- and IL-15-related Fcγ2a fusion proteins selectively bind to activated T cells. The IL-2 component of the IL-2-related fusion protein imparts a signal that triggers activation-induced cell death (AICD) of activated effector but not resting or CD4+CD25+ regulatory T cells. The mutant, high-affinity, antagonist IL-15 component of the other fusion protein blocks the delivery of IL-15-mediated proliferative and potent anti-apoptotic signals to activated IL-15-dependent effector T cells, resulting in passive cell death. The adjunctive use of rapamycin blocks the delivery of IL-2-mediated proliferative, but not AICD triggering, signals. The Fcγ2a domains of the fusion proteins confer IgG-related longevity to the fusion proteins and provide domains that induce complement activation and killing of activated effector T cells by phagocytes expressing Fc receptors. So, the Fcγ2a domains provide a means to exaggerate the apoptotic loss of IL-2 receptor (IL-2R)- and IL-15R-expressing activated T cells. IL-2Rα regulatory T cells are spared, because, unlike activated effector T cells, IL-2 triggers anti-apoptotic signals in regulatory T cells. The consequence of therapy is the early and marked apoptotic loss of effector, but not resting or graft-protecting, regulatory T cells. CD40L, CD40 ligand; TCR, T-cell receptor.
complement and phagocytes that express Fc receptors. Each of these immunoglobulin-related functions is required for optimal activity. This tripartite regimen functions to favourably tip the balance between cytopathic and regulatory T cells. IL-2, which promotes apoptosis of proliferating effectors T cells, is a survival factor for CD4+CD25+ regulatory T cells, so that IL-2–immunoglobulin fusion protein selectively destroys pathogenic, but not regulatory, T cells during an allograft rejection. The induction of peripheral tolerance depends on maintaining, if not exaggerating, the integrity and action of CD4+CD25+–graft-protecting regulatory T cells. The mutant, antagonist-type IL-15–immunoglobulin fusion protein blocks IL-15–triggered anti-apoptotic signals that are normally delivered to activated effector T cells and the Fc portion amplifies the apoptotic loss of activated effector T cells. So, this treatment biases the allograft response towards domination of immunoregulatory over pathogenic T cells. As proliferating not resting T cells are targeted, the treated hosts do not become lymphopaenic. Moreover, non-proliferating memory T cells are not destroyed (T.B.S., unpublished observations). It is notable that this method was successful in the NOD islet-allograft model. Transplantation of allogeneic islets into NOD mice is challenging, as both rejection and autoimmune must be overcome. Moreover, genetic traits that have not been linked so far to the genes encoding IL-2 or IL-15 seem to render NOD mice resistant to tolerance induction. Preliminary experiments in a non-human primate model of islet transplantation are encouraging, and there is no evidence so far of opportunistic infection in this model, which would be associated with the possible risk of over-suppression of the immune response (M. Koulmanda, X.X. Zheng and T.B.S., unpublished observations).

**Targeting co-stimulation.** Additional new and promising, albeit insufficiently tested, strategies include the search for new co-stimulatory pathways that are crucial for tolerizing CD4+ independent CD8+ T-cell–activation pathways and reactivation of memory CD4+ and CD8+ T cells. Promising data have emerged from several laboratories using blockade of canonical plus new co-stimulatory pathways as a means of producing allograft tolerance in non-autoimmune hosts. For example, the CD134–CD134 ligand (CD134L, also known as OX40L) pathway is important in maintaining the survival of memory T cells. Combined CD28–CD80/CD86 and CD134–CD134L blockade prolongs cardiac allograft survival in presensitized rats. Moreover, CD28–/−CD154–/− double-knockout mice or mice treated with OX40L–specific monoclonal antibody or CTLA4–immunoglobulin fusion protein and CD154–specific monoclonal antibody, but not monoclonal antibodies specific for CD70, inducible co-stimulatory molecule (ICOS) or 4–1BB, had long-term survival of skin allografts, identifying CD134–CD134L as a key alternative co-stimulatory pathway involved in CD28/CD154-independent rejection. So far, long-term engraftment of allogeneic islets has not been reported with CTLA4–immunoglobulin fusion protein and CD154–specific monoclonal antibody, or OX40L–specific monoclonal antibody in the clinically relevant model in which allogeneic islets are placed into overtly diabetic NOD hosts. Moreover, attempts to improve therapeutic strategies aimed at establishing long-lasting mixed chimerism in humans, and tolerance to allogeneic islets in overtly diabetic hosts are being further pursued.

**Non-human primate islet-transplant models.** With the availability of non-diabetic, efficacious therapies to prevent graft rejection, extended islet-allograft survival and insulin independence has been obtained with several experimental regimens in non-human primate models. Non-human primate models enable the testing of biological agents that bind to the non-human primate orthologue of human target antigens. Agents that interfere with co-stimulation (CD28 blockade and CD154–specific antibodies, as well as a CD3–immunotoxin–based strategy), have proven effective in non-human primate models. Long-term islet survival and function has been achieved and can extend beyond the discontinuation of therapy, but reproducible tolerance remains elusive. An animal previously made tolerant to a renal allograft, with a protocol involving extensive conditioning and donor bone-marrow infusion, was shown to accept islets from the original kidney donor without immunosuppression. Treatment with RAD (a rapamycin analogue), FTY720 and IL-2R-specific antibody has proven effective for the prevention of rejection in non-human primate models, as long as therapy is continued.

**Concluding remarks.** We hope that a new immunotherapeutic strategy will soon provide a means to produce tolerance to islet allografts for patients with autoimmune type 1 diabetes, so that they can be protected from the long-term complications of this disease. It seems probable that the best results will be achieved through the use of regimens that selectively destroy or inactivate donor-reactive allo- and autoimmune T cells without the induction, even temporarily, of an immunosuppressed, lymphopaenic state that might lead to an unacceptable incidence of infectious disease-related complications.

In the event that tolerance to islet allografts can be achieved, however, there will still be the problem of the short supply of islets for transplantation. In the future, greater emphasis will be placed on efforts to use stem cell technology or xenotransplants to supply islets for transplantation, as well as efforts to alter the islets so that they are more resilient to ischemia–reperfusion and immune injury, thereby enabling a reduction in the number of islets required for successful transplantation. The transplantation of islets is 'a work in progress', but the exciting successes in the clinic have raised hope that it might not be impossible to accelerate progress in this field.
This was the original paper discussing the islet-isolation technology that was used in the first successful trials of islet transplantation.

References 44–46 demonstrate the importance of regulatory T cells to create tolerance to MHC-mismatched alloantigens. References 33–35 show that T helper 1 (TH1) to TH2 immune responses and the balance of CD4 T cells are critical in the development of allograft tolerance. The role of co-stimulatory pathways in transplant tolerance.

Favorably tipping the balance between regulatory T cells and alloreactive T cells to achieve tolerance,References 74–77 show that T cell regulatory mechanisms can prevent co-stimulation blockade-resistant rejection.

References 66–68 demonstrate the importance of CD28/B7 system of T cell co-stimulation.

References 19–21 show that the CD28 pathway is involved in the regulation of T cell survival and proliferation, and that the regulation of T cell survival is important in the development of tolerance to allografts.

References 17–18 show that T cell receptor signaling and co-stimulatory molecules are involved in the regulation of T cell activation and proliferation, and that the regulation of T cell activation and proliferation is important in the development of tolerance to allografts.

References 16–17 show that T cell receptor signaling and co-stimulatory molecules are involved in the regulation of T cell survival and proliferation, and that the regulation of T cell survival and proliferation is important in the development of tolerance to allografts.

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Competing interests statement
The authors declare that they have no competing financial interests.

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FURTHER INFORMATION
Diabetes Research Institute homepage: http://www.drinet.org
Access to this interactive links box is free online.
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At a glance
- Clinical islet allotransplantation has progressed slowly since improved islet-isolation technologies resulted in the achievement of insulin independence in the first series of successful islet allografts in 1990.
- Insulin-independence rates have markedly improved as new and more effective combinations of immunosuppressive drugs have been introduced.
- Because of the risks associated with life-long recipient immunosuppression, islet transplants are now limited to the most severe cases of type 1 diabetes.
- For islet transplantation to become widely applicable, successful strategies for tolerance induction need to be developed.

Links
CD25
CD28
CD134
CD134L
CD154
CTLA4
IFN-γ
IL-2
IL-4
IL-5
IL-10
IL-12
IL-13
IL-15
IL-12p40
IL-12p70
IFN-γ
IL-2
IL-4
IL-5
IL-10
IL-12
IL-13
IL-15