Islet cell transplant and the incorporation of Tregs

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Purpose of review
T regulatory cells (Tregs) play a central role in maintaining immune homeostasis and peripheral tolerance to foreign antigens in humans. The immune response to alloantigens and recurrence of autoimmunity contribute to pancreatic islet transplant dysfunction, hence the adoptive transfer of Tregs has the potential to significantly improve islet graft survival. In this review, we provide an in-depth analysis of challenges associated with the application of ex-vivo expanded Tregs therapy in pancreatic islet transplant.

Recent findings
Tregs administered systemically may poorly migrate to the site of transplantation, which is critical for tolerance induction and graft protection. Intraportal administration of pancreatic tissue exerts some limitations on the ability to cotransplant Tregs at the same site of islet transplantation. In order to maximize therapeutic potential of Tregs, islet transplantation protocols may need additional refinement. Further to this, the Tregs may require cryopreservation in order to make them readily available at the same time as islet transplant.

Summary
On the basis of current experience and technology, the combination of islet and Treg cotransplantation is feasible and has great potential to improve islet graft survival. The possibility to wean off, or withdraw, traditional immunosuppressive agents and improve patient quality of life makes it an interesting avenue to be pursued.

Keywords
pancreatic islet, tolerance, transplant, T regulatory cell

INTRODUCTION

Allogeneic pancreatic islet transplantation and whole pancreas transplant are currently the only therapeutic options to achieve insulin independence in patients with type 1 diabetes mellitus (T1DM). β-Cell replacement therapy is recommended in patients with severe complications, such as hypoglycemia unawareness. Initial attempts of pancreatic islet transplant were hardly successful in reaching insulin independence and long-term graft function. Currently, over 50% of patients remain insulin independent 5 years after transplant because of recent advancements in the field of islet transplantation [1]. Additionally, such results are comparable to those of whole organ transplantation, but it is associated with lower procedure-related morbidity and mortality. Therefore, islet transplantation has the ability to become the primary β-cell mass replacement therapy. This potential can be expanded, thanks to different approaches that may prolong graft function, such as sequential islet infusions or pancreatic islet encapsulation [2▪]. An emerging approach is to apply ex-vivo expanded autologous T regulatory cells (Tregs) as an immunomodulatory therapy for improved islet graft function [3▪]. Tregs are a relatively recently described subpopulation of lymphocytes responsible for maintaining immune homeostasis and promoting tolerance to foreign and self-antigens [4]. Initially, they were considered homogenous; however, it has soon appeared that these are various cell populations that exhibit immunoregulatory properties. The naturally occurring $CD4^+CD25^{hi}CD127^{lo}FoxP3^+$ Tregs appear to be the predominant subpopulation [5▪,6]. Although

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Curr Opin Organ Transplant 2014, 19:610–615
DOI:10.1097/MOT.0000000000000130

KEY POINTS

- Tregs could be particularly useful in allogeneic pancreatic islet transplant settings because they are capable of regulating both alloresponses and autoimmunity.
- Use of alternative islet transplant sites could enable local transplantation of Tregs and the pancreatic tissue, which is required for induction of tolerance by the cells.
- Tregs migration to the islet graft could be induced by creation of chemokines gradient around transplanted islets.
- Despite the fact that Tregs have been proven to be well tolerated in clinical trials, use of antigen-specific Tregs could eliminate the chance of any possible side-effects that may be associated with this kind of treatment.

these cells are found in very low numbers in the peripheral blood, they can be expanded *ex vivo* and adoptively transferred to patients. Initial clinical trials have demonstrated the safety and efficacy of therapy with Tregs in the treatment and prophylaxis of graft-versus-host disease and T1DM [7–9,10▪▪]. Other clinical trials that are currently in progress will reveal more data concerning immunotherapeutic potential of Tregs in the near future [11,12]. In this short review, we will take a closer look at therapeutic potential of Tregs in the treatment and prevention of pancreatic islet rejection. We will also identify technical challenges that might be associated with this procedure and indicate possible solutions based on recent developments in the field.

PANCREATIC ISLET TRANSPLANT AND T REGULATORY CELLS

Currently, pancreatic islets are isolated from deceased donor pancreas and infused intraportally. Subsequently, they localize in small blood vessels of the liver, revascularize and initiate production of endogenous insulin [13▪]. Intraportal islet infusion imparts significant implications on the simultaneous administration of Tregs. Studies in the animal model demonstrate that administration of Tregs at the site of pancreatic islet graft (under the kidney capsule) significantly prolongs islet function *in vivo* compared to systemic administration of the cells. Moreover it was shown that following intravenous administration, Treg migration to the inflamed graft is poor and they could not fully exert their immunosuppressive function [14]. Therefore, in order to maximize the immunomodulatory effect of Tregs on islets, they should be colocalized either in the liver by simultaneous intraportal infusion or utilize an alternative site. Another option is to induce migration of infused Tregs to the site of islet transplantation using chemotactic factors, such as CCL-22 [15▪].

Recently, our group developed the method of anchoring human ex-vivo expanded Tregs to the surface of human pancreatic islets in order to create an immune barrier. Using this approach, we achieved decreased immunogenicity of the islets *in vitro* [16▪]. In this method, Tregs were anchored to the islets using stable binding, however, allowing cells to detach from the graft some time after implantation [17]. The temporary coating of the islets would facilitate the Tregs to be at the site of transplantation and on subsequent release can migrate to the draining lymph nodes to induce immunologic tolerance. This approach requires further testing and optimization in animal models before translation into clinical application. Furthermore, even if Tregs on the surface of the islets could provide sufficient protection from immune rejection, they can hardly protect the graft from instant blood-mediated inflammatory reaction. This sudden and dramatic phenomenon is related to the activation of innate immunity and coagulation pathway resulting from direct contact of pancreatic tissue with peripheral blood. It is postulated that instant blood-mediated inflammatory reaction is responsible for damage of over 50% of intraportally infused islets within the first hours after transplant [18]. However, such reactions could be limited by implanting the islets into the tissue, in which there is no direct contact with blood. Although several alternative transplant sites are currently being explored, only a few have the potential to be suitable. For example, kidney capsule, which is widely used as site of transplant in mice, has demonstrated to be inferior to intraportal administration in humans [19]. Other promising alternative sites include bone marrow [20▪], the gastrointestinal wall [21▪], skeletal muscles [22] and pancreas [13▪]. Although cotransplantation of ex-vivo expanded Tregs is feasible in these alternative sites, accumulation of greater than physiological concentrations of insulin in the direct vicinity of implanted islets may compromise the function of Tregs. A recent report by Han *et al.* [23▪▪] demonstrated that insulin selectively inhibits the secretion of IL-10 by Tregs in mice and activates mTOR kinase, blunting important immunoregulatory mechanism of Tregs function. It is well established that IL-10 plays a central role in the induction of tolerance to transplants and is secreted by both naturally occurring Tregs and induced T regulatory cells (Tr1). It suppresses activation of immune cells and induces development of new Tregs that can mediate the long-term tolerance

of transplanted pancreatic islets [5▪,15▪,24]. The importance of IL-10 has been confirmed in settings of islet transplantation not only in animal models but also in humans [25]. Potential administration of exogenous insulin during the early stages after islet transplant could lower the insulin secretion by transplanted islets and decrease the detrimental effect of higher concentrations of endogenous insulin on IL-10 secretion by colocalized Tregs. Currently, exogenous insulin is used routinely in order to give freshly transplanted islets time to implant and revascularize, so the demand for insulin would not become too much of a metabolic challenge to the β-cells [26]. By the time exogenous insulin is weaned off or withdrawn completely, Tregs could have already migrated from the site of the islet transplant to peripheral lymphoid tissue to promote tolerance of the graft.

Another alternative approach to cotransplant of the islet and Tregs simultaneously is to stimulate the migration of the Tregs to the islet transplantation following systemic administration. During carcinogenesis, Tregs are recruited to the tumor site by tumor-producing chemokines, such as CCL22, and promote tumor growth by suppressing tumor-specific T-cell response [27▪]. In long-surviving allografts, Treg recruitment also seems to play an important role in creating local immunosuppression [28]. This mechanistic principle has been successfully utilized to treat autoimmune disorders [29▪,30] and in mouse models of transplantation [15▪]. Montane *et al.* [30] reported that overexpression of CCL22 in islets transduced by an adenoviral vector delayed diabetes onset in the nonobese diabetic mouse model and also improved syngeneic islet graft survival. Efficacy of Treg recruitment to protect the islet graft from early immune attack was confirmed in intramuscular islet cotransplants with plasmids encoding *CCL22* to MHC-mismatched mice recipients [15▪]. Such strategy could be an alternative to high doses of immunosuppressive drugs used at early stages after islet transplantation.

Of particular interest would be to artificially reproduce gradients of chemokines to increase the number of endogenous or infused Tregs at the islet graft site. Islets could theoretically be encapsulated or coinfused with bioengineered polymers capable of steadily releasing Treg recruiting factor. Such bioinspired vehicles have already been shown to efficiently induce Treg migration *in vivo* [29▪,31▪] and could now be tested in islet transplantation settings.

Finally, the above-described approaches could be beneficial in terms of decreasing the minimal Treg numbers neccessary for reaching therapeutic effect. Experimental studies in murine models demonstrate that adoptive transfer of Tregs at a ratio of 2 : 1 or as high as 5 : 1 to effector T cells can induce effective immunological tolerance. In absolute numbers, we would require an adoptive transfer of 53×10^9 Tregs to achieve transplantation tolerance in a normal individual. However, in islet transplant recipients, who currently undergo induction therapy with T-cell- depleting antithymocyte globulin, 90% reduction in the T cell numbers can be observed. In this case, theoretical tolerance can be induced by adoptively transferring only 5×10^9 Tregs [32▪]. This number could be significantly decreased if Tregs are transplanted locally with the pancreatic tissue.

Among major challenging aspects in cotransplantation of islets with Tregs is the logistics in clinical settings. Although the isolation and ex-vivo expansion of Tregs from the recipient patient could be planned ahead of time, it is impossible to schedule islet isolation from the deceased donor. Hence, design of the clinical islet and Treg transplant protocol should consider the freezing and cryobanking of Tregs after expansion to keep them available as immunosuppressive therapy at the time of islet transplant. Unfortunately, on the basis of the present experience, Treg cryopreservation and thawing may have a negative influence on their function. For example, the procedure of cryopreservation of Tregs decreases the expression of L-selectin (CD62L) and the chemokine receptor CCR5 [33]. These two receptors are critical for Treg function *in vivo* by regulating their trafficking between graft and lymphoid tissues, which is necessary to exert tolerance [34▪]. Moreover, it has been shown that cryopreservation affects the response to antigens [35] and cytokine production [35,36] in frozen/thawed peripheral blood mononuclear cells. Impaired IL-10 secretion was shown after cryopreservation, which may substantially affect function of Tregs [36]. However, cryopreservation, even considering its drawbacks, still appears to be the only option to logistically coordinate Treg infusion with the pancreatic islet transplant.

RECURRENCE OF AUTOIMMUNITY AND DIABETES

As alloreactivity might be relatively well controlled with current immunosuppressive regimens, one of the major concerns after islet transplantation in patients with T1DM is the recurrence of autoimmunity. Particularly, the risk seems to be significant, when islets are infused into muscle or the pancreas. Intramuscularly transplanted islets are very quickly rejected by the immune system despite immunosuppressive treatment of the patients, with

a strong indication of the recurrence of autoimmune response against β-cells [37]. In patients with T1DM, one could expect a high risk of autoimmune reactivation when islets are transplanted into the pancreas, as lymph nodes associated with the pancreas might be the source of β-cell-specific immune cells. The fact that even strong pharmacological immunosuppression used in allogeneic pancreatic islet transplant settings is currently insufficient to induce long-term tolerance makes it also highly unlikely that Treg therapy alone will be sufficient. Indeed, several reports from animal models and human trials further demonstrate that Treg adoptive therapy alone could not achieve long-lasting therapeutic effects in transplant settings [7,38▪▪]. The remedy may be a combination of routine immunosuppressive induction therapy with activated T cell-depleting agents, which also facilitate Treg function *in vivo* [38▪▪]. The introduction of anti-LFA-1 antibody – Efalizumab, which targets activated T cells – into the immunosuppression protocol of islet transplantation gave very good results [39], and this may even be enhanced with the application of ex-vivo expanded Tregs. By definition, this maneuver also reduces alloimmunity. However, lymphopenic state, by induction of homeostatic proliferation, activates islet-specific T memory cells and memory-like T cells, which may paradoxically lead to loss of β-cells due to autoimmunity [38▪▪,40▪]. It is then necessary to tailor an adoptive therapy with Tregs to not only cover tolerance to foreign antigens but also to β-cell autoantigens. Initial clinical results, reported by our group, are very encouraging as far as controlling autoreactivity in patients with early onset of T1DM. Systemic administration of polyclonal Tregs delayed or even inhibited the progression of T1DM in prediabetic patients [41▪▪]. An even more advanced approach currently being pursued is the use of antigen-specific Tregs [42▪]. Many studies have shown that antigen-specific T regulatory cells are much more effective in evoking an immunomodulatory effect than the polyclonal population [5▪,38▪▪,43,44]. Hence, preparation of alloantigen and β-cell-specific T regulatory cells might present a potential opportunity to promote long-term islet graft survival without reactivation of autoimmunity.

SAFETY

As in other immunosuppressive treatment regimens, Treg application may also lead to possible side-effects, including infection and carcinogenesis [45▪]. However, initial clinical reports indicate that adoptive therapy with Tregs is well tolerated. In patients treated with ex-vivo expanded Tregs, there were no adverse events, such as significantly increased susceptibility to infection or decreased response to vaccination. There are no reports of neoplasmic disease, including skin cancer, which is the most common neoplasm attributed to traditional immunosuppressive treatments [10▪▪]. Moreover, Di Ianni *et al.* [8] have reported an improved resistance to cytomegalovirus infection after Treg transfer in hematopoietic stem-cell transplantation patients. Despite the good safety profile of Treg therapy emerging from initial clinical trials, it is evident, on the basis of numerous reports from both human and animal studies, that Tregs are associated with the progression of tumors and inhibition of cancer-specific immune reactions. Tumor-associated Tregs may be efficiently recruited by many types of tumors as chemokines attracting Tregs, such as CCL22, can be secreted by cancer cells or other tumor-associated cells [27▪,46]. Moreover, a recently released report suggests that ex-vivo expanded Tregs transfer may be associated with promotion and accelerated development of a tumor, but only in susceptible individuals [47▪]. Tregs at the tumor site are fully functional and after being activated, promote tolerance to the neoplasmic antigens [48▪]. Elevated numbers of tumor-associated Tregs were found to be a negative prognostic factor of different cancer types [49–51]. However, these data should be treated with caution, as it was often obtained from relatively small patient cohorts and could be misleading, as it was shown by Nosho *et al.* [52] in the case of colon cancer. Interestingly, in normal individuals, Tregs may protect from carcinogenesis by decreasing inflammation [53]. Further to this, in some lymphomas, Tregs are even believed to limit the disease relapses [54]. The latest work of Martelli *et al.* [55▪] is in agreement with those findings; in this work, they showed that coinfusion of conventional T cells with Tregs decreased leukemia relapses. It is important to remember that the risk of side-effects might be greater when Tregs are applied together with current pharmacological immunosuppressive treatment.

It should be highlighted that clinical Treg application is a new therapy, and long-term follow-up reports are not yet published. Therefore, patients with genetic susceptibility to tumors or with records of neoplasm should be excluded from the first clinical trials in order to limit such adverse events. It is probable that the risk may be further limited by utilizing adoptive transfer antigen-specific Tregs instead of polyclonal Tregs.

CONCLUSION

Treg therapies are becoming a reality in clinical settings. Both autoimmunity and transplant rejection can be alleviated with ex-vivo expanded and

adoptively transferred Tregs. There is already a large body of evidence suggesting that the treatment might be well tolerated and effective in humans. Patients with T1DM undergoing islet transplantation could especially benefit from Treg therapy as those cells can control both allogeneic rejection and autoimmune destruction of β-cells of transplanted islets. Although, there are still several questions and major challenges related to the procedure, there is sufficient rationale and data to initiate first clinical trials to test the safety and effectiveness of the combined Treg and islet transplant application and for further efforts and research to optimize the approach.

Acknowledgements

This study was supported by the National Centre for Research and Development (grant Strategmed Tregs), National Centre of Science (funding decision no. UMO-2011/01/B/NZ6/00322), University of Chicago DRTC Grant # P30 DK020595 and CRC – National Center for Advancing Transitional Sciences of the NIH Grant # UL1TR000430. EU COST Action BM1305 http://www.afactt.eu.

Conflicts of interest

The authors have no financial conflict of interest.

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