Published in final edited form as:
Curr Opin Organ Transplant. 2014 December ; 19(6): 610–615. doi:10.1097/MOT.0000000000000130.

Islet cell transplant and the incorporation of Tregs

Adam Krzystyniak[1,2], **Karolina Gołąb**[1,3], **Piotr Witkowski**[3], and **Piotr Trzonkowski**[1]

[1]Department of Clinical Immunology and Transplantology, Medical University of Gdańsk, Debinki 7, 80-210 Poland [2]Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, 80-822 Gdańsk, ul. Kładki 24 [3]Department of Surgery, Section of Transplantation, The University of Chicago, 5841 S. Maryland Ave. MC5027, Chicago, 60637 IL USA

Abstract

Purpose of the 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 co-transplant 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—Based on current experience and technology, the combination of islet and Treg co-transplantation 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

Treg; pancreatic islet; transplant; tolerance

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

Corresponding author: Piotr Trzonkowski, Department of Clinical Immunology and Transplantology, Medical University of Gdańsk, Address: Debinki 7, 80-210 Poland, Tel: 0048 58 349 15 90, ptrzon@gumed.edu.pl.

Conflicts of interest: The authors have no financial conflict of interest.

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 post-transplant due to 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, like sequential islet infusions or pancreatic islet encapsulation [2*]. An emerging approach is to apply *ex-vivo* expanded autologous T regulatory cells (Tregs) as an immuno-modulatory 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 which exhibit immunoregulatory properties. The naturally occurring $CD4^{+}CD25^{hi}CD127^{lo}FoxP3^{+}$ Tregs appear to be the predominant subpopulation [5*,6]. Although 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 (GVHD) and T1DM [7–10**]. Other clinical trials 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 Tregs

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. Recent reports also demonstrate 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 co-localized 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 (IBMIR). 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 IBMIR 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 where 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, have 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*]. Though co-transplantation 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. 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 [23**]. 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 T regulatory cells 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 co-localized 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 co-transplant 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 like 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. reported that over-expression of CCL22 in islets transduced by an adenoviral vector, delayed diabetes onset in the non-obese diabetic (NOD) mouse model and also improved syngeneic islet graft survival [30]. Efficacy of Treg

recruitment to protect the islet graft from early immune attack was confirmed in intramuscular islet co-transplants 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 co-infused with bio-engineered polymers capable of steadily releasing Treg recruiting factor. Such bio-inspired vehicles have already been shown to efficiently induce Treg migration *in vivo* [29*,31] and could now be tested in an islet transplantation settings.

Finally, the above – described approaches, which could be beneficial in terms of Treg numbers. 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 anti-thymocyte 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 co-transplantation 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 planed 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, based on 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 (PBMCs). 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, providing a strong indication of the recurrence of autoimmune response against β-cells [37].

In T1DM patients, one could expect a high risk of autoimmune reactivation when islets are transplanted into the pancreas, as lymph nodes associated with the pancreas are 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 auto-reactivity in patients where new onset of T1DM is concerned. 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 safe. 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 has reported an improved resistance to cytomegalovirus infection after Treg transfer in hematopoietic stem-cell transplantation patients [8*]. Despite the good safety profile of Treg therapy emerging from initial clinical trials, it is evident, based on 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 since 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, this data should be treated with caution, since it was often obtained from relatively small patient cohorts, and could be misleading as it was shown by Nosho et al. in the case of colon cancer [52]. 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 Di Ianni et al. is in agreement with those findings, in this work they showed that co-infusion of conventional T cells with Tregs decreased leukemia relapses [55*]. 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 have 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.

Conclusions

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 safe 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.

Acknowledgments

The 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

References

1. Barton FB, Rickels MR, Alejandro R, et al. Improvement in outcomes of clinical islet transplantation: 1999-2010. Diabetes Care. 2012; 35(7):1436–45. [PubMed: 22723582]

2*. Weir GC. Islet encapsulation: advances and obstacles. Diabetologia. 2013; 56(7):1458–61. different strategies of islet encapsulation allow for their immunoprotection. [PubMed: 23636639]

3*. Xiao F, Ma L, Zhao M, et al. Ex vivo expanded human regulatory T cells delay islet allograft rejection via inhibiting islet-derived monocyte chemoattractant protein-1 production in {CD34+} stem cells-reconstituted {NOD-scid} {IL2rγnull} mice. {PloS} one. 2014; 9(3) study showing that ex vivo expanded Tregs can delay rejection of pancreatic islets.

4. Sakaguchi S, Sakaguchi N, Asano M, et al. Immunologic self-tolerance maintained by activated T cells expressing {IL-2} receptor alpha-chains (CD25). Breakdown of a single mechanism of self-

tolerance causes various autoimmune diseases. J Immunol. 1995; 155(3):1151–64. [PubMed: 7636184]

5*. Wan X, Zaghouani H. Antigen-specific therapy against type 1 diabetes: mechanisms and perspectives. Immunotherapy. 2014; 6(2):155–64. review concerning immunotherapy including Treg therapy. [PubMed: 24491089]

6. Burrell BE, Nakayama Y, Xu J, et al. Regulatory T cell induction, migration, and function in transplantation. J Immunol. 2012; 189(10):4705–11. [PubMed: 23125426]

7. Trzonkowski P, Bieniaszewska M, Juścińska J, et al. First-in-man clinical results of the treatment of patients with graft versus host disease with human ex vivo expanded {CD4+CD25+CD127-} T regulatory cells. Clin Immunol. 2009; 133(1):22–6. [PubMed: 19559653]

8. Di Ianni M, Falzetti F, Carotti A, et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. Blood. 2011

9. Brunstein CG, Miller JS, Cao Q, et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. Blood. 2011; 117(3): 1061–70. [PubMed: 20952687]

10**. Natalia MT, Myśliwiec M, Dobyszuk A, et al. Therapy of type 1 diabetes with {CD4(+)CD25(high)CD127-regulatory} T cells prolongs survival of pancreatic islets - Results of one year follow-up. Clin Immunol. 2014; 153(1):23–30. important follow up after clinical application of ex vivo expanded Tregs, showing effectiveness and safety of the procedure in humans. [PubMed: 24704576]

11. Wood KJ, Bushell A, Hester J. Regulatory immune cells in transplantation. Nat Rev Immunol. 2012; 12(6):417–30. [PubMed: 22627860]

12. Gitelman, SE.; Bluestone, JA.; Herold, KC. T1DM Immunotherapy Using CD4+ CD127lo/-CD25+ Polyclonal Tregs. http://clinicaltrials.gov/show/nct01210664

13*. Molnár C, Essand M, Wennberg L, et al. Islet engraftment and revascularization in clinical and experimental transplantation. Cell Transplant. 2013; 22(2):243–51. work describes the fate of islets after infusion into the portal vein. [PubMed: 22584061]

14. Zhang N, Schröppel B, Lal G, et al. Regulatory T cells sequentially migrate from inflamed tissues to draining lymph nodes to suppress the alloimmune response. Immunity. 2009; 30(3):458–69. [PubMed: 19303390]

15*. Vågesjö E, Christoffersson G, Waldén TB, et al. Immunological shielding by induced recruitment of regulatory T lymphocytes delays rejection of islets transplanted to muscle. Cell Transplant. 2014 immunomodulatory potential of Tregs at the site of pancreatic islet transplant.

16*. Golab K, Kizilel S, Bal T, et al. Improved Coating of Pancreatic Islets with Regulatory T cells (Tregs) to Create Local Immunosuppression by Using Biotin-Polyethylene glycol-Succinimidyl Valeric Acid Ester Molecule. Transplant Proc. **Accepted for publication 2013.** (method of transient anchoring Tregs to Pancreatic islets).

17. Marek N, Krzystyniak A, Ergenc I, et al. Coating human pancreatic islets with {CD4(+)CD25(high)CD127(-)} regulatory T cells as a novel approach for the local immunoprotection. Ann Surg. 2011; 254(3):512–8. [PubMed: 21865948]

18. Nilsson B, Ekdahl KN, Korsgren O. Control of instant blood-mediated inflammatory reaction to improve islets of Langerhans engraftment. Curr Opin Organ Transplant. 2011; 16(6):620–6. [PubMed: 21971510]

19. Jindal RM, Sidner RA, McDaniel HB, et al. Intraportal Vs Kidney Subcapsular Site for Human Pancreatic Islet Transplantation. Transplant Proc. 1998; 30(2):398–9. [PubMed: 9532100]

20*. Maffi P, Balzano G, Ponzoni M, et al. Autologous Pancreatic Islet Transplantation in Human Bone Marrow. Diabetes. 2013 successful clinical islet transplant to site other than liver.

21*. Wszola M, Kwiatkowski A, Berman A, et al. Case of successful Endoscopic Pancreatic Islets auto-transplantation into gastric sub-mucosa in patient with chronic pancreatitis - preliminary report. IPIT 14th World Congr Monterey USA. 2013 another succesful clinical islet transplant to site other than liver.

22. Lau J, Mattsson G, Carlsson C, Nyqvist D, Kohler M, Berggren PO, et al. Implantation {Site-Dependent} Dysfunction of Transplanted Pancreatic Islets. Diabetes. 2007; 56(6):1544–50. [PubMed: 17400931]

23**. Han JM, Patterson SJ, Speck M, et al. Insulin inhibits {IL-10-mediated} regulatory T cell function: implications for obesity. J Immunol. 2014; 192(2):623–9. important work showing possible detrimental effect of insulin on Treg cell function. [PubMed: 24323581]

24. Yi S, Ji M, Wu J, et al. Adoptive transfer with in vitro expanded human regulatory T cells protects against porcine islet xenograft rejection via interleukin-10 in humanized mice. Diabetes. 2012; 61(5):1180–91. [PubMed: 22403295]

25. Berney T, Ferrari-Lacraz S, Bühler L, et al. Long-term insulin-independence after allogeneic islet transplantation for type 1 diabetes: over the 10-year mark. Am J Transplant. 2009; 9(2):419–23. [PubMed: 19120085]

26. Gaba RC, Raquel GR, Oberholzer J. Pancreatic islet cell transplantation: an update for interventional radiologists. J Vasc Interv Radiol. 2012; 23(5):583–94. [PubMed: 22417970]

27*. Li YQQ, Liu FFF, Zhang XMM, et al. Tumor secretion of {CCL22} activates intratumoral Treg infiltration and is independent prognostic predictor of breast cancer. {PloS} one. 2013; 8(10) naturally occurring gradient of Treg attracting chemokine induce tolerance.

28. Lee I, Wang L, Wells AD, et al. Recruitment of Foxp3+ T regulatory cells mediating allograft tolerance depends on the {CCR4} chemokine receptor. J Exp Med. 2005; 201(7):1037–44. [PubMed: 15809349]

29*. Glowacki AJ, Yoshizawa S, Jhunjhunwala S, V, et al. Prevention of inflammation-mediated bone loss in murine and canine periodontal disease via recruitment of regulatory lymphocytes. Proc Natl Acad Sci U S A. 2013; 110(46):18525–30. induction of Treg trafficking into site of inflammation alleviates autoimmune reaction. [PubMed: 24167272]

30. Montane J, Bischoff L, Soukhatcheva G, et al. Prevention of murine autoimmune diabetes by {CCL22-mediated} Treg recruitment to the pancreatic islets. J Clin Invest. 2011; 121(8):3024–8. [PubMed: 21737880]

31. Jhunjhunwala S, Raimondi G, Glowacki AJ, et al. Bioinspired controlled release of {CCL22} recruits regulatory T cells in vivo. Adv Mater. 2012; 24(35):4735–8. artificially prepared system for creation of Treg attracting chemokine gradient. [PubMed: 22821823]

32*. Tang Q, Bluestone JA. Regulatory T-cell therapy in transplantation: moving to the clinic. Cold Spring Harb Perspect Med. 2013 Nov 1.3(11) A review that takes a closer look at technical aspects of clinical application of Tregs.

33. Costantini A, Mancini S, Giuliodoro S, et al. Effects of cryopreservation on lymphocyte immunophenotype and function. J Immunol Methods Elsevier. 2003; 278(1):145–55.

34*. Golab, K.; Leveson-Gower, D.; Wang, XJ., et al. Challenges in cryopreservation of regulatory T cells (Tregs) for clinical therapeutic applications Int Immunopharmacol. Vol. 16. Elsevier B.V.; 2013. p. 371-5.review article depicting challenges connected with cryopreserving Treg cells for therapy

35. B BW, Tree T, Mannering SI, et al. Comparison of cryopreservation methods on T-cell responses to islet and control antigens from type 1 diabetic patients and controls. Diabetes Metab Res Rev. 2011; 27(8):737–45. [PubMed: 22069253]

36. Kvarnström M, Jenmalm MC, Ekerfelt C. Effect of cryopreservation on expression of Th1 and Th2 cytokines in blood mononuclear cells from patients with different cytokine profiles, analysed with three common assays: an overall decrease of interleukin-4. Cryobiology. 2004; 49(2):157–68. [PubMed: 15351687]

37. Stegall MD. Monitoring human islet allografts using a forearm biopsy site. Ann Transplant. 1997; 2(3):8–11. [PubMed: 9869858]

38**. Lee K, Nguyen V, Lee KMM, et al. Attenuation of donor-reactive T cells allows effective control of allograft rejection using regulatory T cell therapy. Am J Transplant. 2014; 14(1):27–38. comprehensive study showing a method for tolerance induction in mice model of pancreatic islet transplant, with the use of Tregs. [PubMed: 24354870]

39. Posselt AM, Bellin MD, Tavakol M, et al. Islet transplantation in type 1 diabetics using an immunosuppressive protocol based on the {anti-LFA-1} antibody efalizumab. Am J Transplant. 2010; 10(8):1870–80. [PubMed: 20659093]

40*. Monti P, Piemonti L. Homeostatic T cell proliferation after islet transplantation. Clin Dev Immunol. 2013; 2013:217934. review describing the influence of homeostatic proliferation induction on Treg cells. [PubMed: 23970924]

41**. Natalia MT, Myśliwec M, Siebert J, Trzonkowski P. Clinical application of regulatory T cells in type 1 diabetes. Pediatr Diabetes. 2013; 14(5):322–32. very important work describing, for the first time, use of ex vivo expanded Tregs in treatment of T1DM in humans. [PubMed: 23627860]

42*. Juvet SC, Whatcott AG, Bushell AR, Wood KJ. Harnessing regulatory T cells for clinical use in transplantation: the end of the beginning. Am J Transplant. 2014; 14(4):750–63. review on antigen specific Tregs. [PubMed: 24592900]

43. Sagoo P, Ali N, Garg G, Nestle FO, Lechler RI, Lombardi G. Human regulatory T cells with alloantigen specificity are more potent inhibitors of alloimmune skin graft damage than polyclonal regulatory T cells. Sci Transl Med. 2011; 3(83)

44. Golshayan D, Jiang S, Tsang J, Garin MI, Mottet C, Lechler RI. In vitro-expanded donor alloantigen-specific {CD4+CD25+} regulatory T cells promote experimental transplantation tolerance. Blood. 2007; 109(2):827–35. [PubMed: 17003369]

45*. Chapman JR, Webster AC, Wong G. Cancer in the transplant recipient. Cold Spring Harb Perspect Med. 2013; 3(7) review talking about side effects of traditional immunotherapy associated with transplant patients.

46. Qin XJ, Shi HZ, Deng JM, et al. CCL22 recruits CD4-positive CD25-positive regulatory T cells into malignant pleural effusion. Clin Cancer Res. 2009; 15(7):1. 2231–7. [PubMed: 19118025]

47*. Janakiram NB, Mohammed A, Bryant T, et al. Adoptive transfer of regulatory T cells promotes intestinal tumorigenesis and is associated with decreased {NK} cells and {IL-22} binding protein. Mol Carcinog. 2014 work highlighting possible side effects of therapy with Treg cells.

48*. Amedei A, Niccolai E, Benagiano M, et al. Ex vivo analysis of pancreatic cancer-infiltrating T lymphocytes reveals that ENO-specific Tregs accumulate in tumor tissue and inhibit Th1/Th17 effector cell functions. Cancer Immunol Immunother. 2013; 62(7):1249–60. in this work Tregs were shown to inhibit antitumour immune responses in an antigen specific manner. [PubMed: 23640603]

49. Petersen RP, Campa MJ, Sperlazza J, et al. Tumor infiltrating Foxp3+ regulatory T-cells are associated with recurrence in pathologic stage I NSCLC patients. Cancer. 2006; 107(12):2866–72. [PubMed: 17099880]

50. Shindo M, Yoshida Y. Regulatory T cells and skin tumors. Recent Pat Inflamm Allergy Drug Discov. 2010; 4(3):249–54. [PubMed: 20673223]

51. Preston CC, Maurer MJ, Oberg AL, et al. The ratios of CD8+ T cells to CD4+CD25+ FOXP3+ and FOXP3- T cells correlate with poor clinical outcome in human serous ovarian cancer. PLoS One. 2013; 8(11):e80063. [PubMed: 24244610]

52. Nosho K, Baba Y, Tanaka N, et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. J Pathol. 2010; 222(4):350–66. [PubMed: 20927778]

53. Gounaris E, Blatner NR, Dennis K, et al. T-regulatory cells shift from a protective anti-inflammatory to a cancer-promoting proinflammatory phenotype in polyposis. Cancer Res. 2009; 69(13):5490–7. [PubMed: 19570783]

54. Carreras J, Armando LG, Fox BC, et al. High numbers of tumor-infiltrating {FOXP3-positive} regulatory T cells are associated with improved overall survival in follicular lymphoma. Blood. 2006; 108(9):2957–64. [PubMed: 16825494]

55*. Martelli MF, Di Ianni M, Ruggeri L, et al. HLA-haploidentical transplantation with regulatory and conventional T-cell adoptive immunotherapy prevents acute leukemia relapse. Blood. 2014; 124(4):638–44. new clinical trial showing the possible beneficial effect of Treg and conventional T cells adoptive transfer in protection from leukemia relapse. [PubMed: 24923299]

Key Points

- Tregs could be particularly useful in allogeneic pancreatic islet transplant settings since 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 safe 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