CellPress

Regulatory T Cell Immunotherapy for Type 1 Diabetes: A Step Closer to Success?

Jagadeesh Bayry[1,2,3,4,*] and Jean-François Gautier[1,2,3,5,6]
[1]Institut National de la Santé et de la Recherche Médicale Unité 1138, Paris 75006, France
[2]Sorbonne Universités, UPMC Univ Paris 06, UMR S 1138, Paris 75006, France
[3]Université Paris Descartes, Sorbonne Paris Cité, UMR S 1138, Paris 75006, France
[4]Centre de Recherche des Cordeliers, Equipe - Immunopathologie et immuno-intervention thérapeutique, Paris 75006, France
[5]Centre de Recherche des Cordeliers, Equipe - Pathogenèse cellulaire et clinique du diabète, Paris 75006, France
[6]Service de Diabétologie, Endocrinologie, Nutrition, Centre Universitaire du Diabète et de ses Complications, Hôpital Lariboisière, University Paris-Diderot Paris-7, Paris 75010, France
*Correspondence: jagadeesh.bayry@crc.jussieu.fr
http://dx.doi.org/10.1016/j.cmet.2016.01.010

Regulatory T cell (Treg) therapy has shown promises in experimental models of type 1 diabetes (T1D) and other autoimmune diseases. Now, Bluestone et al. (2015) report in a phase 1, dose-escalation study that ex vivo-expanded autologous polyclonal Treg therapy is safe and well tolerated in adult patients with recent-onset T1D.

Type 1 diabetes (T1D), one of the most common childhood chronic diseases, results from a cell-mediated autoimmune attack against pancreatic β cells that causes insulin deficiency. One-fourth of cases are also diagnosed in adults. The established treatment is either frequent daily injections or continuous subcutaneous infusion of insulin to control blood sugar. β cell preservation is another important aspect of T1D management. Many clinical trials have revealed how immunointervention can help prevent residual β cell loss either by blocking the autoimmune response via prednisone, azathioprine, cyclosporine, heat shock protein, and anti-CD20 antibody or by re-establishing immune tolerance using anti-CD3 antibody, IL-2, or anti-thymocyte globulin (Atkinson et al., 2014; Hartemann et al., 2013). Autologous hematopoietic stem cell transplantation is another approach used to improve immune tolerance. Although these therapies induce a slower decline or improve C-peptide levels, almost all patients still require exogenous insulin. Generally, benefits of immunomodulatory therapies are associated with improved functions of $CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs) that are critical for the immune tolerance. The experimental models of T1D and other autoimmune diseases have established the potential therapeutic use of Tregs. Several Treg-based clinical trials have been recently conducted, mostly for graft-versus-host disease (Gregori et al., 2015), revealing that Treg therapy is not only safe, but also effective in reducing inflammation.

In a recent study published in *Science Translational Medicine*, Bluestone et al. (2015) conducted a phase 1, dose-escalation immunotherapy trial by using ex vivo-expanded autologous polyclonal Tregs in recent-onset adult T1D patients. They FACS purified Tregs using a combination of CD4, CD25, and CD127 markers and expanded these cells ex vivo using anti-CD3/CD28 antibody-coated beads in the presence of IL-2 (Figure 1). These expanded Tregs maintained their phenotype, polyclonality, and suppressive functions and acquired CD45RO, CCR7, and CD38 markers, indicating memory phenotype, potential lymphoid tissue homing capacity, and enhanced functioning of expanded Tregs, respectively. Bluestone et al. (2015) then adoptively transferred Tregs to the patients. As low Treg dose might not effectively suppress the pathogenic immune responses, and an excessive dose might alter metabolic processes and predispose patients to infections and cancers due to prolonged immunosuppression, it was critical to identify optimum Treg dose. Therefore, they tested four different dose regimes to obtain a clear pointer toward clinical benefits and safety related to Treg immunotherapy. Patients were monitored for primary endpoints (adverse events, laboratory abnormalities, toxicity, infusion reactions, infection complications, and impact on the course of diabetes), and the Treg therapy met with all the safety criteria. ≈25% of transferred Tregs persisted in the circulation a year later. However, metabolic data at 78 or 110 weeks showed that, while insulin use, HbA1c levels, and C-peptide remain unchanged in most of the patients, 75% of the high-dose Treg cohort patients showed >50% decline in C-peptide. Also, except for the significant reduction in NK cells early after Treg transfer, neither effector T cells nor GAD65 and ICA512 autoantibodies were significantly modified. While encouraging, the number of patients (14) would need to be increased to draw clear conclusions regarding the overall metabolic benefits of Treg immunotherapy.

Notably, another clinical trial has reported the safety and positive outcomes of autologous Treg therapy in newly onset T1D children with prolonged survival of pancreatic islets and reduced exogenous insulin use in the majority of the patients at one year, with two patients totally insulin free (Marek-Trzonkowska et al., 2014, 2012). Marek-Trzonkowska et al. (2014) indicated that disease duration and initial C-peptide serum level might determine Treg immunotherapy success. In fact, Marek-Trzonkowska et al. (2014; 2012) included children within 2 months of diagnosis, while the Bluestone et al. (2015) trial involved adults diagnosed within 14–104 weeks. Thus, alterations in the immune system caused by pathological responses and past therapies might have

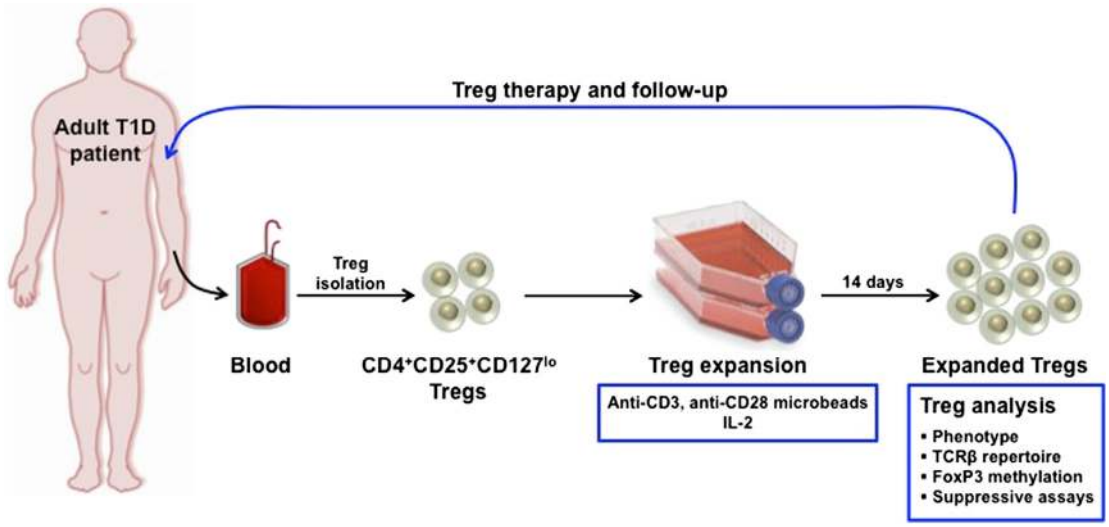

Figure 1. Scheme of Ex Vivo-Expanded Autologous Polyclonal Regulatory T Cell Therapy in Adult Type 1 Diabetes
Bluestone et al. (2015) collected 400 ml of blood from patients with recent-onset adult T1D. $CD4^+CD25^+CD127^{lo}$ regulatory T cells (Tregs) were sorted and expanded for 14 days. Following detailed analysis of expanded Tregs, patients received a single dose of Tregs. During the follow up, patients were assessed for safety and immunological and metabolic parameters. Tracking of adoptively transferred Tregs was also performed.

played a role in the outcome of the Bluestone et al. (2015) study, despite both clinical trials using similar Treg isolation and expansion methods. The course of β cell auto-destruction remains subclinical until the amount of β cell mass is insufficient to maintain glucose homeostasis and, at the time of clinical diagnosis, the β cell mass is approximately decreased by 80%. Therefore, future clinical trials could explore how the extent of β cell mass destruction and ketoacidosis may predict the response to Treg immunotherapy. Both trials also used different doses of Tregs: body weight-dependent Treg dose versus fixed dose. Additionally, Marek-Trzonkowska et al. (2014) included a second dose of Tregs for certain patients based on the metabolic function and suggested that two doses of Treg were better than the single injection. Together, polyclonal Treg therapy appears to be useful for newly onset T1D in children. However, for adult T1D, it might require further adjustments.

How can we further improve polyclonal Treg therapy? Analysis of transferred Tregs in the adult T1D patients' blood indicated no major phenotypic changes, suggesting lack of trans-differentiation into effector T cells. However, the fate of Tregs in the pancreas and lymphoid tissues remains unknown and would need to be explored in the future since the polyclonal Treg preparations used in both trials included $CD127^{lo}CD45RA^{-}FoxP3^{lo}$ non-Treg population (Miyara et al., 2009). An option would be to use CD45RA and CD25 in combination with CD127 to isolate Tregs. As the proportion of antigen-specific Tregs is low in the polyclonal preparation, using antigen-specific Tregs as in experimental models should yield better results (Tang et al., 2004).

Another aspect to consider is that Tregs isolated from the patients subjected to immunotherapy are functionally superior due to neutralization of inflammatory mediators that suppress Treg functions. For example, TNF-α renders Tregs functionally defective by TNFRII-mediated signaling that decreases FoxP3. IL-1β and IL-6 promote Th17 responses at the expense of Tregs. Importantly, these inflammatory cytokines are vital in T1D pathogenesis (Nepom et al., 2013). Therefore, neutralization of inflammatory mediators before the isolation, expansion, and adoptive transfer of Tregs would enhance the half-life of injected Tregs, their functional integrity, and the efficacy of Treg therapy (Bayry et al., 2007). Additional cytokine therapy such as IL-2 might further improve Treg functions and survival (Hartemann et al., 2013).

Several positives can be taken away from the clinical trial by Bluestone et al. (2015), as it reveals that very large numbers of functionally competent, stable, therapeutic-grade Tregs can be obtained by ex vivo expansion. Second, specific defects in Tregs, such as low phosphorylation of STAT5, can be rectified by ex vivo expansion method. Third, the therapy is well tolerated by the patients with minimal side effects. Fourth, transferred Tregs in the circulation can be tracked for analyzing their stability and pharmacokinetics. The richness of this study should ignite further improved randomized clinical trials with Tregs.

REFERENCES

Atkinson, M.A., Eisenbarth, G.S., and Michels, A.W. (2014). Lancet *383*, 69–82.

Bayry, J., Sibéril, S., Triebel, F., Tough, D.F., and Kaveri, S.V. (2007). Drug Discov. Today *12*, 548–552.

Bluestone, J.A., Buckner, J.H., Fitch, M., Gitelman, S.E., Gupta, S., Hellerstein, M.K., Herold, K.C., Lares, A., Lee, M.R., Li, K., et al. (2015). Sci. Transl. Med. *7*, 315ra189.

Gregori, S., Passerini, L., and Roncarolo, M.G. (2015). Front. Immunol. *6*, 593.

Hartemann, A., Bensimon, G., Payan, C.A., Jacqueminet, S., Bourron, O., Nicolas, N., Fonfrede, M., Rosenzwajg, M., Bernard, C., and Klatzmann, D. (2013). Lancet Diabetes Endocrinol. *1*, 295–305.

Marek-Trzonkowska, N., Mysliwiec, M., Dobyszuk, A., Grabowska, M., Techmanska, I., Juscinska, J.,

Wujtewicz, M.A., Witkowski, P., Mlynarski, W., Balcerska, A., et al. (2012). Diabetes Care *35*, 1817–1820.

Marek-Trzonkowska, N., Myśliwiec, M., Dobyszuk, A., Grabowska, M., Derkowska, I., Juścińska, J., Owczuk, R., Szadkowska, A., Witkowski, P., Młynarski, W., et al. (2014). Clin. Immunol. *153*, 23–30.

Miyara, M., Yoshioka, Y., Kitoh, A., Shima, T., Wing, K., Niwa, A., Parizot, C., Taflin, C., Heike, T., Valeyre, D., et al. (2009). Immunity *30*, 899–911.

Nepom, G.T., Ehlers, M., and Mandrup-Poulsen, T. (2013). Clin. Immunol. *149*, 279–285.

Tang, Q., Henriksen, K.J., Bi, M., Finger, E.B., Szot, G., Ye, J., Masteller, E.L., McDevitt, H., Bonyhadi, M., and Bluestone, J.A. (2004). J. Exp. Med. *199*, 1455–1465.