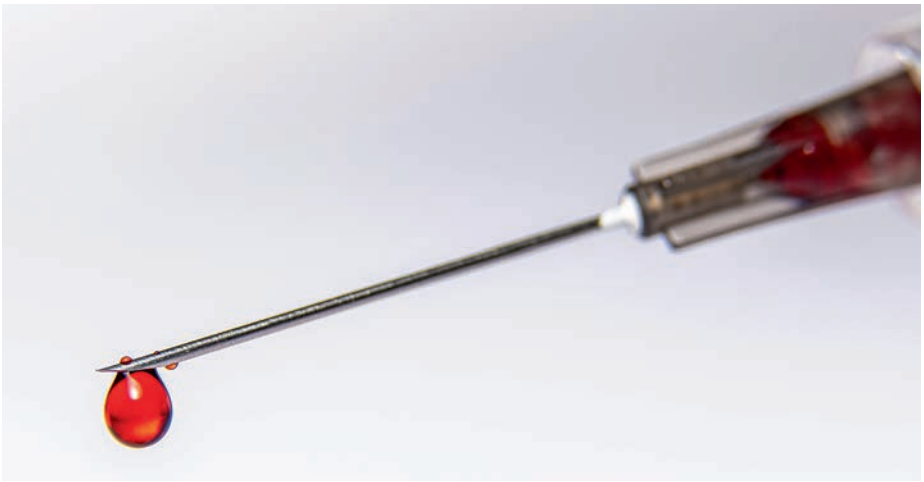
Milestone 20



T_{reg} cells to the rescue: the first clinical studies

Type 1 diabetes (T1D) is an autoimmune disease in which genetically susceptible individuals, influenced by environmental and stochastic events, eventually develop pathogenic T cells that destroy the β-cells of the islets of Langerhans in the pancreas. Several immunomodulatory therapies have shown promise, including CD3-targeted antibodies (Milestone 17), LFA3Ig, thymoglobulin and bone marrow transplantation. Although none of these approaches has induced permanent immune tolerance, it emerged that their efficacy is largely due to a relative increase in regulatory T (T_{reg}) cells versus effector T cells — leading to efforts to use T_{reg} cells as 'living drugs'.

The importance of T_{reg} cells in T1D pathology had previously been established in NOD mice (Milestone 4), where the depletion of $CD4^+CD25^+$ T_{reg} cells greatly accelerates the development of T1D. Similarly, the removal of crucial co-stimulatory or proliferative signals that are necessary for T_{reg} cells, such as IL-2 or CD28, exacerbates T1D in this model. As early as 2004, it was shown that T_{reg} cells amplified ex vivo and adoptively transferred to NOD mice can tame autoreactive T cells and halt disease development.

The first studies in humans demonstrating that T_{reg} cells are impaired in T1D date back to 2005, when Tree and colleagues showed that $CD4^+CD25^+$ T cells from patients with T1D have a reduced ability to suppress T cell proliferation in vitro. Later studies showed that T_{reg} cells from patients with T1D have impaired signalling through their IL-2 receptor. Together with the mouse studies, these findings made therapy with ex vivo-expanded autologous T_{reg} cells an attractive proposition.

> **"administration of T_{reg} cells was safe and tolerable and led to a decrease in requirement for exogenous insulin"**

A big challenge was that T_{reg} cells in humans are relatively rare and the markers and methods for their isolation were yet to be worked out. In humans, isolating T_{reg} cells on the basis of CD4 and CD25 expression risks contamination with potentially autoreactive effector cells. Progress in the field was facilitated by the discovery that human T_{reg} cells can be isolated using a combination of antibodies targeted at CD4, CD25 and CD127 and that they can be expanded ex vivo on a clinical scale using beads coated with antibodies for CD3 and CD28 in the presence of recombinant IL-2.

The first clinical trial results of autologous polyclonal ex vivo-expanded T_{reg} cells in patients with T1D were published in 2012 and 2014 by Marek-Trzonkowska et al. A small trial in children with recent-onset T1D found that administration of T_{reg} cells was safe and tolerable and led to a decrease in requirement for exogenous insulin. After 1 year, the authors reported that repeated treatment was safe and that it prolonged the survival of β-cells. This follow-up also demonstrated statistically lower insulin requirements and higher C-peptide levels (which are indicative of higher insulin levels) than a matched control group.

A second trial (Bluestone et al., 2015) enrolled 14 adult patients with recent-onset T1D who were infused with T_{reg} cells. Several patients had stable C-peptide levels and insulin use for up to 2 years after therapy, although the study was not powered to determine efficacy. Infused cells were labelled with deuterium, which allowed the investigators to follow them in the circulation. They showed that their levels in the blood peaked in the first 2 weeks after injection, followed by a loss of 75% of the peak level in the circulation (owing to either cell death or extravasation to inflamed tissues) during the first 3 months, before numbers stabilized for at least a year. Phenotypic and functional data suggested that the ex vivo expansion protocol increased not only the number of T_{reg} cells but also their suppressive capacity. There was no evidence of transdifferentiation into effector cells in vivo.

Further steps to optimize adoptive therapy with T_{reg} cells may include the addition of T_{reg} cell-promoting therapies, such as low-dose IL-2, and/or strategies to deplete effector T cells, for example with LFA3Ig. Also, efforts are under way to develop islet-specific T_{reg} cells using genetic engineering, either with chimeric antigen receptors or transgenic T cell receptors. The ultimate goal is an off-the-shelf product for adoptive therapy that would forego the need to isolate and individually expand patient-specific T_{reg} cells.

Alexandra Flemming Chief Editor, *Nature Reviews Immunology*

Milestone studies

Marek-Trzonkowska, N. et al. Administration of $CD4^+CD25^{high}CD127^-$ regulatory T cells preserves β-cell function in type 1 diabetes in children. *Diabetes Care* **35**, 1817–1820 (2012) | Marek-Trzonkowska, N. 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.* **153**, 23–30 (2014) | Bluestone, J. A. et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Sci. Transl Med.* **7**, 315ra189 (2015)

Further reading

Please visit the online article for a full list of further reading.