In vivo neutralization of inflammatory cytokines might not be necessary for regulatory T-cell immunotherapy

Manuela Battaglia and Maria-Grazia Roncarolo

We thank Dr Bayry and colleagues for their comments on our recently published Review¹. The authors argue that the naturally occurring CD4⁺CD25⁺ regulatory T (T_{Reg})-cell population is defective in rheumatoid arthritis and possibly in other autoimmune inflammatory diseases (such as multiple sclerosis and type 1 diabetes) and that therefore adoptive immunotherapy with defective T_{Reg} cells isolated from autoimmune patients and expanded *ex vivo* is unlikely to be efficacious. It is important to highlight that, to date, there is still no formal proof that *in vivo* T_{Reg} cells have an intrinsic defect in chronic inflammatory and autoimmune diseases. As highlighted in our Review, studies investigating the percentages and function of circulating CD4⁺CD25⁺ T cells in patient samples have led to conflicting data. These discrepancies might be because the CD4⁺CD25⁺ T-cell population can comprise activated T cells that do not display regulatory function, and therefore do not represent a pure T_{Reg}-cell population. This is the case in studies performed in patients with type 1 diabetes. The existence of T_{Reg} cells with defective suppressor function in patients with type 1 diabetes has been demonstrated by some investigators^{2–4}, whereas others⁵ have shown that T_{Reg} cells from such patients are as suppressive as those from normal healthy donors. This latter study was carried out using CD4⁺CD25^{hi} T cells that were isolated using a fluorescence-activated cell sorter (FACS)⁵, whereas the former studies used magnetic beads to isolate CD4⁺CD25⁺ T cells^{2–4}. It is therefore possible that patients with type 1 diabetes do not have defective T_{Reg} cells *per se*, but instead have a higher frequency of contaminating effector T cells in bead-sorted CD4⁺CD25⁺ T cells compared with those of healthy individuals. The reported increase in expression of activation markers (such as CD69 and HLA-DR) on CD4⁺ and CD8⁺ T cells from patients with type 1 diabetes, both at onset

and during the course of the disease, is consistent with this hypothesis^{6,7}. So, only highly purified FACS-sorted CD4⁺CD25^{hi} T cells from patients with type 1 diabetes display suppressive function *in vitro*⁵. Importantly, we have shown that *ex vivo* culture of T_{Reg} cells isolated from type 1 diabetic patients in the presence of rapamycin leads to expanded cells with *in vitro* regulatory activity that is identical to those of normal donors. These data suggest that, for cells from type 1 diabetic subjects, specific cell-culture conditions can selectively expand functional T_{Reg} cells over contaminating effector CD4⁺CD25⁺ T cells⁸.

As an alternative to the *ex vivo* expansion of T_{Reg} cells we also propose in our Review the use of adoptive immunotherapy with inducible regulatory T cells, such as T_R1 cells, which are generated *in vitro* in the presence of the antigen and interleukin-10. This approach overrides the need for isolating and expanding T_{Reg} cells from patients with ongoing acute and chronic disease¹.

We agree with Dr Bayry and colleagues that it is still completely uncertain whether adoptive transfer of *ex vivo* expanded or generated regulatory T cells *per se* is sufficient to downregulate inflammation and revert active diseases. We indeed suggest that “Regulatory T-cell-based immunotherapy should not be envisaged as an all-or-nothing approach to re-establish immunological tolerance on its own.”¹ An alternative approach that combines regulatory T-cell-based adoptive immunotherapy with other therapeutic interventions that are able to counteract the non-tolerogenic inflammatory milieu and the function of activated effector T cells might result in a better clinical outcome.

The different method proposed by Bayry and colleagues to first induce functionally fit regulatory T cells *in vivo* in the patients followed by their *ex vivo* expansion could

also be considered. The observations that treatment of rheumatoid arthritis patients with tumour-necrosis factor blockade leads to a significant increase in the number of circulating T_{Reg} cells^{9,10} and to the generation of a newly differentiated population of regulatory T cells¹¹ are clearly interesting. However, these cells do not mediate long-term tolerance *in vivo*¹². Furthermore, the demonstration that these *in vivo* re-fit regulatory T cells can be isolated and expanded *in vitro* while maintaining their regulatory activity is still missing.

Manuela Battaglia is at the San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Via Olgettina 58, 20132 Milan, Italy and at the San Raffaele Scientific Institute, Immunology of Diabetes Unit, 20132 Milan, Italy

Maria-Grazia Roncarolo is at the San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Via Olgettina 58, 20132 Milan, Italy and at the Vita-Salute San Raffaele University, 20132 Milan, Italy

doi:10.1038/nri2138-c2

- Roncarolo, M. G. & Battaglia, M. Regulatory T-cell immunotherapy for tolerance to self antigens and alloantigens in humans. *Nature Rev. Immunol.* **7**, 585–598 (2007).
- Brusco, T. M., Wasserfall, C. H., Clare-Salzler, M. J., Schatz, D. A. & Atkinson, M. A. Functional defects and the influence of age on the frequency of CD4⁺CD25⁺ T cells in type 1 diabetes. *Diabetes* **54**, 1407–1414 (2005).
- Glisic-Milosavljevic, S. *et al.* At-risk and recent-onset type 1 diabetic subjects have increased apoptosis in the CD4⁺CD25⁺ T-cell fraction. *PLoS ONE* **2**, e146 (2007).
- Lindley, S. *et al.* Defective suppressor function in CD4⁺CD25⁺ T cells from patients with type 1 diabetes. *Diabetes* **54**, 92–99 (2005).
- Putnam, A. L., Vendrame, F., Dotta, F. & Gottlieb, P. A. CD4⁺CD25^{hi} regulatory T cells in human autoimmune diabetes. *J. Autoimmun.* **24**, 55–62 (2005).
- Gessl, A. & Waldhausl, W. Increased CD69 and human leukocyte antigen-DR expression on T lymphocytes in insulin-dependent diabetes mellitus of long standing. *J. Clin. Endocrinol. Metab.* **83**, 2204–2209 (1998).
- Roncarolo, M. G. *et al.* Interleukin-2 production and interleukin-2 receptor expression in children with newly diagnosed diabetes. *Clin. Immunol. Immunopathol.* **49**, 53–62 (1988).
- Battaglia, M. *et al.* Rapamycin promotes expansion of functional CD4⁺CD25⁺FOXP3⁺ regulatory T cells of both healthy subjects and type 1 diabetic patients. *J. Immunol.* **177**, 8338–8347 (2006).
- Ehrenstein, M. R. *et al.* Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNF α therapy. *J. Exp. Med.* **200**, 277–285 (2004).
- Valencia, X. *et al.* TNF downmodulates the function of human CD4⁺CD25⁺ T regulatory cells. *Blood* **108**, 253–261 (2006).
- Nadkarni, S., Mauri, C. & Ehrenstein, M. R. Anti-TNF- α therapy induces a distinct regulatory T cell population in patients with rheumatoid arthritis via TGF- β . *J. Exp. Med.* **204**, 33–39 (2007).
- Buch, M. H., Marzo-Ortega, H., Bingham, S. J. & Emery, P. Long-term treatment of rheumatoid arthritis with tumour necrosis factor α blockade: outcome of ceasing and restarting biologicals. *Rheumatology (Oxford)* **43**, 243–244 (2004).