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

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We thank Dr Bayry and colleagues for their comments on our recently published Review[1]. 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 others[5] 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 cells[8].

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 disease[1].

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 cells[11] 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.

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doi:10.1038/nri2138-c2

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