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NEWS AND COMMENTARY

Tregalizumab (BT-061) increases regulatory T cell function

Boosting regulatory T-cell function with the humanized CD4-specific humanized monoclonal antibody Tregalizumab (BT-061)

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Regulatory T cells (Tregs) represent a subset of $CD4^+$ T cells that express the forkhead box protein 3 factor (FOXP3) in their nuclei[1] and the high-affinity interleukin 2 (IL-2) receptor CD25 on their cell surface as they are dependent on IL-2 for their homeostasis.[2] Since their discovery 20 years ago,[3] Tregs have proven to be indispensable for maintaining immunological self-tolerance. Indeed, Treg depletion results in a wide spectrum of autoimmune manifestations,[3] whereas mutations of *FOXP3* lead to fatal autoimmunity both in mice (scurfy mice) and humans (Immune dysfunction, polyendocrinopathy, enteropathy, X-linked syndrome).[1] Further, restoring the T-cell balance in favor of Tregs has allowed the control of autoimmunity in a number of animal rheumatologic models.[4]

On the basis of these preclinical data, clinical trials aimed at boosting Tregs in order to promote tolerance have been conducted in the context of autoimmune diseases or allogeneic hematopoietic stem cell transplantation. Specifically, boosting Treg function by administration of low doses of IL-2 has shown promising activities in patients with hepatitis C virus-induced vasculitis[5] and in those suffering from chronic graft-versus-host-disease (a redoubtable complication of allogeneic hematopoietic cell transplantation caused by donor immune cells contained in the graft reacting against recipient healthy tissues that shares many clinical features with autoimmune diseases).[6,7] Further, recent studies have suggested that transfusion of fresh or *in vitro*-expanded Tregs could prevent graft-versus-host disease after allogeneic hematopoietic cell transplantation[8] or prevent type 1 diabetes evolution.[9] However, treatment with low-dose IL-2 might be limited by the observation that Treg number/function return to baseline quickly after IL-2 discontinuation,[7] whereas the administration of sufficient numbers of relatively pure Tregs has remained technically challenging.

Over the last decades, there has been substantial interests in the use of non-depleting anti-CD4 antibodies for tolerance induction.[10] Although Treg activity has been suggested to be the main mechanism of anti-CD4-induced tolerance,[10] recent data have demonstrated that CD4 blockade induces tolerance also in the absence of Tregs, by attenuating the co-stimulatory signals.[11]

In the current issue of the journal, Helling *et al.*[12] report that, in contrast to other anti-CD4 monoclonal antibodies, the humanized non-depleting anti-CD4 monoclonal antibody tregalizumab (BT-061) selectively activates the suppressive properties of Tregs, *in vitro*, by binding to a unique conformational epitope on the domain 2 of CD4. Following binding to CD4, tregalizumab induces a T-cell receptor (TCR) signaling pathway that is incomplete and unique when compared with OKT3 and other anti-CD4 antibodies, but that is similar in Treg and in conventional $CD4^+$ T cells. Specifically, in comparison to what is observed with OKT3 or other anti-CD4 antibodies, tregalizumab induces similar phosphorylation levels of Lck, PLC-γ and SLP-76, but lower phosphorylation levels of SHP-2, ZAP70, LAT, MEK, PyK2 and MAPK. In addition, in contrast to what is observed with OKT3 and other anti-CD4 antibodies, tregalizumab does not lead to the phosphorylation of ItK, Akt, ERK, JNK, PKC, IKK and NF-κB. Further, the duration of phosphorylation induced by tregalizumab is significantly shorter than what is observed with OKT3 or other anti-CD4 antibodies. Given previous observations demonstrating that a suboptimal mode of T-cell activation leads to the generation of Treg,[1] it is likely that the specific incomplete engagement of the TCR pathway by tregalizumab explains the enhanced Treg suppressive function. This might also be in line with the recent data demonstrating that TCR signaling is essential for maintenance of Treg immunoregulatory function.[13]

Interestingly, Treg activation by tregalizumab was achieved without the modulation of typical Treg activation markers such as CD25, CD39, CTLA-4, GARP, LAG-3 or HLA-DR. However, latency-associated peptide (LAP) expression was upregulated by tregalizumab (Figure 1). It is worth mentioning that cell surface expression of LAP is associated with increased TGF-β signaling and secretion. Given than TGF-β is an important mechanism of Treg inhibition, tregalizumab-induced overexpression of LAP by Treg is probably one of the mechanisms explaining the higher inhibitory capacity of Treg when boosted with tregalizumab. Indeed, the same group of investigators recently observed that tregalizumab induces TGF-β secretion in addition to increasing cycling adenosine monophosphate levels, both selectively in Tregs.

Combined with previous observations that human immunodeficiency virus-1 envelope protein gp120 (a molecule that binds with a high affinity to domain 1 of human CD4) also mediates Treg activation and prevents graft-versus-host disease in humanized mice,[14] the current article by Helling *et al.*[12] supports the clinical use of CD4-mediated manipulation of

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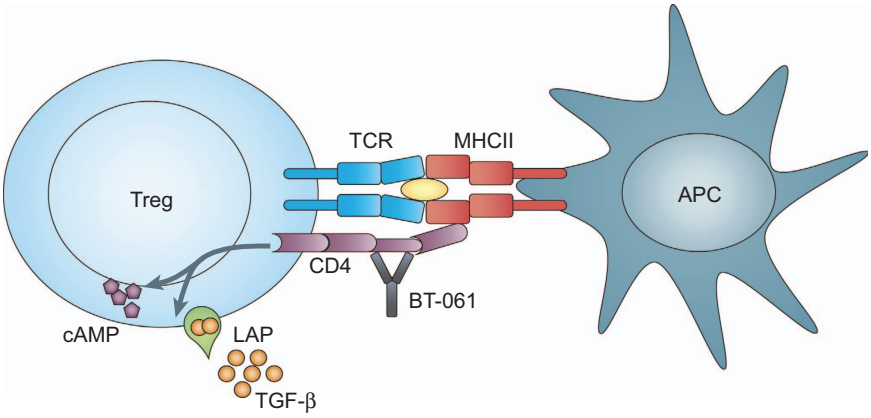

Figure 1 Tregalizumab (BT-061) activates Treg *in vitro*. Tregalizumab binds the extracellular domain 2 (D_2) of the CD4 molecule. Treg suppression activity is then enhanced, possibly through increased LAP expression and increased secretion of TGF-β. Increases in Treg cycling adenosine monophosphate levels following tregalizumab binding on CD4 have also been demonstrated by the same group of investigators (Czeloth *et al.*, manuscript submitted).

Tregs for tolerance induction in patients with autoimmune disease or with graft-versus-host disease. Such a phase IIb clinical trial is currently assessing the impact of tregalizumab in combination with methotrexate in subjects who have active rheumatoid arthritis, and an inadequate response to methotrexate alone (clinicaltrial.gov# NCT01999192). In summary, the study by Helling *et al.*[12] provides novel insights into the mechanism of action of tregalizumab, a clinically relevant immunomodulatory antibody.

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