

LETTER TO THE EDITOR

The role of GITR single-positive cells in immune homeostasis

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Dear Editor:

In 2003, Uraushihara et al. demonstrated that CD4⁺CD25⁻GITR⁺ T cells express cytotoxic T lymphocyte-associated protein (CTLA)-4, are anergic, produce interleukin (IL)-10 and transforming growth factor (TGF)- β , and exhibit regulatory activity [1]. In 2011, we demonstrated in human peripheral blood the existence of CD4⁺CD25^{low}GITR⁺ T lymphocytes isolated from a CD4⁺ population depleted of CD25⁺ cells and positively selected after GITR (TNF receptor superfamily member 18) staining [2]. The cells were designated CD25^{low} (although flow cytometry suggested they were CD25⁻) to convey that they expressed higher levels of CD25 than the effector cells did. In a subsequent publication, however, these cells were designated CD4⁺CD25^{low/-}GITR⁺ to avoid confusion [3]. CD4⁺CD25^{low/-}GITR⁺ T lymphocytes express higher levels of FOXP3 than effector cells, but much lower levels of FOXP3 than CD4⁺CD25⁺ cells (thymus-derived T regulatory cells, tTregs). Moreover, almost all CD4⁺CD25^{low/-}GITR⁺ T lymphocytes expressed the memory marker CD45RO, produced high levels of IL-10 and TGF- β , were anergic, and demonstrated suppressive activity even when cell-to-cell contact was prevented, as observed in other peripheral Treg (pTreg) subsets [2–4]. Therefore, despite the slightly different characteristics of the murine and human subsets, it is reasonable to conclude that CD4⁺CD25^{low/-}FOXP3^{low}GITR⁺ cells are present in both mice and humans. Currently, we refer to these cells as “GITR single-positive” (GITRsp) to emphasize the manner in which they can be sorted. As these cells express CD45RO and do not express CD25, we concluded that they are derived from activated effector T cells and are therefore pTregs, resembling type 1 Treg (Tr1) and T helper (Th)3 cells, the

main pTreg subsets. However, Tr1 cells express IL-10 and are FOXP3⁻; and Th3 cells express TGF- β and are FOXP3⁺ [5, 6]. Consequently, GITRsp cells appear to be neither Tr1 nor Th3 cells.

Some data suggest that GITRsp cells play a relevant functional role in maintaining immune homeostasis. Uraushihara et al. demonstrated that CD4⁺GITR⁺ T cells regulate the mucosal immune responses and intestinal inflammation irrespective of CD25 expression [1]. In particular, CD4⁺CD25⁻GITR⁺ cells inhibit the development of classic CD4⁺CD45RB^{high} transfer colitis in severe combined immunodeficiency mice. Ono et al. compared the effect of transferred GITR- and CD25-depleted T cells in nude mice [7]. Ninety percent of mice that received GITR-depleted cells died due to autoimmune diseases, whereas no mice that received CD25-depleted cells died, suggesting the presence of a GITR⁺CD25⁻ Treg population that is crucial for maintaining immune homeostasis.

Our group observed a potential homeostatic role of GITRsp cells in humans. In fact, we demonstrated the expansion of the GITRsp Treg subpopulation in the peripheral blood of patients with inactive Sjögren's syndrome, but not in patients with active disease [4]. A similar result was observed in patients with systemic lupus erythematosus (SLE). In patients with SLE with a SLE Disease Activity Index (SLEDAI) score of 0, the mean proportion of GITRsp Tregs among CD4⁺ cells was >4%, whereas it was about 1% in patients with SLEDAI scores >0 (i.e., active disease), similar to the percentage of GITRsp Tregs in healthy controls [3]. Interestingly, the attempt to use CD25 as a predictive marker in patients with SLE was unsuccessful [3]. Therefore, it is reasonable to assume that GITRsp Treg expansion impedes disease development and

that a treatment favoring GITRsp Treg expansion could be curative [8, 9].

A recently published paper by Yoshida *et al.* in this journal demonstrates that GITRsp Treg expansion slowed disease progression and prevented relapse in a murine model of arthritis [10]. Mice showing disease signs 9–10 days after glucose-6-phosphate isomerase (GPI) peptide treatment were co-treated with GPI and fingolimod for 5 days. The combined treatment not only decreased the arthritis clinical score during treatment, it also prevented relapse upon re-treatment with GPI on day 32 (resensitization). Mice treated with fingolimod alone or GPI alone had clinical scores similar to placebo-treated mice and were sensitive to resensitization. As no differences in self-antibody concentrations were observed among the four groups, the authors hypothesized that the combined treatment altered the balance between effector T cells and Tregs in the combined treatment group as compared to the fingolimod-treated group. The authors did not find any difference in the percentage of FOXP3⁺ cells (e.g., tTregs and Th3 cells) in the inguinal lymph nodes of the mice. On the contrary, they found that CD4⁺CD25⁻FOXP3⁻GITR⁺ cells (i.e., GITRsp) had increased. Moreover, they demonstrated that GITRsp Tregs express high levels of CTLA-4 similar to that expressed by CD4⁺CD25⁺FOXP3⁺GITR⁺ tTregs, produce much more IL-10 than CD4⁺CD25⁺FOXP3⁺GITR⁺ tTregs, and have regulatory activity. Therefore, the findings of Yoshida *et al.* further illustrate the role GITRsp cells might play in decreasing T cell activation/proliferation and controlling autoimmune diseases. Indeed, we demonstrated that the number of activated CD4⁺ T cells (CD4⁺CD25⁺GITR⁻) was inversely correlated with the number of GITRsp cells in patients with SLE [3].

Although fingolimod promoted permanence of activated T cells in the lymph nodes in the experimental model used by Yoshida *et al.*, it induced the expansion of GITRsp cells only in association with antigenic stimulation. We may hypothesize that the fingolimod-dependent increase in T cell density in the lymph nodes favors GITR triggering by the membrane-bound GITR ligand (GITRL) expressed by activated T cells and antigen-presenting cells (APC) and that increased GITR triggering favors the expansion and/or differentiation of activated/antigen-specific GITRsp cells. Indeed, the expansion of GITRsp cells has been described in GITRL transgenic mice [11].

In summary, Yoshida *et al.* suggest that increased GITRsp Tregs can prevent relapse following resensitization, supporting the hypothesis that drug-induced expansion of GITRsp Tregs is a viable treatment strategy for patients with autoimmune diseases and that GITRsp pTregs can be used in cell-based therapeutic approaches [8, 12]. Several studies in murine models have demonstrated that pTreg infusion is more effective than tTreg infusion in the treatment of

autoimmune diseases. For example, Th3 pTregs suppress lupus-like chronic graft-versus-host disease (GVHD), and the adoptive transfer of antigen-specific Th3 pTregs before the induction of collagen-induced arthritis (CIA) decreases the incidence of CIA [13, 14]. In that model, the CIA score was decreased when the infusion was performed 14 days after CIA induction, while no improvement was observed after tTreg infusion. Indeed, pTregs maintain their regulatory activity in an inflammatory microenvironment while tTregs do not [15]. Some studies on both murine and human tumor-infiltrating lymphocytes (TIL) have suggested that GITRsp cells are activated tissue- and antigen-specific pTregs [9]. For example, GITR expression in Tregs residing in melanoma was approximately 10-fold higher than that of Tregs in the spleens of the same animals [16]. In patients with head and neck squamous cell carcinoma, TIL Tregs producing IL-10/TGF- β are best detected by GITR, which is expressed in 83% of TIL Tregs and in about 5% of peripheral blood mononuclear cell (PBMC) Tregs [17]. For these reasons, GITRsp pTregs are candidate cell-based therapeutic approaches for treating autoimmune diseases [8, 12].

Before GITRsp pTregs are tested in humans, however, several issues should be addressed. For example, it is crucial to determine whether GITRsp Tregs have a stable phenotype and whether they continue to expand over time. Our observation of disease inactivity in patients with SLE and the 17-day interval between the end of GPI/fingolimod co-treatment and resensitization suggests a certain phenotype stability, but future studies should address this question better.

Disclosure statement

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