In this issue of *Clinical Cancer Research*, Mutis et al. (1) use a model of xenogeneic graft-versus-host disease to assess the suppressive function of human regulatory T cells *in vivo*. This model is based on the transfer of human peripheral blood cells into RAG-2−/−γc−/− mice that are deficient for natural killer cells as well as T and B cells. On transfer, human immune cells, especially T cells, induce a robust xenogeneic response to host antigens that kill the animals within 2 weeks. This model was first described by the same team in a previous article published in *Clinical Cancer Research* (2). In the present study, the authors investigate the effect of depletion or enrichment of CD4+CD25+ regulatory T cells in the graft inoculum on the development and severity of xenogeneic graft-versus-host disease after transplant.

Regulatory T cells (Treg) have been characterized as a distinct subset of CD3+CD4+ T cells constitutively expressing the α chain of the high-affinity interleukin (IL)-2 receptor, CD25 (3). At first, these cells could not readily be distinguished from activated CD4+ effector T cells because activated T cells transiently express a wide range of receptors also found on naturally occurring Treg. Subsequently, a unique transcription factor, *forkhead box P3* (FOXP3), was found to be specifically expressed in murine and human Treg (4). Studies have also shown that forced ectopic expression of FOXP3 in CD4+ T cells was sufficient to confer suppressive capabilities to CD4+CD25− T cells (4). To date, no other marker has been identified that defines Treg more accurately than FOXP3. Functionally, CD3+CD4+FOXP3+ Treg act as natural inhibitors of normal immune responses. These cells control and likely channel effect T cells responding to external and internal pathogens (5). They also restrain autoreactive immune cells from mounting destructive reactions to self-antigens (6). Likewise, Treg can also limit natural antitumor immune responses from rejecting transformed cells. A series of articles published in *Clinical Cancer Research* and elsewhere have reported the poor prognosis of patients when tumors are densely infiltrated with Treg (7–10). This is commonly interpreted as evidence to support an important role for Treg as mediators of resistance to effector T cells, although the role of Treg in tumor immunity is likely more complex (11).

Defects in Treg have been associated with a variety of immune pathologies. Single point mutations in the FOXP3 gene are known to cause a lethal systemic autoimmune syndrome, immune dysregulation, polyendocrinopathy, enteropathy X-linked (12). Subtle alterations in Treg functions have also been reported in patients with multiple sclerosis (13), rheumatoid arthritis (14), systemic lupus erythematosus (15), type 1 diabetes (16), autoimmune polyglandular syndromes (17), as well as allergic reactions (18). As knowledge of Treg biology grows, it becomes apparent that these cells participate in virtually all immune responses. Artificially modulating their function could thus provide ways to enhance or reduce immune responses and lead to novel therapies. One of the contexts in which interventions to modulate Treg may first be used is after allogeneic hematopoietic stem cell transplantation (HSCT). Several retrospective clinical studies have suggested that the reconstitution of Treg after HSCT plays an important role in controlling alloimmunity and preventing graft-versus-host disease (GVHD; refs. 19–21). Conversely, poor reconstitution of Treg likely leads to a persistent immune imbalance that results in chronic GVHD (22). It is also possible that Treg, by channeling effect effector cells, are required for any given immune response to reach its full capacity. This would explain the apparent immunodeficiency that correlates with reduced Treg pools and chronic GVHD after allogeneic HSCT.

Several mouse studies have previously shown the potential use of Treg to prevent or treat GVHD across minor or major histocompatibility barriers (23–26). In most models, Treg were administered to the recipient together with the graft and were able to reduce or prevent GVHD. Interestingly, prevention of GVHD was obtained while retaining the beneficial graft-versus-leukemia effect, suggesting that Treg-based therapeutic interventions could be particularly useful in patients with hematologic malignancies undergoing allogeneic HSCT (23, 24, 26). In humans, studies have shown the feasibility of purifying Treg from leukopheresis products from normal donors and expanding them *in vitro* for subsequent infusion in stem cell recipients (27, 28). *Ex vivo*–expanded Treg share a distinct phenotype with their naturally occurring counterparts and have the capacity to suppress a wide range of immune effector cells *in vitro*, including CD4+CD25+ T cells and CD8+ T cells. Taken together, these preclinical studies set the stage for Treg-based therapeutic trials. Yet, without an adequate experimental model, the behavior of human Treg had not been studied *in vivo* until now. In this respect, the experimental model described by Mutis et al. (1) provides a unique opportunity to explore several aspects of the biological function of human Treg *in vivo*.

Xenogeneic mouse transplantation models have previously been used by various research groups to study GVHD mediated by human effector cells. In 2003, van Rijn et al. (2) described the effects of transplanting human T cells into RAG-2−/−γc−/− mice. Compared with the widely used nonobese diabetic-severe combined immunodeficient mice, RAG-2−/−γc−/− double-knockout mice that received i.v. infusions of human peripheral
blood mononuclear cells developed xenogeneic GVHD (x-GVHD) that matched more closely the immune pathology seen in allogeneic HSCT patients. Some animals even developed what seemed to mimic chronic GVHD. In this issue of *Clinical Cancer Research*, the same research team used this model to examine the capacity of human Treg to control x-GVHD in vivo (1). Previous experiments had shown the capacity of human Treg to suppress proliferation of autologous CD4+CD25− T cells in response to porcine xenogeneic stimulation (29). No model had yet tested the function of these cells in vivo, however. As shown in Fig. 1, Mutis et al. (1) modulated the number of human CD4+CD25+ Treg transplanted along with peripheral blood mononuclear cells into RAG-2−/− γc−/− mice and examined the influence of these cells on the onset and severity of x-GVHD. Animals that received grafts depleted of CD25+ cells developed higher-grade x-GVHD. In contrast, restoring purified CD25+ cells to the graft reduced the severity of disease or led to protection from x-GVHD. Interestingly, animals surviving >30 days showed elevated plasma levels of IL-10 and IFN-γ. They also displayed higher CD4/CD8 T-cell ratios during the first week after transplantation. This latter result likely reflected the expansion of CD4+CD25+ T cells early after transplantation that proved critical for later outcome.

The functional effects of human Treg in the x-GVHD model have several potential clinical implications. *(a)* Results from these experiments indicate that human Treg display functional activities similar to those of mouse Treg, thereby validating several mouse studies. It is therefore possible that the differential effects of mouse Treg on graft-versus-leukemia and GVHD can also apply to humans. *(b)* In xenogeneic transplantation, immunologic disparities between recipient and donor are greater than in human allogeneic HSCT; hence, xenogeneic immune reactions are usually stronger than allogeneic responses and consequently more difficult to control. The observation that simple enrichment of transplanted cells with human Treg is sufficient to control such a vigorous x-GVHD underscores the potency of these cells in vivo. *(c)* Using this experimental model, one can now examine different aspects of human Treg function in vivo. For example, a recent article by Zeiser et al. (30) suggests that common immunosuppressive treatments, including cyclosporine, mycophenolate mofetil, and rapamycin, have differential effects on mouse effector cells and Treg. The selectivity of these drugs can now also be tested on human Treg in vivo using the x-GVHD model. In another example, the critical role of IL-2 in the maintenance of Treg pools has recently been shown in several animal studies (31, 32). In humans, administration of recombinant IL-2
induces the expansion of Treg in vivo (33–35). This effect is partly mediated through a direct signaling pathway linking activation of the IL-2 receptor and expression of the FOXP3 gene through the phosphorylation of signal transducers and activators of transcription proteins (STAT; ref. 35). In previous studies, using severe combined immunodeficient mice transplanted with human peripheral blood lymphocytes, Roychowdhury et al. (36) showed that IL-15 exacerbated x-GVHD, whereas administration of IL-2 did not. In this xenogenic model, however, it was unclear whether IL-2 had an effect on Treg. The current xenogenic model developed by Mutis et al. (1) will be very helpful in further characterizing these effects and specifically defining the consequences of IL-2-driven Treg expansion in vivo. Moreover, this in vivo model could also be used to identify additional cytokines that potentially influence Treg homeostasis.

The studies by Mutis et al. (1) also highlight several questions that remain unanswered. First, the mechanism whereby human Treg exert their suppressive activity on xenogenic effector immune cells is still unclear. In this model, the controlling effect of human Treg seems to correlate with elevated plasma levels of IFN-γ, suggesting the in vivo induction of TR1-like cells producing large amounts of this cytokine. Subsequent experiments using the x-GVHD model will be necessary to determine whether transforming growth factor-β, which is also secreted by TR1 cells in conjunction with IL-10, also plays an important role. Unexpectedly, mice that survived xenotransplantation without evidence of x-GVHD had higher plasma levels of IFN-γ. IFN-γ is a key cytokine in the homeostatic control of effector T-cell pools during immune responses, being responsible for both their expansion and subsequent contraction (37). Similarly, it will be interesting to determine whether, in this model, Treg control of effector T-cell proliferation also uses IFN-γ. Another open question relates to the duration of the xenogeneic tolerance achieved in this model and whether late infusion of human Treg could be sufficient to reestablish x-GVHD. Addressing these questions is likely to generate valuable data and help define the functional activity of human Treg in vivo ahead of clinical trials.

References


Studying Human Regulatory T Cells \textit{In vivo}

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