

Bone marrow provides an environment that prevents suppression of therapeutic graft-vs.-tumor immunity by regulatory T cells

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Abbreviations: FOXP3, forkhead box P3; GvHD, graft- vs. -host disease; GvT, graft-*versus*-tumor; IL, interleukin; Treg, regulatory T cell

Regulatory T cells (Tregs) can prevent graft- vs. -host disease as induced by the infusion of donor lymphocytes to cancer patients, but often they also suppress therapeutic antitumor immunity. We discuss an exception to this phenomenon, exemplifying how the milieu provided by the bone marrow may neutralize Tregs to allow local immune responses against cancer.

Over the past decades, the characterization of the mechanisms whereby T lymphocytes eradicate malignant cells coupled to the identification of a large panel of tumor-associated antigens have opened novel promising avenues for anti-cancer immunotherapy by vaccination or adoptive T-cell transfer. Unfortunately, the success of immunotherapy is often hampered by naturally occurring CD4⁺CD25⁺FOXP3⁺ regulatory T cells (Tregs), which have been shown to suppress the activity of tumor-reactive T-cells in multiple solid neoplasms.¹ The depletion of Tregs can significantly improve the outcome of anticancer therapy, as recently shown by robust graft- vs. -tumor (GvT) effects obtained upon the elimination of Tregs from infused donor lymphocytes.² However, a non-specific depletion of the whole Treg population also entails several drawbacks, as Tregs are critical for preventing severe inflammation and autoimmunity. Such a regulatory function of Tregs is fully exploited by the co-infusion of donor cells with extra Tregs to prevent the graft-vs.-host disease (GvHD),³ a severe and often lethal inflammatory

condition induced by allogeneic stem cell transplantation and donor lymphocyte infusion. Thus, Tregs can be disadvantageous for cancer therapy, but they are at the same time required for preventing lethal inflammation. This consideration suggests that successful immunotherapies require advanced methods to selectively neutralize Tregs in the tumor microenvironment but not in other tissues, where the homeostatic and anti-inflammatory functions of Tregs are beneficial.

At least two lines of evidence indicate that Tregs are not terminally differentiated cells. Rather, it seems that the immunosuppressive functions of Tregs can be altered depending on environmental factors, notably cytokines. The first line of evidence in support of this notion originates from recent studies demonstrating that Tregs possess a high degree of plasticity *in vitro*. The inflammatory cytokines interleukin (IL)-1 β and IL-6, which are involved in the differentiation of IL-17-producing T_H17 cells, were shown to confer T_H17-like characteristics to Tregs⁴ and to abolish their immunosuppressive functions.⁵ The second line of circumstantial

evidence comes from early studies by Edinger et al. based on a murine model of GvHD and GvT.⁶ In this setting, the administration of murine Tregs together with tumor-reactive T cells did not abrogate the GvT effect against a B-cell neoplasm developing in the bone marrow, yet the GvHD was effectively prevented. In other studies, however, murine Tregs were shown to abrogate the GvT reaction against the same cancer when they were located outside the bone marrow.^{7,8} Taken together, these observations suggest that Tregs exert a differential activity in different tissues and that the suppression of the GvT effect by Tregs can be prevented in the bone marrow.

We consider our recent findings as a third line of evidence demonstrating that Tregs can be conditionally neutralized in the tumor microenvironment to allow for robust GvT effects while preserving full immunosuppressive functions in other tissues.⁹ Our study was based on a fully humanized GvT model, in which we extensively investigated the impact of Tregs on the GvT effect mediated by T cells against different types of multiple

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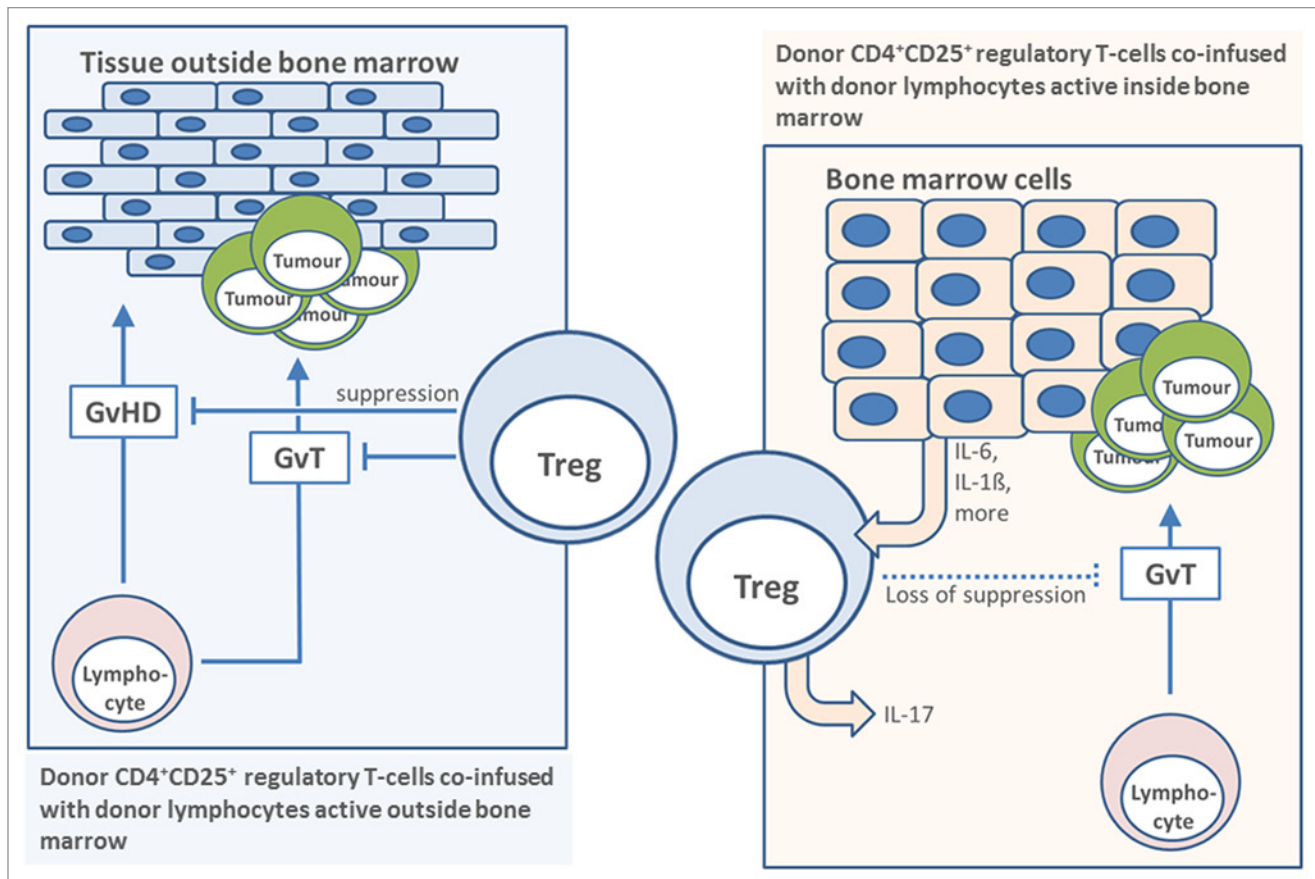


Figure 1. Differential immunosuppressive activity of regulatory T cells in distinct microenvironmental conditions. Normally, donor CD4⁺CD25⁺FOXP3⁺ regulatory T cells (Tregs) suppress the graft- vs. -host disease (GvHD) but also limit curative graft- vs. -tumor (GvT) effects mediated by donor lymphocytes (left). However, when tumor cells are located in the bone marrow, donor Tregs lose their ability to suppress GvT effects but not GvHD (right). This local effect is mainly mediated by bone marrow cells, causing Tregs to lose their immunosuppressive functions.

myeloma.⁹ In tumor-bearing immunodeficient mice, the infusion of human T cells not only induced a robust GvT effect but also a xenogeneic GvHD, due to the attack of murine tissue by infused lymphocytes (Fig. 1). We discovered that the co-infusion of human Tregs with effector T cells does not suppress the GvT effect against human multiple myelomas developing within the bone marrow, yet significantly control the concomitant GvHD. The growth rate and immunogenicity of tumors did not significantly alter the absent impact of Tregs on the GvT effects. Remarkably, Tregs did abrogate the GvT effect against multiple myelomas growing outside the bone marrow, implying that the immunosuppressive effects of Tregs are specifically neutralized within the bone marrow (Fig. 1). Elaborating on

these findings, we discovered that Tregs co-cultured with human bone marrow stromal cells partly lose their immunosuppressive functions. Moreover, we observed that Tregs produce elevated levels of IL-17 when cultured in the presence of bone marrow stromal cells (in vitro) as well as in a humanized bone marrow microenvironment (in vivo). Since bone marrow cells are known for their ability to secrete extremely high amounts of IL-1 β and IL-6, we evaluated the involvement of these cytokines in the loss of immunosuppressive functions by Tregs upon co-culture with stromal cells. Indeed, the neutralization of these cytokines with monoclonal antibodies partly restored the immunosuppressive function of Tregs. Therefore, IL-1 β and IL-6 produced by the bone marrow microenvironment are

very likely to contribute to the maintenance of the GvT effect by neutralizing Tregs.

In our view, the selective neutralization of Tregs may be achieved via the expression of IL-1 β and IL-6 at the tumor site. This may provide a unique opportunity to improve the efficacy of T cell-based immunotherapies against cancer while avoiding unwarranted side effects. This new concept needs to be further developed and explored in convenient tumor models, not only to provide a formal proof-of-principle, but also to identify the conditions that may underpin optimal anticancer responses in this setting.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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