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Autochthonous T cells to the rescue

IL-10 directly activates tumor-resident CD8+ T cells

Jan Emmerich,[1] John B. Mumm[2] and Martin Oft[2,*]

[1]TriMod Therapeutics; Dublin, Ireland; [2]Targenics; San Francisco, CA USA

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Successful cancer immunotherapy is thought to require de novo priming of tumor specific CD8+ T cells in lymphatic organs. Contrasting these beliefs, cancer therapy based on interleukin-10 (IL-10) results in tumor rejection without a requirement for T-cell trafficking from lymphatic organs. Rather, IL-10 directly activates autochthonous, tumor-resident CD8+ T cells.

Successful immune responses against a tumors, be it endogenous or therapeutically induced, consist of three distinct phases.[1] First, dendritic cells (DCs) must take up tumor antigens and mature. These activated DCs then induce an effective anti-tumor T-cell response in the lymph node by the priming and expansion of CD4+ T cells first, and then of cytotoxic CD8+ T cells. Lastly, activated T cells eventually migrate into the tumor and kill antigen-presenting malignant cells. Accordingly, a high number of CD8+ T cells in the tumor correlates with improved prognosis for cancer patients.[2] Cancer vaccines and the adoptive transfer of tumor-reactive T cells expanded ex vivo can result in large numbers of effector T cells in the lymph node and blood, but the therapeutic effects of such therapies have not been as consistent or significant as anticipated.[3]

Two major reasons underlying this lack of efficacy may be the poor infiltration of T cells into the tumor and the immunosuppressive tumor microenvironment. Tumor cells often express low levels of MHC molecules as well as of tumor-associated antigens (TAAs), making them poor targets for cytotoxic CD8+ T cells. Moreover, the tumor environment is often characterized by the accumulation of immunosuppressive cells such as regulatory T cells and myeloid-derived suppressor cells. In addition, tumors actively suppress immune response via inhibitory molecules such as PD-L1 or transforming growth factor β (TGFβ).[1]

Another molecule that can be produced by regulatory T cells, myeloid-derived suppressive cells and tumor cells is interleukin-10 (IL-10), which is thought to contribute to the immunosuppressive tumor microenvironment. In vitro and under inflammatory conditions, IL-10 inhibits the expression of MHC Class II molecules, co-stimulatory molecules, and pro-inflammatory cytokines by antigen-presenting cells (APCs).[4] Inhibition of APC function in turn impairs T-cell responses. In addition, IL-10 directly inhibits the in vitro activation and cytokine secretion of CD4+ T cells and macrophages.[4] IL-10 was shown to impair the efficacy of a tumor vaccine when administered during vaccination. However, when given after vaccination, IL-10 enhanced vaccine-mediated antitumor functions.[5]

Contrasting its immunosuppressive function, IL-10 activates CD8+ T cells in vitro and more importantly, the treatment of tumor-bearing mice with IL-10 leads to tumor rejection in multiple tumor models.[6] The antitumor efficacy of IL-10 depend on the presence of CD8+ T cells, and the IL-10 treatment increased the number of CD8+ T cells within the tumor. Similar to human tumors prior to therapy, CD8+ T cells poorly infiltrated the cancer models employed in our studies.

Contrary to expectations, IL-10 treatment induces interferon γ (IFNγ) expression by CD8+ T cells, which in turn increased the levels of MIG and IP10 in the tumor and serum.[7] These chemokines act as chemoattractants for T cells, suggesting a positive feedback loop of IFNγ-producing CD8+ T-cell recruitment into the tumor initiated by IL-10. Indeed, such a feedback loop has been postulated in mice bearing mammary tumors and treated with IL-10.[8] Surprisingly, however, we found that mice deficient for CXCR3, the receptor for both MIG and IP-10, respond normally to IL-10. Furthermore, even a broad inhibition of T-cell migration from lymphoid organs using the S1P inhibitor FTY720 showed no effect on IL-10 efficacy.[7] Therefore, IL-10 induces the accumulation of activated CD8+ T cells in tumors in the absence of de novo migration from lymph nodes.

These data indicate that the expansion and activation of autochthonous tumor-resident CD8+ T cells is sufficient to induce the rejection of well-established tumors. This comes as a surprise, since most approaches of cancer immunotherapy aim at inducing the priming of naïve TAA-reactive CD8+ T cells or the expansion of TAA-specific T-cell reservoirs in lymphatic organs.[1]

The IL-10 receptor (IL-10R) was upregulated on CD8+ T cells upon stimulation of the T cell receptor (TCR).

*Correspondence to: Martin Oft; Email: martinoft@gmail.com
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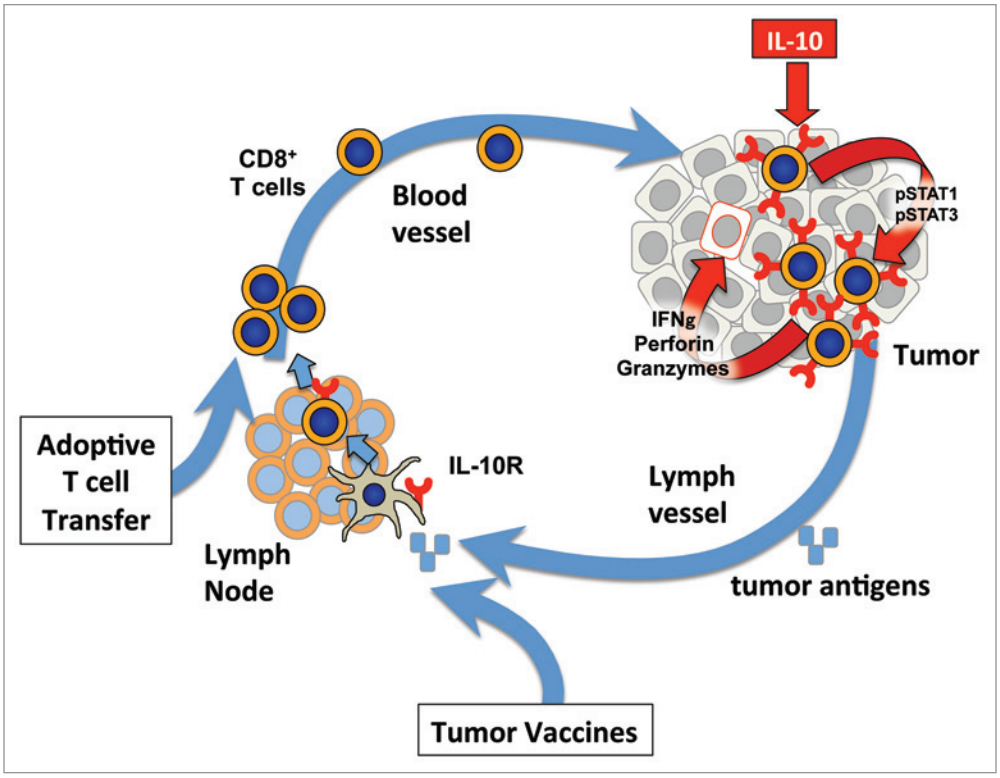

Figure 1. Most immune therapies of cancer increase the number of tumor specific CD8+ T cells by ex vivo amplification or stimulate the amplification of tumor specific CD8+ T cells in lymphoid organs through vaccine strategies (blue pathways). In contrast, treatment with pegylated interleukin-10 (IL-10) induces simultaneously the amplification of autochthonous tumor-specific CD8+ T cells and their cytotoxic activation within the tumor, in the absence of trafficking to and from lymphoid organ (red pathway). To achieve tumor rejection, IL-10 signals are required only within CD8+ T cells and not in other cells of the immune system.

Also, CD8+ TILs showed a higher surface expression of IL-10R than T cells from other locations, suggesting that they are well equipped to directly respond to IL-10. Accordingly, in tumor-infiltrating lymphocytes, IL-10 treatment induced a high degree of phosphorylation not only of STAT3 but also of STAT1. This pattern of STAT activation was unique to tumor-resident CD8+ T cells and was not seen in other T-cell subsets, not even in CD8+ T cells from lymphoid organs.[7] IL-10-activated CD8+ T cells were characterized by increased expression of the cytotoxic molecules granzyme B and perforin as well as of the effector cytokine IFNγ. Analysis of wild type and IL-10R-deficient CD8+ T cells within the same tumor-bearing mouse showed that IL-10 directly and specifically increases the activity of IL-10R-proficient CD8+ T cells without the requirement of other host cells being able to respond to IL-10. Reciprocally, IL-10 treatment did not stimulate IL-10R-deficient CD8+ T cells in an IL-10R-proficient host, confirming the direct nature of IL-10 signaling in controlling the activity of CD8+ T cells. Finally, the administration of IL-10 increased the proliferation of IL-10R-proficient CD8+ T cells, leading to their relative increase within the tumor, while IL-10R-deficient CD8+ T cells were strongly suppressed.

Our data indicate IL-10 directly activates tumor-resident CD8+ T cells increasing their activity, prevalence and proliferation, hence enabling a potent antitumor T-cell response leading to tumor rejection. Since it has already been shown that IL-10 treatment increases the production of IFNγ and granzymes in human peripheral blood mononuclear cells,[9,10] IL-10 might hold promises for the immunotherapy of cancer patients. (**Fig. 1**).

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