Interleukin-10 (IL-10) is considered to be an immunosuppressive cytokine. However, the continuous administration of pegylated IL-10 (PEG-IL10) leads to the rejection of large, firmly established and metastatic syngeneic tumors. PEG-IL10 therapy induces the expansion and activation of intratumoral, tumor antigen-specific CD8+ T cells, leading to interferon-γ (IFN-γ)-mediated Th1-like immunity and tumor rejection.

A limited amount of T cells, notably CD8+ cytotoxic T lymphocytes (CD8+ CTLs), can infiltrate human tumors. However, thanks to extensive research performed over several decades, the isolation of tumor-associated antigen (TAA)-specific CD8+ CTLs, as they develop in many cancer patients, has become possible. This implies that, far from being completely suppressed, the immune system of cancer patients has recognized TAAs enough to allow for the amplification of memory T cells. Human tumors may evolve to inhibit the activity of intratumoral tumor-specific CTLs rather than avoiding recognition altogether. Retrospective analyses of human tumor tissues have shown that patients with 3-fold increased numbers of intratumoral CD8+ CTLs have a better prognosis, and that the intratumoral expression of CD8+ CTLs has been largely neglected. CD8+ CTLs within the tumor tissue nor if they maintain an activity once inside the tumor. The identification of therapies that activate tumor specific CD8+ CTLs within the tumor mass in the absence of severe peripheral toxicity has been challenging.

Due to its anti-inflammatory properties, interleukin-10 (IL-10) is considered an immunosuppressive cytokine. IL-10 inhibits the expression of MHC Class II molecules, co-stimulatory molecules and pro-inflammatory cytokines in APCs, and directly antagonizes the functional activation of CD4+ T cells. In the absence of IL-10, IL-12 production and tumor clearance in response to Toll-like receptor (TLR) stimulation is enhanced, as is the T-cell immunity induced by antitumor vaccines. It has been suggested that IL-10 also contributes to the establishment of an intratumoral immunosuppressive environment.

However, IL-10 enhances the cytotoxicity of CD8+ CTLs, and tumor cell lines transfected with IL-10 are rejected upon transplantation into mice by CD8+ CTLs. In the context of the well-established anti-inflammatory properties of IL-10, its stimulatory activity toward CD8+ CTLs has been largely neglected. In addition, chemically-induced endogenous tumors have been shown to be multiplied and more aggressive in IL10−/− mice, while IL-10-overexpressing animals appear to be resistant to tumor induction, warranting a deeper exploration of the immunostimulatory functions of IL-10 in vivo.

To continuously expose tumor-bearing mice to moderately elevated IL-10 levels, we developed a pegylated form of IL-10 (PEG-IL10). Surprisingly, PEG-IL10 exhibited an unparalleled antitumor activity in the most challenging murine models of human cancer. PEG-IL10 was able to overcome long-established and metastasizing breast cancers and melanomas (in transplantable settings) as well as large endogenous breast cancers developing in MMTV-Her2/neu transgenic mice.

Prior to treatment with PEG-IL10, these tumors were poorly infiltrated by CD8+ CTLs, similar to human cancers. In addition, most T cells within the tumor as well as in the bloodstream did not respond to T cell receptor (TCR) stimulation, a phenomenon known as T-cell anergy. Upon administration of PEG-IL10, the intratumoral expression of cytotoxic enzymes and IFNγ increased within hours. Surprisingly, PEG-IL10 treatment also augmented the number of CD8+ CTLs found within the tumor tissue by several folds and the expression of IFNγ in intratumoral CD8+ CTLs. With regard to this, it is important to note that the intratumoral expression of cytotoxic enzymes and IFNγ as well as the accumulation of CD8+ CTLs in the tumor tissue all correlate with increased survival in cancer patients.

Reflecting the situation of human solid neoplasms, tumor-specific CD8+ CTLs...
Presentation mediate the rejection of tumor masses triggered by PEG-IL-10 (Fig. 1). PEG-IL-10 appears to promote several properties of the intratumoral immune response that are associated with a better prognosis in humans. At the core of these changes are the induction of tumor-specific, intratumoral CD8+ CTLs, the induction of cytotoxic enzymes and IFNγ in these cells, and the upregulation of antigen presentation in the tumor. Given the close similarity (with respect to the immune biology) of our murine cancer models to human cancers, we suggest that the treatment of cancer patients with PEG-IL-10 may similarly induce a potentially therapeutic immune response.

**Figure 1.** 3-fold action of interleukin-10 (IL-10). In pro-inflammatory diseases involving bacterial products and damaged cells, pattern recognition receptors induce inflammatory reactions characterized by the production of specific cytokines (left box). Such reactions are normally reduced in the presence of interleukin-10 (IL-10). However, the cytotoxicity of CD8+ T cells is directly stimulated by IL-10 (right box). IL-10 stimulates the production of interferon γ (IFNγ) by TCR-activated CD8+ T cells, leading to the IFNγ-mediated upregulation of antigen presenting molecules (MHC Class I and II) in situ (middle box). Pegylated IL-10 (PEG-IL-10) treatment induces a strong local release of IFNγ, facilitating antigen presentation and the killing of tumor cells by antigen-specific CD8+ cytotoxic T lymphocytes (CD8+ CTLs).

are rare in our tumor models prior to therapeutic interventions, and short-term PEG-IL-10 treatment did not rescue this response. However, after two weeks of PEG-IL-10 administration, a large percentage of tumor-infiltrating CD8+ CTLs and their circulating counterparts were tumor reactive. Rather than providing a general proliferation and activation signal for all T-cell populations, PEG-IL-10 treatment expanded the TAA-specific CD8+ CTL subsets. Accordingly, PEG-IL-10-induced tumor shrinkage coincided with the appearance of tumor-specific T-cell populations.

IL-10 suppresses the expression of MHC Class II molecules and reduces presentation mediate the rejection of tumor masses triggered by PEG-IL-10 (Fig. 1).

PEG-IL-10 appears to promote several properties of the intratumoral immune response that are associated with a better prognosis in humans. At the core of these changes are the induction of tumor-specific, intratumoral CD8+ CTLs, the induction of cytotoxic enzymes and IFNγ in these cells, and the upregulation of antigen presentation in the tumor. Given the close similarity (with respect to the immune biology) of our murine cancer models to human cancers, we suggest that the treatment of cancer patients with PEG-IL-10 may similarly induce a potentially therapeutic immune response.
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