

Could the properties of IL-27 make it an ideal adjuvant for anticancer immunotherapy?

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We have recently been the first to demonstrate that interleukin (IL)-27 protects against the emergence and progression of autochthonous tumors. Accumulating evidence suggests that IL-27 might be uniquely well positioned to amplify beneficial T_H1 anticancer immune responses while suppressing the unwanted accumulation of regulatory T cells.

Mouse models have affirmed the importance of several cytokines in the elicitation of beneficial anticancer immune responses. Unfortunately, the use of these factors in clinical settings has proven difficult for a range of reasons, including their rather heterogeneous effects and serious toxicities that in some instances develop at therapeutically relevant doses. A case in point is interleukin (IL)-2, which has made its way into the clinic (in a limited sense). High-dose IL-2 is indeed currently used to (re)activate anticancer effector T cells in individuals affected by metastatic melanoma and renal cell carcinoma. Complete regressions have been achieved in around 6–9% of these patients (bearing aggressive end-stage disease), with an overall response rate of around 15–20%.^{1,2} These remarkable results have promoted the use of IL-2 in selected patient subsets, despite a considerable incidence of severe side effects. The pleiotropic nature of IL-2, stimulating immune effectors but also supporting the expansion of immunosuppressive regulatory T cells (Tregs), might contribute to the need for such high (and hence sometimes toxic) doses to achieve therapeutic responses. To be effective, indeed, any immunotherapy needs to circumvent the problem caused by the accumulation of immunosuppressive cells in the tumor microenvironment. As

discussed below, the properties of IL-27 might place it in a preferential position for directing effective antitumor immune responses while suppressing the differentiation of Tregs (Fig. 1).

IL-27 shares structural similarities with IL-6, IL-12, and IL-23, all of which play important roles in the biology of helper T cells and have established, albeit diverse, roles in oncogenesis and tumor progression. IL-27 is widely regarded as an immunosuppressive cytokine as it can stimulate the secretion of IL-10 and directly suppress the differentiation of T_H2 and T_H17 cells. However, IL-27 has also been shown to suppress the differentiation of FOXP3⁺ inducible Tregs (iTregs) and to promote T_H1 immune responses, thereby exerting pro-inflammatory effects in some circumstances.³ Recombinant IL-27 induces the expression of the T-cell-specific T-box transcription factor Tbet and of the β 2 subunit of the IL-12 receptor by CD4⁺ T cells in vitro and potentiates the production of interferon γ (IFN γ). However, mice lacking the α subunit of the IL-27 receptor (*Il27ra*^{-/-} mice) demonstrated robust T_H1 responses in most infectious models. These data belied the importance of IL-27 for T_H1 responses and, together with evidence in a range of inflammatory in vivo models, pointed to a predominantly immunosuppressive role for IL-27.

Despite this, multiple groups have shown that IL-27 exerts antineoplastic effects through various mechanisms, including CD8⁺ T and/or natural killer T (NKT) cell activation, IFN γ production, antibody-dependent cellular cytotoxicity, antiangiogenesis, direct suppression of tumor growth, and cyclooxygenase-2 (COX-2) inhibition.⁴ All previous studies relied on grafted tumor models and, in the majority of cases, malignant cells were genetically engineered to express IL-27 prior to engraftment. Studies investigating the importance of an IL-27-related cytokine, IL-23, demonstrated a profound difference in effects of overexpressed IL-23 on grafted models and physiological levels of IL-23 on autochthonous tumors.⁴ We therefore considered of critical importance to test the physiological role of IL-27 in the development and progression of primary cancers. Moreover, tumors that develop in situ allowed us to investigate the contribution of IL-27 to protective immunosurveillance.

Thus, *Il27ra*^{-/-} mice were investigated in the context of two diverse models of in situ carcinogenesis: 3-methylcholanthrene (MCA)-induced fibrosarcomas and polyomavirus middle T antigen (PyMT)-induced mammary carcinomas. The MCA model has been extensively used to characterize the immunological

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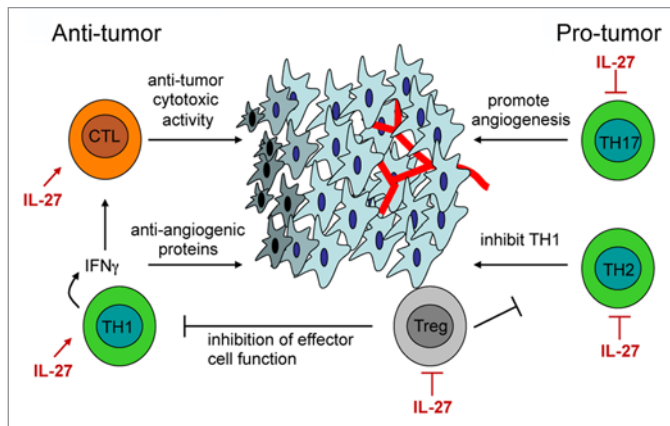


Figure 1. Effects of interleukin-27 on T-cell responses in the course of oncogenesis. Interleukin-27 (IL-27) stimulates the differentiation of naïve CD4⁺ T cells into interferon γ (IFN γ)-producing T_H1 cells and promotes the activity of cytotoxic CD8⁺ T cells, both of which contribute to antitumor immune responses. In addition, IL-27 suppresses the conversion of naïve CD4⁺ T cells into pro-tumorigenic T_H2, T_H17, and regulatory T cells.

control of oncogenesis and tumor progression.⁵ In this system, Tregs suppress anticancer immune responses and a loss of T_H1-relevant signals, such as IL-12, IFN γ , IFN receptors, signal transducer, and activator of transcription 1 (STAT1) and IL-12p40, increases the susceptibility of mice to carcinogenesis.⁶ The role of the immune system in controlling PyMT-induced carcinomas is less well defined. This said, the modulation of cytokine and chemokine expression levels in PyMT transgenic mice has been shown to alter tumor growth.^{7,8} We found that *Il27ra*^{-/-} mice develop malignant lesions more rapidly than their wild-type counterparts in both tumor models, confirming the protective effect of endogenous IL-27 against carcinogen- and transgene-driven autochthonous neoplasms.⁹

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The evaluation of T-cell activity in the neoplastic lesions and peripheral lymphoid organs of tumor-bearing mice revealed that *Il27ra*^{-/-} animals bear increased numbers of Tregs. Surprisingly, we also observed very low levels of IFN γ secretion by *Il27ra*^{-/-} CD4⁺ T cells as compared with their wild-type counterparts. This result contrasts with previous observations obtained in models of infection, a setting in which IL-27 appears to be dispensable for IFN γ production. This difference may originate from the presence of pathogen-associated molecular patterns and high levels of inflammatory mediators during infection, which may compensate for the lack of IL-27-transmitted signals. Interestingly, *Il27ra*^{-/-} CD8⁺ T cells manifested only a minor defect in IFN γ production as compared with their wild-type counterparts.

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We are currently evaluating the tumor-specific cytotoxicity of wild-type and *Il27ra*^{-/-} CD8⁺ cells in these models.

Exciting observations by Salcedo and colleagues,¹⁰ who studied a murine neuroblastoma model (TBJ cells), indicate a potent synergism between IL-27 and IL-2. In this setting, the co-administration of IL-27 and IL-2 completely suppressed the IL-2-induced elevation in Tregs levels and mediated a more than 30-fold increase in serum IFN γ as compared with either cytokine administered individually. Moreover, the combined use of IL-27 and IL-2 had a powerful protective influence on disseminated TBJ neuroblastoma in mice. Thus, IL-27 and IL-2 may constitute a particularly effective combination therapy.

Further studies on the antineoplastic potential IL-27 are warranted in view of potential clinical applications, as this cytokine significantly alters the progression of murine cancers as a standalone immunotherapeutic agent. Moreover, IL-27 might be advantageous over other cytokines as it appears to have been associated with reduced toxicity.⁴ However, the synergistic combination with IL-2, which is already employed in clinical settings, might represent the best chance for IL-27 to successfully move from the bench to the bedside. Our study is an important piece of this picture, validating the protective role of physiological levels of IL-27 against the development and progression of carcinogen- and transgene-driven neoplasms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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