

Review Article

The role of IL-27 in the induction of anti-tumor cytotoxic T lymphocyte response

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Abstract: Cytotoxic T lymphocyte (CTL) response is a critical component of the immune response to tumors, therefore optimal induction of CTL responses to tumor antigens is highly desired for developing efficient cancer immunotherapy. IL-27 is a member of the IL-12 family of cytokines that is comprised of an IL-12 p40-related protein subunit, EBV-induced gene 3 (EBI3), and a p35-related subunit, p28. IL-27 functions through IL-27R and has been shown to have potent anti-tumor activity via activation of a variety of immune components, including anti-tumor CD8⁺ T cell responses. However, the exact mechanisms of how IL-27 enhances anti-tumor CD8⁺ T cell responses are not fully understood. In this paper we mainly discuss the evidences that suggest novel mechanisms by which IL-27 enhances anti-tumor CTL responses, including IL-27 inhibition of activation-induced cell death; the phenotypes of IL-27-stimulated CTLs; IL-27-induced CTL IL-10/IL-21 production and IL-27-mediated suppression of regulatory T cell responses. These evidences suggest that IL-27 may have a great potential to be utilized in boosting anti-tumor CTL responses in human cancer patients.

Keywords: IL-27, anti-tumor cytotoxic T lymphocyte (CTLs), cancer, p28, p35, p53, IL-21

Introduction

Cytotoxic T lymphocyte (CTL) response is a critical component of the immune response to tumors, therefore optimal induction of CTL responses to tumor antigen is highly desired for developing efficient cancer immunotherapy. Previous studies have revealed that some cytokines, such as interleukin-12 (IL-12) and a variety of other cytokines [1-12], are potent stimulators of anti-tumor CTL responses. IL-12 is recognized as a master regulator of Th1/Tc1 responses [13, 14]. However, in contrast to its significant anti-tumor and anti-metastatic activities documented in preclinical studies, clinical trials with IL-12 used as a single agent, or as a vaccine adjuvant, have shown limited efficacy in most cases [14, 15]. It has recently been found that IL-12 up-regulates Tim-3 expression in T cells and induces T cell exhaustion, and thus IL-12-induced T cell responses could not be sustained to resulting in tumor rejection [16]. Thus, more effective application of IL-12, and of newly identified IL-12 family members such as IL-27, should be evaluated as therapeutic

agents, as it may have more potential in the treatment of cancer patients.

Since IL-27 was first reported having anti-tumor activity in 2004 [8, 9], the potent antitumor activity of IL-27 has been verified in various tumor models, and many studies have shown CD8⁺ T cell-dependent tumor rejection [8-10, 12, 17]. The enhancing roles of endogenous IL-27 in the generation of anti-tumor CTL responses were also demonstrated using IL-27R α -deficient mice [18-20]. Although these studies provide a strong case for the role of IL-27 in inducing anti-tumor CTL responses, it is not fully understood how IL-27 enhances anti-tumor CTL responses. In this paper we review the evidences that suggest novel mechanisms by which IL-27 enhances anti-tumor CTL responses.

IL-27 biology

IL-27 is a heterodimeric cytokine composed of two subunits: EBI3 and p28 [21]. Since it is structurally and functionally similar to IL-12, it was classified into the IL-12 cytokine family. Co-expression of EBI3 and p28 within the same

cell is required for IL-27 to employ its biological functions [21]. Early studies demonstrated that activated antigen-presenting cells are the main source of both human and murine IL-27. The expression of human IL-27 has been detected in monocytes, dendritic cells, endothelial cells, and trophoblast cells [21, 22], while the expression of murine IL-27 has been detected in activated macrophages and microglia cells [21, 23]. IL-27 receptor (IL-27R) is also a heterodimeric molecule that consists of WSX-1 (IL-27R α) and gp130. They have been shown to coexpress on a variety of immune cell types, including CD4⁺ T cells, CD8⁺ T cells, NK cells, monocytes, mast cells, neutrophils, and B cells [24, 25]. Therefore, IL-27 has pleiotropic functions that depend on the cell type on which IL-27R is expressed. Although WSX-1 has high affinity to IL-27 in the absence of gp130 [21], it is insufficient to mediate IL-27 signal transduction [24]. In contrast, IL-27 does not bind to gp130 in the absence of WSX-1 [26]. Thus, coexpression of WSX-1 and gp130 in the same cell is pivotal for IL-27 to execute its biological activities.

The interaction of IL-27 with IL-27R has been shown to activate Janus Kinase (JAK) 1-2, Tyrosine kinase (TYK) 2, STAT 1-5 in naïve CD4⁺ T cells [27]. Co-immunoprecipitation studies and pull-down assays show that the intracellular domain of WSX-1 is constitutively associated with JAK1 and contributes to tyrosine phosphorylation of STAT1 [28], while gp130 constitutively interacts with JAK1/TYK2 and elicits a strong activation of STAT3 [24, 29]. By studying STAT1^{-/-} and STAT3^{-/-} murine T cells, STAT3 was shown to be indispensable for IL-27-mediated proliferative effect on naïve CD4⁺ T cells, while STAT1 was found to be dispensable [29]. In contrast, Yoshimura et al. reported that IL-27 preferentially induces STAT3 in fully activated CD4⁺ T cells, while both STAT1 and STAT3 are activated in early activated T cells upon IL-27 stimulation [30]. Since IFN- $\alpha/\beta/\gamma$ utilize JAK1/2, TYK1/2, and STAT1 [31, 32], IL-6 has the ability to activate JAK1/2, TYK2, STAT1/3 [33, 34], IL-27 was considered to have similar biological actions to IFN- $\alpha/\beta/\gamma$ and IL-6. IL-27 is a pleiotropic cytokine capable of regulating Th1, Th2, Th17, and FoxP3⁺ Treg responses [35]. IL-27 was initially found to induce T-bet expression and promote Th1 responses [36]; later studies using IL-27R α ^{-/-} mice and pathogen/autoimmune disease models have revealed that IL-27

actually inhibits Th1 responses [37, 38]. However, the molecular mechanisms of IL-27-mediated Th1 suppression remain unclear. It is well established that IL-27 inhibits Th2 and Th17 responses via blocking the expression of transcription factors GATA-3 (Th2) and RoR γ t (Th17) [27, 39]. IL-27 also appears to inhibit CD4⁺ CD25⁺ Foxp3⁺ regulatory T cell (Treg) responses [40, 41]. Another notable function of IL-27 is its ability in inducing IL-10 production in essentially all subtypes of T lymphocytes [42-46]. A variety of mechanisms, including activation of STAT1/STAT3 [42], induction of ICOS, c-Maf and IL-21 [47-49] have been proposed. IL-27 induces IL-21 production mainly by CD4⁺ T cells [47, 50, 51].

The multi-faced anti-tumor activities of IL-27

In the past decade, IL-27 has attracted considerable interest as a potent antitumor cytokine due to its similarities to IL-12. IL-12 has been proven to be effective in controlling tumor growth and metastases. The mechanisms responsible for the anti-tumor effects of IL-12 include: promoting IFN- γ production, anti-angiogenesis, triggering programmatic changes in suppressive cellular components within tumors, and enhancing lytic abilities of CTLs, NK cells and NKT cells to eradicate tumors [52, 53]. However, IL-12 treatment of human cancer patients is associated with severe side effects, which may be due to its excessive pro-inflammatory effects. Since IL-27 was discovered in 2002, the potent anti-tumor effect of IL-27 has come into agreement in almost all literatures with multiple mechanisms involved [19, 54]. However, the mechanisms by which IL-27 exerts its anti-tumor activity vary among different types of tumors. The potent antitumor activity of IL-27 has been verified in various immunogenic tumor models, and many studies have shown CD8⁺ T cell-dependent tumor rejection [8-10, 12, 17]. While in other tumor models, IL-27 also exerted its antitumor activity through various other mechanisms. For instance, in poorly immunogenic tumors such as B16F10 melanoma, IL-27 can enhance NK cell response [55], inhibit angiogenesis [56], and directly suppress tumor cell proliferation [57]. IL-27 can also suppress NK-resistant head and neck squamous cell carcinoma through inducing antibody-dependent cellular cytotoxicity [58]. In lung cancer model, over-expression of IL-27 or treatment with recombinant IL-27 can decrease expressions of vimentin, COX-2, and

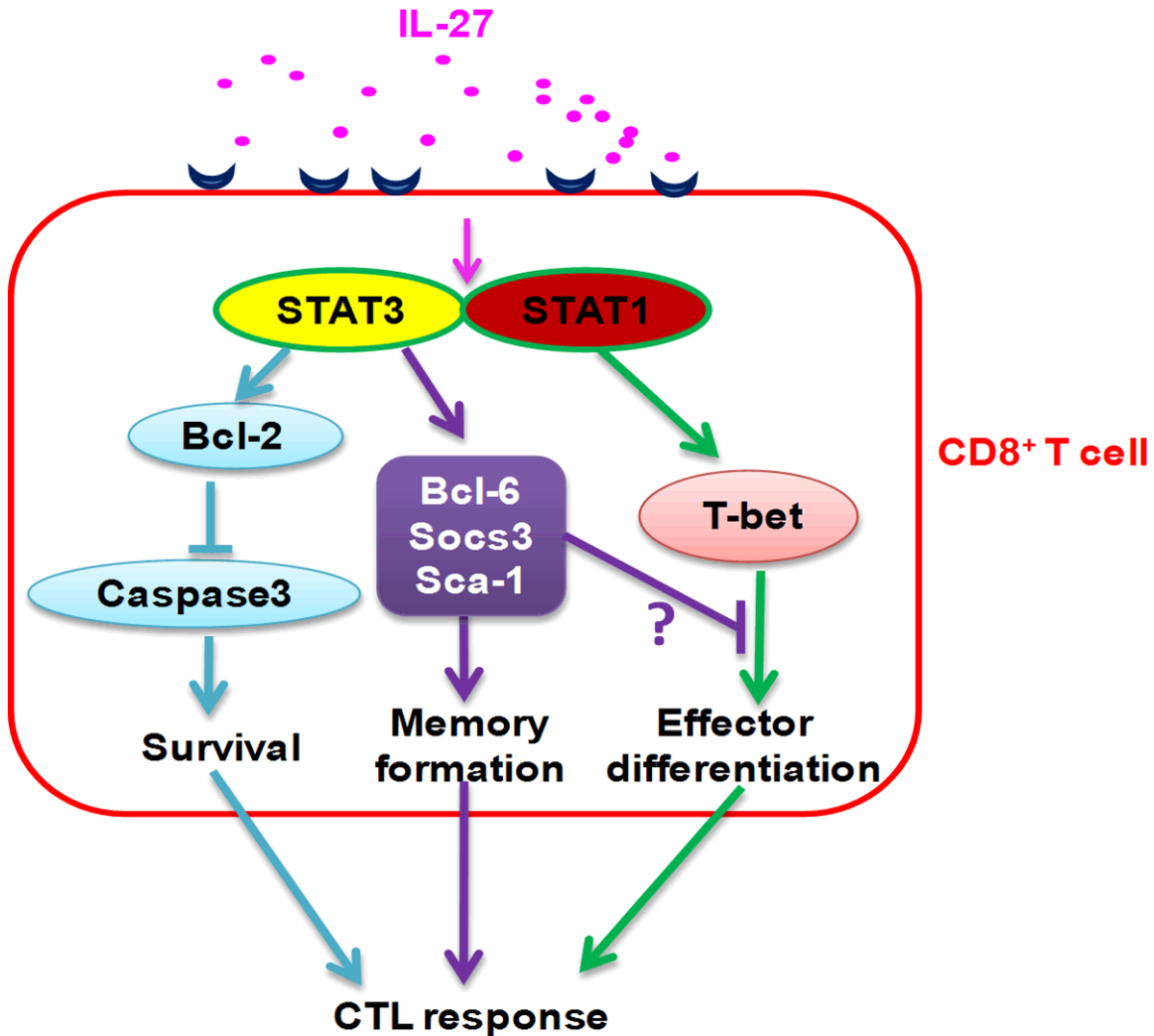


Figure 1. IL-27 directly stimulates CD8⁺ T cells and programs them into memory effector CTL cells.

its metabolite (PGE₂) in lung cancer cells [59], which results in the reduction of cancer cell migration and invasion. IL-27 is also capable of inhibiting human multiple myeloma cell growth as well as osteoclast activity [60]. Collectively, these studies suggest that IL-27 can exert its anti-tumor activity through direct inhibition cancer cell growth, proliferation and migration; inhibiting tumor angiogenesis, enhancing NK activity and more importantly, enhancing tumor-specific CTL responses.

The roles of IL-27 in CTL differentiation and survival

Although previous studies [19, 54] provide a strong case for the role of IL-27 in inducing anti-tumor CTL responses, it is not fully understood how IL-27 enhances anti-tumor CTL responses.

In the literature, IL-27 has been shown to be able to directly stimulate CD8⁺ T cells. IL-27 was shown to activate STAT1 and subsequently augmented the expression of two related T-box transcriptional factors: T-bet and Eomes [19, 61], enhance proliferation and IFN-γ production by CD8⁺ T cells [19], and promote CD8⁺ T cells to express IL-12Rβ₂ and granzyme B [61, 62]. IL-27-induced IL-21 was shown to be pivotal for CD8⁺ T cells to produce granzyme B [63]. We recently [64] analyzed the direct effects of IL-27 on tumor antigen specific CD8⁺ T cells using the tumor P1CTL T cell receptor (TCR) transgenic model [65]. As outlined in **Figure 1**, we have found that IL-27 exerts three STAT1/STAT3 activation-mediated effects on tumor antigen specific CD8⁺ T cells, which provides new explanations for why IL-27 enhances anti-tumor CTL

responses. *First*, IL-27 up-regulates anti-apoptotic molecules such as Bcl-2 and inhibits activation of Caspase 3 in activated CD8⁺ T cells, which leads to improved survival of activated CD8⁺ T cells. The survival enhancing effect of IL-27 on CD8⁺ T cells is consistent with a recent study showing that IL-27 can inhibit activation-induced cell death and enhance the survival of CD4⁺ T cells [66]. The strong anti-apoptosis effect of IL-27 could explain why, in tumor models, more CTLs accumulate in the IL-27-positive tumor microenvironment. *Second*, IL-27 induces a unique memory precursor cell (MPC)/effector phenotype in activated CD8⁺ T cells, characterized by up-regulation of SOCS3, Bcl-6, Sca-1, T-bet, Blimp1 and Perforin, and down-regulation of Eomes and IFN- γ . SOCS3 and Bcl-6 have recently been shown to be critical in establishing CD8⁺ T cell memory [67]. Sca-1 is a cell membrane molecule usually expressed on self-renewing stem cells and is a marker of central memory CD8⁺ T cells [68, 69]. Up-regulation of T-bet and down-regulation of Eomes have been demonstrated in IL-12-stimulated CD8⁺ T cells previously [52], and are associated with CD8⁺ T cell differentiation into effector but not memory T cells [52, 67]. The controversy of up-regulation of T-bet with down-regulation of IFN- γ in P1CTL can be explained by IL-27 induction of Bcl-6, which has been shown to inhibit T-bet induced IFN- γ production [70]. Unlike previous studies, we actually observed decreases of Granzyme B and IFN- γ by CD8⁺ T cells after IL-27 stimulation. Collectively, these results suggest that IL-27 programs tumor antigen specific CD8⁺ T cells into MPC-like effector cells. This phenotype can potentially increase CTL “stemness”, without affecting their effector functions such as cytotoxicity [64]. Consistent with the anti-tumor effects of IL-27, recent other studies [71, 72] have revealed that T cells with concomitant memory and terminally differentiated phenotype are more efficient in rejecting tumors than T cells that are extreme in Th1/Tc1 differentiation. *Third*, IL-27 induces high amount of IL-10 production by CTL and our study suggests that IL-27-induced CTL IL-10 production contributes to MPC phenotype, T cell memory and tumor rejection [64].

The roles of IL-27/IL-21/IL-10 axis in anti-tumor CTL responses and CTL memory

A notable function of IL-27 is its ability in inducing IL-10 production in essentially all subtypes

of T lymphocytes [42-46]. Although the role of IL-10 in tumor immunity is often controversial, increasing evidence suggests a positive role of IL-10 in the induction of anti-tumor CTL responses. For instance, in IL-10-deficient mice, anti-tumor CTL responses were weakened [3] and increased numbers of FoxP3⁺ Treg cells were found [73]; whereas in IL-10 transgenic mice, anti-tumor CTL responses were primed and shown to be responsible for tumor rejection [3, 74]. In our recent study, we have shown that IL-27-induced CTL IL-10 production contributes to CTL-mediated tumor rejection [64]. This conclusion is supported by three lines of evidence. First, adoptive transfer of IL-27-stimulated P1CTL cells, which produce high amounts of IL-10, had better therapeutic efficacy than IL-27-stimulated IL-10-deficient P1CTL. Second, adoptive transfer of P1CTL or IL-10^{-/-} P1CTL cells into Rag2^{-/-} or Rag2^{-/-} IL-10^{-/-} mice with established J558-IL-27 tumors shows that P1CTL cells can reject tumors better than IL-10^{-/-} P1CTL cells. Third, J558-IL-27 tumor cells, which failed to grow into tumors in BALB/c mice due to a potent anti-tumor CTL response, could grow into tumors in IL-10^{-/-} BALB/c mice. IL-10 producing CD8⁺ T cells are usually considered as suppressor cells that down-regulate T cell responses [75]. However, some studies showed that IL-10 producing CTLs were more highly activated and cytolytic than IL-10-deficient CTLs [3, 76]. In our recent study, we found that IL-27-stimulated IL-10-deficient CTLs expressed similar levels of IFN- γ , Granzyme B and perforin, and exhibited similar levels of cytotoxicity to target cells compared to their WT counterparts. However, IL-10-deficiency significantly reduced the expression of IL-27-induced survival molecules, such as SOCS3, Bcl-2, and Bcl-6 in CTLs. Thus, IL-27 induced CTL IL-10 production increases their survival potential, which can lead to stronger CTL responses. Our finding that IL-27 induced CTL IL-10 production contributes to their efficacy in tumor rejection suggests that IL-10 producing CTLs are better effectors rather than suppressor cells.

IL-27 also induces IL-21 production by CD4⁺ [47, 50, 51] and CD8⁺ T cells [63]. IL-21 is a member of the IL-2 family of cytokines. It is mainly produced by CD4⁺ T cells and acts on T cells, B cells, NK cells and dendritic cells [77, 78]. IL-21 signals through IL-21R and activates Jak1 and Jak3, leading to activation of STAT1, 3 and 5; however, the activation of STAT3 appears to be

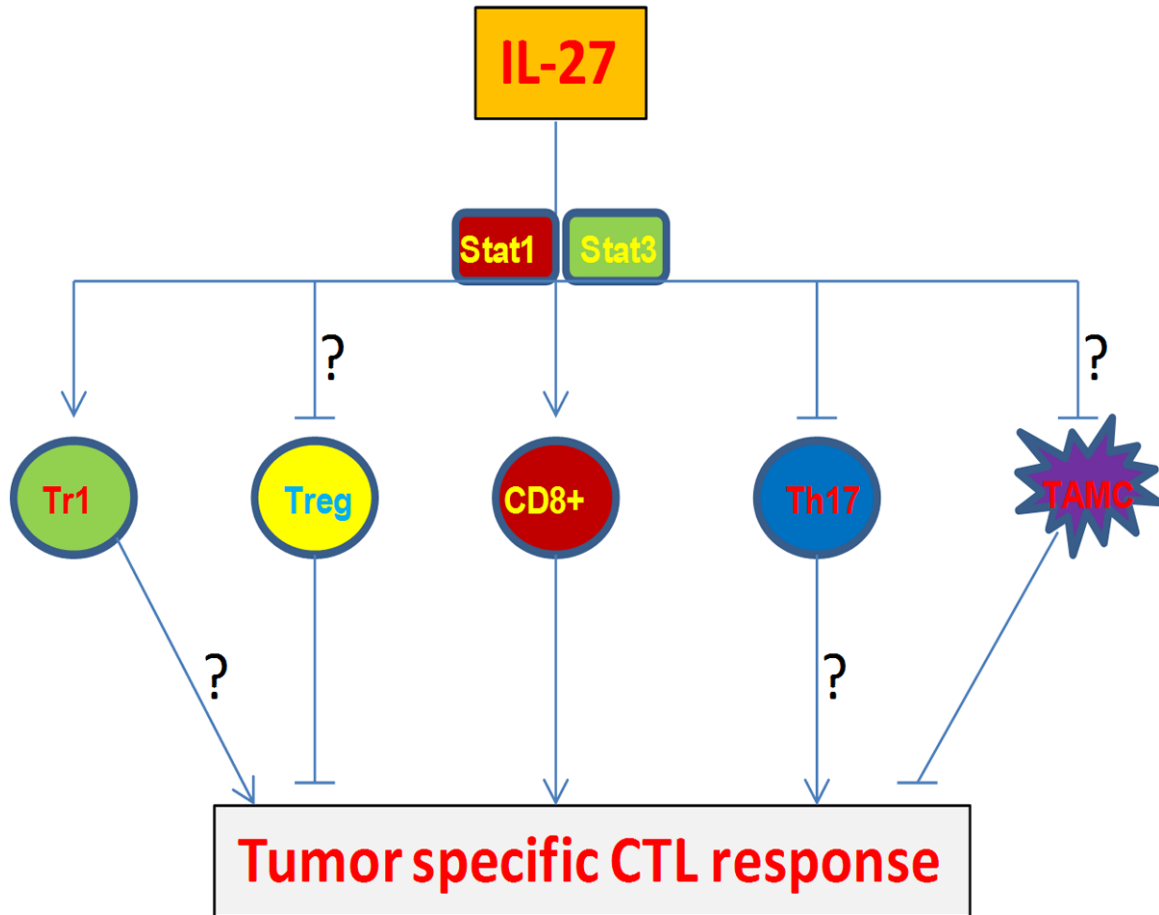


Figure 2. IL-27 affects tumor specific CTL responses via a variety of mechanisms. TAMC: tumor associated myeloid cells.

the most important STAT protein for IL-21 signaling [79]. IL-21 induces Granzyme B expression in CD8⁺ T cells [63] and enhances anti-viral CTL responses [80-83] and CTL-mediated tumor rejection [4-7, 84]. Recently, IL-21-induced STAT3 activation has been shown to play a critical role in the survival and memory responses of CD8⁺ T cells [67, 85]. Given that IL-21 has similar effects of IL-10 in anti-tumor immunity, we expect that IL-27-induced CTL IL-21 production by T cells also contribute to the induction of anti-tumor CTL responses. However, this hypothesis remains to be tested. The roles of IL-27-induced IL-10 and IL-21 production by CD4⁺ T cells also remain to be determined.

IL-10 has also been shown to play a vital role in CD8⁺ T cell memory [86], presumably via induction of SOCS3 [67]. In our recent study we find that in the absence of IL-10, the capacity for

IL-27 induction of MPC phenotype in CD8⁺ T cells is greatly diminished, which is mainly reflected by reduced expression of SOCS3, Bcl-2, and Bcl-6. Adoptive transfer experiments suggest that IL-10-deficient CD8⁺ T cells do not give rise to good memory response compared to IL-27-stimulated WT P1CTL cells. Thus, IL-27 induced CTL IL-10 production contributes to CTL memory response.

IL-27 suppresses treg responses in the tumor microenvironment

A few lines of evidence suggest that IL-27 has a profound impact on the homeostasis of Foxp3⁺ regulatory T cells. IL-27 has been shown to inhibit the conversion of inducible T regulatory cells (iTreg) by inhibiting the expression of Foxp3, CD25 and CTLA4 [40, 41]. IL-27R α -deficient (WSX-1^{-/-}) [87] and IL-27 EB13^{-/-} mice [88] exhibit increased Treg conversion and

expansion during autoimmune responses. IL-27 transgenic mice are deficient for Treg cells and develop systemic inflammation at 8-11 week of age [89]. However, recent evidences also suggest that IL-27 may be needed for Treg survival [66, 90]. These features of IL-27 suggest that Treg responses, and subsequently CTL responses in the tumor microenvironment may be affected by IL-27. Indeed, studies using tumor models have revealed reduced numbers of Foxp3⁺ Treg cells and increased CTL responses in the tumor microenvironment of IL-27-positive tumors [12, 20, 59]. Our recent study (Liu et al, submitted) shows that the frequencies of Foxp3⁺ CD4⁺ Treg cells in tumors are significantly decreased in IL-27-overexpressed tumors, while Treg numbers increased in tumors from EB13^{-/-} mice. While these results suggest negative correlation of Treg response and IL-27, it remains unclear if IL-27 directly mediate Treg suppression in the tumor microenvironment or alternatively via suppression of IL-2 production [89]. Regardless of the mechanisms, it is likely that IL-27 can enhance anti-tumor CTL responses via inhibition of Treg responses.

Concluding remarks and future directions

Accumulating evidences suggest that IL-27 promotes anti-tumor CTL responses in general [19, 54]. As demonstrated in **Figure 1**, IL-27 can enhance anti-tumor CTL responses via directly stimulating CD8⁺ T cells, inhibiting activation induced cell death and programming CTLs into memory effector cells with unique features [64]. Some of the features, such as enhancing CTL survival without reducing cytolytic activity, can clearly benefit anti-tumor CTL response; whereas other features, such as inhibition of CTL IFN- γ production, may affect CTL-mediate tumor rejection. IL-27-induced CTL IL-10 production actually benefits CTL survival and CTL-mediated tumor rejection. IL-27 is also likely to influence CTL responses via affecting Treg, Th17, Tr1 (producing IL-10 and IL-21) and myeloid cells [46, 54] (**Figure 2**). Recently, IL-27 is shown to induce the expression of PD-L1 in CD4⁺ T cells [91], a major mediator of T cell exhaustion [92, 93]. It remains unclear if IL-27 induces PD-L1 expression in CTL cells; if so, it may represents another negative factor for the generation of CTL responses. Selective induction positive features and blocking negative molecules such as PD-L1 will further enhance IL-27-mediated anti-tumor CTL response.

Currently, cytokine based cancer therapy are mainly used in the following ways, namely, systemic administration; local delivery; cytokine-secreting tumor cell vaccination; and the use of cytokines as adjuvant for cancer vaccine and adoptive cell therapy. A recent study [94] suggests that irradiated, IL-27-positive cancer cells induced protective T cell responses against subsequent tumor cell challenge. However, our recent experiments using irradiated, IL-27-secreting B16F10 cells failed to generate protective CTL responses (Liu et al, unpublished). This finding is supported by another report that using IL-12 and IL-27 combined gene therapy. It was found that potent CTL responses can only be induced by delivery of IL-12 first, followed by IL-27 delivery [17]. Thus, IL-27 does not induce, but amplify existing CTL responses. As such, IL-27 should be used with other vaccines to amplify CTL responses. It is also expected that IL-27 can enhance cancer vaccine induced CTL memory. Future research should be focused on identification of the most appropriate ways to use IL-27 as a therapeutic for the generation of anti-cancer CTL responses.

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Disclosure of conflict of interest

None.

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