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Review Article

A Pivotal Role for Interleukin-27 in CD8$^+$ T Cell Functions and Generation of Cytotoxic T Lymphocytes

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Cytotoxic T lymphocytes (CTLs) play a critical role in the control of various cancers and infections, and therefore the molecular mechanisms of CTL generation are a critical issue in designing antitumor immunotherapy and vaccines which augment the development of functional and long-lasting memory CTLs. Interleukin (IL)-27, a member of the IL-6/IL-12 heterodimeric cytokine family, acts on naive CD4$^+$ T cells and plays pivotal roles as a proinflammatory cytokine to promote the early initiation of type-1 helper differentiation and also as an antiinflammatory cytokine to limit the T cell hyperactivity and production of pro-inflammatory cytokines. Recent studies revealed that IL-27 plays an important role in CD8$^+$ T cells as well. Therefore, this article reviews current understanding of the role of IL-27 in CD8$^+$ T cell functions and generation of CTLs.

1. Introduction

A functional CD8$^+$ T cell response is an essential component of the adaptive immune response to various cancers, and bacterial and viral pathogens [1]. Upon engagement with antigen (Ag), naive CD8$^+$ T cells rapidly expand and differentiate into effector CD8$^+$ T cells, producing cytokines such as interferon (IFN)-γ and the effector molecules, perforin and granzyme B. Effector cytotoxic T lymphocytes (CTLs) play a key role in the host defense, using at least two distinct mechanisms to mediate direct killing of target cells. CTLs lyse targets by perforin-mediated release of granzyme B, which is a serine protease to induce apoptosis, and also express Fas ligand (FasL) to engage Fas on a target cell resulting in apoptosis.

The T-box transcription factor T-bet is a master regulator of type-1 helper (Th1) differentiation [2] and cell-mediated immunity capable of controlling the expression of genes encoding effector molecules in CD4$^+$ and CD8$^+$ T cells [3], as well as natural killer (NK) cells [4]. In addition to regulating the effector genes of cell-mediated immunity, T-bet functions in the maturation and homeostasis of NK T cells (NKT cells) and NK cells [4] and contributes to the induction of CD8$^+$ T cell memory [5, 6]. Despite its possible involvement in the development and function of the cytotoxic lineages, there seems to be a substantial T-bet-independent component of CD8$^+$ T cell and NK cell effector function and homeostasis [7]. Eomesodermin (EOMES) is another T-box transcription factor that is highly homologous to T-bet and is expressed in activated CD8$^+$ T cells as well as resting and activated NK cells [7]. EOMES plays a critical role during vertebrate development, and EOMES deficiency in mice shows embryonic death [8]. Dominant negative EOMES expression in CD8$^+$ T cells results in loss-of-function of CD8$^+$ T cells, whereas ectopic expression of EOMES was shown to induce expression of IFN-γ, perforin,

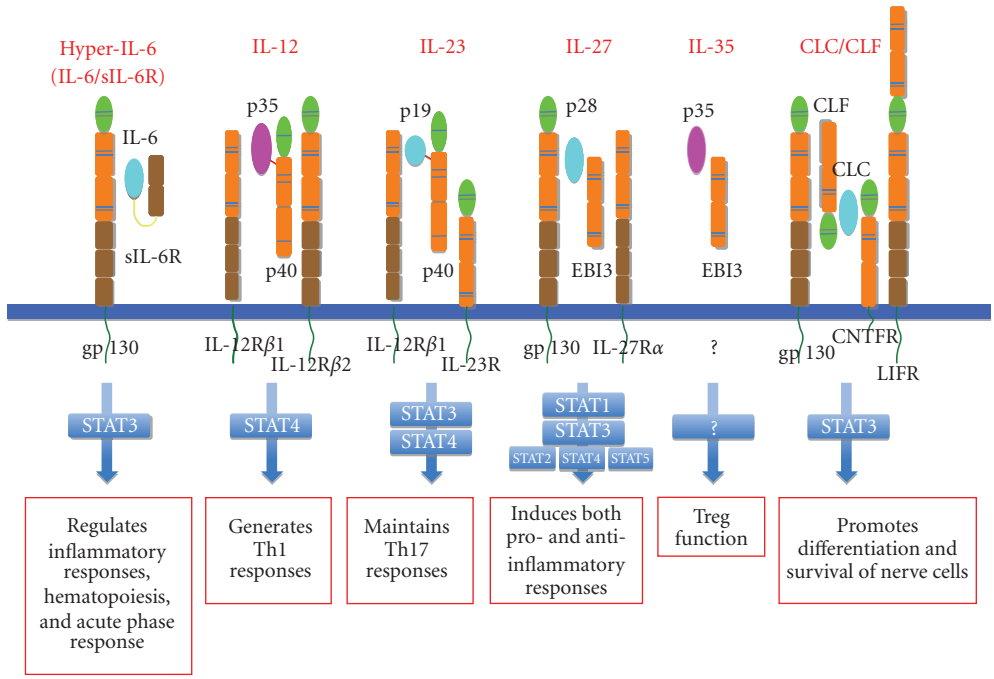

Figure 1: The IL-6/IL-12 heterodimeric cytokine family. IL-27 plays pivotal roles as a pro-inflammatory cytokine to promote the early induction of Th1 differentiation and also as an anti-inflammatory cytokine to limit the T cell hyperactivity and production of pro-inflammatory cytokines.

and granzyme B, suggesting that this transcription factor is important in differentiation of naive $CD8^+$ T cells into effector CTLs [7]. Furthermore, recent studies revealed that $CD8^+$ T cells deficient in both T-bet and EOMES, but not either one, fail to differentiate into functional killers required for defense against lymphocytic choriomeningitis virus (LCMV) [9]. Thus, T-bet and EOMES redundantly activate a transcriptional network required for $CD8^+$ T cell participation in defense against intracellular pathogens.

T-bet expression is induced by IFN-γ through activation of signal transducer and activator of transcription (STAT)1 [10, 11]. In addition to IFN-γ and type I IFNs, STAT1 phosphorylation and T-bet expression can also be induced by other cytokines including interleukin (IL)-27, an IL-6/IL-12 family cytokine [12–14]. Several recent reports suggest that IL-27 may play an important role in induction of $CD8^+$ T cell functions and generation of CTLs [15–21]. This review focuses on the critical role for IL-27 in $CD8^+$ T cells.

2. The IL-6/IL-12 Cytokine Family

The IL-6/IL-12 cytokine family has a unique characteristic that it is a heterodimeric cytokine composed of two different subunits (Figure 1) [22, 23]. IL-12 is composed of p35 and p40 subunits; its receptor (R) consists of two subunits IL-12Rβ1 and β2, and IL-12 activates STAT4, which binds to cytoplasmic region of IL-12Rβ2 [22]. The p40 subunit is also covalently bound with an IL-12 p35-related protein p19 to form IL-23 [24]. Receptor for IL-23 is composed of one of IL-12R subunits IL-12Rβ1, and an IL-12Rβ2-like receptor subunit designated IL-23R [25]. IL-23 activates STAT3 and STAT4, and STAT3 activation is required for IL-17 production by T cells with IL-23 [26]. IL-27 consists of an IL-12 p35-related protein p28, and an IL-12 p40-related protein, Epstein-Barr virus (EBV)-induced gene 3 (EBI3), which has been previously identified as one of molecules induced by EBV infection [27–29]. IL-27R is composed of the IL-27Rα (WSX-1/T-cell cytokine receptor, TCCR), which has a WSXWS sequence and is homologous to the IL-12Rβ2 subunit, and gp130, a common receptor subunit for IL-6 family cytokines [30]. EBI3 was previously reported to associate with p35 as well to form the heterodimeric molecule EBI3/p35, whereas its function had remained unknown [31, 32]. Recently, the EBI3/p35 was demonstrated to be produced by regulatory T (Treg) cells and contribute to their suppressive activity [33, 34]. Therefore, it was named IL-35, while its signaling cascade and its receptor have not been identified yet.

Although IL-27 has been reported to activate STATs1–5, several biological activities are attributed to STAT1 and/or STAT3, which bind to distinct IL-27R subunits, IL-27Rα and gp130, respectively (Figure 2) [12–14, 35, 36]. The contribution of other STATs such as STATs2, 4, and 5 largely remains unknown. The role of IL-27 in regulating immune response is complex with its stimulatory and inhibitory effects acting on various kinds of cells including T cells, B cells, macrophages, and dendritic cell (DC) [37]. IL-27 plays a role in the early induction of Th1 differentiation [12, 38, 39]. IL-27 up-regulates the expression of intercellular adhesion molecule (ICAM)-1, T-bet and subsequent IL-12Rβ2, and

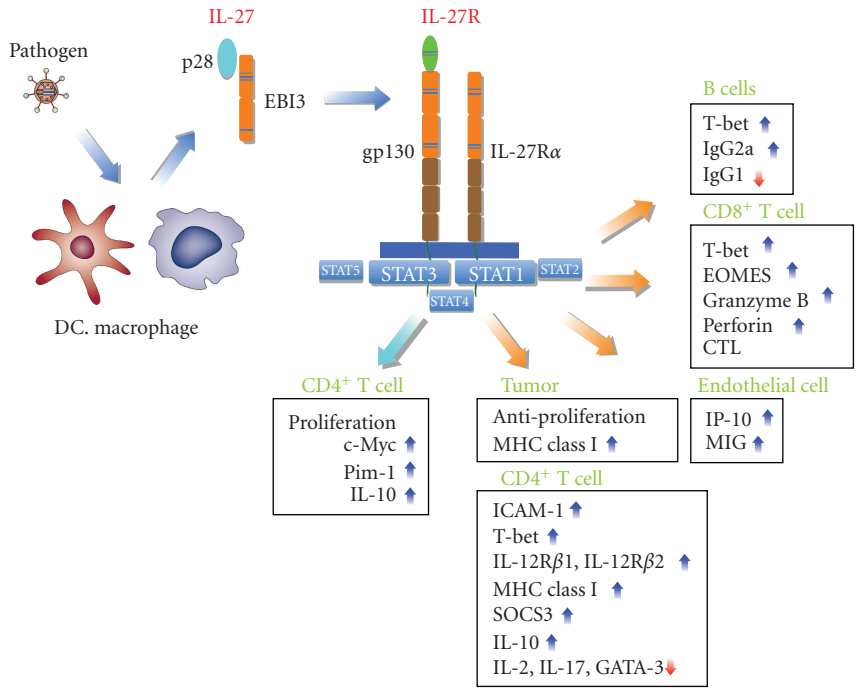

Figure 2: IL-27 is a multifunctional cytokine that mainly activates both STAT1 and STAT3 together with STAT2, STAT4, and STAT5. IL-27 mediates its several biological functions by selectively utilizing these STAT1 and STAT3, which bind to distinct IL-27R subunits, IL-27Rα and gp130, respectively. IL-27 acts on various types of cells including $CD4^+$ and $CD8^+$ T cells, B cells, NK cells, macrophages, mast cells, and endothelial cells.

synergies with IL-12 in primary IFN-γ production [12, 13, 27, 39–41]. In contrast, IL-27 down-regulates the expression of a Th2-specific transcriptional factor, GATA3 [14, 42]. In agreement with these in vitro studies, IL-27Rα-deficient mice have enhanced susceptibility to infection with several intracellular pathogens [28, 38, 43]. However, IL-27Rα is not essential to develop the protective Th1 responses [38, 43], and recent studies revealed that IL-27 regulates not only pro-inflammatory responses including the early initiation of Th1 responses, but also anti-inflammatory responses including the suppression of cellular activation and pro-inflammatory cytokine production in certain infections with *Toxoplasma gondii* [44, 45] and *Trypanosoma cruzi* [46]. Moreover, several lines of evidence demonstrated that IL-27 suppresses Th17 differentiation and the development of experimental autoimmune encephalomyelitis (EAE) [45, 47, 48]. IL-27 also induces the production of one of the immunosuppressive cytokines, IL-10, by activated T lymphocytes, and IL-10 is considered to be involved in the immunomodulatory function of IL-27 [49–51]. In addition, it was recently demonstrated that IL-27 together with transforming growth factor (TGF)-β plays a dominant function in generating IL-10-producing anti-inflammatory T regulatory type 1 (Tr1) cells [52]. IL-27 drives the expansion and differentiation of Tr1 cells by inducing three key elements, the transcription factor c-Maf, the cytokine IL-21, and the costimulatory receptor inducible costimulatory (ICOS) [53]. IL-27-driven c-Maf expression transactivates IL-21 production, which acts as an autocrine growth factor for the expansion and/or maintenance of IL-27-induced Tr1 cells, and IL-27-enhanced ICOS expression further promotes IL-27-driven Tr1 cells.

3. IL-27 Augments Antigen-Specific CTL Generation

The effect of IL-27 on $CD8^+$ T cells in vitro was investigated by us [15]. In a manner similar to $CD4^+$ T cells [12–14, 35, 36, 40], IL-27 activated STATs1–5, and augmented the expression of not only T-bet and IL-12Rβ2 but also effector molecules such as granzyme B, and perforin in naive $CD8^+$ T cells stimulated with anti-CD3 and anti-CD28. IL-27 induced synergistic IFN- γ production with IL-12 and proliferation of naive $CD8^+$ T cells. IL-27 also enhanced proliferation of $CD4^+$ T cell-depleted spleen cells stimulated by allogeneic spleen cells and augmented the generation of CTL. Both T-bet and EOMES are required to generate functional CTLs and to induce their effector molecules such as perforin and granzyme B [5–7, 9]. Therefore, the role of STAT1 and T-bet for the IL-27-mediated functions in $CD8^+$T cells was examined using deficient mice in STAT1 and T-bet [15]. In STAT1-deficient naive $CD8^+$ T cells, IL-27-induced proliferation was not reduced but synergistic IFN-γ production with IL-12 was diminished with decreased expression of T-bet, IL-12Rβ2, granzyme B, and perforin. In T-bet-deficient naive $CD8^+$ T cells, IL-27-induced proliferation was hardly reduced but synergistic IFN-γ production with IL-12 was diminished

with decreased expression of IL-12Rβ2, granzyme B and perforin. However, IL-27 still augmented the generation of CTL from T-bet-deficient $CD4^+$ T cell-depleted spleen cells stimulated by allogeneic spleen cells with increased EOMES and granzyme B expression. Thus, IL-27 induces the generation of functional CTLs by augmenting both T-bet and EOMES.

There are a number of evidences showing that CTLs play a central role in the clearance of pathogenic viruses [54]. In case of hepatitis C virus (HCV) infection, vigorous HCV-specific CTL responses exist in the persons resolving acute HCV infection, and enhancement of HCV-specific CTL induction in HCV-infected individuals is considered to be one of the strategies to clear the virus [55]. DNA vaccination has been proven to be a useful strategy for inducing both humoral and cellular immune responses, and safely mimics the effect of live, attenuated virus-based vaccine to generate a long-lasting CTL response [56]. Therefore, adjuvant effects of IL-23 and IL-27 were evaluated by the prime-boost immunization consisting of priming and the first boosting with the HCV-core expression plasmid, followed by a second boosting with recombinant adenovirus expressing HCV core for induction of HCV core-specific CTLs in HLA-A*0201 transgenic BALB/c mice [16]. To circumvent uneven gene expression of the two subunits, genetically linked, single-chain (sc)IL-12, scIL-23, and scIL-27 were used in theses experiments. Coadministration of either an IL-23 or an IL-27 expression plasmid, as well as an IL-12 expression plasmid, in a prime-boost immunization enhanced induction of HCV-specific CTLs and led to dramatic increases in the numbers of IFN-γ-producing, HCV-specific $CD8^+$ cells [16]. Furthermore, preinjections of IL-12, IL-23, or IL-27 expression plasmids before immunization resulted in great increases in the number of IFN-γ-producing, HCV-specific $CD8^+$ cells in response to immunization with recombinant adenovirus. These data revealed that both IL-23 and IL-27, as well as IL-12, have potent adjuvant activity for induction of epitope-specific CTL.

4. IL-27 Induces Antitumor Activity via Augmenting Tumor-Specific CTL Generation

IL-12 is considered to be one of the most effective cytokines against various tumors, because it activates NK cell, promotes Th1 polarization, and, thereby, promotes cellular immune responses and proliferation of CTL [57, 58]. The promising data obtained in the preclinical models have raised much hope that IL-12 could be a powerful therapeutic agent against cancers [57, 58]. However, excessive clinical toxicity and modest clinical response in the clinical trials have limited the IL-12 therapy [59]. Since IL-27 has several similarities to IL-12, it plays a role in the initiation of Th1 differentiation [12, 39, 41], and enhances generation of CTL [15, 16] as described above, we evaluated the antitumor activity of IL-27 and demonstrated for the first time that IL-27 has a potent antitumor activity [17]. Since then, IL-27 has been evaluated in various preclinical tumors, and a number of reports revealed that IL-27 exerts potent antitumor effects against various tumor models such as colon carcinoma [17, 20, 21], neuroblastoma [18], melanoma [60–62], head and neck squamous cell carcinoma [63], and lung cancer [64] via different mechanisms depending on the characteristic of each tumor. These include mechanisms through not only $CD8^+$ T cells [17, 18, 21], but also NK cells [21, 61, 63], antiangiogenic activity [60], direct antiproliferative activity [62], and suppression of cyclooxygenase-2-mediated activities [64].

Hisada et al. have first evaluated the antitumor activity of IL-27 against a murine tumor model of colon carcinoma colon 26 (C26) [17]. C26 tumor cells, which were transduced with the linked scIL-27 cDNA and became secreting IL-27 (C26-IL-27), exhibited a minimal tumor growth in vivo, and all mice inoculated with these tumor cells survived with a complete tumor remission. Inoculation of mice with C26-IL-27 tumors induced enhanced IFN-γ production and CTL activity against C26 tumors in spleen cells. Recovered mice from the inoculation showed a tumor-specific protective immunity to the following challenge with parental C26 tumors. The antitumor activity of IL-27 was almost diminished in nude mice, and depletion of $CD8^+$ T cells and neutralization of IFN-γ in immunocompetent mice reduced the antitumor activity. These results suggest that IL-27 has potent abilities to induce tumor-specific antitumor activity and protective immunity, which is mediated through mainly $CD8^+$ T cells. Moreover, the antitumor activity was greatly reduced in T-bet-deficient mice, but not in STAT4-deficient mice [17]. Since STAT4 is essential for IL-12 to show its biological activities [65, 66], this result implies that IL-27 exerts the antitumor activity even in the absence of endogenous IL-12. This is consistent with the fact that IL-27 induces Th1 differentiation even in the absence of IL-12 in vitro [39, 41]. Of note, during the IL-27 treatment, any apparent adverse effects such as, splenomegaly and liver injury with elevated serum glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities and intensive mononuclear cell infiltration into liver were not observed, which were seen with IL-12 treatment [67–69]. Similar $CD8^+$ T cell-mediated antitumor effects were also reported by Chiyo et al. using C26 transduced with the heterodimeric IL-27 cDNA (p28-IRES-EBI3) [20, 21]. Taken together, these studies suggest that IL-27 exerts $CD8^+$ T cell-mediated antitumor effects.

Salcedo et al. have also reported that IL-27 exerts $CD8^+$ T cell-mediated potent antitumor activity using other tumor model, TBJ neuroblastoma, which was engineered to overexpress scIL-27 (TBJ-IL-27) [18]. TBJ-IL-27 tumors showed markedly delayed growth compared with control mice, and complete durable tumor regression was observed in almost all of mice bearing either subcutaneous or orthotopic intraadrenal tumors, and in half of mice bearing induced metastatic disease. Consistent with the above other reports [17, 20, 21], the majority of mice cured of TBJ-IL-27 tumors were resistant to tumor rechallenge. Moreover, TBJ-IL-27 tumors were heavily infiltrated by $CD8^+$ T cells. Draining lymph node-derived lymphocytes from mice bearing subcutaneous TBJ-IL-27 tumors were primed to proliferate more readily when cultured ex vivo

with anti-CD3/anti-CD28 compared with lymphocytes from mice bearing control tumors, and to secrete higher levels of IFN-γ. In addition, marked enhancement of local IFN-γ gene expression and potent up-regulation of cell surface major histocompatibility complex (MHC) class I expression were noted within TBJ-IL-27 tumors compared with control tumors. Similar direct effects of IL-27 on tumors to up-regulate MHC class I molecule were reported [62]. Moreover, depletion experiments using specific antibodies revealed that the induction of TBJ tumor regression by IL-27 is mediated via mechanisms that are critically dependent on $CD8^+$, but not $CD4^+$T cells or NK cells. Thus, $CD8^+$ T cells are critically involved in the exertion of antitumor activity by IL-27.

5. Endogenouse IL-27 in $CD8^+$ T Cells Is Important for CTL Generation

IL-27 thus facilitates tumor-specific CTL induction against various tumors. However, IL-27 also suppresses cytokine production of lymphocytes and Ag-presenting function of DCs [70]. Therefore, to examine the role of endogenous IL-27 in generation of CTL and antitumor immunity, IL-27-mediated antitumor effects were examined using IL-27Rα (WSX-1)-deficient mice [38]. In IL-27Rα-deficient mice, in which host cells cannot receive IL-27-mediated signaling, inoculated with B16F10 melanoma cells, tumor growth was higher than in wild-type (WT) mice [19]. These results are consistent with the fact that IL-27 favors establishment of antitumor immunity as described above [17, 18, 20, 21]. Failure of successful inhibition of tumor growth in IL-27Rα-deficient mice could be attributable to impaired generation of CTL or impaired function of other types of cells, such as Ag-presenting cells (APCs). To address this issue, tumor Ag TRP2 peptide-pulsed WT DCs were adoptively transferred into WT or IL-27Rα-deficient mice and examined the effects on tumor growth [19]. In WT mice, transfer of peptide-pulsed DCs induced further protective immunity against B16F10 tumor as compared with the PBS-treated control WT mice. Even in IL-27Rα-deficient mice, transfer of WT DCs pulsed with Ag peptide conferred protective immunity. However, IL-27Rα-deficient mice transferred with DCs still showed excessive growth of tumors as compared with DC-transferred WT mice. These data indicate that the impaired generation of antitumor immunity in IL-27Rα-deficient mice may be attributable to effector populations but not APCs. Consistent with this idea, IL-27Rα-deficient mice with WT DC transfer generated still lower killing activity than WT mice with DC transfer, although DC transfer induced tumor-specific killing activity over PBS control [19]. Perforin expression in generated CTLs from IL-27Rα-deficient mice was also lower than in those from WT mice, which is consistent with the previous report [15]. Moreover, DC transfer augmented expression of perforin by generated CTLs from WT mice, while there was little augmentation by DC transfer in CTLs from IL-27Rα-deficient mice. These results demonstrate that endogenous IL-27, through IL-27Rα signaling, favors generation of CTL with enhanced perforin expression. Of note, when transferred into tumor-bearing mice, IL-27Rα-deficient DCs pulsed with Ag peptide was more potent than WT DCs in tumor growth inhibition and generation of CTLs [19]. This indicates that IL-27Rα signaling has suppressive effects on DC function, as reported previously [70]. Thus, endogenous IL-27 promotes tumor specific CTL generation in $CD8^+$ T cells, while suppressing APC function in DCs, during generation of tumor immunity.

6. IL-27R Signaling Is Indispensable for T-bet-Dependent IFN-γ Production in $CD8^+$ T Cells

$CD8^+$ T cells are one of the major sources of IFN-γ, a key effector cytokine in immune responses against many viruses and protozoa [71]. The diverse effects of IFN-γ on numerous immune cells are mediated through the widely expressed IFN-γ R and the activation of STAT1 [71]. In $CD4^+$ T cells, STAT1 appears to be critical for the activation of T-bet and IFN-γ, suggesting an IFN-γ-dependent positive feedback loop, that is, IFN-γ-dependent STAT1-mediated induction of IFN-γ [10, 11]. However, STAT1 can also be activated by other cytokines, including IL-27. To examine the expression of IFN-γ by $CD8^+$ T cells directly in vivo, Yeti IFN-γ reporter mice were used to bypass the need for in vitro restimulation [72, 73]. In Yeti mice, the IFN-γ-YFP reporter is faithfully expressed only under conditions known to induce IFN-γ, and the reporter fluorescence intensity correlates directly with both the abundance of IFN-γ transcripts and the production of IFN-γ upon restimulation [72, 73]. Using the Yeti IFN-γ reporter mice, the $CD8^+$ T cell-intrinsic roles of IFN-γ R, IL-27R, and T-bet for IFN-γ expression in response to viral and protozoan infection were examined by direct in vivo staining [74]. To directly compare T-bet-deficient or IFN-γ R-deficient T cells with WT T cells in the same animal in a T-bet- or IFN-γ R-sufficient environment, mixed bone marrow (BM) chimeras in which lethally irradiated C57BL/6 mice (CD45.1 or CD45.2) were reconstituted with BM from WT Yeti mice (CD45.1) mixed 1 : 1 with either T-bet-deficient Yeti or IFN-γ R-deficient Yeti CD45.2 donors. Mixed T- bet-deficient/WT BM chimeras were then infected with influenza virus, Sendai virus, or *Toxoplasma gondii*, and various organs were analyzed for IFN-γ response within the population of Ag-specific cells generated by the respective donor BM using MHC class I tetramers specific for each Ag. Very few Ag-specific T-bet-deficient $CD8^+$ T cells in respectively infected mixed BM chimeras expressed IFN-γ, while the vast majority of Ag-specific WT cells were YFP^+ [74]. Importantly, the defective IFN-γ response was not due to a failure of the T-bet-deficient compartment to prime, expand, or disseminate a population of Ag-specific $CD8^+$ T cells, because the frequency of Ag-specific cells was comparable to that of the internal WT control [74]. Although T-bet is reportedly induced by the IFN-γ R/STAT1 pathway [10, 11], IFN-γ expression in IFN-γ R-deficient $CD8^+$ T cells was not impaired in mixed IFN-γ R-deficient/WT BM chimeras infected with influenza virus or Sendai virus as compared with the internal WT control cells. These observations demonstrate that direct IFN-γ

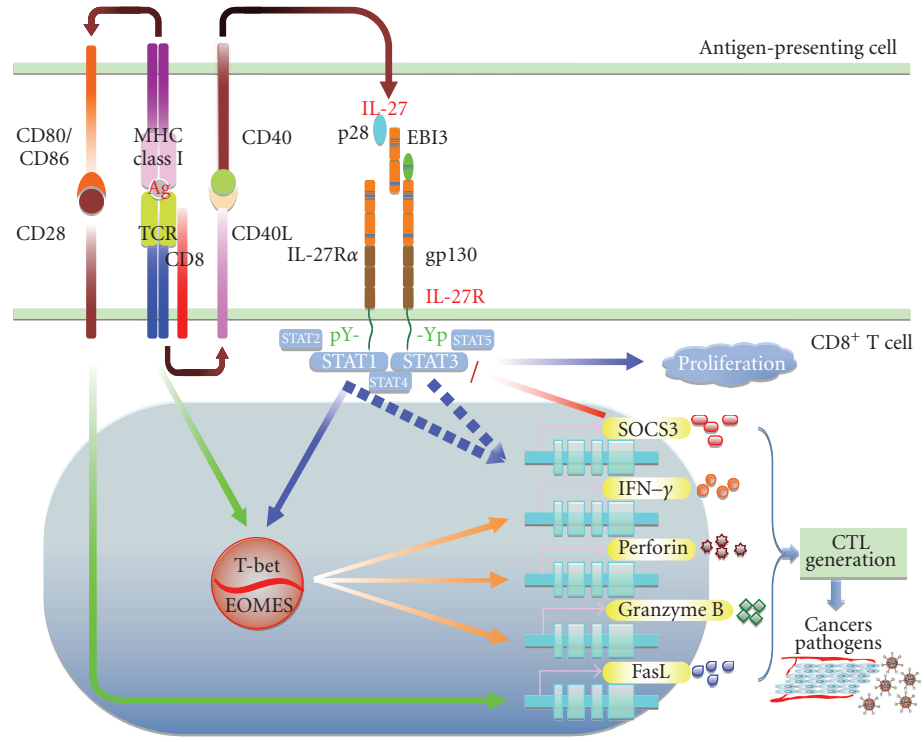

Figure 3: IL-27 plays a pivotal role in CD8$^+$ T cell functions and generation of CTLs. In naive CD8$^+$ T cells, IL-27 activates STAT1 and induces expression of T-bet and EOMES, which are critical transcriptional factors for generation of CTL by transcriptionally up-regulating IFN-γ and effector molecules such as perforin and granzyme B. IL-27 also induces not only proliferation but also SOCS3 expression to limit the excessive proliferation mediated through gp130 of IL-27R.

R signaling is dispensable for the priming, expansion, or dissemination of Ag-specific CD8$^+$ T cells. However, these results are in striking contrast to a recent adoptive transfer study suggesting that direct IFN-γ R signaling is critical for the expansion of a CD8$^+$ T cell response during acute viral infection [75].

Since T-bet reportedly functions downstream of STAT1 [10, 11, 13, 76] and the IFN-γ R is dispensable for T-bet-dependent IFN-γ expression, it was then explored whether the STAT1-activating IL-27R signaling regulates T-bet-dependent IFN-γ expression by CD8$^+$ T cells in vivo [74]. Mixed BM chimeras were generated from IL-27Rα (TCCR)-deficient Yeti (CD45.2) and WT Yeti (CD45.1) donors (IL-27Rα-deficient/WT) and infected with influenza virus or *Toxoplasma gondii*. The frequency of IFN-γ-expressing cells was profoundly reduced within Ag-specific IL-27Rα-deficient CD8$^+$ T cells as compared with the internal WT control [74]. The IFN- γ response of activated CD8$^+$ T cells was similarly impaired in the IL-27Rα-deficient population of IL-27Rα-deficient/WT mixed BM chimeras, while the frequency of activated cells was not reduced. Taken together, these studies suggest that T-bet is critical for the in vivo IFN-γ production by CD8$^+$ T cells upon infection of mice with diverse pathogens. Whereas IFN-γ R signaling is dispensable for the T-bet-dependent IFN-γ production, direct IL-27R signaling is critical for the IFN-γ response in CD8$^+$ T cells.

7. CD8$^+$ T Cell Proliferation Is Regulated by SOCS3 through Inhibition of IL-27R Signaling

Suppressor of cytokine signaling (SOCS) proteins regulates the intensity and duration of cytokine responses [77]. SOCS3 is expressed in peripheral T cells, and recent reports have suggested that overexpression of SOCS3 modulates Ag- and/or costimulation-induced T-cell activation [78]. Other studies in which SOCS3 was deleted in the liver or macrophages have also shown SOCS3 to be a critical regulator of IL-6 signals [79–81]. On a molecular level, this has been attributed to the binding of SOCS3 to phosphorylated Tyr757 (Y757) of the gp130 receptor chain, thereby bringing SOCS3 into proximity to and then inhibiting receptor-associated JAKs [82]. The association of SOCS3 with gp130, the common receptor subunit for the IL-6 family of cytokines, suggests that SOCS3 may regulate activities of other members of this family. IL-27, one of these gp130 ligands, induces SOCS3 expression [36, 83, 84], and it was shown previously that IL-27 suppresses IL-2 production and the development of Th17 cells [36, 45, 47, 83–85]. Therefore, it was proposed that SOCS3 mediates these inhibitory effects of IL-27 [47, 83]. However, studies with SOCS3-deficient T cells have shown that SOCS3 is not essential for IL-27-induced suppression of Th17 differentiation [45, 86].

To investigate the role of SOCS3 in T-cell function and homeostasis in vivo, conditional SOCS3-deficient mice, in which the SOCS3 gene was deleted specifically within T and NKT cells, were used [86, 87]. SOCS3-deficient T cells were revealed to be hyperproliferative in response to a TCR stimulus [88]. This effect was seen in both $CD4^+$ and $CD8^+$ T cells but was most pronounced in SOCS3-deficient $CD8^+$ T cells. Hyperproliferation in the absence of SOCS3 is consistent with previous studies that showed that overexpression of SOCS3 in T cells results in reduced proliferation in response to TCR [89] and antigenic stimulation [90]. However, SOCS3-deficient T cells responded normally to TCR stimulation in combination with costimulation (anti-CD3 plus anti-CD28), which suggests that costimulatory pathways are unaffected by the absence of SOCS3 [88]. In addition, signaling pathways downstream of the TCR including calcium-signaling pathways were activated to a normal intensity and duration in the absence of SOCS3, although responses to TCR ligation were enhanced [88]. Thus, T-cell defects are most probably caused by enhanced cytokine signaling in the absence of SOCS3. Previous studies have shown that SOCS3 regulates gp130 cytokines [79–81, 91]. Indeed, $CD8^+$T cells from $gp130^{Y757F/Y757F}$ mice, which lack the SOCS3-binding site on gp130 [92], also proliferated strongly in response to TCR ligation despite the TCR-signaling pathways being unaffected in these cells [88]. This result suggests that hypersensitivity to a gp130 cytokine drives the hyperproliferative phenotype of SOCS3-deficient T cells. Consistent with the idea, SOCS3-deficient T cells had prolonged STAT activation in response to both IL-6 and IL-27, the main gp130 cytokines that act on T cells [88]. While the absence of IL-6 had no effect, inhibition of IL-27 limited anti-CD3-induced proliferation in $CD8^+$ T cells. Taken together, these data suggest that enhanced proliferation to TCR ligation by SOCS3-deficient $CD8^+$T cells is not caused by aberrant TCR-mediated signaling, but, rather, by increased IL-27R-mediated signaling in the absence of SOCS3 [88].

8. Concluding Remarks

Collectively, by directly acting on naive $CD8^+$ T cells, IL-27 activates STAT1 and induces two related T-box transcription factors, T-bet and EOMES, critical for induction of effector molecules such as perforin and granzyme B to generate functional CTLs (Figure 3). Consistent with the in vitro results, DNA vaccination with IL-27 increases the numbers of IFN-γ-producing Ag-specific $CD8^+$ T cells in vivo, and IL-27-transduced tumor cells show $CD8^+$ T cell-mediated antitumor activity via augmentation of IFN-γ production and tumor-specific CTL generation. Moreover, endogenous IL-27 promotes CTL generation with enhanced perforin expression, while it suppresses APC function in DCs due to its anti-inflammatory functions, during generation of antitumor immunity. Endogenous IL-27R signaling, but not IFN-γR signaling, is also required for T-bet-dependent IFN-γ production by $CD8^+$ T cells upon infection with various pathogens. Moreover, IL-27 together with TCR stimulation induces not only proliferation but also SOCS3 expression to control excessive proliferation in $CD8^+$ T cells. Thus, the recent studies as described in the present review clearly indicate that IL-27 may have a nonredundant role in induction of $CD8^+$ T cell functions and generation of functional CTLs against various tumors and infectious pathogens. Since IL-27 plays pivotal roles in not only the early induction of Th1 differentiation but also the generation of CTL, leading to augmentation of type 1 cell-mediated immunity, IL-27 could be an attractive candidate as an agent applicable to the immunotherapy against cancers and pathogens.

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