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Review Article

Potential clinical application of interleukin-27 as an antitumor agent

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Cancer immunotherapies such as sipuleucel-T and ipilimumab are promising new treatments that harness the power of the immune system to fight cancer and achieve long-lasting remission. Interleukin (IL)-27, a member of the IL-12 heterodimeric cytokine family, has pleiotropic functions in the regulation of immune responses with both pro-inflammatory and anti-inflammatory properties. Evidence obtained using a variety of preclinical mouse models indicates that IL-27 possesses potent antitumor activity against various types of tumors through multiple mechanisms without apparent adverse effects. These mechanisms include those mediated not only by $CD8^+$ T cells, natural killer cells and macrophages, but also by antibody-dependent cell-mediated cytotoxicity, antiangiogenesis, direct antiproliferative effects, inhibition of expression of cyclooxygenase-2 and prostaglandin E_2, and suppression of epithelial–mesenchymal transition, depending on the characteristics of individual tumors. However, the endogenous role of IL-27 subunits and one of its receptor subunits, WSX-1, in the susceptibility to tumor development after transplantation of tumor cell lines or endogenously arising tumors seems to be more complicated. IL-27 functions as a double-edged sword: IL-27 increases IL-10 production and the expression of programmed death ligand 1 and T-cell immunoglobulin and mucin domain-3, and promotes the generation of regulatory T cells, and IL-27 receptor α singling enhances transformation; IL-27 may augment protumor effects as well. Here, we review both facets of IL-27, antitumor effects and protumor effects, and discuss the potential clinical application of IL-27 as an antitumor agent.

Cancer Immunotherapy

Cancer immunotherapy has recently come into the spotlight as a promising new treatment that harnesses the power of the immune system; sipuleucel-T and ipilimumab were approved by the US Food and Drug Administration for patients with cancer in 2010 and 2011, respectively.[1,2] Sipuleucel-T is a therapeutic vaccine using dendritic cells (DC) stimulated by a tumor-specific antigen combined with granulocyte-macrophage colony-stimulating factor.[1] Ipilimumab is an inhibitory monoclonal antibody specific to one of the immune checkpoints, cytotoxic T-lymphocyte antigen 4.[2] Currently, various therapeutic strategies to overcome tumor immune evasion and enhance antitumor immunity are also undergoing investigation.

Interleukin-12 Cytokine Family

The interleukin (IL)-12 cytokine family is unique in that it is a heterodimeric cytokine composed of two different subunits, and one subunit is often shared by another cytokine in the same family.[3,4] These member cytokines are mainly produced by antigen-presenting cells such as DC and play critical roles in the regulation of differentiation into respective helper T (Th) cells and their functions. IL-12 is composed of p35 and p40 subunits and induces the differentiation of naive $CD4^+$ T cells into Th1 cells to enhance the cellular immunity by augmentation of interferon (IFN)-γ production from natural killer (NK) cells and T cells and generation of cytotoxic T lymphocytes (CTL).[3] The p40 subunit is also covalently bound with an IL-12 p35-related protein, p19, to form IL-23, which plays an important role in developing tissue-specific autoimmune diseases and inflammatory diseases by enhancing IL-17 production and maintaining pathogenic Th17 cells.[4] IL-27 consists of an IL-12 p35-related protein, p28, which is also called IL-30, and an IL-12 p40-related protein, Epstein–Barr virus-induced gene 3 (EBI3)[4–7] (Fig. 1). IL-27 receptor (R) is composed of IL-27Rα (WSX-1/TCCR) and glycoprotein (gp) 130, a common receptor subunit for the IL-6 family of cytokines. EBI3 was previously reported to associate with p35 to form

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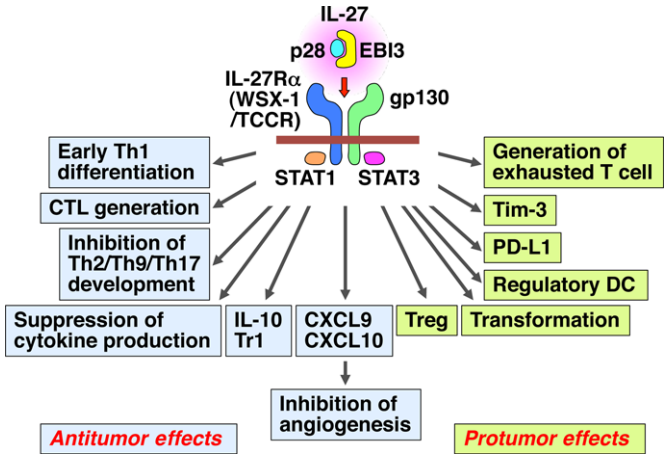

Fig. 1. Antitumor and protumor effects of IL-27 depending on the type of cells that IL-27 stimulates and the tumor context. CTL, cytotoxic T lymphocyte; DC, dendritic cell; EBI3, Epstein–Barr virus-induced gene 3; gp, glycoprotein; IFN, interferon; IL, interleukin; NK, natural killer; PD-L1, programmed death ligand 1; R. receptor; STAT, signal transducer and activator of transcription; Th, helper T; Tim-3, T-cell immunoglobulin and mucin domain-3; Tr1, IL-10-producing regulatory T; Treg, regulatory T.

heterodimeric molecule EBI3/p35, and EBI3/p35 was recently found to be produced by regulatory T (Treg) cells and to play a suppressive role in the Treg cells; therefore, it is named IL-35.

Pro-Inflammatory and Anti-Inflammatory Properties of Interleukin-27

Interleukin-27 is one of the pleiotropic cytokines with both pro-inflammatory and anti-inflammatory properties acting on various types of cells, such as T cells, B cells, macrophages and DC, depending on the context (Fig. 1).[4–6] IL-27 efficiently activates both signal transducer and activator of transcription (STAT) 1 and STAT3, which bind to distinct IL-27R subunits WSX-1 and gp130, respectively. IL-27 promotes the early induction of Th1 differentiation and generation of CTL,[8,9] but it inhibits the differentiation of naive $CD4^+$ T cells into Th2 cells, Th9 cells and Th17 cells, and suppresses the production of pro-inflammatory cytokines, leading to amelioration of allergic diseases and autoimmune diseases. In addition, IL-27 augments IL-10 production from these Th cells and generation of IL-10-producing regulatory (Tr1) T cells,[10–12] whereas the role of IL-27 in induction of Treg cells is still controversial. IL-27 was initially shown to suppress the differentiation of inducible Treg cells from naive $CD4^+$ T cells,[13–15] and transfer of TCCR-deficient $CD4^+CD45RB^{hi}$ cells in a colitis model was demonstrated to reduce development of colitis with an increased percentage of Treg cells in the gut.[16] Contrary to the antagonistic effects of IL-27 on Treg cells, recent reports have paradoxically revealed that IL-27 does not inhibit the generation of Treg cells, but rather promotes it and produces a distinct Treg cell population expressing T-cell-specific T-box transcription factor (T-bet) and CXCR3 to control Th1-mediated immunity at the local sites of inflammation.[17] Moreover, IL-27 was recently demonstrated to exert immunosuppressive functions via induction of the programmed death ligand 1 (PD-L1) on $CD4^+$ T cells and DC.[18–20] IL-27-mediated induction of PD-L1 inhibits Th17 cell differentiation and reduces pathology in experimental autoimmune encephalomyelitis.[20]

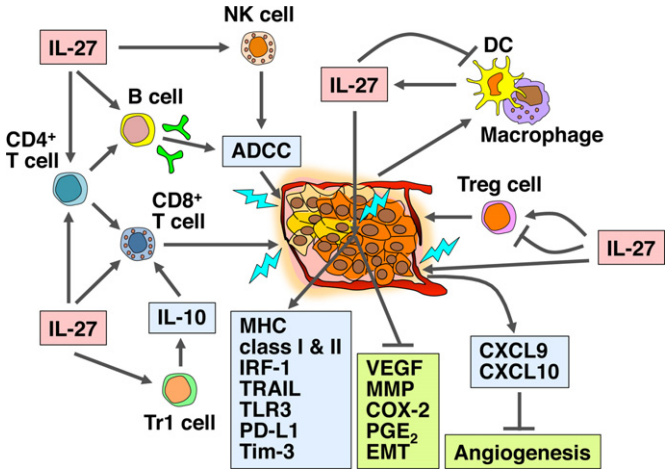

Fig. 2. Potent antitumor activity of IL-27 through multiple mechanisms that are mediated by $CD8^+$ T cells, NK cells, macrophages, macrophages, ADCC, anti-angiogenesis, direct anti-proliferative effect, inhibition of COX-2 and PGE_2 expression, and suppression of EMT, depending on the characteristics of individual tumors. ADCC, antibody-dependent cell-mediated cytotoxicity; COX-2, cyclooxygenase-2; CTL, cytotoxic T lymphocyte; DC, dendritic cell; EBI3, Epstein-Barr virus–induced gene 3; EMT, epithelial–mesenchymal transition; IRF, interferon regulatory factor; IL, interleukin; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; NK, natural killer; PGE_2, prostaglandin E_2; PD-L1, programmed death ligand 1; Tim-3, T-cell immunoglobulin and mucin domain-3; TLR, Toll-like receptor; Tr1, IL-10-producing regulatory T; TRAIL, tumor necrosis factor–related apoptosis-inducing ligand; Treg, regulatory T; VEGF, vascular endothelial growth factor.

Therapeutic Potential of Interleukin-27 as an Antitumor Agent

Antitumor effects of interleukin-27 against transplanted tumors genetically engineered to secrete interleukin-27. The antitumor efficacy of IL-27 was first demonstrated in 2004 using a transplanted mouse tumor genetically engineered to secrete IL-27 before transplantation as a preclinical tumor model.[21] Since then, accumulating evidence has revealed that IL-27 possesses potent antitumor activity against a variety of tumor models through multiple mechanisms without apparent adverse effects.[21,22] The mechanisms are those mediated by $CD8^+$ T cells,[21,23–30] NK cells[22,24,25,31] and macrophages,[32] as well as antibody-dependent cell-mediated cytotoxicity (ADCC),[33] anti-angiogenesis,[34–40] direct antiproliferative effects,[36,41–43] inhibition of expression of cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2),[44] and suppression of epithelial–mesenchymal transition (EMT),[32,39] depending on the characteristics of individual tumors (Fig. 2 and Table 1). Transfection of highly immunogenic tumors such as mouse colon carcinoma (colon 26) with IL-27 expression vector greatly reduced tumor growth, which was mainly mediated by $CD8^+$ T cells.[21] IL-27 plays a particularly important role in the generation of CTL by enhancing the survival of tumor-specific $CD8^+$ T cells and the differentiation into effector and memory cells.[8,9] In contrast, the antitumor effects of IL-27 against poorly immunogenic tumors such as mouse melanoma B16F10 were mediated by multiple mechanisms via angiogenesis,[34] NK cells,[22,25,31] and its direct antiproliferative effects on tumors.[32,39,41,44] IL-27 stimulated human umbilical vein endothelial cells and induced production of anti-angiogenic chemokines such as CXCR9 and CXCR10 to elicit strong anti-angiogenic activity against melanomas, which contributed to its antitumor and antimetastatic activi-

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Table 1. Susceptibility of mice transplanted with tumors expressing IL-27 and mice injected with IL-27 to development of tumors

Treatment	Susceptibility	Tumor	Reference
IL-27 transfectant	Reduced tumor growth *in vivo* by augmented antitumor CTL	Mouse colon carcinoma (Colon 26)	Hisada *et al.* 2004[21]
IL-27 transfectant	Reduced tumor growth *in vivo* by augmented antitumor CTL	Mouse orthotopic primary and metastatic neuroblastoma (TBJ)	Salcedo *et al.* 2004[23]
IL-27 transfectant	Reduced tumor growth *in vivo* by enhanced T-cell-dependent and NK-cell-dependent antitumor immunity	Mouse colon carcinoma (Colon 26)	Chiyo *et al.* 2004[24]
IL-27 transfectant	Reduced tumor growth *in vivo* by upregulation of CXCL9 and CXCL10 and inhibition of angiogenesis	Mouse melanoma (B16F10) lung metastasis	Shimizu *et al.* 2006[34]
IL-27 transfectant	Reduced tumor growth *in vivo* by NK-cell-mediated antitumor immunity independent of IFN-γ	Mouse melanoma (B16F10)	Oniki *et al.* 2006[22]
IL-27 transfectant	Reduced tumor growth in nude mice by NK cells through Fas/FasL pathway	Human esophageal carcinoma (Eca109)	Liu *et al.* 2008[45]
In vitro treatment	Reduced tumor growth *in vitro* by direct antiproliferative activity through WSX-1/STAT1/IRF-1 signaling	Mouse melanoma (B16F10) Human melanoma (SK-MEL-13, 28, 37)	Yoshimoto *et al.* 2008[41]
IL-27 transfectant	Reduced tumor growth in nude mice by augmented IFN-γ production and NK activity	Human esophageal carcinoma (Eca109)	Liu *et al.* 2008[31]
IL-27 transfectant	Reduced tumor growth *in vivo* by NK cell–mediated ADCC	Mouse head and neck squamous cell carcinoma (SCCVII)	Matsui *et al.* 2009[33]
IL-27 transfectant	Reduced tumor growth *in vivo* by $CD8^+$ and $CD4^+$ T cells, IFN-γ and NK cells	Mouse hepatocellular carcinoma (MM45T.Li)	Hu *et al.* 2009[25]
IL-27 plasmid injection	Therapeutic synergy of hydrodynamic injection of IL-27 plasmid and IL-2 by augmented generation of tumor-specific CTLs but suppressed expansion of Treg cells	Mouse disseminated metastatic neuroblastoma (TBJ) in liver	Salcedo *et al.* 2009[26]
IL-27 transfectant	Restraint of tumorigenicity *in vivo* by suppressing the expression of COX-2 and PGE_2	Mouse Lewis lung carcinoma (LLC)	Ho *et al.* 2009[44]
IL-27 plasmid injection	Elimination of distal aggressive tumor by IL-12 and IL-27 sequential gene therapy via intramuscular electroporation through T and NK cells	Mouse colon carcinoma (Colon 26) Mouse adenocarcinoma (4T1)	Zhu *et al.* 2010[27]
IL-27 protein injection	Damped tumorigenicity of human multiple myeloma cell lines in NOD/SCID mice through inhibition of angiogenesis	Human multiple myeloma (NCI-H929 and U266)	Cocco *et al.* 2010[35]
IL-27 protein injection	Inhibition of leukemia cell spreading in NOD/SCID/IL-2R$\gamma^{-/-}$ mice through suppression of proliferation, angiogenesis, miR-155 expression and induction of apoptosis	Human B-ALL	Canale *et al.* 2011[36]
IL-27 gene therapy	Inhibition of tumor growth by sonoporation delivery of IL-27 gene therapy through enhanced accumulation of effector cells	Mouse prostate cancer (TCR2)	Zolochevska *et al.* 2011[28]
IL-27 protein injection	Inhibition of tumor growth in NOD/SCID mice through suppressed angiogenesis	Human follicular lymphoma and diffuse large B-cell lymphoma (SU-DHL-4)	Cocco *et al.* 2012[37]
IL-27 transfectant by retroviral infection	Inhibition of tumor growth in nude mice through induction of apoptosis and cell-cycle arrest	Human pancreatic carcinoma (AsPC1)	Liu *et al.* 2012[42]
IL-27 protein injection	Inhibition of tumor growth in NOD/SCID/IL-2R$\gamma^{-/-}$ mice through suppression of angiogenesis and spreading related genes	Human AML	Zorzoli *et al.* 2012[38]
IL-27 transfectant	Enhanced antitumor CTL responses *in vivo* via programming tumor antigen-specific $CD8^+$ T cells into IL-10-producing memory precursor-like effector cells characterized by a greater survival advantage	Mouse plasmacytoma (J558)	Liu *et al.* 2013[29]
IL-27 transfectant vaccine	T-cell-mediated antitumor effects by tumor vaccine engineered to secrete IL-27 by means of cationic liposome	Mouse Lewis lung carcinoma (LLC)	Zhang *et al.* 2013[30]
IL-27 protein injection	Inhibition of tumor growth in NOD/SCID mice by upregulation of TRAIL and TLR3 in cooperation with a TLR3 agonist poly(I:C) in a partly TRAIL-dependent manner	Human melanoma (SK-ME-13, 28, 37)	Chiba *et al.* 2013[43]

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Table 1 (continued)

Treatment	Susceptibility	Tumor	Reference
In vitro treatment	Inhibition of EMT and angiogenic factor production in a STAT1-dominant pathway	Human non-small cell lung cancer	Kachroo *et al.* 2013[39]
IL-27 protein injection	Inhibition of tumor growth in athymic nude mice through reduced proliferation and vascularization by downregulation of pro-angiogenesis-related genes (FLT1, PTGS1/COX-1, FGFR3) and upregulation of anti-angiogenesis-related genes (CXCL10, TIMP3)	Human prostate cancer (PC3 and DU145)	Di Carlo *et al.* 2014[40]
IL-27 protein injection	Inhibition of tumor growth in athymic nude and NOD/SCID mice through granulocyte-driven and macrophage-driven colliquative necrosis, CXCL3 production, and reduced pluripotency-related and EMT-related gene expression	Human non-small cell lung cancer, particularly adenocarcinoma (Calu-6) and squamous cell carcinoma (SK-MES)	Airoldi *et al.* 2015[32]

ADCC, antibody dependent cell-mediated cytotoxicity; AML, acute myeloid leukemia; B-ALL, B-acute lymphoblastic leukemia; COX-2, cyclooxygenase-2; CTL, cytotoxic T lymphocyte; EBI3, Epstein–Barr virus-induced gene 3; EMT, epithelial–mesenchymal transition; FGFR, fibroblast growth factor receptor; FLT, fms-related tyrosine kinase; IFN, interferon; IRF, interferon regulatory factor; IL, interleukin; NK, natural killer; LLC, Lewis lung carcinoma; NOD/SCID, nonobese diabetic/severe combined immunodeficient; PGE_2, prostaglandin E_2; poly(I:C), polyinosinic-polycytidylic acid; PTGS1, prostaglandin G/H synthase 1; STAT, signal transducer and activator of transcription; TLR, Toll-like receptor; TRAIL, tumor necrosis factor–related apoptosis-inducing ligand; Treg, regulatory T; TIMP3, tissue inhibitor of metalloproteinase 3.

ties.[34] In addition, IL-27 has potent direct antiproliferative activity on melanomas through WSX-1/STAT1 and, in part, through interferon regulatory factor (IRF)-1 and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL).[41,43] It also directly restrained tumorigenicity of mouse Lewis lung carcinoma LLC by suppressing expression of COX-2 and PGE_2.[44] Moreover, IL-27 activated NK cells and augmented tumor-specific immunoglobulin production, which cooperatively exerted ADCC activity against mouse head and neck squamous cell carcinoma SCCVII,[33] and inhibited EMT and angiogenic factor production in a STAT1-dominant pathway in human non-small cell lung cancer.[32,39] Similar suppression of tumor growth was observed with human tumors such as esophageal carcinoma[45] and pancreatic carcinoma.[42]

Antitumor effects of interleukin-27 protein injection against human tumors transplanted in immunodeficient mice. Moreover, IL-27 has demonstrated potent antitumor activity in a variety of human therapeutic models through injection of IL-27 protein into immunodeficient mice such as nude mice, nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice and NOD/SCID/IL-2R$\gamma^{-/-}$ mice after transplantation of human tumors. The human tumors reported on so far are melanoma,[43] multiple myeloma,[35] B-acute lymphoblastic leukemia (B-ALL),[36] follicular lymphoma,[37] diffuse large B-cell lymphoma,[37] acute myeloid leukemia (AML),[38] prostate cancer[40] and non-small cell lung cancer[32,39] (Table 1). IL-27 strongly inhibited *in vitro* tumor growth of human melanoma, multiple myeloma, follicular lymphoma and diffuse large B-cell lymphoma through suppression of angiogenesis and induction of apoptosis, and the tumorigenicity of these tumors transplanted in NOD/SCID mice was greatly hampered by IL-27.[35,37,43] Similarly, IL-27 suppressed leukemic spreading of B-ALL cells and leukemia dissemination of AML cells transplanted in NOD/SCID/IL-2R$\gamma^{-/-}$ because of significant reduction of angiogenic and spreading-related genes, including vascular endothelial growth factors, angiopoietins and matrix metalloproteinases (MMP), and also because of upregulation of angiostatic molecules, such as tissue inhibitor of MMP.[36,38] Direct antiproliferative effects of IL-27 in collaboration with polyinosinic-polycytidylic acid [poly(I:C)], one of the Toll-like receptor 3 (TLR3) ligands whose expression was revealed to be upregulated by IL-27, was also observed in NOD/SCID mice transplanted with human melanoma.[43] In addition, IL-27 was recently shown to inhibit tumor growth of human prostate cancers in athymic nude mice through reduced proliferation and vascularization by downregulation of pro-angiogenesis-related genes and upregulation of anti-angiogenesis-related genes.[40] Inhibition of tumor growth of human non-small cell lung cancers was also demonstrated to be mediated by granulocyte-driven and macrophage-driven colliquative necrosis, CXCL3 production, and reduced pluripotency-related and EMT-related gene expression.[32,39]

Endogenous Role of IL-27 in the Susceptibility to Development of Tumors

Antitumor and protumor effects of endogenous WSX-1. To gain further insight into the antitumor effects of IL-27, it is important to clarify its endogenous role in the exertion of antitumor effects or protumor effects. Therefore, mice deficient in IL-27 subunits and receptor subunits were analyzed for susceptibility to tumor development (Table 2). WSX-1-deficient mice overall showed more excessive tumor growth of melanoma B16 injected subcutaneously than did WT mice.[46] However, this phenotype appears to be the sum of the effects of lacking WSX-1 in different immune responses, such as generation of CTL and antigen-presenting capacity of DC after maturation. Tumor-specific CTL generation was lower in WSX-1-deficient mice than in WT mice, and CTL induction in WSX-1-deficient mice was not restored by transfer of WT DC pulsed with tumor antigen, indicating that IL-27 is directly required for generation of tumor-specific CTL.[46] In contrast, when transferred into tumor-bearing mice, WSX-1-deficient DC pulsed with tumor antigen were more potent than WT DC in the inhibition of tumor growth and generation of CTL, indicating the suppressive effects of IL-27 on DC function.[46] It is also reported that WSX-1-deficient mice had reduced resistance to endogenously arising mouse tumor models, 3-methylcholanthrene (MCA)-induced fibrosarcoma and polyoma middle T antigen (PyMT)-induced mammary carcinoma.[47] This reduced resistance was accompanied by decreased IFN-γ production from $CD4^+$ and $CD8^+$ T cells and an increased number of Treg

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Table 2. Susceptibility of mice deficient in WSX-1, EBI3 and p28 to development of tumors

Deficient mice	Susceptibility	Tumor	Reference
WSX-1	Overall reduced resistance to tumor growth due to impaired antitumor CTL generation but accompanied with augmented antitumor immunity by DC	Melanoma (B16)	Shinozaki *et al.* 2008[46]
	Reduced resistance to endogenously arising tumor growth due to decreased IFN-γ production by $CD4^+$ and $CD8^+$ T cells and increased numbers of Treg cells	MCA-induced fibrosarcoma PyMT-induced mammary carcinoma	Natividad *et al.* 2013[47]
	Augmented resistance to tumor growth due to reduced number of the most exhausted Tim-3^+PD-1^+CD8^+ T cells among TILs	Melanoma (B16F10) Lewis lung carcinoma (LLC)	Zhu *et al.* 2015[48]
EBI3	Augmented resistance to tumor growth due to increased numbers of IFN-γ-producing killer DC with T-bet-mediated antitumor $CD8^+$ T-cell responses in the lung	Melanoma (B16F10) lung metastasis	Sauer *et al.* 2008[49]
DC-specific conditional p28	Reduced resistance to tumor growth due to impairment of IL-27-mediated CXCL10 expression in MDSCs accompanied by reduced recruitment and activation of NK and NKT cells	MCA-induced fibrosarcoma Melanoma (B16)	Wei *et al.* 2013[53]
	Enhanced antitumor immune responses due to impairment of IL-27-mediated CCL22 expression in DC with reduced infiltration of Treg cells into tumors	Melanoma (B16F10) Lymphoma (EL-4) MCA-induced fibrosarcoma	Xia *et al.* 2014[54]

CTL, cytotoxic T lymphocyte; DC, dendritic cells; EBI3, Epstein–Barr virus-induced gene 3; IFN, interferon; IL, interleukin; MCA, 3-methylcholanthrene; MDSC, myeloid-derived suppressor cell; NK, natural killer; PD-1, programmed death-1; PyMT, polyoma middle T antigen; T-bet, T-cell-specific T-box transcription factor; TIL, tumor-infiltrating lymphocyte; Tim-3, T-cell immunoglobulin and mucin domain-3; Treg, regulatory T.

cells. In marked contrast, however, it was recently demonstrated that WSX-1-deficient mice showed more attenuated tumor growth of B16F10 and LLC than did WT mice.[48] This augmented antitumor effect was explained by the reduced number of T-cell immunoglobulin and mucin domain-3 (Tim-$3)^+$ programmed death-1 (PD-$1)^+$ $CD8^+$ T cells, which are the most exhausted T-cell population among tumor-infiltrating lymphocytes, indicating IL-27 signaling as a key regulator of effector T-cell responses via induction of Tim-3.[48] Taken together, these results suggest that IL-27/WSX-1 signaling plays critical roles in both generation and exhaustion of CTL, together with suppression of DC function.

Protumor effects of endogenous Epstein–Barr virus-induced gene 3. In contrast to both the antitumor and protumor effects of WSX-1, whose deficiency seems to highly agree with loss of representative IL-27 functions, EBI3-deficient mice showed augmented antitumor activity against lung metastasis of B16F10 (Table 2).[49] This effect was induced by expansion of a newly described cell subset called IFN-γ-producing killer DC in the lung of EBI3-deficient mice, and these DC then activated $CD8^+$ T cells to produce IFN-γ and TNF-α, resulting in tumor apoptosis in a T-bet-dependent manner. This phenotype is consistent with increased EBI3 expression in serum and tumor tissue correlating with higher risk of cancer progression.[50,51] Although EBI3-deficient mice showed abrogated IL-35-mediated Treg activity[52] and also may be devoid of IL-27-mediated regulation of Treg cells, if any, the endogenous role of EBI3 likely contributes more strongly to the protumor activity mediated by EBI3 alone than the antitumor activity by IL-27.

Both antitumor and protumor effects of endogenous p28. So far, there have been two seemingly opposite articles regarding the endogenous role of p28 in the induction of antitumor immune responses[53,54] (Table 2). DC-specific conditional p28-deficient mice were initially demonstrated to have reduced resistance to progression of tumors such as MCA-induced fibrosarcoma and transplanted melanoma B16.[53] These effects were due to impairment of IL-27-mediated CXCL10 expression in myeloid-derived suppressor cells (MDSC) and IL-12 production from DC, which led to recruitment and activation of NK and NKT cells, resulting in immunological control of tumors.[53] In contrast, using the same p28-deficient mice, the same group also reported that IL-27 plays a critical role in tumor infiltration of Treg cells.[54] In tumor-associated DC, IL-27 promoted the expression of CCL22, which is critical for recruitment of peripheral Treg cells into tumors, and the p28-deficient mice showed reduced tumor infiltration of Treg cells.[54] Because IL-27, but not p28, restored the respective phenomena in the p28-deficient mice, the endogenous role of p28 seems to strongly agree with IL-27 functions. However, the role of IL-27 in the induction of Treg cells still appears to be controversial in the tumor microenvironment, as described later.

Potential Protumor Effects of Interleukin-27

Contribution of interleukin-27-mediated upregulation of interleukin-10 to its antitumor effects. Because IL-27 possesses both pro-inflammatory and anti-inflammatory activities, some such as IL-27-mediated augmentation of IL-10 production[10–12] and upregulation of PD-L1[18–20] and Tim-3[48] may function against its antitumor effects. However, the role of IL-10 in the induction of antitumor immune responses is often controversial, and increasing evidence supports a positive role of IL-10 for induction of antitumor CTL responses, which were demonstrated in IL-10-deficient mice and IL-10 transgenic mice.[55–57] IL-27 was recently demonstrated to significantly enhance the survival of activated tumor-specific $CD8^+$ T cells *in vitro* and *in vivo* and to induce a unique memory precursor cell phenotype in tumor antigen-specific $CD8^+$ T cells, characterized by, for instance, upregulation of IL-10.[29] The IL-27-mediated IL-10 production from CTL contributed to the induction of memory precursor cell phenotype, CTL memory and, consequently, tumor rejection.[29]

Contribution of interleukin-27-mediated upregulation of programmed death ligand 1 and T-cell immunoglobulin and mucin

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domain-3 to its protumor effects. Exhausted T cells are characterized by their sustained expression of inhibitory receptors such as PD-1 and Tim-3.[58–60] The PD1/PD-L1 pathway has emerged as a central player in immune regulation, and cancer cells that express PD-L1 promote tumor progression through inhibition of PD-1 expressing effector cells.[61] Co-expression of PD-1 and other inhibitory receptors such as Tim-3 contributes to the induction of T-cell exhaustion and defines T cells with more deeply exhausted phenotype.[60] IL-27 was recently shown to induce expression of PD-L1 in not only T cells and DC[18–20] but also tumors.[62] Histological analyses revealed that when IL-27 was detected in primary cutaneous melanomas, its expression was maintained in metastatic lesions.[62] In addition, IL-27 induced PD-L1 expression in melanoma cell lines and the in situ expression of IL-27 in melanoma correlated with those of PD-L1 and IL-10.[62] Thus, it is highly conceivable that IL-27-induced PD-L1 expression in T cells and tumors could contribute to the protumor effects of IL-27, whose possibility remains to be directly clarified. Moreover, IL-27 was recently demonstrated to induce the expression of the transcription factor nuclear factor, IL-3 regulated (NFIL3), which cooperates with T-bet, to induce the expression of Tim-3.[48] IL-27 signaling was required for the induction of Tim-3$^+$ exhausted T cells and promotion of tumor growth.[48] Thus, IL-27-mediated upregulation of inhibitory molecules such as PD-L1 and Tim-3 likely contributes to tumor progression.

Positive and negative effects of interleukin-27 on Treg cells. The role of IL-27 in the induction of Treg cells in the context of tumorigenesis is also controversial. The induction of Treg cells[54,63] and the suppression of them[47] by IL-27 have both been reported. Initially, apoptotic tumor cells were demonstrated to induce IL-27 release from human DC to activate Treg cells that express CD69 and CD39 to generate adenosine, thereby suppressing cytotoxicity of CD73-expressing CD8$^+$ T cells and limiting antitumor immunity.[63] Moreover, the effect of endogenous IL-27 on Treg cells in the tumor microenvironment was investigated using IL-27p28 conditional knockout mice. IL-27 induced the expression of CCL22 on tumor-associated DC, which mediates tumor infiltration of Treg cells, resulting in tumor progression.[54] In contrast, it was also demonstrated that tumor development and growth are accelerated in WSX-1-deficient mice.[47] The enhanced tumor growth in both carcinogen-induced fibrosarcoma and oncogene-driven mammary carcinoma was associated with increased number of Treg cells and decreased IFN-γ-production by CD4$^+$ and CD8$^+$ T cells.[47] Collectively, if IL-27 were to promote the generation of Treg cells, the antitumor effects of IL-27 could be weakened. In contrast, if IL-27 were to inhibit it, the antitumor effects of IL-27 could be augmented, but side effects such as development of autoimmune diseases might be expected. Further studies are necessary to elucidate the precise effect of IL-27 on Treg cells in the tumor microenvironment.

Potential transforming activity of interleukin-27Rα. A point mutation in Janus kinase (JAK)2 (JAK2-V617F), which has an increased inherent kinase activity, was identified in a number of neoplastic myeloproliferative disorders.[64] From a patient with acute myeloid leukemia, IL-27Rα was recently identified as a gene that can induce ligand-independent transformation of hematopoietic cells via JAK-dependent pathways.[65] In addition, it was demonstrated that IL-27Rα induces the tyrosine phosphorylation of JAK2 and its downstream target STAT5, and transforms cytokine-dependent hematopoietic cells to cytokine independence when co-expressed with mutated JAK2-V617F but not wild-type JAK2.[65,66] This activating effect on JAK2-V617F by IL-27Rα was dependent on a functional IL-27Rα Box 1 motif but was independent of the gp130 co-receptor, suggesting that IL-27Rα may be functioning as a homodimer.[65] Intriguingly, we have previously shown that IL-27 stimulates and expands hematopoietic stem cells to differentiate into myeloid cells in IL-27 transgenic mice.[67] Taken together, aberrant IL-27Rα signaling may enhance myeloid cell growth, potentially leading to myeloproliferative neoplasms, although higher incidence of development of spontaneous tumors was not observed in the IL-27 transgenic mice.[67]

Future Prospects and Concluding Remarks

Cytokine-based cancer immunotherapies currently used include systemic administration, local delivery, vaccination with tumor cells engineered to secrete cytokine, adjuvants for cancer vaccine and adoptive therapy of immune cells expanded by cytokines. Because IL-27 is a multifunctional cytokine acting with a double-edged sword, both antitumor and protumor effects of IL-27 are conceivably expected depending on the type of cells that IL-27 stimulates and the tumor context. Currently, antibody drugs have become very popular and are one of the most promising cancer immunotherapies. Therefore, to overcome the protumor effects of IL-27, it is highly possible that IL-27-mediated antitumor effects could be further augmented by antibodies against immune checkpoints, such as PD-L1 and Tim-3, and also by antibodies against Treg cells, such as cytotoxic T-lymphocyte-associated protein 4. These possibilities remain to be investigated in the near future. In addition, IL-27 was shown to have lower toxicity in mouse models, probably because of low induction of IFN-γ;[21,22] therefore, the combination of IL-27 treatment with other cancer immunotherapies to overcome the protumor effects of IL-27 could be a promising therapeutic strategy against cancer.

Disclosure Statement

The authors have no conflict of interest to declare.

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