Interleukin 27: a double-edged sword for offense and defense

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RECEIVED JUNE 30, 2009; REVISED SEPTEMBER 6, 2009; ACCEPTED SEPTEMBER 8, 2009. DOI: 10.1189/jlb.0609445

ABSTRACT
Cytokine-mediated immunity plays a crucial role in the pathogenesis of various diseases including infection and autoimmune diseases. IL-27, along with IL-12, -23, and -35, belongs to the IL-12 cytokine family. These family members play roles in regulation of Th cell differentiation. IL-27 is unique in that it induces Th1 differentiation, the same cytokine suppresses immune responses. In the absence of IL-27-mediated immunosuppression, hyperproduction of various proinflammatory cytokines concomitant with severe inflammation is observed. The immunosuppressive effects of IL-27 depend on IL-2 suppression, inhibition of Th17 development, and induction of IL-10 production. Administration of IL-27 suppresses some diseases of autoimmune or allergic origin, demonstrating its potential in therapy of diseases mediated by inflammatory cytokines. In this review, we discuss recent studies about the role of IL-27 in immune regulation in view of its pro- and anti-inflammatory properties and possible therapeutic application. J. Leukoc. Biol. 86: 000–000; 2009.

Introduction
Cytokines are critical players in the regulation of immune responses. With the help of these soluble factors, immune cells undergo proliferation, activation, differentiation, and inactivation or even cell death. Among various types of cells involved in immune responses, CD4+ T cells play central roles in immune regulation. It has been understood that naive CD4+ T cells differentiate into two distinct subsets: Th1 cells for cellular immunity and Th2 cells for humoral immunity for a long time [1]. Recently, Th17 and follicular Th cells, for inflammatory responses and B cell differentiation, respectively, have been identified and their roles elucidated. In addition, Tregs have gathered attention for their immune-suppressive function. Differentiation of these T cell populations is affected by various cytokines.

IL-12 was identified as a potent inducer of IFN-γ production by T cells, NK cells, and other types of lymphocytes and was shown later to be an essential inducer of Th1 differentiation [2]. Recently, IL-23, IL-27, and IL-35 were identified as heterodimeric cytokines structurally related to IL-12. These four cytokines, along with two other cytokines with unknown function, make up a family of heterodimeric cytokines [3, 4]. These IL-12 family members have abilities to help differentiation and/or maintenance of Th1 cells.

In addition to the promotion of Th1 differentiation, some of the family members play unique roles in regulation of Th differentiation. Although IL-23 promotes differentiation of Th17 cells, a Th cell population critically involved in various inflammatory diseases by production of IL-17 and other cytokines, IL-27 suppresses Th17 differentiation and IL-17 production. Immunosuppressive and anti-inflammatory effects of IL-27 have also been demonstrated in various experimental settings. Thus, IL-27 seems to have two distinct functions in immune responses: one as an initiator of Th1-type immune responses and the other as an attenuator of immune/inflammatory responses. In this review, the dual roles of IL-27 in immune responses, molecular basis for the effects, and therapeutic implication of IL-27 will be discussed.

IL-27 AND IL-12 CYTOKINE FAMILY
Cytokines produced by Th1 cells, especially IFN-γ, are critical for the macrophage activation and NO production required for elimination of intracellular pathogens such as Leishmania major [5]. IL-12, a heterodimeric cytokine composed of two subunits, p35 and p40, is critical in this Th1 differentiation [2]. Recently, IL-23 and IL-27 were identified as heterodimeric cytokines functionally and structurally related to IL-12 [6, 7]. In addition, IL-35 was identified as a new IL-12 family member.

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[8, 9]. IL-23 is composed of the p40 subunit of IL-12 and p19. IL-27 is composed of EBI-3, a p40-related molecule, and p28, a p35-related molecule. IL-35 is composed of EBI-3 and p35 [9].

IL-12R is composed of two subunits: β1 and β2. Downstream of IL-12R, STAT4 is activated for direct induction of the IFN-γ gene. IL-27R uses unique IL-23R subunits and shares IL-12Rβ1 subunits. IL-27R is made with WSX-1 (also called the IL-27R of IL-12R, STAT4 is activated for direct induction of the IFN-γ gene). Lower panes show their roles in regulation of inflammatory responses. Although expression of WSX-1 is enhanced, expression of gp130 is down-regulated in activated T cells over naïve T cells [11, 22]. Expression of WSX-1 is also enhanced in DCs upon LPS stimulation [23].

**REGULATION OF IL-27 PRODUCTION**

DCs produce IL-12, -23, and -27 when stimulated by pathogen-associated molecular patterns through TLRs. EBI-3 is strongly induced by TLR2, -4, and -9 stimulation [12]. MyD88 is required for induction of EBI-3 downstream of TLR stimulation. Following TLR stimulation, NF-κB complexes bind to a promoter region of the EBI-3 gene. PU.1 binding to the promoter region is also required for induction of the EBI-3 gene. Although MyD88-dependent TLR4 stimulation is important for expression of p28 in macrophages [13], it was reported that activation of TRIF downstream of TLR3 or -4 is critical in the induction of p28 [14]. IRF3, activated downstream of TRIF, and c-Rel bind to a promoter region of the p28 gene. Type I IFN stimulation also contributes to the induction of p28 through activation of IRF1 and -3 [14, 15]. Interestingly, commensal gram-negative but not gram-positive bacteria induces expression of p28 in human DCs [16]. The transcription of the two subunits of IL-27 is thus regulated differentially, and as such, dissociation of the expression of the two subunits may occur. In vivo, augmented expression of IL-27 is observed in inflamed tissues [17, 18]. As up-regulation of IL-27 is also detected at the decidual placental interface of fetuses born to a specific combination of strains of mice, IL-27-mediated immune regulation is implicated in maintenance of pregnancy [19], although no evidence has been shown from gene-deficient mice. Transcriptional regulation of IL-12 and -23, in addition to IL-27, is well-summarized by Goriely et al. [20] and by Stumhofer and Hunter [21].

Although less well-elucidated, the expression of IL-27R also seems to be regulated during immune responses. Although expression of WSX-1 is enhanced, expression of gp130 is down-regulated in activated T cells over naïve T cells [11, 22]. Expression of WSX-1 is also enhanced in DCs upon LPS stimulation [23].

**IL-27 IN Th1 DIFFERENTIATION**

Although IL-12 is the most potent inducer of Th1 differentiation and IFN-γ production acting on effector Th1 cells, chronologically differential roles and differential use of IL-12, -23, and -27 have been proposed for Th1 differentiation: First, IL-27 commits naïve CD4+ T cells to differentiate into Th1 cells by inducing IL-12Rβ2, and then, IL-12 acts on committed effector Th1 cells for IFN-γ production, followed by IL-23 mediating the proliferation of memory Th1 cells (see Yoshida et al. [24] for review).

IL-27 is a key cytokine to drive naïve cells into the Th1 subset at the initial step of differentiation. The role of WSX-1 in Th1 differentiation was analyzed in L. major −/− mice. In two independent reports by us [25] and by Chen et al. [26], WSX-1 −/− mice, as compared with wild-type mice, showed impaired IFN-γ production. Accordingly, WSX-1 −/− mice showed remarkable susceptibility to L. major [25], an intracellular pathogen, whose clearance depends largely on a Th1 response. Interestingly, impaired production of IFN-γ was observed only at early phases of L. major infection, and the IFN-γ production in WSX-1 −/− mice was restored to the wild-type level at late phases. Thus, IL-27/WSX-1 signaling is only required at the initial step of Th1 differentiation.

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**Figure 1. Structure and roles of IL-12 family members.** Subunit combinations and receptor structures of IL-12 family members are shown. IL-27, -12, and -23 are produced by activated APCs, and IL-35 is produced by Tregs. Major signal transduction pathways for each receptor complex are also shown. The receptor for IL-35 has not been identified. Upper panes show roles of each cytokine for Th1 differentiation. Lower panes show their roles in regulation of inflammatory responses. Th1eff, Effector Th1 cells; Th1mem, memory Th1 cells.
IL-27 stimulation induces STAT1 activation, followed by induction and activation of T-bet, the Th1-specific transcription factor. Stimulation of naive CD4\(^+\) T cells with IL-27 does not induce IFN-γ production, but additional stimulation with IL-12 is required for proper IFN-γ production by naive CD4\(^+\) T cells. The role of WSX-1, therefore, is to render the naive CD4\(^+\) T cells reactive to IL-12 stimulation for IFN-γ production by induction of T-bet and subsequent IL-12Rβ2 [27]. A more recent report indicated that IL-27/WSX-1 suppresses the expression of GATA-3, a transcription factor pivotal for Th2 differentiation, in a STAT1-dependent or -independent way, favoring Th1 differentiation [28].

**THE ANTI-INFLAMMATORY PROPERTIES OF IL-27**

IL-27 also has an immunosuppressive function and suppresses production of inflammatory cytokines. When infected with Trypanosoma cruzi, an intracellular protozoa, WSX-1\(^+\) mice produced more IFN-γ than wild-type mice and demonstrated cytokine-mediated liver damage during the infection [29]. In addition to IFN-γ, CD4\(^+\) T cells isolated from *T. cruzi*-infected WSX-1\(^+\) mice showed hyperproduction of various proinflammatory cytokines including IL-6 and TNF-α. WSX-1\(^+\) mice demonstrated similar phenotypes when infected with another protozoa, Toxoplasma gondii [30]. The WSX-1\(^+\) mice were also susceptible to Mycobacterium tuberculosis by cytokine-mediated severe inflammation in the liver and in the lung [31, 32]. Similarly, WSX-1\(^+\) mice demonstrated hyperproduction of various cytokines in the allergen-induced airway hypersensitivity model or the Con A-induced hepatitis model as well [33, 34]. As expected, IL-27 suppressed cytokine production of activated T lymphocytes in vitro [11]. This immunosuppressive role of IL-27 is particularly important to prevent excessive inflammation, which may result in fatal organ damage or subsequent autoimmunity. From the standpoint of the IL-27-mediated immunosuppression, the aforementioned susceptibility of WSX-1\(^+\) mice to *L. major* infection with impaired Th1 responses may also be interpreted in an alternative way. According to a report by Artis et al. [35], in the absence of IL-27 signaling, IL-4 production, at the early phase of *L. major* infection (“early burst of IL-4” [36]), is augmented concomitant with suppression of STAT1 activation to counter the IL-4-mediated, Th2-promoting effects. Thus, lack of “direct” suppression of Th2 responses by IL-27 will result in the augmented Th2 responses and reciprocal suppression of Th1 responses with subsequent susceptibility to *L. major* infection. Actually, Artis et al. [37] also reported enhancement of Th2 responses with normal Th1 responses in WSX-1\(^+\) mice infected with the gastrointestinal helminth *Trichuris muris*. In an experimental asthma model, also, WSX-1\(^+\) mice exhibited enhanced Th2 responses with simultaneous enhancement of Th1 responses [34]. These data demonstrate that IL-27 suppresses a variety of immune responses, irrespective of Th1/Th2 differentiation.

IL-27 suppresses immune responses and inflammation by various mechanisms. Villarino et al. [38] reported that IL-27 suppressed IL-2 production by CD4\(^+\) T cells, which explains the high levels of IL-2, which are detected in the culture of WSX-1\(^+\) T cells. However, overproduction of IL-2 by WSX-1\(^+\) CD4\(^+\) T cells is not the sole mechanism of the hyperproliferation of WSX-1\(^+\) cells, as WSX-1\(^+\) cells still showed hyperproliferation, even in the presence of anti-IL-2 antibodies.

IL-27 also suppresses inflammation by inhibiting differentiation of Th17 cells. IL-23 plays a critical role in maintenance of the Th17 cells, whose differentiation is initiated by IL-6 plus TGF-β. By producing a group of proinflammatory cytokines, Th17 cells are involved in various inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel diseases [39]. In WSX-1\(^+\) mice, encephalomyelitis of autoimmune origin (EAE) or of infectious origin (*T. gondii* infection) was exacerbated, concomitant with augmented Th17 differentiation [40, 41]. These reports demonstrated that IL-27/WSX-1 inhibits Th17 differentiation and functions to limit autoimmune inflammatory diseases. IL-27 suppresses Th17 differentiation effectively in vitro too. The inhibition of Th17 development by IL-27 is dependent on STAT1 activity, as in STAT1\(^-/-\) mice, IL-27-mediated suppression of Th17 differentiation in vitro was abolished almost completely [40, 41]. The suppression, however, was not dependent on T-bet [40], suggesting that IL-27-mediated suppression of Th17 development was not a result of IL-27-mediated Th1 development. Recently, suppression of Th17-specific transcription factor RORγt by IL-27 has been reported [42].

IL-10 is generally regarded as one of the most immunosuppressive cytokines. Three recent reports demonstrated that IL-27 promotes IL-10 production by CD4\(^+\) T cells [43–45]. Fitzgerald et al. [44] reported that IL-27, in a splenocyte-activation culture, elicited IL-10 production by not only CD4\(^+\) T cells but also by CD8\(^+\) T cells. In a study by Awasthi et al. [45], IL-27, in combination with TGF-β, induced IL-10-producing anti-inflammatory Treg [46]-like cells, which produced IL-10 and IFN-γ but not IL-4, -5, and -13 in vitro. IL-6 also acts in synergy with TGF-β to promote IL-10 production [43]. Interestingly, IL-27 induced IL-10 production by T cells in Th1 and Th2 conditions but not in Th17 conditions [43]. IL-10-producing CD4\(^+\) T cells elicited by IL-27 are T-bet+ FoxP3+ IFN-γ+ [43–45, 47]. Induction of IL-10 by IL-27 is also dependent on STAT1 activity, but STAT3 is also involved in this event, at least partially [43]. Recently, induction of c-Maf, IL-21, and ICOS has been proposed as a mechanism of IL-27-mediated, T1r-like cell differentiation [48].

Although the IL-10 induction is critical in IL-27-mediated immune suppression, IL-10-independent anti-inflammatory mechanisms have also been indicated [43, 44]. For instance, IL-27 showed its suppressive effects in an IL-10-deficient milieu, and it even suppressed IL-10 production in some experimental conditions [11], indicating the presence of an IL-10-independent, more “general” suppression mechanism(s). Recently, we reported that IL-27 inhibited osteoclast differentiation induced by receptor activator of NF-κB stimulation [49]. This inhibition was mediated through c-Fos suppression, accompanied with reduction of NF-κB transcription factor for osteoclastogenesis, it is also crucial for transactivation of various cytokine genes. This c-Fos suppression may be a key to the general suppression by IL-27. Figure 2 illustrates sig-
Figure 2. Role of IL-27 in regulation of immune cell functions. Downstream of the IL-27R, STAT1 and -3 are activated. For Th1 differentiation, STAT1 activation induces T-bet activation. For negative regulation of inflammatory/immune responses, IL-27 stimulation suppresses Th17 differentiation; induces IL-10 production by Th0/1/2 cells through c-Maf induction; and suppresses c-Fos activation. These suppression mechanisms depend largely on STAT1 activation, although STAT3 activation may be involved at least partially.

**Effects of IL-27 on Other Types of Cells**

IL-27 enhances cytotoxic activity against tumors of CD8⁺ T cells. IL-27R signaling is critical in STAT1- and T-bet-induced IFN-γ production by CD8⁺ T cells [52]. Hisada et al. [53] reported that CD8⁺ cells engineered to produce IL-27 conferred protective immunity in a CD8⁺ T cell- and IFN-γ-dependent manner when inoculated in immunocompetent mice. The effect was also dependent on T-bet activation. IL-27 produced by tumor cells also enhanced IFN-γ production by NK cells. STAT1- and T-bet-dependent augmentation of IL-12Rβ2, granzyme B, and perforin expression in CD8⁺ T cells by tumor-derived IL-27 was observed in vitro [54].

IL-27 also has an impact on B cell differentiation and Ig production. IL-27 induced proliferation of activated naïve B cells but not memory B cells, indicating that IL-27 exerts differential effects on B cells depending on activation/differentiation status of the cells [55]. IL-27 stimulation of mouse B cells induced STAT1 activation and T-bet expression followed by class-switching of B cells to IgG2a (Th1-dependent Ig subtype)-producing cells [56]. In contrast, IL-27 inhibited IgG1 (Th2-dependent Ig subtype) class-switching. Interestingly, IL-27 up-regulates IL-4-induced IgE production by naïve B cells without affecting Cε promoter activity. STAT1- and T-bet-dependent activation of B cells and Th1-related Ig production are also reminiscent of the effects of IL-27 on naïve CD4⁺ T cells.

IL-27 augments NK cell activity. Although enhancement of IFN-γ production in NK cells by inoculation of IL-27-producing tumor cells was reported [57, 58], Oniki et al. [59] reported IFN-γ-independent enhancement of NK cell-mediated anti-tumor immunity by IL-27. In contrast, WXS⁻/⁻ NK, IL-1⁺ CD3⁺ T (so-called NKT) cells showed hyperproduction of IL-4 and IFN-γ in response to Con A or α-galactosylceramide stimulation, in vivo and in vitro [33]. Contrary to this report, Siebler et al. [60] reported that EBI-3-deficient mice were resistant to Con A-induced hepatitis. In that report, reduced IFN-γ production concomitant with reduced STAT1 and T-bet activation was observed. The reason for these apparently contrary results is currently unknown.

Effects of IL-27 on macrophage/monocyte are complicated too. Feng et al. [61] reported IL-27-mediated augmentation of MHC class I/II expression as well as transporter associated with antigen processing 1 and β2-microglobulin expression in a THP-1 human monocyte cell line. Expression of costimulatory molecules CD80 and CD86 and an adhesion molecule CD54 was also augmented by IL-27. Thus, IL-27 may enhance immune responses by induction of genes involved in antigen-presenting activity of THP-1 cells. IL-27-stimulated monocytes showed enhanced TLR responsiveness and produced more IL-6 and TNF-α as compared with cells without IL-27 stimulation. However, Holscher et al. [31] reported IL-27-mediated suppression of IL-12 and TNF-α production by activated macrophages and that WXS⁻/⁻ mice succumbed to death by cachexia as a result of overproduction of IL-12 and TNF-α during M. tuberculosis infection. Thus, IL-27 signaling may function as a feedback mechanism to inhibit excessive inflammation. Alternatively, aa-macrophages develop in a Th2 milieu and play critical roles in down-regulation of immune responses. Interestingly, aa-macrophages express WSX-1 [62]. Although IL-27 did not induce aa-macrophage activation, IL-4
strongly up-regulates the expression of WSX-1 on macrophages and enhanced IL-27-mediated signaling, implicating IL-27 in immunosuppression through activation of aa-macrophages. IL-27 has anti-inflammatory effects on DCs too. Wang et al. [23] reported that DCs from WSX-1<sup>-/-</sup> mice were hyper-reactive to LPS stimulation by expression of CD80 and CD86 co-stimulatory molecules and Th1-related cytokine genes. When adoptively transferred in vivo, WSX-1<sup>-/-</sup> DCs pulsed with L. major antigens conferred the recipient mice with strong protective immunity against parasite infection with rapid induction of Th1-biased immune responses. As discussed above, IL-27 enhances CTL activity of CD8<sup>+</sup> T cells. Thus, the combination of wild-type CD8<sup>+</sup> T cells (with positive effects of IL-27) plus WSX-1<sup>-/-</sup> DCs (without suppressive effects of IL-27) resulted in efficient induction of tumor-specific CTLs [63].

**THERAPEUTIC IMPLICATIONS**

The pro- and anti-inflammatory effects of IL-27 have gathered much attention in terms of its therapeutic application toward some immune-related diseases. Effects of augmentation or blocking of IL-27 signaling have been examined extensively in various disease models. The proinflammatory or IFN-y-inducing property of IL-27 could be used for therapy of some infections or for augmentation of anti-tumor immunity. As discussed before, IL-27 enhances CTL activity against tumors, and IL-27-producing autologous tumors could be used as vaccines to augment anti-tumor immunity in vivo. In addition, in vivo administration of an IL-27-expressing vector could augment anti-tumor CTL activity [64]. IL-27 could also be used for induction of CTL in vitro. IL-27 significantly enhanced proliferation and killing activity of alloreactive CTL generation [54]. Given the immunosuppressive effects of IL-27 on APCs, such as macrophages and DCs [23, 31], it is possible that IL-27 would have a detrimental effect on generation of CTL. However, the combination of IL-27-reactive, wild-type CD8<sup>+</sup> T cells and WSX-1<sup>-/-</sup> DCs induced antigen-specific CTLs more effectively than other possible combinations [63]. Autologous DCs with blocked IL-27 signaling may be used as potent APCs in vitro or as a potent adjuvant to induce antigen-specific CTLs in vivo.

IL-27-mediated immunosuppression would be more attractive as a target for treatment of various inflammatory diseases. As discussed previously, WSX-1<sup>-/-</sup> mice developed severe encephalomyelitis of autoimmune or infection origin [40, 41, 44]. IL-27 administered in vivo suppressed CNS inflammation and disease development of EAE induced by adoptive transfer of encephalitogenic Th17 cells or by active immunization [44, 65]. Wang et al. [66] reported that bone marrow stromal cell-derived IL-27 showed a suppressive effect on rat EAE development. Similarly, Amadi-Obi et al. [67] reported suppression of EAU, an experimental model of human uveoretinitis, by administration of IL-27. In this report, IL-27 induced Th1 development, which in turn, suppressed inflammatory Th17 development. IL-27 thus may have therapeutic potential in autoimmune diseases, such as multiple sclerosis or uveitis in Behêchê’s disease.

In addition to EAE/EAU, IL-27 has a suppressive effect on CIA [68]. IL-27 treatment suppressed arthritis severity, inflammation in the joints, and production of IL-17, IFN-y, and IL-6. Inhibition of osteoclastogenesis by IL-27 may contribute to the suppression of arthritis development [49]. However, Cao et al. [69] reported proinflammatory effects of IL-27 in proteoglycan-induced arthritis, another model of rheumatoid arthritis. In WSX-1<sup>-/-</sup> mice, development of proteoglycan-induced arthritis was delayed, and severity was reduced. Relative contribution of Th1 and Th17 responses to the disease pathogenesis may differ in various disease models. IL-27, administered topically, showed its anti-inflammatory effects in the DTH model [70], demonstrating its therapeutic potential against some diseases of allergic origin.

IL-27 is also involved in the pathogenesis of IBD. Although EBI-3 is constitutively expressed by human intestinal epithelium, p28 expression is augmented in intestinal areas of patients with IBD [71–73], implicating IL-27 in the development of the diseases. Initially, Nieuwenhuis et al. [74] reported that EBI-3-deficient mice developed Th1-mediated colitis induced by administration of trinitrobenzene sulfonic acid, just as wild-type mice. Honda et al. [75], however, reported suppression of the development of DSS-induced colitis with reduced production of inflammatory cytokines, including IFN-y in WSX-1<sup>-/-</sup> mice. Contrary, Troy et al. [76] reported exacerbation of DSS-induced colitis with augmented Th17 responses in WSX-1<sup>-/-</sup> mice. As the dose of DSS used in each study was different, these reports, taken together, indicate that IL-27 can promote or suppress colitis depending on the etiology, severity, and surrounding cytokine milieu during the development of IBD.

Given the observation that IL-27 suppresses cytokine production in strongly inflammatory environments [76], IL-27 signaling is a candidate target for treatment of IBD.

MRL/lpr mice carry a mutation of the fas gene and spontaneously develop autoimmune disease resembling human SLE. Representative manifestations of autoimmunity in the mice include GN caused by immune complex-induced inflammation and cellular proliferation inside glomeruli. Effects of IL-27 signaling on autoimmune responses in MRL/lpr mice are intriguing. Although the (auto-) immune response in wild-type MRL/lpr mice is Th1-dependent, MRL/lpr WSX-1<sup>-/-</sup> mice showed Th2-skewed immune responses [77]. Concomitantly, instead of diffuse, proliferative GN (type IV of the World Health Organization classification of lupus nephritis) with Th1-type Ig deposition in wild-type MRL/lpr mice, WSX-1<sup>-/-</sup>/MRL/lpr mice developed membranous GN (type V) with Th2-type Ig deposition. MRL/lpr mice deficient for the EBI-3 gene showed essentially the same phenotypes [78]. In contrast, transgenic overexpression of the WSX-1 gene in T cells of MRL/lpr mice suppressed development of GN completely and improved the survival rate and the kidney function of the mice [79]. Expression of inflammatory cytokines, including IFN-y and IL-17, was suppressed significantly in the transgenic mice. Augmentation of IL-27 signaling holds potential for treatment of systemic autoimmune diseases. These results confirmed the two-sided roles of IL-27 in immune and autoimmune regulation. **Table 1** summarizes effects of administration, blockade, or augmentation of IL-27 signaling in various disease models.

When clinical application of IL-27 is taken into consideration, it would be intriguing to compare IL-27 with other cytokines currently used for therapy of various diseases. Especially,
comparison with type I IFNs will shed some lights to possible merits and demerits of targeting IL-27 signaling for disease treatment. Type I IFNs and IL-27 induce STAT1 and STAT3 phosphorylation and are involved in Th1 differentiation of naïve CD4⁺ T cells [81]. Despite the same intracellular signaling and the similar effects on Th1 differentiation and Th17 inhibition [82], both cytokines have different functions in regulation of immune responses. In addition to the Th1-promoting effects on naïve T cells, type I IFNs enhance the ability of DCs to stimulate T cell proliferation by up-regulating costimulatory and MHC class I and II molecules [83]. As such, type I IFNs are considered as a major player in the pathogenesis of SLE and type 1 diabetes [84]. In sharp contrast to these stimulatory effects of type I IFNs, IL-27 shows suppressive effects largely on macrophages and DCs [23, 31, 63]. Augmentation of IL-27 signaling by transgenic expression of WSX-1 in MRL/lpr mice ameliorated the autoimmune phenotypes of the mice [79]. Although blocking of type I IFN signaling would suppress development of SLE [85], enhancement of IL-27 signaling would inhibit disease development.

Interestingly, type I IFNs are also recognized as inhibitors and inducers of autoimmune disease. In multiple sclerosis, type I IFN therapy appears beneficial in terms of prolonged remission and lower relapse rate [86], in part as a result of its suppression of inflammatory Th17 cell responses, chemokine production, and MHC class II expression by macrophages and microglial cells [87, 88]. In addition, type I IFNs display anti-inflammatory properties in an arthritis model [89]. As IL-27 also shows similar protective effects in EAE and arthritis models [49, 65, 66, 68], type I IFNs and IL-27 would have similar beneficial potential in treatment of these diseases. As IL-27 is produced by DCs in response to type I IFN stimulation in an IRF1/-3-dependent manner [14, 90], IL-27 may be responsible in part for type I IFN-mediated immunosuppression. As the type I IFN-mediated, suppressive mechanisms are not fully elucidated, it is also possible that IL-27 works through a distinct cascade. IL-27 may show additional therapeutic effects on the diseases and may be used when disease in some patients is refractory to type I IFN treatment. Additionally, various side-effects, such as fever, malaise, arthralgia/myalgia, myelosuppression, and psychiatric disorders, have been reported for clinical use of type I IFNs. These side-effects are a result of a wide range of receptor expression in various organs and tissues. In this context, IL-27 may be more specific in regulating cell function. Although the expression of WSX-1 is detected in various types of cells, it is very weak even in CD4⁺ T cells and is

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*CLP, Cecal ligation and puncture.

TABLE 1. Effects of IL-27 and Its Signal Modification on Disease/Therapy Models
up-regulated upon cell activation. It is likely, therefore, that IL-27 exerts its suppressive effects only on activated cells in an inflammatory milieu with minimum impact on quiescent cells. Effects of long-term or high-dose administration of IL-27, however, have yet to be elucidated.

JAKs and STATs have been identified as crucial elements in cytokine signaling. These molecules hold potentials as a target for immunosuppression—especially JAK3, a protein kinase activated downstream of the common γc of a group of cytokine receptors, which emerged as an excellent target for negative regulation of immune responses mediated by “γc cytokines” [91]. Although various JAK3-specific inhibitors have been developed with promising effects [92], these compounds are less specific than have been expected. For instance, even CP-690,550, one of the most specific JAK3 inhibitors [93], is associated with anemia, as a result of inhibition of JAK2 associated with the erythropoietin receptor. In terms of specificity, enhancement of IL-27 signaling may have fewer side-effects. WSX-1-transgenic mice or IL-27-transgenic mice developed no apparent developmental or homeostatic abnormality. Topical or systemic administration of IL-27 into naive mice also showed no toxic effects (Y. Miyazaki and H. Yoshida, unpublished observations). However, as WSX-1 is expressed in non-immune cells, such as epithelial cells and endothelial cells, albeit at lower levels [10], effects of high-dose and/or long-term administration of IL-27 have yet to be elucidated. A drawback of augmenting IL-27 signaling as compared with JAK inhibitors may be developable forms of drugs. Although JAK inhibitors are small molecules and may be available in oral form, development of biologics will be necessary for enhancement of IL-27 signaling, administration of an active form of IL-27, or a humanized agonistic type of anti-IL-27R antibodies.

There are a number of issues that have to be fully solved about the therapeutic application of IL-27. One is obviously the Th1-promoting feature of IL-27. Although administration of IL-27 attenuated CIA, it exacerbated proteoglycan-induced arthritis [68, 69]. Depending on the pathogenesis of diseases, such as differential involvement of Th1 and Th17 responses, IL-27 may exhibit different effects. Indications for IL-27-mediated immunosuppression should be determined with careful review and investigation. Another issue is that there is little proof-of-concept of IL-27-mediated immune suppression in humans. Expression of IL-27 subunits and WSX-1 genes in human tissues, especially in inflammatory conditions, has been reported [17, 71, 72, 94]. In addition, IL-27 expression has been implicated in asthma and IBD in a Korean population [95, 96]. However, little is known about the immunosuppressive effects of human IL-27. Further work is required, particularly in elucidation of the precise effects of human IL-27 and also, its role in human health and disease, for understanding the diverse role of IL-27 and its clinical application.

**CONCLUDING REMARKS**

Immune responses must be controlled to guarantee pathogen elimination and simultaneously prevent tissue damage by excessive inflammation, which can also lead to autoimmune diseases. IL-12 family members play critical roles in the control of T cell developmental pathways. Among the family members, IL-27 is unique in that it has two distinct roles: one as an initiator and the other as an attenuator of immune responses [24]. Therapeutic implications of the pro- and anti-inflammatory effects of IL-27 have gathered much attention for therapeutic applications.

The major issue to be elucidated before the IL-27 signal becomes a promising target of immune therapy is the clear demonstration of the pro- and anti-inflammatory signal pathways downstream of the IL-27R. Precise roles of STAT1 and -3, their transcriptional targets, and regulation of other signals, such as c-Fos and c-Maf, downstream of IL-27R signaling, in dictating the pro- and anti-inflammatory effects of IL-27, have yet to be clarified. In addition, detailed characterization of human IL-27 has yet to be carried out in light of the comparison with murine IL-27. Only a few studies revealed the pathophysiological roles of human IL-27. With knowledge obtained in eventual studies, development of IL-27, engineered to last long in vivo, discovery of small chemical compounds that mimic IL-27/WSX-1 signaling for immunosuppression, and establishment of antagonistic as well as agonistic anti-IL-27R antibodies will no doubt contribute to the development of new therapeutic approaches to manage complex immune diseases, such as autoimmune diseases and infection-induced pathology.

**REFERENCES**


**ACKNOWLEDGMENTS**

H. Y., M. N., and Y. M. were supported in part by grants from the Ministry of Education, Science, Technology, Sports and Culture of Japan. H. Y. was also supported by Grant-in-Aid of The Japan Medical Association. The authors thank Drs. Christopher Hunter, Hitoshi Nakashima, Sinobu Suzuki, and Hitomi Mimura for helpful comments.


