Modulation of inflammation by interleukin-27

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ABSTRACT

A growing body of evidence suggests an essential role of the heterodimeric cytokine, IL-27, for regulating immunity. IL-27 is composed of two subunits (p28 and EBI3) and is classified as a member of the IL-12 family of cytokines. APCs have been recognized as a major cellular source of IL-27 following activation with microbial products or IFNs (types I and II). In this review, we describe the current knowledge of the implications of IL-27 during the pathogenesis of infectious and autoimmune diseases. Experimental studies have used genetically targeted IL-27RA$-/-$ mice, EBI3$-/-$ mice, and p28$-/-$ mice or involved study designs with administration of bioengineered IL-27/IL-27RA homologs. Whereas many reports have described that IL-27 suppresses inflammation, we also review the current literature, suggesting promotion of inflammation by IL-27 in some settings. Recent advances have also been made in understanding the cross-talk of cleavage products of the complement system with IL-27-mediated immune responses. Additional data on IL-27 have been obtained recently by observational studies in human patients with acute and chronic inflammatory diseases. Collectively, the findings from the past decade identify IL-27 as a critical immunoregulatory cytokine, especially for T cells, whereas some controversy is fueled by results challenging the view of IL-27 as a classical silencer of inflammation. *J. Leukoc. Biol.* **94:** 1159–1165; 2013.

IL-27 was first described by Pflanz and colleagues [1] in 2002 as a cytokine with activity on T cells. Notably, the discovery of IL-27 may have been complicated by the fact that the different protein chains of IL-27 are encoded on independent gene loci. Over the past decade, scientific efforts have aimed to analyze structure, signaling, and biological effects of IL-27. Despite accumulating literature on IL-27, several aspects of this cytokine remain enigmatic. In this review, we will provide a perspective of the current knowledge of IL-27 functions and implications during autoimmune and infectious diseases. We present C5a as a selective regulator of IL-27 gene expression. Furthermore, we dissect the available literature, classifying IL-27 as a proinflammatory or anti-inflammatory factor during acute and chronic models of inflammation.

STRUCTURE OF IL-27

The heterodimeric, glycosylated protein IL-27 is assembled by the subunits p28 (also known as IL-27p28, IL-27A, IL-30) and EBI3 [1]. EBI3 is a 229-aa protein (mouse: 228 aa) encoded on human chromosome 19 (mouse: chromosome 17). The subunit p28 consists of 243 aa (mouse: 234 aa) and is located on human chromosome 16 (mouse: chromosome 7). Whereas the predicted structure of p28 is a four-helical cytokine, EBI3 is considered to be a soluble receptor [2]. The predicted interface of p28/EBI3 is composed of noncovalent bonds of distinct hydrophobic and polar properties involving specific amino acid residues of p28 (Trp^{97}) and EBI3 (Phe^{97}, Asp^{210}, Glu^{159}) [2]. IL-27 displays sequence homology with members of the IL-6/IL-12 family of cytokines. Genes encoding for homologs of IL-27 subunits have been identified in nearly 20 mammalian species, whereas little information is available for other vertebrates.

In humans and rodents, other binding partners for p28 or EBI3 exist (**Fig. 1**). EBI3 can associate with the subunit p35 (IL-12A), resulting in the cytokine IL-35 [3]. In addition, p28 appears to have another binding partner, CLF-1 [4]. The existence and biological relevance of homodimers (EBI3/EBI3; p28/p28) and isolated secreted subunits of p28 or EBI3 are not entirely clear, but such molecules may act as natural antagonists of heterodimeric IL-27 [5].

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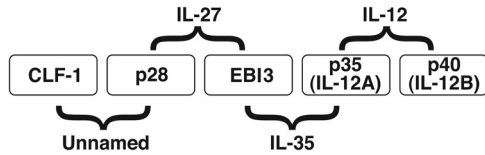
Figure 1. Subunits of IL-12 family members and their known associations as heterodimers. p35, IL-12A; p40, IL-12B.

IL-27R AND SIGNALING

IL-27 binds to a heterodimeric receptor complex, which is composed of a ligand-binding chain, IL-27RA (WSX-1, TCCR), and an additional signal-transducing chain, gp130 [6, 7]. Of note, IL-27RA includes a cytoplasmatic domain with Box 1 motif binding sites for JAK1/2, which contributes to signal transduction of the IL-27RA/gp130 dimer [6]. The common gp130 chain is shared by several other receptors for signaling of cytokines, such as IL-6, IL-11, and leukemia inhibitory factor [8]. The situation becomes even more complex with heterodimers of CLF-1/p28 binding to a trimeric receptor complex composed of IL-27RA/gp130/IL-6RA [9].

Whereas gp130 is found widely in various tissues, the expression of IL-27RA appears to be more restricted to immune cells, such as T, B, NK, plasma, and possibly cells of the myeloid lineage [7, 10, 11]. However, IL-27RA may also be expressed on transformed, malignant, nonimmune cells [12].

IL-27, via IL-27RA/gp130, activates JAK and STAT1/STAT3. STAT1 and STAT3 modulate the activity of downstream T cell lineage-specific transcription factors, such as T-bet (Th1), GATA-3 (Th2), and retinoic acid receptor-related orphan receptor γt (Th17). In addition, IL-27 signaling activates p38 MAPK and recruits the transcription factors c-Maf and the AhR [13, 14].

REGULATION OF IL-27 GENE EXPRESSION BY MICROBIAL PRODUCTS AND ENDOGENOUS FACTORS

IL-27 is expressed mainly by APCs (macrophages and DCs) after encountering microbial products. Abundant quantities of IL-27 are secreted after activation of PRRs, such as TLR3 (polyinosinic:polycytidylic acid), TLR4 (LPS), and in some reports, TLR7 (loxoribine) and TLR9 (CpG) [15–17]. Irradiated gram-negative bacteria (e.g., *Escherichia coli*) induced expression of p28 in human monocyte-derived DCs [18]. Similar results were obtained for IL-27 induction by viable *Salmonella enteritidis* [19]. Production of IL-27 has been noted during viral, bacterial, protozoan, and fungal infections in vivo [17, 20–22] (**Fig. 2**).

TLRs recruit the adaptor molecules MyD88 and TRIF for initiation of downstream signaling cascades. After activation of TLR4 with LPS, MyD88 and TRIF are required for full activation of IL-27 gene expression [23, 24]. Subsequently, transcription factors, such as NF-κB (c-Rel), IRF-1, IRF-3, and IRF-9, bind to the promoter region of p28 [23–25]. An important amplifying circuit for IL-27 production has been identified as endogenous types I

and II IFNs [23–25]. IFNs may also be a relevant trigger to initiate IL-27 production during autoimmune diseases.

Another pathway to activate IL-27 production in myeloid cells is activation of the costimulatory molecule, 4-1BB (CD137), a member of the TNFR family, using agonistic antibodies [26].

Extracellular ATP, which is viewed as a "danger signal" following tissue injury, mediates suppression of p28 and less potently of EBI3 [27]. These effects involve signaling via purinergic (P2) receptors (e.g., $P2Y_{11}$ receptor) [27]. In addition, IL-27 appears to be silenced following activation of the complement system.

IL-27 AND THE COMPLEMENT SYSTEM

The complement system is part of innate immune defenses and is required for immediate clearance of pathogens [28, 29]. In addition, complement activation products, such as C3a, C4a, and C5a, act as potent anaphylatoxins to orchestrate the activation and chemotaxis of various immune cells [29]. Complement cleavage products induce complex signaling cascades to maintain immune surveillance and tissue homeostasis [28]. For instance, the classical receptor for C5a (C5aR) is expressed abundantly on PMN leukocytes and to a lesser extent, on macrophages [15]. Ligation of C5a with C5aR suppresses gene expression and release of p28 from $F4/80^+$ macrophages [15, 30, 31]. Likewise, the C5a degradation product, $C5a_{desArg}$, has retained some functionality to antagonize p28 production from macrophages, presumably with less potency than C5a [15]. During endotoxic shock, higher amounts of p28 are generated in C5aR−/− mice compared with WT mice, whereas no such effects have been observed in mice deficient in the second C5a receptor, C5L2 [15]. In cell culture experiments, lower frequencies of F4/80+p28+ macrophages were observed, when LPS (TLR4 activation) was combined with C5a treatment. The inhibitory effects of C5a on p28 gene expres-

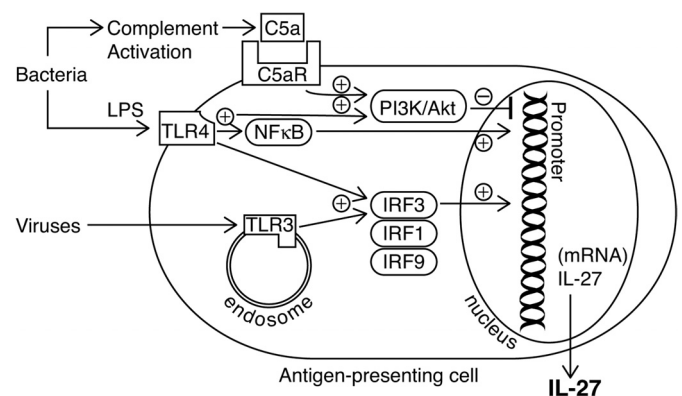**Figure 2. Regulation of IL-27 p28 gene expression by the complement system in the context of infection.** Viral infections activate the TLR3 pathway, whereas bacterial infections predominantly activate the TLR4 pathway. Simultaneous, complement activation results in generation of the anaphylatoxin, C5a, which recruits PI3K-Akt for selective inhibition of the TLR4 pathway to limit IL-27 production.

sion are linked to activation of PI3K/Akt signaling by C5a [15]. An in vivo blockade of Akt, using the small molecule inhibitor, wortmannin, resulted in threefold higher plasma concentrations of p28 during endotoxic shock [15]. Interestingly, suppression of IL-27 by C5a was specific when IL-27 was induced via TLR4, whereas TLR3-induced IL-27 was unaffected [15]. This demonstrates that the IL-27 appearance may be specifically regulated by C5a, depending on the context of bacterial (TLR4) as opposed to viral (TLR3) infections (Fig. 2). In the future, it may be important to evaluate to what extent complement-driven regulation of IL-27-dependent immune responses participates in development and progression of chronic diseases and inflammatory clinical conditions.

ACTIVATION OF INFLAMMATION BY IL-27

In first reports, IL-27 was described as promoting a rapid, clonal expansion of naive CD4$^+$ T cells and triggering IFN-γ production by CD4$^+$ T cells [1]. Since then, many investigations into the role of IL-27 in diseases have used IL-27RA−/− mice (disruption of IL-27 signaling; **Table 1**). In a T cell-transfer model of colitis, IL-27RA−/− mice were protected from colonic inflammation and weight loss [40]. Such mice showed reduced frequencies of Foxp3+ Tregs and reduced IFN-γ production [40]. In another study, CD4$^+$CD45RB$^+$ T cells from IL-27RA−/− mice failed to induce colitis in recombination-

activating gene−/− recipient mice, but on the other side, IL-27 was indispensable for prevention of colitis by Tregs [41]. Apparently, IL-27 confers long-term survival of T cells by down-regulation of Fas ligand and up-regulation of antiapoptotic caspase 8 homologue Fas-associated death domain-like IL-1β-converting enzyme-like inhibitory protein [41].

IL-27 was essential for production of IL-21 by Th follicular cells, via STAT3, in germinal centers [42]. In IL-27RA−/− mice, the production of high-affinity antibodies/autoantibodies was reduced, resulting in diminished severity of glomerulonephritis [42]. In proteoglycan-induced arthritis, IL-27RA−/− mice were protected, including delayed onset and reduced severity of disease, as documented by less cartilage destruction compared with WT mice [35]. The diminished arthritis in IL-27RA−/− mice was associated with fewer IFN-γ-producing T cells and Th17 cells [35]. On the other hand, the implications of IL-27 in arthritis are puzzling, given other reports that administration of bioengineered EBI3-L-p28-Fc fusion protein ameliorated joint inflammation in collagen-induced arthritis (ref. [36]; also reviewed elsewhere in ref. [43]). EBI3−/− mice were protected from Con A-induced T cell-dependent hepatitis, a disease model associated with IFN-γ production, along with pSTAT1 and T-bet activation [44]. STAT1 was reported to mediate IL-27-induced NO production from peritoneal-elicited macrophages [45].

It has been reported that in lung fibroblasts, IL-27 induces the expression of CXCL10 (IFN-γ-inducible protein 10) via

Table 1. Effects of IL-27 in Experimental Models of Disease

Disease model	IL-27 strategy	Major findings	Ref.
EAE	IL-27RA−/−	IL-27 suppressed development of Th17 cells.	[32]
Toxoplasma gondii infection	IL-27RA−/−	IL-27 antagonized Th17 cell functions during neuroinflammation.	[33]
T. gondii infection	IL-27RA−/−	Absence of IL-27 mediates lethal T cell-mediated inflammatory disease; excessive production of IFN-γ.	[21]
T. gondii infection	IL-27RA−/−	IL-27 induced IL-10 in T cells via STAT3.	[34]
Proteoglycan-induced arthritis	IL-27RA−/−	IL-27 promoted severity of arthritis; induction of Th1 response.	[35]
Collagen-induced arthritis	EBI3-L-p28-Fc	IL-27 attenuated histology scores and joint inflammation; less proinflammatory cytokines.	[36]
Listeria monocytogenes	IL-27RA−/−	IL-27 promoted IL-10 production; lower frequency of IL-10+ T cells in IL-27RA−/− mice.	[37]
Trypanosoma cruzi infection	IL-27RA−/−	IL-27 antagonized parasitemia, severe liver injury, mortality, and proinflammatory cytokines (IL-6, TNF-α, IFN-γ).	[38]
Septic peritonitis	IL-27RA-Fc soluble receptor	There was an improved survival with blockade of IL-27 during sepsis.	[20]
TNBS-induced colitis	Single-chain human IL-27	IL-27 treatment improved disease scores and inflammation in colon and LNs; proinflammatory cytokines (IL-17) were suppressed.	[39]
T cell-dependent colitis	IL-27RA−/−	IL-27RA−/− showed less weight loss and colonic inflammation, including IFN-γ production; more Foxp3+ T cells.	[40]
N.A.	p28-Transgenic mice	There was overexpression of p28 (in T and B cells), independent of EBI3; p28 antagonized interaction of IL-6 with gp130.	[5]

p38 MAPK and PI3K-Akt signaling pathways [46]. Proinflammatory CXCL10 plays an important role for chemoattraction of activated T cells to sites of inflammation. CXCL10−/− mice display impaired T cell responses, including proliferation after antigen challenge, secretion of IFN-γ, and pathogen clearance [47].

Finally, IL-27 promotes protective tumor surveillance against endogenous murine tumors [48]. In settings of mammary carcinoma or fibrosarcoma, the development and tumor growth in IL-27RA−/− mice were accelerated, and IL-27RA−/− mice displayed a defective IFN-γ response and higher frequencies of Tregs [48].

INHIBITION OF INFLAMMATION BY IL-27

Several reports have suggested that IL-27 may play a minor role for clearance of intracellular pathogens during infection but may be essential for immune down-modulation during the chronic phases of inflammation, which is critical for reduced intensity of tissue injury and organ dysfunction. For instance, IL-27RA−/− mice infected with *T. gondii* were still capable of developing a protective T cell response and control of parasite replication [21]. However, infected IL-27RA−/− mice failed to silence these protective responses, and mice consecutively developed a lethal, T cell-mediated inflammation, including exuberant IFN-γ production and T cell proliferation [21]. This is, in part, contradictory to earlier findings of IL-27 synergizing with IL-12 to induce IFN-γ from naive CD4+ cells [1]. Following protozoan infection with *T. cruzi*, IL-27RA−/− mice displayed prolonged parasitemia, severe liver injury, and increased mortality compared with WT mice [38]. Splenocytes from IL-27RA−/− mice secreted enhanced levels of Th2 cytokines, as well as enhanced IFN-γ, IL-6, and TNF-α production in liver [38].

It is well established that IL-27 can act with anti-inflammatory function via induction of IL-10 from various T cell subsets, including Tregs [49]. IL-27 used the transcription factors STAT1 and STAT3 to induce IL-10 in Th1, Th2, and Th17 cells [34]. In another study, IL-27-mediated gene expression of IL-10 in IFN-γ+ Foxp3-Th1 cells, via STAT1 and IL-27RA−/− mice, displayed fewer IL-10+ T cells after *L. monocytogenes* infection or EAE [37]. Likewise, IL-27 regulated IL-10 production by CD4+ cells following infection with *Leishmania major* [50]. Mechanistically, IL-27 appears to induce the transcription factor AhR, which partners with c-Maf to transactivate the IL-10 gene promoter region, at least in Tregs (Tr1) [13]. Additional transcription factors involved in IL-27-mediated expression of IL-10 have been identified as Egr-2 and B lymphocyte-induced maturation protein-1 [51]. IL-27 failed to induce IL-10 in CD4+ T cells derived from Egr-2−/− mice [51]. The loss of gp130 expression on CD8+ memory cells was associated with limited IL-27 responsiveness and diminished IL-10 production during antigen rechallenge [52].

It is worth mentioning that several anti-inflammatory effects of IL-27 are independent of IL-10, such as IL-27-mediated suppression of Th17 cells during EAE [53]. In addition, IL-27 blocks the pathway for Th9 cell differentiation when added to cultures of naive Th0 cells and also abrogates the encephalitogenic potential of Th9 cells during EAE [54].

Whereas the nature of IL-27 effects on Th1 cell responses (and IFN-γ) may vary in the context of different diseases, there is clear evidence that IL-27 is a suppressor of Th2 and Th17 cells [11, 38, 49]. IL-27 promotes a population of Tregs (T-bet+CXCR3+) with distinct transcriptional activation profiles compared with Tregs induced by IFN-γ [55]. In Tr1 cells, metallothioneins appear to act as natural antagonists of IL-27 [56]. For example, in Tr1 cells from metallothionein-deficient mice, IL-27 mediated more abundant STAT1/STAT3 phosphorylation, resulting in higher IL-10 production, together with more efficient suppression of effector T cell proliferation [56].

Findings in several other studies have suggested anti-inflammatory properties of IL-27. IL-27RA−/− mice were highly susceptible in the recovery phases following *Plasmodium berghei* infection, and IL-27RA−/− mice developed exuberant CD4+ Th1 cell-associated liver pathology in an IL-10-resistant manner [57]. IL-27A signaling, during experimental malaria infection, suppressed the development of killer cell-like receptor group 1-expressing, terminally differentiated Th1 cells by desensitizing these cells to the effects of IL-12 and IL-2 [58]. CD4+ T cells from spleen expressed higher numbers of the CCR5 receptor (ligands: CCL4, CCL5) when genetically deficient of IL-27RA [59]. This suggests that during malaria, infection IL-27 restricts the chemotactic response of CD4+ T cells, although this appears to be contradictory to the findings that IL-27 can induce CXCL10 (see above) [46].

In collagen-induced arthritis, using a bioengineered EBI3-L-p28-Fc protein ameliorated joint inflammation, histology scores, and release of IL-6, IL-17, and IFN-γ [36]. TNBS-induced colitis was improved, including suppression of IL-17, following administration of IL-27 [39]. In contrast to EBI3−/− mice [44], conditional CD11c-p28(flox/flox)−/− mice were highly susceptible to Con A-induced liver injury [60]. In keratinocytes and fibroblasts, IL-27 induced the production of anti-inflammatory IL-18-binding protein, a natural antagonist of IL-18 [61]. Finally, transgenic mice, overexpressing p28 in T and B cells, were found to suppress IL-6R signaling by direct interaction of p28 with the gp130R chain [5].

IMPLICATIONS OF IL-27 DURING HUMAN DISEASE

There is accumulating evidence suggesting various roles of IL-27 during autoimmune diseases in humans. Elevated concentrations of IL-27 were found in synovial fluids but not plasma of patients with rheumatoid arthritis [62]. Gene expression of p28 and EBI3 was elevated in colonic mucosa of patients with active Crohn's disease [63]. In patients with psoriasis, the serum concentrations of IL-27 were elevated compared with healthy controls, correlating with disease severity and release of IFN-γ [64]. It has been suggested that endogenous release of IL-27 mediates the clinical response following therapeutic administration of IFN-β in patients with multiple sclerosis [65]. IL-27 concentrations were also elevated in patients with moderate or severe aplastic anemia, correlating with the release of IFN-γ [66]. SNPs, in the gene encoding for

IL-27, have been associated with the incidence of several auto-immune diseases, such as asthma [67], chronic obstructive pulmonary disease [68], and inflammatory bowel diseases [68], all studied in Asian populations. One of these SNPs (g.-964A>G) is located in the promoter region of p28 and may influence the binding affinity of a yet-to-be-identified transcription factor [67].

IL-27 has been evaluated recently as a novel, diagnostic biomarker for bacterial infections [69], whereas production of IL-27 appears to be suppressed during HIV infection [70]. However, a functional IL-27 response would likely be helpful to control HIV replication [71]. IL-27 was found recently to confer resistance of macrophages to HIV by inhibiting spectrin β nonerythrocyte 1, a required host factor for HIV-1 infection [72]. Furthermore, IL-27 induced the expression of novel microRNAs (miR-SX1, -SX2, -SX3, and -SX6) in human macrophages, which antivirally target the open reading frames of a number of viruses (HSV-1, HSV-2, and HHV-8) [73]. On the other hand, whereas infections with hepatitis B virus mediate the release of IL-27 [74], quantification of IL-27 may not be useful in determining pathologic stages and progression of liver disease [75]. In addition, IL-27 in serum was elevated significantly in patients with seasonal H1N1 virus subtype (H3N2) or the 2009 pandemic H1N1 infection [76].

CONCLUDING REMARKS

During the past decade, our knowledge of the biology of IL-27 has evolved considerably. Accumulating literature indicates the potential of IL-27 as an essential regulator of adaptive immunity, most notably for T cell functions. One hallmark of IL-27 research was to identify its capability to induce anti-inflammatory IL-10 from various T cell subsets via STAT1/STAT3 pathways. However, the versatile properties of IL-27 as an enhancer or silencer of inflammation are still incompletely understood. Recently, the involvement of the complement systems as a specific regulator of IL-27 production has added a new facet to the puzzling picture of IL-27 biology. Conflicting data for IL-27, promoting or antagonizing inflammation, may, in part, be explained by differences in the disease models, transgenic mouse strains, or bioengineered IL-27/IL-27RA proteins. Future scientific efforts will need to analyze further the molecular mechanisms involved in expression and biological activity of IL-27 in different cell types and models of disease.

To identify potential therapeutic approaches of targeting IL-27 in humans with infectious or autoimmune diseases will be important. Nevertheless, the complex roles of IL-27 may complicate the search for effective therapeutic exploitation of the IL-27 system. One risk, shared by many immunomodulatory pharmaceuticals, may be that manipulation of IL-27 during autoimmune diseases may increase the occurrence of infections or even malignancies. Moreover, it is currently not entirely clear if IL-27 administration should be favored over a blockade of IL-27 in certain autoimmune diseases, such as rheumatoid arthritis. In the future, another potential area of interest may be to evaluate further the roles of IL-27 in cardiovascular diseases and tumor biology. Any therapeutic interventions regarding IL-27 and IL-27RA will require a careful medical evaluation before clinical studies can be considered.

AUTHORSHIP

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DISCLOSURES

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