

Chapter 1

IL-27: Structure, Regulation, and Variability

Abstract Interleukin 27 (IL-27) is a novel cytokine secreted by stimulated antigen-presenting cells. Initial studies on the biology of IL-27 provided evidence for its role in the initiation of Th1 responses; however, subsequent work has indicated that IL-27 has broad stimulatory as well as inhibitory effects on Th1, Th2, Th17, Treg, and Tr1 subsets of T cells as well as NK cells and B cells. Together with other members of IL-12 family, it orchestrates initiation of inflammatory processes and sustains immune polarization. IL-27 is a heterodimer composed of IL-27p28 and EBI3. EBI3 has been found to form a biologically active complex with IL-12p35 (known as IL-35) and IL-23p19 (known as IL-39), while IL-27p28 can form a dimer with CLF. IL-27p28 has also its own inherent activity and can act on its own as IL-30. Here, we have discussed the variability of IL-27 gene, protein structure of IL-27 and its receptor, and molecular mechanisms regulating IL-27 expression.

Keywords IL-27 · p28 · EBI3 · IL-27R α · sIL-27R α · TCCR · WSX-1

1.1 IL-27 and Its Receptors

1.1.1 *The IL-27 Protein*

IL-6/IL-12 family of cytokines, apart from IL-27, includes IL-11, IL-23, IL-31, IL-35, leukemia inhibitory factor (LIF), oncostatin M, ciliary neurotrophic factor (CNTF), cardiotrophin-like cytokine (CLC), neuropoietin, and cardiotrophin-1. Those cytokines share structural makeup consisting of α -subunit with a four-helix bundle fold and β -subunit characterized by a ~ 200 residue-long cytokine-binding homology region (CHR). The CHR module consists of two fibronectin type III (FNIII) domains connected by a linker (Wang et al. 2009). IL-27 is a heterodimer consisting of the Epstein–Barr-induced gene 3 product (EBI3) and IL-27p28 (Pflanz et al. 2002). By comparison with other IL-12-related cytokines, IL-27 differs in that its subunits are not covalently linked.

Translation of an IL-27p28 mRNA results in a precursor protein product of 243 amino acid residue length, molecular weight of 24.5 kDa, and isoelectric point of 6.21. The first 28 amino acids of the N terminus form a signal peptide crucial for secretion of IL-27 heterodimer to the extracellular space. EBI3 precursor protein consists of 229 acid residues with 25,396.35 g/mol molecular weight and has isoelectric point of 9.43.

There is no crystal structure of IL-27p28 nor EBI3. The IL-27p28 structure model built by Rousseau et al. adopts a four-helix bundle fold, with two additional short α -helices located in the AB loop of the cytokine subunit. The loop between C and D helices of IL-27p28 contains a stretch of 13 consecutive glutamic amino acid residues (amino acids in positions 164–176), which is unique among IL-12 family cytokines and possibly contributes to the functional activity of the cytokine (Rousseau et al. 2010).

The EBI3 structure, typical for soluble cytokine receptors, consists of a tandem pair of modified FNIII domains. This domain typically contains two pairs of cysteine residues forming disulfide bridges and a characteristic WSXWS signature motif (Dagil et al. 2012). In EBI3, the actual sequence of WSXWS motif located in the loop between *F'* and *G'* β -strands is LSDWS (amino acid positions 214–218). Ebi3 is unique among IL-6/IL-12 family members in that it is lacking the N-terminal immunoglobulin domain (Jones and Vignali 2011).

In silico and site-directed mutagenesis studies have shown that the predicted IL-27p28/EBI3 interface consists of two areas with distinct hydrophobic and polar properties. IL-27p28 Trp97 and EBI3 Phe97 residues form an aromatic hydrophobic cluster surrounded by polar area abundant in salt bridges composed of positively charged IL-27p28 arginine residues Arg55, Arg67, and Arg219 and negatively charged EBI3 Glu124, Glu159, and Asp210 residues.

By molecular modeling, further confirmed by alanine-scanning mutagenesis, several EBI3 residues (Phe77, Glu139, Phe141, Lys144, Asp187, Asp190, Tyr191) were found to take part in the formation of heterodimer with IL-27p28 (Rousseau et al. 2010; Jones and Vignali 2011). However, no single mutation in aforementioned sites disrupts completely the dimer formation.

The binding of IL-6/IL-12 family of cytokines to their receptors depends on three contact sites. Site 1 of IL-27 is formed by the C-terminal parts of the IL-27p28 AB loop and the α D helix and by residues in loops located at the boundary of the two FNIII domains of EBI3 protein. The critical residues are IL-27p28 Trp97, Arg55, Arg67, Arg219, Arg216 and EBI3 Phe97, Glu124, and Asp210, respectively. IL-27p28 Arg141, Arg145, Arg149, Arg152, and EBI3 Arg191 were identified, but not further analyzed, as candidate residues for site 2. Site 3 is located at the N terminus of the IL-27p28 α D helix and engages the Ig domain of gp130 subunit of IL-27 receptor. Trp197 has been shown to be crucial for this interaction (Rousseau et al. 2010).

1.1.2 Cell Membrane IL-27 Receptor

IL-27 receptor (IL-27R) is a heterodimer. It is composed of the IL-27R α also known as T cell cytokine receptor (TCCR) or WSX-1 and the protein gp130, a signal transducing chain that is utilized by several other cytokines, including IL-6, IL-11, CNTF, LIF, CLC, cardiotrophin-1, and oncostatin M (Batten and Ghilardi 2007; Jones et al. 2011; Pflanz et al. 2002; Pflanz et al. 2004). IL-27R α has two isoforms of ~ 95 and ~ 115 kDa molecular weight. These isoforms corresponded to two different *N*-glycosylated variants, as *N*-glycanase treatment results in a single ~ 80 -kDa product (Dietrich et al. 2014).

IL-27R is capable of binding EBI3/IL-27p28 heterodimer, but not individual subunits. IL-27 binds to IL-27R α in the absence of gp130; however, the both subunits are required for signal transduction (Pflanz et al. 2004).

The expression of gp130 is almost ubiquitous; therefore, responsiveness to IL-27 is limited by IL-27R α expression. The expression of IL-27R α was reported on T cells, B cells, NK cells, monocytes, neutrophils, and mast cells. To a much lesser extent, it has also been observed in macrophages, hepatocytes, vascular endothelium, keratinocytes, and Langerhans cells (Kanda and Watanabe 2008; Shimizu et al. 2006; Hibbert et al. 2003; Pflanz et al. 2004; Villarino et al. 2005; Larousserie et al. 2006; Batten and Ghilardi 2007; Yoshida et al. 2009; Swaminathan et al. 2013).

Mouse neuronal cells express an alternatively spliced IL-27R α isoform of 33 kDa molecular weight, lacking exons 7–14, that encodes part of the extracellular domain (Hashimoto et al. 2009b). This truncated IL-27R α isoform was reported to associate with ciliary neurotrophic factor receptor and gp130, similarly to full-length membrane IL-27R α , to form a tripartite functional receptor for a neurotrophic peptide humanin (Hashimoto et al. 2009a).

1.1.3 Soluble IL-27 Receptor

Extracellular portion of IL-27R α receptor is spontaneously released from cells through proteolytic cleavage by metalloproteases and can be detected in sera of healthy humans (Dietrich et al. 2014). Shedding of soluble form of IL-27R α (sIL-27R α) was detected after stimulation of T cells with phytohaemagglutinin or beads coated with CD2/CD3/CD28, whereas human tonsillar B cells and CD14+ monocytes released sIL-27R α after stimulation with anti-CD40 antibody and LPS, respectively. Amount of sIL-27R α secreted by B cells and monocytes was notably lower compared to T cells. Mature monocyte-derived dendritic cells that constitutively express high amounts of IL-27R α mRNA were found to produce sIL-27R α constitutively as well.

Inhibition of IL-27R α cleavage by metalloprotease inhibitors did not substantially increase its cell surface level, indicating that only a small proportion of

IL-27R α was spontaneously shed from the cell surface, as commonly observed for cytokine receptors (Dietrich et al. 2014).

sIL-27R α binds recombinant IL-27, but not EBI3, consistent with the findings for cellular IL-27R α (Pflanz et al. 2002); however, binding to IL-27p28 was not verified.

sIL-27R α acts as an inhibitor of IL-27 signaling and prevents IL-27-mediated phosphorylation of STAT1 and STAT3 in IL-27-responsive BL2 cell line. STAT1 is activated in BL2 cells by low concentrations of IL-27, whereas higher concentrations are necessary for STAT3 activation. However, sIL-27R α inhibits phosphorylation of both STAT proteins at low concentration. This latter finding indicates that IL-27 complexed with its soluble receptor does not activate the gp130/STAT3 axis by trans-signaling, as has been described for IL-6/IL-6R α complex. sIL-27R α is most likely a competitive inhibitor of IL-27 binding to its cell surface receptor. sIL-27R α at equimolar dose as IL-27 induces a 50 % inhibition of IL-27-mediated STAT1 activation, whereas a complete inhibition could be observed at fourfold molar excess. The inhibitory effect of sIL-27R α is specific to IL-27, and it does not inhibit STAT activation by another STAT1-inducing cytokine, interferon γ (IFN- γ) (Dietrich et al. 2014).

1.2 The Landscape of IL-27-Related Proteins

The marked difference between EBI3^{-/-} and IL-27R α ^{-/-} mice, corroborated by differences between IL-27R α ^{-/-} and IL-27p28^{-/-} cell lines, has led to the discovery of several IL-27-related cytokines. Shared signaling pathways among them complicate the analysis of biologic activity of IL-27. EBI3, secreted readily by itself, also forms an alternative biologically active heterodimer with IL-12p35, forming IL-35 (Collison et al. 2012). Early studies suggested that IL-27p28 is not secreted unless it is coexpressed with EBI3. However, forced expression of IL-27p28 revealed that it is also secreted as a monomer (Shimozato et al. 2009) and acts as a self-standing cytokine. IL-27p28 is able to form a trans-signaling composite cytokine with soluble IL-6 receptor α (sIL-6R α). Moreover, IL-27p28 can form heterodimer with cytokine-like factor 1 (Crabe et al. 2009). IL-27-related cytokines and their respective receptors are depicted in Fig. 1.1.

1.2.1 IL-30

Monomeric IL-27p28 acts as a self-standing cytokine endowed with its own functional properties and in this form is referred to as IL-30. In the absence of EBI3, IL-30 binds to the IL-6 receptor α (IL-6R α) and a homodimer of gp130. Low concentrations of IL-30 induce STAT1/3 phosphorylation and proliferation of cytokine-dependent cell line expressing gp130 and IL-6R α but fail to do so in

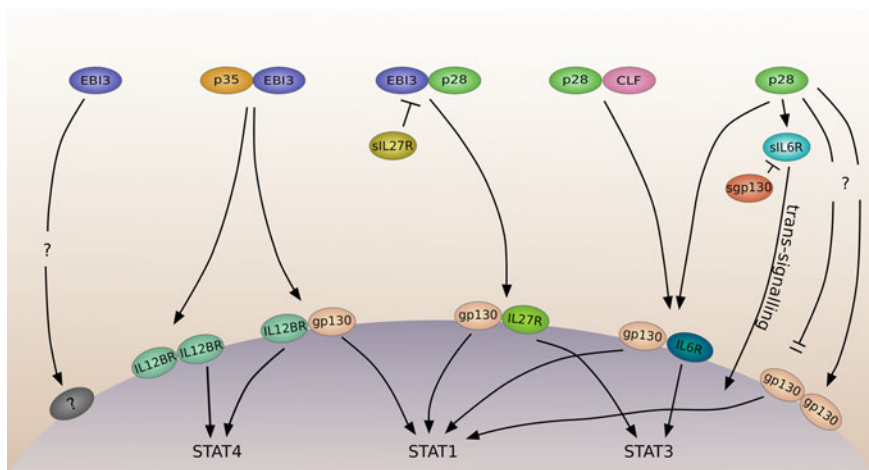


Fig. 1.1 IL-27, related cytokines, and their receptors. *Lines with arrowheads* stimulatory effect, *blunt-ended lines* inhibitory effect, *sIL-27R*-soluble IL-27 receptor α , *sgp130*-soluble gp130 protein. Graphic by Marta Jakubowska

IL-6R α -negative cells. IL-30 binds IL-6R α by the use of the same binding site as IL-6 since tocilizumab, a monoclonal antibody that prevents binding of IL-6 to the IL-6R α via binding site 1 of IL-6, blocks IL-30- and IL-6-induced proliferation of cytokine-dependent cells (Garbers et al. 2013). IL-27R α is not involved in IL-30/IL-6R α -mediated receptor complex formation.

IL-30, as IL-27, is capable of inhibiting Th17 differentiation and IL-17 production by CD4 $^{+}$ T cells in vitro (Stumhofer et al. 2006; Zhang et al. 2015a).

Apart from immune cells, IL-6R α is expressed mainly on hepatocytes. Not surprisingly, most of currently available data on IL-30 concerns hepatoprotective role of IL-30. IL-30 prevented liver injury by downregulating the proinflammatory cytokines TNF- α , IL-1 β , IL-6, and IFN- γ in lipopolysaccharide/D-galactosamine-induced mouse model (Liu et al. 2013). Hepatoprotective effect of IL-30 has been observed also in IL-12-, IFN- γ -, and concanavalin A-induced liver toxicity models (Dibra et al. 2012). IL-30 ameliorates liver fibrosis by recruiting natural killer-like T cells to the liver to remove activated hepatic stellate cells (Mitra et al. 2014).

Recently, IL-30 expression by prostatic cancerous epithelia, CD68 $^{+}$ macrophages, CD33 $^{+}$ /CD11b $^{+}$ myeloid cells, and CD14 $^{+}$ monocytes has been reported in prostate cancer tumor microenvironment (Di Meo et al. 2014). Expression of IL-30 by prostate cancer or tumor-infiltrating leukocytes correlates with advanced disease grade and stage. IL-30 also stimulates in vitro proliferation of PC3 prostate cancer cell line that expresses both gp130 and IL-6R α . Cancer cell proliferation was also higher in IL-30-expressing human prostate primary tumors and metastasis than in IL-30-lacking samples (Di Meo et al. 2014). IL-30 suppresses the expression of selective leukocyte chemoattractants such as chemokine ligand 16, tumor necrosis

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