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REVIEW

Cytokines Focus

Historical overview of the interleukin-6 family cytokine

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Interleukin-6 (IL-6) has been identified as a 26-kD secreted protein that stimulates B cells to produce antibodies. Later, IL-6 was revealed to have various functions that overlap with other IL-6 family cytokines and use the common IL-6 signal transducer gp130. IL-6 stimulates cells through multiple pathways, using both membrane and soluble IL-6 receptors. As indicated by the expanding market for IL-6 inhibitors, it has become a primary therapeutic target among IL-6 family cytokines. Here, we revisit the discovery of IL-6; discuss insights regarding the roles of this family of cytokines; and highlight recent advances in our understanding of regulation of IL-6 expression.

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Introduction

Cytokines are small (15–20-kD) soluble proteins that transduce signals in adjacent cells or transmit signals to distant organs. Most cytokines associate with specific receptors, through which they transmit intercellular signals to their target cells (Dinarello, 2007). Cytokines play diverse roles in the regulation of immunity, development, metabolism, aging, and cancer. Multifunctional cytokines, of which the IL-6 family members define the paradigm, exhibit functional pleiotropy and redundancy.

The IL-6 family consists of 10 ligands and 9 receptors (Fig. 1). The members of this cytokine family have a common core structure and share a signal transducer in their receptor complex, which plays highly diverse roles in the body. Among the family members, the IL-6/IL-6R axis contributes to the progression of several diseases, and inhibition of this axis is highly effective against diseases such as rheumatoid arthritis (RA), Castleman disease, and cytokine release syndrome (Kang et al., 2019). Additionally, several molecules that interact with the cytoplasmic domains of these receptors have also been identified: the JAK family of tyrosine kinases and members of the STAT family. Indeed, inhibitors targeting IL-6 itself, IL-6R α chain (IL-6Rα), or JAK family proteins are efficacious against various immune disorders (Narazaki and Kishimoto, 2018).

Here, we revisit the discovery of the IL-6 cytokine family and discuss the signaling events mediated by members of this family and their receptors, with a particular emphasis on IL-6 itself. We discuss current issues regarding the regulation of IL-6 family gene expression and the potentials as therapeutic targets.

Historical perspectives: From the discovery of IL-6 to development of an IL-6R blocking antibody

IL-6 is the most prominent example of a cytokine that is relevant to inflammatory diseases. In the 1970s, IL-6 was originally identified by Kishimoto's group as a soluble protein produced by T cells that activates the differentiation of B cells into antibody-producing cells; accordingly, it was initially known as B cell stimulatory factor 2 (BSF-2; Kishimoto and Ishizaka, 1976). In 1986, IFN-β2 and a 26-kD protein were identified in fibroblasts; they were shown to be identical to BSF-2 (Haegeman et al., 1986; Zilberstein et al., 1986). Simultaneously, cDNA of the human BSF-2 gene was successfully cloned (Hirano et al., 1986). Later, hepatocyte-stimulating factor and plasmacytoma growth factor were cloned and also shown to be IL-6, highlighting the protein's diverse biological activities (Gauldie et al., 1987). The molecule was first designated IL-6 in 1988 at a conference entitled "Regulation of the Acute Phase and Immune Responses: A New Cytokine" (Sehgal et al., 1989).

Following molecular cloning of IL-6, its receptor and signaling molecules were cloned one after another. The human IL-6R was first cloned in 1988 (Yamasaki et al., 1988). It comprises an Ig-like domain; a cytokine receptor family domain with tryptophan-serine-X-tryptophan-serine (WSXWS) motif, which is

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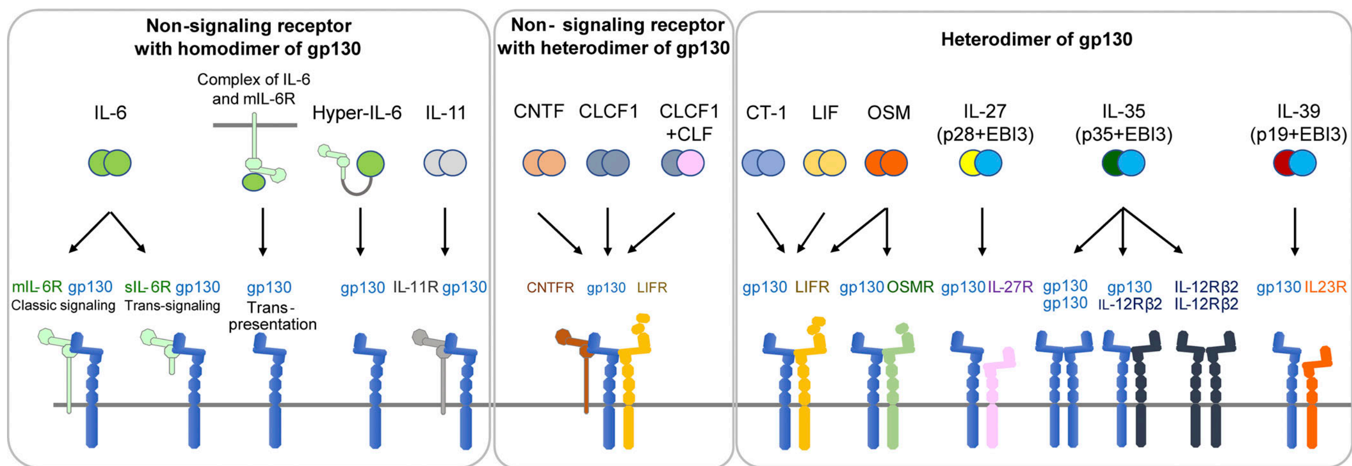

Figure 1. **Receptor composition of IL-6 family cytokines.** IL-6 family cytokines use gp130 to transduce their signals through gp130 homodimers or gp130-containing heterodimers. IL-6, IL-11, CNTF, CLCF1, and CLCF1/CLF require binding of their nonsignaling receptor to transduce signals. A new group of members (IL-27, IL-35, and IL-39) are heterodimeric cytokines: IL-27 consists of IL-27/p28 (IL-27α) and EBI3 (also known as IL-27β); in conformity to IL-12 containing IL-12p40 (IL-12α) and IL-12p35 (IL-12β), IL-23 (IL-23p19 [IL-23α] and IL-23p40 [IL-23β]), IL-35 (IL-23p40 and EBI3), and IL-39 (IL-23p19 and EBI3; Hunter, 2005; Ning-Wei, 2010; Wang et al., 2016a). These new members activate heterodimers of gp130. IL-6R exerts its biological effects via three different signaling modes. IL-6R expression is restricted to hepatocytes and several types of immune cells, whereas gp130 is ubiquitously expressed, reflecting the diverse roles of IL-6. In the classical mode of IL-6 signaling, the cytokine interacts with mIL-6R in cells that also express gp130 (Hunter and Jones, 2015). IL-6 also binds soluble IL-6R (sIL-6R), which is shed from cells following cleavage by ADAM metalloprotease 17 (ADAM17), and is also created by alternative mRNA splicing (Lust et al., 1992). The IL-6–sIL-6R complex binds to gp130, forming a dimer that initiates intracellular signaling, a process referred to as trans-signaling. Recently, a third mode of IL-6 signaling was identified: IL-6 trans-presentation (Heink et al., 2017). This mode is specific to dendritic cells, in which the IL-6–mIL-6R complex is presented to gp130 expressed on T cells to prime pathogenic Th17 cells. These alternative modes of IL-6 signaling contribute to multiple cellular processes.

predicted to function as a ligand interaction site (Bazan, 1990a,b); a membrane-spanning region; and a short cytoplasmic domain that is dispensable for signal transduction. In 1990, Kishimoto's group discovered a 130-kD glycoprotein (gp130, also known as CD130) as another receptor component that functions as signal transducer of IL-6 (Hibi et al., 1990). It consists of 918 amino acids with a single Ig-like domain and 5 fibronectin type III domains, of which the second and third constitute the cytokine receptor family module. Cloning of two receptor components led to clarification of the mechanism of the IL-6 receptor system, in which IL-6 binds to IL-6R alone, and this complex associates with gp130 to induce signaling (Hibi et al., 1990; Taga et al., 1989; Fig. 1). Only the complex of IL-6 and IL-6R, but not either protein alone, exhibits measurable affinity for gp130 (Hibi et al., 1990; Taga et al., 1989). These findings led to the targeting strategy for IL-6 signals by the development of inhibitory antibodies (Tanaka et al., 2014). Some cytokines show functional redundancy with IL-6 and share gp130 as a signal transduction molecule in their receptor systems; thus, the concept of the IL-6 family cytokines was proposed. Members of the IL-6 family cytokine include IL-11 (Paul et al., 1990), oncostatin M (OSM; Malik et al., 1989), leukemia inhibitory factor (LIF; Gearing et al., 1987), cardiotrophin 1 (CT-1; Pennica et al., 1995), ciliary neurotrophic factor (CNTF; Lin et al., 1989), cardiotrophin-like cytokine factor 1 (CLCF; Vlotides et al., 2004), IL-27 (Pflanz et al., 2002), IL-35 (Niedbala et al., 2007), and IL-39 (Wang et al., 2016b).

In the 1990s, research aimed at characterizing intracellular signaling by gp130 intensified. Initiation of IL-6 signaling through gp130 is mainly mediated by phosphorylation of JAK family kinases, which are constitutively associated with the cytoplasmic region of gp130. JAK1 elicits phosphorylation and homodimerization of STAT3, and then induces translocation into the nucleus and its transcriptional activity (Kang et al., 2019). The JAK–STAT pathway is a common pathway of receptors for hematopoietic factor, IFN, and endocrine hormones such as growth hormone and prolactin (Lütticken et al., 1994).

Identification of two different IL-6 receptor proteins clarified the role of signaling through IL-6 and cognate receptors in various diseases and led to the development of several inhibitors targeting IL-6 or IL-6R, as well as selective blockade of IL-6R signaling. In 1991, a trial of murine anti–IL-6 monoclonal antibody was first performed in a patient with myeloma (Klein et al., 1991). This therapy improved tumor outcome and suppressed acute-phase responses. Throughout treatment, however, IL-6 accumulated in the patient's plasma due to formation of an immune complex with anti–IL-6 antibody (Lu et al., 1992). This immune complex prevented the elimination of IL-6 and led to high levels of IL-6 in the serum. Consequently, treatment with IL-6 inhibitor was stopped, highlighting the superiority of anti–IL-6R therapy over anti–IL-6 agents.

At the same time, Kishimoto's group revealed a critical role of IL-6 in inflammatory diseases (Hirano et al., 1988; Hirano et al., 1987) and detected augmented IL-6 levels in sera of patients with cardiac myxoma, who develop a broad range of inflammatory symptoms that disappear after their tumors are removed (Jourdan et al., 1990). Kishimoto's group reported high levels of IL-6 production in the synovium of patients with RA. A decade later, the humanized anti–IL-6R antibody, tocilizumab, which blocks binding of IL-6 to the IL-6R and thereby blocks the IL-6R

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signaling cascade, was developed by Kishimoto and Chugai Pharmaceutical Co. This agent is now used around the world as a therapy for chronic and acute inflammatory diseases (Kang et al., 2019). Moreover, substantial pipelines of therapies targeting IL-6 or IL-6R signaling molecules have been established for several diseases. Consequently, IL-6 targeting is considered to be a promising therapeutic approach in patients with inflammatory diseases and provides an example of a case in which targeting an individual cytokine has dominant or nonredundant activities (Narazaki and Kishimoto, 2018).

Overview of the IL-6 family cytokines and receptor system: Pleiotropy and redundancy

The original IL-6 family cytokines consist of seven cytokines: IL-6, LIF, CNTF, CLCF1, OSM, CT-1, and IL-11 (Jones and Jenkins, 2018). All seven members contain a four-helix bundle structure and associate with gp130 in the presence of their cognate receptor. IL-6 and IL-11 signals are transduced by a homodimer of gp130, whereas other family members transduce their signals through gp130 and an alternative β subunit (Fig. 1). Notably, three members, IL-27, IL-35, and IL-39, have recently been added to the family (Collison et al., 2012; Wang et al., 2016b). These new members are heterodimeric cytokines consisting of p28, p35, and p19; their common subunit is the protein encoded by Epstein-Barr virus–induced gene 3 (EBI3). EBI3 belongs to the cytokine receptor family; hence, IL-27, IL-35, and IL-39 have structural similarities to the IL-6/sIL-6R (soluble form of IL-6R) complex and IL-12 family cytokines (Fig. 1).

The pleiotropic functions of IL-6 and IL-6–related cytokines are summarized in Table 1. To understand the mechanisms of pleiotropy and redundancy of IL-6 family cytokines, we must first understand the receptor system for the IL-6 cytokine family. Among receptors for IL-6 family cytokines, IL-6R is a specialized receptor for IL-6. Interestingly, when IL-6R is cleaved from the cell surface to yield sIL-6R, it can form a complex with IL-6, explaining the cytokine's pleiotropic function (Mackiewicz et al., 1992; Fig. 1). IL-6 engages either the membrane-bound form of IL-6R (mIL-6R) or sIL-6R, along with two subunits of gp130, to form a hexamer (Boulanger et al., 2003), thus mediating classic signaling or trans-signaling, respectively (Fig. 1). Although cells expressing gp130 respond to IL-6, IL-6 trans-signaling affects more target cells, because this mechanism activates even those cells that do not express mIL-6R. A similar structure facilitates formation of the IL-11–IL-11R complex (Barton et al., 2000).

Gp130, a receptor component shared by the IL-6 cytokine family, is ubiquitously expressed in several organs including the spleen, lung, heart, and liver. Its expression pattern is not parallel to that of IL-6R, suggesting that gp130 is involved in signal transduction of other cytokines. Some cytokines, including IL-6, IL-11, CNTF, CLCF1, and the CLCF1/CLF heterodimer, use nonsignaling receptors. Although CNTF first associates with CNTFR, it also binds to IL-6R with lower affinity than the IL-6–IL-6R interaction (Schuster et al., 2003). Additionally, IL-6 family members transduce signals though gp130 homodimers or heterodimers. The CLCF1/CLF heterodimer binds to CNTFR and transmits their signals through gp130—LIFR. On the other hand, LIF, OSM, and viral IL-6 bind directly to different types of gp130 complexes without nonsignaling receptors: gp130–LIFR, gp130–OSMR, and gp130–gp130, respectively (Aoki et al., 2001). Structural analysis demonstrated the redundancy of gp130 and LIFR, showing that the cytoplasmic regions of these receptors contain specific YXXQ, YXPQ, or YXXV motifs that are essential for recruitment and activation of SH2 domain–containing molecule, STAT3, STAT1, or SHP2, respectively (Stahl et al., 1995). These factors have activities to transmit signal transduction.

New members of the group, IL-27, IL-35, and IL-39, use gp130 heterodimers in specialized cells. IL-27 binds to IL-27R (also known as WSX-1) and gp130, which has an activity opposite to its function in T cells: T cell–derived IL-27 inhibits differentiation of T helper type 17 (Th17) cells but promotes production of regulatory T (T reg) cells. In this context, it is noteworthy that IL-27 predominantly induces STAT1 activity rather than IL-6, which mainly promotes STAT3 transcriptional activity (Hirahara et al., 2015). IL-27/p28 transgenic mice have reduced levels of antigen-specific antibody production in vivo, demonstrating that IL-27/p28 inhibits IL-6–gp130 signaling independently of EBI3 (Stumhofer et al., 2010). IL-35 is mostly produced by T reg cells and has regulatory activity (Collison et al., 2007). A reconstitution study of receptor genes revealed that IL-35 utilizes three different receptor modes: gp130–IL-12Rβ2, gp130–gp130, and IL-12Rβ2–IL-12Rβ2.

IL-39 is the most recently identified member of the IL-6 family, and consists of IL-12p19 and EBI3 and transmits signals through the complex of IL-23R and gp130, which is expressed by B cells and has proinflammatory functions (Hasegawa et al., 2016). Thus, promiscuity within the IL-6/IL-12 family cytokines complicates structural and functional categorization of individual cytokines.

IL-6 family cytokines primarily activate JAK1 and JAK2 to drive signal transduction; the JAK proteins phosphorylate conserved tyrosine residues in the cytoplasmic domains of signal transducers such as gp130, OSMR, LIFR, and IL-27Rα (Heinrich et al., 2003). In turn, STAT family proteins, the MAPK cascade, PI3K-Akt signaling, and the YAP–NOTCH pathway are activated (Taniguchi et al., 2015). Although signaling by IL-6 family cytokines is broadly similar, the strength of specific activated pathways depends on the cell type and cytokines: OSMR recruits an adaptor protein, SHC, that drives activation of MAPK pathways upon OSM binding, whereas IL-6 triggers the association of SHP-2 to gp130 (Heinrich et al., 2003). Moreover, unlike IL-6, IL-27 predominantly activates STAT1. Thus, despite their many similarities, IL-6 family cytokines use different receptors, signaling pathways, and expression patterns to achieve functional pleiotropy.

Soluble receptors of the IL-6 family: Agonistic and antagonistic forms

The soluble receptors for the IL-6 cytokine family are present in human serum and are involved in cytokine signaling. Among IL-6 family cytokines, soluble types of nonsignaling and ligand-binding receptors acting as agonists of the corresponding cytokines, including sIL-6R, sIL-11R, and sCNTFR, have been identified. Notably, sIL-6R is produced by proteolytical cleavage of the cell-surface receptor or, to a minor extent, by

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Table 1. **Nomenclature of IL-6 family cytokines and their functions**

Approved symbol (ligand)	Approved name	Gene symbol (alias)	Cellular expression	Function	Approved symbol (receptor)	Approved name	Gene symbol (alias)	Cellular expression
IL6	IL-6	IL-6, BSF2, HGF, HSF	Macrophage, DC, lymphocyte, epithelial cell, osteoclast, hepatocyte	Acute responses, angiogenesis, osteoclastogenesis, differentiation of Th17 subset and B cell, glucose metabolism	IL6R	IL-6 receptor	CD126	Macrophage, monocyte, DC, hepatocyte, adipocyte
					IL6ST	IL-6 signal transducer	GP130, CD130, sGP130	Ubiquitous expression
IL11	IL=11	IL-11, AGIF	Stromal cell line, fibroblast, chondrocytes, various cancers	Hematopoiesis, adipogenesis, neuronal differentiation, bone metabolism, cell proliferation, invasiveness	IL11RA	IL-11 receptor subunit α		Various cancers
OSM	Oncostatin M	MGC20461	Monocyte, macrophage, neutrophil, T cell	Hematopoiesis, bone turnover, lipid metabolism, liver regeneration	OSMR	Oncostatin M receptor	OSMRB, OSMRβ	Nonhematopoietic cell; hepatocyte, epithelial cell, endothelial cell, stromal cell, fibroblast cell
LIF	Leukemia inhibitory factor	CDF, DIA, HILDA	T cell, activated monocyte, fibroblast, endothelial cell	Bone remodeling, neural regeneration,	LIFR	LIF receptor subunit alpha	CD118	skeletal muscle cell, cardiomyocyte
CTF1	Cardiotrophin 1	CT-1, CT1	Cardiac myocyte	Apoptosis				
CNTF	Ciliary neurotrophic factor	HCNTF	Osteoblast, osteocyte, osteoclast, chondrocyte	Bone metabolism, glucose metabolism	CNTFR	Ciliary neurotrophic factor receptor		Skeletal muscle cell
CLCF1	Cardiotrophin-like cytokine factor 1	NNT1, BSF3, CLC, NR6, CISS2, BSF-3, NNT-1	Activated Jurkat human T cell lymphoma cell	Development of nervous system				
IL27	IL-27	IL-27, p28, IL27p28, IL-27A, IL27A, MGC71873	Antigen-presenting cell	Differentiation of T cell subsets	IL27RA	IL-27 receptor subunit α	WSX-1, CCR, CRL1, WSX1, zcytor1, IL-27R	Macrophage, DC, T cell, B cell
EBI3	Epstein-Barr virus–induced 3	IL27B, IL35B	B cell					
IL12A	IL-12A	CLMF, IL-12A, p35, NFSK	Macrophage, DC, neutrophil	Differentiation of Th1, Th2 subset	IL12RB	IL-12 receptor subunit β	CD212	T cell, NK cell
IL23A	IL-23 subunit α	SGRF, IL23P19, IL-23, IL-23A, p19			IL23R	IL-23 receptor	IL-23R	

DC, dendritic cell; NK, natural killer.

alternative splicing of receptor mRNA. In healthy human serum, sIL-6R is present at a concentration of 79 ng/ml and mediates trans-signaling of IL-6 (Fig. 1). The designed protein, “hyper-IL-6,” is a fusion protein in which IL-6 is covalently attached to sIL-6R; it mimics trans-signaling and stimulates cells expressing gp130. Soluble forms of nonsignaling receptors have agonistic function, whereas soluble forms of signaling receptors have antagonistic function. In its natural state,

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soluble gp130 (sgp130) is present in human serum at a concentration of 390 ng/ml and functions as an inhibitor of IL-6/sIL-6R signaling (Narazaki et al., 1993). In line with this, the Rose-John group (Jostock et al., 2001) generated sgp130-Fc, in which dimerized soluble gp130 is conjugated to the Fc portion of human Ig; the fusion protein inhibits IL-6 trans-signaling but not classic signaling. Indeed, specific blockade of IL-6 trans-signaling by sgp130-Fc improved survival in a cecal ligation puncture sepsis model (Barkhausen et al., 2011). Moreover, sgp130 also inhibited the activities of CNTF, LIF, and OSM, although less efficiently than IL-6 trans-signaling (Narazaki et al., 1993). Preclinical trials of sgp130-Fc in several inflammation murine models were discussed in a review (Rose-John, 2018). In addition to sgp130, sLIFR and sIL-27R also have inhibitory function against their corresponding cytokines (Dietrich et al., 2014; Layton et al., 1992).

Mutations of IL-6 family cytokine receptors in humans

IL-6 and its family members have been linked to the pathogenesis of several diseases. The cell types expressing human IL-6 family cytokines or receptors are well characterized and are summarized in Table 1.

Various mutations in genes for IL-6, its family members, and its receptors have been identified in humans. These mutations manifest as an alteration of either phenotype or function. For example, a next-generation sequencing analysis indicated that mutations in the human *IL11R* gene cause a craniosynostosis syndrome characterized by bicoronal synostosis alone with occasional pansynostosis, hypertelorism, and other symptoms (Keupp et al., 2013).

A homozygous mutation of *IL6ST*, encoding Gp130 p.N404Y, results in immunodeficiency with skeletal abnormalities including craniosynostosis. Loss of function in the *IL6ST* gene leads to severe defects in IL-6, IL-11, IL-27, and OSM signaling (Schwerd et al., 2017). A somatic mutation in human gp130 that constitutively activates ligand-independent signaling causes inflammation-related carcinogenesis in the liver (Rebouissou et al., 2009). Mice lacking the *gp130* gene exhibit myocardial and hematological defects and ultimately die prematurely (Yoshida et al., 1996). Moreover, mice with a conditional deficiency of gp130 experience dysfunction and damage in the liver and heart during acute-phase responses, leading to the development of emphysema and increased susceptibility to infection (Betz et al., 1998).

In 1994, a single-nucleotide polymorphism (SNP) in the human *IL-6R* gene at the proteolytic cleavage site (Asp358) first underlined the importance of the cleavage site in induction of signaling (Müllberg et al., 1994). The Asp358Ala allele of IL-6R increases the serum levels of sIL-6R and is associated with a reduced risk of coronary heart disease (Sarwar et al., 2012; Swerdlow et al., 2012). IL-6R containing this mutation is shed more effectively by proteolytic cleavage from the cell surface of hepatocytes, macrophages, and monocytes; consequently, classic IL-6 signaling activity is reduced. Alternatively, the higher level of sIL-6R caused by the SNP may increase its buffering capacity. sIL-6R forms a complex with endogenous sgp130, resulting in reduced IL-6 activity. Therefore, the lower risk of coronary heart disease in carries of the Asp358Ala SNP may be due to improved IL-6 buffering capacity by the sIL-6/sgp130 complex. Notably in this regard, a recent report described two patients with homozygous mutations in the *IL-6R* gene. Both patients exhibited defects in acute-phase responses and immune functions, severe skin infections, and allergic symptoms such as asthma and atopic dermatitis, with high levels of serum IgE and eosinophilia (Spencer et al., 2019). These observations suggest that IL-6 signaling is involved in inflammation, self-defense, and suppression of allergic responses.

Spatiotemporal regulation of IL-6: Transcription and posttranscription

Transcriptional regulation

Whereas IL-6 family cytokines have redundant activities, the expression patterns of each member in response to stimuli differ (Guillet et al., 1995; Quinton et al., 2008). Among all family members, the transcriptional regulation of IL-6 has been studied most extensively (Fig. 2). The promoter and enhancer regions of IL-6 contain multiple cis-regulatory elements for various trans-acting transcription factors (TFs). Several of these TFs, such as NF-κB, NF-IL6 (also known as CAAT/enhancer-binding protein β), activator protein 1 (AP-1), specificity protein 1 (SP-1), and IFN regulatory factor 1 (IRF1), activate IL-6 transcription (Akira and Kishimoto, 1992). Upon stimulation by IL-1 and IL-6, *IL-6* transcription is activated primarily through NF-IL6 (Akira, 1997). Additionally, viral products such as the human T-lymphotropic virus 1–derived transactivator protein can also activate the transcriptional activities of NF-κB and NF-IL-6. On the other hand, peroxisome proliferator–activated receptor α, estrogen receptor, glucocorticoid receptor, and aryl hydrocarbon receptor (Ahr) repress IL-6 transcription (Delerive et al., 1999). Particularly, in complex with Ahr and STAT1, NF-κB suppresses IL-6 transcription in macrophages; consequently, deficiency in Ahr induces abnormal immune responses by either enhancing robust IL-6 production or inhibiting Th17 cell differentiation (Kimura et al., 2009; Nakahama et al., 2011).

The promoter regions of each IL-6 family cytokine contain different TF binding motifs. The promoters of LIF, OSM, and p28 contain putative NF-κB binding sites, whereas those of CNTF and CT-1 do not. Indeed, LPS-stimulated macrophages express p28 through NF-κB and IRF1 (Liu et al., 2007). In tumor cells, TGF-β elevates IL-11 expression via two different pathways, Runx2 and AP-1 (Zhang et al., 2015). The promoter region of human OSM contains NF-IL-6 and several GC-rich regions that promote basal activity, whereas GM-CSF stimulation induces STAT5 to bind to its cis-element in the OSM promoter (Ma et al., 1999). Thus, the different expression patterns of IL-6 family cytokines are mediated by several transcriptional regulatory elements, dependent on cell type and stimulus.

Posttranscriptional regulation by microRNA (miRNA) and RNA-binding protein (RBP)

So far, posttranscriptional regulatory mechanisms have extensively been studied in IL-6 expression among IL-6 family cytokines. Most of these factors dampen IL-6 expression by targeting the 3′ untranslated region (UTR) of the mRNA and promoting its

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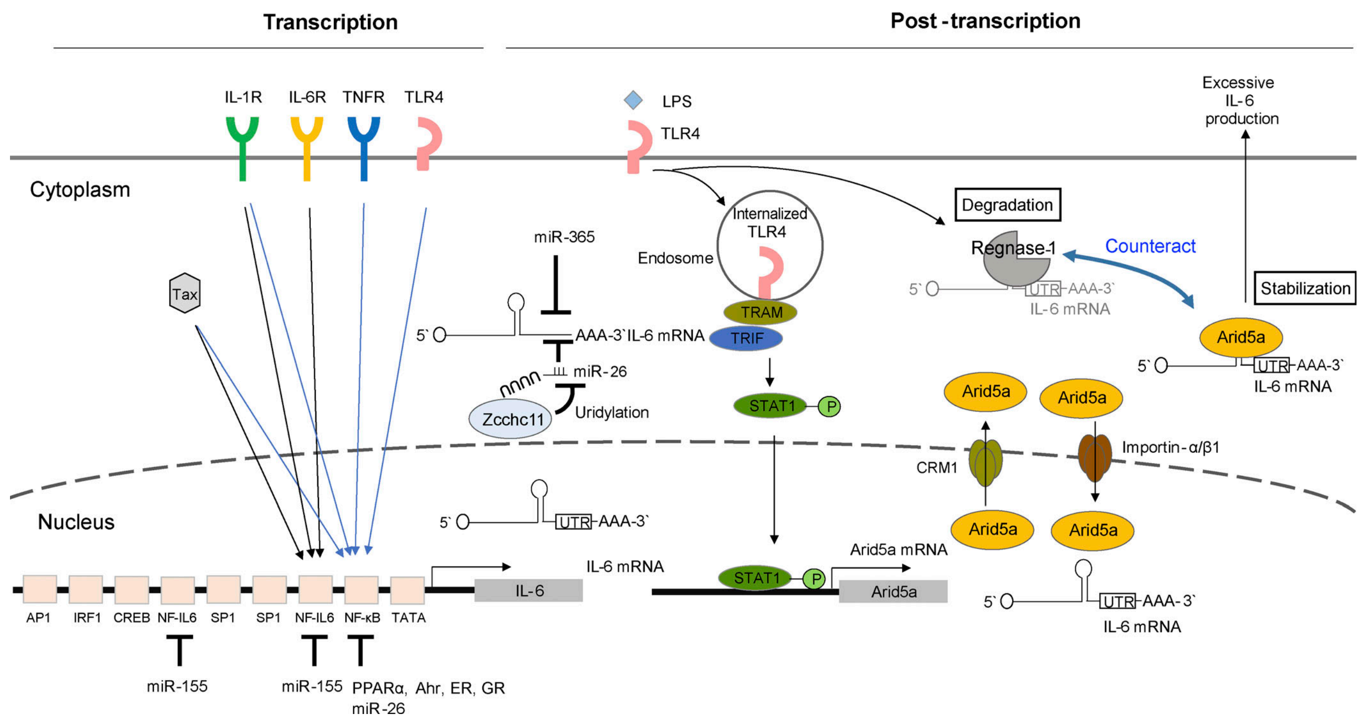

Figure 2. **Spatiotemporal regulation of the *IL-6* gene.** Infections activate TLR and cytokine receptor signaling. NF-κB and NF-IL6 act primarily as TFs in *IL-6* mRNA transcription. Moreover, some miRNAs target *IL-6* mRNA to dampen its expression. *IL-6* mRNA is posttranscriptionally regulated in macrophages. Engagement of TLR4 promotes *IL-6* and *Arid5a* transcription through distinct pathways. Arid5a stabilizes *IL-6* mRNA and counteracts Regnase-1 activity. Zcchc11 stabilizes IL-6mRNA by uridylation of miR-26. TRAM, TRIF-related adaptor molecule; CRM1, chromosomal region maintenance 1.

degradation, and they consist primarily of miRNAs and RBPs (Tanaka et al., 2016). Several miRNAs inhibit IL-6 by targeting its 3′ UTR or indirectly suppressing it via an upstream activator. For instance, miRNA-26 targets both the IL-6 and NF-κB 3′ UTRs, miRNA-155 targets the NF-IL6 3′ UTR, and miRNA-365 targets the IL-6 3′ UTR (Chen et al., 2016; He et al., 2009; Song et al., 2015; Fig. 2).

RBPs, which are key regulators of gene expression in the immune system, contain RNA-binding zinc-finger domains that modulate mRNA stability via distinct mechanisms. RBPs recognize cis-elements such as AU-rich elements (AREs) and stem-loop structures in the 3′ UTRs of mRNAs. Mechanistically, following the recognition of cis-elements in the 3′ UTR, miRNA- or RBP-mediated decay of *IL-6* mRNA occurs in stress granules or processing bodies, to which the components of the mRNA decay machinery are recruited (Anderson and Kedersha, 2008). The CCR4–NOT deadenylase complex removes the poly(A) sequence from the 3′ UTR of *IL-6* mRNA, followed by removal of the 7-methyl-guanosine cap from its 5′ UTR by decapping enzymes, allowing degradation of the mRNA (Anderson, 2010). The *IL-6* mRNA 3′ UTR contains cis elements for multiple posttranscriptional regulators, and the cooperative interactions between these regulators determine the half-life of *IL-6* mRNA. Multiple RBPs, including ARE/poly-(U) binding degradation factor 1 (AUF1), tristetraprolin (TTP), Zc3h12a (also known as Regnase-1), Roquin-1, and AT-rich interactive domain–containing 5a (Arid5a), modulate the stability of the *IL-6* mRNA by binding to AREs or stem-loop structures in its 3′ UTR (Kang et al., 2019; Mino and Takeuchi, 2018).

TTP, one of the best-characterized zinc-finger proteins, regulates the *IL-6* mRNA stabilization. Specifically, through its zinc-finger domain, TTP interacts with AREs and destabilizes the *IL-6* mRNA (Stoecklin et al., 2008). In mice, TTP deficiency leads to robust IL-6 expression and longer mRNA half-life (Zhao et al., 2011).

Regnase-1, an endonuclease also known as Zc3h12a, is another zinc-finger protein that dampens IL-6 expression by recognizing a stem-loop structure in the IL-6 3′ UTR, resulting in its cleavage (Fig. 2; Yoshinaga and Takeuchi, 2019). The importance of IL-6 dysregulation has been demonstrated in *Regnase-1*–deficient (*Regnase-1*$^{-/-}$) mice, which spontaneously develop autoimmune diseases accompanied by splenomegaly and lymphadenopathy (Matsushita et al., 2009). Thus, Regnase-1 plays critical roles in the regulation of both the innate and adaptive immune responses. *Regnase-1*$^{-/-}$ macrophages express high levels of IL-6 upon TLR ligand stimulation (Matsushita et al., 2009). In CD4 T cells, deficiency in Regnase-1 increases *Icos*, *Il2*, *Ox40*, and *c-Rel* expression, resulting in abnormal Th populations including Th1, Th2, and Th17 (Uehata et al., 2013). Although these RBPs recognize overlapping sites on the mRNA, Regnase-1 and Roquin-1 play nonredundant functions in control of the immune system. Upon LPS stimulation, Regnase-1 and Roquin-1 digest *IL-6* mRNAs at different time points: Regnase-1 in the early phase and Roquin-1 in the late phase (Mino et al., 2015); however, deficiency of either Regnase-1 or Roquin-1 in CD4 T cells exacerbates inflammation (Cui et al., 2017). Mechanistically, Regnase-1 localizes to the ribosome and endoplasmic reticulum to promote digestion of target mRNA, which requires the RNA

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helicase activity of UPF1, whereas Roquin-1 functions in mRNA degradation through recruitment of the CCR4–NOT deadenylase complex in stress granules and P bodies (Popp and Maquat, 2013). These findings suggest that Regnase-1 and Roquin-1 control overlapping sets of mRNA targets through spatiotemporally distinct mechanisms, which mediates fine-tuned regulation of inflammatory gene expression.

In contrast to the destabilizing role of many zinc-finger proteins and their ability to dampen IL-6 expression, the zinc-finger protein Zcchc11 stabilizes *IL-6* mRNA by modification of miRNA. Zcchc11 has uridyltransferase activity that allows it to add uridine residues to the 3′ UTR of miRNA-26, resulting in its inactivation (Jones et al., 2009).

Regulation of IL-6 mRNA by the balance between Arid5A and Regnase-1

Arid5a is an RBP that directly binds to a stem–loop element in the 3′ UTR (Masuda et al., 2013). Arid5a possesses an AT-rich interaction domain, also known as the DNA-binding domain (Wilsker et al., 2002). Recent work revealed the critical roles of Arid5a in innate and adaptive immune responses. In macrophages stimulated with LPS, IL-6, or IL-1β, Arid5a recognizes the stem-loop structure on *IL-6* mRNA, which is also the target site of Regnase-1, and stabilizes *IL-6* mRNA by counteracting Regnase-1–mediated decay of *IL-6* mRNA (Iwasaki et al., 2011; Masuda et al., 2013). Moreover, IL-6 enhances its own mRNA stability by promoting Arid5a expression via a positive feedback loop (Nyati et al., 2017). Indeed, *Arid5a*-deficient (*Arid5a*$^{-/-}$) mice exhibit impairment of IL-6 and IFN-γ expression upon LPS injection and are resistant to lethal endotoxin sepsis (Masuda et al., 2013; Zaman et al., 2016). Interestingly, spatiotemporal regulation of the balance between Regnase-1 and Arid5a plays a key role in regulating the half-life of *IL-6* mRNA in macrophages. At steady state, Regnase-1 is localized mainly in the cytoplasm, where it degrades *IL-6* mRNA, thereby preventing its aberrant expression (Matsushita et al., 2009). During the early phase of LPS stimulation, the inhibitor of NF-κB (IκB) kinase α/β complex phosphorylates Regnase-1 at Ser435 and Ser439. Phosphorylated Regnase-1 undergoes ubiquitin/proteasome-mediated degradation, thereby relieving inhibition of IL-6 expression. Regnase-1 is reexpressed during the late phase of LPS stimulation to dampen *IL-6* mRNA production (Iwasaki et al., 2011). Notably, recent work by our group showed that MyD88-independent TRIF (Toll-interleukin-1 receptor homology domain–containing adaptor-inducing IFN-β) signaling promotes Arid5a-mediated IL-6 expression during the late phase of LPS stimulation, and that noncanonical phosphorylation of STAT1 induced by endosomal TLR4 is required for Arid5a transcription (unpublished data). Moreover, we revealed that the localization of Arid5a plays critical roles in the development of inflammation. In the resting state, Arid5a resides in the nucleus, but upon engagement of TLR4, it is translocated into the cytoplasm through association with chromosomal region maintenance 1 (CRM1; Fig. 2; Higa et al., 2018). In the opposite direction, Arid5a is imported into the nucleus via the importin-α/β–mediated pathway. Consistent with this, mice overexpressing Arid5a exhibit more robust IL-6 production than wild-type mice, although they have comparable IL-6 levels under unstimulated conditions. Thus, the dynamic subcellular localization of Arid5a regulates inflammatory responses. Additionally, in CD4 T cells, Arid5a also binds to mRNA of STAT3 and T-box–containing protein expressed in T cell (T-bet). Th1 or Th17 responses, which have been associated with development of experimental autoimmune encephalomyelitis, are impaired in Arid5a-deficient mice (Masuda et al., 2016). In fact, Arid5a deficiency inhibits the development of experimental autoimmune encephalomyelitis (Masuda et al., 2013). Additionally, Arid5a is involved in *Il17* mRNA stabilization by association with the eukaryotic translation initiation complex, which also counteracts degradation by Regnase-1 (Amatya et al., 2018). These findings reveal that these two RBPs control *IL-6* mRNA stabilization through spatiotemporal and subcellular dynamics. A high ratio of Arid5a to Regnase-1 may contribute to the pathogenesis of IL-6–related immune diseases.

Future of IL-6 research

From the studies described here, it is clear that IL-6 and its family members constitute a very broad field of research that has opened up numerous possibilities for treatment of a variety of human diseases. The number of publications on the members of this family, as well as their involvement in human chronic diseases, indicate their potential as therapeutic agents. It is becoming increasingly evident that members of the IL-6 family can mediate acute inflammatory diseases, such as sepsis and macrophage activation syndrome, and therefore need to be tightly regulated. To cure acute inflammation, we require a deeper understanding of the pathways activated by these ligands and how to selectively interrupt these pathways.

Clinical trials of several inhibitors of IL-6, IL-6R, or gp130, or intracellular molecules such as JAK, using antibodies or small compounds, have been conducted for various diseases. Given that these inhibitors exhibit high efficacy against several immune-related disorders, it is plausible that manipulation of the activities of RBPs, Arid5a, or Regnase-1 could control immune responses, especially those mediated by macrophages. Development of therapeutic reagents targeting these RBPs might be beneficial for immune-related diseases.

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