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Review Article

Interleukin-6 and its receptors: A highly regulated and dynamic system

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ABSTRACT

Interleukin-6 (IL-6) is a multifunctional cytokine with well-defined pro- and anti-inflammatory properties. Although only small amounts in the picogram range can be detected in healthy humans, IL-6 expression is highly and transiently up-regulated in nearly all pathophysiological states. IL-6 induces intracellular signaling pathways after binding to its membrane-bound receptor (IL-6R), which is only expressed on hepatocytes and certain subpopulations of leukocytes (classic signaling). Transduction of the signal is mediated by the membrane-bound β-receptor glycoprotein 130 (gp130). In a second pathway, named trans-signaling, IL-6 binds to soluble forms of the IL-6R (sIL-6R), and this agonistic IL-6/sIL-6R complexes can in principle activate all cells due to the uniform expression of gp130. Importantly, several soluble forms of gp130 (sgp130) are found in the human blood, which are considered to be the natural inhibitors of IL-6 trans-signaling. Most pro-inflammatory roles of IL-6 have been attributed to the trans-signaling pathway, whereas anti-inflammatory and regenerative signaling, including the anti-bacterial acute phase response of the liver, is mediated by IL-6 classic signaling. In this simplistic view, only a minority of cell types expresses the IL-6R and is therefore responsive for IL-6 classic signaling, whereas gp130 is ubiquitously expressed throughout the human body. However, several reports point towards a much more complex situation. A plethora of factors, including proteases, cytokines, chemical drugs, and intracellular signaling pathways, are able to modulate the cellular expression of the membrane-bound and soluble forms of IL-6R and gp130. In this review, we summarize current knowledge of regulatory mechanisms that control and regulate the dynamic expression of IL-6 and its two receptors.

1. Introduction: IL-6, IL-6R and gp130

Biomedical research has made impressive progress during the last two decades in identifying signaling cascades and individual proteins that can be targeted therapeutically in order to treat human diseases. One prominent example in inflammatory disorders is the cytokine Interleukin (IL)-6, a small secreted glycoprotein that activates cells via a heterodimeric signaling complex consisting of the IL-6 α-receptor (IL-6R) and the signal-transducing β-subunit glycoprotein 130 (gp130), which is shared with several other cytokines [1]. The IL-6R exists as soluble as well as membrane-bound forms, which allows the discrimination between IL-6 classic (via the membrane-anchored IL-6R) and IL-6 trans-signaling (via the soluble IL-6R). Gp130 expression can be detected in every tissue and cell type of the human body, whereas the IL-6R is predominantly expressed in hepatocytes, megakaryocytes and several leukocyte subpopulations. Evidence suggests that IL-6 trans-signaling via the soluble IL-6R accounts for the pro-inflammatory properties of IL-6. Thus, specific inhibition of this pathway, which does not compromise the anti-inflammatory activities of IL-6 via classic signaling, could be a valuable therapeutic tool to treat human inflammatory diseases [2–4].

A number of excellent reviews has been published over the last years which dealt with different aspects of IL-6. Their content ranges from the biological functions of IL-6 and its other family members [1,5,6] and detailed descriptions of the activated signaling pathways [1,7–9] to therapeutic strategies through which IL-6 can be specifically blocked [4,10–12].

In the present review, we will focus on an aspect of IL-6 signaling that is often overlooked. A number of papers has been published which show that IL-6R, gp130 and its soluble forms are highly dynamic and extensively regulated. Several mechanisms have been identified over the years that increase or decrease the membrane-bound expression of gp130 and IL-6R, thereby shaping the cellular cytokine receptor composition. We will discuss these

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findings and assemble evidence that also sIL-6R and the soluble form of gp130 (sgp130) are regulated by diverse mechanisms and discuss their value as prognostic markers.

Thus, we present the IL-6/(s)IL-6R/(s)gp130 system as highly dynamic and regulated, which has to be taken into account when studied *in vitro* and *in vivo*.

2. Signaling of IL-6

Cytokines are glycosylated proteins with immunoregulatory functions and important roles during infection and inflammation. Well-known representatives of cytokines are the members of the IL-6 family comprising among others IL-6, IL-11, IL-27, Oncostatin M (OSM), Cardiotrophin-1 (CT-1) and Neuropoeitin (NP-1) [1]. They are characterized by performing their biological effect via homo- or heterodimerization of the signal-transducing β-receptor gp130, which is ubiquitously expressed. The further transfer of the signal is performed by the Janus-kinase/Signal transducer and activator of transcription (Jak/STAT)-, mitogen-activated protein kinase (MAPK)- and phosphatidyl-inositol-3-kinase (PI3K)-pathway [7].

In regard to IL-6 signaling, this cytokine binds to its specific membrane-bound α-receptor IL-6R followed by formation of the signaling complex via a gp130 homodimer. In contrast to gp130, IL-6R is only expressed on a limited number of cell types, as there are hepatocytes, megakaryocytes and some leukocytes, namely monocytes, macrophages, B cells and subtypes of T cells [13,14]. This fact facilitates the selective activation of definite target cells, therefore differentiating between two different signaling-pathways (Fig. 1). The cellular activation via binding of IL-6 to its membrane-bound receptor IL-6R is named classic signaling. All other cells, which do not express the membrane-bound IL-6R, obtain their IL-6 signals by trans-signaling. Hereby, IL-6 binds to the soluble form of IL-6R (sIL-6R), and this complex forms the signaling complex with gp130 on the cell surface.

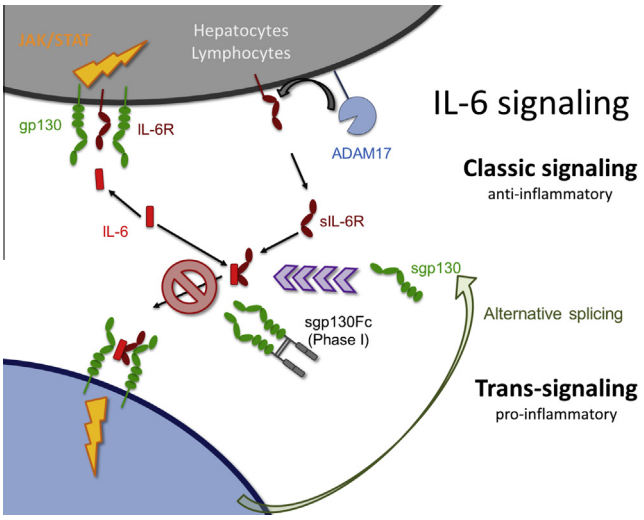

Fig. 1. Interleukin-6 classic and trans-signaling. The Interleukin-6 receptor (shown in dark red) is expressed on hepatocytes and lymphocytes, where IL-6 (shown in red) can activate Jak/STAT signaling via a gp130 (shown in green) homodimer (classic signaling). The metalloprotease ADAM17 can cleave the IL-6R, and the resulting sIL-6R can form an agonistic complex with IL-6 that can activate other cells via trans-signaling. A soluble form of gp130 (sgp130) is generated via alternative mRNA splicing that inhibits IL-6 trans-signaling. Sgp130Fc, which is sgp130 dimerized via the Fc part of a human IgG antibody, efficiently blocks IL-6 trans-signaling and is currently tested in phase I clinical trials. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The sIL-6R is found at about 25–35 ng/ml in human plasma, and can be generated by two different mechanisms [15]. The majority of the sIL-6R is generated by proteolytic cleavage (“shedding”) of the membrane bound form [16]. Minor amounts (1–10%) are produced via alternative splicing by omission of the transmembrane domain coding exon [17]. In contrast to classic signaling with anti-inflammatory features, trans-signaling is responsible for the pro-inflammatory effects of IL-6 [6]. The soluble form of gp130 (sgp130) has been shown to be a natural inhibitor of trans-signaling [18]. Based on this, the artificial fusion protein sgp130-Fc, in which two sgp130-monomers are dimerized by the Fc-part of a human IgG antibody, was developed and optimized [19]. As a possible drug for treatment of inflammatory diseases, an optimized version of this inhibitor (FE999301) is currently tested in clinical phase I studies by Ferring Pharmaceuticals [4,20].

3. Regulation of IL-6 expression

The first two papers describing IL-6 originate from 1980, when two labs independently identified a novel mRNA, which arose after fibroblasts where stimulated with interferon β, and termed it interferon β 2 [21,22]. In the following years, groups worldwide identified several proteins (for example 26 kDa protein [23,24], B cell stimulatory factor 2 (BSF-2) [25], hybridoma growth factor (HGF) [26], or hepatocyte-stimulating factor (HSF) [27]), which all later turned out to be the same protein, which was finally termed IL-6 [28].

The IL-6 mRNA encodes a protein of 212 amino acids including a 29 amino acids long signal peptide, resulting in a secreted protein which consists of 184 amino acids. Although this would translate into a protein of around 21 kDa, IL-6 is found in several isoforms of 21–28 kDa due to different N-linked glycosylation [29]. However, none of these modifications has been shown to be essential for IL-6 signaling. Recombinant IL-6 produced in *Escherichia coli*, and thus devoid of any glycosylation, is perfectly functional and widely used for *in vitro* and *in vivo* experiments. Although not necessary for function, IL-6 glycosylation might be important for stability or half-life of the protein. IL-6 consists of four α-helices, which are arranged in a bundle of the typical up–up–down–down topology found in all IL-6 type cytokines, and activates its receptor complex of IL-6R and gp130 through three distinct contact sites (called ‘site I’, ‘site II’ and ‘site III’, Fig. 2A) [6].

IL-6 is found in the blood of healthy humans at very low concentrations of about 1–5 pg/ml. IL-6 concentrations rise dramatically during inflammatory conditions, and can reach concentrations in the range of μg/ml in cases of sepsis [30]. Elevated IL-6 level can be found in most, if not all, human diseases which have an inflammatory component. These include autoimmune diseases like Rheumatoid Arthritis (RA), Crohn’s disease and Systemic Lupus Erythematosus, as well as chronic inflammatory diseases like Castleman’s disease, Behçet’s disease or Systemic Juvenile Idiopathic Arthritis (the role of IL-6 and its therapeutic inhibition is described in detail elsewhere [4,11,31–34]). The expression of the cytokine is tightly controlled at multiple levels to prevent overshooting systemic conditions.

Several factors have been described that control generation and fate of the IL-6 mRNA either at the transcriptional or post-transcriptional level (Fig. 2B). The IL-6 promoter region contains upstream of its TATA box sequence motifs for the binding of AP-1 [35,36], a cyclic AMP (cAMP)-responsive element [37], a binding site for CCAAT enhancer binding protein β (C/EBPβ, also termed NF-IL6 [38]) and a binding motif for NF-κB [39–41]. A key role has been attributed to the transcription factor NF-κB. The expression of IL-6 can be induced by lipopolysaccharide (LPS) stemming from bacterial infections [42], the pro-inflammatory cytokines TNFα