Review

Impact of interleukin-6 classic- and trans-signaling on liver damage and regeneration

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ABSTRACT

Interleukin-6 (IL-6) has been suggested to play a pivotal role in liver regeneration. IL-6 on target cells activates a receptor complex consisting of the IL-6 receptor (IL-6R) and the signal transducing receptor subunit gp130. Not all cells in the body express the IL-6R on the cell surface. IL-6 can signal via two different pathways: classical signaling via the membrane bound IL-6R and IL-6 trans-signaling via a naturally occurring soluble IL-6R (sIL-6R). This second pathway widens the scope of IL-6 signaling since also cells expressing no membrane bound IL-6R can be stimulated by the trans-signal pathway. Mimicking IL-6 trans-signaling via a designer molecule, Hyper-IL-6 has been shown to accelerate liver regeneration. Another designer molecule, sgp130Fc, specifically blocks IL-6 trans-signaling. Using these proteins we investigated the contribution of IL-6 classic- and trans-signaling in the liver. Here we review the role of IL-6 signaling in response to liver damage and during liver regeneration.

1. Introduction

The liver has an enormous capability to regenerate after injury. After liver damage or resection of parts of the liver, the human liver regains its original mass within 2–3 weeks. This regenerative process is clearly structured involving different types of liver cells: hepatocytes and non-parenchymal cells (NPCs). The latter fraction includes Kupffer cells, which are the macrophages of the liver, endothelial cells that line the liver sinusoids, and stellate cells (Ito cells) the fat storing cells of the liver. Also blood cells are present in the liver. In case of cell damage all liver cells are involved in the regenerative process [1]. Neutrophils and macrophages are the first cells that are activated and recruited into the inflamed liver. Upon activation, NPCs secrete immunoregulatory and proinflammatory cytokines such as IL-6, TNFα, chemokines, prostaglandins, and reactive oxygen species (ROS).

The regenerative process is clearly structured and can be divided into three different phases [2]. Under normal conditions, hepatocytes rest in the G_0 Phase. In the first phase of liver regeneration hepatocytes are primed to re-enter the cell cycle. This initiation phase is regulated by cytokines such as IL-6 and TNFα. Thereafter follows the expansion phase. Cells divide until the original liver mass is reached again. This stage is primarily regulated by mitogenic factors such as Hepatocyte Growth Factor (HGF). In the termination stage, mitosis stops. Sometimes the reached liver mass exceeds the original mass. In such cases, apoptosis can occur for a fine regulation of liver mass [1–3]. In this review, we focus on the role of IL-6 in liver damage and regeneration.

IL-6 is a pleiotropic cytokine originally identified as an antigen-nonspecific B-cell differentiation factor that activates B-cells to produce immunoglobulins [4]. IL-6 is important in immune regulation. The IL-6 protein is secreted by T-cells, B-cells, endothelial cells, fibroblasts, monocytes and macrophages. In the liver, IL-6 is secreted by Kupffer cells, the tissue-resident macrophages. Upon TNFα stimulation, Kupffer cells produce IL-6 and activate hepatocytes in a paracrine mode of action. Both factors, TNFα and IL-6, are key regulators of liver regeneration, since liver regeneration is impaired in their absence [1,3,5].

From a structural point of view, IL-6 belongs to the family of gp130 cytokines. This family consists of IL-6, IL-11, ciliary neutrophic factor (CNTF), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), leukaemia inhibitory factor (LIF), neuropoietin (NPN, in humans only found as a pseudogene), Oncostatin M (OSM), and IL-27. All family members share a four-helical protein structure and they exert their signals via a receptor complex containing at least one subunit of the signal transducing receptor glycoprotein gp130 [6–8]. IL-6 first binds to the IL-6 receptor (IL-6R) and this IL-6/IL-6R complex binds to gp130 leading to homodimerization and subsequent activation of the Jak/Stat- and Ras/Map/Akt-signal transduction pathway.

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2. IL-6 classic and IL-6 trans-signaling

Two different signaling pathways for IL-6 have been described. In classical IL-6 signaling pathway, IL-6 binds to the membrane bound IL-6R, which leads to dimerization and activation of the signal transducing protein gp130 (Fig. 1A) [6,9]. Whereas gp130 is expressed on all cells of the body, the IL-6R is only expressed on some cell populations, mainly on hepatocytes, neutrophils, macrophages and some lymphocytes. Therefore, the effect of IL-6 through the classical IL-6 signal pathway is restricted to these cells. There exists, however, an alternative IL-6 pathway. In this, IL-6 binds to a naturally occurring soluble IL-6R (sIL-6R) and this IL-6/sIL-6R complex activates gp130 (Fig. 1B). Therefore, cells lacking the membrane bound IL-6R can respond to IL-6. This alternative IL-6 pathway was called IL-6 trans-signaling [10].

IL-6 trans-signaling has important biological effects. It enlarges the spectrum of IL-6 target cells because cells that do not express a membrane bound IL-6R can be stimulated by IL-6 and the sIL-6R. However, IL-6 trans-signaling affects also cells that express the membrane bound IL-6R, e.g. hepatocytes. In this setting an activation of IL-6 trans-signaling can enhance stimulatory effects of IL-6 [11]. Since hepatocytes express far more gp130 than IL-6R, the total number of activated gp130 molecules is higher when IL-6 and sIL-6R are present. This has been shown in cell culture [12] as well as in *in vivo* experiments [13]. In *in vivo* experiments it has been shown that injection of IL-6 together with sIL-6R has a more pronounced effect on proliferation of hepatocytes than IL-6 alone as will be reviewed below.

3. Generation of the soluble IL-6 receptor

Many cytokine receptors exist in a soluble form. The soluble receptors consist of the extracellular part of the transmembrane receptor. They have similar binding affinities to their ligands and can be found in different body fluids. Interestingly, most soluble cytokine receptors such as the sTNFR normally act as antagonists, competing with the membrane bound receptors for their cognate ligands. In contrast, the sIL-6R acts as an agonist that enhances IL-6 effects [14].

Two mechanisms of generation of the sIL-6R are described in humans. The sIL-6R can be formed via limited proteolysis from the membrane bound receptor. This reaction, called shedding, is carried out by transmembrane metalloproteinases, like the ADAM-proteases [15]. Different stimuli induce shedding of the IL-6R by ADAM-17: phorbol esters that activate protein kinase C [16], the acute phase protein C-reactive protein [17], bacterial toxins [18], apoptosis [19], Cholesterol depletion [20], P2X7-receptor- [21], and G protein-coupled receptor-activation [22]. The second mechanism by which soluble receptors can be generated is translation from alternatively spliced mRNA that lack the coding region of the transmembrane domain [23].

In humans, the sIL-6R protein is found in the blood of healthy individuals at concentrations of about 50–80 ng/ml [24]. sIL-6R levels increase in some inflammatory diseases such as peritonitis [25] and rheumatoid arthritis [26] by a factor of two, suggesting that the sIL6R plays an important role in inflammatory diseases. It is noteworthy, however, that IL-6 levels under these conditions are elevated up to several hundred fold [25,26].

4. Hyper-IL-6 and sgp130Fc: Two designer proteins for the *in vivo* analysis of IL-6 trans-signaling

The IL-6 pathways can be influenced via activation or specific blockade of the classical- and/or the IL-6 trans-signaling pathway. To investigate the IL-6 trans-signal pathway in more detail, we have developed two designer proteins: Hyper-IL-6, which mimics activated IL-6 trans-signaling and sgp130Fc, which specifically blocks the IL-6 trans-signal pathway without interfering with IL-6 signaling via the membrane bound IL-6R [27]. These two designer proteins could be also used as therapeutic agents to modulate IL-6 trans-signaling.

Hyper-IL-6 is a fusion-protein that consists of the human sIL-6R covalently bound to human IL-6 via a flexible peptide linker (Fig. 2A) [28]. Hyper-IL-6 is 100–1000 times more potent to stimulate cells than the combination of the two separate proteins IL-6 and sIL-6R [28]. We produced recombinant Hyper-IL-6 in stably transfected CHO cells and purified the recombinant protein via
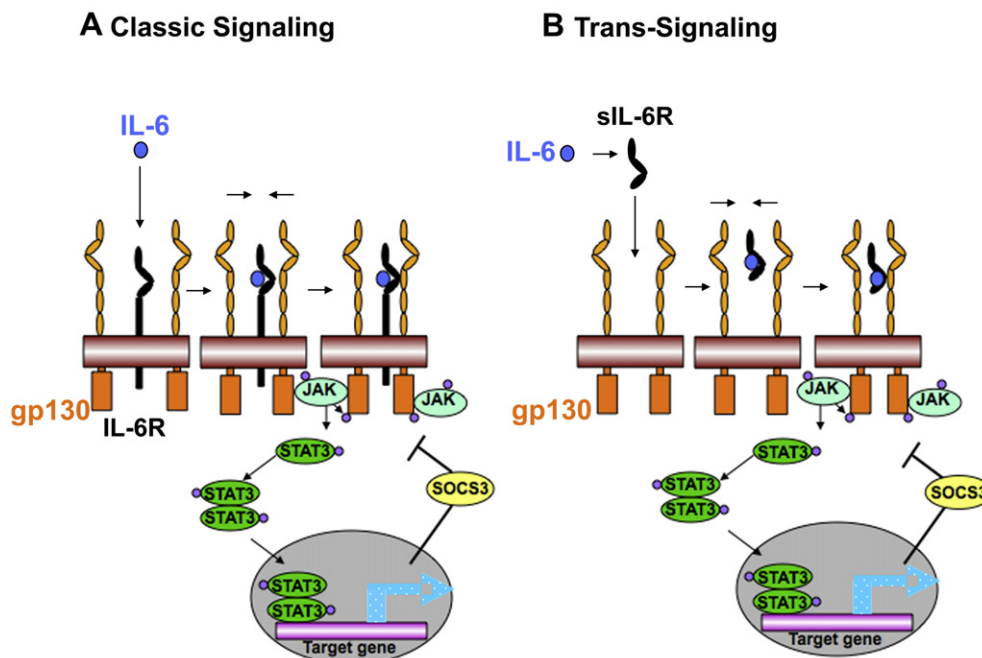
Fig. 1. Classic and IL-6 trans-signaling (A) In the classical IL-6 signaling pathway, IL-6 binds to the membrane bound IL-6R which leads to dimerization and activation of the signal transducing protein gp130. (B) IL-6 trans-signaling: IL-6 binds to a soluble IL-6R (sIL-6R) and this IL-6/sIL-6R complex activates gp130.