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Review

Interleukin-6 biology is coordinated by membrane bound and soluble receptors★

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Cytokine receptors exist in membrane bound and soluble form. Both forms bind their ligands with comparable affinity. While most soluble receptors are antagonists in that they compete for the ligands with their membrane counterparts, some soluble receptors are agonists. In this case, the complex of ligand and soluble receptor binds on target cells to a second receptor subunit and initiates signal transduction. Soluble receptors of the IL-6 family of cytokines are agonists. *In vivo,* the IL-6/soluble IL-6R complex stimulates several types of target cells not stimulated by IL-6 alone, since they do not express the membrane bound IL-6R. This process has been named transsignaling. We have shown that in several chronic inflammatory diseases like chronic inflammatory bowl disease, peritonitis and rheumatoid arthritis, transsignaling *via* the soluble IL-6R complexed to IL-6 is a crucial point in the transition from the acute to the chronic state of the disease. The mechanism by which the IL-6/ soluble IL-6R complex regulates the inflammatory state is discussed.

The interleukin-6 (IL-6) family of cytokines acts *via* receptor complexes that contain at least one subunit of the signal transducing protein gp130 (Taga & Kishimoto, 1997). The family comprises IL-6, IL-11, ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1),

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Abbreviations: ALS, amytrophic lateral sclerosis; CLC, cardiotrophin-like cytokine; CNTF, ciliary neurotrophic factor; CT-1, cardiotrophin-1; GPI, glycosylphosphatidylinositol; IL, interleukin; LIF, leukemia inhibitory factor; OSM, oncostatin M; R, receptor; s, soluble; v, viral.

cardiotrophin-like cytokine (CLC), leukemia inhibitory factor (LIF), and oncostatin M (OSM) (Taga & Kishimoto, 1997). IL-6, IL-11, and CNTF first bind to specific receptors, and these complexes associate with a homodimer of gp130 in the case of IL-6 and IL-11 or, alternatively, with a heterodimer of gp130 and the related protein LIF receptor (LIF-R) in the case of CNTF. OSM and LIF first bind directly to gp130 and LIF-R, respectively, and form heterodimers with LIF-R and gp130. Recently, a gp130-related protein was described that can heterodimerize with gp130 and that acts as an alternative OSM receptor (Mosley *et al.,* 1996). CT-1 binds directly to the LIF-R and induces gp130/LIF-R heterodimer formation (Pennica *et al.,* 1996). Recently, the presence of a specific glycosylphosphatidylinositol (GPI)-anchored CT-1 receptor on neuronal cells was implicated (Pennica *et al.,* 1996).

On target cells IL-6 first binds to the IL-6 receptor (IL-6R). The complex of IL-6 and IL-6R associates with the signal transducing membrane protein gp130, thereby inducing its dimerization and initiation of signaling (Rose-John, 2001; Taga & Kishimoto, 1997). gp130 is expressed by all cells in the body whereas IL-6R is mainly expressed by hepatocytes, monocytes/macrophages and some lymphocytes. A naturally occurring soluble form of the IL-6R (sIL-6R), which has been found in various body fluids, is generated by two independent mechanisms, limited proteolysis of the membrane protein and translation from an alternatively spliced mRNA (Hundhausen *et al.,* 2003; Lust *et al.,* 1992; Matthews *et al.,* 2003; Müllberg *et al.,* 2000a; Rose-John & Heinrich, 1994). Interestingly, the sIL-6R together with IL-6 stimulates cells which only express gp130 (Mackiewicz *et al.,* 1992; Taga *et al.,* 1989), a process which has been named transsignaling (Müllberg *et al.,* 2000a; Peters *et al.,* 1998; Rose-John & Heinrich, 1994). Recently, it has been shown that the sIL-6R strongly sensitizes target cells (Peters *et al.,* 1996). Embryonic stem cells (Rose-John, 2002), early hematopoietic progenitor cells (Peters *et al.,* 1998; Peters *et al.,* 1997), many neural cells (März *et al.,* 1998; März *et al.,* 1999), smooth muscle cells (Klouche *et al.,* 1999) and endothelial cells (Romano *et al.,* 1997), among others, are only responsive to IL-6 in the presence of sIL-6R (Jones & Rose-John, 2002).

Most cytokine receptors exist in membrane bound and soluble form. Interestingly, cytokines bind to both receptor forms with comparable affinity. While most soluble receptors are antagonists in that they compete with their membrane counterparts for the ligands, some soluble receptors are agonists. In this case, the complex of ligand and soluble receptor binds on target cells to a second receptor subunit and initiates signal transduction. Soluble receptors of the IL-6 family of cytokines are agonists (Althoff *et al.,* 2001; Althoff *et al.,* 2000; Müllberg *et al.,* 2000a). *In vivo,* the IL-6/sIL-6R complex stimulates several types of target cells, which are not stimulated by IL-6 alone, since they do not express the membrane bound IL-6R. Such cells include embryonic stem cells (Rose-John, 2002), endothelial cells (Romano *et al.,* 1997), hematopoietic progenitor cells (Audet *et al.,* 2001; Hacker *et al.,* 2003), osteoclasts (Tamura *et al.,* 1993) and neuronal cells (Brunello *et al.,* 2000; Sun *et al.,* 2002).

Interestingly we could recently show that CNTF not only acts *via* the membrane bound and soluble CNTF-R. CNTF can also use the membrane bound and soluble IL-6R (Schuster *et al.,* 2003). This fact might have important implications for the use of CNTF as a therapeutic agent. The use of CNTF as a drug in amytrophic lateral sclerosis (ALS) had to be discontinued due to severe peripheral side effects. This was surprising since the receptor for CNTF is not expressed outside of the central nervous system. The fact that CNTF can also signal *via* the IL-6R may explain these side effects and may be the basis for the construction of CNTF variants which only bind to the CNTF-R but not to the IL-6R (Schuster *et al.,* 2003).

THE CONCEPT OF DESIGNER CYTOKINES

Using the structural information available on membrane bound and soluble cytokine receptors, we have constructed chimeric proteins in which receptor recognition modules have been altered or exchanged and in which cytokines have been fused to their soluble cytokine receptors. Furthermore, chimeric receptor proteins have been constructed which contain cytokine binding modules of gp130, LIFR or OSMRβ. This approach has allowed the definition of cytokine binding modules on receptor proteins (Aasland *et al.,* 2002; Aasland *et al.,* 2003; Kallen *et al.,* 1999; Kallen *et al.,* 2000).

Furthermore, we have constructed a fusion protein consisting of the domains of IL-6 and sIL-6R which are necessary for biological function. The two proteins are covalently fused by a flexible polypeptide linker (Fig. 1). The recombinant protein was folded correctly and showed biological activity. The fusion protein, which we call Hyper-IL-6 is 100–1000 times more active than the separate proteins IL-6 and sIL-6R. Many cells including hematopoietic progenitor cells, neuronal cells, endothelial cells, smooth muscle cells which do not respond to IL-6 alone show a remarkable response to IL-6/sIL-6R (Fischer *et al.,* 1997; Hacker *et al.,* 2003; Jones & Rose-John, 2002; Renné *et al.,* 1998). Recently, this approach has also been used to construct a fusion protein between IL-11 and the soluble IL-11R (Pflanz *et al.,* 1999). A designer cytokine consisting of CNTF fused to the soluble CNTF-R was shown to exhibit high neurotrophic activity on primary hippocampal neurons (Sun *et al.,* 2002).

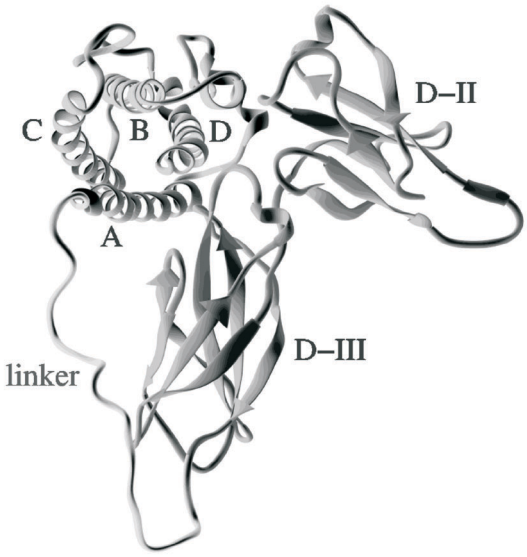

Figure 1. Hyper-IL-6 is: a highly active designer cytokine consisting of IL-6 and soluble IL-6R.

Molecular model of the fusion protein of IL-6 and sIL-6R (Hyper-IL-6) consisting of IL-6 and sIL-6R fused by a flexible peptide linker. A, B, C, D denote the four helices of IL-6; D-II and D-III are the two cytokine-binding receptor domains of the sIL-6R which were used for the construction of the fusion protein.

VIRAL INTERLEUKIN-6

The genome of HHV8 codes for several proteins with significant homologies to human antiapoptotic proteins, chemokines, and cytokines including a viral form of Interleukin-6 (vIL-6) with 25% homology to human IL-6 (Moore *et al.,* 1996; Neipel *et al.,* 1997). vIL-6 has been demonstrated to have biologic activities reminiscent of human IL-6, i.e. stimulation of proliferation of murine hybridoma and human myeloma cells (Burger *et al.,* 1998; Molden *et al.,* 1997; Moore *et al.,* 1996). More recently it was shown in mice, injected with vIL-6 transfected NIH3T3 cells, that vIL-6 induced angiogenesis and hematopoiesis. It was concluded that through these functions vIL-6 played an important role in the pathogenesis of HHV8-associated disorders (Aoki *et al.,* 1999).

We have recently shown that purified recombinant vIL-6 binds directly to gp130 and stimulates primary human smooth muscle cells and primary human Kaposi sarcoma cells. IL-6R fails to bind vIL-6 and is not involved in its signaling. Our data demonstrate that vIL-6 is the first cytokine which directly binds and activates gp130. This property points to a pos-

sible role of this viral cytokine in the pathophysiology of HHV8 (Hoischen *et al.,* 2000; Klouche *et al.,* 2002; Müllberg *et al.,* 2000b). In Fig. 2 we show the vIL-6 stimulation of HepG2 cells which have been engineered not to express the IL-6R on the cell membrane (Mackiewicz *et al.,* 1992). On these cells, human IL-6 does not lead to STAT3 activation whereas vIL-6 and Hyper-IL-6 activate STAT3 activity. The activation of STAT3 can be completely inhibited by a neutralizing gp130 antibody (Fig. 2). As can be seen in Fig. 3, vIL-6

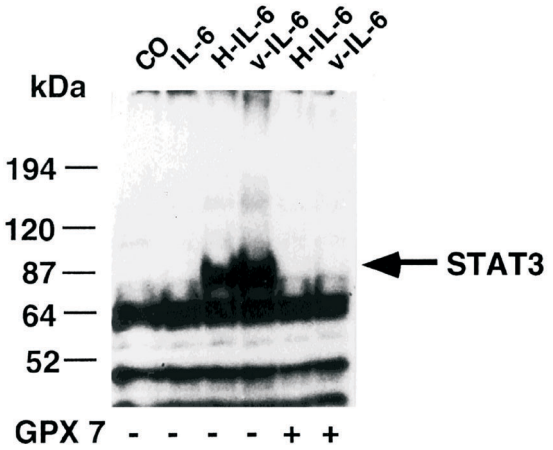

Figure 2. STAT3 activation by vIL-6 *via* direct stimulation of gp130 on human hepatoma cells.

HepG2-IL-6 cells were stimulated with 100 ng/ml IL-6, HyperIL-6, and vIL-6 in the presence or absence of the neutralizing gp130 mAB GPX7 (1 mg/ml) for 15 min. Cells were lysed and proteins were separated by SDS-PAGE and blotted onto nitrocellulose. Phosphorylated STAT3 protein was detected by Western blotting using a phosphospecific STAT3 mAB.

stimulates the proliferation of BAF/3 cells which only express gp130 but no IL-6R (Fig. 3A). The presence of the IL-6R in BAF/3 cells does not lead to a change in the observed dose response curve indicating that the IL-6R is not used by the viral cytokine (Fig. 3B).

The fact that vIL-6 forms a functional complex with gp130 without the need for the IL-6R (Hoischen *et al.,* 2000; Müllberg *et al.,* 2000b) has been exploited to crystallize the complex of the extracellular portion of gp130 together with vIL-6. This led to the first structural in-

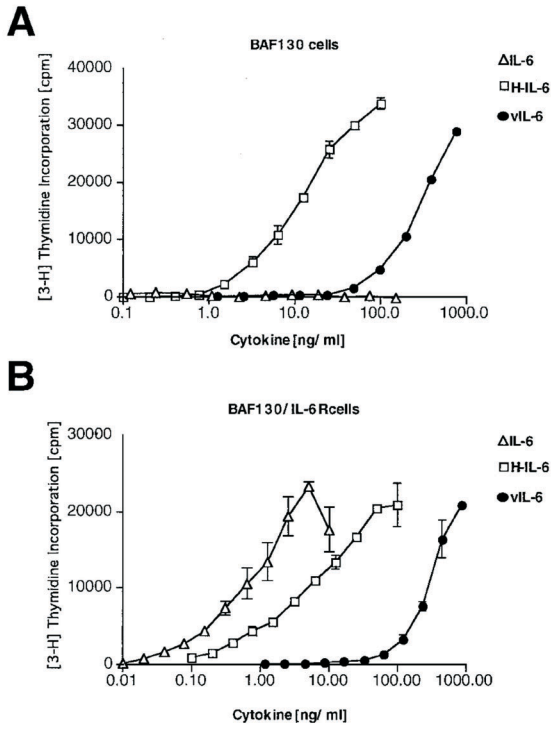

Figure 3. Biological activity of vIL-6 is mediated by gp130 directly.

BAF/3 cells stably transfected with a human gp130 cDNA (A) and BAF/3 cells stably transfected with human gp130 and IL-6R cDNAs (B) were stimulated with increasing amounts of HyperIL-6 (H-IL-6), human IL-6, and vIL-6. Proliferation of the cells was assessed by measuring [^{3}H]thymidine incorporation into DNA.

formation of a member of the complex type cytokines together with its receptor (Chow *et al.,* 2001). Complex type cytokines require the interaction with three cytokine receptor subunits to induce cellular signaling. In the case of IL-6 there is an interaction of the cytokine with the IL-6R and two molecules of gp130. In the case of CNTF the cytokine would interact with the CNTF-R, gp130 and the LIF-R protein (Taga & Kishimoto, 1997). Members of this cytokine family comprise besides IL-2 and IL-15 several members of the gp130 cytokine family like IL-6, IL-11, CNTF, CT-1 and CLC. Structural information on the simple type cytokine family which comprises (among others) growth hormone and prolactin has been available for more than 10 years (De Vos *et al.,* 1992).

THE ROLE OF SOLUBLE gp130

The role of a soluble form of gp130 (sgp130) was analyzed using two soluble gp130 fusion proteins. In the first version, the extracellular portion of gp130 was fused to a COOH-terminal hexahistidine tag. In a second version, the extracellular portion of gp130 was fused to the constant portion of a human IgG1 antibody protein. As can be seen in Fig. 4, sgp130

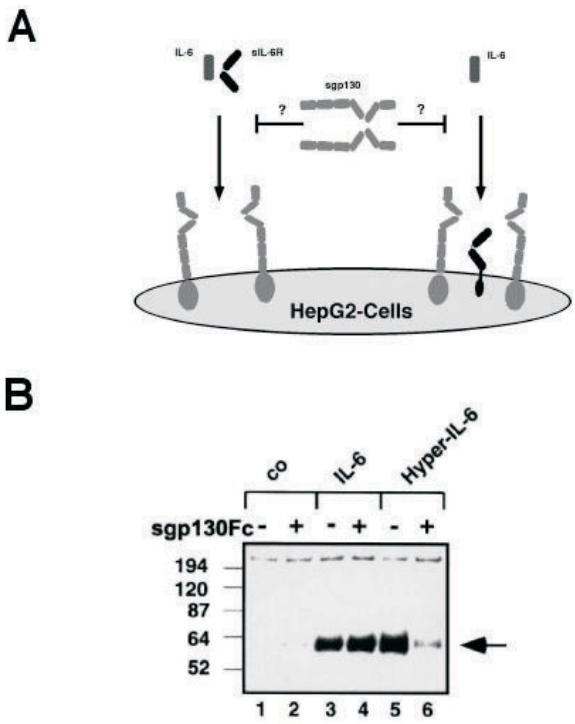

Figure 4. gp130 selectively inhibits signaling *via* IL-6/sIL-6R without affecting signaling *via* the membrane bound IL-6R.

HepG2 cells were stimulated with IL-6 or IL-6/sIL-6R in the presence or absence of soluble gp130. (A) Schematic representation of the experiment. (B) The induction of the acute phase protein antichymotrypsin on HepG2 cells was only inhibited by soluble gp130 when the cells were stimulated with IL-6/sIL-6R but not with IL-6 alone. These data indicate that sgp130 selectively inhibits biologic responses of IL-6/sIL-6R but not of IL-6 acting on the membrane bound IL-6R.

only inhibited the expression of the acute phase protein antichymotrypsin in HepG2 cells, which had been treated with Hyper-IL-6. The inductin of acute phase protein expression of HepG2 cells by human IL-6 is unaffected by soluble gp130 (Fig. 4B). It turned out that sgp130 exclusively inhibited IL-6 responses mediated by the sIL-6R without interfering with responses *via* the membrane bound IL-6R (Atreya *et al.,* 2000; Hurst *et al.,* 2001; Jostock *et al.,* 2001). Therefore we postulated that sgp130 acts as the natural inhibitor of IL-6/sIL-6R complexes. Our model of the molecular mechanism by which soluble gp130 exerts specific inhibitory towards the IL-6/sIL-6R complex is depicted in Fig. 5. IL-6 does not bind to soluble gp130. So IL-6 binds to the membrane bound IL-6R and form a complex with membrane bound gp130. The soluble gp130 protein does not have access to

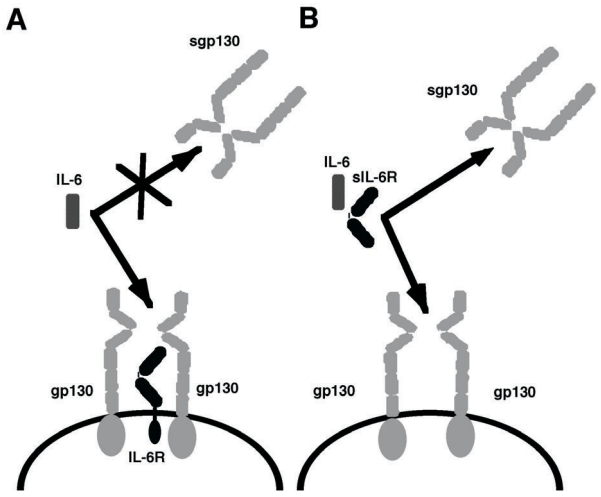

Figure 5. Schematic view of the inhibitory mechanism of sgp130.

(A) sgp130 has no access to IL-6 complexed by membrane bound IL-6R and two molecules of membrane bound gp130. (B) The IL-6/sIL-6R complex can bind to both, membrane bound and sgp130. Consequently, a molar excess of sgp130 leads to competitive inhibition of the IL-6/sIL-6R response.

this complex which therefore is not inhibited (Fig. 5A). The IL-6/sIL-6R complex binds as well to soluble and membrane bound gp130. Therefore, a molar excess of sgp130 leads to inhibition of the biologic response (Fig. 5B).

A functional role of sIL-6R has recently been demonstrated in chronic inflammatory bowl disease (Crohn's disease). We could show that

T-cells of Crohn's disease patients are extremely resistant to apoptosis and show activation of the JAK-STAT signal transduction pathway. These T-cells produce large amounts of IL-6 but lack membrane bound IL-6R. Surprisingly, treatment of these cells with a neutralizing monoclonal antibody to IL-6R induced apoptosis. Moreover treatment of the cells with sgp130 showed the same effect (Fig. 6). These results clearly demonstrate

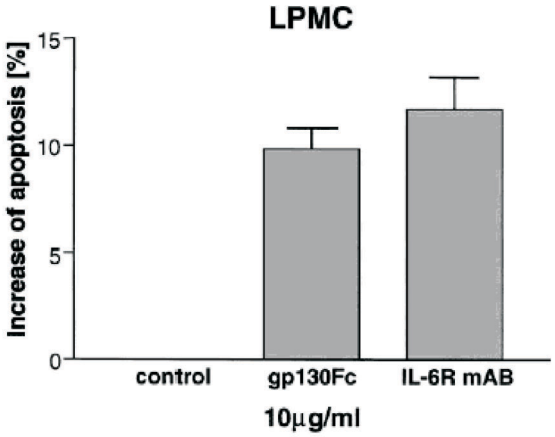

Figure 6. Apoptosis of lamina propria mononuclear cells (LPMC) of Crohn's disease patients upon treatment with sgp130.

LPMCs were isolated and cultured for 48 h in the presence or absence of 10 μg/ml of a neutralizing mAB specific for human IL-6R or 10 μg/ml sgp130Fc. Subsequently, cells were stained for annexin V and propidium iodide and analyzed by FACS. The increase in apoptotic (annexin V positive and propidium iodide negative) cells is shown. The data presented are means of triplicate measurements with standard errors shown as vertical bars.

that IL-6 is involved in apoptotic resistance of T-cells of Crohn's disease patients. Moreover, the data demonstrate that sIL-6R and not the membrane bound IL-6R is responsible for T-cell stimulation. Most likely, the sIL-6R is produced by lamina propria macrophages or neutrophiles (Atreya *et al.,* 2000; Jostock *et al.,* 2001).

The fact that in Crohn's disease the chronic inflammatory state is maintained with the help of IL-6/sIL-6R signaling seems to be a more general phenomenon. It was recently shown in a murine peritonitis model that the transition between the acute phase, which is governed by neutrophils to the chronic state which is characterized by massive mononuclear cell infiltration is regulated by the level of the soluble IL-6R complex. The sIL-6R in the peritoneum is presumably generated by shedding from neutrophilic cells. Therefore the transition of the neutrophil to the mononuclear cell phase could be inhibited by the addition of soluble gp130 protein (Hurst *et al.,* 2001).

CONCLUSION

We conclude that sgp130 is the natural inhibitor of IL-6 responses which are dependent on sIL-6R. Furthermore, recombinant sgp130 is expected to be a valuable therapeutic tool to specifically block disease states in which sIL-6R transsignaling responses exist, e.g. in Crohn's disease and other chronic inflammatory diseases.

NOTE

There are inclusions in this text from a number of different articles which are cited in the reference section.

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