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THEORETICAL ARTICLE

sIL-6R: more than an agonist?

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On target cells, interleukin-6 (IL-6) interacts with its receptor complex consisting of the membrane-bound IL-6 receptor (IL-6R) and the signal transducing protein gp130. IL-6R can exist as a soluble protein (sIL-6R), which binds the ligand IL-6. This soluble complex can bind to gp130 on cells that lack the membrane-bound IL-6R and initiate signaling. This process is named transsignaling. The significance of transsignaling via sIL-6R is underlined by different publications and exceeds very probably the significance of the membrane-bound IL-6R. It is the general assumption that sIL-6R acts as an agonist in combination with IL-6 resulting in an enhancement of the IL-6 effects. In this article, we suppose 'non-agonistic' properties. There are several publications that give reasons to speculate that sIL-6R (a) has IL-6-antagonistic effects, (b) has orphan properties and (c) interacts with yet unknown binding partners different from IL-6. Knowledge about additional properties of sIL-6R will enlarge the biologic understanding of this molecule and might give an explanation for the sometimes contrasting effects of the cytokine IL-6.
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PREREQUISITE FOR IL-6 SIGNALING

In order that interleukin-6 (IL-6) signaling is possible, different receptors are required. On target cells, IL-6 first binds to the IL-6 receptor (IL-6R). In order that signaling may take place a second subunit is required. The complex of IL-6 and IL-6R associates with the signal-transducing membrane protein gp130, inducing its dimerization and initiation of signaling.[1] It does not bind IL-6 by itself. Dimerization of gp130 is followed by the rapid activation of tyrosine kinases of the Janus kinase (Jak) family and subsequent activation of transcription factors of the STAT family, STAT3 and STAT1.[2,3] STAT factors play important roles in transcriptional regulation of genes containing STAT-specific binding sites. Gp130 is expressed by all cells in the body, whereas IL-6R is mainly expressed by hepatocytes, neutrophils, monocytes/macrophages and some lymphocytes. Besides the membrane-bound IL-6R, a naturally occurring soluble form of the IL-6R (sIL-6R) is generated by two independent mechanisms, limited proteolysis of the membrane protein and translation from an alternatively spliced mRNA.[4–9] Interestingly, the sIL-6R together with IL-6 stimulates cells that only express gp130,[10–12] a process named transsignaling. This process implicates that cells which were originally unresponsive to IL-6 (because of the lack of membrane-bound IL-6R) become now responsive via the soluble sIL-6R/IL-6 complex. Embryonic stem cells,[13,14] early hematopoietic progenitor cells,[15] colon cancer cells in transgenic mice,[16] many neural cells,[17] smooth muscle cells[18] and endothelial cells[19] are responsive to IL-6 only in the presence of sIL-6R. The IL-6 transsignaling is depicted in Figure 1.

SIGNIFICANCE OF TRANSSIGNALING VIA SIL-6R

The full impact of IL-6 transsignaling has begun to be appreciated within the past few years. The importance of sIL-6R, using a mathematical model, is underlined by the fact that about 70% of the circulating IL-6 forms a complex with the sIL-6R in the blood and binds directly to membrane gp130.[20] The other 30% is supposed to have only a transient existence in the blood, or binds to the membrane-bound receptor (IL-6R).[20,21] The study of Peters *et al.*[22] clearly show in transgenic mice that sIL-6R functions as a carrier protein for its ligand, thereby markedly prolonging the plasma half-life of IL-6 indicating that IL-6 signaling is increased by sIL-6R. The significance of the soluble IL-6 receptor is further underlined by a study done by Vermes *et al.*[23] analyzing human osteoblasts. They found the cells to express cell surface IL-6R but the membrane-bound IL-6R was unable to transmit IL-6-induced signals (tyrosine phosphorylation of gp130 is an essential step in the IL-6-induced signaling mechanism) until it is shed into its soluble form sIL-6R. The results of Modur *et al.*[24] show that for the IL-6 signaling process the limiting factor is the soluble IL-6R, not the cytokine IL-6. Endothelial cell activation depended on the concentration of sIL-6R. The study of Becker *et al.*[16] using a colon tumor mice model show that IL-6 signal transduction was mediated by the soluble rather than the membrane-bound IL-6R, indicating that tumor growth is controlled by IL-6 transsignaling via the soluble form of the IL-6R. Concerning acute inflammation, Hurst *et al.*[25] could show that sIL-6R-mediated signaling is an important intermediary in the resolution of inflammation and supports the transition between the early predominantly neutro-

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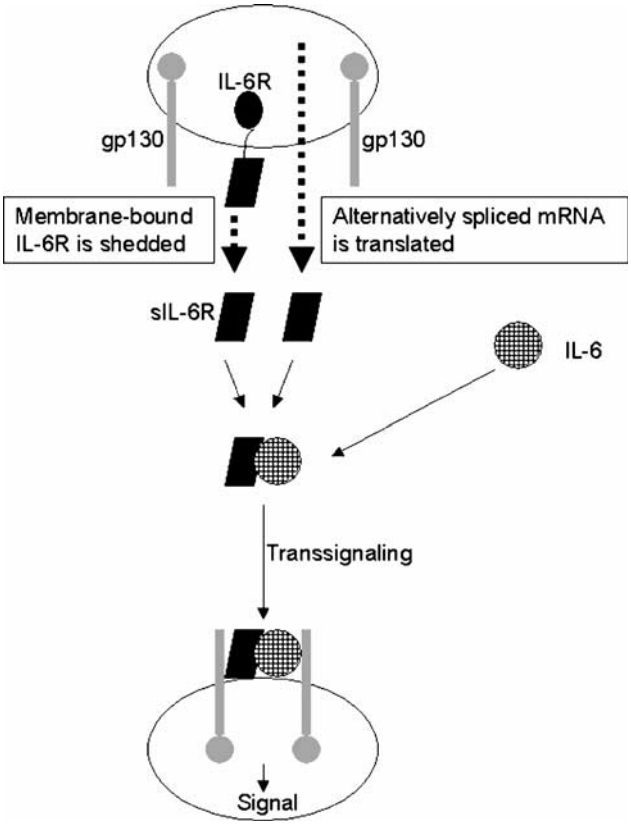

Figure 1 IL-6 transsignaling. By shedding or alternative splicing, the soluble IL-6 receptor (sIL-6R) is released. sIL-6R binds the cytokine IL-6, induces homodimerization of gp130 on target cells that lack membrane-bound IL-6R and IL-6 signaling may take place.

philic stage of an infection and the more sustained mononuclear cell influx. Furthermore, they provide evidence that the biological activities of sIL-6R serve as a potential explanation for the apparent paradox of IL-6 being able to act as both a pro- and anti-inflammatory mediator. In chronic intestinal inflammation blockade of the IL-6 transsignaling caused T-cell apoptosis, indicating that the IL-6–sIL-6R system mediates the resistance of T cells to apoptosis in Crohn's disease.[26]

AGONISTIC AND 'NON-AGONISTIC' PROPERTIES OF SIL-6R

On the one hand, it is well known and a general assumption that sIL-6R is agonistic with IL-6, indicating an enhanced sensitivity of cells to IL-6. On the other hand, there are hints indicating that sIL-6R also possesses 'non-agonistic' properties, for example antagonistic properties against IL-6. Furthermore, there are some interesting publications dealing with a relatively new assumption that sIL-6R alone (without combination with IL-6) induces effects on different cells indicating an 'orphan' property of sIL-6R. Last but not least one can speculate that sIL-6R interacts with yet unknown binding partners, which have to be identified.

Agonistic properties of sIL-6R

Activation of STAT3 is one of the essential steps in the IL-6 signaling pathway.[27,28] Nakanishi *et al.*[29] could show using the pleomorphic malignant fibrous histiocytoma cell line MIPS-2 that the treatment of the cells with a combination of IL-6 (100 ng/ml) and sIL-6R (100 ng/ml) robustly increased the phosphorylation level of STAT3 in a time-dependent manner with a peak at 30 min. The conditioned medium from MIPS-2 cells (containing IL-6) and sIL-6R, or the treatment with IL-6 alone also induced phosphorylation of STAT3 but to a lesser extent. Similar results were worked out for the phosphorylation level of p44/p42 mitogen-activated protein kinase. Furthermore, both IL-6 and sIL-6R demonstrated the growth inhibitory effects on proliferation in a dose-dependent manner. The effect of sIL-6R was less pronounced but enhanced by the addition of IL-6. They also showed a diminished invasiveness of MIPS-2 cells using an *in vitro* Matrigel invasion assay with sIL-6R, and again a further decreased invasiveness when the combination of IL-6/sIL-6R was added. These results support the accepted hypothesis that sIL-6R acts agonistically with IL-6. The study of Sundelin *et al.*[30] analyzed the effects of different cytokines (among others IL-6 and sIL-6R) on matrix metalloproteinase-1 and -9 in two oral squamous-cell carcinoma cell lines. Matrix metalloproteinases play an important role in enhanced intratumoral proteolytic activity, promoting angiogenesis and invasion by acting on extracellular matrix substances. The authors showed a moderate stimulatory effect on matrix metalloproteinase-1 secretion in both cell lines for IL-6, whereas sIL-6R had no effect. When these cytokines were added together, a dose-dependent, synergistic stimulatory effect was observed. Another study, done by Franchimont *et al.*[31] tested the relevance of IL-6 and sIL-6R in regard to collagenase 3 production in osteoblast-enriched cells from fetal rat calvariae. IL-6 caused a small increase in collagenase mRNA levels, but in the presence of IL-6 soluble receptor, IL-6 caused a marked increase in collagenase transcripts after 2–24 h. In addition, sIL-6R increased collagenase mRNA when tested alone indicating both molecules to be involved in bone resorption.

Yuan *et al.*[32] found IL-6 to suppress Fas-mediated apoptosis, an increase in Bcl-xl expression and a prominent STAT1 tyrosine phosphorylation and a minimal STAT3 activation. Furthermore, they examined whether butyrate (a short-chain fatty acid produced in the lumen of the colon after the ingestion of fiber) might interfere with IL-6-induced signaling events. Butyrate treatment led to the loss of the IL-6-induced STAT1 activation. Introduction of the sIL-6R led to a restoration of the STAT1 activation; furthermore, butyrate treatment inhibited the antiapoptotic effects of IL-6 and led to a loss of IL-6R (gp80) mRNA expression. sIL-6R could restore resistance to apoptosis in butyrate-treated cells, but restoration was not complete indicating sIL-6R to function as a resistance mechanism in these cells. The study of Dowdall *et al.*[33] analyzed whether sIL-6R increases tumor cell adherence to the endothelium. In their study, treatment of colon tumor cells with sIL-6R did not alter the degree of transmigration across the endothelium monolayer, but it did significantly increase the number of adhering cells indicating that sIL-6R might influence the genesis of early metastasis.

Using a cDNA expression array the mRNA levels of about 580 human genes were tested by Holub *et al.*[34] Comparing the effects of IL-6 and IL-6 plus sIL-6R, they found that in the case of some genes the presence of sIL-6R greatly enhances the effectiveness of IL-6 (fos-related antigen). In other genes IL-6 alone has no or only slight effect; however, if given together with sIL-6R the enhancing effects are very high. Expression of large number of genes is modulated if the cells were treated with sIL-6R in the presence of IL-6 (for example, the cells had a possibility to use more IL-6R). These data suggest that presence of soluble cytokine receptors affects actual gene expression profile of corresponding cells and support the agonistic property of sIL-6R.

'Non-agonistic': antagonistic properties of sIL-6R

On the one hand, the agonistic properties of sIL-6R by enhancing IL-6 signaling are well documented. On the other hand, there are results

that are in opposite to this general assumption indicating also antagonistic properties of sIL-6R.

Besides a soluble form of the IL-6R, a soluble form of gp130 (sgp130) has been detected in healthy human sera containing 390±72 ng/ml sgp130 [35] with antagonistic properties. The study of Müller-Newen *et al.*[36] show that the antagonistic activity of sgp130 is markedly enhanced in the presence of sIL-6R. Among others, they investigated the antagonistic activity of sgp130 on cells expressing membrane-bound IL-6R. These cells respond to IL-6 without the requirement of sIL-6R. They tested HepG2 cells that respond to IL-6 with the synthesis of the acute-phase protein α1-antichymotrypsin. sgp130 was antagonistic to the effects of IL-6. Most surprisingly, they found out that sIL-6R increased the antagonistic effect of sgp130. They repeated this experiment with physiologic concentrations of IL-6, sIL-6R and sgp130 and again, an inhibitory effect of sIL-6R in the presence of sgp130 was observed. The authors speculate that in cases in which cells are stimulated with IL-6 alone, the cytokine first binds to the cell surface IL-6R before it interacts with sgp130 or with membrane-bound gp130. The IL-6/IL-6R complexes, due to their membrane location, have to find membrane-bound gp130 only in a two-dimensional space. When sIL-6R is added, IL-6/sIL-6R complexes can be trapped by sgp130 in the soluble high-affinity ternary complexes and are thereby efficiently neutralized before they bind to the cell surface receptors. These results are supported and further enlarged by the results of Jostock *et al.*[37] They recombinantly expressed the entire extracellular portion of sgp130 with a C-terminal hexahistidine tag as well as with a C-terminal Fc moiety of a human IgG antibody. They assayed the activity of sgp130 on cells that only expressed gp130 and on cells that expressed both membrane-bound IL-6R and gp130. It turned out that sgp130 completely abrogated the proliferation of gp130 expressing cells, which had been stimulated with IL-6/sIL-6R or the fusion protein Hyper-IL-6 (designer cytokine). sgp130 exclusively inhibited IL-6 responses mediated by the sIL-6R without interfering with responses via the membrane-bound IL-6R. Therefore, the authors concluded that sgp130 selectively inhibits IL-6 transsignaling without affecting classic signaling via the membrane-bound IL-6R. In this context, sIL-6R acts antagonistically in combination with sgp130. This paper also gives a schematic view and explanation of the inhibitory mechanism of sgp130. The complex of IL-6 and membrane-bound IL-6R associates with two molecules of gp130. Due to steric hindrance, sgp130 has no access to the IL-6/IL-6R complex associated with membrane-bound gp130. IL-6 complexed with sIL-6R can bind to both the membrane-bound and sgp130. Therefore, a molar excess of sgp130 leads to competitive inhibition of the IL-6/sIL-6R response.

The studies of Igaz *et al.*[38] also shows antagonistic activity of sIL-6R. Addition of IL-6 resulted in an induction of junB mRNA expression using HepG2 cells and primary rat hepatocytes. The presence of sIL-6R markedly decreased the induction of junB mRNA expression (by 50%). Furthermore, the sIL-6 receptor significantly decreased the IL-6-induced fibrinogen production in both cell types indicating sIL-6R to be an antagonistic player. The authors did not analyze the significance of the antagonistic player sgp130. Therefore, it cannot be excluded that the antagonistic activity of sIL-6R is mediated via sgp130.

One explanation for the antagonistic property of sIL-6R is the interaction of sgp130 with sIL-6R. The review of Peters *et al.*[15] offers a further explanation how different ratios between IL-6R and gp130 on the cell surface might result in agonistic and antagonistic properties of the soluble IL-6R. The number of gp130 signal transducers is constant on all cells of the body, whereas the number of IL-6R molecules expressed on the surface of target cells may vary form one cell type to another. Cells that do not express any IL-6R on their surface can be stimulated only by the IL-6/sIL-6R complex and are insensitive to IL-6 alone. Cells that express fewer IL-6R molecules on their surface than gp130 signal transducers respond toward IL-6 alone, and this response can be enhanced by the presence of the sIL-6R. Cells that show a balanced expression of IL-6R and gp130 on their surface respond toward IL-6, and this response is not altered by the sIL-6R. Theoretically, on cells that express more IL-6R molecules than gp130 proteins, the addition of the soluble IL-6R might inhibit the IL-6 response, because the formation of inactive complexes containing only one gp130 molecule would be favored.

'Non-agonistic': 'orphan' properties of sIL-6R

The relatively new assumption that sIL-6R itself, without IL-6, might exert signaling on cells is supported by three studies. It could be shown that the presence of sIL-6R induced the expression of junB 6.66-fold relative to the control in HepG2 cells (which are known not to produce endogenous IL-6[38]). Another study done by Holub *et al.*,[34] also showed surprising effects of sIL-6R on HepG2 cells. For gene expression studies HepG2 cells (5×10^6) were kept in serum-free medium for 24 h, then incubated for 2 h in the presence of medium, 100 ng/ml IL-6, sIL-6R (5 µl 68 pg/ml) or sIL-6R plus IL-6 using the same concentrations. In their macroarray approach, expression of 578 human genes was studied. Their data show that upon treatment of the HepG2 cells with sIL-6R alone the expression of most genes are not changing. Nevertheless, there is one gene (cAMP-response element binding protein) which is notably upregulated, 10 others are strongly decreased when IL-6R alone was applied. The inhibition is rather high (70–100%), particularly for IAP3, an apoptosis inhibitor, hEGR1 (early growth response protein) and an ets transcription factor (NER F2).

The relatively old study of Diamant *et al.*[39] also gives reason to speculate that sIL-6R has orphan properties. Addition of sIL-6R resulted in a dose-dependent stimulatory effect on the proliferation of B9 cells. This stimulatory effect could not be neutralized by the addition of anti-IL-6 ab. The authors concluded that these results indicate a stimulatory effect of sIL-6R by itself.

These results let speculate that sIL-6R may act as 'orphan' molecule on cells without complexing with IL-6. Furthermore, the 'orphan' properties of sIL-6R are eventually mediated via an yet unknown binding partner of sIL-6R which is to be identified.

'Non-agonistic': novel binding properties of sIL-6R

Our speculation of an eventually not yet defined reaction partner is underlined by a study done by Su *et al.*[40] They used a relatively new method, termed phage peptide display library screening. With this method they found a novel synthetic peptide, S7, with specifically bound to soluble IL-6R. Immunofluorescence staining revealed phage 7 binds to the plasma membrane of C33A cervical cancer cells and other IL-6R-expressing cell lines. Further confirmation of the specificity of S7 binding to IL-6R was obtained by laser scan confocal microscopic observation of C33A cells double stained with anti-IL-6R and anti-M13 antibodies. Furthermore, S7 peptide prevents IL-6-mediated survival signaling and sensitizes cervical cancer cells to chemotherapeutic compounds *in vitro*. The analysis of antiangiogenic activity showed that S7 peptide substantially inhibits IL-6-induced vascular endothelial growth factor-A expression and angiogenesis in different cancer cell lines. Furthermore, S7 peptide was bioavailable *in vivo*, leading to a significant suppression of IL-6-induced vascular endothelial growth

factor-mediated cervical tumor growth in severe combined immunodeficient mice.

The study of Schuster *et al.*[41] clearly demonstrates that human ciliary neurotrophic factor can use both the membrane bound and the soluble human IL-6R as a substitute for its cognate α-receptor and thus widen the target spectrum of human ciliary neurotrophic factor. Binding of human ciliary neurotrophic factor to the human IL-6R takes place with an affinity roughly 50-fold lower than that of IL-6 binding to the IL-6R. An important result of this study is that human ciliary neurotrophic factor can induce cellular responses after binding to the soluble IL-6R.

The possibility of an unknown binding partner for sIL-6R is indirectly supported by recent studies of Novotny-Diermayr *et al.*[42] Their aim was to identify new binding partners of the activated gp130 and the associated Janus kinases. In their assay using the relatively new 'yeast two-hybrid screening' method they found LMO4, a LIM domain-containing protein that belongs to a family of oncogenes, mediates protein–protein interactions and is highly expressed in breast cancer cell lines,[43] to directly interact with the intracellular region of the gp130 receptor. The effect of LMO4 in IL-6 signaling was subsequently examined. Overexpression of LMO4 enhanced the transcriptional activity and target gene expression of Stat 3. Consistent with this, silencing LMO4 expression in stable cell lines expressing the small interfering RNA of LMO4 decreased Stat3 activity. Furthermore, the half-life of gp130 was shortened, and the production of acute phase proteins induced by IL-6 was reduced.

CONCLUSION

It is a general assumption that sIL-6R acts agonistically in combination with IL-6 indicating an enhancement of IL-6 effects. In this article, we summarize the literature which give reason to speculate that sIL-6R possesses also 'non-agonistic' properties. Further experiments are necessary to rule out the significance of the 'non-agonistic' signaling in regard to the sometimes contrasting biological effects of IL-6.

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1 Taga T, Kishimoto T. Gp130 and the interleukin-6 family of cytokines. *Annu Rev Immunol* 1997; **15**: 797–819.
2 Hibi M, Murakami M, Saito M, Hirano T, Taga T, Kishimoto T. Molecular cloning and expression of an IL-6 signal transducer, gp130. *Cell* 1990; **63**: 1149–1157.
3 Rakemann T, Niehof M, Kubicka S, Fischer M, Manns MP, Rose-John S *et al.* The designer cytokine hyper-interleukin-6 is a potent activator of STAT3-dependent gene transcription *in vivo* and *in vitro*. *J Biol Chem* 1999; **274**: 1257–1266.
4 Lust JA, Donovan KA, Kline MP, Greipp PR, Kyle RA, Maihle NJ. Isolation of an mRNA encoding a soluble form of the human interleukin-6 receptor. *Cytokine* 1992; **4**: 96–100.
5 Rose-John S, Heinrich PC. Soluble receptors for cytokines and growth factors: generation and biological function. *Biochem J* 1994; **300** (Part 2): 281–290.
6 Mullberg J, Althoff K, Jostock T, Rose-John S. The importance of shedding of membrane proteins for cytokine biology. *Eur Cytokine Netw* 2000; **11**: 27–38.
7 Althoff K, Reddy P, Voltz N, Rose-John S, Mullberg J. Shedding of interleukin-6 receptor and tumor necrosis factor alpha. Contribution of the stalk sequence to the cleavage pattern of transmembrane proteins. *Eur J Biochem* 2000; **267**: 2624–2631.
8 Althoff K, Mullberg J, Aasland D, Voltz N, Kallen K, Grotzinger J *et al.* Recognition sequences and structural elements contribute to shedding susceptibility of membrane proteins. *Biochem J* 2001; **353** (Part 3): 663–672.
9 Matthews V, Schuster B, Schutze S, Bussmeyer I, Ludwig A, Hundhausen C *et al.* Cellular cholesterol depletion triggers shedding of the human interleukin-6 receptor by ADAM10 and ADAM17 (TACE). *J Biol Chem* 2003; **278**: 38829–38839.
10 Taga T, Hibi M, Hirata Y, Yamasaki K, Yasukawa K, Matsuda T *et al.* Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell* 1989; **58**: 573–581.
11 Mackiewicz A, Schooltink H, Heinrich PC, Rose-John S. Complex of soluble human IL-6-receptor/IL-6 up-regulates expression of acute-phase proteins. *J Immunol* 1992; **149**: 2021–2027.
12 Rose-John S, Schooltink H. Cytokines are a therapeutic target for the prevention of inflammation-induced cancers. *Recent Results Cancer Res* 2007; **174**: 57–66.
13 Nichols J, Chambers I, Smith A. Derivation of germline competent embryonic stem cells with a combination of interleukin-6 and soluble interleukin-6 receptor. *Exp Cell Res* 1994; **215**: 237–239.
14 Rose-John S. GP130 stimulation and the maintenance of stem cells. *Trends Biotechnol* 2002; **20**: 417–419.
15 Peters M, Muller AM, Rose-John S. Interleukin-6 and soluble interleukin-6 receptor: direct stimulation of gp130 and hematopoiesis. *Blood* 1998; **92**: 3495–3504.
16 Becker C, Fantini MC, Schramm C, Lehr HA, Wirtz S, Nikolaev A *et al.* TGF-beta suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. *Immunity* 2004; **21**: 491–501.
17 Marz P, Otten U, Rose-John S. Neural activities of IL-6-type cytokines often depend on soluble cytokine receptors. *Eur J Neurosci* 1999; **11**: 2995–3004.
18 Klouche M, Bhakdi S, Hemmes M, Rose-John S. Novel path to activation of vascular smooth muscle cells: up-regulation of gp130 creates an autocrine activation loop by IL-6 and its soluble receptor. *J Immunol* 1999; **163**: 4583–4589.
19 Romano M, Sironi M, Toniatti C, Polentarutti N, Fruscella P, Ghezzi P *et al.* Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity* **6**: 315–325.
20 Gaillard J, Pugniere M, Tresca J, Mani J, Klein B, Brochier J. Interleukin-6 receptor signaling. II. Bio-availability of interleukin-6 in serum. *Eur Cytokine Netw* 1999; **10**: 337–344.
21 Ndubuisi MI, Patel K, Rayanade RJ, Mittelman A, May LT, Sehgal PB. Distinct classes of chaperoned IL-6 in human blood: differential immunological and biological availability. *J Immunol* 1998; **160**: 494–501.
22 Peters M, Jacobs S, Ehlers M, Vollmer P, Mullberg J, Wolf E *et al.* The function of the soluble interleukin 6 (IL-6) receptor *in vivo*: sensitization of human soluble IL-6 receptor transgenic mice towards IL-6 and prolongation of the plasma half-life of IL-6. *J Exp Med* 1996; **183**: 1399–1406.
23 Vermes C, Jacobs JJ, Zhang J, Firneisz G, Roebuck KA, Glant TT. Shedding of the interleukin-6 (IL-6) receptor (gp80) determines the ability of IL-6 to induce gp130 phosphorylation in human osteoblasts. *J Biol Chem* 2002; **277**: 16879–16887.
24 Modur V, Li Y, Zimmerman GA, Prescott SM, McIntyre TM. Retrograde inflammatory signaling from neutrophils to endothelial cells by soluble interleukin-6 receptor alpha. *J Clin Invest* 1997; **100**: 2752–2756.
25 Hurst SM, Wilkinson TS, McLoughlin RM, Jones S, Horiuchi S, Yamamoto N *et al.* Il-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation. *Immunity* 2001; **14**: 705–714.
26 Atreya R, Mudter J, Finotto S, Mullberg J, Jostock T, Wirtz S *et al.* Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis *in vivo*. *Nat Med* 2000; **6**: 583–588.
27 O'Shea JJ, Gadina M, Schreiber RD. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. *Cell* 2002; **109** (Suppl): S121–S131.
28 Rane SG, Reddy EP. JAKs STATs and Src kinases in hematopoiesis. *Oncogene* 2002; **21**: 3334–3358.
29 Nakanishi H, Yoshioka K, Joyama S, Araki N, Myoui A, Ishiguro S *et al.* Interleukin-6/soluble interleukin-6 receptor signaling attenuates proliferation and invasion, and induces morphological changes of a newly established pleomorphic malignant fibrous histiocytoma cell line. *Am J Pathol* 2004; **165**: 471–480.
30 Sundelin K, Roberg K, Grenman R, Hakansson L. Effects of cytokines on matrix metalloproteinase expression in oral squamous cell carcinoma *in vitro*. *Acta Otolaryngol* 2005; **125**: 765–773.
31 Franchimont N, Rydziel S, Delany AM, Canalis E. Interleukin-6 and its soluble receptor cause a marked induction of collagenase 3 expression in rat osteoblast cultures. *J Biol Chem* 1997; **272**: 12144–12150.
32 Yuan H, Liddle FJ, Mahajan S, Frank DA. IL-6-induced survival of colorectal carcinoma cells is inhibited by butyrate through down-regulation of the IL-6 receptor. *Carcinogenesis* 2004; **25**: 2247–2255.
33 Dowdall JF, Winter DC, Andrews E, Laug WE, Wang JH, Redmond HP. Soluble interleukin 6 receptor (sIL-6R) mediates colonic tumor cell adherence to the vascular endothelium: a mechanism for metastatic initiation? *J Surg Res* 2002; **107**: 1–6.
34 Holub MC, Hegyesi H, Igaz P, Polgar A, Toth S, Falus A. Soluble interleukin-6 receptor enhanced by oncostatin M induces major changes in gene expression profile of human hepatoma cells. *Immunol Lett* 2002; **82**: 79–84.
35 Narazaki M, Yasukawa K, Saito T, Ohsugi Y, Fukui H, Koishihara Y *et al.* Soluble forms of the interleukin-6 signal-transducing receptor component gp130 in human serum possessing a potential to inhibit signals through membrane-anchored gp130. *Blood* 1993; **82**: 1120–1126.
36 Muller-Newen G, Kuster A, Hemmann U, Keul R, Horsten U, Martens A *et al.* Soluble IL-6 receptor potentiates the antagonistic activity of soluble gp130 on IL-6 responses. *J Immunol* 1998; **161**: 6347–6355.
37 Jostock T, Mullberg J, Ozbek S, Atreya R, Blinn G, Voltz N *et al.* Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. *Eur J Biochem* 2001; **268**: 160–167.
38 Igaz P, Toth S, Rose-John S, Madurka I, Fejer G, Szalai C *et al.* Soluble interleukin 6 (IL-6) receptor influences the expression of the protooncogene junB and the production

of fibrinogen in the HepG2 human hepatoma cell line and primary rat hepatocytes. *Cytokine* 1998; **10**: 620–626.
39 Diamant M, Hansen MB, Rieneck K, Svenson M, Yasukawa K, Bendtzen K. Stimulation of the B9 hybridoma cell line by soluble interleukin-6 receptors. *J Immunol Methods* 1994; **173**: 229–235.
40 Su JL, Lai KP, Chen CA, Yang CY, Chen PS, Chang CC *et al*. A novel peptide specifically binding to interleukin-6 receptor (gp80) inhibits angiogenesis and tumor growth. *Cancer Res* 2005; **65**: 4827–4835.
41 Schuster B, Kovaleva M, Sun Y, Regenhard P, Matthews V, Grotzinger J *et al*. Signaling of human ciliary neurotrophic factor (CNTF) revisited. The interleukin-6 receptor can serve as an alpha-receptor for CTNF. *J Biol Chem* 2003; **278**: 9528–9535.
42 Novotny-Diermayr V, Lin B, Gu L, Cao X. Modulation of the interleukin-6 receptor subunit glycoprotein 130 complex and its signaling by LMO4 interaction. *J Biol Chem* 2005; **280**: 12747–12757.
43 Racevskis J, Dill A, Sparano JA, Ruan H. Molecular cloning of LMO41, a new human LIM domain gene. *Biochim Biophys Acta* 1999; **1445**: 148–153.