REVIEW ARTICLE

Soluble receptors for cytokines and growth factors: generation and biological function

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INTRODUCTION

Cytokines and growth factors

Cytokines and growth factors are hormone-like molecules which are secreted by many different cells (e.g. monocytes/macrophages, endothelial cells, fibroblasts, B- and T-cells), mostly after stimulation. They play important regulatory roles in the control of the immune response, haematopoiesis, acute-phase response, inflammatory reactions, cell proliferation and tumour growth (Yarden and Ullrich, 1988; Thomson, 1991). Cytokines and growth factors exert their actions on target cells via specific cell-surface receptors; as they show such potent biological effects, their activity is strictly controlled.

Control of receptor function

The availability of cytokines and growth factors is controlled by their expression. There are multiple ways to regulate receptor function: (i) expression of receptors may be restricted to specific cell types; (ii) the receptor number on the plasma membrane may vary due to receptor mRNA regulation or receptor internalization, leading to either degradation or recycling; (iii) the receptor affinities may be modulated on some cells by additional membrane proteins, which interact with the ligand-binding protein; or (iv) the membrane-bound receptor may be transformed into a soluble form by limited proteolysis (shedding).

Interestingly, there is a second important biochemical pathway leading to the generation of soluble receptors: soluble receptors can be translated from differentially spliced mRNAs lacking transmembrane and cytoplasmic regions. To date, many soluble forms of membrane-bound cytokine and growth-factor receptors, which have retained their ability to specifically bind their ligands, have been identified in biological fluids and biochemically characterized.

It should be noted that not only cytokines and growth factors have soluble counterparts of their receptors, but so also do other transmembrane proteins such as adhesion molecules (e.g. ICAM-1/CD54) (Seth et al., 1991), the transferrin receptor (Chitambar et al., 1991), the receptor for complexes between lipopolysaccharide (LPS) and LPS-binding protein (CD14) (Landmann et al., 1992), the low-affinity receptor for IgE (CD23) (Alderson et al., 1992), and the membrane glycoproteins CD27 and CD40 expressed on B- and T-cells (Loenen et al., 1992).

In this review we will discuss the present knowledge about soluble cytokine and growth-factor receptors with emphasis on biochemical mechanisms involved in the generation and physiological actions of these molecules. Until now few reviews on this subject have been published (Fernandez-Botran, 1991; Tedder, 1991). We have not reviewed the many studies carried out on the regulation of the soluble interleukin (IL)-2 receptor in various disease states.

SOLUBLE RECEPTORS FOR CYTOKINES AND GROWTH FACTORS

Table 1 presents a synopsis of the presently known soluble cytokine and growth-factor receptors. We define soluble receptors as the soluble forms of their membrane counterparts which bind their ligands with similar affinities (not more than a 10-fold decrease in affinity) to those of the membrane-bound receptors. Furthermore, all soluble receptors summarized in Table 1 correspond to single transmembrane proteins. The soluble receptors are smaller in size than their respective membrane-bound counterparts, as they consist only of the extracellular region of the membrane-bound receptor. In some instances it has been documented that the release of soluble receptors from the membrane-bound precursor form is stimulated by physiological mediators, probably after exogenous disturbances of the physiological homoeostasis. This is also reflected in increased levels of soluble receptors in serum and urine from patients suffering from infections, or inflammatory or autoimmune diseases. It is shown in Table 1 that measurable constitutive levels have been found for most soluble receptors.

As shown in Table 1, the naturally occurring concentrations of soluble receptors are rather low. Purification from body fluids would therefore not lead to recovery of the substantial amounts necessary for studies on receptor–ligand interactions. Therefore the extracellular domains of most of the cytokine and growth-factor receptors have been expressed in bacterial, mammalian or insect cells, in order to obtain sufficient quantities to study ligand–soluble-receptor interactions. Although it is widely believed that the recombinant soluble receptors have identical properties with the naturally occurring soluble receptors, this is not necessarily the case. It will be discussed later that in some instances the natural soluble receptor proteins have never been detected. As shown in Table 2. the affinities of the soluble receptors for their respective ligands are in the nanomolar range throughout and are comparable with the affinities determined for membrane-bound receptors. In the past it has been difficult to elucidate the stoichiometry of ligand–receptor complexes for membrane-bound receptors. The availability of large amounts of soluble cytokine and growth-factor receptors allowed the performance of cross-linking and even crystallographic studies in order to work out the structures of ligand–receptor complexes.

It can be seen from Table 2 that various stoichiometric ratios of ligand–receptor complexes exist: 1:1, 1:2, 2:1 and 3:3. In the cases of growth hormone and tumour necrosis factor (TNF) α, the molecular structures have been solved by X-ray analysis after co-crystallization with the respective soluble receptors. Figure 1(a) shows that in the case of growth hormone one ligand is bound to two soluble receptor molecules (De Vos et al., 1992); Figure 1(b) demonstrates that a TNFα trimer interacts with three soluble TNFα-receptor molecules (Banner et al., 1993).

Abbreviations used: CNTF, ciliary neurotrophic factor; HIV, human immunodeficiency virus; IL, interleukin; LIF, leukaemia inhibitory factor; LPS, lipopolysaccharide; PMA, 4β-phorbol 12-myristate 13-acetate; TNF, tumour necrosis factor.

Table 1 Characteristics of presently known soluble receptors for cytokines and growth factors

Abbreviations: s, soluble; R, receptor; RA rheumatoid arthritis; OSM, oncostatin M; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; EGF, epidermal growth factor; TGF, transforming growth factor; PDGF, platelet-derived growth factor; NGF, nerve growth factor; FMLP, formylmethionyl-leucyl-phenylalanine; IFN, interferon.

Soluble receptor	Approx. molecular mass (kDa)	Mechanism of generation		Release stimulant	Site of protein detection		Possible function(s)	References
		Differential splicing	Shedding		Normal subjects	Disease states		
sIL-1-RI								
sIL-1-RII	45	–	+	IL-4	Serum	Synovial fluid (RA)	IL-1 scavenger	Colotta et al. (1993)
sIL-2-Rα	43							Baran et al. (1988); Honda et al. (1990)
sIL-2-Rβ								
sIL-4-R	30–40	+	–		Serum*		IL-4 carrier and depot	Mosley et al. (1989); Fernandez-Botran and Vitetta (1990)
					Ascites*			
					Urine*			
sIL-5-R	Protein not detected	+	–					Takaki et al. (1990); Devos et al. (1993)
sIL-6-Rα	50–60	+	+	PMA	Serum (76 ng/ml)		Complex of IL-6 and sIL-6-R	Novick et al. (1989); Taga et al. (1989); Honda
				HIV	Urine (> 350 ng/ml)		acts agonistically	et al. (1992); Lust et al. (1992); Mackiewicz et al. (1992); Müllberg et al. (1993a); Suzuki et al. (1993)
sIL-6-Rβ	90/110	–	+		Serum (390 ng/ml)		IL-6, IL-11, LIF, OSM, CNTF antagonist	Müllberg et al. (1993b); Narazaki et al. (1993)
sIL-7-R	Protein not detected	+	–					Goodwin et al. (1990)
sIL-9-R	Protein not detected	+	–					Renauld et al. (1992)
s-mpl	55	+	–					Souyri et al. (1990); Skoda et al. (1993)
	Protein detected only intracellularly							
sLIF-R	90*	+	–		1000 ng/ml (normal)*		Antagonist	Gearing et al. (1991); Layton et al. (1992)
					500 ng/ml (neonatal)*			
					32 μg/ml (pregnant)*			
sCNTF-R	68	–	+†	Peripheral nerve injury	Cerebrospinal fluid (10 ng/ml)	Upon nerve injury 500 ng/ml	Complex of CNTF and sCNTF-R acts agonistically	Davis et al. (1991, 1993a,b)
sG-CSF-R	Protein not detected	+	–					Fukunaga et al. (1990)
sGM-CSF-R		+	–					Raines et al. (1991)
sEGF-R	93/100	+	–	EGF				Khire and Das (1990); Günther et al. (1990)
sTGFβ-R	100–120	–	+					López-Cassilas et al. (1991)
sPDGF-R	90	–	+		Plasma		Possible PDGF antagonist	Tiesman and Hart (1993)
					Serum			
sNGF-R	50/52	–	+		Urine			Zupan et al. (1989); Barker et al. (1991); Zupan and Johnson (1991)
					Serum			
sTNF-RI	28	–	+	PMA, FMLP, C5a, GM-CSF, Ca-ionophore	Plasma (2.5–35 ng/ml)	Elevated (3-fold) in infected hepatic ascites	Protection against proteolysis and depot	Porteu and Nathan (1990); Aderka et al. (1991); Andus et al. (1992); Pennica et al. (1992); Van Zee et al. (1992); Howard et al. (1993); Roux-Lombard et al. (1993)
					Ascites (1.5–60 ng/ml)			
					Synovial fluid (6 ng/ml)			
sTNF-RII	32	–	+	PMA	Plasma	Elevated (2–3-fold) in infected and malignant ascites	Protection against proteolysis and depot	Porteu et al. (1991)
					Ascites			
					Synovial fluid (20 ng/ml)			
sIFNα	55				Urine (0.5–1 ng/ml)	Serum of hairy-cell-leukaemia patients (10–100 ng/ml)		Novick et al. (1989, 1992a)
sIFNγ	40							

* Mouse.
† Hydrolysis of glycosyl-phosphatidylinositol anchor.

Table 2 Dissociation constants and stoichiometric organization of soluble cytokine/growth-factor receptors

Abbreviations: s, soluble; R, receptor; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; NGF, nerve growth factor; IFNγ, interferon γ; GH, growth hormone; CL, determined by cross-linking; X-ray, determined by X-ray analysis.

Soluble receptor	Binding affinity (K_d) (nM)	Stoichiometry of ligand–receptor complexes (L/R)	References
sIL-1-R	5.1		Giri et al. (1990)
	2.7		Symons et al. (1991)
sIL-2-Rα	2	Receptor homodimer	Baran et al. (1988); Jacques et al. (1990)
sIL-4-R	0.07*	1:1* (CL)	Fernandez-Botran and Vitetta (1990)
sIL-5-R	20	2:1 (CL)	Devos et al. (1993)
sIL-6-R	4.9		Yasukawa et al. (1990)
	1.5	1:1 and/or 2:2 (CL)	Stoyan et al. (1993)
sLIF-R	0.6		Layton et al. (1992)
sEGF-RT	100	1:1	Günther et al. (1990); Hurwitz et al. (1991)
		1:2 (CL)	
sPDGF-R	0.4	1:1 and/or 2:2	Duan et al. (1991)
sNGF-R	1		Zupan et al. (1989)
sTNFα-R		3:3 (X-ray)	D'Arcy et al. (1993)
sIFNγ-R		2:1	Fountoulakis et al. (1990, 1991)
sGH-R	0.34	1:2 (X-ray)	Cunningham et al. (1989); De Vos et al. (1992)

* Mouse.

MECHANISMS OF GENERATION OF SOLUBLE RECEPTORS

Thus far two different mechanisms have been found to be responsible for the formation of soluble cytokine or growth-factor receptors. First, soluble receptors can be translated from differentially spliced pre-mRNA molecules lacking the transmembrane domain (Figure 2a). The second mechanism involves limited proteolysis (shedding) in the extracellular region of the membrane-bound receptor (Figure 2b).

Differential splicing

Table 3 summarizes the presently known soluble cytokine and growth-factor receptors generated by differential splicing. The difference between the nucleotide sequences of the membrane-bound and the soluble receptors results from either insertion or deletion of nucleotide sequences, reflecting two types of differential splicing. The soluble receptors differ from the extracellular regions of their membrane-bound counterparts by missing extracellular amino acids and by having unique amino acids added at the C-terminus. The additional unique amino acids are the consequence of the insertion or deletion of nucleotide sequences, often involving a reading frame shift. In most cases only a few amino acids are missing from the extracellular region of the soluble receptors. In the cases of the soluble IL-9 receptor, mpl receptor and leukaemia inhibitory factor (LIF) receptor, 57, 55 and 109 amino acids respectively are missing at the C-terminal ends of the extracellular region. In the cases of the receptors for IL-9 and mpl these deletions result in the loss of the Trp-Ser-Xaa-Trp-Ser motif (WSXWS), which is essential for ligand binding (Yawata et al., 1993). Since the extracellular domain of the soluble IL-9 receptor contains only one WSXWS box, we would predict that the soluble protein is not able to bind IL-9. In the case of the mpl receptor two WSXWS motifs are present in the extracellular part, leaving one WSXWS box in the soluble protein, which is probably sufficient for the binding of the as yet unidentified ligand.

It should be noted that the presence of mRNA for soluble receptors within the cell does not necessarily imply that the corresponding protein is synthesized. This has been clearly shown for the IL-5 and the mpl receptors, where in spite of high mRNA levels for the soluble receptors no proteins could be detected (Devos et al., 1993; Skoda et al., 1993).

The situation is even more complicated for the soluble IL-6 receptor. From the existence of a differentially spliced receptor mRNA, Lust et al. (1992) postulated generation by differential splicing. This evidence, however, is based only on PCR techniques. No measurements of the soluble IL-6-receptor mRNA or protein levels have been performed. In our laboratory (see below) it has been found that a soluble IL-6 receptor is generated by shedding of the membrane form of the IL-6 receptor. We have determined the cleavage site at the C-terminal end (Figure 3a); this does not correspond to the C-terminus proposed by Lust et al. (1992) (Figure 3b). It should be noted, however, that in our system cells transfected with a cDNA coding for the membrane form of the IL-6 receptor have been used. In a pulse–chase experiment we could demonstrate that the radioactivity incorporated in the membrane-bound receptor could be chased completely into its soluble form. From these results we concluded that the shedding reaction occurred quantitatively (Müllberg et al., 1992, 1993a). Figure 3 shows a comparison between the C-terminus predicted by the experiments of Lust et al. (1992) and the cleavage site determined by Müllberg et al. (1994).

Shedding

As shown in Table 1, the soluble forms of the receptors for IL-1, IL-2, IL-6, ciliary neurotrophic factor (CNTF), transforming growth factor α, platelet-derived growth factor, nerve growth factor and TNFα are generated by limited proteolysis (shedding), i.e. a proteinase cleaves a juxtamembraneous peptide bond in the extracellular domain of the membrane receptor leaving the ligand-binding domain intact. At present there is no general amino acid sequence (consensus sequence) known which is recognized by the proposed shedding proteinase. Table 4 shows the cleavage sites for the IL-6 and TNFα receptors, the only two examples presently known. Since several cytokines and growth factors themselves are synthesized as membrane-bound precursors, which are proteolytically processed and secreted, the

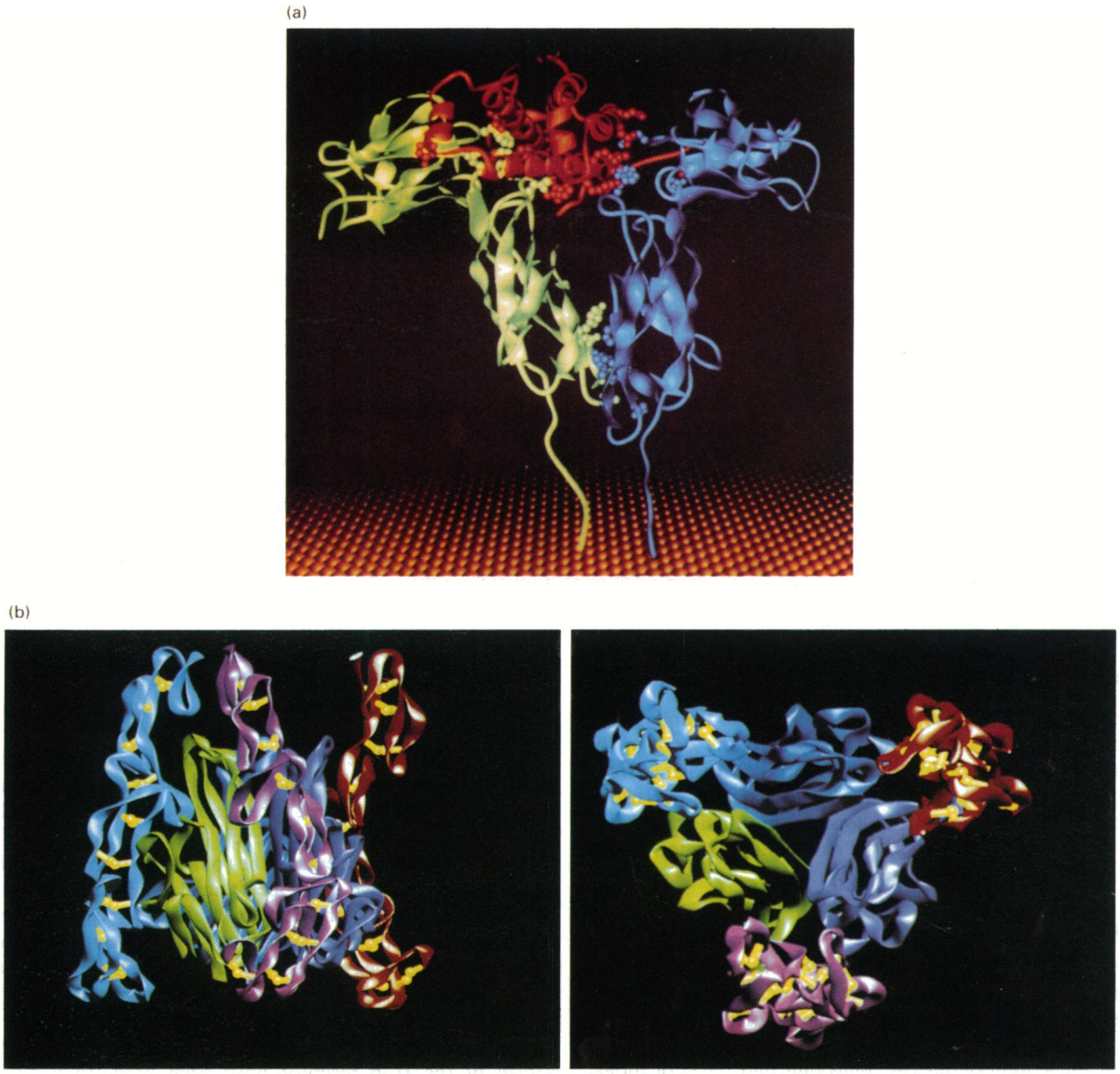

Figure 1 X-ray structures of ligand–soluble-receptor complexes

(**a**) Model of the complex between human growth hormone and the extracellular domain of its receptor. One ligand molecule (red) is bound to two receptor molecules (blue and green). (**b**) One TNFβ trimer (green, indigo and dark blue) is complexed to three soluble receptor molecules (magenta, cyan and red), viewed from the side (left) and from the top (right). Reproduced from De Vos et al. (1992) (copyright 1992 by the AAAS) and Banner et al. (1993) (copyright Cell Press), with permission.

cleavage sites of four representative members of this family have been included in Table 4.

There is no obvious sequence similarity between the two receptor cleavage sites and the four membrane-bound ligand cleavage sites. Therefore it is not surprising that point mutations in this region had essentially no effect on the susceptibility to proteolytic cleavage. Only deletions of between five and 12 amino acids resulted in the impairment of shedding (Massagué and Pandiella, 1993; Müllberg et al., 1994).

Not all membrane proteins are subject to quantitative shedding. In the case of the human IL-6 receptor we have clearly shown that only the α-subunit is quantitatively released from cells which have been transfected with a cDNA coding for the membrane form of the IL-6 receptor. Proteolytic cleavage of the second subunit (gp130) in the same experimental system is only marginal (Müllberg et al., 1993b).

The limited proteolysis leading to the formation of soluble cytokine receptors cannot be inhibited by any of the known proteinase inhibitors, indicating that the shedding proteinase does not belong to one of the four classical specificity groups of proteolytic enzymes (serine, acidic, cysteine and metalloproteinases). Despite the general importance of the shedding phenomenon, no shedding proteinase has so far been identified and characterized.

In contrast to the lack of specific inhibitors for the shedding proteinase, phorbol esters have been found to be potent inducers of the shedding reaction. As shown in Table 1, 4β-phorbol 12-myristate 13-acetate (PMA) stimulates shedding of the membrane

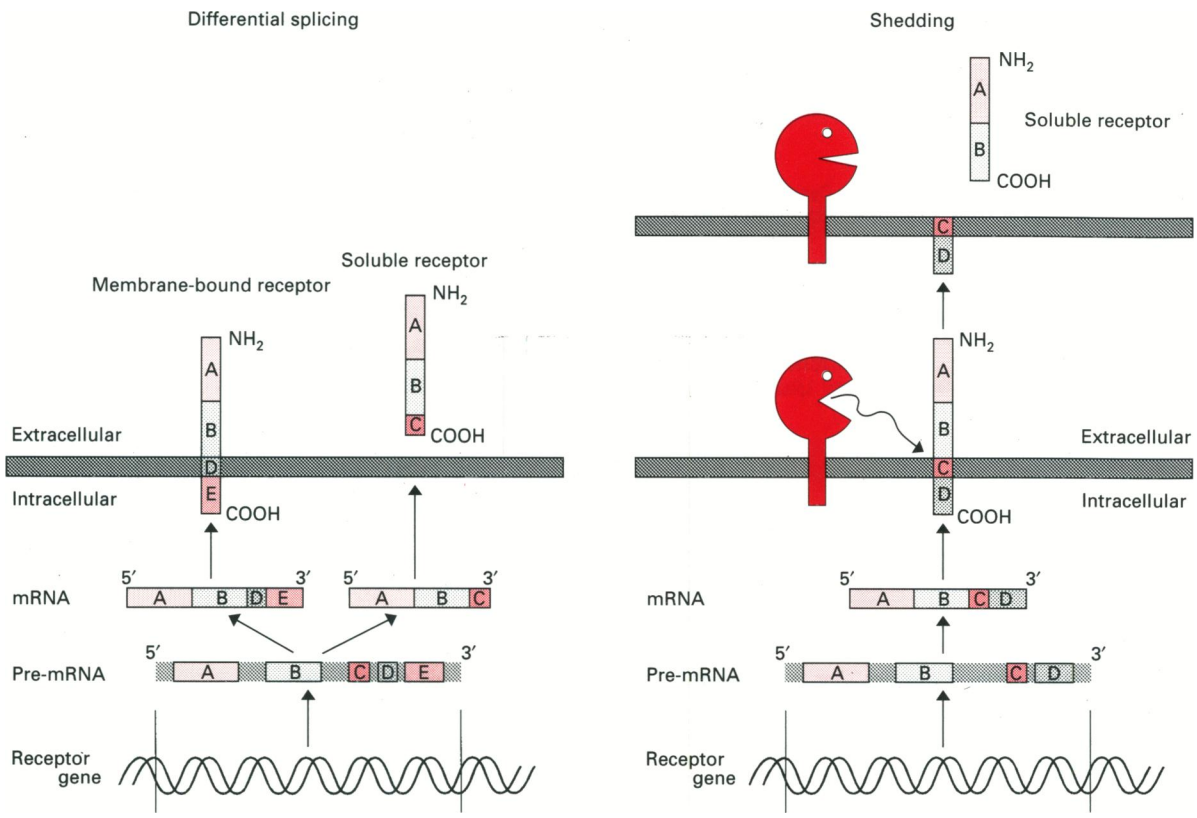

Figure 2 Mechanisms of soluble receptor generation

(**a**) Membrane-bound and soluble forms of receptors are generated by translation of differentially spliced pre-mRNA. Exons are represented by boxes labelled A, B, C, D and E. (**b**) The soluble form of the receptor is generated by limited proteolysis of a single transmembrane receptor protein. The presumed proteinase is shown in red.

Table 3 Soluble cytokine and growth-factor receptors generated by differential splicing

Abbreviations: R, receptor; nt, nucleotide; mpl, oncogene product of the murine myeloproliferative leukaemia virus; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; GH, growth hormone.

Receptor	Alteration in receptor mRNA	Extracellular amino acids missing	Unique amino acids of soluble receptor	References
Il-4-R	114-nt insertion	9	26	Mosley et al. (1989)
IL-5-R	94-nt deletion	10	62	Takaki et al. (1990)
	179-nt deletion	11	4	
IL-6-R	94-nt deletion	3	10	Lust et al. (1992)
IL-7-R	94-nt deletion	4	27	Goodwin et al. (1990)
Mouse IL-9-R	1071-nt deletion	57	24	Renauld et al. (1992)
mpl-R	257-nt deletion	55	30	Skoda et al. (1993)
Mouse LIF-R	No deletion	109	0	Gearing et al. (1991)
G-CSF-R	88-nt deletion	6	150	Fukunaga et al. (1990)
GM-CSFα-R	97-nt deletion	3	16	Ashworth and Kraft (1990); Raines et al. (1991)
Rat GH-R	Replacement of 3′ sequences	3	17	Baumbach et al. (1989)

receptors for IL-1, IL-6 and TNF. It is known that phorbol esters specifically activate protein kinase C, a key enzyme involved in many signal-transduction pathways (Nishizuka, 1988). The physiological stimulators leading to the activation of protein kinase C have only been identified in very few cases (Table 1). A role for direct phosphorylation of the cytoplasmic region of the IL-6 receptor, and of TNF receptors, can be excluded because deletion of the cytoplasmic domains does not lead to an abrogation of shedding (Brakebusch et al., 1992; Müllberg et al., 1994). It is therefore likely that the shedding proteinase involved is a transmembrane protein, the cytoplasmic portion of which directly or indirectly interacts with protein kinase C. We and other workers have shown that PMA-induced shedding is a very rapid process (20–40 min), and does not require *de novo* protein synthesis, implying that not too many additional steps can be involved in the regulation of the shedding reaction.

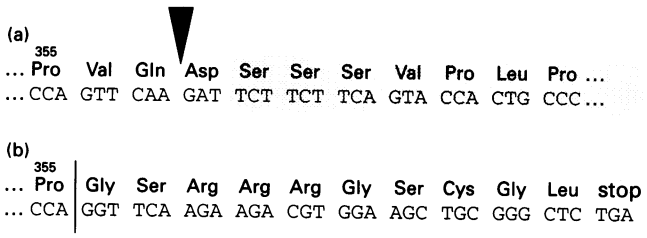

Figure 3 Nucleotide and amino acid sequences of the soluble IL-6 receptor generated by shedding or proposed differential splicing

(**a**) Cleavage site of the membrane-bound IL-6 receptor. (**b**) Proposed C-terminal amino acid sequence of the soluble IL-6 receptor generated by differential splicing. The shaded area represents part of the putative transmembrane domain.

It should be noted that the CNTF receptor is attached to the plasma membrane by a phospholipid anchor. Release of a soluble form of the CNTF receptor is mediated, therefore, by a phosphatidylinositol-specific phospholipase C (Davis et al., 1993a). Interestingly, soluble CNTF receptor is released after peripheral nerve injury from skeletal muscle cells (Davis et al., 1993a).

There is, at present, no evidence that soluble cytokine and growth-factor receptors are generated by internalization of the membrane-bound receptors and subsequent 'clipping' in vesicles followed by exocytosis. Furthermore, no stimulatory effect of the ligands on the release of soluble receptors has been observed.

BIOLOGICAL FUNCTIONS OF SOLUBLE RECEPTORS

Antagonistic and carrier functions

Although soluble receptors for most cytokines and growth factors have been discovered in human body fluids, there is no clear concept regarding their physiological role. Fernandez-Botran and Vitetta (1991) presented evidence that the soluble IL-4 receptor has a potential role as a carrier protein for IL-4 *in vivo*. These authors also reported that IL-4 complexed with its soluble receptor is less susceptible to proteolytic degradation by added trypsin. Since under physiological conditions trypsin is rather

Table 4 Comparison of extracellular cleavage sites in membrane-bound cytokine receptors and growth factors

Cytokine receptor/growth factor	Cleavage site	References
IL-6 receptor	LPVQDDSSS	Müllberg et al. (1994)
TNF receptor	PLAN↓VTNP	Himmler et al. (1990)
Epidermal growth factor	WELR↓HAGH	Bell et al. (1986)
Transforming growth factor α	DLLR↓VVAA	Derynck et al. (1984)
Amphiregulin	CGEK↓SNKT	Shoyab et al. (1989); Plowman et al. (1990)
Kit ligand	PPVA↓ASSR	Huang et al. (1990); Williams et al. (1990)

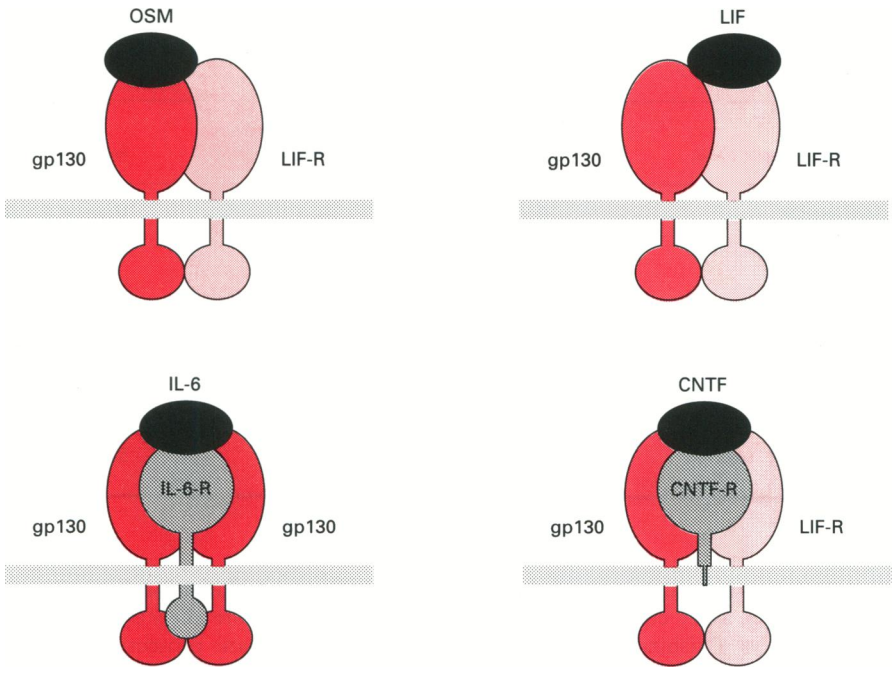

Figure 4 Receptor complexes for LIF, oncostatin M (OSM), IL-6 and CNTF sharing the signal-transducing subunit gp130

OSM binds directly to gp130; LIF binds to the LIF-receptor protein. IL-6 and CNTF first bind to specific receptors and interact subsequently with a homodimeric gp130 or gp130–LIF-receptor heterodimer respectively. The gp130 is shown in red, with the LIF-binding subunit (LIF-R) in pink.

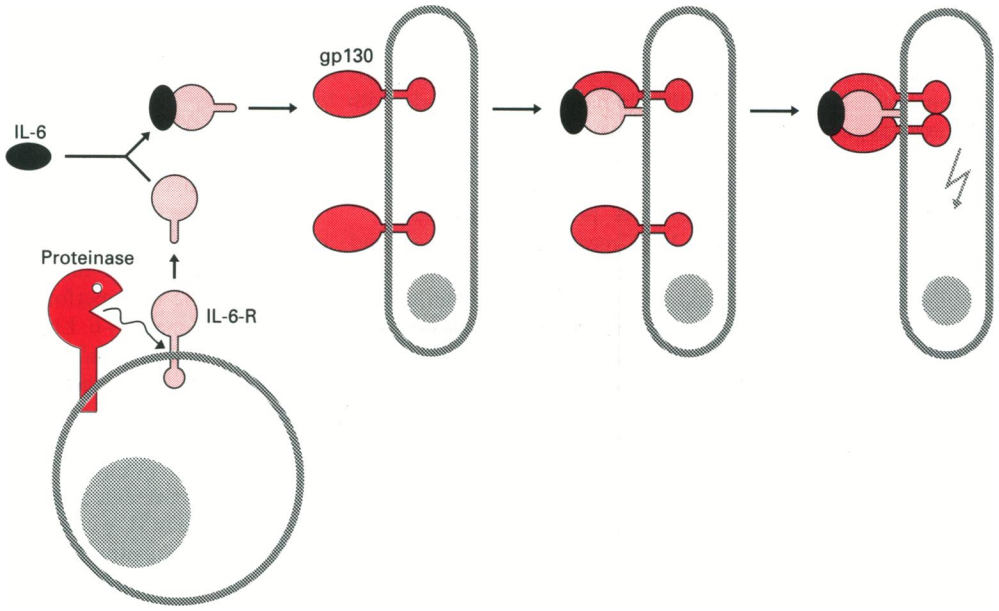

Figure 5 Agonistic action of the IL-6–soluble-IL-6-receptor complex and proposed trans-signalling between IL-6-responsive and non-responsive cells

The soluble IL-6 receptor generated by shedding first complexes with IL-6, and the binary complex then interacts with gp130 on cells which do not express IL-6 receptors on their surface. Such an association, together with a dimerization of gp130, leads to the generation of the biological signal.

unlikely to degrade the cytokine, physiologically relevant proteinases that are involved in such a degradation need to be identified. Aderka et al. (1991) have reported that TNFα bound to both types of soluble TNFα receptor retained its bioactivity for a longer time than the uncomplexed cytokine. As cytokines and growth factors are rather small molecules (< 30 kDa) they are rapidly cleared from the circulation. However, complexed to their soluble receptors these cytokines should circulate for longer times. On the other hand, cytokine–soluble-receptor complexes are in equilibrium with their components, i.e. upon depletion of free ligand, receptor–ligand complexes serve as suppliers of cytokines and growth factors. In order to predict the biological status of a cytokine/growth factor, it is essential to know the actual concentrations of ligand and soluble receptor, as well as the respective dissociation constants. In the case of IL-6 and its soluble receptor, concentrations of 1–10 pg/ml and 70 ng/ml of serum respectively have been reported for healthy individuals (Nijsten et al., 1987; Honda et al., 1992). As a consequence of the estimated dissociation constant for IL-6 and its soluble receptor (approx. 1×10^{-9} M) all IL-6 molecules should be complexed to the soluble receptor. Assuming identical binding constants for soluble and membrane-bound cytokine/growth-factor receptors, the distribution of the ligands depends on the number of soluble and membrane-bound receptors. In reality, the affinity constants of the cytokines/growth factors for their soluble receptors are lower by a factor of 2–10 compared with those for the membrane-bound receptors. Therefore a soluble receptor should act as an antagonist when its number vastly exceeds that of the membrane-bound receptors. Antagonistic activities of soluble receptors have been measured. Maliszewski et al. (1990) reported that soluble receptors specifically inhibit IL-1- and IL-4-induced B-cell activity *in vitro*. For the soluble LIF-receptor protein it has been shown that at the levels present in the circulation it does antagonize the action of LIF (Layton et al., 1992). Gentz et al. (1992) and Ozmen et al. (1993) presented evidence that the soluble receptor for interferon γ neutralizes the antiviral activity of interferon γ *in vitro* and in mice *in vivo*. Interestingly, it has recently been shown that TNFα-induced human immunodeficiency virus (HIV) 1 transcription and expression can be inhibited by a soluble TNFα receptor *in vitro* (Howard et al., 1993).

In this context it should be mentioned that viruses such as vaccinia virus (Alcami and Smith, 1992), cowpox (Spriggs et al., 1992) and Shope fibroma (Smith et al., 1990) carry genes coding for the synthesis of IL-1- and TNFα/β-binding proteins respectively. In all these cases an antagonistic activity of these proteins has been demonstrated.

Agonistic action of soluble receptors

A completely different situation for the action of soluble receptors has been found for IL-6 and CNTF. As shown in Figure 4, the ligand-binding protein interacts with a dimeric form of the signal-transducing subunit gp130 for signalling. Similarly the CNTF–CNTF-receptor complex interacts with gp130 and the LIF receptor. Surprisingly, it turned out that the soluble IL-6 receptor, together with its ligand IL-6, also interacts with gp130 (Figure 5), leading to a biological signal. This has been shown for the inhibition of proliferation of M1 cells (Taga et al., 1989), for the induction of proliferation of BA/F3 cells (Hibi et al., 1990) and for the induction of acute-phase protein synthesis in human hepatoma cells (Mackiewicz et al., 1992). Even in the presence of membrane-bound IL-6 receptors, soluble IL-6-receptor–IL-6 complexes lead to an increase in sensitivity towards IL-6. This has been shown for the growth inhibition of human breast carcinoma cells (Novick et al., 1992b) and for the induction of the acute-phase protein α_1-antichymotrypsin in HepG2 cells (Heinrich and Rose-John, 1993). In addition to the agonistic activity of the IL-6–IL-6-receptor complex, an antagonistic activity of the ternary complex of soluble IL-6 receptor/soluble gp130 and IL-6 has recently been reported by Narazaki et al. (1993). Davis et al. (1993a) recently demonstrated that the

soluble form of the CNTF receptor, together with its ligand CNTF, induces the proliferation of TF1 cells.

It may be speculated that soluble IL-6 or CNTF receptors are released by shedding from one cell type and, after ligand binding, act on cells which only express gp130 and LIF receptors on their cell surface (Figure 5). The latter cells would, by themselves, never react with IL-6 or CNTF. In line with this view of such a 'trans-signalling pathway' is the fact that gp130 is present essentially on all cell types, whereas IL-6 and CNTF receptors are not expressed ubiquitously. As a first approach to such a hypothesis we have engineered human hepatoma cells which do not express IL-6 receptors. These cells are completely unresponsive to IL-6 or soluble IL-6 receptor alone, but show a full IL-6 signal upon treatment with IL-6–soluble-receptor complexes (Rose-John et al., 1993).

An interesting discussion concerning an analogy between the IL-6–soluble IL-6 receptor complex and IL-12 (natural-killer-cell stimulatory factor) has been initiated by Gearing and Cosman (1991). IL-12 is composed of the proteins p35 and p40, which show considerable sequence similarity to IL-6 and the soluble IL-6 receptor. In the light of this observation one could view the IL-6–soluble IL-6 receptor complex as a cytokine heterodimer, exerting its action via the signal-transducing subunit gp130 (Gearing and Cosman, 1991).

SOLUBLE RECEPTORS AND DISEASE

As summarized in Table 1, soluble cytokine and growth-factor receptors are present in astonishingly high concentrations in normal human body fluids. In general, increases in soluble receptor levels have been described for various pathological states. Due to commercially available e.l.i.s.a.s, most studies on serum levels of soluble receptors in disease have been performed for the soluble IL-2 receptor. Increased soluble IL-2-receptor concentrations have been found after infection with human T-cell leukaemia virus (HTLV)-1, HIV, hepatitis and measle viruses, parasitic infection (malaria), bacterial diseases (tuberculosis) and autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and neoplastic diseases (reviewed in Fernandez-Botran, 1991). Suzuki et al. (1993) found, in MRL/lpr mice, one of the representative animal models of human autoimmune disease, an elevated production of soluble IL-6 receptors during the development of the autoimmune disease in these animals. An increase in the serum IL-6 concentration has also been observed. An enhanced release of human soluble IL-6 receptors has also been reported after HIV infection (Honda et al., 1992).

THERAPEUTIC POTENTIAL OF SOLUBLE RECEPTORS

As already mentioned, most soluble receptors compete with their membrane-bound counterparts for the cytokine/growth-factor ligands and therefore act antagonistically. It was therefore speculated that soluble receptors might be useful molecules with which to neutralize those cytokines/growth factors which cause diseases. Fanslow et al. (1990) have demonstrated in mice that the survival of heterotropic heart allografts was prolonged upon treatment with soluble IL-1 receptor. Soluble IL-1 receptors have also been used to suppress the growth of acute myelogeneous leukaemia blast cell progenitors (Estrov et al., 1992). Soluble type-I/type-II IL-1 receptors impair DNA synthesis in human astrocytes *in vitro* (Barna et al., 1993). Soluble TNF receptors have been found to inhibit HIV activation *in vitro* (Clouse et al., 1993). In mice it has been shown that soluble interferon γ receptors suppress auto-antibody formation (Garotta et al., 1993). Since soluble receptors bind the cytokine/growth-factor ligands with lower affinity than the respective membraneous receptors, the amounts of soluble receptors to be applied in order to achieve therapeutic inhibition of the cytokine/growth-factor response have to be in a range which can hardly be achieved in patients.

A second, even more severe, drawback of using soluble receptors as cytokine/growth-factor inhibitors can be deduced from experiments which have been performed with neutralizing monoclonal antibodies to human IL-6. Lu et al. (1992) and Klein et al. (1991) have shown that after administration of anti-IL-6 monoclonal antibodies to a patient with plasma-cell leukaemia, a complete blockage of myeloma-cell proliferation was observed for 1.5 months. In addition, the production of the acute-phase protein C-reactive protein was completely inhibited. Surprisingly, at the end of the anti-IL-6 treatment, high levels of bioactive IL-6 were detected. This increase in IL-6 can be explained by the fact that the antibody–IL-6 complexes are cleared from the circulation much slower than the ligand alone, thus leading to an enormous accumulation of IL-6–antibody complexes. These complexes represent a depot form of IL-6 (Klein et al., 1991; Lu et al., 1992). On the basis of these findings the use of cytokine/growth-factor-receptor antagonists for therapy seems to be more appropriate than using monoclonal antibodies or antagonistic soluble receptors.

Receptor antagonists are proteins which bind to the cytokine/growth-factor receptors with high affinity without generating a biological response. In fact, a natural receptor antagonist has been discovered for IL-1 (Seckinger et al., 1987; Arend, 1991; Eisenberg et al., 1991). Recombinant IL-1-receptor antagonist has been effective in animal models of rheumatoid arthritis, sepsis, inflammatory bowel disease, graft-versus-host disease and asthma, and it is presently being studied in clinical trials of these diseases (Catalano, 1993). Thus far, no other natural cytokine/growth-factor-receptor antagonists are known. In the cases of human IL-4, IL-6 and growth hormone, antagonists have been obtained by amino acid replacements of the recombinant proteins. Such proteins have been shown to bind to their respective receptors without eliciting a signal (Kruse et al., 1992; Fuh et al., 1992; Brakenhoff et al., 1994). Therefore these molecules were capable of antagonizing the action of the natural cytokines IL-4, IL-6 and growth hormone.

CONCLUSIONS

In this review we have summarized the present knowledge on soluble cytokine/growth-factor receptors. It seems that essentially all receptors with a single transmembrane domain also exist as soluble forms. Although the soluble cytokine/growth-factor receptors are found in appreciable quantities in body fluids, it is completely unclear which cells in the organism are responsible for their release. Little is known about the physiological stimuli responsible for the generation of soluble receptors. It will certainly be rewarding to identify the physiological pathways leading to the activation of protein kinase C which have been mimicked in several experiments by phorbol ester administration, resulting in shedding. Although, in this review, we have discussed in detail the ligand-binding capability of soluble receptors, it should be emphasized that the pharmacokinetics of soluble-receptor–cytokine/growth factor complexes still have to be established. Such studies might help to approach the problem of the function of soluble receptors *in vivo*. Presently the biological role of soluble receptors remains enigmatic. Two types of possible

functions for soluble receptors exist. For most soluble receptors a more or less antagonistic activity has been described, whereas at present an agonistic mode of action is found for only two soluble cytokine receptors. It will be interesting to find out whether more soluble receptors belonging to the latter group will be discovered.

PERSPECTIVES

Meanwhile, however, soluble receptors are already being widely used in the search for cytokine/growth-factor antagonists. Due to molecular-biology techniques, soluble receptors have become available in milligram quantities, allowing ligand–receptor-interaction studies in cell-free systems. Such studies have led to the definition of contact points between ligand and receptor subunits (ligand-binding and signal-transducing subunits). This information in turn can be used for the design of inhibitors, superbinders and finally the construction of low-molecular-mass agonists/antagonists. As the crystallization of membrane-bound proteins is still a formidable task, the soluble-receptor–ligand complexes have recently been employed to solve the tertiary structures. During the next few years more ligand–soluble-receptor complex structures will become available as a result of the pioneering work on the growth hormone and TNFβ soluble-receptor–ligand complexes. Such information will definitely have a great impact on the development of specific therapeutic agents in inflammatory and autoimmune diseases in the future.

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