The soluble interleukin 6 receptor: mechanisms of production and implications in disease

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ABSTRACT **Interleukin 6 (IL-6) performs a prominent role during disease and has been described as both a pro- and anti-inflammatory cytokine. A key feature in the regulation of IL-6 responses has been the identification of a soluble interleukin 6 receptor (sIL-6R), which forms a ligand–receptor complex with IL-6 that is capable of stimulating a variety of cellular responses including proliferation, differentiation and activation of inflammatory processes. Elevated sIL-6R levels have been documented in numerous clinical conditions indicating that its production is coordinated as part of a disease response. Thus, sIL-6R has the potential to regulate both local and systemic IL-6-mediated events. This review will outline the central role of sIL-6R in the coordination of IL-6 responses. Details relating to the mechanisms of sIL-6R production will be provided, while the potential significance of sIL-6R during the development of clinical conditions will be emphasized. We want to convey, therefore, that when thinking about the inflammatory capability of IL-6, it is essential to consider not only the action of IL-6 itself, but also the effect sIL-6R may have on cellular processes.—Jones, S. A., Horiuchi, S., Topley, M. Yamamoto, N., Fuller, G. M. The soluble interleukin 6 receptor: mechanisms of production and implications in disease. *FASEB J.* 15, 43–58 (2001)**

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INTERLEUKIN 6 AND INTERLEUKIN 6 RECEPTOR SIGNALING

Interleukin 6 (IL-6) is produced by a variety of cell types during infection, trauma, and immunological challenge (1); elevated IL-6 concentrations have been associated with disease states (2–8). The functional properties of IL-6 are extremely varied and this is reflected by the terminology originally used to describe the activities of this cytokine [interferon β2 (IFN-β2, hepatocyte-stimulating factor, cytotoxic T cell differentiation factor, B cell differentiation factor, and B cell stimulatory factor 2]. Indeed, IL-6 promotes inflammatory events through the expansion and activation of T cells, differentiation of B cells, and the induction of acute-phase reactants by hepatocytes. In contrast, IL-6 also performs a protective role during disease and counteracts the manifestation of certain inflammatory responses. In septic shock, for example, IL-6 suppresses acute neutrophil accumulation caused by intratracheal administration of endotoxin (9, 10). This is paralleled by studies showing that IL-6 down-regulates proinflammatory cytokine expression while simultaneously inducing the expression of IL-1 receptor antagonist and the soluble p55 tumor necrosis factor α (TNF-α) receptor (11–13). Thus, IL-6 acts not only as a pro- but also as an anti-inflammatory cytokine, and as a result serves a pivotal role during disease.

The receptor complex mediating the biological activities of IL-6 consists of two distinct membrane-bound glycoproteins, an 80 kDa cognate receptor subunit (IL-6R, CD126) and a 130 kDa signal-transducing element (gp130, CD130). Expression of the *trans*-membrane-spanning gp130 is found in almost all organs, including heart, kidney, spleen, liver, lung, placenta, and brain (14). In contrast, cellular distribution of the cognate IL-6R is limited and its expression is predominantly confined to hepatocytes and leukocyte subpopulations (monocytes, neutrophils, T cells, and B cells). Although gp130 was initially identified as the signal-transducing component of the IL-6 receptor, it is now apparent that cognate receptors for interleukin 11 (IL-11), oncostatin-M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), leukemia inhibitory factor (LIF), and novel neurotrophin-1/B cell-stimulating factor-3 all transmit activation signals via gp130 (15–18). As a consequence, each of these cytokines possess overlapping activities, and the phenotypic characteristics of mice lacking either IL-6, IL-11, LIF, or CNTF (19–21) are less severe than the apparent pleiotropic properties of these mediators would suggest. In contrast, targeted disruption of the gp130 gene is embryonically lethal (22).

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Interleukin 6 signaling is facilitated through the homodimerization of gp130 to the ligand–receptor complex. Intracellular signaling is subsequently triggered via activation of gp130-associated cytoplasmic tyrosine kinases (JAK1, JAK2, and TYK2) and phosphorylation of STAT1 and STAT3 (23, 26). In contrast, the high-affinity receptors of LIF, OSM, and CNTF activate cells by a heterodimerization between gp130 and a gp130-related protein (the LIF receptor) (27). Such homo- or heterodimers activate distinct but overlapping patterns of tyrosine phosphorylation through the Jak-Tyk family of cytoplasmic tyrosine kinases (28). This may contribute to the different cellular responses associated with this family of proteins. Interleukin 6 also activates the Ras-Raf signaling cascade, which regulates phosphorylation of MAP-kinase and ultimately activation of the transcription factors NF-IL-6 (a C/EBP family member) and AP-1 (c-Jun and c-Fos) (29–31). Stimulation of this Ras-dependent MAP kinase cascade has been suggested to perform an important role in IL-6-mediated proliferation, since activation of this pathway was associated only with cell types that proliferate in response to IL-6 (31). Subsequent reports have also confirmed the involvement of Sak, Hck, Fes, Btk, and Tec-kinases in IL-6 signaling (32–35). However, the significance of their activation remains to be determined. For a more comprehensive review of signaling via gp130, the reader is directed elsewhere (36).

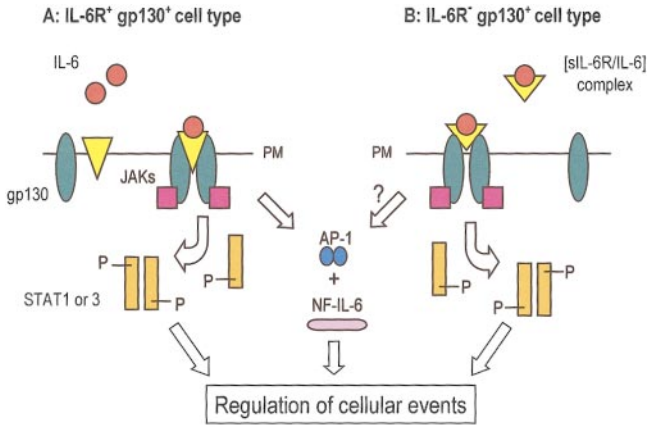

Figure 1. Interleukin 6 signaling via the cognate IL-6R and sIL-6R. *A)* Cellular activation by IL-6 requires binding to its cognate receptor and the resulting dimerization of gp130. This mediates phosphorylation of gp130-associated JAKs, which facilitates docking of STAT-1/STAT-3 factors to gp130, and their phosphorylation. Monomeric STAT subunits form homo- and heterodimers and translocate into the nucleus were they initiate gene expression. Activation of the Ras-Raf pathway leads to the recruitment of the transcription factors AP-1 and NF-IL-6. *B)* Signaling through gp130 via the [sIL-6R/IL-6] complex activates a similar series of cellular events. Although there is no direct evidence for sIL-6R facilitating Ras-Raf signaling, since activation of this pathway is associated with IL-6-mediated proliferation it is possible that the [sIL-6R/IL-6] complex may stimulate this cascade (31).

THE SOLUBLE IL-6 RECEPTOR

In addition to the membrane-bound receptor, a soluble form of the IL-6R (sIL-6R) has been purified from human serum and urine (37, 38). This soluble receptor binds IL-6 with an affinity similar to that of the cognate receptor (0.5–2 nM) (39, 40) and prolongs its plasma half-life (41). More important, the [sIL-6R/IL-6] complex is capable of activating cells via interaction with membrane-bound gp130 (**Fig. 1**). This feature makes the [sIL-6R/IL-6] complex an agonist for cell types that although they express gp130, would not inherently respond to IL-6 alone. Hence, the sIL-6R has the ability to widen the repertoire of cell types that are responsive to IL-6. This is in contrast to the function of most soluble cytokine receptors that bind their ligand and antagonize cellular signaling by preventing the interaction of the cytokine with their respective plasma membrane-bound cognate receptor (42). However, it of interest that genetically engineered forms of soluble IL-11 and CNTF receptors (the LIF-receptor) can also mimic the stimulatory properties of the [sIL-6R/IL-6] complex once associated with their ligands (43–45).

The importance of the sIL-6R as a regulator of IL-6 responses is illustrated by the increasing number of studies that have now been published describing the agonistic properties to this soluble receptor. Consequently, the [sIL-6R/IL-6] complex should be thought of as being an heterodimeric cytokine that exerts its action through gp130. In terms of structure, this may be analogous to the heterodimeric IL-12, whose p40 subunit shares extensive amino acid homology with the entire extracellular domain of the IL-6R (46).

ACTIVITIES MEDIATED THROUGH THE ACTION OF sIL-6R

Recombinant forms of the sIL-6R were initially shown to bind IL-6 and associate with gp130 to induce gene expression in cells that lack the cognate IL-6R (47–49). It was later confirmed that sIL-6R purified from human plasma also retain similar biological activities (38, 50); roles for sIL-6R in cellular proliferation, differentiation, and the activation of inflammatory responses have now been described.

Cellular proliferation and differentiation

Initials experiments observed that sIL-6R could bind IL-6 and efficiently suppress the proliferation of peripheral blood mononuclear cells activated with concanavalin A (38). Subsequently, it has been reported that the [sIL-6R/IL-6] complex promotes the growth of many cell types including Kaposi's sarcoma cell lines (51), hematopoietic progenitor cells (52), and synovial fibroblasts (53). Indeed, the potential effect of the sIL-6R on proliferative events was recently emphasized by a study of hepatocyte proliferation in double-transgenic mice expressing high levels of human IL-6 and sIL-6R (54,

55). These mice spontaneously develop nodules of hepatocellular hyperplasia around periportal spaces, which is consistent with regenerative hyperplasia events commonly associated with myeloproliferative and immunological conditions (55). This implies that the [sIL-6R/IL-6] complex may act as a primary stimulus for hepatocyte proliferation and as a promoter of hepatocellular transformation.

The sIL-6R has also been implicated in cellular differentiation. For example, IL-6 alone is unable to induce osteoclast formation in cocultures of mouse bone marrow and osteoblastic cells, but when combined with the sIL-6R, osteoclast formation is promoted (56–58). Further evidence for sIL-6R acting as a mediator of differentiation events has also been obtained by comparing the phenotypic characteristics of transgenic mice expressing either human IL-6 or sIL-6R alone with animals coexpressing both IL-6 and sIL-6R. The main difference between these mice is the dramatic increase in extramedullary hematopoiesis that occurs within the liver and spleen of double-transgenic adult mice (59, 60). This feature of sIL-6R/IL-6 double-transgenic animals is highlighted by the elevated numbers of granulocytes, macrophages, B cells, and hematopoietic progenitor cells in the liver and spleen, and by a massive increase in circulating leukocytes and red blood cells within these animals. For a detailed review of sIL-6R and hematopoiesis events, see ref 60.

Based on recent studies, it has been questioned whether IL-6 not only acts as a cytokine, but also as a neurotrophic factor (61). Trophic factors are broadly defined by their capacity to promote neuronal survival and development. Although many neuronal cells are capable of producing IL-6, they remain unresponsive to stimulation by IL-6 itself. Differentiation and survival of neuronal cells can, however, be mediated through the action of sIL-6R. For example, sympathetic and sensory neurons from neonatal superior cervical ganglia and embryonic dorsal root ganglia both show a marked increase in survival and neurite outgrowth when stimulated by the [sIL-6R/IL-6] complex (62, 63). In addition, sIL-6R may perform a role in axon growth from dorsal root ganglia and in the development of Schwann cell progenitors, which express myelin basic protein after activation with a combination of IL-6 and sIL-6R (64). Thus, sIL-6R in conjunction with IL-6 may be important in nerve regeneration through the promotion of remyelination events.

In many cases, the cellular responses coordinated by the [sIL-6R/IL-6] complex can also be controlled by the action of other gp130-stimulating cytokines. For instance, stimulation of cardiomyocyte proliferation and the development of pathological ventricular hypertrophy in response to the [sIL-6R/IL-6] complex (65, 66) have been described as functional properties of CT-1 (67). Similarly, sIL-6R-mediated expression of neuropeptides and transmitter biosynthetic enzymes by sympathetic neuronal cells (63) can also be mimicked by the action of LIF, CNTF, OSM, and CT-1 (67–69), whereas osteoclast formation is mediated by IL-11, LIF, and OSM alone or via sIL-6R in conjunction with IL-6 (58, 70). This raises the following question: if a given cell type, which was naturally devoid of the cognate IL-6R, were to express the IL-6R, would activation of these cells by IL-6 alone be able to fulfill many of the functions assigned to the [sIL-6R/IL-6] complex? Predicting the answer to this question may not be as straightforward as initially thought, and is highlighted by studies of the proliferative capability of human $CD34^+$ cells after stimulation by [sIL-6R/IL-6] complex (71, 72). Although $CD34^+$ cells bear gp130, expression of IL-6R is limited to only 30–50% of the total cell population (71). Subsequent analysis of sorted $CD34^+$ cells showed that $CD34^+$ $IL\text{-}6R^-$ cells expand into a variety of hematopoietic progenitor and erythroid cells in the presence of IL-6, sIL-6R, and stem cell factor (71). Under identical experimental conditions, stimulation of $CD34^+$ $IL\text{-}6R^+$ cells with IL-6 alone promoted the expansion of granulocyte/macrophage colonies (71). These data may provide initial evidence for potential functional differences between the activity of IL-6 and the [sIL-6R/IL-6] complex or, alternatively, that IL-6R expression reflects a progenitor cell type at a different stage of differentiation and is more committed to expansion into a specific cell lineage.

Regulation of inflammatory mediators

Examination of IL-6-deficient ($IL\text{-}6^{-/-}$) mice has recently suggested that sIL-6R regulates leukocyte recruitment in a subcutaneous (s.c.) air pouch model of inflammation (73). It was subsequently shown that although endothelial cells lack the cognate IL-6R, they could be activated to phosphorylate STAT3, produce chemokines (IL-8, MCP-1, and, to a lesser extent, MCP-3), and up-regulate adhesion molecule (ICAM-1, VCAM-1) expression in response to the [sIL-6R/IL-6] complex (73, 74). This activation likely occurs via direct interaction with the gp130 signal-transducing element. Thus, the [sIL-6R/IL-6] complex plays a significant role in regulation of leukocyte recruitment and may serve a positive role in the prothrombotic/proinflammatory activation of endothelial cells. Consistent with this finding, it was recently reported that the degree of leukocyte infiltration into arthritic joints correlates with elevated sIL-6R levels in synovial fluid (75). Furthermore, activation of skin fibroblasts (76), smooth muscle cells (77), thyroid follicular cells (78), and astrocytes (79) by the [sIL-6R/IL-6] complex has been shown to promote cytokine (IL-6) and chemokine production. In contrast, the [sIL-6R/IL-6] complex does not appear to stimulate chemokine production by astrocytes, but has the capacity to block TNF-α-mediated expression of VCAM-1 by these cells (80, 81). In addition to enhanced chemokine and adhesion molecule expression, the sIL-6R has also been shown to regulate expression of certain proteases and protease inhibitors (82, 83).

In vivo properties of the sIL-6R

Initial *in vivo* experiments designed to examine the functional properties of sIL-6R were based on the observation that intracerebroventricular administration of IL-6 caused an increase in body temperature and a decrease in locomotory activity and food intake. When these animals were treated with IL-6 and an accompanying injection of sIL-6R, all of these central effects were enhanced and prolonged (84), suggesting that sIL-6R not only heightens IL-6 responsiveness, but also extends the circulating half-life of IL-6.

Subsequent studies have now confirmed and extended these observations through the use of transgenic mice overexpressing either human sIL-6R alone or together with human IL-6 (54, 59, 60, 85). In all these cases, the model system exploits the species specificity of human IL-6 and its receptor, which are capable of transmitting signals via interaction with murine IL-6R and murine gp130, respectively. In contrast, murine IL-6 is unable to bind the human IL-6R, allowing sIL-6R bioactivity to be reconstituted only through the presence of human IL-6. In general, double-transgenic mice (overexpressing sIL-6R and IL-6) are smaller than their wild-type littermates and have reduced body weight, whereas autopsy and histopathological analysis show that these animals have markedly reduced fat deposits, enlarged spleen, and shrunken disfigured livers (54, 59, 85). These latter morphological characteristics may be accounted for by increases in hepatic and splenic hematopoiesis, hepatocellular hyperplasia, and plasma cell proliferation associated with the double-transgenic mice (54, 59). Consequently, the sIL-6R appears to represent a major stimulator of cellular growth and hematopoietic cells *in vivo*.

Although these studies emphasize the significance of sIL-6R *in vivo*, detection of high circulating levels of human IL-6 (10–20 ng/ml) and sIL-6R (4–8 μg/ml) in these transgenic mice populations (59) infers that this murine model reflects the influence of systemically elevated sIL-6R. Conversely, when considering the involvement of sIL-6R in disease progression, it is also important to appraise the effect of locally produced sIL-6R in eliciting cellular events. To date, few studies have examined the biological consequence of locally produced sIL-6R. Nevertheless, the significance of locally produced sIL-6R has been demonstrated through the use of IL-6/sIL-6R double-transgenic mice (85). In this instance, human IL-6 and sIL-6R were coexpressed in mice under the respective control of metallothionein and PEPCK promoters. Although both promoters were functional within the liver, their activities were confined to distinct regions of the organ. Using *in situ* hybridization techniques, the authors showed that IL-6-induced expression of the acute-phase reactant haptoglobin was localized to areas of the liver were sIL-6R was expressed (85). Thus, sIL-6R has the potential to act as a paracrine mediator whose effects are elicited close to its site of generation. In addition, sIL-6R release by leukocyte subpopulations (86–88) suggests that activation of infiltrating leukocytes may also contribute to locally elevated sIL-6R levels that are commonly associated with several inflammatory disorders. However, a direct link between infiltrating leukocytes and enhanced local concentrations of sIL-6R remains unsubstantiated.

MECHANISMS OF SOLUBLE IL-6 RECEPTOR PRODUCTION

Since the bioactivity of IL-6 is modulated through binding the sIL-6R, it is essential to delineate the functional properties of IL-6 itself from those mediated via formation of the [sIL-6R/IL-6] complex. Consequently, understanding the regulation of sIL-6R generation is central to determining the pathological relevance of this receptor in disease.

Soluble forms of transmembrane proteins that retain bioactivity have now been identified in biological fluids. These include soluble adhesion molecules and receptors for cytokines and growth factors. Two independent cellular processes have been identified as controlling their production (42). First, differential mRNA splicing can lead to the expression of soluble factors that lack cytoplasmic and membrane-spanning domains of the cell-associated protein. Examples include receptors for IL-4, epidermal growth factor (EGF), and LIF (42). The second mechanism involves proteolytic cleavage of a membrane-anchored protein at a site close to the cell surface. Regulatory proteins processed in this manner include soluble receptors for IL-1, IL-2, TNF-α, platelet-derived growth factor, and the adhesion molecule L-selectin (CD62L). In the case of the sIL-6R, both processes regulate release (**Fig. 2**). Thus, two distinct isoforms of the sIL-6R contribute to the overall properties of this soluble receptor. These isoforms will be referred to here as either DS-sIL-6R or PC-sIL-6R to denote whether the receptor is released as the product of differential mRNA splicing (DS) or shed after proteolytic cleavage (PC). The information detailed below reviews the evidence for sIL-6R release through both mechanisms and the manner in which they are regulated.

Differential mRNA splicing

Examination of IL-6R expression by monocytic and tumor cell lines shows that these cells encode two distinct IL-6R mRNA transcripts (89–92). The shorter of the two transcripts lacks 94 base pairs [nucleotides 1504 (T) to 1597 (G)] encoding for the putative transmembrane region of the cognate IL-6R (89, 90). A role for differential mRNA splicing in the construction of this shorter transcript was indicated by the identification of consensus sequences for splicing donor (nucleotides 1501–1503, CAG) and splicing acceptor (nucleotide 1598, G) motifs that flank the deleted region (90) (**Fig. 3**). Absence of this 94 base pair section

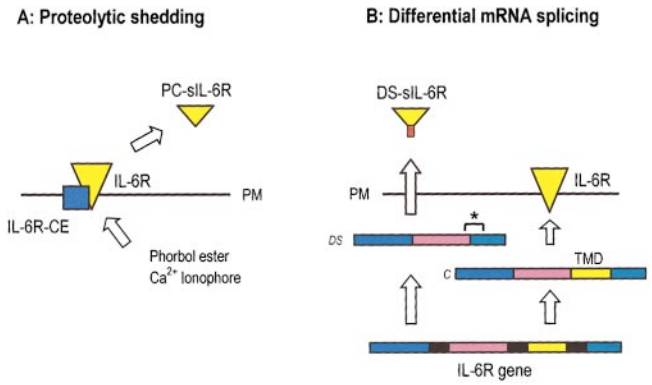

Figure 2. Mechanisms of sIL-6R generation. Diagrammatic representations of sIL-6R release through proteolytic shedding (*A*) and differential mRNA splicing (*B*) are shown. *A)* Upon appropriate stimulation, an unidentified IL-6R cleavage enzyme (IL-6R-CE), shown as a blue box, promotes release of PC-sIL-6R. *B)* Differential mRNA splicing accounts for two distinct IL-6R mRNA transcripts: one encodes for DS-sIL-6R (*DS*), the other the cognate membrane-bound IL-6R (*C*). The DS-sIL-6R transcript lacks the coding sequence for the transmembrane domain (TMD) and as the result of DNA rearrangement, there is a reading frameshift, which is highlighted by the asterisk. This leads to the introduction of a unique COOH-terminal sequence [GSRRRGSCGL] shown in red.

predicts the introduction of a reading frameshift and the subsequent incorporation of a novel 10 amino acid (GSRRRGSCGL) sequence at the COOH-terminal tail of this sIL-6R isoform (90) (Fig. 3). Antibodies specifically raised against this putative peptide sequence have been used to demonstrate expression of DS-sIL-6R both *in vitro* (88, 90) and *in vivo* (92, 93). Examination of sIL-6R levels in plasma of healthy individuals shows that the predominant circulating form of sIL-6R is the product of differential mRNA splicing (94). More recently, we have established that plasma levels of DS-sIL-6R decrease with age (93). For example, mean circulating levels of DS-sIL-6R in individuals 21–30, 31–40, and 41–60 years of age were found to be 18.3 ng/ml, 7.2 ng/ml, and 3.1 ng/ml, respectively. Hence, regulation of DS-sIL-6R release appears to be complex. To date, there is a lack of information pertaining to the transcriptional regulation of the splicing mechanism, whereas very few activators of this process have been described. In particular, T cells release DS-sIL-6R upon stimulation with phytohemoagglutinin (90), whereas IL-1α (94) and oncostatin-M (94, 95) promote DS-sIL-6R secretion by hepatocyte cell lines.

Proteolytic cleavage (shedding)

A second mechanism of sIL-6R generation involves shedding of the cognate membrane-bound receptor from the cell surface after proteolytic cleavage (86, 96–98). Examination of COS cells stably expressing full-length IL-6R showed that phorbol esters could activate sIL-6R release (96). Although this indicated that protein kinase C regulates generation of sIL-6R, it is unknown whether this phorbol ester response represents an increase in the activity of the cleavage protease, increased expression of the receptor, or increased trafficking of IL-6R to the plasma membrane. Purification and COOH-terminal amino acid analysis of the liberated sIL-6R identified the proteolytic cleavage site as being immediately prior to the putative transmembrane domain (residues Gln^{357}-Asn^{358} (98; Fig. 3).

Initial attempts to identify the class of protease responsible for the liberation of PC-sIL-6R using known protease inhibitors were fruitless (86, 95) and suggested the involvement of a novel protease. Subsequent studies showed that hydroxamic acid-based metalloprotease inhibitors, such as those known to block pro-TNF-α processing (e.g., the TNF-α (processing) protease inhibitor or TAPI) (99), could prevent the phorbol ester- and ionomycin-induced shedding of

A

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IL-6R      agtggagccc ggaggccatg ggcacgcctt ggacagaatc caggagtcct
1401       ccagctgaga acgaggtgtc caccccccatg caggcactta ctactaataa
           agacgatgat aatattctct tcagagattc tgcaaatgcg acaagcctcc
                                  Transmembrane encoding region
1501       cagTGCAAGA TTCTTCTTCA GTACCACTGC CCACATTCCT GGTTGCTGGA
DS-sIL-6R  cagttcaaga agacgtggaa gctgcgggct ctga
           GGGAGCCTGG CCTTCGGAAC GCTCCTCTGC ATTGCCATTG TTCTGAGgtt
1601       caagaagacg tggaagctgc gggctctgaa ggaaggcaag acaagcatgc
           atccgccgta ctctttgggg cagctggtcc cggagaggcc tcgacccacc
1701       ccagtgcttg ttcctctcat ctccccaccg gtgtcccccа gcagcctggg
           gtctgacaat acctcgagcc acaaccgacc agatgccagg gacccacgga
1801       gcccttatga catcagcaat acagactact tcttccccag atag
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B

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IL-6R      MLAVGCALLAALLAAPGAALAPRRCPAQEVARGVLTSLPGDSVT
           LTCPGVEPEDNATVHWVLRKPAAGSHPSRWAGMGRRLLLRSVQL
           HDSGNYSCYRAGRPAGTVHLLVDVPPEEPQLSCFRKSPLSNVVC
           EWGPRSTPSLTTKAVLLVRKFQNSPAEDFQEPCQYSQESQKFSC
           QLAVPEGDSSFYIVSMCVASSVGSKFSKTQTFQGCGILQPDPPA
           NITVTAVARNPRWLSVTWQDPHSWNSSFYRLRFELRYRAERSKT
           FTTWMVKDLQHHCVIHDAWSGLRHVVQLRAQEEFGQGEWSEWSP
           EAMGTPWTESRSPPAENEVSTPMQALTTNKDDDNILFRDSANAT
                              Transmembrane domain
           SLPVQDSSSVPLPTFLVAGGSLAFGTLLCIAIVLRFKKTWKLRA
DS-sIL6R   SLPGSRRRGSCGL
PC-sIL6R   SLPVQ
           LKEGKTSMHPPYSLGQLVPERPRPTPVLVPLISPPVSPSSLGSD
           NTSSHNRPDARDPRSPYDISNTDYFFPR
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Figure 3. Nucleotide and amino acid sequences for sIL-6R isoforms. *A)* Partial nucleotide sequence for the cognate IL-6R (accession number: X12830, M20566) (159) showing residues 1351–1844 that includes the transmembrane encoding region (underlined sequence) and the 94 base pair deletion of the spliced variant (capital letters). The proximal 3′ nucleotide sequence for DS-sIL-6R is shown, as is the location of this sequence within the complete IL-6R sequence. The underlined *tag* and *tga* nucleotides identify the stop codons for the cognate IL-6R and DS-sIL-6R, respectively. *B)* Entire amino acid sequence for the cognate IL-6R. The putative transmembrane domain is underlined. Proximal COOH-terminal sequences for both DS- and PC-sIL-6R is shown. The novel COOH-terminal residues of DS-sIL-6R are identified in blue

IL-6R from monocytic cells (86, 100) and multiple myeloma cell lines (101). Although hydroxamic acid-based metalloprotease inhibitors also block shedding of the TNF-α receptors (86, 102), L-selectin (103, 104), CD14 (105), and the processing of protransforming growth factor-α (TGF-α) (106) and pro-EGF (107), no conserved proteolytic cleavage site has been identified within any of these proteins. In addition to the inhibitory action of hydroxamic acid-based metalloprotease inhibitors, TIMP-3 (101) and high concentrations of 1,10-phenanthrolene (105, 108) have also been reported to prevent sIL-6R shedding.

The cleavage enzyme responsible for liberating sIL-6R remains unidentified. Based on the inhibitory properties of hydroxamic acid-based metalloprotease inhibitors, it would follow that the IL-6R cleavage enzyme is closely related to the recently cloned protease responsible for the processing of pro-TNF-α. This enzyme, termed the TNF-α-cleavage enzyme (TACE, also known as ADAM-17), is a zinc binding metalloprotease disintegrin and belongs to the adamalysin family (109, 110). Although TACE has been implicated in the processing of pro-TGF-α as well as L-selectin and p75 TNF-α-receptor shedding (111), a role for TACE in sIL-6R shedding has not been proposed. A second metalloprotease disintegrin (ADAM-10) has been reported to mediate processing of pro-TNF-α, but again this enzyme was unable to cleave peptides representing the processing sites in various proteins, including IL-6R (112). Although IL-6R shedding via an ADAM directed proteolytic event remains to be fully substantiated, bacterial-derived metalloproteases, which are sensitive to agents such as TAPI, have been shown to release sIL-6R (108). The cleavage site where these proteases act, however, is distinct from that used by the endogenous cellular protease (98, 108).

Since the initial observation that IL-6R shedding can be promoted by stimulation with phorbol esters, very few physiologically relevant activators of sIL-6R shedding have been identified. Bacterial-derived, pore-forming toxins streptolysin-O and hemolysin-A have been shown to promote shedding of functionally active sIL-6R from monocytic cells via a TAPI-sensitive mechanism (105), while activation of human neutrophils with f-Met-Leu-Phe causes a significant loss of surface-bound IL-6R as detected by flow cytometry (74). In a study of sIL-6R production by monocytic THP-1 cells, a series of cytokines (IL-1β, TNF-α, IFN-γ, IL-4, IL-6, IL-10), chemokines (RANTES, MCP-1), and growth factors such as platelet-derived growth factor (PDGF-AA, PDGF-BB) were tested for their ability to induce sIL-6R production (100). However, none of these agents significantly elevated sIL-6R production by these cells. Subsequent studies revolved around the rationale that IL-6R shedding may be activated by factors known to coordinate release of other soluble receptors/adhesion molecules (e.g., soluble TNF-α and soluble L-selectin) or agents known to modulate IL-6 activity or to be regulated by IL-6. Based on these criteria human neutrophils were found to shed IL-6R after stimulation by the acute-phase reactant C-reactive protein (CRP) (88). During the onset of inflammation or tissue injury, plasma concentrations of CRP are dramatically elevated from ~1 μg/ml in healthy individuals to as much as 500 μg/ml during the acute-phase response (113). *In vitro* studies show that control of this response is primarily regulated by IL-6 (114). CRP plays a significant role in host defense against pathogens and also binds specific receptors on human neutrophils, causing neutrophil responses, such as chemotaxis (115) and the activation of superoxide generation and degranulation by chemoattractants (116), to be diminished. Recently, the major receptor for CRP on leukocytes has been identified as Fcγ receptor-IIa (CD32) (117). CRP and biologically relevant CRP-peptides have been shown to prevent neutrophil adhesion to endothelial cells via induction of L-selectin shedding (118), a process that is prevented by a hydroxamic acid based metalloprotease inhibitor (118). Surprisingly, the CRP-induced shedding of sIL-6R by neutrophils was largely unaffected by the hydroxamic acid-based metalloprotease inhibitor TAPI (88). Thus, an alternative shedding mechanism may be responsible for the CRP-induced release of sIL-6R from human neutrophils. Indeed, it has been suggested that certain leukocyte-derived proteases may facilitate the release of a variety of surface proteins (119–121), with cathepsin-G being implicated in IL-6R shedding (122).

DIFFERENTIAL RELEASE OF SOLUBLE IL-6 RECEPTOR ISOFORMS

Soluble IL-6R appears to be released from cell types that are known to express the cognate receptor, namely, hepatocytes and leukocyte subpopulations. This raises the possibility that a given IL-6R$^+$ cell type has the capacity to release both isoforms. In general, release of PC- and DS-sIL-6R differ with respect to the kinetics of their production. Activators of IL-6R shedding events are typically rapid, and significant increases in sIL-6R levels can be detected within the first 30–120 min of stimulation (88, 100, 105). In contrast, secretion of DS-sIL-6R is relatively slow, and enhanced concentrations of this isoform are typically seen 8–24 h after activation (95). The delay in DS-sIL-6R release presumably occurs because of the necessity for *de novo* synthesis, since cycloheximide substantially diminishes production of this isoform (95). We infer from this that sIL-6R derived via alternate mRNA splicing is not under the same control as that released through proteolytic cleavage. Credence for this statement is provided by both *in vitro* and *in vivo* studies. Examination of human monocytic THP-1 cells, hepatoma HepG2 cells, and myeloma cells has shown that these lines release both PC- and DS-sIL-6R. (95, 100, 123). Indeed, it has been shown that basal sIL-6R production by THP-1 cells occurs through differential mRNA splicing, whereas phorbol ester and ionomycin stimulated release of sIL-6R results from activation of proteolytic shedding

(100). A differential pattern of sIL-6R isoform production is also apparent *in vivo* (92, **Fig. 4**); however, the significance of this has not been considered fully.

Since PC- and DS-sIL-6R may independently contribute to the action of sIL-6R, the relevance of both isoforms needs to be considered when determining the overall properties of sIL-6R. Thus, it is necessary to consider how production of these isoforms is coordinated and also the potential biological significance of both forms. At present, there is no antibody available for the specific detection of PC-sIL-6R. Consequently, patterns of sIL-6R isoform production can only be monitored by comparing levels of total sIL-6R (detected using antibodies that recognize the extracellular portion of IL-6R and are unable to distinguish between the isoforms) with sIL-6R concentrations measured using antibodies specific for the unique COOH-terminal sequence of DS-sIL-6R (88, 92, 93, 100). Using such an approach, it was initially shown that the predominant circulating isoform in normal healthy individuals was derived from differential mRNA splicing [93, Fig. 4*A*]. However, it has now been established that serum levels of this isoform substantially decrease with age whereas total circulating concentrations of sIL-6R remain largely unaltered (Fig. 4*B*). This may reflect either a switch from DS-sIL-6R secretion to PC-sIL-6R release or conceivably a processing of the COOH-terminal sequence of DS-sIL-6R rendering the sIL-6R undetectable with anti-DS-sIL-6R antibodies. Few studies have examined the temporal production of sIL-6R isoforms during disease. However, based on the limited information currently available, it is clear that PC- and DS-sIL-6R release is differentially regulated during specific disease processes. This is most apparent in conditions linked with retroviral infections such as AIDS and adult T cell leukemia or human T cell leukemia virus-1 (HTLV-1)-associated myelopathy. Although circulating sIL-6R levels are significantly elevated in each of these diseases (38, 92), closer inspection of the isoform composition reveals that the increased levels associated with HTLV-1 infection are derived from differential mRNA splicing whereas concentrations of DS-sIL-6R in AIDS patients remain unaltered from those observed in healthy volunteers (92). Similarly, levels of DS-sIL-6R in rheumatoid arthritis patients do not account for the increase in sIL-6R concentrations associated with this disease (Fig. 4*A*; 92), suggesting that proteolytic shedding may be accounting for the increases detected.

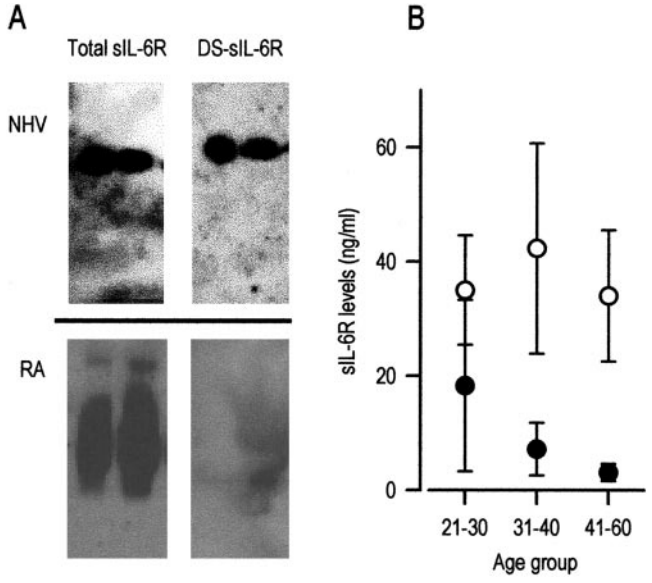

Figure 4. Differential patterns of sIL-6R release. *A)* Western blot analyses of affinity-purified sIL-6R from the sera of normal healthy volunteers (NHV) and rheumatoid arthritis (RA) patients (two independent donors are shown). Sera were isolated after coagulation of whole blood. Cellular deposits were removed by centrifugation (10,000 *g* for 20 min at 4°C). The sera was diluted 1:1 with PBS (pH 7.4) containing 50 mM EDTA and filter sterilized. Soluble IL-6R was affinity purified from the sera by passage through an anti-IL-6R antibody (AB-227-NA from R&D Systems) column. The column was washed and proteins eluted with 100 mM acidic-glycine (pH 2.7). Purified fractions were separated by SDS-PAGE and transferred to a PVDF membrane. Blots were probed with either anti-IL-6R (AB-227-NA, total sIL-6R) or monoclonal anti-DS-sIL-6R (1F10) and protein bands were visualized. *B)* Quantification of total circulating sIL-6R and DS-sIL-6R concentrations in the plasma of normal healthy volunteers. Individuals were clustered into the various age groups and total sIL-6R concentrations quantified as described previously by ELISA (92, 100). For detection of DS-sIL-6R, plates were coated with the mAb 2F3 raised against the COOH-terminal sequence of DS-sIL-6R (92, 100). DS-sIL-6R determinations were performed using an HRP-conjugated polyclonal anti-IL-6R antibody. Values represent the mean sIL-6R concentration ± SD for each population (n=15, n=9, n=10 for each of the respective age groups).

So far it is unclear why two distinct mechanisms are used for the production of sIL-6R. By coupling the differential release of the two sIL-6R isoforms with the presence of a unique COOH-terminal sequence in DS-sIL-6R, it is tempting to question whether structurally distinct forms of sIL-6R may differ in their capacity to activate cellular events. The ability of the [sIL-6R/IL-6] complex to signal via gp130 highlights that the *trans*-membrane region of the IL-6R is not required for the functional interaction of IL-6R with gp130. Furthermore, the NH_2-terminal domain of the IL-6R is dispensable for ligand recognition/signaling, whereas proximal cytokine receptor domains (termed D2 and D3) are required for IL-6 binding and receptor interaction with gp130 (124). It remains to be determined whether the extended COOH-terminal sequence of DS-sIL-6R influences in some way the binding properties of these proximal cytokine receptor domains. An initial comparison of the biological activities of the two isoforms revealed no apparent difference in their capacity to signal (125). However, it is unclear whether responses facilitated via PC- and DS-sIL-6R differ with respect to their potency or efficacy.

ANTAGONISM OF SIL-6R ACTIVITY

To ensure that the agonistic properties of the sIL-6R are tightly regulated, the activity of the [sIL-6R/IL-6]

complex is counteracted by the presence of a soluble form of gp130 (sgp130) (126). Relatively high circulating levels of sgp130 are detected in human blood and sgp130 associates with the [sIL-6R/IL-6] complex to inhibit signaling via membrane-bound gp130 (126, 127). Indeed, sgp130 has been shown to inhibit sIL-6R-mediated proliferation of Kaposi's sarcoma cells (51), STAT activation and the expression of α1-antichymotrypsin (40). However, the inhibitory capacity of sgp130 is not solely restricted to preventing sIL-6R signaling, since sgp130 also inhibits the action of LIF and OSM (128), which may in part account for the high levels of sgp130 detected in the plasma of healthy and diseased individuals.

Recently it was hypothesized that sIL-6R acts as an antagonistic molecule that enhances the inhibitory capacity of sgp130 (40). This conclusion was based on the observation that although sgp130 could not block IL-6-induced activation of IL-6R$^+$ cells, inclusion of sIL-6R in the assay setup enhanced the antagonistic properties of sgp130 by promoting formation of an [IL-6-sIL-6R-sgp130] tertiary complex. Hence, sgp130 and sIL-6R may conspire to prevent IL-6 signaling through the cognate receptor. Consequently, when considering the effect of sIL-6R *in vivo*, it is also necessary to consider the time frame of sIL-6R and sgp130 production during disease progression. In this respect, examination of a recently developed SCID mouse model for multiple myeloma revealed that release of sIL-6R levels preceded those of sgp130 (129).

Several studies have examined the mechanism of sgp130 release and, like sIL-6R production, both differential mRNA splicing and proteolytic cleavage have been implicated in sgp130 release (127, 130–132). Although only a few physiological activators of sgp130 release have been identified (127), the [sIL-6R/IL-6] complex was recently shown to up-regulate gp130 expression in smooth muscle cells (77). However, it is unclear whether enhanced expression of gp130 was also paralleled by an increase in sgp130 concentrations.

SOLUBLE IL-6 RECEPTOR AND DISEASE

Elevated sIL-6R levels have been described in various conditions, which infers that sIL-6R performs an important role in the regulation of IL-6 responses and consequently disease progression. Although changes in sIL-6R concentrations have been determined in numerous clinical disorders (**Table 1**), in most instances the biological significance of sIL-6R within these pathologies remains unclear. We have selected a few disorders in which currently available information from clinical studies and *in vitro* and *in vivo* investigations highlights the potential involvement of sIL-6R in disease progression.

Arthritic lesions

High IL-6 concentrations have been documented in the serum and synovial fluids of rheumatoid arthritis and juvenile rheumatoid arthritis patients (6, 133–135), whereas a prominent role for IL-6 participation in the clinical manifestation of rheumatoid arthritis has been emphasized by several *in vivo* studies (136–139). Indeed, administration of an anti-IL-6 monoclonal antibody to arthritic patients in a limited trial proved to be clinically beneficial (140). Similarly, suppression of arthritic bone destruction was associated with inhibition of IL-6 production after adenovirus transfer of the *csk* gene (which blocks Src kinase activity) into rat ankle joints susceptible to adjuvant-induced arthritis (141). Taken together, these studies appear to contradict the anti-inflammatory properties assigned by some to IL-6 (9–13) and accentuate the necessity to understand the regulation of IL-6-mediated events and the effect of sIL-6R on arthritic lesions.

Several studies have shown that sIL-6R levels are elevated in both rheumatoid arthritis and juvenile rheumatoid arthritis (57, 142, 143); although sIL-6R concentrations are raised in osteoarthritic patients, increases in sIL-6R are more pronounced in rheumatoid arthritis (57). Indeed, levels of sIL-6R and IL-6 have been shown to be highest in the more progressive stages of rheumatoid arthritis development (143). Through a series of *in vitro* approaches, sIL-6R has been implicated in a variety of cellular consequences typically associated with arthritis—for instance, the severe destruction of cartilage and bone, which is a characteristic hallmark of rheumatoid arthritis. Examination of both IL-6 and sIL-6R concentrations in synovial fluid from arthritic patients showed that the extent of joint destruction correlated with the increased concentration of these mediators (57). Furthermore, synovial fluids from rheumatoid arthritis patients containing high levels of IL-6 and sIL-6R promoted osteoclast-like cell formation when added to cocultures of osteoblastic cells and bone marrow cells (57). Inclusion of a blocking anti-IL-6R antibody in these experiments significantly reduced the cellular differentiation, confirming an involvement of sIL-6R in this process. A role for sIL-6R in bone matrix degradation and resorption is also supported by the observation that rat osteoblasts release collagenase-3 after costimulation with IL-6 and sIL-6R (82). In addition to bone remodeling, the [sIL-6R/IL-6] complex may partially account for the loss of proteoglycan commonly associated with arthritic lesions since its activity is associated with suppression of proteoglycan synthesis (144). Together, these findings indicate that sIL-6R contributes, at least in part, to joint destruction. In contrast to these proinflammatory responses, it has been suggested that sIL-6R performs a protective role in cartilage metabolism. Experiments using isolated human articular synovial fibroblasts and chondrocytes revealed that stimulation of these cells by the [sIL-6R/IL-6] complex induced expression of TIMP-1 and prevented the collagenolytic activity of conditioned medium from IL-1-induced synoviocytes (83).

The presence of elevated sIL-6R levels in arthritic episodes strongly suggests that sIL-6R production is

TABLE 1. *Soluble IL-6R and soluble gp130 levels in disease*

Clinical condition	sIL-6R	sgp130	Comments on sIL-6R levels	Refs
Inflammatory disorders				
Rheumatoid arthritis	▲		Elevated sIL-6R levels correlate with the degree of leukocyte recruitment and joint destruction	57, 75
Juvenile chronic arthritis	▲	▲▼	Significant increases in both IL-6 and sIL-6R, which correlated with fever	142
Osteoarthritis	▲		Elevated levels, but not to the extent associated with rheumatoid arthritis	57
Sepsis	▼		In sepsis patients, median serum sIL-6R concentrations were lower than in normal healthy volunteers	160
Asthma	▲		Asthmatic patients have high serum sIL-6R levels and levels increase after allergen challenge	161
Interstitial lung disease	▲		Raised sIL-6R levels contribute to systemic and local responses in pneumonia and sarcoidosis patients	162
Inflammatory bowel disease	▲		Elevated IL-6 and sIL-6R levels contribute to the pathogenesis of chronic intestinal inflammation	156–158
Systemic sclerosis	▲	▲▼	sIL-6R levels correlate with the severity of pulmonary fibrosis associated with systemic sclerosis	163
Intraocular inflammation	▲		Elevated sIL-6R and IL-6 levels in the aqueous humor of uveitis patients	164
Graves' disease	▲		High serum sIL-6R levels in Graves' disease patients, especially with thyroid-associated ophthalmopathy	165
Endometriosis	▲		Peritoneal and serum sIL-6R levels significantly higher than in other benign gynecologic conditions	166
Neurological conditions				
Alzheimer's disease	▼/▲▼	▲▼	Decreased or unaltered levels found in the CSF	167, 168
Depression	▼	▲▼	Significantly decreased sIL-6R and IL-6 concentrations in CSF compared with healthy subjects	169
Multiple sclerosis	▲	▲	Elevated serum sIL-6R concentrations correlate with disease severity	170
Schizophrenia and mania	▲		Higher sIL-6R levels in psychotic patients than healthy volunteers	171
Cerebral trauma	▲		Increased CSF concentrations of sIL-6R and intrathecal sIL-6R release are suggested	172
Psychological stress	▲	▲▼	Elevated in post-traumatic stress disorder, especially in patients with major depression	173
Cancers				
Breast cancer	▼		Serum sIL-6R levels were lower than in normal individuals but elevated after surgery	174
Pancreatic cancer	▲▼		Soluble IL-6R levels unaltered from normal individuals	175
Adult T-cell Leukemia	▲	▲	See HTLV-1 infection and also HTLV-1-associated myelopathy	89
Multiple myeloma	▲		Circulating sIL-6R levels correlate with disease severity	151–153
Pathogen infections				
HIV	▲		Viral infection promotes sIL-6R release, which might contribute to the onset of AIDS-related pathologies	38, 92
HTLV-1	▲	▲	Increased levels of sIL-6R are derived from differential mRNA splicing	92
Cerebral malaria	▲		Elevated IL-6 and sIL-6R levels after *P. falciparum* infection	176
Urinary tract infection	▲		Increased serum sIL-6R concentrations correlate with a loss of glomerular filtration rate	177
Meningococcal infection			sIL-6R levels are low in acute infection, but dramatically increase during recovery and normalize	178

coordinated as part of the inflammatory response. Through comparison of sIL-6R determinations in serum and synovial fluid of patients with rheumatoid arthritis, it is difficult to judge whether the elevated sIL-6R levels associated with this disease are derived from systemic or local sources (75). To date, the cellular origin of sIL-6R in arthritis remains unknown, although structural cells of the joint (chondrocytes, synoviocytes, fibroblasts, and endothelial cells) are unlikely to contribute to the sIL-6R levels seen in this disease (75). It is conceivable that sIL-6R may be released from activated leukocytes. This may account for the observed correlation between leukocyte influx into arthritic joints and the increased concentration of sIL-6R in synovial fluid (75). In the event of such a scenario, it is of interest to consider the capacity of the [sIL-6R/IL-6] complex to induce chemokine production and enhance adhesion molecule expression (73, 74, 77, 78). Thus, sIL-6R release by infiltrating leukocytes would promote further leukocyte recruitment. Conversely, systemic sIL-6R production may also contribute to the regulation of IL-6 responses in arthritis,

since sIL-6R levels are significantly elevated in systemic onset juvenile chronic arthritis (142).

Multiple myeloma

Early studies into the action of IL-6 led investigators to name this cytokine by a variety of functional synonyms, including B cell differentiation factor and/or B cell-stimulating factor-2 (145). Consequently, it is not surprising to find that IL-6 plays a major role in the proliferation and survival of clonal malignant plasma cells in multiple myeloma (146, 147). Indeed, levels of IL-6 are significantly elevated in patients suffering from this condition (148). An active role for the sIL-6R in multiple myeloma was first presented by the observation that physiological concentrations of sIL-6R increase the sensitivity of myeloma cells to IL-6 (149). As a result, numerous studies have now established that sIL-6R levels are significantly elevated in serum from multiple myeloma patients (150–153), whereas the extent of sIL-6R in circulation appears to correlate with the severity/stage of multiple myeloma (151–153). Indeed, circulating levels of sIL-6R may act as a useful prognosis marker, since high serum sIL-6R concentrations were associated with patients who died within 3 years of diagnosis (151).

Although IL-6 has been implicated in the proliferation and survival of myeloma cells, a precise role for sIL-6R in these processes is far from clear. In particular, no correlation could be made between sIL-6R and IL-6 (as a myeloma cell growth factor) levels or thymidine kinase (as a marker of cellular proliferation) activity within a population of 207 multiple myeloma patients (151). Even so, expansion of myeloma cell lines has been reported to be promoted by a combination of IL-6 and sIL-6R (154, 155) as well as by stimulatory monoclonal anti-human gp130 antibodies (129). In this latter study, the authors used these agonistic antibodies to develop a SCID mouse model of human multiple myeloma. Human myeloma cells were transplanted into the mouse peritoneum and tumor growth promoted by the presence of anti-gp130 monoclonal antibodies (129). When circulating levels of human sIL-6R were periodically tested within these mice, elevated serum concentrations of sIL-6R were detected prior to formation of a palpable tumor mass (129). In contrast, elevated sgp130 levels were raised some 2–3 wk after the initial observation of tumor expansion, suggesting that sIL-6R activity remains unchecked during the early stages of tumor growth (129). The significance of this finding remains to be determined.

Crohn's disease

Crohn's disease is a chronic inflammatory condition of the gastrointestinal tract that typically manifests itself by the formation of bowel strictures, ileus, and fistulas. Development of this condition appears to be controlled by type-1 T-helper cells (Th1) and is associated with elevated concentrations of Th1-polarizing cytokines (e.g., IFN-γ). In addition, elevated concentrations of IL-6 and sIL-6R have been described in both Crohn's disease and ulcerative colitis (156), suggesting a role for these mediators in the pathogenesis of these conditions. Using a SCID mouse model of colitis, it was recently demonstrated that treatment of mice with a blocking anti-IL-6R monoclonal antibody significantly impaired disease progression (157). Specifically, mice treated with an anti-IL-6R antibody retained normal growth, whereas sham mice administered with a non-immune IgG control lost weight. This observation was paralleled by suppression of colonic ICAM-1, VCAM-1, IFN-γ, TNF-α, and IL-1β expression after anti-IL-6R antibody treatment (157). These results point to a critical role for IL-6 in the pathogenesis of colitis episodes and are supported by the finding that sIL-6R in combination with IL-6 prevents T cell apoptosis in experimental colitis (158). In particular, activity of the [sIL-6R/IL-6] complex was associated with induced expression of the anti-apoptotic genes bcl-2 and bcl-x1, while blockade of sIL-6R-mediated signaling (using either anti-IL-6R monoclonal antibodies or an inhibitory gp130-Fc fusion protein) promoted apoptosis of lamina propria T cells (158). Furthermore, inhibition of sIL-6R activity in several murine models of colitis suppressed the clinical indices of disease progression (158). Thus, therapeutic measures that target sIL-6R signaling may be of potential importance in the treatment of Crohn's disease.

QUESTIONS FOR THE FUTURE

The confined cellular expression of the cognate IL-6R initially suggested that only a few distinct cell types possessed the capacity to respond to IL-6. However, with the identification of sIL-6R and the agonist properties assigned to the [sIL-6R/IL-6] complex, it is now apparent that IL-6 can activate cell types that are inherently unresponsive to IL-6 alone. As a result, environments such as an arthritic joint where the resident cells appear unable to respond to IL-6 itself (75), the principle mechanism for coordinating IL-6-mediated events would be through the release of sIL-6R. Consequently, it is necessary for future studies to establish the source of sIL-6R. It is essential to consider elevated sIL-6R levels in terms of localized increases in sIL-6R concentrations as well as systemic sIL-6R production. In cases of multiple myeloma, for instance, it is apparent that systemically elevated sIL-6R levels have a pronounced bearing on the outcome of the disease (151–153). In contrast, sIL-6R levels in synovial fluid from rheumatoid arthritis patients correlate with the extent of leukocyte infiltration into the joint (75) and with the degree of bone destruction (57), suggesting that local sIL-6R levels also contribute to disease progression. Thus, a better appreciation of how sIL-6R concentrations are regulated is required. In particular, determining the time frame of sIL-6R production during disease progression is central to our

understanding of when sIL-6R is likely to participate in the disease process. In line with this, it is necessary to consider not only temporal changes in the release of both DS- and PC-sIL-6R, but also the effect sgp130 may have in the balance between the agonistic properties of IL-6 and its soluble receptor and the antagonistic function of sgp130.

Interleukin 6 has been proposed to act as both a pro- and anti-inflammatory cytokine. For instance, examination of the regulatory properties of IL-6 in local and systemic acute inflammation using IL-$6^{-/-}$ mice reveals that the absence of IL-6 correlates with increased expression of TNF-α, IFN-γ, GM-CSF, and MIP-2 (the murine homologue of IL-8) and by significantly elevated neutrophilia (11). Hence, IL-6 has the capacity to limit neutrophil recruitment and suppress the activities of proinflammatory mediators. In contrast, the degree of leukocyte infiltration in an s.c. air pouch inflammation model has been reported to be mediated via sIL-6R (73), whereas *in vitro* studies show that the [sIL-6R/IL-6] complex has the capacity to enhance chemokine and adhesion molecule expression (73, 74). These findings support a proinflammatory role for IL-6 and sIL-6R in disease pathogenesis. Indeed, the detrimental consequences of their action have now been documented in several *in vivo* models of arthritis (136–139) and chronic intestinal inflammation (157, 158). Thus, it would appear that the assignment of inflammatory properties to IL-6 depends on the clinical condition in which it acts. As a result, it is essential that the biological processes controlled by IL-6 alone be distinguished from those mediated via the sIL-6R.

CONCLUDING REMARKS

The emphasis of this review has been to document the central role of sIL-6R in the regulation of IL-6 activity. Elevated IL-6 concentrations have been described in numerous clinical disorders, where they appear to orchestrate a variety of inflammatory responses. Given the agonistic properties of sIL-6R, it is evident that control of many of these IL-6-mediated events is regulated via sIL-6R. Consequently, when considering the role of IL-6 in disease progression, it is equally important to consider how sIL-6R affects its function. Although enhanced sIL-6R levels have been documented in a variety of disease states, we are only now beginning to appreciate the potential contribution sIL-6R may have in these pathologies. As a result, future studies must address this issue if the functional properties of IL-6 are to be fully understood. FJ

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