Introduction

Interleukin-6 (IL-6) is a cytokine that was originally identified as a factor that could induce B lymphocytes to differentiate into immunoglobulin-producing plasma cells [1]. Subsequent studies have shown IL-6 to be a multifunctional cytokine that is produced by, and can affect, a range of different cells [2,3]. IL-6 is the original member of a family of cytokines that now include leukaemia inhibitory factor, interleukin 11, oncostatin M, ciliary neurotropic factor and cardiotrophin 1. The receptors for these molecules share a common signal transducing component, which accounts for their overlapping biological activities.

Abbreviations used: IL, interleukin; IL-6R, IL-6 receptor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumour necrosis factor; sIL-6R, soluble IL-6R.

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The IL-6/IL-6 receptor (IL-6R) system

IL-6 initiates its biological response via the IL-6R complex, which consists of two subunits (Figure 1). IL-6 initially binds to the cell-surface IL-6R (also known as gp80) [4] and the resulting complex then associates with a second, non-ligand-binding, signal-transducing subunit known as gp130. This in turn leads to homodimerization of gp130 and the activation of signalling pathways within the cell [5]. More recent studies have suggested that the functional signalling complex is likely to be a hexamer consisting of two molecules each of IL-6, IL-6R and gp130 [6]. Early studies demonstrated that a truncated form of the IL-6R, lacking the cytoplasmic domain, retained biological activity, suggesting that the cytoplasmic region was not required for signalling [4].

In addition to the membrane-bound form, a soluble IL-6R (sIL-6R) has been identified in both serum and urine [7,8]. sIL-6R can bind...
IL-6 with a similar affinity to that of the membrane-bound form of the receptor. In addition, sIL-6R is unusual among soluble cytokine receptors in that after binding IL-6 it retains the ability to interact with membrane-bound gp130 and allows normal IL-6-mediated responses in the cell (Figure 1) [9]. Because gp130 is expressed ubiquitously [10], cells that do not express the membrane-bound IL-6R are still able to respond to IL-6 in the presence of the soluble form of the receptor. This ‘trans-signalling’ might prove to be an important means by which IL-6 or related cytokines induce a cellular response [11]. Indeed, at physiological concentrations, sIL-6R has been shown to increase the sensitivity of myeloma cells to IL-6 by up to 10-fold [12].

A soluble form of gp130 (sgp130) has also been found in human serum and shown to be released from cells transfected with a gp130 cDNA [9,13]. Soluble gp130 is thought to act as an antagonist to IL-6 because it has been shown to bind to the IL-6/sIL-6R complex and inhibit the effect of this complex on cell proliferation in vitro (Figure 1) [9].

Generating sIL-6R
Soluble cytokine receptors can be generated by either alternative mRNA splicing or release of the extracellular domain of the membrane-bound form by the action of a proteinase [14]. Studies have shown that an alternatively spliced form of the IL-6R exists that lacks residues predicted to encode the transmembrane region [15]. Furthermore, recent studies have demonstrated this form of the receptor in human plasma [16]. However, several reports have shown that sIL-6R can also be generated by shedding from the cell surface. Early studies by Mullberg et al. [17,18] demonstrated that IL-6R could be released from the cell membrane of COS-7 cells transiently transfected with an IL-6R cDNA. Subsequently a range of different cells, including human peripheral blood monocytes, a monocytic cell line (THP-1) and human myeloma cells, have been reported to release IL-6R from the cell surface [19,20]. Shedding of IL-6R from transiently transfected cells and cells that express the receptor constitutively can be significantly upregulated by treatment with PMA, suggesting that the activation of protein kinase C might be important in the release of this receptor [17–20]. PMA-induced shedding of IL-6R occurs rapidly, with an increase being observed within minutes of treatment, indicating that this is an acute response rather than an effect that requires new gene transcription and protein synthesis de novo [19,20]. Although the mechanism of PMA-stimulated shedding is unclear, this treatment might lead to modifications within the cytoplasmic region of the receptor, resulting in alterations to the three-dimensional structure of the extracellular domain and exposing a site for proteolytic cleavage. Alternatively, treatment with PMA might lead to the stimulation of a proteinase activity, or a cascade of proteinases, that are responsible for IL-6R shedding. A combination of these possibilities could operate simultaneously.

Extensive analysis of the site of proteolytic activity within IL-6R has shown that cleavage occurs at Gln-357/Asp-358, which is one residue proximal to the transmembrane region [21]. However, the sequence surrounding this site shows no identity with cleavage sites found in other membrane-bound proteins known to be released by proteolysis. Attempts to identify the protease responsible for shedding of IL-6R have proved unsuccessful. Inhibitors of serine, cysteine and aspartic proteinases, and several metalloproteinase inhibitors, including tissue inhibitor of metalloproteinase 1 and 2 (TIMP-1 and -2), are unable to prevent release of IL-6R (Table 1) [19,20]. However, hydroxyamate-based metalloproteinase inhibitors have been shown to suppress both constitutive and PMA-induced shedding of IL-6R from a range of different cells [19,20,22,23]. Furthermore, we have recently demonstrated that TIMP-3 can also inhibit shedding of the IL-6R from human myeloma cells [20].

Several studies have suggested that sgp130 can also be generated by both alternative mRNA splicing and proteolytic activity [13,24]. A gp130 mRNA transcript containing an additional exon in the region predicted to encode the extracellular domain has been identified [24]. This results in a change in the reading frame, introducing a stop codon immediately before the transmembrane region. Cells transfected with this cDNA have been reported to release increased concentrations of sgp130 into the culture supernatant. Mullberg et al. [13] have demonstrated that Madin–Darby canine kidney cells transfected with a gp130 cDNA also shed gp130. However, the level of shedding seemed to be significantly less than for IL-6R, and was not promoted by PMA. In contrast, we have observed
that a number of human myeloma cell lines release gp130, a process that can be promoted by PMA and suppressed by hydroxamate-based metalloproteinase inhibitors (P. G. Hargreaves, N. Drury and P. I. Croucher, unpublished work). These observations suggest that both IL-6R and gp130 can be shed in a similar manner.

Although sIL-6R and sgp130 can be generated by both alternative mRNA splicing and proteolytic processing, the relative contributions of these mechanisms to the extracellular concentration of these proteins has not been defined. The demonstration that protein synthesis de novo was not required for the generation of sIL-6R in THP-1 cells suggests that the soluble form of the receptor detected in cultures of these cells is predominantly generated by shedding [19]. Furthermore, because PMA-induced production of sIL-6R occurs very rapidly and is inhibited almost completely by hydroxamate-based inhibitors, it is likely that this is also mediated by proteolysis [19, 20]. However, the constitutive release of sIL-6R is not completely blocked by such inhibitors, even at high concentrations, indicating that there might be a contribution from alternative splicing [19, 20]. In support of this the alternatively spliced form of IL-6R has been detected in plasma [16], suggesting that both mechanisms might operate simultaneously. The mechanisms of regulating the two systems are currently unclear.

Possible role for members of the ADAM family in IL-6R shedding

The demonstration that hydroxamate-based metalloproteinase inhibitors and TIMP-3, but not TIMP-1 or TIMP-2, inhibit IL-6R shedding, strongly suggests that a non-matrix-type metalloproteinase is responsible for this event. Although the identity of this protease remains unknown, there is increasing evidence that members of the ADAM ('a disintegrin and metalloproteinase') family of metalloproteinases are involved. The mammalian adamalysin, or ADAM, family is a recently described family of proteins that contain a number of characteristic domains [25]. These include a proregion, a metalloproteinase domain, a disintegrin-like domain, a

Table 1

Effect of proteinase inhibitors on IL-6R shedding

This is not a definitive list of inhibitors, but it includes examples of inhibitors used to characterize the protease involved in IL-6R shedding. Abbreviations: TLCK, 7-amino-1-chloro-3-1-tosylamidoheptan-2-one; TPCK, 1-chloro-4-phenyl-3-1-toluene-p-sulphonamidobutan-2-one; E-64, trans-epoxysuccinyl-l-leucylamido(4-guanidino)butane. RU26156 is the same compound as BB-94.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Class of proteinase inhibited</th>
<th>Effect on IL-6R shedding</th>
<th>References</th>
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<tbody>
<tr>
<td>3,4-Dichloroisocoumarin</td>
<td>Serine</td>
<td>–</td>
<td>[19]</td>
</tr>
<tr>
<td>TLCK</td>
<td>Serine</td>
<td>–</td>
<td>[19]</td>
</tr>
<tr>
<td>TPCK</td>
<td>Serine</td>
<td>–</td>
<td>[19]</td>
</tr>
<tr>
<td>Benazamidine</td>
<td>Serine</td>
<td>–</td>
<td>[20]</td>
</tr>
<tr>
<td>Leupeptin</td>
<td>Trypsin-like serine/some cysteine</td>
<td>–</td>
<td>[19, 20]</td>
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<tr>
<td>E-64</td>
<td>Cysteine</td>
<td>–</td>
<td>[20]</td>
</tr>
<tr>
<td>Phosphoramidon</td>
<td>Metallo</td>
<td>–</td>
<td>[18, 20]</td>
</tr>
<tr>
<td>Captoprol</td>
<td>Metallo</td>
<td>–</td>
<td>[19, 20]</td>
</tr>
<tr>
<td>Actinonin</td>
<td>Metallo</td>
<td>–</td>
<td>[20]</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>Metallo (matrix-type)</td>
<td>–</td>
<td>[19, 20]</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>Metallo (matrix-type)</td>
<td>–</td>
<td>[19, 20]</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>Metallo</td>
<td>+</td>
<td>[20]</td>
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<tr>
<td>Batimastat (BB-94)</td>
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<td>+</td>
<td>[20]</td>
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<tr>
<td>TAPI</td>
<td>Metallo</td>
<td>+</td>
<td>[19]</td>
</tr>
<tr>
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<td>Metallo</td>
<td>+</td>
<td>[23]</td>
</tr>
<tr>
<td>RU26156</td>
<td>Metallo</td>
<td>+</td>
<td>[22]</td>
</tr>
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</table>
Table 2

Members of the ADAM family

This table contains members of the ADAM family that contain a metallocproteinase domain that is predicted to be active, show widespread tissue distribution, and are expressed by cells known to shed IL-6R. Abbreviation TACE, TNF-α-converting enzyme.

<table>
<thead>
<tr>
<th>ADAM family member</th>
<th>Alternative name</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAM9</td>
<td>MDC-9, MCMP</td>
<td>[33,34]</td>
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<tr>
<td>ADAM10</td>
<td>MADM</td>
<td>[35]</td>
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<tr>
<td>ADAM15</td>
<td>Metargidin</td>
<td>[36]</td>
</tr>
<tr>
<td>ADAM17</td>
<td>TACE</td>
<td>[29,30]</td>
</tr>
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</table>

cysteine-rich region containing an epidermal growth factor-like repeat, a transmembrane domain and a cytoplasmic tail. More than 20 members of this family have now been identified. Although the functions of many of the members of the ADAM family are unknown, several lines of evidence are consistent with their involvement in IL-6R shedding.

First, the activity responsible for shedding IL-6R has been identified in a range of cell types. Several members of the ADAM family show a similar widespread distribution (Table 2). Indeed, our own studies have shown that human myeloma cells, which shed IL-6R, express ADAM9, ADAM10, ADAM15 and ADAM17 (M. Zhou, N. Fernandez da Silva, I. Holen and P. I. Croucher, unpublished work), each one of which contains the conserved zinc-binding site and is predicted to be catalytically active. Secondly, Kuzbanian, a member of the ADAM family originally identified in Drosophila [26], has been reported to be responsible for the proteolytic processing of Notch, a membrane receptor that mediates lateral inhibition and regulates neurogenesis [27]. Thirdly, the most compelling evidence is the demonstration that the activity responsible for shedding IL-6R has an identical inhibition profile to that of the proteinase responsible for processing membrane-bound tumour necrosis factor α (TNF-α) to the mature soluble form [28]. This proteinase, the TNF-α-converting enzyme, has recently been identified and shown to be a member of the ADAM family (ADAM17) [29,30]. Furthermore, ADAM10 has also been purified and shown to be able to process TNF-α [31,32]. Interestingly, ADAM10 was unable to cleave peptides spanning the cleavage sites of other membrane-bound proteins known to be proteolytically processed, including IL-6R [32].

Conclusions

IL-6 is an important cytokine with diverse effects on a range of tissues. The effect of IL-6 on a cell reflects the balance that exists between the different components of the IL-6/IL-6R system. One of the most important components of this system is sIL-6R. Although our understanding of the mechanisms of producing sIL-6R is incomplete, proteolytic shedding is an important means of generating this form of the receptor. Although the proteinase responsible for releasing the IL-6R is unknown, members of the ADAM family of metalloproteinases represent strong candidates, especially because ADAMs have been shown to process TNF-α.

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