Anti-inflammatory

gp130-mediated signalling as a therapeutic target

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IL-6 is a pleiotropic cytokine that regulates haematopoiesis, inflammation and the immune response. The IL-6 receptor consists of an α chain and gp130, a common subunit that is shared among the receptors of the IL-6 family of cytokines. The binding of IL-6 to its receptor induces the homodimerisation of gp130, resulting in the activation of JAKs (Janus kinases). A variety of signal transduction pathways, such as those mediated by SHP2 and STAT3 (signal transducer and activator of transcription), are then activated. Because both the overexpression of IL-6 and the aberrant activation of the gp130 signal have been implicated in the pathology of a variety of diseases, including rheumatoid arthritis (RA), juvenile chronic arthritis, multiple myeloma/plasmacytoma, Castleman’s disease and Kaposi’s sarcoma, the development of inhibitors of the IL-6 signalling pathway is a promising avenue for the treatment of these diseases. Several approaches have been taken to inhibit the activation of this pathway. One is to interfere with the formation of the IL-6/IL-6Rα/gp130 complex. This strategy has already been used to improve the symptoms of patients with RA, multiple myeloma and Castleman’s disease. Another is the direct targeting of STAT3 activity. Here, we describe the biological activity of IL-6 and of the signal transduction pathways mediated through the IL-6 receptor, and discuss the possible therapeutic applications of IL-6 inhibitors.

Keywords: Castleman’s disease, CNTF, Crohn’s disease, cytokine, Gab, gp130, IL-6, IL-11, Kaposi’s sarcoma, LIF, MAPK, multiple myeloma, negative regulation, OSM, PIAS, rheumatoid arthritis, SHP2, signal transduction, SOCS, STAT3


1. Introduction

IL-6, cloned in 1986 [1,2], was first identified as one of the factors inducing the differentiation of B-cells into antibody-forming plasma cells [3]. It was also cloned independently as IFNβ2 and 26 kDa protein [4-6]. IL-6 is a typical cytokine that exhibits a variety of biological functions, including immunoglobulin production, the acute phase reaction, gliogenesis and inflammation, by regulating cell growth, differentiation and survival [2,7-9]. The IL-6 receptor consists of an α chain (IL-6Rα) and gp130 (Figure 1) [10-12]. The binding of IL-6 to its receptor initiates the homodimerisation of gp130 and activates JAK tyrosine kinases, which are constitutively
gp130-mediated signalling as a therapeutic target

Table 1: Examples of diseases with deregulated production of IL-6 family cytokines.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Overview of disease: epidemiology, and aetiology</th>
<th>Relevance to gp130 signalling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple myeloma/plasmacytoma/plasmacytosis</td>
<td>Incidence rates for myelomatosis are 3 to 10 per 100,000, higher in males and especially in Africans [176].</td>
<td>anti-IL-6, anti-IL-6Ra therapy</td>
</tr>
<tr>
<td>Kaposi’s sarcoma</td>
<td>Similar distribution to that of Burkitt’s lymphoma [177]. HIV-1 infection increases the risk of Kaposi’s sarcoma [29].</td>
<td>[29]</td>
</tr>
<tr>
<td>Castleman’s disease</td>
<td>Rare disease, also known as angiofollicular lymph node hyperplasia [177].</td>
<td>anti-IL-6Ra therapy</td>
</tr>
<tr>
<td>Cardiac myxoma</td>
<td>Prevalence is 1 to 5 per 10,000 in autopsy series, or 2 per million in the general population [178].</td>
<td>[16]</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Prevalence is remarkably consistent worldwide (approximately 1%) [179].</td>
<td>anti-IL-6, anti-IL-6Ra therapy</td>
</tr>
<tr>
<td>Mesangial proliferative glomerulonephritis</td>
<td>10 - 20% of primary glomerulonephritis, when expressed as a percentage of biopsies. However, it is difficult to be sure of the true incidence [180].</td>
<td>[24]</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>The incidence rate is 1 to 7 per 100,000, and it is increasing in Europe and Scandinavia [181].</td>
<td>IL-11, anti-IL-6Ra therapy</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>In temperate zones psoriasis affects 2% of the Caucasian population [182].</td>
<td>IL-11 therapy</td>
</tr>
<tr>
<td>Asthma</td>
<td>Prevalence of asthma varies between 1.6 - 20.5% depending on age, sex, environment, and respiratory infection [183].</td>
<td>[28]</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>Incidence rates are 1 to 1.5 per 100,000 population. Prevalence rates are 4 to 6 per 100,000 [184].</td>
<td>CNTF therapy</td>
</tr>
</tbody>
</table>

Associated with gp130, resulting in tyrosine phosphorylation of gp130 by the JAKs. These events create the docking sites for signalling molecules, such as SHP2, which is a protein tyrosine phosphatase containing an SH2 domain and STAT3 [13]. gp130 is used not only by the IL-6 receptor but also by the receptors for other members of the IL-6 family of cytokines, such as leukaemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), IL-11, cardiotoxophin-1 (CT-1) and possibly neurotrophin-1/B-cell stimulating factor-3 (NNT-1/BST-3) (Figure 1) [14,15] (for review, see Hirano et al. [13]). Knock-out, transgenic and knock-in mice for these molecules have been generated and it has become evident that both loss- and gain-of-function mutations of these cytokines and the components involved in their signalling result in immunological, neurological and developmental abnormalities.

In the late 1980s, the possible involvement of deregulated IL-6 expression was first demonstrated in patients with cardiac myxoma and RA [16,17]. Since then, much evidence has accumulated to indicate a role for the deregulated expression of IL-6 family cytokines in various diseases, including inflammation, autoimmune diseases and malignancies (Table 1) [2,9]. Common features of the diseases caused by the deregulation of IL-6-related signals are extensive inflammation and cell proliferation. This finding is consistent with the function of IL-6 in cell growth and differentiation, and in the regulation of the immune system. Thus, the control of gp130-mediated signalling may contribute to the development of new therapies for these diseases.

In this review, we summarise the signalling mechanism of gp130, introduce current ongoing clinical trials and discuss gp130 as a possible therapeutic target.

2. Biological functions of IL-6 family cytokines

Besides its role in inducing B-cell differentiation, IL-6 also induces T-cell growth and differentiation, differentiation of the myeloid leukaemic cell line M1 into macrophages, megakaryocyte maturation, neural differentiation of PC12 cells, development of osteoclasts, and acute-phase protein synthesis in hepatocytes. IL-6 acts as a growth factor for
myeloma/plasmacytoma, keratinocytes, mesangial cells, renal cell carcinoma and Kaposi’s sarcoma, and promotes the growth of haematopoietic stem cells. Furthermore, IL-6 inhibits the growth of certain carcinoma cells [2,7-9].

A possible involvement of IL-6 in disease was first seen in patients with cardiac myxoma [16]. These patients have a variety of autoimmune symptoms, such as hypergammaglobulinaemia, the presence of autoantibodies and an increase in acute phase proteins, all of which disappear after the resection of the tumour cells. The finding that myxoma cells produce high levels of IL-6 led us to speculate that overproduction of IL-6 might play a critical role in the development of autoimmune symptoms. RA patients have high levels of IL-6, LIF, IL-11 and OSM in their synovial fluid [17-19] and increased amounts of sIL-6Rα (soluble form of IL-6Rα, which can be released by receptor shedding or secretion after translation of an alternatively spliced mRNA) are implicated in the pathogenesis of juvenile chronic arthritis [20]. Patients with multiple myeloma, a malignant plasma cell tumour, have higher than normal levels of IL-6, sIL-6Rα and OSM [21-23]. IL-6 may be involved in the promotion of mesangial proliferative glomerulonephritis [24], which occurs either as a primary glomerulonephritis or as part of systemic diseases, such as a lupus erythematoses. The overexpression of IL-6 has also been shown in connection with other diseases, including osteoporosis, Castleman’s disease and several autoimmune diseases [9,25,26]. Recently, Atreya et al. reported that lamina propria T-cells and macrophages from patients with Crohn’s disease (CD) or ulcerative colitis (UC) show increased production of IL-6 [27]. They also showed that blocking IL-6 signalling suppressed the experimental colitis in various animal models of CD. IL-11 expression is increased in severe asthma [28]. Interestingly, human herpesvirus 8 (HHV8, or Kaposi’s sarcoma-associated herpesvirus), which has been found in Kaposi’s sarcoma lesions, carries the viral counterpart of IL-6 (vIL-6) and vIL-6 is implicated in promoting the course of Kaposi’s sarcoma [29].

A large body of work using knock-out and transgenic mice has addressed the in vivo functions of IL-6 family cytokines (Table 2). Transgenic mice overexpressing IL-6, sIL-6Rα, LIF or both IL-6 and sIL-6Rα have provided important evidence regarding the
pathogenicity of IL-6 family cytokines. IL-6 transgenic mice of C57BL/6 origin develop massive plasmacytosis, but not plasmacytomas. However, the introduction of the BALB/c genetic background into IL-6 transgenic mice could generate monoclonal transplantaible plasmacytomas with the chromosomal translocation t(12;15) [30,31]. Since gp130 is involved in both cell growth and the differentiation of B-cells into plasma cells through STAT3 activation [32], chronic B-cell activation by deregulated IL-6 expression may be one of the major pathogeneses of these diseases [33]. The overexpression of LIF in T-cells results in B-cell hyperplasia, polyclonal hypergammaglobulinaemia and mesangial proliferative glomerulonephritis [34]. Double transgenic mice co-expressing both IL-6 and sIL-6Rα throughout the body show progressive extramedullary haematopoiesis and liver-specific IL-6-sIL-6Rα double transgenic mice develop nodular regenerative hyperplasia and adenomas of the liver [35,36].

On the other hand, IL-6-deficient mice show impaired antigen-specific antibody production [37,38]. IL-6-deficient mice also display dysfunction in diverse systems, for example, in haematopoiesis [39], the acute-phase reaction [37], Type 1 helper T-cell (Th1) development [40-42] and protection against Listeria monocytogenes infection [43]. The resistance against collagen-induced arthritis (CIA) in the IL-6-deficient mice shows the indispensable role of IL-6 in RA [44,45]. Neutralising anti-IL-6R antibody ameliorates the joint disease in murine CIA [46]. Experimental autoimmune encephalomyelitis was suppressed in the IL-6-deficient mice, showing that IL-6 is important for the activation and differentiation of autoreactive T-cells [47-49]. LIF-deficient mice display defects in haematopoiesis and thymocyte proliferation [50] and CNTF-deficient mice have slightly fewer motor neurones [51].

3. gp130: structure and signalling

The IL-6 receptor consists of an α chain and gp130 (Figure 1). Both the IL-6Rα chain and gp130 are required for the high-affinity binding site for IL-6, but only the cytoplasmic region of gp130 is necessary for the activation of the intracellular signalling pathways. The binding of IL-6 to the receptor induces the homodimerisation of gp130, leading to the activation of the associated JAKs, including JAK1, JAK2 and Tyk2 [13]. gp130 is a single transmembrane glycoprotein with a molecular mass of 130 - 150 kDa [12]. The extracellular region of gp130 is predicted to consist of six individual domains (Figure 1) and several mutagenesis studies have addressed the roles of these domains. The most N-terminal Ig-like domain of gp130 has been shown to be important for forming the stable hexameric receptor complex for IL-6 [52], but is not required for signalling by LIF or OSM [53]. On the other hand, the most N-terminal Ig-like domain of IL-6Rα is important for non-induced receptor shedding [54]. Compared with the Ig-like domain, the roles of the second and third domains of gp130 have been studied extensively and are important for ligand binding [55,56]. Of these, the N-terminal fibronectin Type III-like domain contains four conserved cysteine residues and the C-terminal fibronectin Type III-like domain contains a WSXWS sequence and together they form a cytokine-binding module. Based on the presence of these conserved domains, gp130 is defined as belonging to the Type I cytokine receptor superfamily [15]. The other receptors for the IL-6-family cytokines, including the IL-6Rα chain, also belong to the Type I cytokine receptor superfamily, with the exception of CNTFRα, which is a GPI-anchored receptor. The fourth to sixth domains of the extracellular region of gp130 are important for the coupling of ligand binding with the activation of gp130 [57]. In its intracellular region, gp130 contains regions known as box1 (I551WPNVD of human sequence) and box2 (V691SVWEANDKKP), which are conserved among the members of the Type I cytokine receptor superfamily. The JAKs are activated through these two regions (Figure 2).

Functional redundancy is one of the characteristic features of cytokines. For example, IL-6, LIF, or IL-11 can induce acute-phase protein production in hepatic cells or the differentiation of the mouse leukaemic cell line, M1. Sharing the common signal transducer gp130 is one of the mechanisms through which the functional redundancy of the IL-6 family cytokines is mediated. IL-6, IL-11, CNTF and CT-1 initially bind to their specific receptors – IL-6Rα, IL-11Rα, CNTFRα and CT-1Rα. The binding of IL-6 and IL-11 then leads to the homodimerisation of gp130. In contrast, the binding of CNTF, LIF, OSM, CT-1 and NNT-1/BSF-3 leads to the heterodimerisation of gp130 with other gp130-related receptors (i.e., the LIF receptor and the OSM receptor) (Figure 1). IL-6Rα, IL-11Rα and CNTFRα are not thought to transmit signals, since they have very few or no amino acid residues in their cytoplasmic domains. The homodimerisation or
Table 2: Phenotypes of genetically engineered mice.

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethality</td>
<td>Antibody response</td>
</tr>
<tr>
<td>IL-6 KO</td>
<td>TD response ↓ Mucosal IgA response ↓</td>
</tr>
<tr>
<td>IL-6 TG</td>
<td>plasmacytosis, plasmacytoma</td>
</tr>
<tr>
<td>sIL-6Ra TG</td>
<td>↑</td>
</tr>
<tr>
<td>IL-6/IL-6Ra double TG</td>
<td>plasmacytoma</td>
</tr>
<tr>
<td>IL-11 TG (lung, airway specific)</td>
<td>B cell hyperplasia</td>
</tr>
<tr>
<td>LIF TG (T-cell specific)</td>
<td>CFU-S ↓</td>
</tr>
<tr>
<td>LIF KO</td>
<td>perinatal</td>
</tr>
<tr>
<td>LIFR KO</td>
<td>perinatal</td>
</tr>
<tr>
<td>CNTF TG</td>
<td>motor neurone ↑, gliosis in CNS lesion</td>
</tr>
<tr>
<td>CNTF KO</td>
<td>mild motor neurone deficit</td>
</tr>
<tr>
<td>CNTFRa KO</td>
<td>perinatal</td>
</tr>
<tr>
<td>gp130 (dominant negative) TG</td>
<td>TD response ↓</td>
</tr>
<tr>
<td>gp130 KO</td>
<td>d12.5-perinatal</td>
</tr>
<tr>
<td>gp130 (postnatally) KO</td>
<td>not severe defect</td>
</tr>
<tr>
<td>gp130 (heart-specific) KO</td>
<td>normal heart development</td>
</tr>
<tr>
<td>gp130 (SHP2 signal deficient) KI</td>
<td>TD response ↓ Th1</td>
</tr>
</tbody>
</table>
**Table 2:** Phenotypes of genetically engineered mice *(continued).*

<table>
<thead>
<tr>
<th>Lethality</th>
<th>Antibody response</th>
<th>Th balance</th>
<th>APR</th>
<th>Haematopoiesis</th>
<th>Others</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp130 (STAT3 signal perinatal deficient) KI</td>
<td>TD response ↓</td>
<td>Th2</td>
<td>normal</td>
<td></td>
<td></td>
<td>[65]</td>
</tr>
<tr>
<td>gp130 (all signal deficient) KI</td>
<td>perinatal</td>
<td>TD response ↓</td>
<td>normal</td>
<td></td>
<td></td>
<td>[65]</td>
</tr>
<tr>
<td>Jak1 KO</td>
<td>perinatal</td>
<td>lymphoid ↓</td>
<td>response through gp130 ↓</td>
<td></td>
<td></td>
<td>[197]</td>
</tr>
<tr>
<td>Jak2 KO</td>
<td>d12 -d13</td>
<td>BFU-E, CFU-E ↓</td>
<td></td>
<td></td>
<td></td>
<td>[198,199]</td>
</tr>
<tr>
<td>SHP2 KO</td>
<td>d14 -term</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[200]</td>
</tr>
<tr>
<td>STAT3 KO</td>
<td>before d8.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[201]</td>
</tr>
<tr>
<td>STAT3 (T-cell specific) KO</td>
<td></td>
<td></td>
<td>IL-6 dependent T-cell proliferation ↓</td>
<td></td>
<td></td>
<td>[121]</td>
</tr>
<tr>
<td>STAT3 (macrophage specific) KO</td>
<td></td>
<td>Th1</td>
<td>abnormal activation of macrophages</td>
<td></td>
<td></td>
<td>[161]</td>
</tr>
<tr>
<td>Gab1 KO</td>
<td>d12.5 -d17.5</td>
<td></td>
<td>defect in heart, placenta, and skin</td>
<td></td>
<td></td>
<td>[90]</td>
</tr>
<tr>
<td>SOCS1 KO</td>
<td>perinatal</td>
<td></td>
<td>IFN-γ response ↑</td>
<td></td>
<td></td>
<td>[142,143,202]</td>
</tr>
<tr>
<td>SOCS3 KO</td>
<td>d12 -d16</td>
<td>BFU-E, CFU-E ↓</td>
<td>erythrocyte shift from immature to mature</td>
<td></td>
<td></td>
<td>[144]</td>
</tr>
</tbody>
</table>

Blanks in the ‘lethality’ column mean that adult homozygotes are obtainable. Most other blanks indicate not tested. When no major phenotypes were observed, columns were left as blanks.

APR: Acute-phase response; BFU-E: Erythroid burst forming unit; CFU-E: Erythroid colony forming unit; CFU-GM: Granulocyte-macrophage colony forming unit; CFU-S: Spleen colony forming unit; d: Days post-coincidum; KI: Knock-in; KO: Knock-out; TD: Thymus dependent; TG: Transgenic.
heterodimerisation of gp130 results in the activation of the gp130-associated JAKs (JAK1, JAK2 and Tyk2) [58,59]. Subsequently, gp130 is phosphorylated on tyrosines and the phosphorylated gp130 recruits signal transducing molecules such as SHP2 and STAT3, and activates them to transmit signals downstream (Figure 2) [60-62].

gp130-deficient mice die as embryos and show hypoplastic ventricular myocardium, greatly reduced haematopoietic progenitors in the liver and severe defects in thymocyte development [63]. In contrast, a line of Cre-loxP-mediated heart-specific gp130-deficient mice showed no obvious defect in the heart structure [64], consistent with our recent results showing that the gp130-mediated signal is not required for development of the heart [65]. Furthermore, some gp130-deficient mice come to term, showing that gp130 is not essential for intra-uterine development [66]. gp130 was implicated to mediate myocyte survival pathway that acts to block the onset of myocyte apoptosis during the pressure overload [64]. Transgenic mice expressing a dominant-negative form of gp130 show impaired production of antigen-specific antibodies [67]. Postnatally-induced inactivation of the gp130 gene using the Cre-loxP system confirmed or led to the identification of roles for gp130 in Schwann cell development, antigen-specific antibody production and protection from viral and bacterial infections, in addition to haematopoiesis [68]. These findings are consistent with the results of previous knock-out studies that showed IL-6-family cytokines to be important for the production of antigen-specific antibodies.

Recently, we generated knock-in mouse lines in which the gp130-mediated SHP2 signal and/or STAT3 signal are selectively disrupted [65]. The phenotypes of these knock-in mice, which express mutant gp130 without its transmembrane or cytoplasmic regions and thus are deficient for all of the gp130-mediated signals, were somewhat milder than those of conventional gp130-deficient mice. The knock-in mice died perinatally without any apparent defects in their organs. Their haematopoiesis was normal, as far as could be assessed by in vitro colony assays and peripheral blood profiles. Their stomachs contained no milk, suggesting they could not suck. These phenotypes are rather similar to those of CNTFR-deficient and LIFR-deficient mice [69-71].

3.1 SHP2 signalling

gp130 contains six tyrosines in its cytoplasmic region [12]. Tyr<sup>759</sup> (of human gp130) is required for the tyrosine phosphorylation of SHP2 (Figure 2) [60,61]. SHP2 is thought to be a positive regulator of signals, although SHP1, a close relative of SHP2, is predominantly a negative regulator [72]. SHP2 contains a YXXN motif, which is the consensus sequence for Grb2 binding, in its C-terminal region [73,74]. Consistently, upon stimulation of gp130, SHP2 is tyrosine phosphorylated and interacts with Grb2, which is constitutively associated with Sos, a GDP-GTP exchanger for Ras [60,75,76]. These findings suggest that SHP2 may act as an adapter molecule for transmitting signals to the extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK).

On the other hand, mutating Tyr<sup>759</sup> of gp130 or overexpressing a SHP2 mutant with an inactive catalytic domain enhances the STAT3-mediated biological actions in hepatocytes and neuroblastoma cells [77,78], suggesting a role for SHP2 in attenuating gp130-mediated signals. Protein interaction through the SH2 domain of SHP2 enhances its phosphatase activity [79]. The expression of an inactive phosphatase mutant of SHP2 suppresses endothelial growth factor (EGF), fibroblast growth factor (FGF) and insulin-dependent MAPK activation [80-82]. However, the phosphoprotein substrate for SHP2 in gp130 signalling is still unclear. Gab family proteins are good candidates. Gab1 was originally isolated as a binding protein for Grb2 [83]. DOS (daughter of sevenless), which is a Drosophila homologue of Gab1, is a substrate for the Drosophila SHP2 homologue, Corkscrew (CSW). DOS was shown to act downstream of the receptor tyrosine kinase Sevenless and upstream of, or in parallel with, the Ras pathway (Figure 3) [84,85]. Gab family proteins are tyrosine phosphorylated and interact with SHP2 and phosphatidylinositol 3'-kinase (PI-3K) in response to various kinds of stimulation, including gp130 stimulation [86-88] (for review, see Hibi et al. [89]). A mutation of Tyr<sup>759</sup> in gp130 reduces the interactions between SHP2 and Grb2, and SHP2 and Gab1, and diminishes the activation of ERK MAPK [60,87,88], suggesting that SHP2 mediates signals to the ERK MAPKs through Grb2 and the Gab proteins (Figure 2). Gab1-deficient mice show defects in the placenta, epidermis and heart, indicating a positive signalling role of Gab1 in response to various stimuli in vivo [90]. In fact, ERK MAPK activation through gp130, EGF receptor (EGFR) and c-Met is reduced in embryonic fibroblasts.
from Gab1-deficient mice [90]. Taken together, these results indicate that SHP2 acts as a linker to the MAPK pathway and serves as a negative regulator of signals.

The SHP2-mediated signal is obligatory in the proliferation of the mouse pro B cell line BAF-B03 [60,91]. In these cells, the SHP2-mediated signal has been shown to act in concert with the STAT3-mediated signal to support growth [60,91]. Specifically, the SHP2 signal is responsible for the S to G2/M cell cycle transition, and the STAT3 signal is required for the G1-S cell cycle transition and inhibition of apoptosis [60,91]. The SHP2-mediated signal also plays a major role in the neurite outgrowth in PC12 cells [92]. Although knock-in mice that are deficient for the SHP2-mediated signal are apparently normal, they display splenomegaly and lymphadenopathy [65]. These problems are first recognisable in most mice at 11 weeks of age, and the size of their spleen and lymph nodes increases with age. Immunohistological analysis showed that the structures of the lymphoid organs are intact in these mice. These results indicate that gp130 signalling plays a role in maintaining the homeostasis of lymphoid organs and that the SHP2 may play a negative role in regulating gp130 signalling. These knock-in mice also show a variety of immunological disorders. We will discuss these phenotypes and the negative role of the SHP2 signal in the next section.

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3.2 STAT3 signalling

Any one of four tyrosines in the carboxyl-terminus (Y767, Y814, Y905 and Y915) of gp130, all of which have a glutamine at position +3 of the tyrosine motif (YXXQ), are required for the tyrosine phosphorylation of STAT3 ([Figure 2](#)). Y905 and Y915 in the YXPQ motif are required for the tyrosine phosphorylation of STAT1 [93]. After tyrosine phosphorylation, STAT3 forms a homo- or heterodimer with STAT1, enters the nucleus and regulates the expression of a set of genes ([Figure 2](#)) [94-97]. Y905 and Y915 have been shown to be involved in STAT1 activation [93,98].

Although all four tyrosines have been thought to be equivalent in activating STAT3, recent reports by two groups suggest that this is not always the case [99,100]. Both groups showed that the two distal tyrosines are more potent than the proximal ones. The reason for this difference is not yet clear. However, point mutations of individual tyrosines in full-length gp130 may lead to different biological responses, compared...
with deletion of the entire C-terminal region. Unknown signals may originate from the membrane-distal region. For example, a di-leucine motif, which has been shown to mediate receptor internalisation and is thought to play a role in attenuating gp130-mediated signals [101], is located in the membrane-distal region.

STAT3 signalling plays a central role in several biological functions of the IL-6 family cytokines [13,32]. For example, the STAT3-mediated signal plays a central role in the IL-6 family-induced differentiation of the mouse leukaemia cell line, M1 [62,102]. The STAT3 signal is also central to the differentiation of neural stem cells into glial fibrillary acidic protein (GFAP)-positive astrocytes [103,104]. Furthermore, the STAT3-mediated signal maintains the pluripotency of embryonic stem (ES) cells [99]. STAT3 has been shown to be important in cell proliferation. In many human cancers and transformed cell lines, STAT3 is persistently activated and is required for cellular transformation. The involvement of STAT proteins in cell growth is supported by the observation that STATs are constitutively activated in cells transformed with human T-cell leukaemia virus I (HTLV-I) [105], v-src [106,107], abl [108,109], bcr-abl [110], eyk [111] and active Goo [112], and in some multiple myeloma cells [113]. STAT3 activation is required for the transformation of NIH3T3 cells with v-src [107,114] and is also indicated in v-abl-induced plasmacytomasogenes [109]. A constitutively active mutant of STAT3 in immortalised fibroblasts causes cellular transformation, defined as colony formation in soft agar and tumour formation in nude mice, thus acting as an oncogene [115]. Introduction of a dominant-negative form of STAT3 not only suppresses the biological functions of gp130 [102], but also inhibits the cell proliferation of a melanoma cell line [116]. It is not clear whether the activation of STAT3 by gp130 is involved in the process of tumour formation. However, antagonising IL-6 suppressed the growth of prostate carcinoma cells [117]. IL-6 supports the growth of prostate carcinoma cells [117-120].

STAT3 might be a common target for different therapies. The inactivation of the STAT3 gene in T-cells revealed that STAT3 is required for IL-6-mediated anti-apoptosis, independent of bcl-2 [121]. The autonomous proliferation of cells is also associated with STAT3 activation. B-1 cells, a particular subset of peritoneal B-cells with surface CD5 expression, are known to have self-renewal potential. STAT3 is constitutively activated in B-1 cells and is associated with their proliferation [122]. The proto-oncogenes Pim-1 and Pim-2 have been identified as targets for the gp130-mediated STAT3 signal [123]. Although the activation of STAT3 is required for the expression of c-myc [124], c-Myc alone is not sufficient to compensate for the loss of STAT3 in the progression of the cell cycle. In contrast, the constitutive expression of c-Myc and Pim-1 fully compensate for the loss of the gp130-mediated STAT3 signal in cell cycle progression, as well as in cell survival. Pim-1 induces bcl-2 expression, possibly through VCP (valosine containing protein), an AAA-superfamily ATPase, and inhibits c-Myc-mediated apoptosis. Thus, STAT3 mediates the signals of gp130 to regulate the expression of genes that are required for gp130-mediated cell proliferation. STAT3 orchestrates the signalling molecules to control the final output of the signals to the cells.

Knock-in mice that are deficient for STAT3 signalling die perinatally without any apparent organ defects, as do the gp130 signal-deficient knock-in mice [65]. Inability of sucking may be due to some neural deficit, as suggested by the observation that the gp130-mediated STAT3 signal plays a central role in the emergence of GFAP-positive astrocytes in vivo and in vitro [65,104,125].

To explore the roles of gp130-mediated signals in the differentiation of haematopoietic cells and the immune response, foetal liver cells from E14.5 knock-in mice were transplanted into lethally irradiated adult B6C3F1 mice [65]. The STAT3 signal-deficient immune systems were impaired in antigen-specific antibody production, specifically of the IgG2a and IgG2b isotypes, further confirming the central role of STAT3 signalling in antibody production. In contrast, the immune systems of knock-in mice that are specifically deficient in the SHP2-mediated signal exhibited augmented antibody responses of the same immunoglobulin isotypes. Furthermore, the SHP2 signal-deficient knock-in mice showed an enhanced acute-phase response, for which the STAT3 signal is essential. They showed enhanced Th1 type cytokine production by T-cells, which was diminished in the STAT3 signal-deficient immune systems. The immune system of the SHP2 signal-deficient mice also showed enhanced immunoglobulin production.

Taken together, the balance of positive and negative signals, generated through gp130 and depending on its tyrosine residues, regulates a wide variety of
biological responses in vivo. These results are consistent with the observation that SHP2-deficient embryonic fibroblasts showed sustained phosphorylation of STAT3, reflecting the negative regulatory role of SHP2 on STAT3 signals. On the other hand, a gp130 mutant that is defective in SHP2 signalling fails to activate ERK MAPK, confirming the positive role for SHP2 in activating this kinase. The aberrant activation of STAT3 in vivo caused by the uncoupling of SHP2 from gp130 may result in several diseases, such as cancers and autoimmune diseases.

3.3 Negative regulation of signals

Just as SHP2 can act as a negative regulator of gp130 signals both in vivo and in vitro [65,77,78], other molecules can also participate in this negative regulation. PIAS3 (protein inhibitor of activated STAT3) directly interacts with phosphorylated STAT3 and reduces its DNA-binding activity, thus inhibiting the transcription of its target genes (Figure 2) [126]. PIAS1 was also cloned and shown to inhibit STAT1 [127]. However, the molecular characteristics of PIAS proteins are largely unknown.

STAT3 is required for the induction of SOCS1 and SOCS3 expression in gp130 signalling (Figure 2) [128,129]. Members of the SOCS (suppressor of cytokine signalling) family, also referred to as JAK binding (JAB) and as STAT-induced STAT inhibitor (SSI), are characterised by a conserved SOCS box and an SH2 domain [129-131]. CIS is the first identified SOCS family member that acts as an immediate early response gene in EPO signalling [132]. SOCS proteins inhibit the cytokine signal transduction by one or both of the following mechanisms: they suppress the kinase activity of JAKs by masking the activation loop of JAKs with their own SH2 domain [133]; or they may inhibit the entire signalling pathway through direct interactions with the receptors [132]. Recently, it was reported that Tyr ^759 of gp130 also acts as a docking site for SOCS3 [134,135]. SOCS3 may act as a negative regulator by interacting with gp130 directly, whereas SOCS1 inhibits the signalling by interacting with JAKs. As discussed above, mutating Tyr ^759 of gp130 resulted in enhancement of gp130-mediated signalling both in vivo and in vitro, suggesting that, in addition to SHP2, SOCS3 is involved in the Tyr ^759-dependent negative regulation of gp130 signalling. This issue remains to be resolved.

MAPK may also be a negative regulator of part of the JAK/STAT signalling pathway (Figure 2). Sengupta et al. reported that expression of constitutively active MEK1, the kinase that activates ERKs, or overexpression of ERK2 but not [NK1, inhibits Stat3 activation [136]. MAPK kinases (MEKs) and ERKs also inhibit JAK1 and JAK2 [136]. Jain et al. reported that STAT3 activity is negatively regulated by the direct binding of ERK2 to STAT3 [137]. These reports suggest that ERK MAPKs negatively regulate STAT3 activity. STAT3β, an alternatively spliced form of STAT3, has been shown to act in a dominant-negative fashion. This isoform lacks a C-terminal domain, which is necessary for activation. Interestingly, the half-life of STAT3β is about 50% of that of STAT3α [138].

Induction of these negative regulatory proteins are not limited to gp130 signalling. Growth hormone induces SOCS3 [139] and IL-4 induces SOCS1 [140,141]. SOCS1-deficient mice die perinatally because of excessive IFN-γ responses [142,143]. SOCS3-deficient mice die embryonically, possibly because of excessive foetal erythropoiesis [144]. Furthermore, it is well known that SHP2 and MAPK respond to various cytokines and growth factors. Because of their broad range of negative regulatory roles, induction of these proteins will provoke several side effects. However, controlling the expression and activity of these molecules at specific place and time is still a potentially useful therapeutic avenue.

4. The therapies

Despite the great increase of knowledge about the role of IL-6 family cytokines in the pathologies of diseases, not so many clinical trials have been performed. Ideas are roughly classified into two groups. One is blocking the pathological role of IL-6 family cytokines by neutralising proteins. The other is the use of beneficial effects of IL-6 family cytokines.

4.1 Neutralising protein-based approaches

The contact site between IL-6, IL-6Rα and gp130 has been mapped [55,145], and several approaches have been taken to interfere with the formation of the receptor complex to prevent the pathogenic activity of IL-6 family cytokines (also see Kallen et al. and Bravo et al. [146,147]). These approaches include the design of gp130 antagonists based on the mutational studies and the development of monoclonal antibody targeting IL-6 and IL-6Rα. Among various trials, we would like to introduce ongoing clinical trials with promising results.
Anti-IL-6 antibodies have been given to patients with RA, multiple myeloma and Castleman’s disease [148-150]. This strategy led to great improvement in the patients’ clinical status for several weeks. However, the improvement was transient, because the high stability of the complex of IL-6 and the antibodies in plasma increased the circulating levels of endogenous IL-6. Furthermore, the patients generated antibodies against the murine anti-IL-6 antibodies. To overcome these problems, two strategies have been taken. One is the use of a cocktail of three different anti-IL-6 antibodies [151]. This strategy enabled the rapid clearance of serum IL-6, thus decreased the circulating levels of IL-6.

The second strategy has been the development of a chimeric anti-IL-6 antibody consisting of the antigen-binding variable region of the murine anti-IL-6 antibody and the constant region of a human IgG1κ immunoglobulin [152]. Phase I/II trials of this antibody were carried out in patients with multiple myeloma and no human antibodies to the chimeric antibody were induced [152-154]. Endogenous IL-6 production never reached its pre-treatment value during the treatment period and C-reactive protein (CRP) levels decreased to below detection level in almost every patient [154]. Thus, this therapy greatly improved the patients’ disease status with low toxicity, low immunogenicity and a long half-life [152]. A similar approach was taken with a humanised anti-human IL-6Rα antibody. This therapy improved the conditions of patients with Castleman’s disease and certain autoimmune diseases [155-157]. This therapy was effective for 11 months, indicating that the induction of human antimouse antibodies was prevented. Continuous administration of humanised anti-IL-6Rα antibody increased the serum concentration of sIL-6Rα as observed in the anti-IL-6 therapy. However, the maximum sIL-6Rα concentration decreased gradually after 2 months. Therefore, these therapies using chimeric antibodies could be useful tools in treating diseases that are caused by the deregulation of IL-6.

Another approach to interfering with the formation of the ternary complex is the development of antagonists directly targeting gp130 [158]. Renne et al. constructed fusion proteins that consist of the soluble form of human IL-6Rα covalently linked to an IL-6 carrying mutations in the amino acid residues that are responsible for protein interactions. These fusion proteins directly bind gp130 without inducing dimerisation, thereby acting as effective antagonists. These proteins effectively blocked the biological functions not only of IL-6, but also of other IL-6 family cytokines. Although further in vivo experiments need to be carried out, these fusion proteins have therapeutic potential for treating diseases that are related to IL-6 family cytokines.

4.2 Cytokine therapy

Administrations of recombinant cytokine have made great success in the use of granulocyte colony-stimulating factor (G-CSF) and erythropoietin. However, probably because of its inflammatory function, administrations of IL-6 family cytokines frequently resulted in disappointing results.

IL-11 is a unique cytokine that has both inflammatory and anti-inflammatory effects. Clinical trials of rhIL-11 have been performed on patients with psoriasis and CD [159,160]. Subcutaneous injection of rhIL-11 to the patients with psoriasis ameliorated the disease state, as shown by reduced keratinocyte proliferation, cutaneous inflammation, number of infiltrated T-lymphocytes and expression of disease-related genes. IL-11 modulates the functions of macrophages and Th1 cells in cell culture and shows anti-inflammatory activity in animal models. STAT3 activation by IL-11 may be important for attenuating the activation of macrophages or Th1 cells. This result is consistent with the observation that macrophage-specific ablation of the STAT3 gene resulted in the abnormal activation of macrophages [161]. Clinical remission and increase in platelet counts were observed among patients with CD who received rhIL-11 subcutaneously. IL-6 family cytokines were shown to have thrombocytopoietic activity and growth promoting effect on haematopoietic progenitor cells when stimulated in combination with other colony-stimulating factors (e.g., IL-3, M-CSF, SCF).

CNTF is a neuroactive cytokine found in Schwann cells that appears to be released in response to injury. Amyotrophic lateral sclerosis (ALS) is a human neurodegenerative disease, primarily of motor neurons. CNTF has been implicated in the pathogenesis of ALS; thus, a large-scale clinical trial was performed to evaluate the efficacy of CNTF. However, there were no statistically significant treatment effects. Furthermore, side effects, including anorexia, weight loss and coughing, were sufficient to limit dosing in many patients [162]. Enthusiasm for developing CNTF as a drug has diminished. However,
some researchers regard these problems as having been derived from the route of systemic delivery (sc. treatment was adopted in the previous clinical trials) and are investigating a therapeutic role for this cytokine using it. delivery of CNTF [163, 164].

4.3 Development of artificial cytokine based on the ‘receptor conversion model’

The soluble form of the cytokine receptor often functions as a competitive inhibitor for the ligand. However, a sIL-6Rα, when complexed with IL-6, can activate signals in the cells expressing only gp130 receptor subunit, on which IL-6 alone cannot act [11]. Another example is a cytokine, IL-12. It consists of a disulphide heterodimer of a 35 kD (p35) subunit, which is a cytokine, and a 40 kD (p40) subunit, a soluble form of the cytokine receptor [165]. Therefore, IL-12, generally accepted as a kind of cytokine, is actually a complex of a cytokine and a soluble form of its receptor. The complex of soluble CNTFRα and CNTF acts on cells expressing LIFR and gp130 [166]. The complex of IL-11 and the sIL-11R acts through gp130 [167, 168].

Based on these facts, a novel mechanism by which the cytokine system generates functional diversity was proposed [169, 170]. This is called the ‘Receptor Conversion Model’. A complex consisting of a soluble cytokine receptor and its corresponding cytokine ligand acquires different target specificity from the original cytokine, leading to the expression of distinct functions from those of the original cytokine. Actually, double transgenic mice expressing human IL-6 and IL-6Rα showed myocardial hypertrophy [171], extraordinary expansion of haematopoietic progenitor cells [35], and nodular regenerative hyperplasia and adenomas of the liver [36], indicating that the complex of IL-6 and sIL-6Rα acts on heart muscle cells and haematopoietic stem cells that express gp130 but not IL-6Rα, on which IL-6 alone cannot act. Thus, by forming a complex, IL-6 apparently acquires novel biological activities. Thus, the ‘Receptor Conversion Model’ may be applied to a wide range of other receptor systems.

This mechanism contributes to generating the functional diversity of cytokines and probably growth factors. Furthermore, novel drugs could be designed based on this model. A bioactive designer cytokine composed of sIL-6Rα and IL-6 linked to each other by a flexible peptide chain was developed by Rose-John and his colleagues, and it was found to act on the cells expressing gp130 in the absence of IL-6Rα and would be useful to expand haematopoietic stem cells [172, 173].

5. Concluding remarks and Expert Opinion

As NNT-1/BSF-3 was recently identified, there is still a possibility that there are more unidentified IL-6 family cytokines. Consistently, the fact that the phenotypes of CNTFR-deficient mice were more severe than that of CNTF-deficient mice, implicated the existence of an unknown CNTF-like ligand [70]. However, the use of chimeric antibodies in treating diseases associated with deregulated IL-6 family cytokine production has resulted in promising therapies, although further confirmation of their safety is needed. Therapeutic approaches intended to interfere with the formation of the IL-6, IL-6Rα, gp130 complex will shed further light on possible treatments for these diseases.

Signalling mechanisms of gp130 have been extensively studied and the central role of STAT3 in pathogenesis has been emerging. Controlling STAT3 activation will be a major concern in future therapies. Recently, parthenolide, a sesquiterpene lactone found in many medical plants, was shown to inhibit the STAT3 signal stimulated by IL-6 family cytokines [174]. Parthenolide inhibits STAT3 phosphorylation on Tyr705, although it also inhibits IL-1-induced NF-κB activation [175]. The investigation of STAT3-inhibiting drugs, induction of negative regulatory molecules and chimeric antibodies will be continued in the attempt to identify safe and effective treatments for a broad range of diseases.

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