



<http://www.ashley-pub.com>

## Review

1. Introduction
  2. Biological functions of IL-6 family cytokines
  3. gp130: structure and signalling
    - 3.1 SHP2 signalling
    - 3.2 STAT3 signalling
    - 3.3 Negative regulation of signals
  4. The therapies
    - 4.1 Neutralising protein-based approaches
    - 4.2 Cytokine therapy
    - 4.3 Development of artificial cytokine based on the 'receptor conversion model'
  5. Concluding remarks and Expert Opinion
- Bibliography

Anti-inflammatory

# gp130-mediated signalling as a therapeutic target

Takuya Ohtani, Katsuhiko Ishihara, Toru Atsumi, Yuichi Yoshida, Keigo Nishida, Masahiro Narimatsu, Takahiro Shirogane, Masahiko Hibi & Toshio Hirano

*Division of Molecular Oncology (C7), Biomedical Research Center, Osaka University Graduate School of Medicine 2-2, Yamada-oka Suita, Osaka 565-0871, Japan*

IL-6 is a pleiotropic cytokine that regulates haematopoiesis, inflammation and the immune response. The IL-6 receptor consists of an  $\alpha$  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-6R $\alpha$ /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*

*Emerging Therapeutic Targets (2000) 4(4):*

## 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 IFN $\beta$ 2 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  $\alpha$  chain (IL-6R $\alpha$ ) 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

## 2 gp130-mediated signalling as a therapeutic target

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

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

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, cardiotrophin-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.

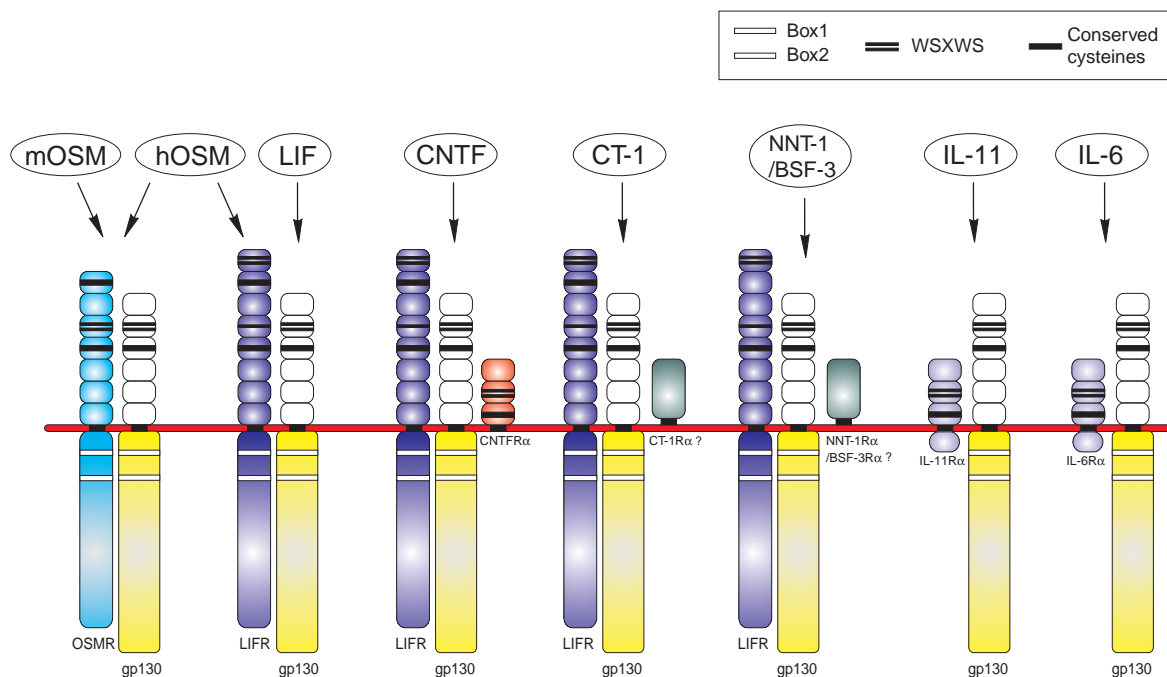
### 2. Biological functions of IL-6 family cytokines

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

**Figure 1:** gp130 is a common signal transducer for IL-6 family cytokines.

Receptor complexes of IL-6 family cytokines consist of a specific  $\alpha$  chain (IL-6R $\alpha$ , IL-11R $\alpha$ , CNTFR $\alpha$ ) and  $\beta$  chain (gp130, LIFR, OSMR), the  $\beta$  chain being the mediator of signalling. Individual domains of the extracellular regions of receptors are presented as globular drawings. CT-1R $\alpha$  and NNT-1/BSF-3R $\alpha$  have not yet been identified.

BSF: B-cell stimulating factor; CNTF: Ciliary neurotrophic factor; CT: Cardiotrophin; h: Human; IL: Interleukin; LIF: Leukaemia inhibitory factor; m: Mouse; NNT: Neurotrophin; OSM: Oncostatin M; R: Receptor.



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-6R $\alpha$  (soluble form of IL-6R $\alpha$ , 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-6R $\alpha$  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-6R $\alpha$ , LIF or both IL-6 and sIL-6R $\alpha$  have provided important evidence regarding the

## 4 gp130-mediated signalling as a therapeutic target

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 transplantable 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-6R $\alpha$  throughout the body show progressive extramedullary haematopoiesis and liver-specific IL-6-sIL-6R $\alpha$  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  $\alpha$  chain and gp130 (**Figure 1**). Both the IL-6R $\alpha$  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-6R $\alpha$  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-6R $\alpha$  chain, also belong to the Type I cytokine receptor superfamily, with the exception of CNTFR $\alpha$ , 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 (I<sup>651</sup>WPNVDP of human sequence) and box2 (V<sup>691</sup>SVVEIEANDKKP), 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-6R $\alpha$ , IL-11R $\alpha$ , CNTFR $\alpha$  and CT-1R $\alpha$ . 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-6R $\alpha$ , IL-11R $\alpha$  and CNTFR $\alpha$  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.

	Phenotypes					Ref.
	Lethality	Antibody response	Th balance	APR	Haematopoiesis	
IL-6 KO	TD response ↓ Mucosal IgA response ↓	Th1 response ↓	↓	BFU-E ↑ CFU-S ↓	bone loss by oestrogen depletion ↓ hepatocyte regeneration ↓	[37,38,40-42, 185-188]
IL-6 TG	plasmacytosis, plasmacytoma		↑			[30,31,189]
sIL-6Ra TG						[190]
IL-6/sIL-6Ra double TG	plasmacytoma			extramedullary	hepatocellular hyperplasia	[35,191]
IL-11 TG (lung, airway specific)					inflammation	[192,193]
LIF TG (T-cell specific)	B cell hyperplasia				defect in thymic epithelium	[34]
LIF KO				CFU-S ↓	sterile female	[50,194]
LIFR KO	perinatal			normal	defect in placenta, bone, and liver	[69,71]
CNTF TG					motor neurone deficit	
CNTF KO					motor neurone ↑, gliosis in CNS lesion	[195,196]
CNTFRa KO	perinatal				mild motor neurone deficit	[51]
gp130 (dominant negative) TG	TD response ↓			normal	severe motor neurone deficit	[70]
gp130 KO	d12.5-perinatal			BFU-E, CFU-GM ↓	heart abnormality	[63,66]
gp130 (postnatally) KO	not severe defect		↓	thrombopoiesis ↓	defect in lung, heart, liver and nerves	[68]
gp130 (heart-specific) KO					normal heart development	[64]
gp130 (SHP2 signal deficient) KI	TD response ↓	Th1	↑	normal		[65]

**Table 2:** Phenotypes of genetically engineered mice (*continued*).

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

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-coincidence; 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

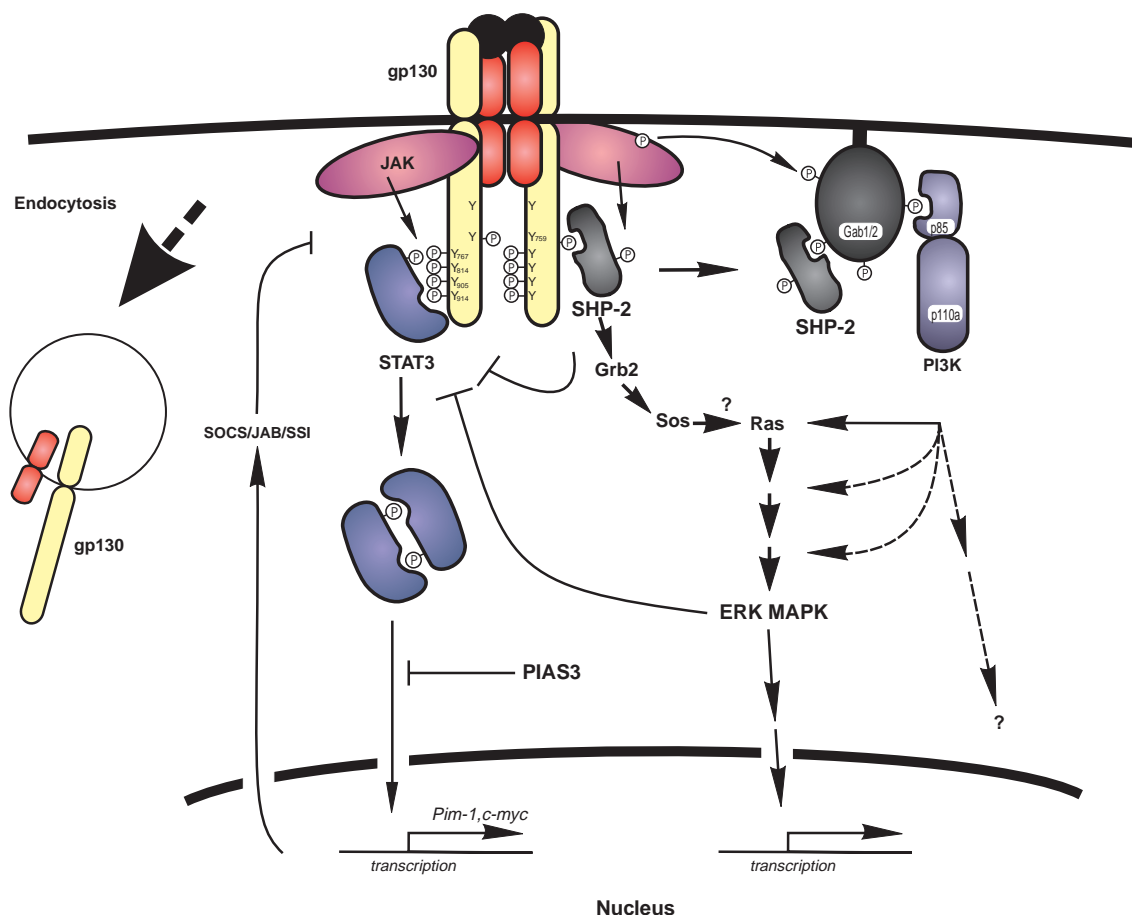
gp130 contains six tyrosines in its cytoplasmic region [12]. Tyr<sup>759</sup> (of human gp130) is required for the tyrosine phosphorylation of SHP2 (**Figure 2**) [60,61]. SHP2 is thought to be a positive regulator of signals, although SHP1, a close relative of SHP2, is predominantly a negative regulator [72]. SHP2 contains a YXNX 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

## 8 gp130-mediated signalling as a therapeutic target

**Figure 2:** Schematic representation of gp130 signalling. Distinct tyrosine residues of gp130 are involved in different signal transduction pathways. Y759 elicits SHP2-mediated pathways, whereas each one of Y767, Y814, Y905 and Y914 can elicit STAT3-mediated pathways. SHP2, MAPK, SOCS and PIAS proteins, as well as a classical receptor internalisation, were shown to be involved in negative regulation of signals.

ERK: Extracellular signal-regulated kinase; **Gab**: ; **Grb**: ; JAB: JAK binding; JAK: Janus kinase; MAPK: Mitogen-activated protein kinase; PI3K: Phosphatidylinositol 3'-kinase; **PIAS3**: ; **Ras**: **SHP**: ; SOCO: Suppressor of cytokine signalling; Sos: Son of sevenless; SSI: STAT-induced STAT inhibitor; STAT3: Signal transducer and activator of transcription.



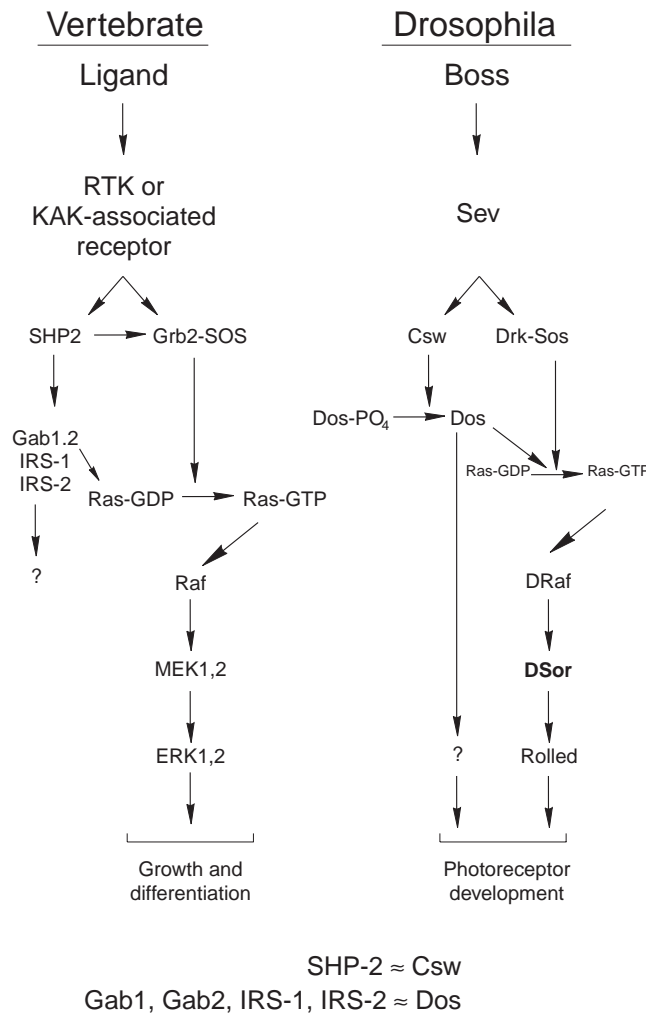
from Gab1-deficient mice [90]. Taken together, these results indicate that SHP2 acts as a linker to the MAPK pathway and serves as a negative regulator of signals.

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.

**Figure 3:** Conserved roles for SHP2, Gab proteins and DOS. Signal transduction cascades are conserved between insects and vertebrates. Receptor tyrosine kinase sevenless (Sev) plays critical roles in *Drosophila* photoreceptor development. Sev signals downstream CSW and/or Drk-SOS in response to sevenless (Boss).

Csw: Corkscrew; Dos: Daughter of sevenless; ERK: Extracellular signal-regulated kinase; **Gab**: ; **GDP**: **GTP**: **Grb**: IRS: Insulin receptor substrate; **KAK**: ; MAPK: Mitogen-activated protein kinase; MEK: MAPK kinase; **Raf**: ; **Ras**: ; RTK: Receptor tyrosine kinase; SHP: Sev: Sevenless; SOS: Son of sevenless.



### 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 G $\alpha$  [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 plasmacytomagenesis [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<sup>759</sup> 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<sup>759</sup> 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<sup>759</sup>-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 JNK1, 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. STAT3 $\beta$ , 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 STAT3 $\beta$  is about 50% of that of STAT3 $\alpha$  [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- $\gamma$  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 pathology 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-6R $\alpha$  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-6R $\alpha$ . 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 IgG1 $\kappa$  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 antihuman IL-6R $\alpha$  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-6R $\alpha$  antibody increased the serum concentration of sIL-6R $\alpha$ , as observed in the anti-IL-6 therapy. However, the maximum sIL-6R $\alpha$  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-6R $\alpha$  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-lymphocyte 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 thrombopoietic 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 neurones. 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-6R $\alpha$ , 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 CNTFR $\alpha$  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-6R $\alpha$  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-6R $\alpha$  acts on heart muscle cells and haematopoietic stem cells that express gp130 but not IL-6R $\alpha$ , 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-6R $\alpha$  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-6R $\alpha$  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-6R $\alpha$ , 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 Tyr<sup>705</sup>, although it also inhibits IL-1-induced NF- $\kappa$ B 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.

#### Bibliography

Papers of special note have been highlighted as:

- of interest
  - of considerable interest
1. HIRANO T, YASUKAWA K, HARADA H *et al.*: **Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin.** *Nature* (1986) **324**(6092):73-76.
    - cDNA cloning of IL-6.
  2. HIRANO T: **Interleukin 6 and its receptor: ten years later.** *Int. Rev. Immunol.* (1998) **16**(3-4):249-284.
    - A review describing the biological functions of IL-6.
  3. TERANISHI T, HIRANO T, ARIMA N, ONOUE K: **Human helper T cell factor(s) (ThF). II. Induction of IgG production in B lymphoblastoid cell lines and identification of T cell replacing factor (TRF)-like factor(s).** *J. Immunol.* (1982) **128**:1903-1908.

4. MAY LT, HELFGOTT DC, SEHGAL PB: **Anti- $\beta$ -interferon antibodies inhibit the increased expression of HLA-B7 mRNA in tumor necrosis factor-treated human fibroblasts: Structural studies of the  $\beta_2$  interferon involved.** *Proc. Natl. Acad. Sci. USA* (1986) **83**:8957-8961.
5. ZILBERSTEIN A, RUGGIERI R, KORN JH, REVEL M: **Structure and expression of cDNA and genes for human interferon- $\beta$ -2, a distinct species inducible by growth-stimulatory cytokines.** *EMBO J.* (1986) **5**(10):2529-2537.
6. HAEGEMAN G, CONTENT J, VOLCKAERT G *et al.*: **Structural analysis of the sequence encoding for an inducible 26-kDa protein in human fibroblasts.** *Eur. J. Biochem.* (1986) **159**:625-632.
7. VAN SNICK J: **Interleukin-6: an overview.** *Ann. Rev. Immunol.* (1990) **8**:253-278.
8. SEHGAL PB, GRIENGER G, TOSATO G: **Regulation of the acute phase and immune responses: interleukin-6.** *Ann. NY Acad. Sci.* (1989) 1-583.
9. HIRANO T: **Interleukin 6.** In: *The Cytokine Handbook (3rd Edition)*. Thomson AW (Ed.), Academic Press (1998):197-228.
10. YAMASAKI K, TAGA T, HIRATA Y *et al.*: **Cloning and expression of the human interleukin-6 (BSF-2/IFN  $\beta$  2) receptor.** *Science* (1988) **241**(4867):825-828.
11. TAGA T, HIBI M, HIRATA Y *et al.*: **Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130.** *Cell* (1989) **58**(3):573-581.
12. HIBI M, MURAKAMI M, SAITO M *et al.*: **Molecular cloning and expression of an IL-6 signal transducer, gp130.** *Cell* (1990) **63**(6):1149-1157.
- cDNA cloning of gp130.
13. HIRANO T, NAKAJIMA K, HIBI M: **Signaling mechanisms through gp130: a model of the cytokine system.** *Cytokine Growth Factor Rev.* (1997) **8**(4):241-252.
- A review focusing on the signalling mechanisms through gp130.
14. SENALDI G, VARNUM BC, SARMIENTO U *et al.*: **Novel neurotrophin-1/B cell-stimulating factor-3: a cytokine of the IL- 6 family.** *Proc. Natl. Acad. Sci. USA* (1999) **96**(20):11458-11463.
15. HEINRICH PC, BEHRMANN I, MULLER-NEWEN G, SCHAPER F, GRAEVE L: **Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway.** *Biochem. J.* (1998) **334**(Pt 2):297-314.
- A comprehensive overview of gp130 signalling.
16. HIRANO T, TAGA T, YASUKAWA K *et al.*: **Human B cell differentiation factor defined by an anti-peptide antibody and its possible role in autoantibody production.** *Proc. Natl. Acad. Sci. USA* (1987) **84**:228-231.
17. HIRANO T, MATSUDA T, TURNER M *et al.*: **Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis.** *Eur. J. Immunol.* (1988) **18**(11):1797-1801.
18. WARING PM, CARROLL GJ, KANDIAH DA, BUIRSKI G, METCALF D: **Increased levels of leukemia inhibitory factor in synovial fluid from patients with rheumatoid arthritis and other inflammatory arthritides.** *Arthritis Rheum.* (1993) **36**(7):911-915.
19. OKAMOTO H, YAMAMURA M, MORITA Y *et al.*: **The synovial expression and serum levels of interleukin-6, interleukin- 11, leukemia inhibitory factor and oncostatin M in rheumatoid arthritis.** *Arthritis Rheum.* (1997) **40**(6):1096-1105.
20. KEUL R, HEINRICH PC, MULLER-NEWEN G, MULLER K, WOO P: **A possible role for soluble IL-6 receptor in the pathogenesis of systemic onset juvenile chronic arthritis.** *Cytokine* (1998) **10**(9):729-734.
21. KAWANO M, HIRANO T, MATSUDA T *et al.*: **Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas.** *Nature* (1988) **332**(6159):83-85.
22. KLEIN B, ZHANG XG, LU ZY, BATAILLE R: **Interleukin-6 in human multiple myeloma.** *Blood* (1995) **85**(4):863-872.
23. WIERZBOWSKA A, URBANSKA-RYS H, ROBAK T: **Circulating IL-6-type cytokines and sIL-6R in patients with multiple myeloma.** *Br. J. Haematol.* (1999) **105**(2):412-419.
24. HORII Y, MURAGUCHI A, IWANO M *et al.*: **Involvement of IL-6 in mesangial proliferative glomerulonephritis.** *J. Immunol.* (1989) **143**(12):3949-3955.
25. YOSHIZAKI K, MATSUDA T, NISHIMOTO N *et al.*: **Pathogenic significance of interleukin-6 (IL-6/BSF-2) in Castleman's disease.** *Blood* (1989) **74**(4):1360-1367.
26. JILKA RL, HANGOC G, GIRASOLE G *et al.*: **Manolagas SC Increased osteoclast development after estrogen loss: mediation by interleukin-6.** *Science* (1992) **257**:88-91.
27. ATREYA R, MUDTER J, FINOTTO S *et al.*: **Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis *in vivo*.** *Nature Med.* (2000) **6**(5):583-588.
28. MINSHALL E, CHAKIR J, LAVIOLETTE M *et al.*: **IL-11 expression is increased in severe asthma: association with epithelial cells and eosinophils.** *J. Allergy Clin. Immunol.* (2000) **105**(2 Pt 1):232-238.
29. JAFFE HW, PELLETT PE: **Human herpesvirus 8 and Kaposi's sarcoma - some answers, more questions.** *N. Engl. J. Med.* (1999) **340**(24):1912-1913.
30. SUEMATSU S, MATSUDA T, AOZASA K *et al.*: **IgG1 plasmacytosis in interleukin 6 transgenic mice.** *Proc. Natl. Acad. Sci. USA* (1989) **86**(19):7547-7551.
- IL-6 transgenic mice develop plasmacytosis.
31. SUEMATSU S, MATSUSAKA T, MATSUDA T *et al.*: **Generation of plasmacytomas with the chromosomal translocation t(12;15) in interleukin 6 transgenic mice.** *Proc. Natl. Acad. Sci. USA* (1992) **89**(1):232-235.
- IL-6 transgenic mice develop plasmacytoma depending on their genetic background.
32. HIRANO T, ISHIHARA K, HIBI M: **Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors.** *Oncogene* (2000) **19**:2548-2556.

33. HIRANO T: **Interleukin 6 (IL-6) and its receptor: their role in plasma cell neoplasias.** *Int. J. Cell Cloning* (1991) **9**(3):166-184.
34. SHEN MM, SKODA RC, CARDIFF RD *et al.*: **Expression of LIF in transgenic mice results in altered thymic epithelium and apparent interconversion of thymic and lymph node morphologies.** *EMBO J.* (1994) **13**(6):1375-1385.
35. PETERS M, SCHIRMACHER P, GOLDSCHMITT J *et al.*: **Extramedullary expansion of hematopoietic progenitor cells in interleukin (IL)-6-sIL-6R double transgenic mice.** *J. Exp. Med.* (1997) **185**:755-766.
- IL-6 and sIL-6R double transgenic mice develop extramedullary haematopoiesis.
36. MAIONE D, DI CARLO E, LI W *et al.*: **Coexpression of IL-6 and soluble IL-6R causes nodular regenerative hyperplasia and adenomas of the liver.** *EMBO J.* (1998) **17**(19):5588-5597.
- Hepatocellular transformation in IL-6 and sIL-6R double transgenic mice.
37. KOPF M, BAUMANN H, FREER G *et al.*: **Impaired immune and acute-phase responses in interleukin-6-deficient mice.** *Nature* (1994) **368**(6469):339-342.
- First report of IL-6-deficient mice showing the importance of IL-6 in immune responses.
38. KOPF M, HERREN S, WILES MV, PEPYS MB, KOSCOVILBOIS MH: **Interleukin 6 influences germinal center development and antibody production via a contribution of C3 complement component.** *J. Exp. Med.* (1998) **188**(10):1895-1906.
- The role of IL-6 in antigen-specific antibody responses.
39. KOPF M, RAMSAY A, BROMBACHER F *et al.*: **Pleiotropic defects of IL-6-deficient mice including early hematopoiesis, T and B cell function and acute phase responses.** *Ann. NY Acad. Sci.* (1995) **762**:308-318.
- A review concerning the phenotypes of IL-6-deficient mice.
40. LADEL CH, BLUM C, DREHER A *et al.*: **Lethal tuberculosis in interleukin-6-deficient mutant mice.** *Infect. Immun.* (1997) **65**(11):4843-4849.
41. ROMANI L, MENCACCI A, CENCI E *et al.*: **Impaired neutrophil response and CD4+ T helper cell 1 development in interleukin 6-deficient mice infected with *Candida albicans*.** *J. Exp. Med.* (1996) **183**(4):1345-1355.
42. SUZUKI Y, RANI S, LIESENFELD O *et al.*: **Impaired resistance to the development of toxoplasmic encephalitis in interleukin-6-deficient mice.** *Infect. Immun.* (1997) **65**(6):2339-2345.
43. DALRYMPLE SA, LUCIAN LA, SLATTERY R *et al.*: **Interleukin-6-deficient mice are highly susceptible to *Listeria monocytogenes* infection: correlation with inefficient neutrophilia.** *Infect. Immun.* (1995) **63**(6):2262-2268.
44. ALONZI T, FATTORI E, LAZZARO D *et al.*: **Interleukin 6 is required for the development of collagen-induced arthritis.** *J. Exp. Med.* (1998) **187**(4):461-468.
45. SASAI M, SAEKI Y, OHSHIMA S *et al.*: **Delayed onset and reduced severity of collagen-induced arthritis in interleukin-6-deficient mice.** *Arthritis Rheum.* (1999) **42**(8):1635-1643.
46. TAKAGI N, MIHARA M, MORIYA Y *et al.*: **Blockage of interleukin-6 receptor ameliorates joint disease in murine collagen-induced arthritis.** *Arthritis Rheum.* (1998) **41**(12):2117-2121.
47. MENDEL I, KATZ A, KOZAK N, BEN-NUN A, REVEL M: **Interleukin-6 functions in autoimmune encephalomyelitis: a study in gene-targeted mice.** *Eur. J. Immunol.* (1998) **28**(5):1727-1737.
48. OKUDA Y, SAKODA S, BERNARD CC *et al.*: **IL-6-deficient mice are resistant to the induction of experimental autoimmune encephalomyelitis provoked by myelin oligodendrocyte glycoprotein.** *Int. Immunol.* (1998) **10**(5):703-708.
49. SAMOILOVA EB, HORTON JL, HILLIARD B, LIU TS, CHEN Y: **IL-6-deficient mice are resistant to experimental autoimmune encephalomyelitis: roles of IL-6 in the activation and differentiation of autoreactive T cells.** *J. Immunol.* (1998) **161**(12):6480-6486.
50. ESCARY JL, PERREAU J, DUMENIL D, EZINE S, BRULET P: **Leukaemia inhibitory factor is necessary for maintenance of haematopoietic stem cells and thymocyte stimulation.** *Nature* (1993) **363**(6427):361-364.
51. MASU Y, WOLF E, HOLTSMANN B *et al.*: **Disruption of the CNTF gene results in motor neuron degeneration.** *Nature* (1993) **365**(6441):27-32.
52. MORITZ RL, WARD LD, TU GF *et al.*: **The N-terminus of gp130 is critical for the formation of the high-affinity interleukin-6 receptor complex.** *Growth Factors* (1999) **16**(4):265-278.
53. HAMMACHER A, RICHARDSON RT, LAYTON JE *et al.*: **The immunoglobulin-like module of gp130 is required for signaling by interleukin-6, but not by leukemia inhibitory factor.** *J. Biol. Chem.* (1998) **273**(35):22701-22707.
54. VOLLMER P, OPPMANN B, VOLTZ N, FISCHER M, ROSE-JOHN S: **A role for the immunoglobulin-like domain of the human IL-6 receptor. Intracellular protein transport and shedding.** *Eur. J. Biochem.* (1999) **263**(2):438-446.
55. HORSTEN U, MULLER-NEWEN G, GERHARTZ C *et al.*: **Molecular modeling-guided mutagenesis of the extracellular part of gp130 leads to the identification of contact sites in the interleukin-6 (IL-6). IL-6 receptor.gp130 complex.** *J. Biol. Chem.* (1997) **272**(38):23748-23757.
56. OLIVIER C, AUGUSTE P, CHABBERT M *et al.*: **Identification of a gp130 cytokine receptor critical site involved in oncostatin M response.** *J. Biol. Chem.* (2000) **275**(8):5648-5656.
57. KURTH I, HORSTEN U, PFLANZ S *et al.*: **Importance of the membrane-proximal extracellular domains for activation of the signal transducer glycoprotein 130.** *J. Immunol.* (2000) **164**(1):273-282.
58. LUTTICKEN C, WEGENKA UM, YUAN J *et al.*: **Association of transcription factor APRF and protein kinase Jak1**

- with the interleukin-6 signal transducer gp130. *Science* (1994) **263**(5143):89-92.
59. STAHL N, BOULTON TG, FARRUGGELLA T *et al.*: **Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6  $\beta$  receptor components.** *Science* (1994) **263**(5143):92-95.
60. FUKADA T, HIBI M, YAMANAKA Y *et al.*: **Two signals are necessary for cell proliferation induced by a cytokine receptor gp130: involvement of STAT3 in anti-apoptosis.** *Immunity* (1996) **5**(5):449-460.
- Biological importance of SHP2- and STAT3-mediated signals through gp130.
61. STAHL N, FARRUGGELLA TJ, BOULTON TG *et al.*: **Choice of STATs and other substrates specified by modular tyrosine- based motifs in cytokine receptors.** *Science* (1995) **267**(5202):1349-1353.
62. YAMANAKA Y, NAKAJIMA K, FUKADA T, HIBI M, HIRANO T: **Differentiation and growth arrest signals are generated through the cytoplasmic region of gp130 that is essential for Stat3 activation.** *EMBO J.* (1996) **15**(7):1557-1565.
63. YOSHIDA K, TAGA T, SAITO M *et al.*: **Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders.** *Proc. Natl. Acad. Sci. USA* (1996) **93**(1):407-411.
- gp130-deficient mice displayed multiple disorders and led to embryonic lethal phenotype.
64. HIROTA H, CHEN J, BETZ UA *et al.*: **Loss of a gp130 cardiac muscle cell survival pathway is a critical event in the onset of heart failure during biomechanical stress.** *Cell* (1999) **97**(2):189-198.
65. OHTANI T, ISHIHARA K, ATSUMI T *et al.*: **Dissection of signaling cascades through gp130 *in vivo*: reciprocal roles for STAT3- and SHP2-mediated signals in immune responses.** *Immunity* (2000) **12**(1):95-105.
- Reciprocal *in vivo* roles for signalling via STAT3 (positive) and SHP2 (negative) were found by knock-in techniques.
66. KAWASAKI K, GAO YH, YOKOSE S *et al.*: **Osteoclasts are present in gp130-deficient mice.** *Endocrinology* (1997) **138**(11):4959-4965.
67. KUMANOGOH A, MARUKAWA S, KUMANOGOH T *et al.*: **Impairment of antigen-specific antibody production in transgenic mice expressing a dominant-negative form of gp130.** *Proc. Natl. Acad. Sci. USA* (1997) **94**(6):2478-2482.
- This paper describes the importance of gp130 in immune responses.
68. BETZ UAK, BLOCH W, VAN DEN BROEK M *et al.*: **Postnatally induced inactivation of gp130 in mice results in neurological, cardiac, hematopoietic, immunological, hepatic and pulmonary defects.** *J. Exp. Med.* (1998) **188**(10):1955-1965.
- Postnatally, gp130 knock-out mice using Cre-loxP system showed multiple defects.
69. LI M, SENDTNER M, SMITH A: **Essential function of LIF receptor in motor neurons.** *Nature* (1995) **378**(6558):724-727.
70. DECHIARA TM, VEJSADA R, POUYEMIROU WT *et al.*: **Mice lacking the CNTF receptor, unlike mice lacking CNTF, exhibit profound motor neuron deficit at birth.** *Cell* (1995) **83**:313-322.
71. WARE CB, HOROWITZ MC, RENSHAW BR *et al.*: **Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death.** *Development* (1995) **121**(5):1283-1299.
72. TONKS NK, NEEL BG: **From form to function: signaling by protein tyrosine phosphatases.** *Cell* (1996) **87**(3):365-368.
73. FENG GS, HUI CC, PAWSON T: **SH2-containing phosphotyrosine phosphatase as a target of protein-tyrosine kinases.** *Science* (1993) **259**(5101):1607-1611.
74. VOGEL W, LAMMERS R, HUANG J, ULLRICH A: **Activation of a phosphotyrosine phosphatase by tyrosine phosphorylation.** *Science* (1993) **259**(5101):1611-1614.
75. BENNETT AM, TANG TL, SUGIMOTO S, WALSH CT, NEEL BG: **Protein-tyrosine-phosphatase SHPTP2 couples platelet-derived growth factor receptor  $\beta$  to Ras.** *Proc. Natl. Acad. Sci. USA* (1994) **91**(15):7335-7339.
76. LI W, NISHIMURA R, KASHISHIAN A *et al.*: **A new function for a phosphotyrosine phosphatase: linking GRB2-Sos to a receptor tyrosine kinase.** *Mol. Cell. Biol.* (1994) **14**(1):509-517.
77. KIM H, HAWLEY TS, HAWLEY RG, BAUMANN H: **Protein tyrosine phosphatase 2 (SHP-2) moderates signaling by gp130 but is not required for the induction of acute-phase plasma protein genes in hepatic cells.** *Mol. Cell. Biol.* (1998) **18**(3):1525-1533.
- SHP2 acts as a negative regulator of gp130 signalling.
78. SYMES A, STAHL N, REEVES SA *et al.*: **The protein tyrosine phosphatase SHP-2 negatively regulates ciliary neurotrophic factor induction of gene expression.** *Curr. Biol.* (1997) **7**(9):697-700.
- SHP2 acts as a negative regulator of gp130 signalling.
79. HOF P, PLUSKEY S, DHE-PAGANON S, ECK MJ, SHOELSON SE: **Crystal structure of the tyrosine phosphatase SHP-2.** *Cell* (1998) **92**(4):441-450.
80. BENNETT AM, HAUSDORFF SF, O'REILLY AM, FREEMAN RM, NEEL BG: **Multiple requirements for SHPTP2 in epidermal growth factor-mediated cell cycle progression.** *Mol. Cell. Biol.* (1996) **16**(3):1189-1202.
81. NOGUCHI T, MATOZAKI T, HORITA K, FUJIOKA Y, KASUGA M: **Role of SH-PTP2, a protein-tyrosine phosphatase with Src homology 2 domains, in insulin-stimulated Ras activation.** *Mol. Cell. Biol.* (1994) **14**(10):6674-6682.
82. TANG TL, FREEMAN RM, JR., O'REILLY AM, NEEL BG, SOKOL SY: **The SH2-containing protein-tyrosine phosphatase SH-PTP2 is required upstream of MAP kinase for early *Xenopus* development.** *Cell* (1995) **80**(3):473-483.
83. HOLGADO-MADRUGA M, EMLET DR, MOSCATELLO DK, GODWIN AK, WONG AJ: **A Grb2-associated docking**

- protein in EGF- and insulin-receptor signalling.** *Nature* (1996) **379**(6565):560-564.
84. RAABE T, RIESGO-ESCOVAR J, LIU X *et al.*: **DOS, a novel pleckstrin homology domain-containing protein required for signal transduction between sevenless and Ras1 in Drosophila.** *Cell* (1996) **85**(6):911-920.
  85. HERBST R, CARROLL PM, ALLARD JD *et al.*: **Daughter of sevenless is a substrate of the phosphotyrosine phosphatase Corkscrew and functions during sevenless signaling.** *Cell* (1996) **85**(6):899-909.
  86. GU H, PRATT JC, BURAKOFF SJ, NEEL BG: **Cloning of p97/Gab2, the major SHP2-binding protein in hematopoietic cells, reveals a novel pathway for cytokine-induced gene activation.** *Mol. Cell* (1998) **2**(6):729-740.
    - Gab family proteins act downstream of various cytokines and growth factors.
  87. NISHIDA K, YOSHIDA Y, ITOH M *et al.*: **Gab-family adapter proteins act downstream of cytokine and growth factor receptors and T and B cell antigen receptors.** *Blood* (1999) **93**(6):1809-1816.
    - Gab family proteins act downstream of various cytokines and growth factors.
  88. TAKAHASHI-TEZUKA M, YOSHIDA Y, FUKADA T *et al.*: **Gab1 acts as an adapter molecule linking the cytokine receptor gp130 to ERK mitogen-activated protein kinase.** *Mol. Cell. Biol.* (1998) **18**(7):4109-4117.
    - First report describing Gab1 action in the gp130 signalling.
  89. HIBI M, HIRANO T: **Gab-family adapter molecules in signal transduction of cytokine and growth factor receptors and T and B cell antigen receptors.** *Leukemia Lymphoma* (2000) **37**(3-4):299-307.
  90. ITOH M, YOSHIDA Y, NISHIDA K *et al.*: **A role of Gab1 for heart, placenta and skin development and growth factors- and cytokines-induced ERK MAP kinase activation.** *Mol. Cell. Biol.* (2000) **20**(10):3695-3704.
  91. FUKADA T, OHTANI T, YOSHIDA Y *et al.*: **STAT3 orchestrates contradictory signals in cytokine-induced G1 to S cell-cycle transition.** *EMBO J.* (1998) **17**(22):6670-6677.
  92. IHARA S, NAKAJIMA K, FUKADA T *et al.*: **Dual control of neurite outgrowth by STAT3 and MAP kinase in PC12 cells stimulated with interleukin-6.** *EMBO J.* (1997) **16**(17):5345-5352.
  93. HEMMANN U, GERHARTZ C, HEESEL B *et al.*: **Differential activation of acute phase response factor/Stat3 and Stat1 via the cytoplasmic domain of the interleukin 6 signal transducer gp130. II. Src homology SH2 domains define the specificity of stat factor activation.** *J. Biol. Chem.* (1996) **271**(22):12999-13007.
  94. DARNELL JE, JR., KERR IM, STARK GR: **Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins.** *Science* (1994) **264**(5164):1415-1421.
  95. DARNELL JE, JR.: **STATs and gene regulation.** *Science* (1997) **277**(5332):1630-1635.
  96. IHLE JN, KERR IM: **Jaks and Stats in signaling by the cytokine receptor superfamily.** *Trends Genet.* (1995) **11**(2):69-74.
  97. IHLE JN: **STATs: signal transducers and activators of transcription.** *Cell* (1996) **84**(3):331-334.
  98. GERHARTZ C, HEESEL B, SASSE J *et al.*: **Differential activation of acute phase response factor/STAT3 and STAT1 via the cytoplasmic domain of the interleukin 6 signal transducer gp130. I. Definition of a novel phosphotyrosine motif mediating STAT1 activation.** *J. Biol. Chem.* (1996) **271**(22):12991-12998.
  99. NIWA H, BURDON T, CHAMBERS I, SMITH A: **Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3.** *Genes Dev.* (1998) **12**(13):2048-2060.
  100. SCHMITZ J, DAHMEN H, GRIMM C *et al.*: **The cytoplasmic tyrosine motifs in full-length glycoprotein 130 have different roles in IL-6 signal transduction.** *J. Immunol.* (2000) **164**(2):848-854.
  101. DITTRICH E, HAFT CR, MUYS L, HEINRICH PC, GRAEVE L: **A di-leucine motif and an upstream serine in the interleukin-6 (IL-6) signal transducer gp130 mediate ligand-induced endocytosis and down-regulation of the IL-6 receptor.** *J. Biol. Chem.* (1996) **271**(10):5487-5494.
  102. NAKAJIMA K, YAMANAKA Y, NAKAE K *et al.*: **A central role for Stat3 in IL-6-induced regulation of growth and differentiation in M1 leukemia cells.** *EMBO J.* (1996) **15**(14):3651-3658.
  103. NAKASHIMA K, YANAGISAWA M, ARAKAWA H *et al.*: **Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300.** *Science* (1999) **284**(5413):479-482.
  104. BONNI A, SUN Y, NADAL-VICENS M *et al.*: **Regulation of gliogenesis in the central nervous system by the JAK-STAT signaling pathway.** *Science* (1997) **278**(5337):477-483.
  105. MIGONE TS, LIN JX, CERESETO A *et al.*: **Constitutively activated Jak-STAT pathway in T cells transformed with HTLV-I.** *Science* (1995) **269**(5220):79-81.
  106. YU CL, MEYER DJ, CAMPBELL GS *et al.*: **Enhanced DNA-binding activity of a Stat3-related protein in cells transformed by the Src oncogene.** *Science* (1995) **269**(5220):81-83.
  107. TURKSON J, BOWMAN T, GARCIA R *et al.*: **Stat3 activation by Src induces specific gene regulation and is required for cell transformation.** *Mol. Cell. Biol.* (1998) **18**(5):2545-2552.
    - STAT3 and cellular transformation.
  108. DANIAL NN, PERNIS A, ROTHMAN PB: **Jak-STAT signaling induced by the v-abl oncogene.** *Science* (1995) **269**(5232):1875-1877.
  109. HILBERT DM, MIGONE TS, KOPF M, LEONARD WJ, RUDIKOFF S: **Distinct tumorigenic potential of abl and raf in B cell neoplasia: abl activates the IL-6 signaling pathway.** *Immunity* (1996) **5**(1):81-89.
  110. ILARIA RL, JR., VAN ETEN RA: **P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members.** *J. Biol. Chem.* (1996) **271**(49):31704-31710.

## 18 gp130-mediated signalling as a therapeutic target

111. BESSER D, BROMBERG JF, DARNELL JE, JR., HANAFUSA H: **A single amino acid substitution in the v-Eyk intracellular domain results in activation of Stat3 and enhances cellular transformation.** *Mol. Cell. Biol.* (1999) **19**(2):1401-1409.
112. RAM PT, HORVATH CM, IYENGAR R: **Stat3-mediated transformation of NIH-3T3 cells by the constitutively active Q205L G $\alpha$ o protein.** *Science* (2000) **287**(5450):142-144.
113. CATLETT-FALCONE R, LANDOWSKI TH, OSHIRO MM *et al.*: **Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells.** *Immunity* (1999) **10**(1):105-115.
- Constitutive activation of STAT3 in human cancers.
114. BROMBERG JF, HORVATH CM, BESSER D, LATHEM WW, DARNELL JE, JR.: **Stat3 activation is required for cellular transformation by v-src.** *Mol. Cell. Biol.* (1998) **18**(5):2553-2558.
115. BROMBERG JF, WRZESZCZYNSKA MH, DEVGAN G *et al.*: **Stat3 as an oncogene.** *Cell* (1999) **98**(3):295-303.
- STAT3 and cellular transformation.
116. NIU G, HELLER R, CATLETT-FALCONE R *et al.*: **Gene therapy with dominant-negative Stat3 suppresses growth of the murine melanoma B16 tumor *in vivo*.** *Cancer Res.* (1999) **59**(20):5059-5063.
117. BORSELLINO N, BONAVIDA B, CILIBERTO G *et al.*: **Blocking signaling through the Gp130 receptor chain by interleukin-6 and oncostatin M inhibits PC-3 cell growth and sensitizes the tumor cells to etoposide and cisplatin-mediated cytotoxicity.** *Cancer* (1999) **85**(1):134-144.
118. HOBISCH A, EDER I.E., PUTZ T *et al.*: **Interleukin-6 regulates prostate-specific protein expression in prostate carcinoma cells by activation of the androgen receptor.** *Cancer Res.* (1998) **58**(20):4640-4645.
119. QIU Y, RAVI L, KUNG HJ: **Requirement of ErbB2 for signalling by interleukin-6 in prostate carcinoma cells.** *Nature* (1998) **393**(6680):83-85.
120. QIU Y, ROBINSON D, PRETLOW TG, KUNG HJ: **Etk/Bmx, a tyrosine kinase with a pleckstrin-homology domain, is an effector of phosphatidylinositol 3'-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells.** *Proc. Natl. Acad. Sci. USA* (1998) **95**(7):3644-3649.
121. TAKEDA K, KAISHO T, YOSHIDA N *et al.*: **Stat3 activation is responsible for IL-6-dependent T cell proliferation through preventing apoptosis: generation and characterization of T cell-specific Stat3-deficient mice.** *J. Immunol.* (1998) **161**(9):4652-4660.
122. KARRAS JG, WANG Z, HUO L *et al.*: **Signal transducer and activator of transcription-3 (STAT3) is constitutively activated in normal, self-renewing B-1 cells but only inducibly expressed in conventional B lymphocytes.** *J. Exp. Med.* (1997) **185**(6):1035-1042.
123. SHIROGANE T, FUKADA T, MULLER JM *et al.*: **Synergistic roles for Pim-1 and c-Myc in STAT3-mediated cell cycle progression and antiapoptosis.** *Immunity* (1999) **11**(6):709-719.
- Pim-1 and c-Myc synergistically act downstream of STAT3.
124. KIUCHI N, NAKAJIMA K, ICHIBA M *et al.*: **STAT3 is required for the gp130-mediated full activation of the c-myc gene.** *J. Exp. Med.* (1999) **189**(1):63-73.
125. NAKASHIMA K, WIESE S, YANAGISAWA M *et al.*: **Developmental requirement of gp130 signaling in neuronal survival and astrocyte differentiation.** *J. Neurosci.* (1999) **19**(13):5429-5434.
126. CHUNG CD, LIAO J, LIU B *et al.*: **Specific inhibition of Stat3 signal transduction by PIAS3.** *Science* (1997) **278**(5344):1803-1805.
127. LIU B, LIAO J, RAO X *et al.*: **Inhibition of Stat1-mediated gene activation by PIAS1.** *Proc. Natl. Acad. Sci. USA* (1998) **95**(18):10626-10631.
128. NICHOLSON SE, WILLSON TA, FARLEY A *et al.*: **Mutational analyses of the SOCS proteins suggest a dual domain requirement but distinct mechanisms for inhibition of LIF and IL-6 signal transduction.** *EMBO J.* (1999) **18**(2):375-385.
129. STARR R, WILLSON TA, VINEY EM *et al.*: **A family of cytokine-inducible inhibitors of signalling.** *Nature* (1997) **387**(6636):917-921.
130. ENDO TA, MASUHARA M, YOKOUCHI M *et al.*: **A new protein containing an SH2 domain that inhibits JAK kinases.** *Nature* (1997) **387**(6636):921-924.
131. NAKA T, NARAZAKI M, HIRATA M *et al.*: **Structure and function of a new STAT-induced STAT inhibitor.** *Nature* (1997) **387**(6636):924-929.
132. YOSHIMURA A, OHKUBO T, KIGUCHI T *et al.*: **A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors.** *EMBO J.* (1995) **14**(12):2816-2826.
133. YASUKAWA H, MISAWA H, SAKAMOTO H *et al.*: **The JAK-binding protein JAB inhibits Janus tyrosine kinase activity through binding in the activation loop.** *EMBO J.* (1999) **18**(5):1309-1320.
134. SCHMITZ J, WEISSENBACH M, HAAN S, HEINRICH PC, SCHAPER F: **SOCS3 exerts its inhibitory function on interleukin-6 signal transduction through the SHP2 recruitment site of gp130.** *J. Biol. Chem.* (2000) **275**(17):12848-12856.
- SOCS3 interacts with gp130.
135. NICHOLSON SE, DE SOUZA D, FABRI LJ *et al.*: **Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130.** *Proc. Natl. Acad. Sci. USA* (2000) **97**(12):6493-6498.
- SOCS3 interacts with gp130.
136. SENGUPTA TK, TALBOT ES, SCHERLE PA, IVASHKIV LB: **Rapid inhibition of interleukin-6 signaling and Stat3 activation mediated by mitogen-activated protein**

- kinases. *Proc. Natl. Acad. Sci. USA* (1998) **95**(19):11107-11112.
- MAPK acts as a negative regulator of STAT3.
137. JAIN N, ZHANG T, FONG SL, LIM CP, CAO X: **Repression of Stat3 activity by activation of mitogen-activated protein kinase (MAPK)**. *Oncogene* (1998) **17**(24):3157-3167.
- MAPK acts as a negative regulator of STAT3.
138. SIEWERT E, MULLER-ESTERL W, STARR R, HEINRICH PC, SCHAPER F: **Different protein turnover of interleukin-6-type cytokine signalling components**. *Eur. J. Biochem.* (1999) **265**(1):251-257.
139. ADAMS TE, HANSEN JA, STARR R *et al.*: **Growth hormone preferentially induces the rapid, transient expression of SOCS-3, a novel inhibitor of cytokine receptor signaling**. *J. Biol. Chem.* (1998) **273**(3):1285-1287.
140. LOSMAN JA, CHEN XP, HILTON D, ROTHMAN P: **Cutting edge: SOCS-1 is a potent inhibitor of IL-4 signal transduction**. *J. Immunol.* (1999) **162**(7):3770-3774.
141. DICKENSHEETS HL, DONNELLY RP: **Inhibition of IL-4-inducible gene expression in human monocytes by Type I and Type II interferons**. *J. Leukoc. Biol.* (1999) **65**(3):307-312.
142. MARINE JC, TOPHAM DJ, MCKAY C *et al.*: **SOCS1 deficiency causes a lymphocyte-dependent perinatal lethality**. *Cell* (1999) **98**(5):609-616.
143. ALEXANDER WS, STARR R, FENNER JE *et al.*: **SOCS1 is a critical inhibitor of interferon  $\gamma$  signaling and prevents the potentially fatal neonatal actions of this cytokine**. *Cell* (1999) **98**(5):597-608.
144. MARINE JC, MCKAY C, WANG D *et al.*: **SOCS3 is essential in the regulation of fetal liver erythropoiesis**. *Cell* (1999) **98**(5):617-627.
145. CIAPPONI L, GRAZIANI R, PAONESSA G *et al.*: **Definition of a composite binding site for gp130 in human interleukin-6**. *J. Biol. Chem.* (1995) **270**(52):31249-31254.
146. KALLEN K-J, GALLE PR, ROSE-JOHN S: **New developments in IL-6 dependent biology and therapy: where do we stand and what are the options?** *Exp. Opin. Invest. Drugs* (1999) **8**(9):1327-1349.
- A detailed review about the development of gp130 agonists and antagonists, and the diseases concerning gp130 signalling.
147. BRAVO J, HEATH JK: **New EMBO members' review: receptor recognition by gp130 cytokines**. *EMBO J.* (2000) **19**(11):2399-2411.
148. KLEIN B, WIJDENES J, ZHANG XG *et al.*: **Murine anti-interleukin-6 monoclonal antibody therapy for a patient with plasma cell leukemia**. *Blood* (1991) **78**(5):1198-1204.
- A clinical trial using mouse antihuman IL-6 antibody.
149. BECK JT, HSU SM, WIJDENES J *et al.*: **Brief report: alleviation of systemic manifestations of Castleman's disease by monoclonal anti-interleukin-6 antibody**. *N. Engl. J. Med.* (1994) **330**(9):602-605.
150. WENDLING D, RACADOT E, WIJDENES J: **Treatment of severe rheumatoid arthritis by anti-interleukin 6 monoclonal antibody**. *J. Rheumatol.* (1993) **20**(2):259-262.
151. MONTERO-JULIAN FA, KLEIN B, GAUTHEROT E, BRALLY H: **Pharmacokinetic study of anti-interleukin-6 (IL-6) therapy with monoclonal antibodies: enhancement of IL-6 clearance by cocktails of anti-IL-6 antibodies**. *Blood* (1995) **85**(4):917-924.
152. VAN ZAAANEN HC, KOOPMANS RP, AARDEN LA *et al.*: **Endogenous interleukin 6 production in multiple myeloma patients treated with chimeric monoclonal anti-IL6 antibodies indicates the existence of a positive feed-back loop**. *J. Clin. Invest.* (1996) **98**(6):1441-1448.
- A clinical trial using chimeric anti-IL-6 antibody
153. VAN ZAAANEN HC, LOKHORST HM, AARDEN LA *et al.*: **Blocking interleukin-6 activity with chimeric anti-IL6 monoclonal antibodies in multiple myeloma: effects on soluble IL6 receptor and soluble gp130**. *Leukemia Lymphoma* (1998) **31**(5-6):551-558.
154. VAN ZAAANEN HC, LOKHORST HM, AARDEN LA *et al.*: **Chimaeric anti-interleukin 6 monoclonal antibodies in the treatment of advanced multiple myeloma: a Phase I dose-escalating study**. *Br. J. Haematol.* (1998) **102**(3):783-790.
155. YOSHIZAKI K, NISHIMOTO N, MIHARA M, KISHIMOTO T: **Therapy of rheumatoid arthritis by blocking IL-6 signal transduction with a humanized anti-IL-6 receptor antibody**. *Springer Semin. Immunopathol.* (1998) **20**(1-2):247-259.
156. NISHIMOTO N, KISHIMOTO T, YOSHIZAKI K: **Anticytokine therapy in autoimmune diseases**. *Intern. Med.* (1999) **38**(2):178-182.
157. NISHIMOTO N, SASAI M, SHIMA Y *et al.*: **Improvement in Castleman's disease by humanized anti-interleukin-6 receptor antibody therapy**. *Blood* (2000) **95**(1):56-61.
- A clinical trial using humanized anti-IL-6R antibody.
158. RENNE C, KALLEN KJ, MULLBERG J *et al.*: **A new type of cytokine receptor antagonist directly targeting gp130**. *J. Biol. Chem.* (1998) **273**(42):27213-27219.
- Molecular design of a gp130 antagonist.
159. SANDS BE, BANK S, SNINSKY CA *et al.*: **Preliminary evaluation of safety and activity of recombinant human interleukin 11 in patients with active Crohn's disease**. *Gastroenterology* (1999) **117**(1):58-64.
160. TREPICCHIO WL, OZAWA M, WALTERS IB *et al.*: **Interleukin-11 therapy selectively downregulates Type I cytokine proinflammatory pathways in psoriasis lesions**. *J. Clin. Invest.* (1999) **104**(11):1527-1537.
161. TAKEDA K, CLAUSEN BE, KAISHO T *et al.*: **Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils**. *Immunity* (1999) **10**(1):39-49.
162. **A double-blind placebo-controlled clinical trial of subcutaneous recombinant human ciliary neurotrophic factor (rHCNTF) in amyotrophic lateral sclerosis**. ALS CNTF Treatment Study Group. *Neurology* (1996) **46**(5):1244-1249.

163. AEBISCHER P, SCHLUEP M, DEGLON N *et al.*: **Intrathecal delivery of CNTF using encapsulated genetically modified xenogeneic cells in amyotrophic lateral sclerosis patients.** *Nature Med.* (1996) **2**(6):696-699.
164. PENN RD, KROIN JS, YORK MM, CEDARBAUM JM: **Intrathecal ciliary neurotrophic factor delivery for treatment of amyotrophic lateral sclerosis (phase I trial).** *Neurosurgery* (1997) **40**(1):94-99; discussion 99-100.
165. KOBAYASHI M, FITZ L, RYAN M *et al.*: **Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes.** *J. Exp. Med.* (1989) **170**(3):827-845.
166. DAVIS S, ALDRICH TH, IP NY *et al.*: **Released form of CNTF receptor  $\alpha$  components as a soluble mediator of CNTF responses.** *Science* (1993) **259**:1736-1739.
167. BAUMANN H, WANG Y, MORELLA KK *et al.*: **Complex of the soluble IL-11 receptor and IL-11 acts as IL-6-type cytokine in hepatic and nonhepatic cells.** *J. Immunol.* (1996) **157**(1):284-290.
168. NEDDERMANN P, GRAZIANI R, CILIBERTO G, PAONESSA G: **Functional expression of soluble human interleukin-11 (IL-11) receptor  $\alpha$  and stoichiometry of *in vitro* IL-11 receptor complexes with gp130.** *J. Biol. Chem.* (1996) **271**(48):30986-30991.
169. HIRANO T, MATSUDA T, NAKAJIMA K: **Signal transduction through gp130 that is shared among the receptors for the interleukin 6 related cytokine subfamily.** *Stem Cells* (1994) **12**(3):262-277.
170. HIRANO T: **Molecular basis underlying functional pleiotropy of cytokines and growth factors.** *Biochem. Biophys. Res. Commun.* (1999) **260**(2):303-308.
171. HIROTA H, YOSHIDA K, KISHIMOTO T, TAGA T: **Continuous activation of gp130, a signal transducing receptor component for interleukin 6-related cytokines, causes myocardial hypertrophy in mice.** *Proc. Natl. Acad. Sci. USA* (1995) **92**:4862-4866.
172. FISCHER M, GOLDSCHMITT J, PESCHEL C *et al.*: **I. A bioactive designer cytokine for human hematopoietic progenitor cell expansion.** *Nature Biotechnol.* (1997) **15**(2):142-145.
- A designer cytokine, composed of sIL-6R $\alpha$  and IL-6, effectively expands haematopoietic progenitor cells.
173. PETERS M, MULLER AM, ROSE-JOHN S: **Interleukin-6 and soluble interleukin-6 receptor: direct stimulation of gp130 and hematopoiesis.** *Blood* (1998) **92**(10):3495-3504.
174. SOBOTA R, SZWED M, KASZA A, BUGNO M, KORDULA T: **Parthenolide inhibits activation of signal transducers and activators of transcription (STATs) induced by cytokines of the IL-6 family.** *Biochem. Biophys. Res. Commun.* (2000) **267**(1):329-333.
175. BORK PM, SCHMITZ ML, KUHN M, ESCHER C, HEINRICH M: **Sesquiterpene lactone containing Mexican Indian medicinal plants and pure sesquiterpene lactones as potent inhibitors of transcription factor NF- $\kappa$ B.** *FEBS Lett.* (1997) **402**(1):85-90.
176. DURIE BGM, GILES FJ: **Myeloma and other proteinemias.** In: *Oxford Textbook of Medicine (3rd Edition)*. Weatherall DJ, Ledingham JGG, Warell DA (Eds.), Oxford University Press, New York, USA (1996):3597-3605.
177. BUNCH C, GATTER KC: **The lymphomas.** In: *Oxford Textbook of Medicine (3rd Edition)*. Weatherall DJ, Ledingham JGG, Warell DA (Eds.), Oxford University Press, New York, USA (1996):3568-3587.
178. TRAILL TA: **Cardiac myxoma.** In: *Oxford Textbook of Medicine (3rd Edition)*. Weatherall DJ, Ledingham JGG, Warell DA (Eds.), Oxford University Press, New York, USA (1996):2472-2474.
179. WORDSWORTH BP: **Rheumatoid arthritis.** In: *Oxford Textbook of Medicine (3rd Edition)*. Weatherall DJ, Ledingham JGG, Warell DA (Eds.), Oxford University Press, New York, USA (1996):2953-2965.
180. WILLIAMS DG: **Mesangiocapillary glomerulonephritis.** In: *Oxford Textbook of Clinical Nephrology (2nd Edition)*. Davison AM, Cameron JS, Grunfeld J-P, Kerr DNS, Ritz E, Winearls CG (Eds.), Oxford University Press, New York, USA (1998):591-612.
181. JEWELL DP: **Crohn's disease.** In: *Oxford Textbook of Medicine (3rd Edition)*. Weatherall DJ, Ledingham JGG, Warell DA (Eds.), Oxford University Press, New York, USA (1996):1936-1943.
182. RYAN TJ: **Diseases of the skin.** In: *Oxford Textbook of Medicine (3rd Edition)*. Weatherall DJ, Ledingham JGG, Warell DA (Eds.), Oxford University Press, New York, USA (1996):3705-3811.
183. SEARS MR: **Epidemiology.** In: *Asthma Basic Mechanism and Clinical Management (2nd Edition)*. Barnes PJ, Rodger IW, Thomson NC (Eds.), Academic Press, San Diego, USA (1992):1-19.
184. DONAGHY M: **The motor neurone diseases.** In: *Oxford Textbook of Medicine (3rd Edition)*. Weatherall DJ, Ledingham JGG, Warell DA (Eds.), Oxford University Press, New York, USA (1996):4087-4090.
185. RAMSAY AJ, HUSBAND AJ, RAMSHAW IA *et al.*: **The role of interleukin-6 in mucosal IgA antibody responses *in vivo*.** *Science* (1994) **264**(5158):561-563.
186. BERNAD A, KOPF M, KULBACKI R *et al.*: **Interleukin-6 is required *in vivo* for the regulation of stem cells and committed progenitors of the hematopoietic system.** *Immunity* (1994) **1**(9):725-731.
- The role of IL-6 in haematopoiesis.
187. CRESSMAN DE, GREENBAUM LE, DEANGELIS RA *et al.*: **Liver failure and defective hepatocyte regeneration in interleukin-6- deficient mice.** *Science* (1996) **274**(5291):1379-1383.
- Impaired liver regeneration in IL-6-deficient mice.
188. POLI V, BALENA R, FATTORI E *et al.*: **Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion.** *EMBO J.* (1994) **13**(5):1189-1196.
- The role of IL-6 in bone metabolism.
189. FATTORI E, DELLA ROCCA C, COSTA P *et al.*: **Development of progressive kidney damage and myeloma**

- kidney in interleukin-6 transgenic mice. *Blood* (1994) **83**(9):2570-2579.**
190. PETERS M, JACOBS S, EHLERS M *et al.*: **The function of the soluble interleukin 6 (IL-6) receptor *in vivo*: sensitization of human soluble IL-6 receptor transgenic mice towards IL-6 and prolongation of the plasma half-life of IL-6.** *J. Exp. Med.* (1996) **183**(4):1399-1406.
191. SCHIRMACHER P, PETERS M, CILIBERTO G *et al.*: **Hepatocellular hyperplasia, plasmacytoma formation and extramedullary hematopoiesis in interleukin (IL)-6/soluble IL-6 receptor double- transgenic mice.** *Am. J. Pathol.* (1998) **153**(2):639-648.
192. TANG W, GEBA GP, ZHENG T *et al.*: **Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling and airways obstruction.** *J. Clin. Invest.* (1996) **98**(12):2845-2853.
193. RAY P, TANG W, WANG P *et al.*: **Regulated overexpression of interleukin 11 in the lung. Use to dissociate development-dependent and -independent phenotypes.** *J. Clin. Invest.* (1997) **100**(10):2501-2511.
194. STEWART CL, KASPAR P, BRUNET LJ *et al.*: **Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor.** *Nature* (1992) **359**(6390):76-79.
195. TOLOSANO E, CUTUFIA MA, HIRSCH E *et al.*: **Ciliary neurotrophic factor constitutively expressed in the nervous system of transgenic mice protects embryonic dorsal root ganglion neurons from apoptosis.** *Eur. J. Neurosci.* (1996) **8**(3):521-529.
196. WINTER CG, SAOTOME Y, LEVISON SW, HIRSH D: **A role for ciliary neurotrophic factor as an inducer of reactive gliosis, the glial response to central nervous system injury.** *Proc. Natl. Acad. Sci. USA* (1995) **92**(13):5865-5869.
197. RODIG SJ, MERAZ MA, WHITE JM *et al.*: **Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses.** *Cell* (1998) **93**(3):373-383.
198. NEUBAUER H, CUMANO A, MULLER M *et al.*: **Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis.** *Cell* (1998) **93**(3):397-409.
199. PARGANAS E, WANG D, STRAVOPODIS D *et al.*: **Jak2 is essential for signaling through a variety of cytokine receptors.** *Cell* (1998) **93**(3):385-395.
200. SAXTON TM, HENKEMEYER M, GASCA S *et al.*: **Abnormal mesoderm patterning in mouse embryos mutant for the SH2 tyrosine phosphatase Shp-2.** *EMBO J.* (1997) **16**(9):2352-2364.
201. TAKEDA K, NOGUCHI K, SHI W *et al.*: **Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality.** *Proc. Natl. Acad. Sci. USA* (1997) **94**(8):3801-3804.
- STAT3 is required for the early stages of development.
202. NAKA T, MATSUMOTO T, NARAZAKI M *et al.*: **Accelerated apoptosis of lymphocytes by augmented induction of Bax in SSI-1 (STAT-induced STAT inhibitor-1) deficient mice.** *Proc. Natl. Acad. Sci. USA* (1998) **95**(26):15577-15582.

Takuya Ohtani<sup>1</sup>, Katsuhiko Ishihara<sup>2</sup>, Toru Atsumi<sup>2</sup>, Yuichi Yoshida<sup>2</sup>, Keigo Nishida<sup>2</sup>, Masahiro Narimatsu<sup>2</sup>, Takahiro Shirogane<sup>2</sup>, Masahiko Hibi<sup>2</sup> & Toshio Hirano<sup>2†</sup>

<sup>†</sup>Author for correspondence

<sup>1</sup>Present Address: Dept. of Immunology, Osaka City University Medical School, 1-4-3, Asahimachi, Abeno-ku, Osaka 545-8585, Japan

<sup>2</sup>Division of Molecular Oncology (C7), Biomedical Research Center, Osaka University Graduate School of Medicine 2-2, Yamada-oka Suita, Osaka 565-0871, Japan

Tel.: +81 6 6879 3880; Fax: +81 6 6879 3889;

E-mail: hirano@molonc.med.osaka-u.ac.jp