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A central role for Stat3 in IL-6-induced regulation of growth and differentiation in M1 leukemia cells

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Interleukin-6 (IL-6) induces either differentiation or growth of a variety of cells. Little is known about the molecular basis of this cellular decision. The family of signal transducer and activator of transcription (Stat) proteins are involved in signaling through a variety of cytokine and growth factor receptors, although their biological roles have not been established. To address whether Stat proteins play roles in IL-6-induced growth or differentiation, we introduced two types of mutant Stat3 acting in a dominant-negative manner into M1 leukemic cells which respond to IL-6 with growth arrest and terminal differentiation. We show that dominant-negative forms of Stat3 inhibited both IL-6-induced growth arrest at G_0/G_1 and macrophage differentiation in the M1 transformants. Blocking of Stat activation resulted in inhibition of IL-6-induced repression of c-*myb* and c-*myc*. Furthermore, IL-6 enhanced the growth of M1 cells primarily through shortening the length of the G_1 period when Stat3 was suppressed. Thus IL-6 generates both growth-enhancing signals and growth arrest- and differentiation-inducing signals at the same time. Stat3 may be a key molecule which determines the cellular decision from cell growth to differentiation in M1 cells.

Keywords: differentiation/growth arrest/interleukin 6/M1/Stat3

Introduction

Cytokines regulate cell growth, differentiation and death. Cellular responses to cytokine signals are the consequence of gene expression programs elicited differentially by the array of intracellular signals. Therefore, to understand how cellular responses to cytokine signals are determined, most efforts have been focused on the elucidation of the molecular mechanisms of signal transduction from the cell surface receptor to the nucleus.

Interleukin-6 (IL-6), one of the multifunctional cytokines, causes cell growth in certain cells including multiple myelomas and B cell plasmacytomas/hybridomas, cell differentiation in various cells including normal B lymphocytes and murine M1 leukemic cells, and induces production of acute phase reactants in hepatocytes (Sehgal *et al.*, 1989; Van Snick 1990; Hirano, 1994). The receptor for IL-6 is composed of an IL-6-specific receptor subunit (α chain) and a signal transducer, gp130 (β chain) (Yamasaki *et al.*, 1988; Hibi *et al.*, 1990). Both of them belong to the cytokine receptor superfamily (Bazan, 1989). Gp130 is shared by the receptors for ciliary neurotrophic factor, leukemia inhibitory factor, oncostatin M, IL-11 and cardiotrophin 1 as a signal-transducing receptor subunit (Gearing *et al.*, 1992; Ip *et al.*, 1992; Yin *et al.*, 1993; Pennica *et al.*, 1995; Hibi *et al.*, 1996). These related cytokines are grouped as the IL-6 subfamily. The binding of IL-6 to an α chain resulted in the formation of a hexametric complex containing two molecules of each component: IL-6, α chain and gp130 (Murakami *et al.*, 1993; Paonessa *et al.*, 1995), followed by the activation of Janus kinase (JAK) family protein tyrosine kinase(s) (PTK) (Lütticken *et al.*, 1994; Matsuda *et al.*, 1994; Stahl *et al.*, 1994) and tyrosine phosphorylation of various cellular proteins including gp130 itself (Murakami *et al.*, 1991; Nakajima and Wall, 1991). Of the JAK family kinases, Jak1, Jak2 and Tyk2 associate constitutively with gp130- or gp130-related LIF receptor and are activated by the IL-6 family of cytokines (Lütticken *et al.*, 1994; Matsuda *et al.*, 1994; Stahl *et al.*, 1994). The activated tyrosine kinases, in turn, phosphorylate and activate the signal transducer and activator of transcription (Stat) family proteins, especially Stat3 and Stat1 for IL-6 (Akira *et al.*, 1994; Darnell *et al.*, 1994; Fujitani *et al.*, 1994; Zhong *et al.*, 1994; Guschin *et al.*, 1995; Nakajima *et al.*, 1995; Stahl *et al.*, 1995). This pathway is called the JAK-STAT signal transduction pathway (Darnell *et al.*, 1994) and has been characterized in a variety of cytokine receptor systems including: IFNα/β, IFNγ, IL-2, IL-3/granulocyte-macrophage colony stimulating factor/IL-5, IL-4, IL-6, IL-7, growth hormone (GH), erythropoietin and thrombopoietin (for review see Darnell *et al.*, 1994; Ihle, 1995; Ihle and Kerr, 1995). Stat proteins have been shown to induce the expression of a set of genes in response to those cytokines. The IL-6-mediated JAK-STAT pathway has been shown to activate several genes, including *junB* (Nakajima *et al.*, 1993; Fujitani *et al.*, 1994; Coffer *et al.*, 1995; Nakajima *et al.*, 1995; Kojima *et al.*, 1996), acute phase reactants (Wegenka *et al.*, 1993; Akira *et al.*, 1994) and interferon regulatory factor-1 (IRF-1) (Guschin *et al.*, 1995; Wegenka *et al.*, 1994; Yuan *et al.*, 1994). However, the physiological role of the JAK-STAT pathway or the STAT-mediated pathway are largely unknown.

We have shown recently that gp130 mutants without any of the YXXQ motifs required for Stat3 activation (Stahl *et al.*, 1995) neither activate Stat3 nor induce growth arrest, or terminal differentiation in M1 leukemic cells, and suggested that Stat3 may be a key molecule for IL-6 function (Yamanaka *et al.*, 1996). In this study, we have addressed directly the question as to whether Stat3

mediates IL-6-induced growth arrest, repression of c-*myc* and c-*myb*, and macrophage differentiation, by using Stat3 mutants acting in a dominant-negative manner. Here, we show that suppression of Stat3 activity inhibits both IL-6-induced growth arrest and terminal differentiation. Furthermore, we show that IL-6 enhances, rather than inhibits, cell growth when Stat3 activation is suppressed. The results show that Stat3 plays a key role in IL-6-mediated regulation of cell growth and differentiation.

Results

Stat proteins with mutations either at a critical carboxy-terminal tyrosine residue or at its DNA-binding domain act specifically in a dominant-negative manner

We introduced two types of mutation into Stat3 and one into Stat1 (Figure 1A) and tested whether they could inhibit the activity of endogenous Stat proteins in transient transfection assays using HepG2 cells and reporter luciferase genes driven by the acute-phase response element (APRE) (Wegenka *et al.*, 1993) or the IFNγ response region (the GRR) of the FcgRI gene (Pearse *et al.*, 1993), a target preferential for Stat1 homodimers and Stat3/Stat1 heterodimers (Horvath *et al.*, 1995). The Stat3 mutants carrying either phenylalanine substitution at Tyr705 (Stat3F) or mutations at the positions important for DNA binding (Stat3D) efficiently inhibited IL-6-activated transcription from both APRE (Figure 1B) and GRR elements (data not shown). None of the Stat3 mutants inhibited IFNγ-activated transcription of both reporter genes. In contrast, Stat1F with a Phe substitution for Tyr701 inhibited the IFNγ-activated transcription of the reporter genes with no apparent inhibitory effect on IL-6-activated, APRE-driven expression (Figure 1B). Therefore, these results indicate that Stat3F, Stat3D and Stat1F could all function as dominant-negative Stats in a cytokine- or receptor-specific manner.

Establishment of M1 transformants stably expressing the dominant-negative form of Stats

We then introduced these expression plasmids encoding hemagglutinin peptide (HA)-tagged Stat3F (HA-Stat3F), HA-tagged Stat3D (HA-Stat3D) and HA-tagged Stat1F (HA-Stat1F) into an M1 myeloleukemic cell line. This cell line has been shown to be an excellent model system for studying the mechanisms by which IL-6 induces growth arrest and macrophage differentiation (Miyaura *et al.*, 1988; Shabo *et al.*, 1988; Hoffman and Liebermann 1991a,b; Kimchi, 1992; Liebermann *et al.*, 1995). We established multiple G418-resistant M1 transformants, each expressing either HA-Stat3F (M1-Stat3F), HA-Stat3D (M1-Stat3D) or HA-Stat1F (M1-Stat1F). The expression levels of the transfected Stat3 or Stat1 mutant were determined by immunoprecipitation of HA-Stat with anti-HA monoclonal antibody (12CA5), followed by immunoblot analysis with polyclonal anti-Stat3 antibody for HA-Stat3F and HA-Stat3D or with anti-Stat1 monoclonal antibody for HA-Stat1F (data not shown). Then we selected several transformants that expressed similar levels of HA-Stat proteins and used at least three independent clones for each transformant in the following experiments. Exogenously expressed HA-Stat3D itself, but not HA-

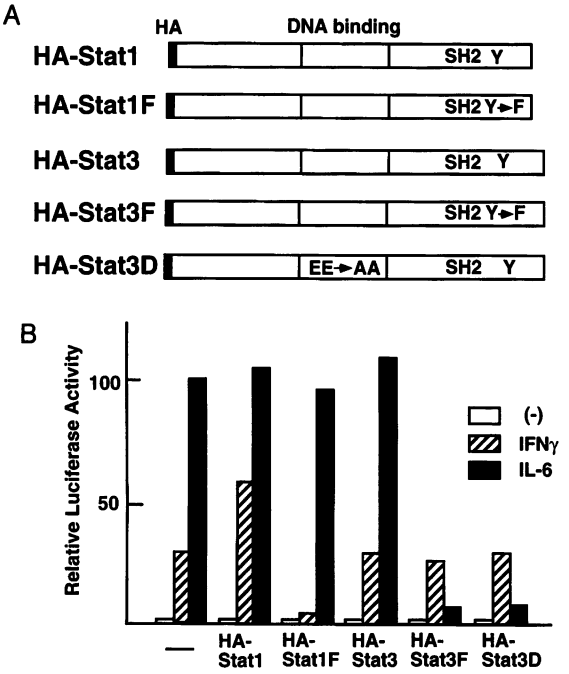

Fig. 1. Specific inhibition of Stat activity by DN-Stat1 and DN-Stat3. (**A**) Dominant negative forms of Stat1 and Stat3 mutants, as well as their wild type, are depicted. (**B**) HepG2 cells were transfected with a plasmid DNA mixture (1.2 μg of reporter genes containing four copies of APRE in front of the minimal *junB* promoter linked to the luciferase gene, 3 μg of either expression vector pCAGGS-Neo, with no insert or with an insert of Stat cDNA encoding either HA-Stat3F, HA-Stat3D or HA-Stat1F and 1 μg of pEF-BOS-lacZ) as described (Nakajima *et al.*, 1993). Forty hours post-transfection, HepG2 cells were stimulated with none (□), 100 ng/ml of IL-6 (■) or IFNγ (▨) for 5 h. Luciferase values were normalized for β-galactosidase activity and expressed relative to the normalized luciferase activity in the extracts from unstimulated cells transfected with the reporter plasmids and a control expression plasmid. Averages of more than three independent experiments are shown.

Stat3F, was tyrosine-phosphorylated and translocated into the nucleus following stimulation with IL-6 (data not shown), as shown by Horvath *et al.* (1995). In the parental M1 cells and M1-Stat1F, IL-6 caused sustained tyrosine phosphorylation of endogenous Stat3 (Figure 2A) and sustained Stat3 DNA-binding activities [Figure 2B, as indicated by A, Stat3 homodimer; B, Stat3 and Stat1 heterodimer; C, Stat1 homodimer, verified by the use of specific antibodies against Stat3 and Stat1 (data not shown), and corresponding to SIF A, SIF B and SIF C described by Sadowski *et al.* (1993)]. DNA-binding complexes containing the Stat1 homodimer were induced very weakly and detected only at 15 min and 24 h following stimulation (Figure 2B). The M1-Stat3F clones had diminished levels of both IL-6-induced Stat3 tyrosine phosphorylation (Figure 2A) and Stat3 DNA-binding activity (Figure 2B), while Stat3D had no effect on IL-6-induced tyrosine phosphorylation of Stat3 (data not shown) but inhibited the appearance of DNA-binding complexes containing Stat3 (complexes A and B). The reduction of activated Stats in the M1-Stat3F shown was not due to the difference in the amount of loaded proteins since the same whole-cell extracts used in Figure 2A showed similar levels of over all tyrosine-phosphorylated proteins except for the markedly reduced levels of tyrosine phosphorylation at the ~90 kDa band most likely corresponding to

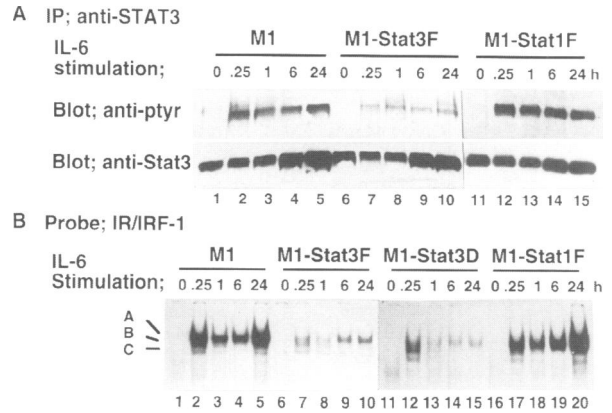

Fig. 2. Inhibition of IL-6-induced Stat3 activation in M1 cells by DN-Stat3. (**A**) IL-6-induced tyrosine phosphorylation of endogenous Stat3 in M1 cells, M1-Stat3F or M1-Stat1F. M1 cells (lanes 1–5) and M1-Stat3F (clone 7, lanes 6–10), or M1-Stat1F (clone 13, lanes 11–15) were stimulated with IL-6 at 100 ng/ml for the indicated period. Whole-cell extracts were subjected to immunoprecipitation with rabbit anti-Stat3 serum (Kojima *et al.*, 1996). The anti-Stat3 immunoprecipitates were analyzed by immunoblotting with anti-phosphotyrosine monoclonal antibody (4G10, UBI) and anti-Stat3 antibody. Results with M1 parents and only representative clones are shown. (**B**) Stat DNA-binding activities in parental M1 cells and transformants. Whole-cell extracts from M1 cells (lanes 1–5), M1-Stat3F (clone 7, lanes 6–10), M1-Stat3D (clone 3, lanes 11–15) and M1-Stat1F (clone 13, lanes 16–20) untreated (lanes 1, 6, 11 and 16) or treated with IL-6 at 100 ng/ml for 15 min (lanes 2, 7, 12 and 17), 1 h (lanes 3, 8, 13 and 18), 6 h (lanes 4, 9, 14 and 19) or 24 h (lanes 5, 10, 15 and 20), were subjected to electromobility shift assay using a ^{32}P-labeled oligonucleotide probe containing an IL-6/IFNγ response element in the murine IRF-1 gene promoter (Harroch *et al.*, 1994). Complexes containing homodimer of Stat3, the heterodimer of Stat3 and Stat1, and the homodimer of Stat1 are indicated on the left by A, B and C.

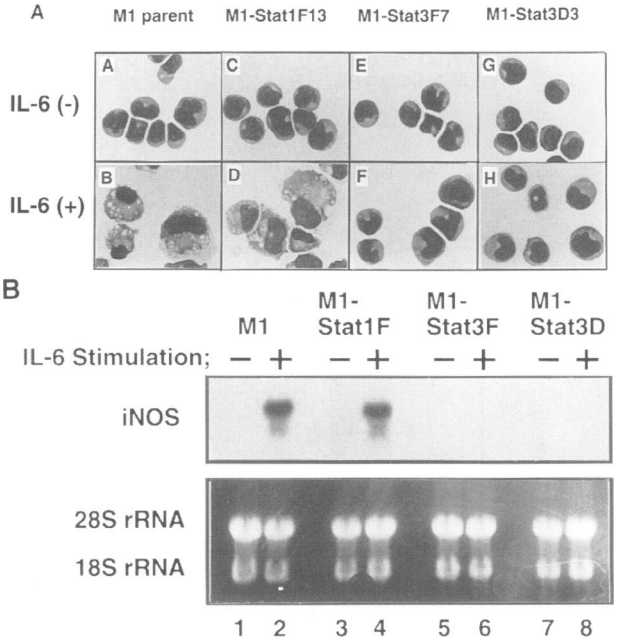

Fig. 3. Inhibition of IL-6-induced differentiation of M1 cells by DN-Stat3. (**A**) Morphological changes in M1 and M1 transformants expressing DN-Stat in response to IL-6. M1 parental cells (A and B), M1-Stat1F clone 13 (C and D), M1-Stat3F clone 7 (E and F) and M1-Stat3D clone 3 (G and H) stimulated with IL-6 at 100 ng/ml (B, D, F and H) for 4 days or left untreated (A, C, E and G) were collected and subjected to May–Grünwald–Giemsa staining. (**B**) Total RNAs extracted from M1 (lanes 1 and 2), M1-Stat1F clone 13 (lanes 3 and 4), M1-Stat3F clone 7 (lanes 5 and 6) and M1-Stat3D clone 3 (lanes 7 and 8), stimulated with 100 ng/ml IL-6 for 3 days (lanes 2, 4, 6 and 8) or unstimulated (lanes 1, 3, 5 and 7), were subjected to Northern blot analysis for iNOS mRNA expression. The loaded RNA stained with ethidium bromide is shown in the lower panel.

Stat3 (data not shown). Apparently, Stat1F had no effect on IL-6-induced DNA-binding complexes (Figure 2B), in spite of the fact that it could inhibit IFNγ-induced Stat1 DNA-binding activity (data not shown). All of these results were consistent with the notion that both Stat3F and Stat3D exerted a dominant-negative effect on IL-6-induced activation of Stat3, although some effects on Stat1 activation were also detected, suggesting a complexity to the interactions and activation mechanisms of Stat proteins.

Dominant-negative Stat3 blocks IL-6-induced growth arrest and terminal differentiation but makes IL-6-induced growth-enhancing signals apparent in M1 transformants

Next we examined the effect of DN-Stat3 on IL-6-induced growth arrest and terminal differentiation in M1 cells. The M1 cells and the M1-Stat1F clones exhibited morphological changes characteristic of terminally differentiated macrophages, and expressed the inducible nitric oxide synthase (iNOS) gene following a 4 day treatment with IL-6. In contrast, neither the M1-Stat3F nor the M1-Stat3D clones exhibited morphological changes or iNOS mRNA expression in response to IL-6 (Figure 3A and B). In accordance with this, all of the clones of M1-Stat1F, as well as the parental M1 cells, gradually stopped proliferating after IL-6 treatment, whereas neither the M1-Stat3F nor the M1-Stat3D clones stopped growing in response to IL-6 (Figure 4A). These results showed that Stat3 was critical for IL-6-induced growth arrest and differentiation. Strikingly, IL-6 enhanced the growth of both the M1-Stat3F and the M1-Stat3D clones in a dose-dependent manner (Figure 4A and B), suggesting that IL-6 also generates the growth-enhancing signal in M1 cells, which is apparent only under the condition in which Stat3 activation is suppressed.

IL-6-induced growth-enhancing signals mainly cause shortening of the G_1 period

Parental M1 cells accumulated in G_0/G_1 phase in response to IL-6 as reported (Resnitzky *et al.*, 1992) (Figure 5A), whereas treatment of M1-Stat3D cells with IL-6 for 60 h resulted in a decrease in the population of cells in G_0/G_1 phase and a concomitant increase in the population of cells in $S/G_2/M$ phase (Figure 5A). This result, together with the fact that IL-6 shortened the doubling time of the M1-Stat3D clone from 20 h to 14 h, suggested that the growth-enhancing signals of IL-6 shorten the duration of the G_1 phase. This possibility was tested directly by cell cycle analysis using synchronized cells. The M1-Stat3D cells synchronized at mitotic metaphase by nocodazole treatment were released to progress through the cell cycle in the presence or absence of IL-6. The appearance of cells in S phase was observed as early as 7 h in the presence of IL-6, while in the absence of IL-6 it was observed at 12 h (Figure 5B). These results imply that in M1 cells, previously thought to be destined to stop proliferating and differentiate to macrophage in response

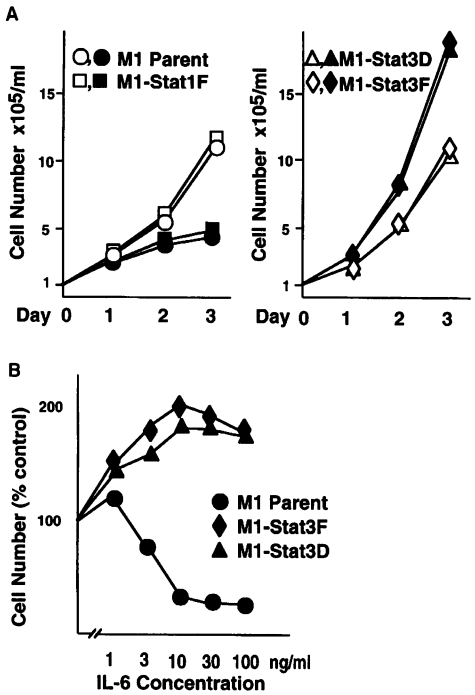

Fig. 4. Negative regulation of M1 cell growth by Stat3. (**A**) M1 parent cells (○, ●), M1-Stat1F clone 13 (□, ■), M1-Stat3F clone 7 (◇, ◆) and M1-Stat3D clone 3 (△, ▲) cells were seeded at 1×10^5/ml on day 0 and cultured in the presence (filled symbol) or absence (open symbol) of IL-6 at 100 ng/ml. Cells were harvested on days 1, 2 and 3 and counted for cell number. (**B**) Cell numbers of M1 parent (●), M1-Stat3F clone 7 (◆) and M1-Stat3D clone 3 (▲) after 3 days culture in the presence of the indicated concentrations of IL-6. Cell number values are expressed relative to the number cultured without IL-6 for 3 days.

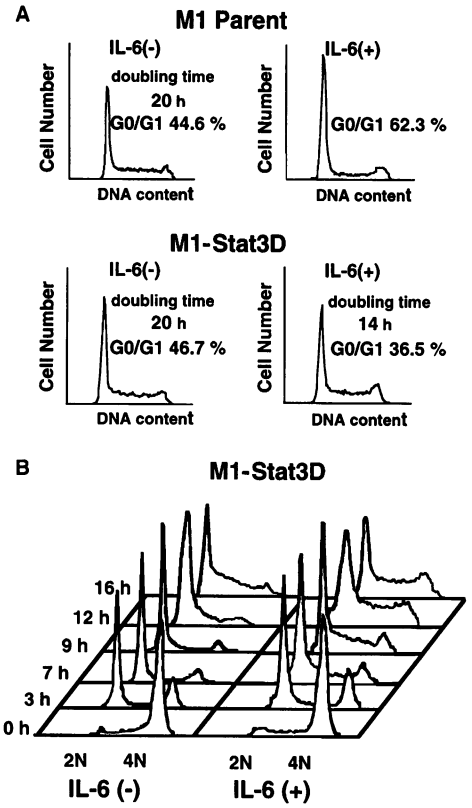

Fig. 5. IL-6-induced shortening of the G_1 period in M1-Stat3D cells. (**A**) DNA contents of M1 cells (upper panel) and M1-Stat3D clone 3 cells (lower panel) after 3 days culture in the presence or absence of IL-6 at 10 ng/ml. The doubling time of M1 and M1-Stat3D clone 3 was determined by the cell numbers counted at days 2 and 3 in parallel cultures. The percentages of cell population in G_0/G_1, S and G_2/M phases were determined by a Mod Fit LT program. For clarity, only the percentage of cells in G_0/G_1 was shown. (**B**) M1-Stat3D clone 3 cells were cultured in the presence or absence of IL-6 at 10 ng/ml for 40 h and treated with 0.2 μg/ml of nocodazole (Aldrich Biochem) for 16 h. The cells synchronized at metaphase were washed four times with DMEM containing 5% horse serum, cultured in the complete medium in the presence or absence of 10 ng/ml IL-6 for the indicated times and harvested for analysis of DNA contents.

to IL-6, IL-6 gives growth-enhancing signals causing primarily shortening of the G_1 period and that these signals are overcome by high level and sustained Stat3 activity.

The growth-enhancing signals may derive, at least partly, from the second tyrosine residue in the gp130

To show further the role of Stat3 in gp130-mediated growth regulation in M1 cells, we used four types of the M1 transformants expressing chimeric receptors containing the extracellular domain of growth hormone receptor (GHR) and the cytoplasmic portion of gp130, a signal transducing subunit of the IL-6 receptor complex, as developed and used in our previous study (Yamanaka *et al.*, 1996). The transformants used here were as follows: M1-GHR133 expressing the GHR chimeric receptor with the membrane-proximal 133 amino acid region of gp130 that is a minimum region for growth arrest and differentiation signals; M1-GHR-133F3 with a Tyr→Phe mutation at Tyr126 of the first YXXQ motif (Stahl *et al.*, 1995) of gp130, required for Stat3 activation; M1-GHR-133F2/3 with Tyr→Phe mutations at both Tyr118 and Tyr126 and M1-GHR-108 with the membrane-proximal 108 amino acid region of gp130 (Yamanaka *et al.*, 1996). As shown in the previous work, GH induced growth arrest (Figure 6) and terminal differentiation of M1-GHR-133, which was the only transformant that had an ability to activate Stat3 in response to GH among the four types of transformants used here (Yamanaka *et al.*, 1996). On the other hand, GH enhanced the growth of M1-GHR-133F3 but not M1-GHR-133F2/3 or M1-GHR-108 (Figure 6). The results suggest that growth-enhancing signals are generated by gp130 in M1 cells through the membrane-proximal 133 amino acids, but not the 108 amino acid region, and that the signals are apparent only when Stat3 is not activated, fully consistent with the results obtained with M1 transformants expressing DN-Stat3. Furthermore, the results showed that a region containing Tyr118 of gp130 initiates, at least in part, the growth-enhancing signal.

IL-6-suppression of c-myb and c-myc expression, but not IL-6 induction of egr-1, is a downstream event of Stat3

Because growth arrest at G_0/G_1 in M1 cells by IL-6 treatment is preceded by repression of c-*myb* and c-*myc* expression (Hoffman and Liebermann, 1991b), and since the deregulated expression of c-*myc* and c-*myb* inhibits IL-6-induced terminal differentiation (Hoffman and Liebermann, 1991a; Selvakumaran *et al.*, 1992), we examined whether expression of DN-Stat3 inhibited IL-6-repression of the genes. Both DN-Stat3s, but not Stat1F,

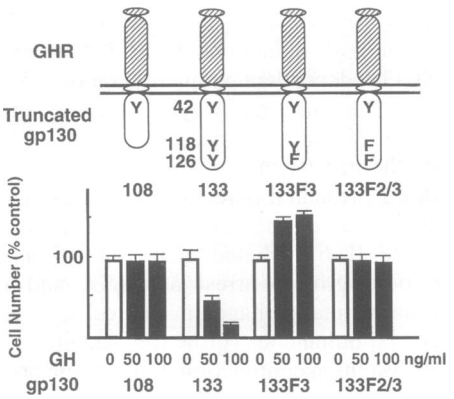

Fig. 6. IL-6-induced growth-enhancing signal(s) is, at least partly, generated by the region containing the second tyrosine (Tyr118) of truncated gp130. M1-GHR-133 clone 2, M1-GHR-133F3 clone 2, M1-GHR-133F2/3 clone 5 and M1-GHR-108 clone 1 (Yamanaka *et al.*, 1996) were seeded at 1×10^5/ml on day 0 and cultured in the presence (filled columns) or absence (blank columns) of GH at 50 or 100 ng/ml. Cell number values with a triplicate standard deviation are expressed relative to the number of M1-GHR-133 cells cultured without GH for 3 days. Three independent experiments were done using three clones for each of M1-GHR133, M1-GHR133F3, M1-GHR133F2/3 and M1-GHR108 with essentially similar results.

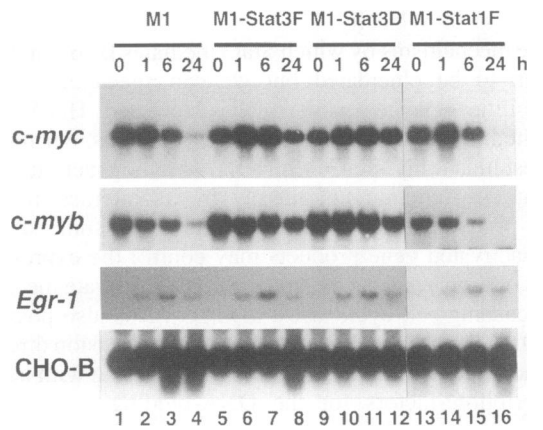

Fig. 7. DN-Stat3s, but not DN-Stat1, inhibited the IL-6-induced repression of c-*myb* and c-*myc* mRNA expression. Total RNAs from M1, M1-Stat1F clone 13, M1-Stat3F clone 7 and M1-Stat3D clone 3 cells cultured for the times indicated in the presence of 100 ng/ml of IL-6 were analyzed for steady state mRNA expression of c-*myb*, c-*myc* and *Egr-1* by Northern blotting using random primer labeled 1.6 kb *Sac*I–*Hin*dIII fragment containing the third exon of the c-*myc* gene, the 2.2 kb *Eco*RI fragment of c-*myb* cDNA and the 0.7 kb *Eco*RI fragment of *egr-1* cDNA. As a control, the expression level of CHO-B was shown. CHO-B cDNA (0.6 kb *Eco*RI–*Bam*HI fragment, a gift from J.Darnell,Jr) was used as a probe.

inhibited IL-6-induced repression of c-*myb* and c-*myc*, although they had no effect on the slight, transient, IL-6-induced increase of *c-myc* and induction of *egr-1* (Figure 7). The intact IL-6 induction of *egr-1* mRNA expression observed in the M1 transformants expressing Stat3D or Stat3F is consistent with the notion that *egr-1* mRNA expression is induced through a Stat3-independent pathway, most likely through a Ras-mitogen-activated protein (MAP) kinase pathway, initiated by molecules which require the second tyrosine residue (Tyr118) of the gp130 cytoplasmic domain in M1 cells (Yamanaka *et al.*, 1996). These observations indicate that IL-6-induced repression of c-*myb* and c-*myc*, but not IL-6 induction of *egr-1*, is a downstream event of Stat3 activation.

Discussion

To address the question as to whether Stat3 plays a role in IL-6-induced growth arrest and terminal macrophage differentiation in M1 cells, we made two forms of Stat3 mutants which were expected to act in a dominant-negative manner. Both mutants of Stat3, Stat3F with phenylalanine substitution at Tyr705, and Stat3D with mutations at the positions important for DNA binding, efficiently and specifically inhibited IL-6-activated reporter gene transcription in transient transfection assays (Figure 1B) and efficiently inhibited Stat3 activity as determined by an electromobility shift assay in stable transformants. The following explanation may be applied to the mechanisms by which Stat3F and Stat3D inhibit endogenous Stat3 activity. Stat3F may inhibit the activation process for both Stat3 and Stat1, presumably by competing with endogenous Stat proteins for their recruitment to the activated gp130 or gp130-JAK kinase complexes and Stat3D may inhibit the DNA-binding activity of Stats by making dimers unable to bind the target DNA. So far, the results obtained are consistent with the expected mechanisms above. However, considering that there was no remarkable increase in the levels of total Stat3 proteins in the presence of transfected Stat3F (Figure 2A) or Stat3D (not shown) and that all of the M1 transformants expressing Stat3F or Stat3D had reduced levels of mRNA for endogenous Stat3 as compared with those of M1 parental cells (data not shown), the protein level of endogenous Stat3 was likely to be reduced. Therefore, the resultant reduction in the expression level of endogenous Stat3 may also contribute to the reduced activity of Stat3 in both the M1-Stat3F and M1-Stat3D cells. This reduction seemed to be specific to the Stat3F or Stat3D clones, since it was not observed in the M1-Stat1F clones, as shown in Figure 2A. Although we do not know the relative expression levels of transfected Stat proteins as compared with those of endogenous Stat proteins, we believe that there must be enough transfected proteins over endogenous proteins, since each M1 transformant contained the mRNA for transfected Stat3 at a level that was several-fold higher than that for endogenous Stat3 (data not shown). There seems to be an autoregulation mechanism on the expression of Stat3 in this cell line. This matter is interesting but beyond the scope of this study. Stat3F, however, may inhibit other uncharacterized pathway(s) through competing with other signaling molecules for binding to the tyrosine-phosphorylated region(s) of the gp130. Since, in this study, we have obtained identical results using two different types of DN-Stat3 with different modes of action, it is appropriate for us to conclude that the inhibitory effect of DN-Stat3 occurs by inhibiting Stat3 function rather than through other non-specific mechanisms. The failure of Stat3F and Stat3D to inhibit the pathway causing induction of *egr-1* gene activation (Figure 7) indicates further the specificity of the dominant-negative function of the two Stat3 mutants. Therefore, we can reasonably conclude that Stat3 is critical for IL-6-induced terminal

differentiation, growth arrest and down-regulation of c-*myc* and c-*myb* in M1 cells.

The mechanisms by which Stat3 mediates IL-6 functions remain to be elucidated but we can speculate several possibilities. Since exogenously expressed HA-Stat3D inhibited, rather than mediated, IL-6 functions, our results suggest that only Stat3 with DNA-binding activity can execute its function not merely by its interaction with other proteins. Therefore, it is likely that some of the Stat3-activated gene products may control the expression levels of c-*myb* and c-*myc*, and eventually activate mechanisms causing cell cycle arrest at G_0/G_1. It is also possible that DNA-bound Stat3 may inhibit gene expression directly instead of activating these genes by competing with active transcription factors for the DNA-binding sites. Since previous reports showed that treatment of M1 cells with IL-6 results in an increase in the level of hypophosphorylated Rb (Resnitzky *et al.*, 1992) and in p21/WAF-1/CIP1, an inhibitor of cyclin-dependent kinases (Steinman *et al.*, 1994), and a decrease of the level of G_1 cyclins (Levy *et al.*, 1993), cyclin A (Resnitzky *et al.*, 1992) and E2F DNA-binding activity (Melamed *et al.*, 1993), molecules controlling cyclin-dependent kinase activities at G_1 and G_1/S or E2F-binding activities are likely downstream targets for Stat3.

As to the function of Stat3, it is also noted that in IL-6-stimulated M1 cells, Stat3 activity persisted for >24 h, as shown in Figures 2 and 3, and in the other extended experiments we observed strong Stat3 activity even after 48 h (data not shown). This activation pattern was not detected in other lineages of cells, including the hepatoma cell line HepG2 and B cell hybridoma cell lines, where Stat3 is activated rapidly and transiently by IL-6 (Wegenka *et al.*, 1993 and data not shown). The sustained nature of Stat3 activity in M1 cells may be important in causing growth arrest and terminal differentiation, as is the case for nerve growth factor-induced sustained activation of MAP kinase and PLCγ in PC12 cell differentiation (Qui and Green, 1992; Traverse *et al.*, 1992). It is also possible that transiently activated Stat3 may have a different and positive role in IL-6-induced cell growth in other lineages of cells, such as plasmacytomas and myelomas. The role of Stat1, a minor component of Stat proteins activated by IL-6, was hard to assess in IL-6 signals in M1 cells in this study, because Stat1 activity was weak and only detected when Stat3 was activated greatly, and there was no apparent inhibitory effect of Stat1F on Stat1 activity (Figure 3).

In addition to the role of Stat3 in growth arrest and differentiation of M1 cells, we showed that IL-6 simultaneously generates both growth-enhancing signal(s) causing shortening of the G_1 period and growth arrest- and differentiation-inducing signals in M1 cells, although the former signals were only apparent when Stat3 activation was suppressed. Although we do not know the precise nature of the growth-enhancing signals, the entity of the signals was substantiated by the use of M1-transformants expressing chimeric receptors (Figure 6). Our results showed that the second tyrosine residue at 118 of the cytoplasmic domain of gp130 was partly responsible for the growth-enhancing signals in M1 cells, because M1 cells expressing the chimeric receptor GHR-133F3, but not GHR-133F2/3, responded to GH by increasing the cell number by 40–50% at day 3 (Figure 6). Tyr118 is also responsible for IL-6 induction of *egr-1* gene expression through a Stat3-independent pathway (Figure 7; Yamanaka *et al.*, 1996). The same pathway initiated by the region containing Tyr118 may enhance the growth of M1 cells and activate the *egr-1* gene.

The findings presented here fully support our previous work (Yamanaka *et al.*, 1996) and establish that Stat3 is crucial for the IL-6-mediated gene regulation program leading to both growth arrest at G_0/G_1 and terminal differentiation to macrophage in M1 cells, overcoming IL-6-induced simultaneous growth-enhancing signal(s). The M1 cell system in conjunction with dominant-negative forms of Stat3 and a series of chimeric receptors should be a very interesting and useful system for studying the mechanisms by which an extracellular signal, such as IL-6, determines cellular fate.

Materials and methods

Cell lines

The murine myeloid leukemia cell line M1 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; GIBCO) supplemented with 10% horse serum, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere with 5% CO_2. M1 transformants expressing chimeric receptors containing the extracellular domain of GHR and the transmembrane and cytoplasmic domains of gp130 were as described previously (Yamanaka *et al.*, 1996). Of those, three clones for each of M1-GHR-108, M1-GHR-133, M1-133F3 and M1-133F2/3 were used in this study.

Construction of expression vectors

Human Stat1 cDNA and murine Stat3 cDNA were obtained by RT–PCR using HepG2 poly(A)$^+$ RNA or by screening a C57BL/6 murine thymus cDNA library (a gift from Dr Ogata), respectively. These full-length cDNAs were subcloned into pBluescriptII (Stratagene). Sequences of both cDNAs were verified by the dideoxy DNA sequencing method. An annealed oligonucleotide encoding the HA tag was added to the amino-terminus of Stat1 and Stat3 cDNA. Oligonucleotide-mediated mutagenesis was performed by using PCR. The tyrosine residue at 701 of Stat1 and that at 705 of Stat3 were replaced with phenylalanine to make HA-Stat1F and HA-Stat3F, respectively. To make HA-Stat3D, E434 and E435 of Stat3 were replaced with alanines (Horvath *et al.*, 1995). Each full-length cDNA encoding HA-Stat1, HA-Stat1F, HA-Stat3 or HA-Stat3F was subcloned into the pEF-BOS expression vector (a gift from Dr Nagata). HA-Stat1F and HA-Stat3F were also inserted into pCAGGS-Neo, in which a *SalI–XhoI* fragment containing the Neo gene linked to the thymidine kinase gene promoter from pMC1-Neo (Clontech) was subcloned at the *SalI* site of pCAGGS (a gift from Dr Miyazaki). HA-Stat3D was subcloned directly into pCAGGS-Neo.

Transient transfection assay

For transfection experiments, HepG2 cells were transfected with DNA by the calcium phosphate co-precipitation method. Typically, 1.2 μg of one of the reporter plasmids containing the fire fly luciferase gene, 1 μg of pEFLacZ [an expression vector containing the *LacZ* gene encoding β-galactosidase (Nakajima *et al.*, 1993)] as an internal control for transfection efficiency were used. Three micrograms of either pEF-BOS or pCAGGS-Neo expression vector system with a cDNA encoding either HA-Stat1, HA-Stat3 or their derivatives were co-transfected. Cells were incubated with DNA co-precipitates for 16 h, washed with phosphate-buffered saline (PBS), re-fed with complete medium for 20–24 h and stimulated with 100 ng/ml of IL-6 or IFNγ for 5 h. Around 40–45 h after transfection, cells were collected in 120 μl lysis buffer, and subjected to the assay for luciferase and β-galactosidase activity as described (Kojima *et al.*, 1996). The reporter genes contain either four copies of APRE or three copies of GRR in front of the minimal *junB* promoter linked to the luciferase gene. The response elements, APRE and GRR, are the APRE of the rat α2-macroglobulin gene, 5′-GCGCC-TTCTGGGAAGATCCTTACGGGAATTCAG-3′ and the IFNγ response region (the GRR) of the FcgRI gene, 5′-GTATTTCCCAGAAAAGG-3′, respectively. Only results with the reporter gene containing four copies

of APRE are shown, since the results with GRR elements were very similar to those of APRE in terms of the inhibitory effects of Stat mutants, except for the magnitude of the responses to IL-6 and IFNγ.

Transformation of M1 cells

To make stable transformants of M1 cells expressing dominant-negative Stat1 and Stat3, pCAGGS-Neo-HA-Stat1F, pCAGGS-Neo-HA-Stat3F and pCAGGS-Neo-HA-Stat3D were transfected into M1 cells by the electroporation technique and transformants were selected with 800 μg/ml of G418. M1 cells were maintained in DMEM containing 10% horse serum. The expression levels of exogenously expressed HA-Stat1F, HA-Stat3F or HA-Stat3D were tested by immunoprecipitation of equal amounts of whole-cell extracts from each clone with an anti-HA monoclonal antibody, 12CA5 (Boehringer Mannheim), followed by immunoblotting with anti-Stat1 monoclonal antibody (Transduction Laboratory) or anti-Stat3 antibody (data not shown). Eight clones for M1-Stat1F, eight for M1-Stat3F and three for M1-Stat3D were obtained. Results with representative clones, M1-Stat1F clone 13, M1-Stat3F clone 7 and M1-Stat3D clone 3 were shown throughout this report.

Immunoprecipitation and immunoblotting

Whole-cell extracts were prepared by lysing cells in ice-cold lysis buffer (50 mM HEPES pH 7.5, 0.1% Tween 20, 250 mM NaCl, 1 mM EDTA, 2.5 mM EGTA, 1 mM DTT, 0.1 mM sodium vanadate, 0.1 mM PMSF, 10 μg/ml leupeptin, pepstatin and aprotinin, and 1 mM sodium fluoride) with sonication. For immunoblotting, the anti-Stat3 immunoprecipitates were electrophoresed on 7.5% SDS–polyacrylamide gels, transferred to Immobilon P membranes (Millipore), probed with antibodies to phosphotyrosine (4G10, UBI) or Stat3 and detected for signals with the ECL system (Amersham).

Electromobility shift assay

Whole-cell extracts (20 μg) were incubated in a final volume of 20 μl [10 mM HEPES, pH 7.9, 80 mM NaCl, 10% glycerol, 1 mM dithiothreitol, 1 mM EDTA, 100 μg/ml poly(dI-dC)poly(dI-dC)] with a ^{32}P-labeled oligonucleotide containing an IR/IRF1 (Harroch *et al.*, 1994): 5′-GCGTGATTTCCCCGAAATGATGAGGCA-3′ (10 000 c.p.m., 0.5–1 ng) for 20 min at room temperature. The protein–DNA complexes were resolved on a 4.5% non-denaturing polyacrylamide gel containing 2.5% glycerol in 0.25× TBE (1× TBE is 0.13 M Tris base, 0.12 M boric acid and 2.0 mM EDTA pH 8.8) at room temperature and autoradiographed.

Morphology

M1 parent cells, M1-Stat1F clone 13, M1-Stat3F clone 7 or M1-Stat3D clone 3 were seeded in a Petri dish at a density of 5×10^4 per ml, and cultured in the presence or absence of 100 ng/ml IL-6 for 4 days. Cells were harvested, spun down on slide glasses by a Cytospin (Shandon) and subjected to May–Grünwald–Giemsa staining.

Cell cycle analysis

DNA contents were analyzed as follows. 1×10^6 cells were washed with PBS, resuspended in 100 μl of PBS and fixed by adding 900 μl of cold ethanol. The fixed cells were incubated with 300 μl of the staining buffer (1 mg/ml RNase, 20 μg/ml of propidium iodide and 0.01 % NP-40 in PBS) for 10 min and analyzed with FACSort (Becton Dickinson) using CELL QUEST software. Data were analyzed further using a Mod Fit LT program.

Northern blot analysis

Total cytoplasmic RNA was prepared from treated cultures by the NP-40–proteinase K method (Sambrook *et al.*, 1989). Fifteen micrograms of total RNA per sample were separated by electrophoresis in 1% agarose formaldehyde gels and transferred to Hybond N$^+$ (Amersham) nylon filters. Filters were hybridized with ^{32}P-labeled cDNA fragment overnight and washed three times with 0.1× SSC, 0.1% SDS at 56°C for 20 min and subjected to autoradiography. The amount of loaded RNA was tested by ethidium bromide staining or by the expression level of CHO-B mRNA. The probes used are as follows: the 0.8 kb *Hin*dIII–*Eco*RI fragment of murine iNOS cDNA (a gift from Dr Fujii), the 1.6 kb *Sac*I–*Hin*dIII fragment containing the third exon of the c-*myc* gene, the 2.2 kb *Eco*RI fragment of c-*myb* cDNA, the 0.7 kb *Eco*RI fragment of *egr-1* cDNA and the 0.6 kb *Eco*RI–*Bam*HI fragment of CHO-B cDNA (a gift from J.Darnell,Jr).

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References

Akira,S. *et al.* (1994) Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. *Cell*, **77**, 63–71.

Bazan,J.F. (1989) A novel family of growth factor receptors: a common binding domain in the growth hormone, prolactin, erythropoietin and IL-6 receptors, and the p75 IL-2 receptor β-chain. *Biochem. Biophys. Res. Commun.*, **164**, 788–795.

Coffer,P., Lütticken,C., van Puijenbroek,A., Klop-de Jonge,M., Horn,F. and Krüijer,W. (1995) Transcriptional regulation of the *junB* promoter: analysis of STAT-mediated signal transduction. *Oncogene*, **10**, 985–994.

Darnell,J.J., Kerr,I.M. and Stark,G.R. (1994) Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science*, **264**, 1415–1421.

Fujitani,Y., Nakajima,K., Kojima,H., Nakae,K., Takeda,T. and Hirano,T. (1994) Transcriptional activation of the IL-6 response element in the *junB* promoter is mediated by multiple Stat family proteins. *Biochem. Biophys. Res. Commun.*, **202**, 1181–1187.

Gearing,D.P. *et al.* (1992) The IL-6 signal transducer, gp130: an oncostatin M receptor and affinity converter for the LIF receptor. *Science*, **255**, 1434–1437.

Guschin,D. *et al.* (1995) A major role for the protein tyrosine kinase JAK1 in the JAK/STAT signal transduction pathway in response to interleukin-6. *EMBO J.*, **14**, 1421–1429.

Harroch,S., Revel,M. and Chebath,J. (1994) Interleukin-6 signaling via four transcription factors binding palindromic enhancers of different genes. *J. Biol. Chem.*, **269**, 26191–26195.

Hibi,M., Murakami,M., Saito,M., Hirano,T., Taga,T. and Kishimoto,T. (1990) Molecular cloning and expression of an IL-6 signal transducer, gp130. *Cell*, **63**, 1149–1157.

Hibi,M., Nakajima,K. and Hirano,T. (1996) IL-6 family cytokine and signal transduction: a model of cytokine system. *J. Mol. Med.*, **74**, 1–12.

Hirano,T. (1994) Interleukin-6. In Thompson,A. (ed.), *The Cytokine Handbook*. 2nd edn. Academic Press, London, pp. 145–168.

Hoffman,L.B. and Liebermann,D.A. (1991a) Interleukin-6- and leukemia inhibitory factor-induced terminal differentiation of myeloid leukemia cells is blocked at an intermediate stage by constitutive c-*myc*. *Mol. Cell. Biol.*, **11**, 2375–2381.

Hoffman,L.B. and Liebermann,D.A. (1991b) Suppression of c-*myc* and c-*myb* is tightly linked to terminal differentiation induced by IL6 or LIF and not growth inhibition in myeloid leukemia cells. *Oncogene*, **6**, 903–909.

Horvath,C.M., Zilong,W. and Darnell,J.E.,Jr (1995) A Stat protein domain that determines DNA sequence recognition suggests a novel DNA-binding domain. *Genes Dev.*, **9**, 984–994.

Ihle,J.N. (1995) Cytokine receptor signalling. *Nature*, **377**, 591–595.

Ihle,J.N. and Kerr,I.M. (1995) Jaks and Stats in signaling by the cytokine receptor superfamily. *Trends Genet.*, **11**, 69–74.

Ip,N.Y. *et al.* (1992) CNTF and LIF act on neuronal cells via shared signaling pathways that involve the IL-6 signal transducing receptor component gp130. *Cell*, **69**, 1121–1132.

Kimchi,A. (1992) Cytokine triggered molecular pathways that control cell cycle arrest. *J. Cell Biochem.*, **50**, 1–9.

Kojima,H., Nakajima,K. and Hirano,T. (1996) IL-6-inducible complexes on an IL-6 response element of the *junB* promoter contain Stat3 and 36 kDa CRE-like site binding protein(s). *Oncogene*, **12**, 547–554.

Levy,N., Yonish,R.E., Oren,M. and Kimchi,A. (1993) Complementation by wild-type p53 of interleukin-6 effects on M1 cells: induction of cell cycle exit and cooperativity with c-myc suppression. *Mol. Cell. Biol.*, **13**, 7942–7952.

Liebermann,D.A., Hoffman,B. and Steinman,R.A. (1995) Molecular controls of growth arrest and apoptosis: p53-dependent and independent pathways. *Oncogene*, **11**, 199–210.

Lütticken,C. *et al.* (1994) Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. *Science*, **263**, 89–92.

Matsuda,T., Yamanaka,Y. and Hirano,T. (1994) Interleukin-6-induced tyrosine phosphorylation of multiple proteins in murine hematopoietic lineage cells. *Biochem. Biophys. Res. Commun.*, **2001**, 821–828.

Melamed,D., Tiefenbrun,N., Yarden,A. and Kimchi,A. (1993) Interferons and interleukin-6 suppress the DNA-binding activity of E2F in growth-sensitive hematopoietic cells. *Mol. Cell. Biol.*, **13**, 5255–5265.

Miyaura,C., Onozaki,K., Akiyama,Y., Taniyama,T., Hirano,T., Kishimoto,T. and Suda,T. (1988) *FEBS Lett.*, **234**, 17–21.

Murakami,M., Narazaki,M., Hibi,M., Yawata,H., Yasukawa,K., Hamaguchi,M., Taga,T. and Kishimoto,T. (1991) Critical cytoplasmic region of the interleukin 6 signal transducer gp130 is conserved in the cytokine receptor family. *Proc. Natl Acad. Sci. USA*, **88**, 11349–11353.

Murakami,M., Hibi,M., Nakagawa,N., Nakagawa,T., Yasukawa,K., Yamanishi,K., Taga,T. and Kishimoto,T. (1993) IL-6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. *Science*, **260**, 1808–1810.

Nakajima,K. and Wall,R. (1991) Interleukin-6 signals activating *junB* and TIS11 gene transcription in a B-cell hybridoma. *Mol. Cell. Biol.*, **11**, 1409–1418.

Nakajima,K., Kusafuka,T., Takeda,T., Fujitani,Y., Nakae,K. and Hirano,T. (1993) Identification of a novel interleukin-6 response element containing an Ets-binding site and a CRE-like site in the *junB* promoter. *Mol. Cell. Biol.*, **13**, 3027–3041.

Nakajima,K., Matsuda,T., Fujitani,Y., Kojima,H., Yamanaka,Y., Nakae,K., Takeda,T. and Hirano,T. (1995) Signal transduction through IL-6 receptor: Involvement of multiple protein kinases, stat factors, and a novel H7-sensitive pathway. *Ann. NY Acad. Sci.*, **762**, 55–70.

Paonessa,G., Graziani,R., De Sero,A., Savino,R., Ciapponi,L., Lahm,A., Salvati,A.L., Toniatti,C. and Ciliberto,G. (1995) Two distinct and independent sites on IL-6 trigger gp 130 dimer formation and signalling. *EMBO J.*, **14**, 1942–1951.

Pearse,R.N., Feinman,R., Shuai,K., Darnell,J.E. and Ravetch,J.V. (1993) Interferon γ-induced transcription of the high-affinity Fc receptor for IgG requires assembly of a complex that includes the 91-kDa subunit of transcription factor ISGF3. *Proc. Natl Acad. Sci. USA*, **90**, 4314–4318.

Pennica,D. *et al.* (1995) Cardiotrophin-1. Biological activities and binding to the leukemia inhibitory factor receptor/gp130 signaling complex. *J. Biol. Chem.*, **270**, 10915–10922.

Qui,M.-S. and Green,S.H. (1992) PC12 cell neuronal differentiation is associated with prolonged p21ras activity and consequent prolonged ERK activity. *Neuron*, **9**, 705–717.

Resnitzky,D., Tiefenbrun,N., Berissi,H. and Kimchi,A. (1992) Interferons and interleukin 6 suppress phosphorylation of the retinoblastoma proteins in growth-sensitive hematopoitic cells. *Proc. Natl Acad. Sci. USA*, **89**, 402–406.

Sadowski,H.B., Shuai,K., Darnell,J.,Jr and Gilman,M.Z. (1993) A common nuclear signal transduction pathway activated by growth factor and cytokine receptors. *Science*, **261**, 1739–1744.

Sambrook,J., Fritsch,I.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sehgal,P.B., Grieninger,G. and Tosato,G. (1989) Regulation of the acute phase and immune responses: interleukin-6. *Ann. NY Acad. Sci.*, **557**, 1–583.

Selvakumaran,M., Liebermann,D.A. and Hoffman,L.B. (1992) Deregulated c-*myb* disrupts interleukin-6- or leukemia inhibitory factor-induced myeloid differentiation prior to c-*myc*: role in leukemogenesis. *Mol. Cell. Biol.*, **12**, 2493–2500.

Shabo,Y., Lotem,J., Rubinstein,M., Revel,M., Clark,S.C., Wolf,S.F., Kamen,R. and Sachs,L. (1988) The myeloid blood cell differentiation-inducing protein MGI-2A is interleukin-6. *Blood*, **72**, 2070–2073.

Stahl,N. *et al.* (1994) Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components. *Science*, **263**, 92–95.

Stahl,N., Farruggella,T.J., Boulton,T.G., Zhong,Z., Darnell,J.,Jr and Yancopoulos,G.D. (1995) Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. *Science*, **267**, 1349–1353.

Steinman,R.A., Hoffman,B., Iro,A., Guillouf,C., Liebermann,D.A. and el-Houseini,M.E. (1994) Induction of p21 (WAF-1/CIP1) during differentiation. *Oncogene*, **9**, 3389–3396.

Traverse,S., Gomez,N., Paterson,H., Marshall,C. and Cohen,C. (1992) Sustained activation of the mitogen-activated (MAP) kinase cascade may be required for differentiation of PC12 cells. *Biochem. J.*, **288**, 351–355.

Van Snick,J. (1990) Interleukin-6: an overview. *Annu. Rev. Immunol.*, **8**, 253–278.

Wegenka,U.M., Buschmann,J., Lütticken,C., Heinrich,P.C. and Horn,F. (1993) Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. *Mol. Cell. Biol.*, **13**, 276–288.

Wegenka,U.M. *et al.* (1994) The interleukin-6-activated acute-phase response factor is antigenically and functionally related to members of the signal transducer and activator of transcription (STAT) family. *Mol. Cell. Biol.*, **14**, 3186–3196.

Yamanaka,Y., Nakajima,K., Fukada,T., Hibi,M. and Hirano,T. (1996) Differentiation and growth arrest signals are generated through the cytoplasmic region of gp130 that is essential for Stat3 activation. *EMBO J.*, **15**, 1557–1565.

Yamasaki,K., Taga,T., Hirata,Y., Yawata,H., Kawanishi,Y., Seed,B., Taniguchi,T., Hirano,T. and Kishimoto,T. (1988) Cloning and expression of the human interleukin-6 (BSF-2/IFN beta 2) receptor. *Science*, **241**, 825–828.

Yin,T., Taga,T., Tsang,M.L., Yasukawa,K., Kishimoto,T. and Yang,Y.C. (1993) Involvement of IL-6 signal transducer gp130 in IL-11-mediated signal transduction. *J. Immunol.*, **151**, 2555–2561.

Yuan,J., Wegenka,U.M., Lütticken,C., Buschmann,J., Decker,T., Schindler,C., Heinrich,P.C. and Horn,F. (1994) The signalling pathways of interleukin-6 and gamma interferon converge by the activation of different transcription factors which bind to common responsive DNA elements. *Mol. Cell. Biol.*, **14**, 1657–1668.

Zhong,Z., Wen,Z. and Darnell,J.,Jr. (1994) Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science*, **264**, 95–98.

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