Many cytokines have dual functions of promoting or inhibiting cell proliferation; however, the molecular mechanism of the dual functions of cytokines is not well understood. Under normal conditions, interleukin (IL)-3 is required for Ba/F3 cell proliferation, whereas interferon (IFN)-γ inhibits Ba/F3 cell proliferation. It is known that Stat1 play a major role in inhibition of cell growth in response to IFN-γ. We have examined the possibility of whether IFN-γ can act as a growth-promoting cytokine if the Stat1 function is selectively blocked. We have established variant Ba/F3 cell lines in which Stat1 function is inhibited by a dominant-negative Stat1 mutant. Intriguingly, once Stat1 function is inhibited, IFN-γ can replace IL-3 acting as an essential growth factor for cell proliferation. To understand the molecular mechanism of regulation of cell proliferation by the cytokines, the signaling pathways and gene induction by IL-3 and IFN-γ are further studied. Although IL-3 activates mitogenic-activated protein kinase and Akt kinase, IFN-γ does not. Interestingly, both IL-3 and IFN-γ induce expression of the c-Myc gene that is not dependent on the Stat1 activity. Expression of a dominant-negative mutant Myc can block IFN-γ-mediated Ba/F3 cell proliferation, suggesting that c-Myc gene induction is required for IFN-γ-mediated cell proliferation. These findings suggest that IFN-γ intrinsically and simultaneously induces specific and conflicting signaling pathways and transcriptional programs that contribute to the potential dual effects of IFN-γ in promoting or inhibiting cell proliferation.

Many essential activities of mammalian cells are controlled by extracellular polypeptide ligands, including soluble cytokines and growth factors. The most notable feature of cytokines is the ability to stimulate cell growth. Many growth factor receptors contain intrinsic protein-tyrosine kinases (PTKs), whereas cytokine receptors are associated with PTK during activation (1, 2). Activation of PTKs can generate mitogenic effects through the RAS-MAPK pathway, phosphatidylinositol 3-kinase-Akt kinase pathway and other signal proteins (3–5).

Cytokines and growth factors can induce expression of proto-oncogenes, such as Myc, Fos, and other transcription factors that may positively regulate cell proliferation (3, 6, 7). Many growth factors and cytokines can strongly promote cell growth, including epidermal growth factor, platelet-derived growth factor, and interleukin (IL)-3. On the other hand, some PTK-activating cytokines can inhibit cell proliferation. For example, interferons are cytokines with growth suppression activities (8–10). We have previously suggested that the PTK-STAT signaling pathway, Stat1 in particular, may play a role in the negative control of cell growth by inducing gene expression of cyclin kinase inhibitors and caspases in response to extracellular stimuli (11–13).

However, it is intriguing that a specific PTK-activating cytokine may exert conflicting (positive and negative) effects on cell proliferation. It is often observed that the same cytokine is capable of stimulating and inhibiting cell growth depending on cell context, cytokine concentration, and other conditions (14). One striking example is epidermal growth factor, which promotes proliferation of keratinocytes and fibroblasts but inhibits hair follicle cells, squamous carcinoma, and some breast cancer cells (14, 15). Similarly, many other cytokines, such as IL-2, IL-4, and IL-6, etc., have routinely been classified as growth factors. However, they also have growth inhibiting activities (16–21). To understand the molecular mechanisms of the dual effects of cytokines on cell growth is one of the most important tasks in the studies of cytokine functions and their medical applications.

A working hypothesis is that every PTK-activating cytokine simultaneously initiates multiple signaling pathways that may have opposite (positive and negative) effects on cell growth. Thus whether a cytokine promotes or inhibits cell growth is determined by the relative strengths of positive or negative signals that may be induced intrinsically by the same cytokine-bound receptor.

In the present study, we have comparatively investigated mechanisms of IL-3- and IFN-γ-mediated cell proliferation and growth suppression. Our results indicate that IFN-γ is a cytokine with both growth stimulating and inhibiting activities, although the growth promoting potential is usually suppressed by Stat1. Intriguingly, IFN-γ induces expression of the c-Myc oncogene and promotes cell proliferation, which is independent of Stat1 activation. Thus, we suggest that cytokine-induced conflicting signaling pathways and gene expression programs are molecular bases for stimulating or inhibiting proliferation of a cell.

EXPERIMENTAL PROCEDURES

Cells and Cell Culture—Ba/F3-6, a subclone of Ba/F3 cell line, is a bone marrow-derived murine IL-3-dependent pre-B cell line (22). WT9 and WT10 are Ba/F3-6-derived stable transformant clones expressing the wild type human Stat1. Y701F1 and Y701F2 are Ba/F3-6-derived stable transformant clones expressing the mutant human Stat1 (see below). MycDN9 and MycDN22 are Y701F1-derived stable transformant clones expressing the mutant human c-Myc-In373 (27). For es-
establishment of these Myc-In373-expressing Y701F1 cells, the cells were selected with cotransfection of 5 μg of hygromycin-resistant gene plasmid (pMiwph) in one electroporation. Then 24 h after transfection, 2 mg/ml hygromycin B were used to select Myc-In373-expressing cells. The Myc expression was confirmed by Western blotting (see Fig. 7A). For Northern blot analysis, 10 μg of total RNA from each cell was prepared by TRIzol® Reagent (Life Technologies, Inc.). For Northern blot analysis, 5 μg of total RNA was electrophoresed on 0.8% agarose formaldehyde gels and transferred onto Zeta-Probe GT blottling membranes (Bio-Rad). Probe was labeled with [3H]thymidine incorporation of Ba/F3-6 cell in the presence of various concentrations of IL-3, B, [3H]thymidine incorporation of Ba/F3-6 cell in the presence of various concentrations of IFN-γ with (closed squares) or without (closed circles) IL-3.

chain reaction using a pair of primers, 5′-AACTCTAGAGGCTTCCACC-CCAGAGTC-3′ and 5′-AGATTCTGTTGTCGTCGTGCGCCCCCTTAC-3′, and first strand cDNA mixture as a template prepared from total RNA of IL-3-stimulated Ba/F3-6 cell with oligo(dT) primer and Moloney murine leukemia virus reverse transcriptase (New England Biolabs, Inc.); for c-Myc, a 0.47-kilobase EcoRI-XhoI fragment excised from pBSMyc that contains mouse c-Myc cDNA prepared by polymerase chain reaction using a pair of primers, 5′-AGAAATCTCACACCCAGA-GGGACTCT-3′ and 5′-AACTTAGAGGATGATAGGGCGTGTTT-3′, and first strand cDNA mixture as a template described above; and for IL-3, a 0.5-kilobase EcoRI-XhoI fragment excised from pBSMIL-3 that contains mouse IL-3 cDNA prepared by polymerase chain reaction using a pair of primers, 5′-AGAATCTGGAGGACAGGAGGAGACGAAAGCAAGGAT-3′ and 5′-TAGAAGAGACGAGCCAGACATC-3′, and first strand cDNA mixture as a template prepared from total RNA of T-cell receptor-stimulated mouse T cell with oligo(dT) primer and Moloney murine leukemia virus reverse transcriptase.

RNA Extraction and Northern blot Analysis—Total cellular RNA from each cell was precipitated with TDEnSeM (Roche Molecular Biochemicals) and hybridized at 65 °C for 16 h. After hybridization, 20 μg of total RNA was electrophoresed on 0.8% agarose formaldehyde gels and transferred onto Zeta-Probe GT blotting membranes (Bio-Rad). Probe was labeled with [α-32P]dCTP using a Random Primed DNA Labeling Kit (Roche Molecular Biochemicals) and hybridized at 65 °C for 16 h. In contrast, cell proliferation in response to IL-3 was inhibited by IFN-γ treatment (Fig. 1B). These results are consistent with the view that IL-3 is a growth factor, whereas IFN-γ is a growth inhibitor. To exclude the
possibility that the cell line Ba/F3-6 may have accumulated specific mutations during selection, or other potential clonal effects, we also isolated a number of other clones, which gave identical results as cell line Ba/F3-6 (data not shown).

Overexpressing of a Mutant Stat1 (Y701F) Blocks Transcriptional Activation of Stat1—Our hypothesis is that the Stat1 may play a critical role in negative control of cell growth in response to IFN-γ. Based on this hypothesis, we anticipate that IFN-γ should lose its growth inhibiting effect if Stat1 activation can be blocked in these Ba/F3 cells. It was known that tyrosine 701 of Stat1 is essential for activation (23). Mutation of this critical site may generate a mutant Stat1 protein that may still bind to phosphotyrosine sites through the SH2 domain (24) but itself is not phosphorylated and activated. Once this mutant Stat1 is overexpressed, it may generate a dominant-negative effect on activation of endogenous Stat1 protein.

We thus made a point mutation that converted tyrosine 701 to phenylalanine (Y701F). We isolated cell clones from Ba/F3-6 cells transfected with this mutant Stat1 or wild type Stat1 (see “Experimental Procedures” for details). Two representative clones expressing the exogenously introduced the mutant Stat1 (Y701F1 and Y701F2) or wild type (WT9 and WT10) Stat1 protein were analyzed (Fig. 2A). These exogenous Stat1 proteins were expressed at the same level (lane 2–5), but it was significantly higher than endogenous Stat1 protein expression (lane 1).

To check the effects of the expressed proteins, Ba/F3-6 and derivative cells expressing wild type (WT9 and WT10) or mutant (Y701F1 and Y701F2) Stat1 were stimulated with IFN-γ for 30 min. Specific STAT DNA binding activity induced by IFN-γ was determined by electrophoresis mobility shift assay using a high affinity m67-SIE probe (Fig. 2B; see “Experimental Procedures” for details). Stat1 activity was significantly increased in cells expressing wild type Stat1 (lane 4 and 7), whereas Stat1 activity was almost completely blocked in cells expressing Y701F mutant Stat1 (lane 10 and 12). These results indicate that overexpression of Y701F mutant inhibits activation of endogenous Stat1. The identification of Stat1 in the complex was confirmed by supershifted bands (SS*) by a specific anti-Stat1 antibody.

IL-3 could weakly activate Stat1 and Stat3 in Ba/F3 cells, and the identification of Stat1 and Stat3 in the complex were confirmed by supershifted bands (SS*, Stat1; SS**, Stat3) by anti-Stat1 or Stat3 antibodies (Fig. 2C, lanes 1–4). Stat1 activation by IL-3 was greatly enhanced in WT9 and WT10 (lanes 5–10) but was inhibited in Y701F1 and Y701F2 (lanes 11–14), suggesting again that Y701F mutant Stat1 acts as an efficient inhibitor of Stat1 activation.

To investigate whether Stat1 function in transcriptional activation was affected in these cells, we examined gene induction mediated by Stat1. The IRF-1 gene is an immediate early gene regulated by Stat1 in response to IFN-γ (25, 26). We found that expression of the IRF-1 gene was induced to a maximal level in the Ba/F3-6 cells in response to IFN-γ, and expressing more wild type Stat1 protein in WT10 cells did not further increase IRF-1 gene expression (Fig. 2D, upper panel). In contrast, in the cells expressing mutant Stat1 (Y701F1), IRF-1 gene induction by IFN-γ was almost completely inhibited (Fig. 2D). This result was consistent with the diminished Stat1 DNA binding activities in the Y701F mutant cells.

Furthermore, correlated with induced Stat1 activities, IL-3 weakly enhanced IRF-1 expression in parental Ba/F3-6 cells, whereas IL-3 greatly enhanced IRF-1 expression in cells expressing the wild type (WT10) but not mutant (Y701F1) Stat1 protein (Fig. 2E). These results further show that the Y701F mutation has a strong dominant-negative effect, and expression of the Y701F mutant protein in these cells is sufficient for blocking Stat1 activation and its function in gene regulation. These results also demonstrate that IL-3 can induce IRF-1 expression in a Stat1-dependent manner.

IFN-γ Stimulates Cell Proliferation in Y701F Cells That Are Defective in Stat1 Function—Based on the above results, we obtained three types of Ba/F3 cells that were derived from the identical genetic background but with different levels of Stat1 activities: the parental Ba/F3-6 clonal cells with “normal” Stat1 activity, the wild type Stat1 expressing cells (WT) with higher Stat1 activity, and the mutant Stat1 expressing cells (Y701F1) in which Stat1 activity was specifically inhibited. Using these different kinds of cells we next addressed the question of possible pleiotropic potentials of IFN-γ in regulation of cell proliferation.

We have hypothesized that IFN-γ may have potential in stimulating cell growth, but this potential may be overridden by the strong anti-proliferative effect of Stat1. Indeed, treatment of Ba/F3-6 cells with IFN-γ resulted in no increase in thymidine incorporation in these cells (Fig. 3A), indicating that IFN-γ did not promote DNA synthesis of Ba/F3-6 cells. However, as anticipated, in Y701F cells in which Stat1 function is defective, IFN-γ significantly promoted DNA synthesis (Fig. 3A). As controls, in wild type Stat1 expressing cells (WT9 and WT10), IFN-γ treatment did not increase DNA synthesis (Fig. 3B). These results suggested that IFN-γ can stimulate DNA synthesis if the negative signaling pathway is specifically inhibited.

On the other hand, IL-3 could greatly promote DNA synthesis in the parental Ba/F3 cells (Fig. 3C, see the curve labeled as Ba/F3-6), whereas inhibition of Stat1 function by expressing Y701F mutant further enhanced DNA synthesis by IL-3 (Fig. 3C, Y701F1). On the contrary, expressing the wild type Stat1 reduced DNA synthesis in response to IL-3 (Fig. 3C, WT10). These results indicate that IL-3, like IFN-γ, has negative signaling potentials that are mediated by Stat1 activation.

To exclude the possibility that these results were affected by clonal differences, Ba/F3-6 cells, which transiently expressed wild type Stat1, Y701F mutant Stat1, or vector alone were measured for the rates of DNA synthesis in the presence of various concentrations of IFN-γ (Fig. 3D). Similarly to the above results from clonal cells, IFN-γ promoted DNA synthesis in the cell population transfected with Y701F mutant but not the vector. In contrast to Y701F mutant, cells transfected with the wild type Stat1 further reduced DNA synthesis.

We next examined whether IFN-γ has a potential for maintaining long term proliferation of these cells (Fig. 4). Ba/F3-6 (closed circle), WT9 (open square), WT10 (open triangle), Y701F1 (closed square), and Y701F2 (closed triangle) cells were cultured in the presence of 100 ng/ml of IFN-γ but not IL-3. Within 7 days the parental Ba/F3-6 and wild type Stat1 expressing cells were all dead in the presence of IFN-γ. However, the mutant Stat1 expressing cells Y701F1 and Y701F2 were surviving and proliferating in response to IFN-γ (Fig. 4A). Strikingly, after longer culturing, these Y701F mutant expressing cells become stable cell lines that could proliferate in presence of either IFN-γ or IL-3 (Fig. 4B). The dependence on either IFN-γ or IL-3 was further shown by the observation that these Y701F cells become dead when none of the cytokines was provided (Fig. 4B, open symbols with solid lines). This dependence on IFN-γ or IL-3 also excludes the possibility that oncogenic mutations were occurred and selected in these Y701F cells. We also confirmed that IFN-γ does not induce IL-3 production in Y701 cells (data not shown). These results indicate that when the STAT signaling pathway is selectively blocked, IFN-γ can replace IL-3, acting as an essential growth factor for
FIG. 2. **Characterization of Ba/F3-6-derived transformants.**

A, equal expression of Stat1 and Stat1 mutant proteins in Ba/F3-6 and derivative cells. 10 μg of cell lysates were analyzed by protein immunoblotting assay with anti-Stat1 antibody (E23). Lanes 6 and 7 are Stat1 controls for cell lines mentioned in Fig. 7. A and B, DNA binding activities of Stat1 and Stat3 in response to IFN-γ and IL-3. Ba/F3-6 cell was stimulated with IFN-γ (B) or IL-3 (C) for 30 min and lysed. Electrophoresis mobility shift assay was performed with m67-SIE probe. Anti-Stat1 antibody (G16930) or anti-Stat3 antibody was added to the reaction mixture as indicated to verify the specificity of shifted bands. Supershifted bands were indicated as SS* for Stat1 or SS** for Stat3. Ba/F3-6 and Ba/F3-6-derived clonal cells WT9, WT10, Y701F1, and Y701F2 were stimulated with IFN-γ (B) or IL-3 (C) for 30 min and lysed. Electrophoresis mobility shift assay was performed with SIEm67 probe (53). Anti-Stat1 antibody (C24, Santa Cruz) was added to the reaction mixture as indicated to verify the specificity of shifted bands. Supershifted bands were indicated as SS*.

D and E, differential expression of the IRF1 gene in Ba/F3-6 and Ba/F3-6-derived transformants. Cells were stimulated with IFN-γ (D) or IL-3 (E) for various periods as indicated. Total RNA was extracted from these cells, and Northern blot analysis was carried out as described under "Experimental Procedures." Membranes were stained with methylene blue. 28S ribosomal RNA is indicated.
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ERK activity, Ba/F3-6, WT10, and Y701F1 cells were stimulated with IFN-γ or IL-3 for 15 min, and the whole cell lysates were analyzed with specific anti-phospho-ERK1/2 antibody (Fig. 5A). In all these cells, despite different STAT activities shown above, ERK1 and ERK2 were significantly phosphorylated and activated by IL-3 treatment, whereas no ERK phosphorylation was observed by IFN-γ treatment. The activation of ERKs in Y701F cells by IL-3 but not IFN-γ was confirmed by the Western blot with anti-ERK1/2 antibody showing shifted phosphorylation (Fig. 5B). Furthermore, because activation of Akt kinase was involved in cell survival (5), we also examine whether this kinase is activated by IL-3 or IFN-γ in mutant Y701F cells. Using a specific anti-phospho-Akt antibody, we found that the Akt kinase was phosphorylated in response to IL-3 but not to IFN-γ (Fig. 5C). The above results are consistent with the observation that IL-3 has a strong mitogenic effect and is required for survival of the Ba/F3 cells, whereas IFN-γ may not activate mitogenic pathways involving ERKs and Akt.

Both IL-3 and IFN-γ Induce Expression of Proto-oncogene Myc, Which Is Necessary for Mitogenic Response of IFN-γ—Although IFN-γ has anti-proliferative and apoptotic activities, we have shown above that IFN-γ also acts as a mitogen when Stat1 activity is inhibited (Figs. 3 and 4). We are specifically interested in finding genes induced by IFN-γ that have cell proliferative functions. After examining many potential genes involved in mitogenic stimulation, we found that both IL-3 and IFN-γ significantly induced c-Myc gene expression (Fig. 6). There are two important features of c-Myc mRNA induction by these two cytokines. First, c-Myc mRNA is induced within 30 min, indicating that c-Myc is probably a primary responsive gene in response to IL-3 and IFN-γ. Second, Unlike IRF-1, expression of c-Myc gene is induced at similar levels by IFN-γ in Ba/F3-6, WT10, and Y701F1 cells, suggesting that Stat1 activity is not required for c-Myc induction. Thus, in Y701F1 cells, the loss of Stat1 activity abolished expression of genes involved in the negative control of cell growth, such as IRF-1, but did not affect expression of c-Myc. Thus the potential mitogenic effect of IFN-γ becomes dominant in Y701F1 cells.

We next asked whether c-Myc function is necessary for IFN-γ-induced cell proliferation in Y701F cells. It was previously shown that an insertion mutation C-terminal to the DNA binding basic domain (In373) results in nonfunctional c-Myc pro-
tein, which can exhibit a dominant-negative effect (27). We expressed this dominant-negative form of c-Myc in Y701F cells. Two representative clones (MycDN9 and MycDN22) were used, and the Myc protein expression was examined by Western blot (Fig. 7A, lanes 3 and 4). The c-Myc expression did not affect expression of mutant Y701F Stat1 in these cells (Fig. 2A, lanes 6 and 7). Consistent with the suggestion that Myc function is essential, expression of the dominant-negative c-Myc protein diminished the cell proliferative effect induced by IFN-γ in these Y701F cells (Fig. 7B), suggesting that c-Myc function is essential for IFN-γ-mediated cell proliferation. These results provide a possible molecular mechanism of IFN-γ-stimulated cell proliferation.

Although this dominant-negative form of c-Myc had inhibited cell proliferation in Y701F cells in response to IFN-γ, it did not have a significant effect on IL-3-induced cell proliferation (data not shown). This is probably because IL-3 can activate other major mitogenic pathways (such as MAPK activation; Fig. 5) or because IL-3 induced Myc to such a high level (Fig. 6). The expression of the dominant-negative form of c-Myc is not sufficient for inhibition of endogenous Myc function.

**DISCUSSION**

Cell proliferation is regulated by extracellular signals. PTKs, which are often activated by cytokines, are believed to mediate positive signals for cell proliferation. Indeed, RAS-MAPK and phosphatidylinositol 3-kinase-Akt signaling pathways that are activated by PTKs promote mitogenic responses. Abnormal activation of these mitogenic pathways is a cause for oncogenesis. However, PTK activation may also induce cell differentiation, which requires cell growth arrest. A model was previ-
ous proposed in which the strength and duration of the MAPK signaling determined the specificities in proliferation or differentiation induced by PTK signaling (28).

We have proposed and examined a new hypothesis in this study in which cytokines simultaneously activate both positive and negative signaling pathways responsible for promoting and inhibiting cell proliferation. Whether a cytokine acts as a growth factor or inhibitor is determined by the relative strengths of the positive or negative signals that are intrinsically induced in a cell. Furthermore, we have presented data indicating specific signaling pathways and transcriptional regulations that could be responsible for the dual and conflicting effects of the cytokines.

A striking example is IFN-γ, which is usually classified as a growth inhibitor of many types of cells. IFN-γ can induce strong activation of Stat1 and expression of IRF-1. Both Stat1 activation and IRF-1 expression have been implicated in inhibition of cell proliferation (11, 26, 29). On the other hand, intriguingly, activation of Stat1 and expression of IRF-1. Both Stat1 activation is sensitive to IFN-γ in Stat1-deficient fibroblasts from Stat1 null mice, which are not sensitive than using simple Stat1-deficient cells, such as NIH3T3 fibroblasts (2). Numerous publications from a number of laboratories have shown similar results that tyrosine 701 is essential for dimer formation and Stat1-mediated gene expression (23). Thus the exogenously expressed STAT1 (WT10 in Fig. 3C) can reduce the rate of DNA synthesis induced by IL-3, whereas inhibition of Stat1 function by the dominant-negative Stat1 mutant (Y701F in Fig. 3C) can further enhance the proliferative effect of IL-3. Therefore, IL-3 has also dual potentials in promoting and inhibiting cell proliferation. In Ba/F3 cells, the Stat1 activation by IL-3 is much weaker than that by IFN-γ, the negative effect caused by Stat1 is not sufficient for overcoming the growth proliferative potential. Thus, IL-3 overall acts as a growth factor.

Our results, in large part, are derived from using the dominant-negative Stat1 mutant Y701F using the Ba/F3 cells. A recent publication has reported an observation that the mutant Y701F may still function in facilitating apoptosis induced by tumor necrosis factor (30), raising the possibility of an unusual and intriguing mechanism of STAT function. However, our results have clearly indicated that Y701F mutant cannot mediate any gene induction but inhibit endogenous Stat1 function in regulating gene expression, such as expression of IRF-1 (Fig. 2). Numerous publications from a number of laboratories have shown similar results that tyrosine 701 is essential for dimer formation and Stat1-mediated gene expression (23). Thus the observation on Y701F function in tumor necrosis factor-induced apoptosis may indicate a function of Stat1 that does not involve gene regulation through Stat1 dimer.

It should be pointed out that using Ba/F3 cells have the unique advantages. These cells are dependent on IL-3 for proliferation and survival; therefore a selection pressure can be used to find the hidden or potential signaling pathways that can mediate cell proliferation. Thus it will be more revealing and sensitive than using simple Stat1-deficient cells, such as Stat1-deficient fibroblasts from Stat1 null mice, which are not sensitive to IFN-γ effect.2

The provocative observation we made in this report is that IFN-γ can mediate cell proliferation through up-regulation of c-Myc gene expression. Unlike the regulation of IRF-1, induction of the c-Myc gene by IFN-γ is independent of Stat1 function (Fig. 6). The importance of c-Myc function in IFN-γ-mediated cell proliferation of Y701F Ba/F3 cells is confirmed by the experiment showing that expression of a dominant-negative mutant c-Myc protein can diminish IFN-γ-mediated cell proliferation (Fig. 7). The c-Myc gene has been previously implicated in cytokine-mediated cell growth (7, 31, 32). We have shown that IL-3 can strongly induce c-Myc expression in the all Ba/F3 cell lines (Fig. 6B), which may further enhance the growth proliferative effect of IL-3. However, we cannot exclude the possibility that IFN-γ induces expression of additional genes that have proliferative effect. Although expression of dominant-negative Myc has significantly diminished IFN-γ-mediated cell proliferation, some proliferative activities remain in these cells, suggesting that factors other than c-Myc may be also involved.

Our results suggest that Stat1 has an anti-proliferative effect in response to cytokine stimulation. This suggestion is consistent with the results from studies of Stat1 null mice, which are significantly more sensitive to tumor induction than those of wild type mice (33), and of other Stat1-deficient cells that are resistant to growth induction in response to IFNs and other cytokines (11, 29). We have previously shown that CDK inhibitor p21/WAF1 can be up-regulated through Stat1 in A431 and other epithelial cells. However, we could not detect changes of levels of p21/WAF1 expression in Ba/F3 cells in response to STAT activation (data not shown). It is possible that induction of CDK inhibitors through the STAT pathway is cell- and tissue-specific, and other members of CDK inhibitors or other cell cycle inhibitors may be involved in the Ba/F3 cells and other lymphocytes (20, 34–36), and growth inhibitory pathways other than Stat1 may exist (37).

Myc protein is a critical factor in regulation of cell proliferation and oncogenesis. It is believed that Myc protein can collaborate with Max, a constitutively expressed protein in regulation of gene expression (38). Src kinase has been shown to be involved in Myc activation in response to some cytokines and other signals (39). It is not known how Myc is regulated at the gene expression level by IFN-γ and other cytokines. However, it is clear from our studies that Stat1 may not have any effect on c-Myc induction in Ba/F3 cells. Besides c-Myc, c-Fos has also been implicated in cell proliferation in response to cytokines (6, 40). We have found that c-Fos is not induced by IFN-γ, although it is strongly induced by IL-3 in Ba/F3 cells.2 Expression of a dominant-negative Myc protein can block IFN-γ-mediated proliferation, suggesting that Myc protein, but not other oncogenes, is mainly responsible for the effect.

It is likely that other STAT proteins may have multiple roles in regulation of cell proliferation. Both Stat3 and Stat5 have been implicated in cell proliferation and transformation in a variety of cell systems (41–48). However, Stat3 and Stat5 are also believed to be responsible for cell differentiation and growth arrest (20, 35, 49–52). These seemingly conflicting results may be attributed to differences caused by specific cells, cytokine concentrations, effects of early or late genes, and stages of development. It is necessary in the future to dissect specific signaling proteins and transcriptional programs induced by these STAT proteins in detail.

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