

Roles of JAK kinases in human GM-CSF receptor signal transduction

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The receptors for human interleukin-3 (IL-3) and human granulocyte-macrophage colony-stimulating factor (GM-CSF), hIL-3R, hGM-CSFR, respectively, consists of two subunits, α and β , both of which are members of the cytokine receptor superfamily. Phosphorylation of tyrosine residues in the hGMR β subunit and several cellular proteins is observed after hGM-CSF stimulation. We analyzed the role of tyrosine residues in the hGMR β subunit and the nature of tyrosine kinase, JAK2, in hGMR signal transduction using several hGMR β subunit mutants. In addition to the box1 region, a membrane distal region (a.a. 544-589) of the hGMR β was required for c-fos activation. Only one tyrosine residue (Tyr577) existed within the region 544 to 589, and substitution of Tyr577 to phenylalanine in GMR β 589 resulted in loss of c-fos activation. In contrast, the same substitution in a wild type receptor did not affect GM-CSF induced activities such as c-fos messenger RNA (mRNA) induction and proliferation, but the substitution abolished Shc phosphorylation. These results suggest that the activation of Shc is not essential for c-fos activation and several tyrosine residues cooperate for c-fos activation. It is well documented that IL-3 or GM-CSF activate JAK2 in BA/F3 cells. The role of JAK2 in IL-3/GM-CSF functions, however, is largely unknown. We examined the role of JAK2 in GM-CSF induced signaling pathways. Dominant negative JAK2 (Δ JAK2) lacking the C-terminus kinase domain suppressed IL-3/GM-CSF induced c-fos activation and c-myc activation and proliferation, suggesting that JAK2 was involved in both signaling pathways. Protein tyrosine phosphatase SHP-2 (also called PTP 1D) and Shc were phosphorylated by IL-3/GM-CSF in BA/F3 cells; however, these phosphorylation events were inhibited by the expression of Δ JAK2. Taken together, these results indicate that JAK2 is a primary kinase regulating all the known activities of GM-CSF. JAK2 mediates GM-CSF induced c-fos activation through receptor phosphorylation and Shc/PTP 1D activation. (J Allergy Clin Immunol 1996;98:S183-91.)

Key words: JAK family kinases, GM-CSF receptor signal transduction

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that regulates the differentiation and proliferation of various hemopoietic cells.¹ The GM-CSF receptor is composed of two subunits, α and β , both of which are members of the cytokine receptor family.^{2,3} In both mice and humans, receptors for GM-CSF, IL-3, and IL-5 share a common β subunit. We previously showed that distinct signaling pathways for the human GM-CSF receptor (hGMR) were involved in c-myc mRNA induction and cell proliferation and in the induction of c-fos/c-jun mes-

Abbreviations used

GM-CSF:	granulocyte-macrophage colony-stimulating factor
hGMR:	human GM-CSF receptor
JAK2:	Janus Kinase 2

senger RNAs (mRNAs). These processes depend on a membrane proximal region (a.a. 455-544) and a more cytoplasmic region (a.a. 544-589) of the β subunit, respectively.⁴ To analyze the signals for the activation of c-myc and cell proliferation, we established protocols to monitor the activities of the c-myc promoter and DNA replication using a polyoma-replicon.^{5,6}

Experiments using these systems revealed that E2F/p107 complexes play an important role in the regulation of c-myc induction. Further analyses to clarify signaling molecules involved in c-myc activation and the initiation of DNA replication are

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necessary. Although GMR has no intrinsic tyrosine kinase activity, phosphorylation of tyrosine residues in GMR β and several cellular proteins are observed with GM-CSF stimulation.

The involvement of JAK family kinases in cytokine signaling has been well documented. The JAK family kinases comprise JAK1, JAK2, JAK3, and Tyk2 in mammalian species,⁷ but their roles in hematopoiesis remain to be determined. Interestingly, a dominant mutation of the *Drosophila* homologue *hop* gene (*hopscotch*^{Tumorous-lethal}) resulted in hematopoietic defects.⁸ Much attention has been directed toward the JAK family kinases because of their recognized roles in interferon (IFN) signals.⁹ Studies with IFN receptor signals revealed that JAK family kinases are involved in IFN-specific gene expression in cooperation with signal transducers and activators of transcription proteins.¹⁰⁻¹² Subsequent studies of IL-6 and mammary gland factor signaling revealed that the JAK-STAT system plays a role in cytokine specific gene expression.^{13,14} It is unclear, however, whether the JAK family kinases are involved in activities shared by many cytokines, such as the induction of cell proliferation or activation of immediate response genes. JAK2 is phosphorylated or activated by many cytokines, including IL-3 and GM-CSF,¹⁵⁻¹⁷ and the association of JAK2 with the common β subunit of IL-3R and GMR has been reported.¹⁸ In the current work, we attempted to determine whether JAK2 is involved in hGMR signals using BA/F3 and COS7 cells. We found that JAK2, which is activated through the box1 region of hGMR β , plays essential roles in both *c-myc* proliferation activation and *c-fos* *c-jun* activation signaling pathways.

RESULTS AND DISCUSSION

The role of tyrosine residues in the hGMR β subunit and *c-fos* activation

Similar to other growth factors such as epidermal growth factor and platelet derived growth factor, GM-CSF induces cell proliferation and transcription of immediate early genes such as *c-fos*, *c-jun*, and *c-myc*.^{4,19} In addition, GM-CSF induces the ID1, cln, and *egr* genes. Using a series of β subunit deletion mutants (Fig. 1A), we used transient analysis to examine the signaling mechanism that induces *c-fos* promoter activation (Fig. 1A).⁴ For this purpose, we used a luciferase plasmid fused with a 0.4 kilobase (kb) fragment of the *c-fos* promoter. As shown in Fig. 1B, deletion up to a.a. 589 did not affect *c-fos* promoter activation by hGM-CSF in BA/F3 cells, but further deletion to a.a. 544 significantly reduced this activity. Mutants 517 and 455 showed almost no

activation by hGM-CSF stimulation. These results indicated the involvement of the region between AA 544 and 589 for activation of the *c-fos* promoter. We had previously shown that the membrane proximal region (a.a. 455-517) containing box1 of the receptor β subunit was required for the activation of *c-myc* transcription and proliferation.^{4,6,20} Taken together, these results indicated that at least two hGMR signaling pathways existed: one pathway leading to *c-myc* proliferation activation, and the other pathway involving a more membrane distal region (a.a. 544-589), leading to activation of *c-fos* and *c-jun* transcription. Because several tyrosine residues are located within the C-terminal region of the β subunit, we examined the roles of tyrosine residues in hGMR β and the involvement of tyrosine kinase in GM-CSF signals. To analyze the requirement of tyrosine residues, we constructed several hGMR β mutants and analyzed signals through these mutants in BA/F3 cells (Fig. 1A). Six tyrosine residues are located in the C-terminal region of hGMR β , but only one tyrosine residue, 577, is located in the region covering 544 to 589, which is essential for *c-fos* activation. We first substituted tyrosine residue 577 to phenylalanine in the mutant 589. As expected, this mutant did not activate the *c-fos* promoter (Fig. 2A). Furthermore, this mutant also did not activate the tyrosine phosphorylation of cellular proteins or the GMR β (Fig. 2B). On the other hand, the same substitution in the wild type hGMR β did not affect these activities. This means that tyrosine 577 plays an essential role in the signaling of mutant 588, but in the wild type receptor, multiple tyrosine residues are involved in the *c-fos* gene activation.

It is tempting to speculate that multiple signals arise from each tyrosine residue. To test this hypothesis, we determined the involvement of tyrosine 577 in Shc and SHP-2 activation. Shc²¹ and SHP-2²² are known to be phosphorylated by IL-3/GM-CSF²³ and their positive roles in the MAP kinase cascade have been discussed.²⁴ We analyzed the hGM-CSF induced phosphorylation of these molecules by immunoprecipitation followed by Western blotting using the anti-phosphotyrosine antibody 4G10. BA/F3 cells expressing wild type α and mutant β subunits were depleted of mIL-3 for 5 hours and stimulated with 5 ng/ml of hGM-CSF for 5 minutes. The cells were harvested and lysed with lysis buffer. Immunoprecipitations were done with either anti-Shc or anti-SHP-2 proteins and Western blotting was performed with either anti-phosphotyrosine, 4G10, anti-Shc, or anti-SHP-2 antibodies. As shown in Figure 2C, Shc was phosphorylated with hGM-CSF stimulation in BA/FGMR cells.

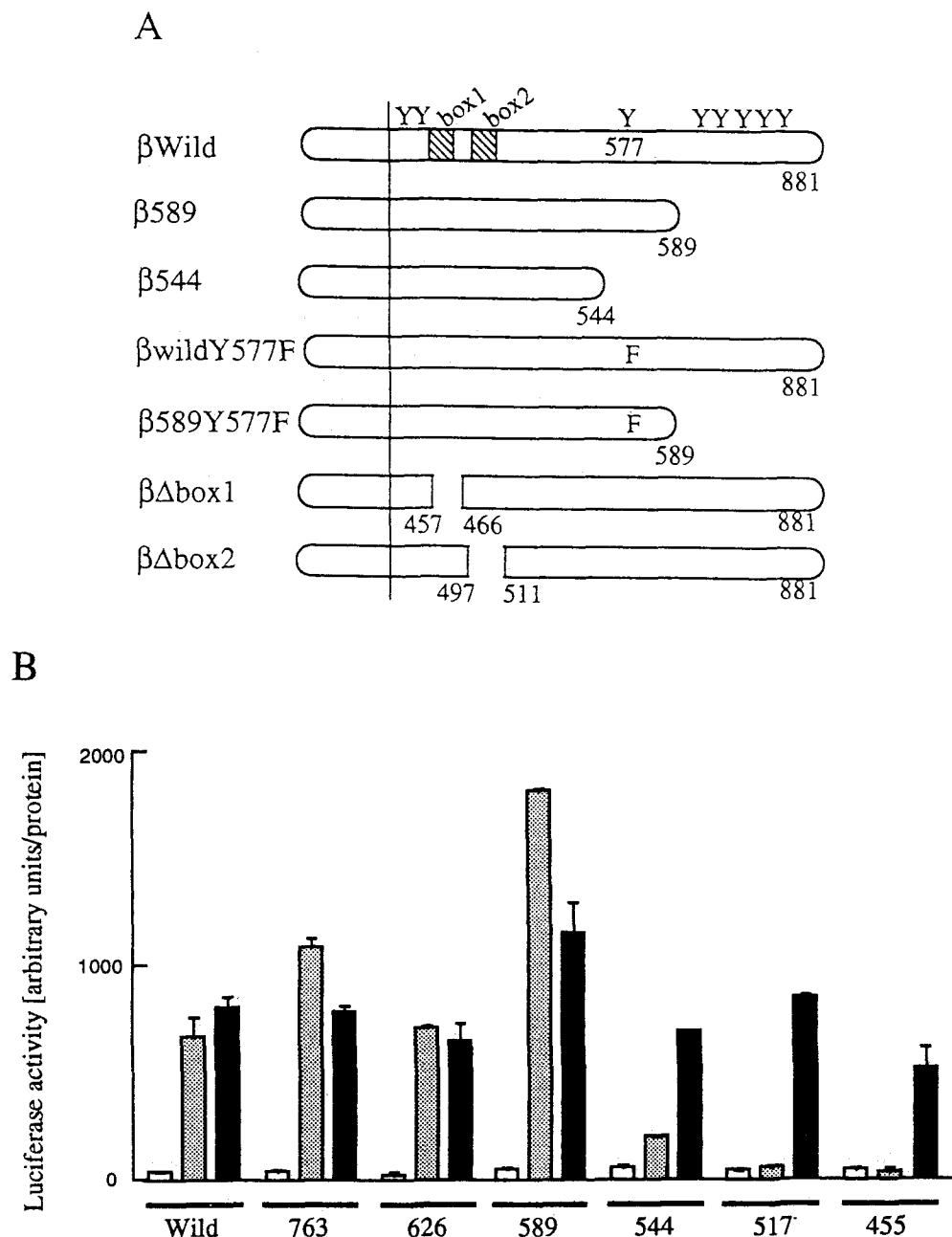


FIG. 1. Analysis of requirements of tyrosine residues of hGMR on various signals. **A**, Schematic drawing of various hGMR β mutants. **B**, Activation of *c-fos* promoter through various C-terminal deletion mutants of hGMR β .

The phosphorylation of Shc was abolished with a phenylalanine substitution of tyrosine 577 in GMR β mutant 588. For the same substitution in the wild type receptor, phosphorylation of Shc could not be observed even though this mutant could induce *c-fos* activation. These results suggested that tyrosine 577 of hGMR β was essential for Shc activation, and activation of Shc was not essential for activation of the *c-fos* promoter.

SHP-2 also was phosphorylated after hGM-CSF stimulation, and substitution of tyrosine 577 to phenylalanine in the 589 mutant abolished GM-CSF induced SHP-2 phosphorylation. In contrast to Shc activation, the wild type receptor containing phenylalanine 577 could phosphorylate SHP-2. These results suggested that tyrosine 577 was not essential for SHP-2 activation. Because dominant negative ras (N17ras) suppressed hGM-CSF in-

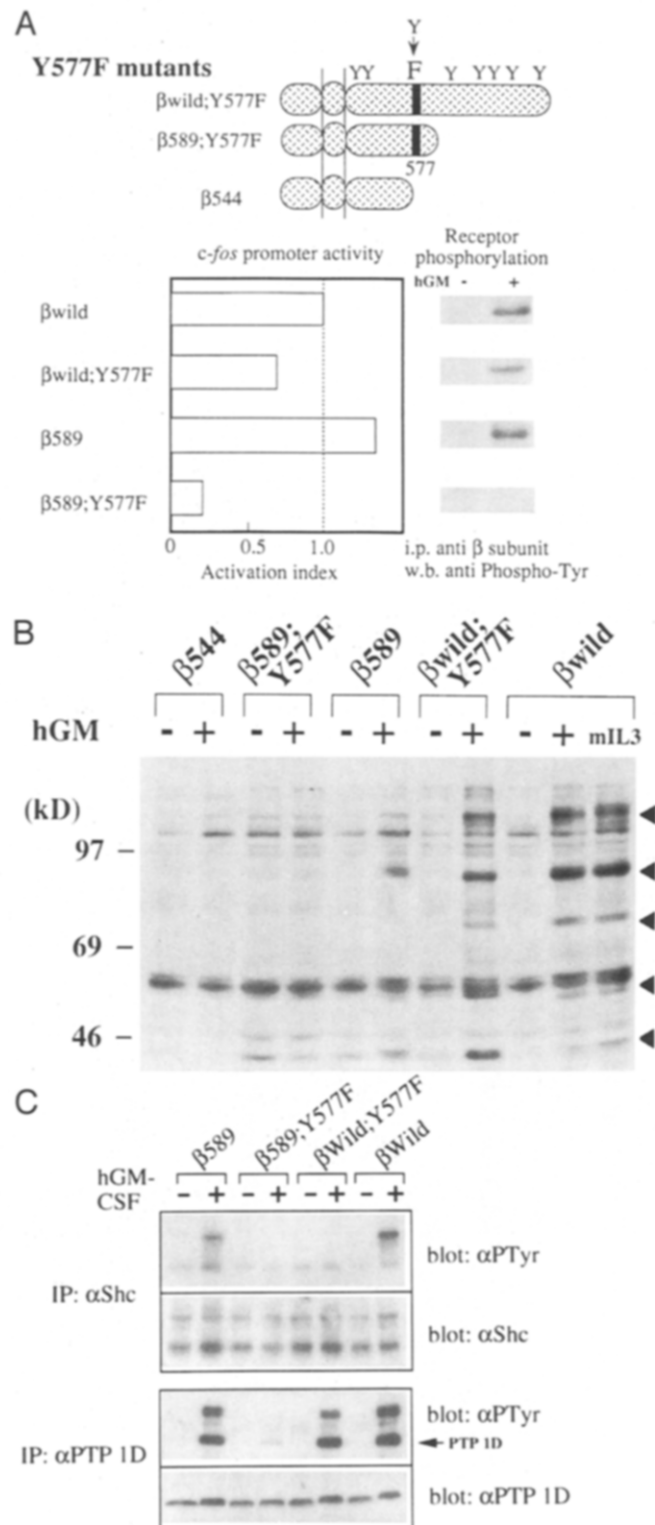


FIG. 2. Effects of tyrosine residue substitution in hGMR β on Shc and SHP-2(PTP 1D) phosphorylation. **A**, *c-fos*-luciferase activity in the BA/F3 cells expressing hGMR α and various mutants of hGMR β . BA/F3 cells expressing hGMR α and various mutants of hGMR β were depleted of mIL-5 for 5 hours and restimulated with hGM-CSF for 5 minutes. Cells were lysed and total proteins (**B**) or proteins immunoprecipitated by either anti-Shc or anti-SHP-2(PTP 1D) antibodies (**C**) were analyzed using Western blotting.

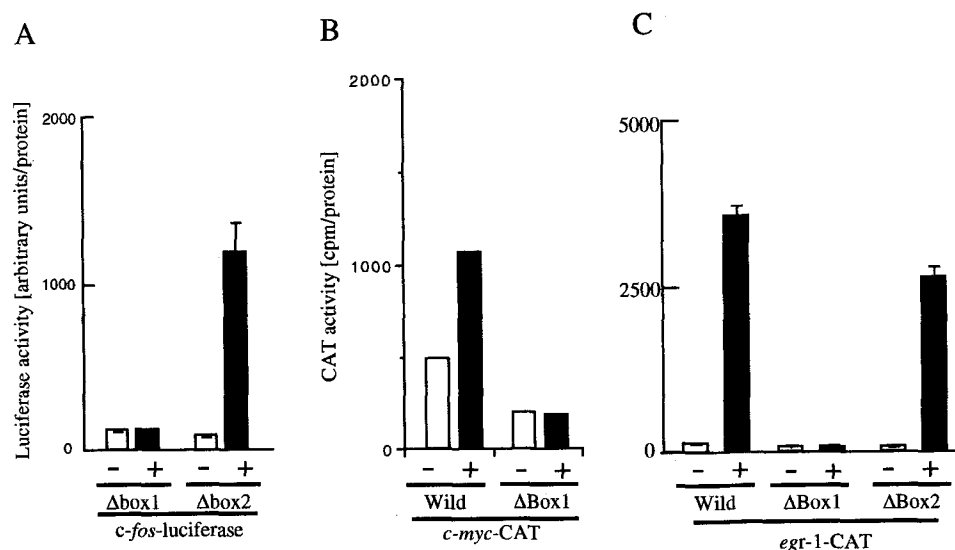


FIG. 3. Requirements of box1, box2 region for various hGM-CSF activities. **A**, Effects of deletion of either box1 or box2 on *c-fos* (**A**) or *c-myc* (**B**) and *egr-1* (**C**) promoter activations by hGM-CSF. BA/F3 cells expressing wild type hGMR α and hGMR β mutants lacking either the box1 or box2 were transfected by *c-fos*-luciferase, *c-myc*-CAT, or *egr-1*-CAT plasmids. Activation of these promoters by hGM-CSF was analyzed.

duced *c-fos* promoter activation,^{24a} we proposed that the signals transduced by Shc and PTP 1D were integrated at Ras and led to activation of *c-fos* transcription. We analyzed the involvement of the tyrosine kinase responsible for these events.

Requirement of box1, box2 regions for various hGM-CSF activities

Deletion analysis from the C-terminus region of hGMR β revealed the importance of the membrane proximal region including the box1 and box2 motifs of the hGMR β subunit. We examined the role of box1 in hGM-CSF signals by using an internal deletion mutant of the box1 region of hGMR β . We first analyzed the role of box1 and box2 in *c-fos*, *c-myc*, and *egr-1* promoter activations. Plasmids containing either *c-myc*-chloramphenicol acetyl transferase, *c-fos*-luciferase, or *egr-1*-CAT were transfected into BA/F3 cells expressing wild type hGMR α subunits and mutant β subunits lacking either the box1 or box2 region. Deletion of the box1 region of hGMR β abolished hGM-CSF induced *c-myc* promoter activation (Fig. 3A). *c-fos* or *egr-1* also were not activated in this mutant (Fig. 3B, C). In contrast, deletion of the box2 region did not affect activation of *c-fos* or *egr-1* promoters by hGM-CSF.

We analyzed the requirement of the box1 and box2 regions for hGM-CSF induced tyrosine residue phosphorylation of hGMR β and cellular pro-

teins. Figure 4 shows the Western blotting patterns with anti-phospho tyrosine antibody 4G10 of hGMR β immunoprecipitant (*a*) and total cellular proteins (*b*). The box1 region was essential for phosphorylation of these proteins and box2 was dispensable. These results suggested that in addition to the C terminal tyrosine residues, the box1 region was required for all the examined activities of hGM-CSF. Because hGMR does not contain a tyrosine kinase sequence, we speculate that tyrosine kinase interaction with the box1 region plays a role in the phosphorylation of hGMR β and further signaling events.

Role of JAK2 in GM-CSF signals

Several tyrosine kinases such as the src family tyrosine kinases or Tec kinase were reported to be activated by GM-CSF stimulation.^{15, 16, 25} JAK2 was activated by IL-3 or GM-CSF and it was reported that the membrane-proximal region, box1 of hGMR β , was required for the phosphorylation of JAK2.^{24a} We analyzed the roles of JAK2 in hGMR signals using a dominant negative JAK2, Δ JAK2. JAK2 lacking the C-terminal kinase domain was constructed. This mutant dominantly inhibited autophosphorylation of JAK2 but not JAK1 or JAK3 in COS7 cells (data not shown). We first analyzed the effect of Δ JAK2 on hGM-CSF induced phosphorylation of JAK2 and STAT5 in BA/F3 cells. hGMR are

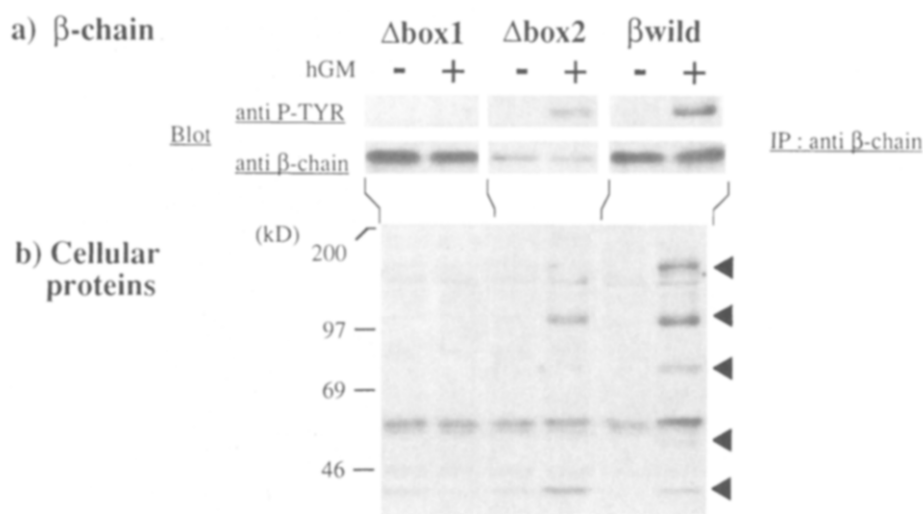


FIG. 4. Phosphorylation of hGMR β and cellular proteins through hGMR β mutants. Total proteins (**b**) or immunoprecipitants of hGMR β (**a**) were electrophoresed and tyrosine phosphorylation was analyzed using Western blotting using anti-phospho-tyrosine antibody or anti β -chain antibody.

transiently expressed in BA/F3 cells with and without Δ JAK2 and phosphorylation of JAK2 and STAT5 was examined by immunoprecipitation followed by Western blotting. As shown in Figure 5A, this phosphorylation was abolished by the coexpression of Δ JAK2. We then analyzed the involvement of JAK2 in the pathway for the *c-myc* gene and cell proliferation activation in BA/F3 cells. We previously established a *c-myc* promoter transient assay⁶ by using the CAT gene as a reporter gene. As shown in Fig. 5B, *c-myc* CAT activities induced by IL-3 or GM-CSF were completely suppressed by coexpression of Δ JAK2. DNA replication was monitored using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide analysis. Plasmids encoding hGMR α and β subunits were transiently expressed in BA/F3 cells and stimulated with 5 ng/ml of hGM-CSF. As shown in Fig. 5B, the hGM-CSF induced proliferation also was abolished by Δ JAK2. These results suggest that JAK2 plays an essential role in signal regulation of *c-myc* promoter activation and cell proliferation.

The signaling pathway regulating *c-fos* activation by GM-CSF is resistant to the tyrosine kinase inhibitor genistein.⁴ Because genistein does not affect GM-CSF induced JAK2 phosphorylation, we tested the possibility that JAK2 was involved in *c-fos* activation using *c-fos*-luciferase in a transient analysis. As shown in Figure 5C, Δ JAK2 completely suppressed *c-fos* promoter activation by IL-3 or GM-CSF in BA/F3 cells. In addition, *egr-1* activation, which required the same receptor domain as *c-fos* activation,

also was suppressed by coexistence of Δ JAK2. The hGM-CSF responsive sequence of the *egr-1* promoter did not contain a STAT binding sequence, and our current results indicate that the *egr-1* transcription mediated by hGM-CSF was driven mainly by serum response element and cAMP response element sites.^{24a} Taken together, these results suggest that JAK2 is involved in a STAT independent pathway for GM-CSF signals.

To analyze the role of JAK2 in the activation of SRE or *c-fos*, we next examined the effect of Δ JAK2 on the signal transducing molecules, Shc and SHP-2, which are known to be involved in the activation of the *c-fos* promoter. Phosphorylation of Shc or SHP-2 through transiently expressed hGMR was completely abolished by coexpression of Δ JAK2 (data not shown). Δ JAK2 apparently interfered with signaling event(s) upstream of Shc or SHP-2 activation, thereby indicating that JAK2 plays an essential role in the activation of both signaling molecules.

hGMR β is tyrosine phosphorylated by JAK2 in COS7 cells

The hGMR β is tyrosine phosphorylated in response to hGM-CSF stimulation, yet the nature of the involved tyrosine kinase is unknown. To determine whether JAK2 can phosphorylate hGMR β , we transiently reconstituted hGMR in COS7 cells with and without JAK2. hGMR α and hGMR β with the control vector JAK2 or Δ JAK2 were transfected into COS7 cells. The cells were cultured for 40 hours, after which the hGMR β was immunoprecipitated and tyrosine phosphorylation was monitored by

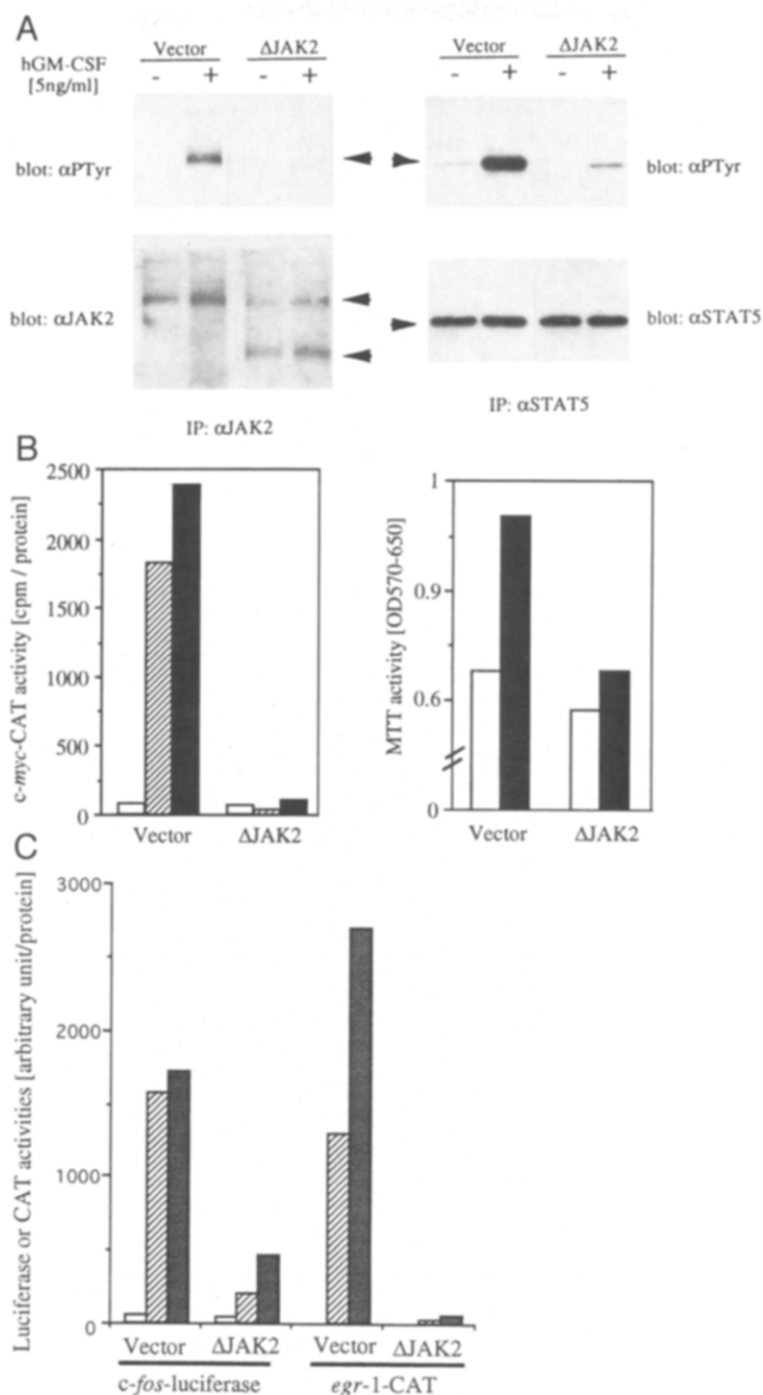


FIG. 5. Effect of dominant negative JAK2 in various activities by hGMR. **A**, Phosphorylation of JAK2 and STAT5 in the presence or absence of Δ JAK2. Plasmids encoding hGMR α and β were transfected to BA/F3 cells with either vector control or Δ JAK2. JAK2 or STAT5 proteins were immunoprecipitated and analyzed with Western blotting using anti-phospho tyrosine antibody 4G10. **B**, Effects of Δ JAK2 for c-myc promoter activation and proliferation. Δ JAK2 was cotransfected with c-myc CAT plasmid in BA/FGMR cells and CAT activity induced by either mIL-3 $\square$ or hGM-CSF $\blacksquare$ was analyzed using diffusion analysis. Plasmids encoding hGMR α and β subunits were transfected to BA/F3 cells with or without Δ JAK2 plasmids and proliferation activity was analyzed using MTT analysis. **C**, hGM-CSF induced c-fos and egr-1 promoter activation in the presence of dominant negative JAK2. c-fos-luciferase or egr-1-CAT plasmids were transfected to BA/FGMR cells with and without Δ JAK2, and luciferase or CAT activities induced by mIL-3 $\square$ or hGM-CSF $\blacksquare$ were analyzed.

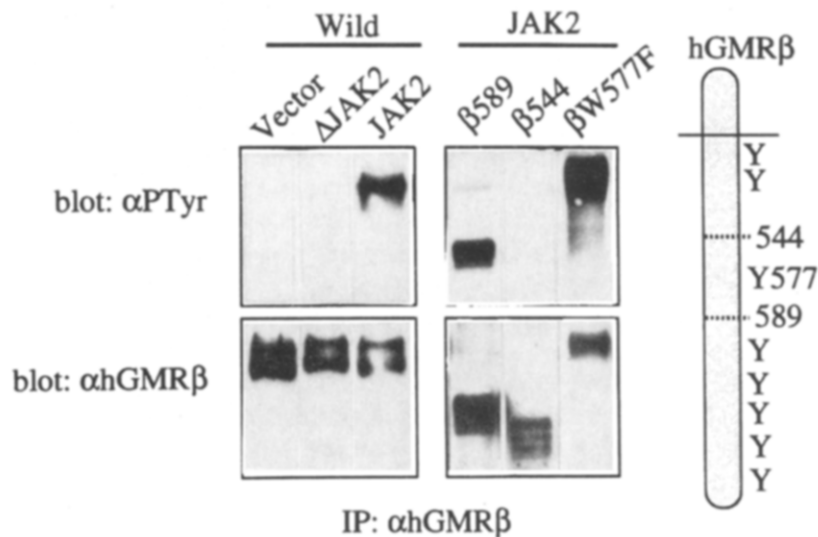


FIG. 6. Phosphorylation of various mutants and wild type of hGMR β subunits in COS7 cells in the presence of JAK2. Plasmids encoding wild type or various mutants of hGMR β were transfected to COS7 cells with JAK2 plasmid and phosphorylation of these receptors was analyzed using immunoprecipitation followed by immunoblotting.

Western blotting with anti-phospho tyrosine antibody 4G10. hGM-CSF failed to induce any detectable tyrosine phosphorylation of the hGMR β in COS7 cells (Fig. 6, left panel). Cotransfected JAK2, but not Δ JAK2, induced tyrosine phosphorylation of hGMR β even without hGM-CSF stimulation. To characterize tyrosine residues of hGMR β phosphorylated by JAK2, we used various hGMR β mutants (Fig. 1A). Wild type hGMR α and hGMR β mutants were cotransfected into COS7 cells with either the control vector or the JAK2 plasmid. Without JAK2, no hGMR mutant was phosphorylated even in the presence of hGM-CSF stimulation (data not shown). JAK2 induced phosphorylation of β 589 but not of β 544, which suggests that one target of phosphorylation is tyr577, and two other tyrosine residues within the membrane proximal region (tyr 450 and 452) were not phosphorylated under this condition. To determine whether tyr577 was the only target of JAK2, we examined whether BW577F, in which tyr577 is replaced by phenylalanine, could be phosphorylated by tyrosine. JAK2 induced phosphorylation of BW577F, showing that the other tyrosine residue(s) located closer to the C terminal side of hGMR β became phosphorylated in COS7 cells. These results are consistent with the pattern of hGMR β phosphorylation in BA/F3 cells.²⁰

CONCLUSION

In summary, we conclude that JAK2 is the primary kinase regulating all the known GM-

CSF signals such as the activation and proliferation of the *c-myc* promoter and *c-fos* promoter. Activation of JAK2 is dependent on the GMR box1 region and is essential for both signaling pathways and for JAK2 phosphorylation. JAK2 may be responsible for hGMR β phosphorylation and activates a signaling pathway including the phosphorylation of PTP 1D, which leads to the expression of the *c-fos* promoter. These results indicate that JAK2 is involved in multiple hGMR signaling pathways in addition to the STAT dependent pathway.

How is JAK2 activated by GM-CSF and its receptor? Because JAK2 reportedly is constitutively bound to the hGMR β subunit, a possible mechanism involves ligand-induced dimerization of the β subunit, leading to the phosphorylation and activation of JAK2 kinase and followed by phosphorylation of the β subunit. To test this possibility, we examined the organization of hGMR subunits using a chemical cross linker. BA/FGMR cells were treated with the chemical cross linker, BS3, and immunoprecipitation was done with anti- β subunit. We found that the β subunit dimer was formed even in the absence of hGM-CSF stimulation, and the α subunit was associated with the β subunit only when the receptor was stimulated.²⁶

In summary, we propose the following model for activation of hGMR: JAK2 forms a homodimer with the β subunit in the absence of hGM-CSF, and its

activation is triggered by hGM-CSF stimulation. Because JAK2 constitutively binds to hGMR β and GMR β forms a homodimer, the mechanism of JAK2 activation seems to be more complex than simple dimerization induced by hGM-CSF. JAK2 is involved in all the known activities, and signals are extinguished by hematopoietic cell specific protein tyrosine phosphatase SHP-1 (also called PTP 1C). We obtained evidence that the C-terminal region is responsible for the activation of hematopoietic cell specific protein tyrosine phosphatase SHP-1 (also called PTP 1C). We currently are working on the mechanism of JAK2 activation and investigating the roles of other tyrosine kinases such as JAK1, Src family kinases, and Tec in GM-CSF signals.

REFERENCES

1. Arai K, Lee F, Miyajima A, Miyatake S, Arai N, Yokota T. Cytokines: coordinators of immune and inflammatory responses. *Ann Rev Biochem* 1990;59:783-836.
2. Miyajima A, Kitamura T, Harada N, Yokota T, Arai K. Cytokine receptors and signal transduction. *Annu Rev Immunol* 1992;10:295-331.
3. Miyajima A, Mui A L-F, Ogorochi T, Sakamaki K. Receptors for granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5. *Blood* 1993;82:1960-74.
4. Watanabe S, Muto A, Yokota T, Miyajima A, Arai K. Differential regulation of early response genes and cell proliferation through the human granulocyte macrophage colony-stimulating factor receptor: selective activation of the *c-fos* promoter by genistein. *Mol Biol Cell* 1993;4:983-92.
5. Watanabe S, Ito Y, Miyajima A, Arai K. Granulocyte macrophage colony stimulating factor dependent replication of Polyoma virus replicon in hematopoietic cells: analyses of receptor signals for replication and transcription. *J Biol Chem* 1995;270:9615-21.
6. Watanabe S, Ishida S, Koike K, Arai K. Characterization of cis-regulatory elements of the *c-myc* promoter responding to human GM-CSF or mouse interleukin 3 in mouse proB cell line BA/F3 cells expressing the human GM-CSF receptor. *Mol Biol Cell* 1995;6:627-36.
7. Ihle JN, Witthuhn BA, Quelle FW, et al. Signaling by the cytokine receptor superfamily: JAKs and STATs. *Trends in Biochemical Science* 1994;19:222-7.
8. Luo H, Hanratty WP, Dearolf CR. An amino acid substitution in the *Drosophila* *hopTum-1* Jak kinase causes leukemia-like hematopoietic defects. *EMBO J* 1995;14:1412-20.
9. Darnell Jr JE, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 1994;264:1415-21.
10. Muller M, Briscoe J, Laxton C, et al. The protein tyrosine kinase JAK1 complements defects in interferon- α/β and - γ signal transduction. *Nature* 1993;366:129-35.
11. Watling D, Guschin D, Muller M, et al. Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon- γ signal transduction pathway. *Nature* 1993;366:166-70.
12. Velazquez L, Fellous M, Stark GR, Pellegrini S. A protein tyrosine kinase in the interferon α/β signaling pathway. *Cell* 1992;70:313-22.
13. Wegenka UM, Lutticken C, Buschmann J, et al. 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* 1994;14:3186-96.
14. Wakao H, Gouilleux F, Groner B. Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. *EMBO J* 1994;13:2182-91.
15. Silvennoinen O, Witthuhn BA, Quelle FW, Cleveland JL, Yi T, Ihle JN. Structure of the murine Jak2 protein-tyrosine kinase and its role in interleukin 3 signal transduction. *Proc Natl Acad Sci U S A* 1993;90:8429-33.
16. Quelle FW, Sato N, Witthuhn BA, et al. JAK2 associates with the βc chain of the receptor for granulocyte-macrophage colony-stimulating factor, and its activation requires the membrane-proximal region. *Mol Cell Biol* 1994;14:4335-41.
17. Taniguchi T. Cytokine signaling through nonreceptor protein tyrosine kinases. *Science* 1995;268:251-5.
18. Brizzi MF, Zini MG, Aronica MG, Blechman JM, Yarden Y, Pegoraro L. Convergence of signaling by interleukin-3, granulocyte-macrophage colony-stimulating factor, and mast cell growth factor on JAK2 tyrosine kinase. *J Biol Chem* 1994;269:31680-84.
19. Watanabe S, Mui A L-F, Muto A, et al. Reconstituted human granulocyte-macrophage colony-stimulating factor receptor transduces growth-promoting signals in mouse NIH 3T3 cells: comparison with signalling in BA/F3 pro-B cells. *Mol Cell Biol* 1993;13:1440-8.
20. Itoh T, Muto A, Watanabe S, Miyajima A, Yokota T, Arai K. Granulocyte-macrophage colony-stimulating factor provokes different modes of signaling leading to Ras activation and transcription of *c-fos* through tyrosine residues of the receptor β subunit. *J Biol Chem* 1996;271:7587-92.
21. Pellici G, Lanfrancone L, Grignani F, et al. A novel transforming protein (Shc) with an SH2 domain is implicated in mitogenic signal transduction. *Cell* 1992;70:93-104.
22. Vogel W, Lammers R, Huang J, Ullrich A. Activation of a phosphotyrosine phosphatase by tyrosine phosphorylation. *Science* 1993;259:1611-4.
23. Welham MJ, Dechert U, Leslie KB, Jirik F, Schrader JW. Interleukin (IL)-3 and granulocyte/macrophage colony-stimulating factor, but not IL-4, induce tyrosine phosphorylation, activation, and association of SHPTP2 with *grb2* and phosphatidylinositol 3'-kinase. *J Biol Chem* 1994;269:23764-8.
24. 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;14:509-17.
- 24a. Watanabe S, et al. *Blood* 1996 (in press).
25. Torigoe T, O'Connor R, Santoli D, Reed JC. Interleukin 3 regulates the activity of the Lyn protein-tyrosine kinase in myeloid-committed leukemic cell lines. *Blood* 1992;80:617-24.
- 25a. Watanabe S, Itoh T, Arai K. Roles of JAK2 in human GM-CSF receptor signals. *Molecular Biology of Hematopoiesis* 1996;453-62.
26. Muto A, Watanabe S, Miyajima A, Yokota T, Arai K. The β subunit of human GM-CSF receptor forms a homodimer and is activated via association with the α subunit. *J Exp Med* 1996;183:1911-6.