An Epidermal Growth Factor Receptor/Jak2 Tyrosine Kinase Domain Chimera Induces Tyrosine Phosphorylation of Stat5 and Transduces a Growth Signal in Hematopoietic Cells*  

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The Jak family of tyrosine kinases and the Stat family of transcription factors have been implicated in transducing signals from the hematopoietic growth factor receptors. To explore the role played by a member of the Jak family, Jak2, in hematopoietic cell growth signaling, we constructed a chimeric cDNA coding for the Jak2 tyrosine kinase domain linked to the extracellular and transmembrane regions of the epidermal growth factor (EGF) receptor (EGFR) and expressed the chimera in an interleukin (IL)-3-dependent cell line, 32D. When deprived of IL-3, EGF prevented apoptosis of the transfectants induced dose-dependent tyrosine phosphorylation of the EGFR/Jak2 chimera and Stat5, which correlated with the EGF dose dependence of cell proliferation. On the other hand, EGF did not induce tyrosine phosphorylation of other factors implicated in cytokine receptor signaling, including the IL-3 receptor; β unit, Jak kinases, Stat proteins other than Stat5, Shc, Syp, and mitogen-activated protein kinases. These results suggest that the activation of Jak2 may be sufficient for transducing a growth signal in hematopoietic cells by activating the Stat5 pathway or previously unidentified signaling pathways. In addition, because EGF induces homodimerization of the EGFR to activate its tyrosine kinase activity, the present study, which shows EGF-dependent activation of the EGFR/Jak2 chimera, implies that Jak2 may also become activated by homodimerization.

The growth and differentiation of hematopoietic cells are controlled by several hematopoietic growth factors, including erythropoietin (Epo), 1 interleukin (IL)-3, granulocyte-macrophage colony-stimulating factor, and granulocyte colony-stimulating factor. The receptors for these hematopoietic growth factors belong to the cytokine receptor superfamily and lack intrinsic tyrosine kinase activity (1, 2). However, recent studies have shown that the activation of all known cytokine receptors induces tyrosine phosphorylation and activation of the Jak family of cytoplasmic tyrosine kinases, which consists of Jak1, Jak2, Jak3, and Tyk2 (3, 4). The Jak2 kinases have a carboxy-terminal tyrosine kinase domain immediately preceded by a kinase-like (or "pseudokinase") domain (5). Although no other recognizable protein motifs, such as the Src homology 2 and Src homology 3 domains, are present, amino-terminal domains, termed the Jak homology (JH)1-5 domains, are conserved in the different family members (6). However, the roles of these domains are unknown. The activated Jak2 kinases induce tyrosine phosphorylation and activation of a class of latent cytoplasmic transcription factors known as signal transducers and activators of transcription (Stats) (3, 4). Six members of the Stat family, Stat1–Stat6, are presently known. The activated Jak2 kinases also induce tyrosine phosphorylation of the cytokine receptor cytoplasmic region and thereby recruit the Src homology 2-containing signaling factors to the activated receptors (3, 4, 7). The tyrosine-phosphorylated cytoplasmic region of the cytokine receptors may play a role in activation of the Ras/mitogen-activated protein (MAP) kinase pathway, which is mediated through tyrosine phosphorylation of Shc as well as Syp and their subsequent association with the Sos-Grb2 complex (3, 4, 8). Nevertheless, the role played by the activated Jak2 kinases or by the activation of these signaling pathways in hematopoietic cell growth signaling has remained to be determined.

Using IL-3-dependent cell lines expressing the transfected Epo receptor (EpoR) or its mutants, we previously showed that Jak2 associates with the membrane proximal region of the EpoR and becomes tyrosine phosphorylated and activated upon binding of the receptor with Epo (9, 10). Since the EpoR is hypothesized to homodimerize upon binding with Epo (11, 12), it is speculated that Jak2 may become activated by homodimerization and intermolecular autophosphorylation through binding with the EpoR homodimer. The region of the EpoR required for binding with Jak2 was also required for induction of cell proliferation (13, 14), thus suggesting that Jak2 may play a critical role in growth signaling from the EpoR. Furthermore, Jak2 also associates with and becomes activated by other cytokine receptors that play important roles in growth regulation of hematopoietic cells, such as the common β subunit of the receptors for granulocyte-macrophage colony-stimulating factor, IL-3, and IL-5 or the granulocyte colony-stimulating factor receptor (3, 15, 16). Jak2 may, therefore, play a particularly important role in the growth control of hematopoietic cells.

To explore the role of Jak2 plays in the growth signaling from the hematopoietic growth factor receptors and to examine the activation mechanism of Jak2, we constructed a chimeric construct in which the intracellular domain of the EGFR was replaced by the tyrosine kinase domain of Jak2. When ex-
pressed in an IL-3-dependent cell line, the chimeric receptor was tyrosine phosphorylated upon EGF binding and transduced an EGF-dependent growth signal. EGF stimulation of the transfectants also induced tyrosine phosphorylation of Stat5. However, other signaling molecules known to be involved in cytokine receptor signaling, including other Stats and those involved in activation of the Ras/MAP kinase pathway, did not show any change in tyrosine phosphorylation status after EGF stimulation. These data suggest that Jak2 may become activated by dimerization and transduces a growth signal in hematopoietic cells by activating Stat5 or previously unidentified signaling pathways.

MATERIALS AND METHODS

Cells and Reagents—A clone of 32D cells, an IL-3-dependent cell line originally isolated from long-term bone marrow cultures, was maintained in RPMI 1640 supplemented with 10% fetal calf serum (FCS) and 10% WEHI-3-conditioned medium as a source of IL-3, as described previously (14). Murine IL-3 and human EGF were purchased from Life Technologies, Inc.

The murine Jak2 cDNA (15) was kindly provided by Dr. J. Ihle (St. Jude Children's Research Hospital, Memphis, TN). The human EGF cDNA (17) was obtained through the Riken Gene Bank (Ibaraki, Japan). The mammalian expression plasmid pcDNA3 was purchased from Invitrogen (San Diego, CA).

Rabbit antiserum against Jak1, Jak2, Jak3, Stat3, Stat4, Stat5a, and Stat6 were kindly provided by Dr. J. Ihle (15, 18–20). An anti-Jak2 antibody raised against a synthetic peptide corresponding to amino acids 1110–1129 mapping at the carboxyl terminus of murine Jak2 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Stat5b, anti-Tyk2, and anti-Syp antibodies were also from Santa Cruz Biotechnology. Monodonal antibodies against phosphotyrosine (4G10) and EGF (LA2Z), as well as antibodies against Shc, Vav, and extra-cellular signal-regulated kinase 1 (erk1/CT), were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). A monoclonal antibody against the mouse IL-3 receptor β subunit (HC) was from MBL (Nagoya, Japan). An anti-Stat1 monoclonal antibody was purchased from Transduction Laboratories (Lexington, KY).

Construction of an Expression Plasmid for an EGF/Jak2 Chimera and Transfection into 32D Cells—To construct an expression plasmid for an EGF/Jak2 chimera containing the Jak2 tyrosine kinase domain linked to the extracellular and transmembrane regions of the EGFR, a portion of the EGF cDNA coding for amino acids 24 to 647 was amplified by the polymerase chain reaction using 5′ and 3′ primers of 5′-CCGTACGGAGGCGGATATCGG-3′ and 3′-CTCTTACGTGGAACGCCGGATATCGG-3′, respectively. The primer sequences were designed to add KpnI and EcoRV recognition sequences at the 5′ and 3′ ends, respectively, of the amplified fragment, and these sites were used for directional subcloning into the KpnI-EcoRV sites of the expression plasmid pcDNA3. The murine Jak2 cDNA was digested with StuI and Nhel in the coding and 3′ noncoding regions, respectively. The 1100-bp base pair Stul-Nhel fragment, coding amino acids 796–1129, was then directionally subcloned in frame into the EcoRV-Xbal site of the pcDNA3 clone containing the 5′ portion of EGF cDNA to create pcD/EG-J2. The structure of pcD/EG-J2 was confirmed by digestion with multiple restriction enzymes. As shown in Fig. 1, the chimera protein EG-J2 coded by pcD/EG-J2 contains the signal peptide, extracellular domain, and transmembrane region of the EGFR followed by an Asp residue resulting from the artificially added EcoRV recognition sequence, the carboxyl-terminal portion of the tyrosine kinase-like domain (JH2) of Jak2, and the entire Jak2 tyrosine kinase domain (JH1).

Transfection of pcD/EG-J2 into 32D cells and isolation of clones expressing the EG-J2 chimera were carried out essentially as described previously (13, 14). In brief, 32D cells were transfected with 10 μg of pcD/EG-J2 by electroporation and selected in medium containing G418. Three clones, isolated by limited dilution, were subjected to the subsequent studies.

Cell Growth Assays—For measurement of the cell number increase, cells were seeded into a 6-well plate at a density of 1 × 10⁶/ml in 10% FCS-containing RPMI 1640 supplemented with the indicated growth factors. Viable cell counts were determined by trypan blue staining. Alternatively, cells were seeded into a 96-well plate at a density of 1 × 10⁶/ml in 10% FCS-containing RPMI 1640 supplemented with the indicated concentrations of EGF or with 10% WEHI-3 conditioned medium. After 2 days, the number of viable cells was measured by the sodium 3-[1-(phenylamino carbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydroxylamine colorimetric assay (Boehringer-Mannheim) according to the manufacturer’s recommendation.

Analysis of Apoptosis of Cells—To detect apoptosis of cells, low molecular weight chromosomal DNA was isolated essentially as described (21). Briefly, 10⁷ cells were cultured for 16 h in 10% FCS-containing RPMI 1640 without growth factor or supplemented with 10% WEHI-3-conditioned medium or with 100 ng/ml EGF. The cells were then lysed in 600 μl of a lysis buffer (10 mM Tris-HCl, pH 7.5, 10 mM EDTA, and 0.2% Triton X-100) and incubated on ice for 10 min. After centrifugation at 15,000 rpm for 10 min, the supernatant was extracted first with phenol and then with phenol:chloroform:isoamyl alcohol (25:24:1) and subjected to ethanol precipitation. The precipitate was dissolved in TE buffer (10 mM Tris-HCl, pH 7.5, and 1 mM EDTA), treated with RNase A (0.6 mg/ml) at 37°C for 30 min, and electrophoresed on a 2% agarose gel. DNA fragments were then visualized by ethidium bromide staining.

Immunoprecipitation and Immunoblotting—For stimulation with EGF or IL-3, cells were starved for 12 h in 10% FCS-containing RPMI 1640 without IL-3. The cells were then left unstimulated as a negative control or stimulated with EGF (100 ng/ml) or IL-3 (300 ng/ml) conditioned medium at 37°C for 5 min. The cells were lysed in a lysis buffer containing 1% Triton X-100, 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml each of aprotinin and leupeptin.

For immunoprecipitation, a relevant antibody was added to the lysates, along with protein A-Sepharose beads, and incubated for 4 h at 4°C. After extensive washing, the immunoprecipitates, each obtained from 1 × 10⁷ cells, were resolved by SDS-polyacrylamide gel electrophoresis (PAGE), transferred to polyvinylidene difluoride membranes, immunoblotted with the indicated antibody, and developed by the enhanced chemiluminescence (ECL) system (Amersham Corp.). Immunoprecipitation of denatured MAP kinasess was carried out as described previously (8). For reprobing with a different antibody, the membranes were treated at 50°C for 30 min with stripping buffer containing 100 mM 2-mercaptoethanol, 2% SDS, and 62.5 mM Tris-HCl (pH 6.7). The results shown are representative of experiments repeated at least twice.

RESULTS

Expression of an EGF/Jak2 Chimera in 32D Cells—An expression plasmid for an EGF/Jak2 chimera, EG-J2, in which the intracellular domain of the EGF was replaced by the Jak2 tyrosine kinase domain, as shown in Fig. 1, was constructed as described in "Materials and Methods." The plasmid was transfected into an IL-3-dependent murine myeloid cell line, 32D, which does not express endogenous EGF (22). Transfectants were selected in G418-containing medium, and three clones, isolated by limited dilution, were subjected to the subsequent studies.

The three clones, named EJ-1, EJ-2, and EJ-3, were first analyzed for expression of the EG-J2 chimera by immunoprecipitation and immunoblotting. The chimera receptor was first immunoprecipitated from cell lysates of these clones or parental 32D cells with an monoclonal antibody against the extracellular domain of human EGF and then immunoblotted with...
a rabbit antibody (anti-jak2c) raised against a synthetic polypeptide corresponding to amino acids 1110–1129 at the carboxy terminus of murine Jak2. As shown in Fig. 2, an indistinct band in the 140-kDa range was observed in samples from the three transfected clones but not in that from the parental clone. This band was not detected by reprobing with an anti-jak2 antibody (anti-jak2m) against amino acids 758–776 in the JH2 region, which are not contained in EG-J2 (data not shown). These data demonstrated that the three transfected clones expressed the EG-J2 chimera. The indistinct nature of the chimeric protein most likely reflects the glycosylation of the EGFR extracellular domain, which may also explain the apparent molecular mass of the chimera that is larger than that predicted from its amino acid sequence. Since the three transfected clones expressed the chimera at comparable levels, a clone, EJ-1, was arbitrarily chosen for further detailed studies.

EGF-dependent Growth of 32D Cells Expressing EG-J2—To examine whether EGF stimulation activates the chimeric receptor to transduce a growth signal, EJ-1 cells were cultured in the absence of growth factors or in the presence of EGF or IL-3. Fig. 3A shows growth curves of EJ-1 cells in 10% FCS-containing RPMI 1640 without any growth factor or supplemented with IL-3 or EGF. EJ-1 cells remained growth factor-dependent and thus could not survive in medium not supplemented with any growth factor. The addition of EGF to medium induced proliferation of EJ-1 cells, although the rate of growth in EGF was considerably slower than that in IL-3. It was also confirmed that EGF supported the long-term growth of EJ-1 cells as well as the other two clones (data not shown).

EGF dose dependence of proliferation of EJ-1 was next examined by the sodium 3-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid colorimetric assay. Each data point represents the mean (bars, S.E.) of triplicate determinations and is expressed as a percentage of maximal stimulation with IL-3. In C, EJ-1 cells were cultivated for 16 h in the absence (C) or presence of the growth factor as indicated. The cells were harvested, and DNA fragmentation of the cells was examined as described under “Materials and Methods.” The sizes of DNA molecular mass markers (M) are indicated and given in base pairs. For the results shown in Fig. 3, two additional experiments gave similar results.

To examine the ability of the EG-J2 chimera to suppress apoptosis, chromosomal DNA fragmentation of EJ-1 cells was then analyzed. The characteristic DNA fragmentation of apoptotic cell death was observed when EJ-1 cells were removed from growth factors for 16 h, as shown in Fig. 3C. When the cells were cultured in the presence of EGF or IL-3, the DNA fragmentation was prevented, thus demonstrating that the EG-J2 chimera also transduced an EGF-dependent antiapoptotic signal.

EGF-induced Tyrosine Phosphorylation of the EG-J2 Chimera—Because the tyrosine phosphorylation of Jak2 has been shown to correlate closely with the tyrosine kinase activity of Jak2 (3, 4, 9, 10), we examined the tyrosine phosphorylation status of EG-J2 in EJ-1 cells. For this purpose, growth factor-starved EJ-1 cells were first stimulated with EGF or IL-3. The cell lysates were then immunoprecipitated with a monodonal antibody against the extracellular domain of EGFR, anti-jak2m, or anti-jak2c. Antiphosphotyrosine blotting of the immunoprecipitates revealed that EGF stimulation, but not IL-3 stimulation, induced tyrosine phosphorylation of the 140-kDa Jak2 (3, 4, 9, 10), we examined the tyrosine phosphorylation status of EG-J2 in EJ-1 cells. For this purpose, growth factor-starved EJ-1 cells were first stimulated with EGF or IL-3. The cell lysates were then immunoprecipitated with a monodonal antibody against the extracellular domain of EGFR, anti-jak2m, or anti-jak2c. Antiphosphotyrosine blotting of the immunoprecipitates revealed that EGF stimulation, but not IL-3 stimulation, induced tyrosine phosphorylation of the 140-kDa EG-J2 chimera, which was immunoprecipitated with anti-EGFR or anti-jak2c but not with anti-jak2m (Fig. 4, upper panel). On the other hand, endogenous 130-kDa Jak2, which was immunoprecipitated with anti-jak2m or anti-jak2c but not with anti-EGFR, was tyrosine phosphorylated only after IL-3 stimulation. Reprobing of the filter with anti-jak2c showed equal loading of the immunoprecipitates and confirmed that EG-J2 or Jak2 was specifically immunoprecipitated with anti-EGFR or anti-jak2c, respectively, whereas both were immunoprecipitated with anti-jak2c. Although the in vitro kinase assay was technically not feasible using anti-jak2c or anti-EGFR that was used in this study (data not shown), these results strongly suggest that EGF activates the tyrosine kinase activity of EG-J2 but not that of endogenous Jak2 in EJ-1 cells.

Substrates of Tyrosine Phosphorylation Induced by EGF Stimulation in EJ-1 Cells—Because the Jak kinases have been
shown to transduce signals to the nucleus by inducing tyrosine phosphorylation and activation of the Stat family of transcription factors, we next examined the tyrosine phosphorylation status of various Stat proteins in EGF-stimulated EJ-1 cells. In accordance with a recent report (23), antiphosphotyrosine blotting of anti-Stat5a and anti-Stat5b immunoprecipitates showed that the tyrosine phosphorylation of these proteins was induced by IL-3 (Fig. 5A). EGF also induced, albeit to a lesser extent, tyrosine phosphorylation of Stat5a and Stat5b. We also examined the tyrosine phosphorylation status of Stat1, Stat3, Stat4, and Stat6 using specific antibodies. However, none of these Stat proteins was induced to be tyrosine phosphorylated by stimulation with EGF or IL-3 (Fig. 5A and data not shown). Inconsistent with our observation, Stat6 was previously reported to be tyrosine phosphorylated upon IL-3 stimulation in another IL-3-dependent cell line, DA3 (20). It is speculated that the difference in cell lines examined might have caused the discrepancy.

In repeated experiments, EGF never induced detectable levels of tyrosine phosphorylation of endogenous Jak2 (Figs. 4 and 5B). Nevertheless, to address the possibility that the EG-J2 chimera may activate other Jak kinases to transduce a mitogenic signal, we examined the tyrosine phosphorylation status of Jak1, Jak3, and Tyk2. Antiphosphotyrosine blotting of immunoprecipitates obtained with specific antibodies indicated were subjected to SDS-PAGE, followed by immunoblotting with an antiphosphotyrosine monoclonal antibody, 4G10 (αPY). The membrane was then stripped and reprobed with the antibody against the Jak2 carboxy terminus (αJak2c), as indicated. Arrows, the positions of EG-J2 and endogenous Jak2.

**Fig. 4.** EGF-induced tyrosine phosphorylation of EG-J2 in EJ-1 cells. EJ-1 cells were washed out from IL-3 for 12 h and left unstimulated (C) or stimulated with EGF or IL-3 as indicated for 5 min before solubilization. Equivalent amounts of the cell lysates were subjected to immunoprecipitation with a monoclonal antibody against EGFR (αEGFR) or antiserum raised against a synthetic peptide corresponding to amino acids 758–776 (αJak2m) or amino acids 1110–1129 (αJak2c) mapping within the JH2 domain or at the carboxy terminus of murine Jak2, respectively. The immunoprecipitates were resolved by 6% SDS-PAGE and subjected to immunoblotting with an antiphosphotyrosine monoclonal antibody, 4G10 (αPY). The membrane was then stripped and reprobed with the antibody against the Jak2 carboxy terminus (αJak2c), as indicated. Arrows, the positions of EG-J2 and endogenous Jak2.

**Fig. 5.** Substrates of tyrosine phosphorylation induced by EGF or IL-3 treatment of EJ-1 cells. A, B, and C, after starvation from IL-3, EJ-1 cells were either left unstimulated (C) or were stimulated for 5 min with a saturating concentration of EGF or IL-3, as indicated, and solubilized. In D, after starved from IL-3, EJ-1 cells were stimulated for the indicated time with EGF or IL-3 and solubilized under denaturing conditions. Immunoprecipitates obtained with the antibodies indicated were subjected to SDS-PAGE, followed by immunoblot analysis with the 4G10 antiphosphotyrosine monoclonal antibody (upper panels). The filters were then stripped and reprobed with the antibody used for immunoprecipitation to demonstrate equivalent loading of the immunoprecipitated proteins in each lane (lower panels). The positions of Stat5a, Stat5b, Syp, Shc, extracellular signal-regulated kinase 1, and extracellular signal-regulated kinase 2 are indicated. Arrow, the position of tyrosine phosphorylated Jak2.

**Fig. 6.** EGF dose dependence of tyrosine phosphorylation of EG-J2 and Stat5—Of all the signal transducing factors we examined, EGF induced tyrosine phosphorylation of only EG-J2 and Stat5 in EJ-1 cells. To explore the significance of tyrosine phosphorylation of these proteins, the EGF dose dependence of tyrosine phosphorylation was then examined. As shown in Fig. 6, the tyrosine phosphorylation of both EG-J2 and Stat5b was faintly detectable at 1 ng/ml of EGF and increased to a near maximal level at 100 ng/ml. The EGF dose dependence of EG-J2 and Stat5b was thus comparable and also correlated with that of cell proliferation. Taken together with the data showing that EG-J2 did not activate the other signaling pathways examined, these results suggest that Stat5 may play a role in transducing a growth signal from the EG-J2 chimera.
The critical role of Jak2 in hematopoietic cell growth signaling has been suggested by several studies. We previously showed that the ability of various mutant EpoRs to activate Jak2 closely correlated with the ability to transduce a growth signal in hematopoietic cells. However, the ability of Jak2 tyrosine kinase domain to transduce a growth signal from the activated Jak2 kinase, because of all signaling molecules we have examined, only Stat5 was shown to be tyrosine phosphorylated by the chimeric receptor activated by EGF. Furthermore, the EGF dose dependence of tyrosine phosphorylation of Stat5 correlated with that of cell proliferation, which also suggests a role for Stat5 in growth signaling. Initially identified as a mediator of prolactin-induced transcription of the β-casein gene (26), Stat5 was shown later to be activated by tyrosine phosphorylation in response to a variety of cytokines, including growth hormone, Epo, IL-2, IL-3, granulocyte-macrophage colony-stimulating factor, IL-5, and thrombopoietin (23, 27–32). Two murine homologues, Stat5a and Stat5b, sharing 96% amino acid sequence identity, have been identified, although no functional differences have yet been observed between these two molecules (23). Stat5 binds a subset of interferon-γ-activated sequence elements that contains a common palindromic element 5′-TTC-NNGAA-3′, which are primarily found in promoters of early-response genes induced by cytokines and interferons. However, a role of Stat5 in growth signaling from cytokine receptors has remained controversial. A previous study showed that the growth signaling from the IL-2 receptor may not require Stat5 activation, because a mutant receptor was reported to trigger cell proliferation without activating Stat5 (30). On the other hand, Damen et al. (33) very recently reported that the activation of Stat5 correlated directly with proliferation in cells expressing several mutant EpoRs. This is in accordance with the present study and provides evidence for the role of Stat5 in growth signaling. However, further studies are obviously required to prove this possibility and to explore target genes of Stat5 that may lead to cell proliferation. The EG-J2 chimera, which selectively activated Stat5 in transfected cells, may prove useful for these studies.

In apparent contradiction with our study which implied that Jak2 may tyrosine phosphorylate Stat5 independently of the cytokine receptor cytoplasmic domains, Damen et al. (33) reported that tyrosine-343 in the EpoR was required for the activation of Stat5 by Epo stimulation. The requirement for this tyrosine residue, however, was not absolute, since a mutant EpoR lacking all of the intracellular tyrosine residues in fact induced, although weakly, the tyrosine phosphorylation and activation of Stat5 when activated by a high concentration of Epo (33). Jak2 may thus directly bind and activate Stat5 under certain circumstances.

Most of the cytokine receptors activate the Ras/MAP kinase pathway by inducing tyrosine phosphorylation of Shc or Syp and their association with the Grb2-Sos1 complex (3, 4). Nevertheless, the significance of the activation of Ras/MAP kinase pathway in hematopoietic cell growth signaling has remained to be determined. We showed previously that a mutant EpoR that was severely impaired in its ability to activate the Ras/MAP kinase pathway normally transduced a proliferation signal when expressed in the IL-3-dependent cell line 32D (8). In contrast, Kinoshita et al. (34) showed that IL-3/granulocyte-macrophage colony-stimulating factor receptor β subunit mutants lacking the ability to activate the Ras/MAP kinase pathway stimulated DNA synthesis but failed to prevent apoptosis of IL-3-dependent Ba/F3 cells in serum-free medium (35). Nevertheless, IL-2 receptor mutants defective in Ras activation have recently been shown to confer Ba/F3 cells the ability to grow in response to IL-2 in serum-free medium. Moreover, very recent study by Terada et al. (36) demonstrated that a dominant-negative form of Ras did not show any inhibitory effect on IL-3-dependent proliferation, thus indicating that the Ras/MAP kinase pathway is dispensable for IL-3-induced growth.
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stabilization. Results in the present study also indicate that the activation of the Ras/MAP kinase pathway is not required for growth signaling in IL-3-dependent hematopoietic cells, because cells expressing the EG-J2 chimera proliferated in response to EGF without showing tyrosine phosphorylation of Shc, Syp, or MAP kinases. The present study further demonstrated the ability of the EG-J2 chimera to transduce an anti-apoptotic signal, which is consistent with the study of Zhuang et al. (37) showing inhibition of the anti-apoptotic effect of Epo by dominant-negative forms of Jak2. Thus, Jak2 may directly mediate growth hormone-induced transcriptional activation of the EGFR/Jak2 chimera possessing the ability to transduce a growth signal.

Acknowledgments—We are grateful to Dr. James N. Ihle for invaluable comments and for the generous gift of the murine jak2 cDNA and antibodies. We thank Drs. Yoji Ikawa, Atsushi Miyajima, and Hiroshi Wakao for critically reading the manuscript.

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