

Roles of the cytoplasmic domains of the α and β subunits of human granulocyte-macrophage colony-stimulating factor receptor

Akihiko Muto, PhD,^a Sumiko Watanabe, MS,^a Tohru Itoh, MS,^a
Atsushi Miyajima, PhD,^b Takashi Yokota, PhD,^a and
Ken-ichi Arai, MD, PhD^a Tokyo, Japan, and Palo Alto, Calif.

The high-affinity and functional granulocyte-macrophage colony-stimulating factor receptor (GMR) is composed of two distinct subunits, α and β ; and the cytoplasmic domain of the β subunit is essential to transduce growth-promoting signals. In contrast to the β subunit, the role of the α subunit is not well characterized. We examined the requirement of the cytoplasmic domain of the α subunit and its functional region by deletion analyses. We demonstrated that the cytoplasmic domain of the α subunit, especially 29 amino acids residues near the transmembrane domain, was absolutely required for various signaling events including activation of immediate early genes, induction of tyrosine phosphorylation of cellular proteins, and cell growth. We further analyzed the role of the cytoplasmic domain of each subunit by constructing chimeric subunits, designated α/β and β/α , by exchanging cytoplasmic domains of the α and β subunits of human (h) GMR. Reconstituted high-affinity chimeric hGMRs, hGMR($\alpha/\beta, \beta/\alpha$) and hGMR($\alpha/\beta, \beta$), transduced signals at levels similar to the wild type hGMR(α, β) in Ba/F3 cells and in NIH3T3 cells. These observations indicate that the original configuration between the extracellular and the cytoplasmic domains of the hGMR(α, β) subunits is not required and that hGMR($\alpha/\beta, \beta$) transduced signals through the cytoplasmic domain of the β subunit in an oligomeric form, without involvement of the cytoplasmic domain of the α subunit. Therefore human granulocyte-macrophage colony-stimulating factor signals are mainly transduced through the β subunit, and the cytoplasmic domain of the α subunit is likely to activate the β subunit in the normal hGMR. (J ALLERGY CLIN IMMUNOL 1995;96:1100-14.)

Key words: GM-CSF receptor, α subunit, β subunit, cytoplasmic domain, receptor oligomerization, signal transduction

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine produced by wide variety of cell types, including activated T lympho-

From ^aThe Department of Molecular and Developmental Biology, The Institute of Medical Science, University of Tokyo; and ^bthe Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto.

Supported in part by a grant-in-aid for scientific research on priority areas and cancer research from the Ministry of Education, Science and Culture of Japan. The DNAX Research Institute of Molecular and Cellular Biology is supported by the Schering-Plough Corporation.

Reprint requests: Ken-ichi Arai, MD, PhD, Department of Molecular and Developmental Biology, The Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo, Japan.

Copyright © 1995 by Mosby-Year Book, Inc.
0091-6749/95 \$5.00 + 0 1/0/68343

Abbreviations used

FCS:	Fetal calf serum
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
GMR:	Granulocyte-macrophage colony-stimulating factor receptor
MTT:	3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide

cytes, macrophages, fibroblasts, and stromal cells.^{1,2} This cytokine plays pivotal roles in proliferation and differentiation of various types of cells, and its effects are exerted through the specific receptor expressed on the cell surface. The GM-CSF receptor (GMR) consists of two distinct subunits termed α and β , and both subunits are

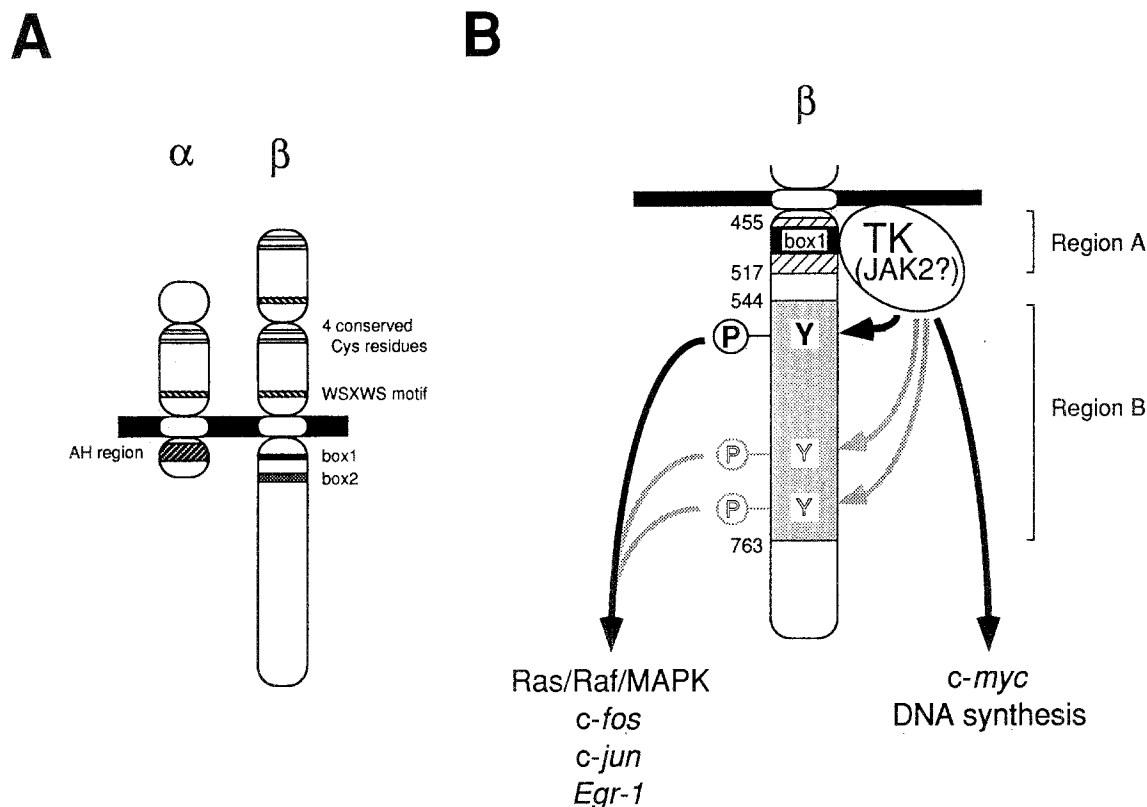


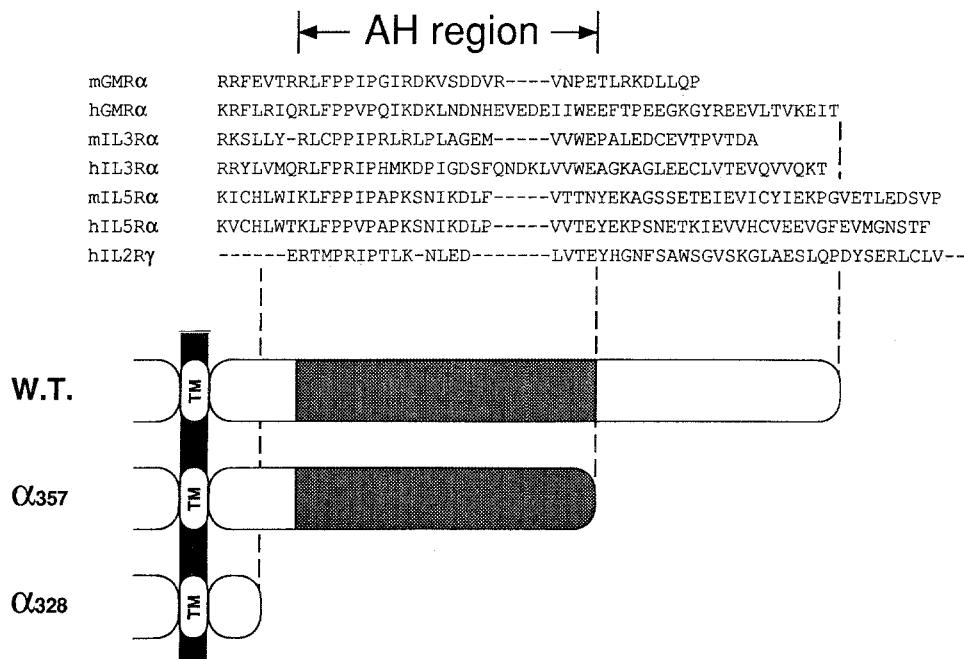
FIG. 1. Structure and signal transduction of GMR.

members of the cytokine receptor superfamily having characteristic features in the extracellular domains (i.e., four conserved cysteine residues and the WSXWS motif) (Fig. 1, A).³⁻⁵ Neither the cytoplasmic domain of the α nor that of the β subunit has known enzymatic activity. The β subunit has both box 1 and box 2 motifs in the cytoplasmic domain, which are conserved among various cytokine receptors. In the cytoplasmic domain of the α subunit a sequence conserved among α subunits for GMR, IL-3R, and IL-5R (which we call the α homology or AH region) is found, and this region contains a short proline rich motif similar to box 1 (Fig. 2).^{4,6,7} Although the α subunit specifically binds to human (h)GM-CSF with low-affinity, it alone does not transduce any signals. On the other hand, the β subunit in itself does not bind any cytokines, including hGM-CSF, but forms a high-affinity and functional hGMR with the α subunit.^{4,5,8} In contrast to the α subunit, which is specific to GMR, the β subunit is shared by high-affinity receptors for IL-3 and IL-5.^{5,6,9,10}

In the mouse system a second β subunit, AIC2A, exists in addition to the common β subunit (AIC2B).¹¹⁻¹³ Despite a high degree of homology between AIC2A and AIC2B (91% identity at

amino acid level), AIC2A is specific to mouse (m) IL-3R; and an IL-3-specific β subunit, corresponding to AIC2A, is not found in the human system.^{5,13}

GM-CSF induces various cellular events including cell proliferation, tyrosine phosphorylation of cellular proteins, and activation of the immediate early genes.¹⁴⁻¹⁷ Reconstitution analyses revealed that the high-affinity hGMR reconstituted on mouse cells transduces signals not only in hematopoietic cells but also in nonhematopoietic cells, such as fibroblasts.^{8,18-20} Further analyses with a series of deletion mutants of the β subunit have indicated that cytoplasmic domain of the β subunit, which can be dispensed to form a high-affinity GMR, is essential to transduce signals.²¹⁻²⁴ In the cytoplasmic domain at least two distinct regions, region A and region B (spanning amino acid positions 456-517 and 545-763, respectively) are required for signal transduction (Fig. 1, B). Region A contains box 1 but not box 2 and is required for cell growth and activation of the *c-myc* oncogene. Region B is required for activation of *c-fos*, *c-jun*, and *Egr-1* genes, activation of the Ras-Raf-MAPK pathway and major tyrosine phosphorylation of cellular proteins, including the β subunit. Studies

FIG. 2. Schematic representation of mutant α subunits.

with inhibitors of tyrosine kinases have revealed that tyrosine phosphorylation is involved in signal transduction through both region A and region B.²³ In region B there are several tyrosine residues, and phosphorylation of some of these sites is involved in activation of *c-fos* and *Egr-1* oncogenes. In contrast to the β subunit, roles of the cytoplasmic domain of the α subunit are not well known.

In this study we examined the role of the cytoplasmic domain of the α subunit in various cellular responses by means of reconstitution analyses with deletion mutants of the α subunit. We found that the cytoplasmic domain of the α subunit is required to transduce various signals activated by hGM-CSF. Furthermore, we used chimeric subunits of hGMR to evaluate the more specific role of the cytoplasmic domain of each subunit. The chimeric subunits, α/β and β/α , were constructed by exchanging the cytoplasmic domains of both subunits of hGMR. In this system the cytoplasmic domains of both subunits are oligomerized by hGM-CSF in various combinations (i.e., homooligomer of the cytoplasmic domain of the α or β subunit alone or hetero-oligomer consisting of both). We also found that the chimeric hGMR(α/β), which had the cytoplasmic domain derived from the β subunit, but not from the α subunit, could transduce signals at a level similar to that transduced through the wild type hGMR.

METHODS

Chemicals, media, genes, and antibodies

Recombinant hGM-CSF produced in *Escherichia coli* was provided by the Schering-Plough Corporation (Kenilworth, N.J.). Mouse IL-3 (mIL-3) produced by silkworm (*Bombyx mori*) was purified as described previously.²⁵ RPMI 1640 medium and Dulbecco's modified Eagle medium (DMEM) were purchased from Nikken BioMedical Laboratories Co. Ltd. (Kyoto, Japan) and fetal calf serum (FCS) was from Biocell Laboratories, Inc. (Rancho Dominguez, Calif.). G418 was purchased from Gibco Life Technologies, Inc. (Gaithersburg, Md.), and hygromycin B was from Wako Chemicals USA, Inc. (Richmond, Va.). Iodine 125-labeled hGM-CSF, [α -phosphorous 32-labeled deoxycytidine triphosphate, horseradish peroxidase-linked sheep anti-mouse immunoglobulin, and the enhanced chemiluminescence system were purchased from Amersham Japan Co. Ltd. (Tokyo, Japan). The luciferase assay system was purchased from Promega Corporation (Madison, Wis.). Anti-phosphotyrosine mouse monoclonal antibody 4G10 was purchased from Upstate Biotechnology Inc. (Lake Placid, N.Y.). Mouse monoclonal antibody against hGMR α subunit for immunoprecipitation and fluorescence-activated cell sorter (FACS) analysis, GMA-1, and rat monoclonal antibody against the human common β subunit, 5A5, were prepared by Dr. H. Kurata (Institute of Medical Science, The University of Tokyo) and by Dr. Y. Watanabe (DNAX Research Institute),²⁶ respectively. Rabbit polyclonal

antibodies against the extracellular domain or the cytoplasmic domain of the human common β subunit were obtained from Medical & Biological Laboratory Co. Ltd. (Nagoya, Japan). Mouse *c-fos* and rat *c-jun* complementary DNAs were kind gifts from Dr. T. Curran (Roche Institute of Molecular Biology). Mouse *c-myc* cDNA was provided by Dr. N. Arai (DNAX Research Institute). Human G3PDH cDNA was purchased from CLONTECH Laboratories, Inc. (Palo Alto, Calif.).

Cell lines and culture conditions

An mIL-3-dependent pro-B-cell line, Ba/F3, was maintained in RPMI 1640 medium containing 10% FCS, 50 U/ml penicillin, 50 μ g/ml streptomycin, and 0.25 ng/ml mIL-3. A mouse embryonic fibroblast line, NIH3T3, was maintained in DMEM containing 10% FCS, 50 U/ml penicillin, and 50 μ g/ml streptomycin.

Construction of chimeric and mutant receptors

Human GMR α subunit and common β subunit cDNAs were originally inserted in pCEV4 and pME18S, respectively, as described previously.⁵ To construct the α/β and β/α chimeric hGMR subunits carrying the extracellular and transmembrane domains of the α and β subunits and the cytoplasmic domains of the β and α subunits, respectively, DNA fragments encoding the cytoplasmic domains of the α and the β subunits were replaced by the corresponding DNA fragments of the β and the α subunits. First, the α subunit cDNA was digested with *Eco*81I at amino acid position 350, followed by a fill-in reaction with the Klenow fragment, then the β subunit cDNA was digested with *Fsp*I at amino acid position 471. Both DNAs were then cleaved at the *Xba*I site in the vector sequence beside the 3' end of cDNA. Isolated blunt-end-*Xba*I DNA fragments encoding the cytoplasmic domain of each receptor cDNA were exchanged and ligated to the vector containing the extracellular and transmembrane regions of the other subunit of the receptor. The resultant chimeric hGMR(α/β) product has one extra amino acid, serine, at the junction of the two polypeptide fragments derived from the α and β subunits, and the hGMR(β/α) product has no additional amino acid at the junction of the two polypeptide fragments. To establish stable transfectants, α and α/β subunit cDNAs were subcloned into pME18S containing a hygromycin-resistant gene, and β and β/α subunit cDNAs were subcloned into the same vector with a neomycin-resistant gene.

The truncated mutants of the α and β subunits, or α_{328} and β_{455} , were constructed as described previously. The α_{357} mutant was generated by insertion of a termination codon at the amino acid position 358 by polymerase chain reaction.

Transient transfection and luciferase assay

Ba/F3 and NIH3T3 cells were transfected with plasmid DNAs by using the diethylaminoethyl cellulose-dextran method and electroporation, respectively, as described elsewhere.²⁰ Five micrograms of *c-fos*-luciferase fusion plasmid and various combinations of 2 μ g of receptor cDNA plasmid or control vector were used for the transfection. After 5 hours of stimulation with the indicated factor or FCS, the transfected cells were lysed and used for luciferase assay.

Establishment of stable transfectants

Ba/F3 cells were transfected by electroporation to establish stable transfectants, essentially as described by Kitamura et al.⁸ Exponentially growing cells were harvested, washed, and resuspended in ice-cold phosphate-buffered saline at 1×10^7 cells/ml. Twelve micrograms of linearized plasmid DNA was added to 0.2 ml of the cell suspension, and the cells were shocked by a Gene Pulser (Bio-Rad Laboratories, Hercules, Calif.) at 250 μ F and 300 V. Selection was initiated with either 1.2 mg/ml hygromycin or 1 mg/ml G418 at 2 days after the transfection. After 2 weeks, expression of the receptor or receptors was confirmed by flow cytometry with FACS-can (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) by using monoclonal antibodies directed against the α (GMA-1) and the β (5A5) subunits of hGMR.

Ligand binding assay

Ligand binding assay was done as described previously.²¹ Continuously growing cells were harvested and resuspended in ice-cold Hanks' balanced salt solution at 4×10^6 cells/ml. Fifty μ l aliquots (2×10^5 cells) were added to an equal volume of Hanks' balanced salt solution containing increasing concentrations of ¹²⁵I-hGM-CSF (20 pmol/L to 10 nmol/L) with and without a 100-fold molar excess of cold hGM-CSF and were then incubated at 4°C for 2 hours. Cell-bound and free ¹²⁵I-hGM-CSF were separated by centrifugation, and radioactivity was measured in a gamma-counter (Auto-Gamma Counter model 5550; Packard Instrument Co., Meriden, Conn.). The number of receptors on the cell surface and the dissociation constant were determined by Scatchard plot analysis.²⁷

Cell proliferation assay

Cell proliferation of Ba/F3 was measured by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay originally described by Mosmann.²⁸ Harvested cells were washed with factor-free medium and resuspended in the same medium at 5×10^4 cells/ml, then 100 μ l aliquots of the cell suspension were seeded to a 96-well plate. After 24-hour culture with various concentrations of mIL-3 or hGM-CSF, 10 μ l of MTT solution (5 mg/ml) was added, and the preparation was incubated for an additional 4 hours at 37°C. Cells

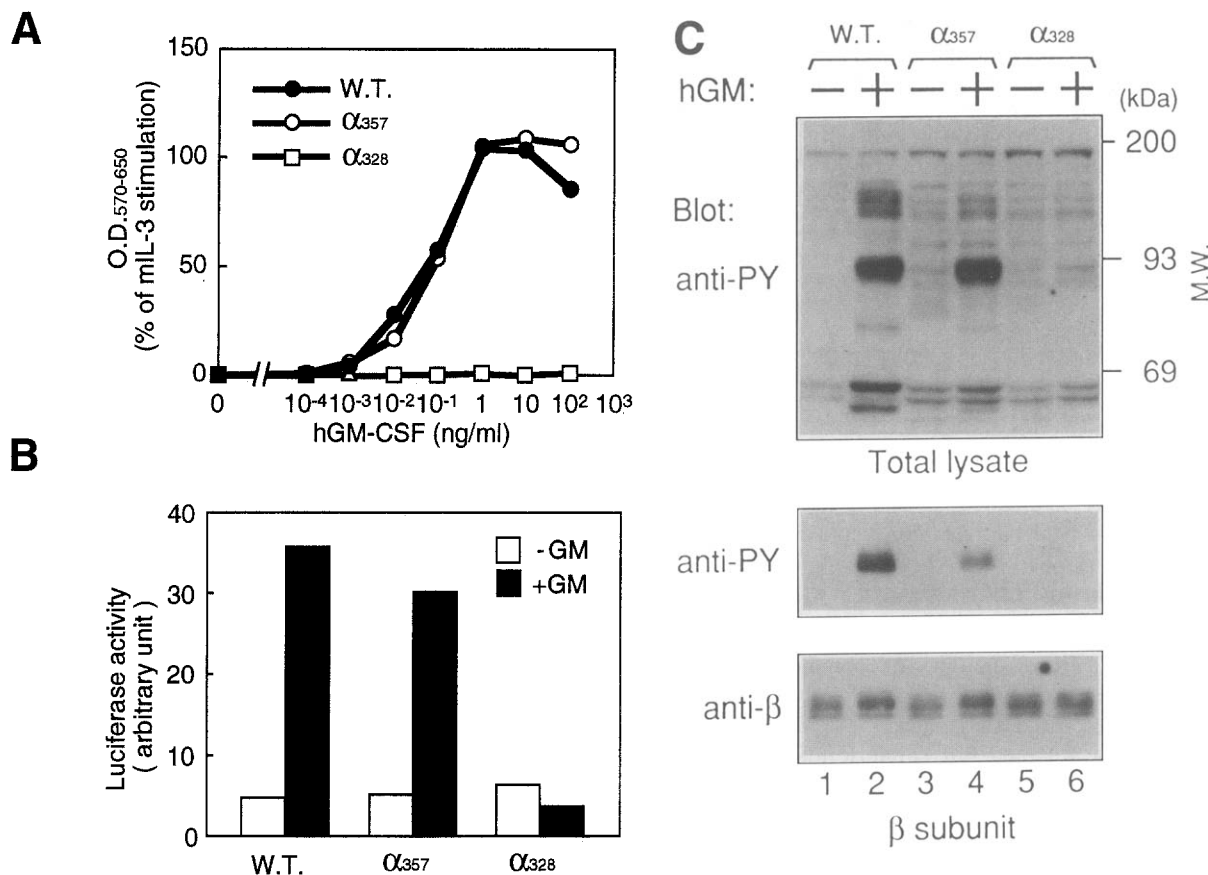


FIG. 3. Functional analyses of the cytoplasmic domain of the α subunit. **A**, hGM-CSF-dependent growth of Ba/F3 transfectants was measured by the MTT assay, as described in Methods. **B**, Activation of the *c-fos* promoter through mutant hGMR was measured by the transient transfection assay. **C**, Induction of tyrosine phosphorylation of cellular proteins in total cell lysate (upper panel) and of the immunoprecipitated β subunit (middle and lower panels). Samples were prepared from cells stimulated with (lanes 2, 4, and 6) or without (lanes 1, 3, and 5) 10 ng/ml hGM-CSF for 5 minutes. Phosphorylated proteins were analyzed by Western blotting with an anti-phosphotyrosine (anti-PY) antibody (4G10) (upper and middle panels) and anti- β antibody (lower panel).

were lysed by addition of 100 μ l of isopropanol containing 0.04N HCl, and the absorbance values (optical density at 570 to 650 nm) were measured with an Emax microplate reader (Wako Chemicals Inc., Osaka, Japan).

Northern blot analysis

The messenger RNAs of immediate early genes were analyzed with Northern blot analysis, as previously described.²⁰ Briefly, cells were incubated in factor-free medium for 5 hours before stimulation with mIL-3 or hGM-CSF. Total cellular RNA was extracted from the cells at indicated time points after stimulation by the acid guanidinium isothiocyanate-phenol-chloroform method.²⁹ Ten micrograms of total RNA was separated by electrophoresis through a 1.2% agarose gel containing 6% formaldehyde and was then transferred onto a nylon membrane by capillary blotting. The RNA blots were hybridized with ³²P-labeled cDNA probes prepared with the random prim-

ing method, washed, exposed to an imaging plate, and analyzed with a FUJIX Bio-imaging analyzer (model BAS-2000; Fuji Film, Tokyo, Japan).

Preparation and analysis of protein samples

After factor depletion for 5 hours, the cells were stimulated with or without either 1 ng/ml mIL-3 or 5 to 10 ng/ml hGM-CSF for 10 minutes at 37°C, rapidly precipitated by centrifugation. To analyze tyrosine-phosphorylated proteins in the total cell lysate, the cells were immediately lysed in Laemmli's sample buffer³⁰ containing 1% 2-mercaptoethanol at 1×10^7 cells/ml and boiled. For immunoprecipitation of the β subunit, the cells were lysed in the lysis buffer containing 20 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1 mmol/L ethylenediaminetetraacetic acid, 1% Nonidet P-40, 50 mmol/L NaF, 100 μ mol/L Na₃VO₄, 1 mmol/L phenylmethylsulfonyl fluoride, 1 μ g/ml pepstatin and 2 μ g/ml

leupeptin for 1 hour at 4° C. After immunoprecipitation with an anti- β subunit antibody (5A5), the immunoprecipitate was dissolved in Laemmli's buffer and boiled. The prepared proteins were analyzed by Western blot analysis as described elsewhere.²¹

RESULTS

Signal transduction by the mutant α subunits

Although the cytoplasmic domain of the α subunit was shown to be required for cell growth, functional regions and requirements of this domain for other signals are not determined. To assess the role of the cytoplasmic domain of the α subunit, we examined the signal transducing ability of two mutant α subunits in Ba/F3 cells. One is α_{328} , which lacks most of the cytoplasmic domain, and the other is α_{357} , which lacks the C terminal half of the cytoplasmic domain but retains the AH region (Fig. 2). As shown in Fig. 3, A, hGMR(α_{357} , β), which consists of the α_{357} subunit and the β subunit, but not hGMR(α_{328} , β) could induce cell growth of Ba/F3 cells in response to hGM-CSF at a level similar to that induced by hGMR(α , β). Therefore the AH region is sufficient to transduce the cell growth signal. Next, we attempted to determine whether the cytoplasmic domain of the α subunit is also required for activation of the *c-fos* promoter, using the transient transfection assay with the *c-fos* promoter-luciferase reporter construct (Fig. 3, B). Human GMR(α_{357} , β), but not hGMR(α_{328} , β), could activate the *c-fos* promoter, as well as cell growth. We also found that the AH region is sufficient to induce tyrosine phosphorylation of cellular proteins including the β subunit (Fig. 3, C). Therefore the cytoplasmic domain of the α subunit, especially the AH region, is required for signal transduction through hGMR.

The α_{328} subunit could associate with the β subunit in the presence of hGM-CSF

The α_{328} subunit does not transduce any signals but can form a high-affinity GMR when the β subunit is coexpressed. We attempted to determine whether the mutant α subunit associates with the β subunit. Western blot analysis with anti- α antibody revealed that the β subunit formed a complex with either the wild type α subunit or the mutant α_{328} subunit only in the presence of hGM-CSF (Fig. 4, A, lanes 2 and 6). However, tyrosine phosphorylation of the β subunit was not induced

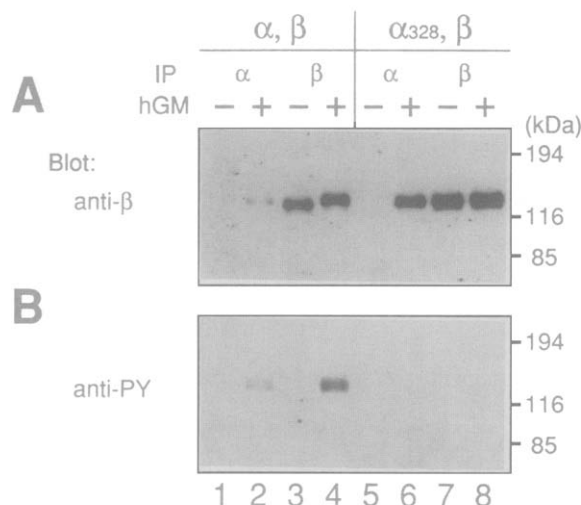


FIG. 4. The cytoplasmic domain of the α subunit is required for activation of but not association with the β subunit. The β subunits expressed in Ba/F3- α , β (lanes 1 to 4) and Ba/F3- α_{328} , β (lanes 5 to 8) cells were immunoprecipitated with an anti- α (lanes 1, 2, 5, and 6) or anti- β (lanes 3, 4, 7, and 8) antibody after stimulation of cells with (lanes 2, 4, 6, and 8) or without (lanes 1, 3, 5, and 7) 10 ng/ml hGM-CSF for 5 minutes at 37° C. The immunoprecipitates were analyzed by Western blotting with (A) anti- β and (B) anti-phosphotyrosine (anti-PY) antibodies. IP, Immunoprecipitate.

in Ba/F3- α_{328} , β cells (Fig. 4, B, lanes 6 and 8). These results indicated that hGM-CSF-dependent association of the α and β subunits is mediated by the extracellular or transmembrane domains of the α subunit.

Human GM-CSF activates the *c-fos* promoter through transiently expressed chimeric hGMRs in Ba/F3 and NIH3T3 cells

To examine the role of each domain of hGMR α and β subunits, we generated the chimeric hGMR subunits α/β and β/α . The predicted structures of the chimeric receptors are shown schematically in Fig. 5. The chimeric subunit α/β consists of extracellular and transmembrane domains of the α subunit and the cytoplasmic domain of the β subunit. The chimeric subunit β/α consists of extracellular and transmembrane domains of the β subunit and the cytoplasmic domain of the α subunit. These chimeric receptor cDNAs were placed downstream from the SR α promoter in a mammalian expression vector. We then performed transient transfection assays with the *c-fos* promoter luciferase as a reporter gene in Ba/F3 or NIH3T3 cells. As shown in Table I, the *c-fos* promoter was activated by hGM-CSF (5 ng/ml) in Ba/F3 cells transfected with the hGMR

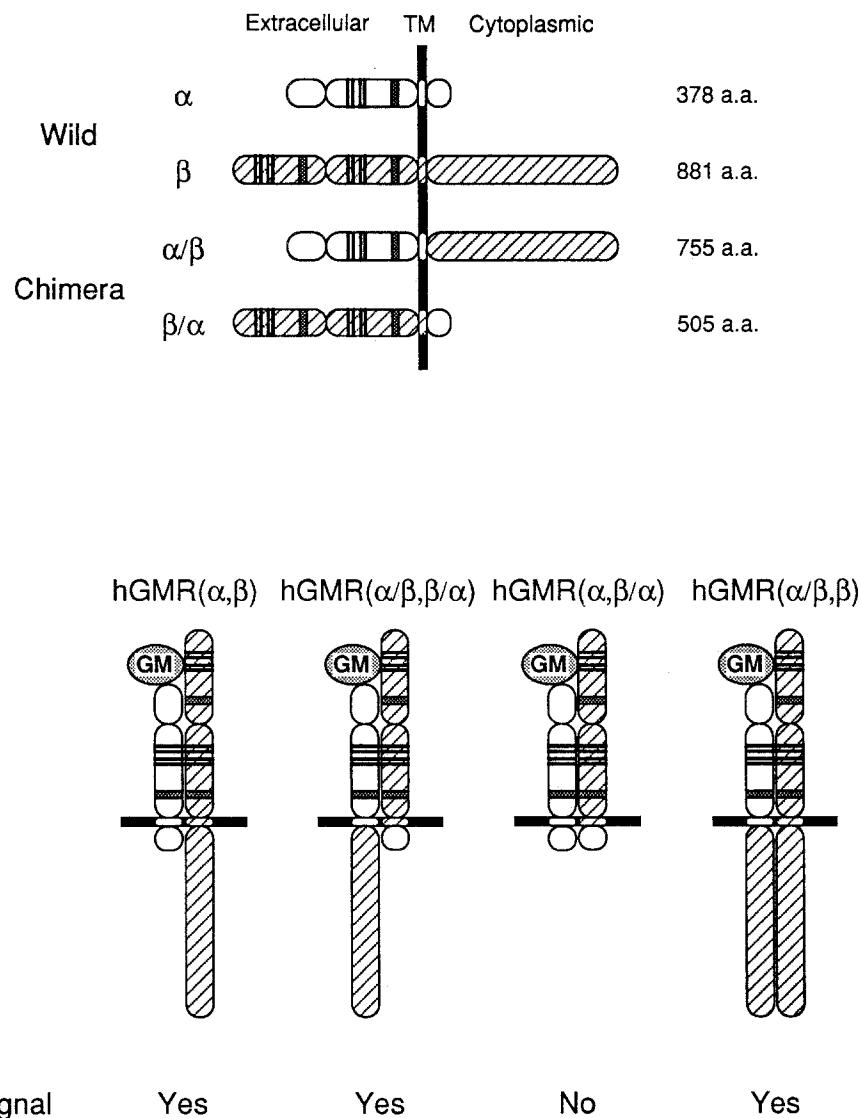


FIG. 5. Schematic representation of chimeric receptors. a.a., Amino acid; TM, transmembrane.

chimeric subunits (α/β and β/α) in a manner similar to that for the wild type hGMR(α,β). Interestingly, hGMR($\alpha/\beta,\beta$), reconstituted by cotransfecting the chimeric α/β subunit and the wild type β subunit, could also transduce signals to activate the *c-fos* promoter in response to hGM-CSF (5 ng/ml). Therefore the signals that activated the *c-fos* promoter could be transduced through chimeric hGMR($\alpha/\beta,\beta$) without involvement of the cytoplasmic domain of the α subunit. With the other combinations of subunits, such as hGMR($\alpha,\beta/\alpha$), or each chimeric subunit alone, hGM-CSF also failed to activate the *c-fos* promoter, even at a high dose (100 ng/ml) (Table I). Because the results were similar to those obtained

when NIH3T3 cells were used, these events were not restricted to hematopoietic cells (Table I).

Activation of immediate early genes in Ba/F3 transfectants stably expressing chimeric hGMRs by hGM-CSF

To examine in more detail the properties of signal transduction by chimeric hGMRs, we established Ba/F3 transfectants expressing the wild type and chimeric hGMR subunits in various combinations. The expression of each subunit was confirmed by FACS analysis with antibodies against the extracellular domain of the hGMR α subunit (GMA-1) or the β subunit (5A5), as described in

TABLE I. Activation of the *c-fos* promoter by hGM-CSF in Ba/F3 and NIH3T3 cells transiently expressing chimeric hGMRs

Stimuli	Receptor†	Relative luciferase activity*	
		Ba/F3 cells	NIH3T3 cells
Control‡	None	100	100
None	None	13.6 ± 2.1	14.6 ± 1.3
hGM-CSF§	None	5.1 ± 0.9	20.0 ± 4.3
	α + β	636.8 ± 23.9	116.9 ± 18.9
	α/β + β/α	330.2 ± 24.6	80.8 ± 4.3
	α + β/α	9.1 ± 1.0	14.6 ± 3.3
	α/β + β	228.9 ± 28.0	83.2 ± 16.2
	α + α/β	12.7 ± 1.2	19.1 ± 0.7
	β/α + β	11.0 ± 0.1	20.0 ± 2.4
	α/β	5.7 ± 0.9	20.4 ± 4.4
	β/α	7.4 ± 0.3	20.8 ± 2.9

*Luciferase activity is expressed as values relative to that of control samples. These experiments were done three times, and essentially the same results were obtained.

†All cDNAs encoding hGMR subunits were inserted into pME18S. Five micrograms of the *c-fos* promoter-luciferase plasmid and various combinations of 2 μg of each receptor plasmid were used for transfection. When the single or no receptor plasmid was used, the total amount of transfected DNA was adjusted with pME18S.

‡Ba/F3 or NIH3T3 cells were depleted for either mIL-3 or FCS, respectively, then Ba/F3 cells were stimulated with 1 ng/ml mIL-3 and NIH3T3 cells were stimulated with 10% FCS, as described in Methods.

§At 5 ng/ml.

Methods. The transfectant expressing the α/β subunit alone, designated Ba/F3-α/β, and transfectants expressing both subunits in various combinations—designated Ba/F3-α,β, Ba/F3-α/β,β/α, and Ba/F3-α/β,β—were used for subsequent studies. First, we characterized the binding properties of hGMR in these receptor transfectants. As shown in Table II, all of these transfectants, except for Ba/F3-α/β cells, expressed high- and low-affinity hGMR. In contrast, Ba/F3-α/β cells exhibited only low-affinity hGMR. These results are consistent with previous observations that the extracellular domains of both the α and β subunits are sufficient to reconstitute high-affinity hGMR and that the cytoplasmic domains did not significantly affect the binding property of hGMR.²¹

Next, using Northern blot analysis, we attempted to determine whether hGMRs containing chimeric subunits could transduce signals to activate endogenous *c-fos*, *c-jun*, and *c-myc* genes (Fig. 6). These genes were activated by hGM-CSF stimulation in Ba/F3 and NIH3T3 cells expressing the wild type hGMR.^{20, 22, 23} In all Ba/F3 transfectants expressing

TABLE II. Ligand binding properties of hGMR expressed on Ba/F3 transfectants

Cells	High-affinity hGMR		Low-affinity hGMR	
	K _d (pmol/L)	No. of sites per cell	K _d (nmol/L)	No. of sites per cell
Ba/F3-α,β	192	11,000	10.8	91,000
Ba/F3-α/β,β/α	158	9,000	7.5	160,000
Ba/F3-α,β/α	159	7,000	6.3	104,000
Ba/F3-α/β,β	107	11,000	8.2	109,000
Ba/F3-α/β	ND	ND	8.2	306,000

Expression of each subunit was primarily confirmed by FACS analysis. Several independent clones of each transfectant expressed comparable levels of each subunit. These experiments were done for one of several independent clones of each transfectant. The specific activity of radiolabeled hGM-CSF was 9×10^5 cpm/pmol. K_d and the number of binding sites were determined by Scatchard plot analysis.

K_d, Dissociation constant; ND, not detected.

the high-affinity chimeric hGMR, with the exception of Ba/F3-α,β/α cells, hGM-CSF induced accumulation of *c-fos*, *c-jun*, and *c-myc* mRNAs with kinetics similar to those of cells expressing the wild type hGMR. Mouse IL-3 stimulation, used as a control, resulted in accumulation of these mRNAs in all the transfectants. These observations are consistent with the results shown in Table I. These experiments were done with at least three independent clones of each transfectant, and the results were reproducible.

Cell growth by hGM-CSF through chimeric hGMRs

As previously reported, proliferation of Ba/F3 cells expressing the wild type hGMR was supported by hGM-CSF in a dose-dependent manner.^{8, 21} We then asked whether the hGMRs containing chimeric subunits would transduce growth-promoting signals in Ba/F3 cells (Fig. 7). We first attempted to determine whether cell proliferation of these transfectants was supported by hGM-CSF. The cells maintained in RPMI 1640 medium supplemented with mIL-3 were transferred to the same medium supplemented with 3 ng/ml hGM-CSF at day 0. Several independent clones of Ba/F3-α/β,β/α and Ba/F3-α/β,β cells but not other types of transfectants grew for at least 7 days, with growth rates similar to that of Ba/F3-α,β cells grown in the presence of mIL-3 or hGM-CSF.

Next, dose requirement for cell proliferation of Ba/F3 cells was measured by using the MTT assay in the presence of various concentrations of hGM-

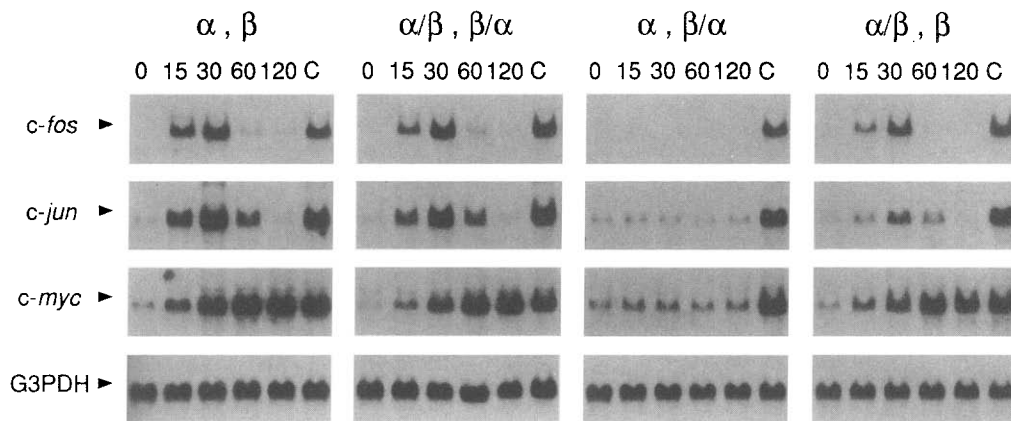


FIG. 6. Northern blot analysis of immediate early genes in Ba/F3 transfectants. Factor-depleted Ba/F3 clones were stimulated with either 1 ng/ml mIL-3 or 5 ng/ml hGM-CSF. After incubation for 30 minutes with mIL-3 as a control (*lane C*) or for indicated times (0, 15, 30, 60, and 120 minutes) with hGM-CSF (*lanes 0 to 120*), total RNA was extracted, and 10 μ g of each RNA was analyzed by Northern blotting, as described in Methods. The names of transfectants and detected mRNAs are given at the top and left of the figure, respectively.

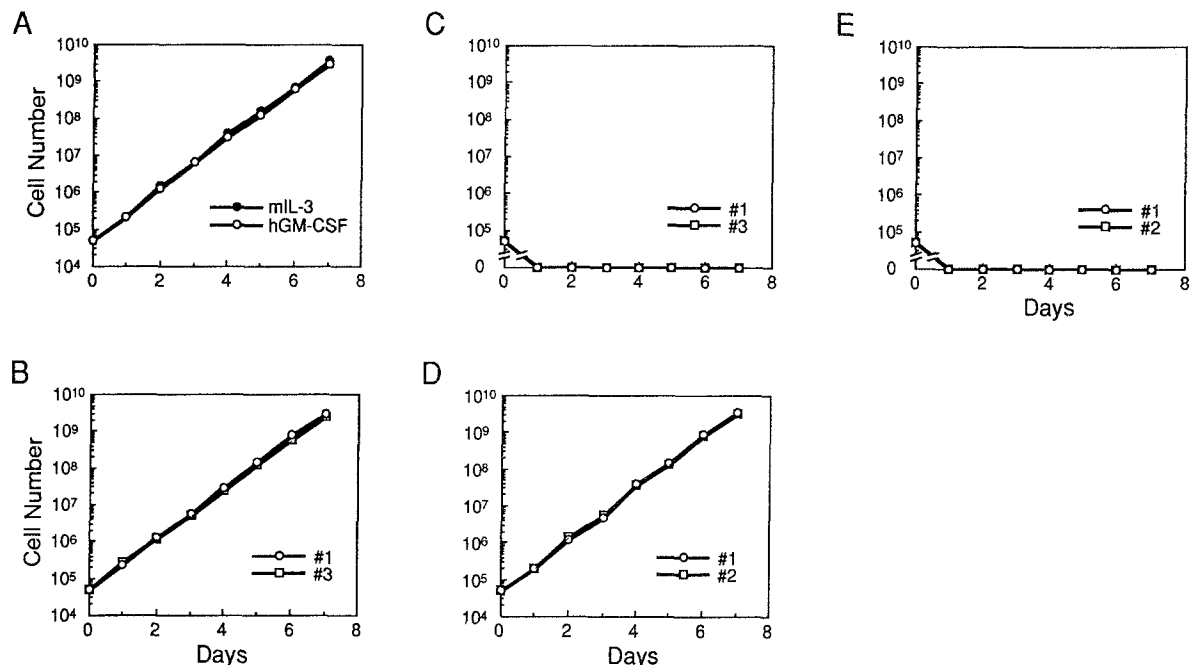


FIG. 7. Long-term growth of Ba/F3 transfectants by hGM-CSF. Continuously growing cells were harvested and washed with factor-free RPMI 1640 medium supplemented with 10% FCS and resuspended in the same medium containing 0.25 ng/ml mIL-3 (*filled symbols*) or 3 ng/ml hGM-CSF (*open symbols*) at 5×10^4 cells/ml at day 0. The number of trypan blue-resistant cells was counted every 24 hours. The vertical and horizontal axes indicate cell number and days of culture, respectively. **A**, Ba/F3- α, β . **B**, Ba/F3- $\alpha/\beta, \beta/\alpha$. **C**, Ba/F3- $\alpha, \beta/\alpha$. **D**, Ba/F3- $\alpha/\beta, \beta$. **E**, Ba/F3- α/β cells. Number sign (#) indicates clone number.

CSF. As shown in Fig. 8, *A* and *B*, Ba/F3- $\alpha/\beta, \beta/\alpha$ cells proliferated in response to a low concentration (1 ng/ml) of hGM-CSF. Likewise, Ba/F3- $\alpha, \beta/\alpha$ cells also proliferated in the presence of 1 ng/ml

hGM-CSF, at a level comparable to that observed with Ba/F3- α, β cells expressing the wild type hGMR (Fig. 8, *D*). In contrast, Ba/F3- $\alpha, \beta/\alpha$ and Ba/F3- α/β (Fig. 8, *C* and *E*) cells did not prolifer-

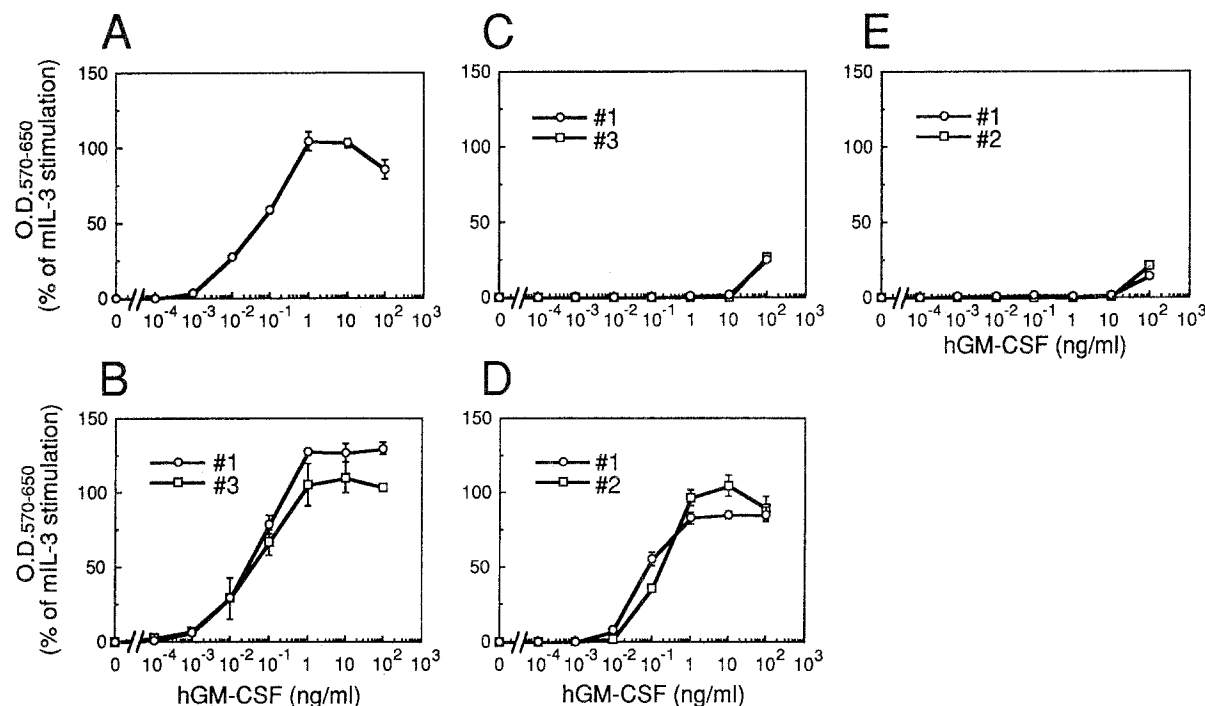


FIG. 8. Dose requirement of hGM-CSF-dependent cell proliferation of Ba/F3 cells. Proliferation of Ba/F3 transfectants was determined by MTT assay after culture for 24 hours in the presence of 0 to 100 ng/ml hGM-CSF. As a control, cells were cultured with 1 ng/ml mL-3. Horizontal axis indicates concentrations of hGM-CSF, and vertical axis indicates relative MTT reduction value normalized to values for cells incubated with 1 ng/ml mL-3. **A**, Ba/F3- α,β . **B**, Ba/F3- $\alpha,\beta/\alpha$. **C**, Ba/F3- $\alpha,\beta/\alpha$. **D**, Ba/F3- $\alpha/\beta,\beta$. **E**, Ba/F3- α/β cells. All values are averages of samples analyzed in triplicate, and standard deviations are shown as error bars. Number sign (#) indicates the clone number.

ate in response to 1 ng/ml hGM-CSF. Proliferation of Ba/F3- $\alpha,\beta/\alpha$ and Ba/F3- α/β cells in response to 100 ng/ml hGM-CSF is likely due to a low-affinity and functional hGMR formed by the α and α/β subunits with endogenous AIC2B protein, respectively, and is consistent with a previous observation.⁸ We performed these experiments at least three times with several independent clones of each transfectant, and we obtained essentially the same results.

Analysis of tyrosine-phosphorylated protein induced by hGM-CSF in Ba/F3 cells expressing chimeric hGMRs

It has been well documented that hGM-CSF induces tyrosine phosphorylation of various intracellular proteins and tyrosine kinase inhibitors suppress cell growth and modulate the level of accumulation of immediate early genes.^{15, 21, 23} Therefore protein tyrosine phosphorylation has important roles in the signal transduction of hGM-CSF. We analyzed the pattern of protein tyrosine

phosphorylation, induced by hGM-CSF through the high-affinity chimeric hGMRs, by Western blotting with the anti-phosphotyrosine antibody 4G10 (Fig. 9). In Ba/F3- α,β cells, hGM-CSF stimulated tyrosine phosphorylation of proteins (130, 95, 93, 76, 66, 55, 45, and 43 kd) in a manner similar to that seen with mL-3 (Fig. 9, lanes 2 and 3). These proteins were also phosphorylated in Ba/F3- $\alpha,\beta,\beta/\alpha$ and Ba/F3- $\alpha/\beta,\beta$ cells in response to hGM-CSF, with a pattern indistinguishable from that for the Ba/F3- α,β cells (Fig. 9, lanes 5 and 9). In contrast to these transfectants, hGM-CSF failed to induce any detectable tyrosine phosphorylation in Ba/F3- $\alpha,\beta/\alpha$ cells (Fig. 9, lane 7). We performed these experiments at least three times with three independent clones of each transfectant, and we obtained essentially the same results.

DISCUSSION

Previous studies in which various mutants of the β subunit of hGMR were used have demonstrated that the cytoplasmic domain of this subunit is

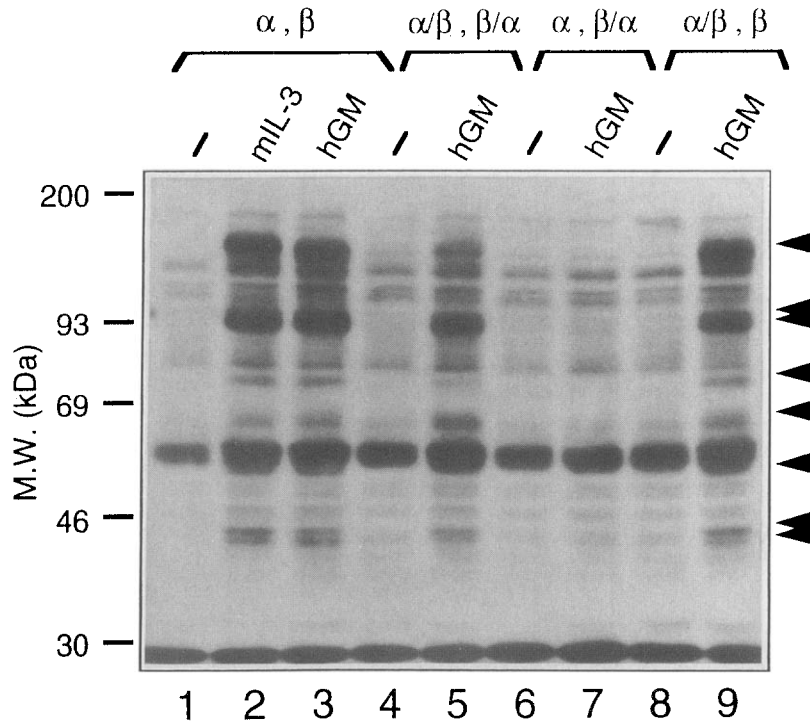


FIG. 9. Induction of tyrosine phosphorylation in response to hGM-CSF in Ba/F3 transfectants. After factor depletion at the concentration of 4×10^6 cells/ml, cultures were additionally incubated for 10 minutes with 1 ng/ml mIL-3 (lane 2), 5 ng/ml hGM-CSF (lanes 3, 5, 7, and 9), or no factor (lanes 1, 4, 6, and 8). The cells were harvested and lysed in Laemmli's solution at 2×10^7 cells/ml. Ten microliters of total cell extracts corresponding to 2×10^5 cells from Ba/F3- α,β (lanes 1 to 3), Ba/F3- $\alpha/\beta,\beta/\alpha$ (lanes 4 and 5), Ba/F3- $\alpha,\beta/\alpha$ (lanes 6 and 7), and Ba/F3- $\alpha/\beta,\beta$ (lanes 8 and 9) were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (8%); and tyrosine-phosphorylated proteins were analyzed by Western blotting with anti-phosphotyrosine antibody 4G10. Sizes of proteins are shown in kilodaltons on the left. Positions of hGM-CSF-induced tyrosine-phosphorylated proteins are indicated by arrowheads on the right.

required for signal transduction in a normal form of the receptor.²¹⁻²⁴ Although the cytoplasmic domain of the α subunit is also necessary for cell growth, it was not clear whether this domain was required to transduce other signals.^{21, 24, 31, 32} In this study, we extended these observations regarding the requirements of the cytoplasmic domain of the α subunit in hGM-CSF signaling, such as tyrosine phosphorylation of cellular proteins, including the β subunit and activation of the *c-fos* promoter (Fig. 3). Therefore cytoplasmic domains of both subunits are absolutely required for various cellular responses induced by hGM-CSF. We also found that only 29 amino acid residues, juxtaposed to the transmembrane domain (or the AH region), were sufficient for signal transduction. A short proline-rich motif (R³³²LFPPVP), which is similar to the box 1 motif of the β subunit, is found in the AH region of the α subunit.⁴ The AH region also has weak similarity to the membrane proximal region in the cytoplasmic domain of the IL-2R γ

(γ_c) subunit (Fig. 2). Because both the β subunit of hGMR and the γ_c subunit have been shown to associate with JAK kinases, it is tempting to speculate that the α subunit associates with a member of JAK family kinase that plays a role in signal transduction. If such a kinase exists, it is unlikely to be JAK2 because association of the α subunit and JAK2 was not detected even under conditions in which both the α subunit and JAK2 were overexpressed.³³

Analyses with various deletion mutants of the α and β subunits demonstrated that the cytoplasmic domains of both subunits are required for various cellular responses induced by hGM-CSF including cell proliferation, activation of immediate early genes, and induction of tyrosine phosphorylation of cellular proteins. However, more specific roles performed by the cytoplasmic domain of each subunit are unknown. To further analyze the roles of the cytoplasmic domains of both subunits, we used the chimeric receptor approach (Fig. 5).

When we constructed chimeric α/β and β/α subunits of hGMR, we found that the high-affinity hGMR($\alpha/\beta, \beta/\alpha$), composed of the chimeric α/β and β/α subunits, was functional and transduced signals in Ba/F3 cells for activation of immediate early genes, tyrosine phosphorylation, and cell proliferation. Thus the original configuration between extracellular and cytoplasmic domains of the hGMR(α, β) subunits was not required to reconstitute an active hGMR. On the basis of this evidence, we did a series of experiments with the wild type (α and β) and chimeric (α/β and β/α) subunits of hGMR. Reconstitution studies indicated that the high-affinity hGMR($\alpha/\beta, \beta$), which is composed of the α/β and β subunits and carries no cytoplasmic portion of the α subunit, was functional; whereas the high-affinity hGMR($\alpha, \beta/\alpha$), which carries only the cytoplasmic domain of the α subunit, failed to transduce any signals. Thus the cytoplasmic domain of the β subunit, in an oligomeric form, is sufficient to transduce growth-promoting signals, and the cytoplasmic domain of the α subunit can be dispensed to form a functional hGMR in the hGMR($\alpha/\beta, \beta$).

With regard to the manner in which hGM-CSF activates hGMR($\alpha/\beta, \beta$) without involving the cytoplasmic domain of the α subunit, at least two possibilities can be considered. One is that the cytoplasmic domain of the chimeric α/β subunit could substitute for the function of the cytoplasmic domain of the α subunit. In this context the cytoplasmic domain of the α/β subunit in hGMR($\alpha/\beta, \beta$) may activate the β subunit by promoting a conformational change of the β subunit in a manner similar to that of the cytoplasmic domain of the α subunit in wild type hGMR. Alternatively, a molecule or molecules associated with the cytoplasmic domain of the α subunit may be functionally mimicked by a molecule or molecules associated with the cytoplasmic domain of the α/β subunit. As already mentioned, it is possible that a JAK or JAK-like kinase associates with the cytoplasmic domain of the α subunit. If this is found to be the case, JAK2, associating with box 1 motif of the β subunit, may substitute for the function of the α -associating kinase. The other possibility for activation of hGMR($\alpha/\beta, \beta$) is that the cytoplasmic domain of the chimeric α/β subunit and cytoplasmic domain of the β subunit formed an active β -dimer-like structure. This is not the case for hGM-CSF-induced dimerization of the β subunit, because the β subunit formed a constitutive homodimer without hGM-CSF, as shown in this study. At present, we have no direct

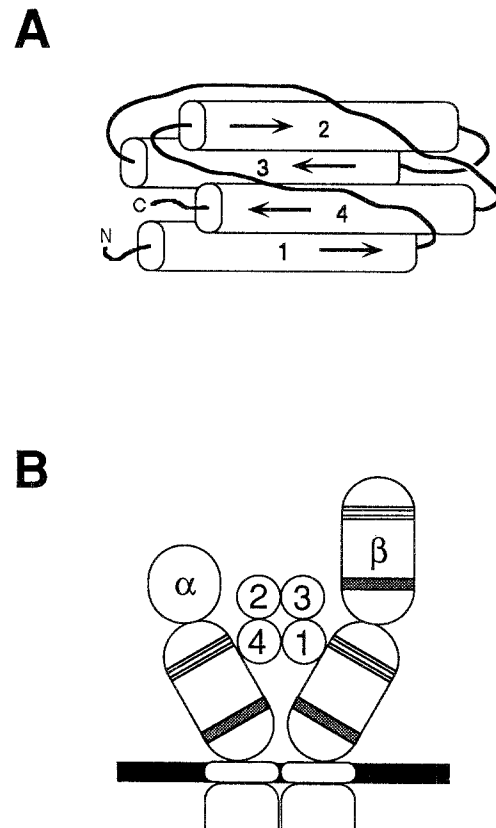


FIG. 10. Structure of GM-CSF and its association with GMR. **A**, Tertiary structure of GM-CSF. GM-CSF, as well as other cytokines, has four α helices, which are numbered from the N-terminal. **B**, Association of GM-CSF with GMR. The first and fourth helices associate with the β and α subunits of GMR, respectively.

evidence to determine which is the most likely possibility. Similar results and interpretations were recently reported in the IL-5R system.³⁴

We showed in this study that growth-promoting signals could be transduced through the cytoplasmic domain of the β subunit without involvement of the cytoplasmic domain of the α subunit in a chimeric hGMR system. This does not exclude the possibility that the cytoplasmic domain of the α subunit may also have alternative functions to transduce specific signals governing cellular responses other than proliferation. The α subunit-specific signals may account for the specificity of GM-CSF, IL-3, and IL-5. An interesting report has demonstrated existence of such a subunit-specific signal by using chimeric receptors.³⁵ Each of these chimeric receptors consisted of the extracellular domain of c-kit and the cytoplasmic domain of the β or γ subunit of IL-2R (kit/2 β or kit/2 γ), and stem cell factor (the ligand of c-kit) induced ho-

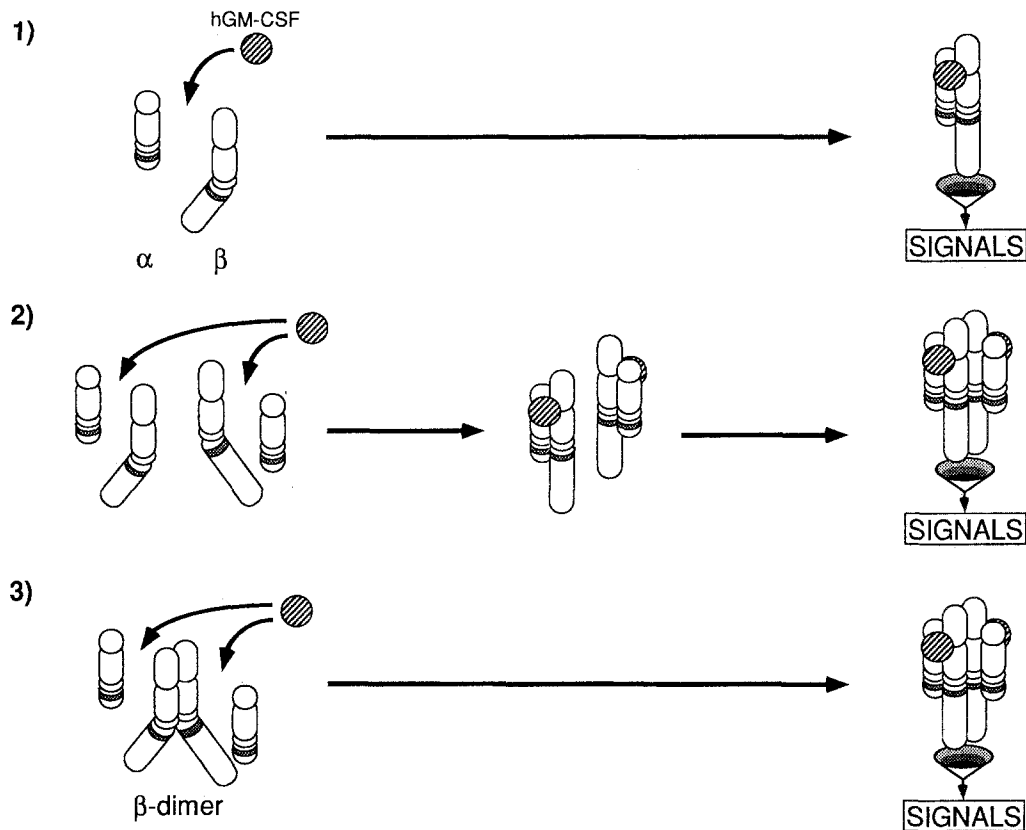


FIG. 11. Possible models of the activation mechanism of GMR.

modimerization or heterodimerization of these chimeric subunits. In this report it was shown that Ba/F3 cells expressing kit/2β alone or both kit/2β and kit/2γ grew in response to stem cell factor, but upregulation of IL-2Rα expression occurred only in the latter cells. The results indicated that γ subunit-specific signals are required for upregulation of IL-2Rα expression but are not required for growth of Ba/F3 cells. Therefore a comparison of signals transduced through hGMR(α,β) with those through hGMR(α/β,β) may shed more light on cellular events specifically induced only in the presence of the cytoplasmic domain of the α subunit.

At present, stoichiometry of the α and β subunits remains to be clarified. Several lines of evidence led to the speculation that the α and β subunits exist in a molar ratio of one to one in a functional hGMR. Most cytokines are composed of four α helices and are similar in tertiary structure (Fig. 10, A).³⁶ The mode of association between a cytokine and a receptor, which has been initially elucidated by crystallography of the GH-GHR complex,³⁷ is that one molecule of ligand binds to two molecules of the receptor and each of

the first and fourth helices of the ligand is a binding site for each of two receptors. Results consistent with this observation were obtained from mutation analyses of hGM-CSF, in which first and fourth helices bind to the β and α subunits, respectively (Fig. 10, B).³⁸

On the basis of these observations, it is possible to propose three models for an active form of hGMR (Fig. 11): (1) a monomeric α subunit and a monomeric β subunit form an active heterodimer in response to hGM-CSF, (2) homodimerization of the β subunit is induced through association with the α subunit (a model similar to that of IL-6R), and (3) the β subunit forms a homodimer, even in the absence of hGM-CSF. This homodimer exists as an inactive form without hGM-CSF and is activated by hGM-CSF-dependent interaction with the α subunit. Although it is unclear which is true, we recently obtained evidence supporting the last possibility. More work is required to clarify the exact mechanism required to activate hGMR.

We thank T. Kinoshita, T. Kitamura, N. Sato, and H. Kurata for collaboration and helpful discussion, and M. Ohara for comments on this manuscript.

REFERENCES

- Baldwin GC. The biology of granulocyte-macrophage colony-stimulating factor: effects on hemetopoietic and non-hematopoietic cells. *Dev Biol* 1992;151:352-67.
- Gasson JC. Molecular physiology of granulocyte-macrophage colony-stimulating factor. *Blood* 1991;77:1131-45.
- Bazan JF. Structural design and molecular evolution of a cytokine receptor superfamily. *Proc Natl Acad Sci USA* 1990;87:6934-8.
- Gearing DP, King JA, Gough NM, Nicola NA. Expression cloning of a receptor for human granulocyte-macrophage colony-stimulating factor. *EMBO J* 1989;8:3667-76.
- Hayashida K, Kitamura T, Gorman DM, Arai K, Yokota T, Miyajima A. Molecular cloning of a second subunit of the receptor for human granulocyte-macrophage colony-stimulating factor (GM-CSF): reconstitution of a high-affinity GM-CSF receptor. *Proc Natl Acad Sci USA* 1990;87:9655-9.
- Kitamura T, Sato N, Arai K, Miyajima A. Expression cloning of the human IL-3 receptor cDNA reveals a shared β subunit for the human IL-3 and GM-CSF receptors. *Cell* 1991;66:1165-74.
- Murata Y, Takaki S, Migata M, Kikuchi Y, Tominaga A, Takatsu K. Molecular cloning and expression of the human interleukin 5 receptor. *J Exp Med* 1992;175:341-51.
- Kitamura T, Hayashida K, Sakamaki K, Yokota T, Arai K, Miyajima A. Reconstitution of functional receptors for human granulocyte/macrophage colony-stimulating factor (GM-CSF): evidence that the protein encoded by the AIC2B cDNA is a subunit of the murine GM-CSF receptor. *Proc Natl Acad Sci USA* 1991;88:5082-6.
- Miyajima A, Mui AL-F, Ogorochi T, Sakamaki K. Receptors for granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5. *Blood* 1993;82:1960-74.
- Tavernier J, Devos R, Cornelis S, Tuypens T, Van der Heyden J, Fiers W, Plaetnick G. A human high affinity interleukin-5 receptor (IL5R) is composed of an IL-5-specific α chain and a β chain shared with the receptor for GM-CSF. *Cell* 1991;66:1175-84.
- Itoh N, Yonehara S, Schreurs J, et al. Cloning of an interleukin-3 receptor gene: a member of a distinct receptor gene family. *Science* 1990;247:324-7.
- Gorman DM, Itoh N, Kitamura T, et al. Cloning and expression of a gene encoding an interleukin 3 receptor-like protein: identification of another member of the cytokine receptor gene family. *Proc Natl Acad Sci USA* 1990;87:5459-63.
- Hara T, Miyajima A. Two distinct functional high affinity receptors for mouse interleukin-3 (IL-3). *EMBO J* 1992;11:1875-84.
- Harel-Bellan A, Farrar WL. Modulation of proto-oncogene expression by colony stimulating factors. *Biochem Biophys Res Commun* 1987;148:1001-8.
- Kanakura Y, Druker B, Cannistra SA, Furukawa Y, Torimoto Y, Griffin JD. Signal transduction of the human granulocyte-macrophage colony-stimulating factor and interleukin-3 receptors involves tyrosine phosphorylation of a common set of cytoplasmic proteins. *Blood* 1990;76:706-15.
- Linnekin D, Farrar WL. Signal transduction of human interleukin 3 and granulocyte-macrophage colony-stimulating factor through serine and tyrosine phosphorylation. *Biochem J* 1990;271:317-24.
- Wang H-M, Collins M, Arai K, Miyajima A. EGF induces differentiation of an IL-3-dependent cell line expressing the EGF receptor. *EMBO J* 1989;8:3677-84.
- Eder M, Griffin JD, Ernst TJ. The human granulocyte-macrophage colony-stimulating factor receptor is capable of initiating signal transduction in NIH3T3 cells. *EMBO J* 1993;12:1647-56.
- Sasaki K, Chiba S, Hanazono Y, Mano H, Yazaki Y, Hirai H. Coordinate expression of the α and β chains of human granulocyte-macrophage colony-stimulating factor receptor confers ligand-induced morphological transformation in mouse fibroblasts. *J Biol Chem* 1993;268:13697-702.
- Watanabe S, Mui AL-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.
- Sakamaki K, Miyajima I, Kitamura T, Miyajima A. Critical cytoplasmic domains of the common β subunit of the human GM-CSF, IL-3 and IL-5 receptors for growth signal transduction and tyrosine phosphorylation. *EMBO J* 1992;11:3541-9.
- Sato N, Sakamaki K, Terada N, Arai K, Miyajima A. Signal transduction by the high-affinity GM-CSF receptor: two distinct cytoplasmic regions of the common β subunit responsible for different signaling. *EMBO J* 1993;12:4181-9.
- 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.
- Weiss M, Yokoyama C, Shikama Y, Naugle C, Druker B, Sieff C. Human granulocyte-macrophage colony-stimulating factor receptor signal transduction requires the proximal cytoplasmic domains of the α and β subunits. *Blood* 1993;82:3298-306.
- Miyajima A, Schreurs J, Otsu K, Kondo A, Arai K, Maeda S. Use of the silkworm, *Bombyx mori*, and an insect baculovirus vector for high-level expression and secretion of biologically active mouse interleukin-3. *Gene* 1987;58:273-81.
- Watanabe Y, Kitamura T, Hayashida K, Miyajima A. Monoclonal antibody against the common β subunit (β_c) of the human interleukin-3 (IL-3), IL-5, and granulocyte-macrophage colony-stimulating factor receptors shows up-regulation of β_c by IL-1 and tumor necrosis factor- α . *Blood* 1992;80:2215-20.
- Scatchard G. The attractions of proteins for small molecules and ions. *Ann NY Acad Sci* 1949;51:660-72.
- Mosmann T. Rapid colorimetric assays for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:55-63.
- Chomczynski P, Sacchi N. Single-step method of RNA isolation by guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
- Polotskaya A, Zhao Y, Lilly ML, Kraft AS. A critical role for the cytoplasmic domain of the granulocyte-macrophage colony-stimulating factor α receptor in mediating cell growth. *Cell Growth Differ* 1993;4:523-31.
- Polotskaya A, Zhao Y, Lilly MB, Kraft AS. Mapping the

- intracytoplasmic regions of the granulocyte-macrophage colony-stimulating factor receptor necessary for cell growth regulation. *J Biol Chem* 1994;269:14607-13.
33. Quelle FW, Sato N, Witthuhn BA, et al. JAK2 associates with the bc 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.
34. Takaki S, Kanazawa H, Shiiba M, Takatsu K. A critical cytoplasmic domain of the interleukin-5 (IL-5) receptor α chain and its function in IL-5-mediated growth signal transduction. *Mol Cell Biol* 1994;14:7404-13.
35. Nelson BH, Lord JD, Greenberg PD. Cytoplasmic domains of the interleukin-2 receptor β and γ chains mediate the signal for T-cell proliferation. *Nature* 1994;369:333-6.
36. Sprang SR, Bazan JF. Cytokine structural taxonomy and mechanisms of receptor engagement. *Curr Opin Structural Biol* 1993;3:815-27.
37. De Vos AM, Ultsch M, Kossiakoff AA. Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. *Science* 1992;255:306-12.
38. Hercus TR, Cambareri B, Dottore M, et al. Identification of residues in the first and fourth helices of human granulocyte-macrophage colony-stimulating factor involved in biologic activity and in binding to the α - and β -chains of its receptor. *Blood* 1994;83:3500-8.