Granulocyte-macrophage colony-stimulating factor (GM-CSF) regulates differentiation, survival, and proliferation of myeloid progenitor cells. The biologic actions of GM-CSF are mediated by its binding to the α and β subunits of the GM-CSF receptor (GM-CSFRα and βc, respectively). To determine whether identical regions of the βc protein mediate both cell growth and differentiation, we expressed cDNA constructs encoding the human wild-type (897 amino acids) and truncated βc (hβc) subunits along with the wild-type human GM-CSFRα subunit in the murine WT19 cell line, an FDC-P1-derived cell line that differentiates toward the monocytic lineage in response to murine GM-CSF. Whereas the WT19 cell line carrying the C-terminal deleted hβc subunit of 627 amino acids was still able to grow in human GM-CSF (hGM-CSF), 681 amino acids of the hβc were necessary for cell differentiation. The addition of hGM-CSF to WT19 cell lines containing the hβc627 subunit stimulated the phosphorylation of ERK (extracellular signal-regulated kinase) and induced the tyrosine-phosphorylation of SHP-2 and STAT5, suggesting that the activation of these molecules is insufficient to mediate the induction of differentiation. A point mutation of tyrosine 628 to phenylalanine (Y628F) within hβc681 abolished the ability of hGM-CSF to induce differentiation. Our results indicate that the signals required for hGM-CSF-induced differentiation and cell growth are mediated by different regions of the hβc subunit.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is secreted by activated T cells, endothelial cells, fibroblasts, mast cells, B cells, and macrophages and plays an important role in promoting differentiation, survival, and proliferation of colony-forming unit-granulocyte-macrophage (CFU-GM) progenitor cells. GM-CSF also enhances the function of mature neutrophils, monocytes, and eosinophils, and stimulates the burst-promoting activity for BFU-E (1–3). In addition, in murine models, alveolar proteinosis, a disease caused by excessive secretion of protein into the alveolar spaces, is associated with the deletion of the GM-CSF gene, suggesting that GM-CSF is essential for alveolar macrophage functions (4, 5).

The biologic actions of GM-CSF are mediated by its binding to a specific receptor consisting of α and β subunits, both of which are members of the type I cytokine receptor family (6, 7). The α subunit (GM-CSFRα) binds GM-CSF with low affinity (6). The β subunit does not bind GM-CSF itself, but it forms a high affinity receptor in combination with the α subunit (7). The β chain is referred to as the common β chain (βc) because it is also utilized by interleukin 3 (IL-3) and interleukin 5 (IL-5) (8, 9).

We have recently reported that the cytoplasmic domain of the human GM-CSFRα (hGM-CSFRα) is indispensable for cellular differentiation induced by human GM-CSF (hGM-CSF) (10). In addition, the cytoplasmic domain of the human βc (hβc) has recently been demonstrated to be essential for hGM-CSF-mediated myeloid cell differentiation (11). However, the exact mechanism of differentiation by GM-CSF has not been clearly elucidated. In the present study, we examined the role of βc in GM-CSF-mediated cell differentiation by using the murine WT19 cell line, an FDC-P1-derived cell line that uniformly differentiates toward the monocytic lineage in response to murine GM-CSF (mGM-CSF), but grows without differentiation in the presence of murine IL-3 (mIL-3) (10, 12). By expressing the wild-type and mutated hβc constructs along with the wild-type hGM-CSFRα in WT19 cells, we demonstrate the following results. 1) Mutation of the two prolines in the box 1 region of hβc, which are necessary for Jak2 activation, results in the loss of hGM-CSF-induced cell differentiation. 2) The hβc C-terminal truncation mutants demonstrate that 681 amino acids of the hβc are sufficient to mediate hGM-CSF-induced differentiation, but the truncation of the hβc subunit to amino acid 627 abolishes this differentiation response. In contrast, 559 amino acids of the hβc are sufficient for hGM-CSF-induced cell growth. 3) Within the hβc681 subunit, a point mutation of tyrosine 628 to phenylalanine abolishes the activity of hβc681 to induce differentiation. 4) The activation of either ERK or STAT5 by hGM-CSF does not correlate with the ability of hGM-CSF to induce myeloid cell differentiation. These results demonstrate that hGM-CSF-mediated cell growth and differentiation require distinct regions of the hβc cytoplasmic domain.
Domains of hβc Causing hGM-CSF-mediated Differentiation

EXPERIMENTAL PROCEDURES

Cells and Cell Culture—The mouse IL-3 (mIL-3)-dependent WT19 cell line derived from the FDC-P1 cell line was a generous gift from Dr. Larry Rohrschneider (Fred Hutchinson Cancer Research Center, Seattle, WA) and was described previously (10, 12). The cells were cultured in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum and 10% WEHI-3B conditioned medium containing mIL-3.

Reagents—Recombinant hGM-CSF was purchased from Immunex Corp. (Seattle, WA). Recombinant mGM-CSF and mIL-3 were obtained from Genzyme (Cambridge, MA). PD98059, a specific inhibitor of ERK kinase (MEK) was purchased from Alexis Corp. (San Diego, CA).

Plasmids—The expression plasmid pEFBSOS-hGM-CSF9a containing the wild-type hGM-CSF RNA was a generous gift of Dr. N. A. Nicola (Walter and Eliza Hall Institute for Medical Research, Victoria, Australia). The wild-type human GM-CSFR β chain (hGM-CSFR) cDNA was obtained from the plasmid pH97 (provided by Dr. A. Miyajima, DNA Research Institute, Palo Alto, CA), and the 2.9-kilobase HindIII-NotI insert was ligated into pCEP4 (Invitrogen, Carlsbad, CA), which contains a hygromycin selection marker giving the plasmid pCEP4-hGM-CSFR. To construct pCEP4-hβc 602, 796, 681, 627, and 592, the wild-type Bgl II-Xhol fragment of pCEP4-hGM-CSFR was replaced with the Bgl II-Xhol fragments of the PCR products generated with 5' primer, GCCCTGAC-AGACTCCAGCTG, and 3' primer, GGAACTCTAGACTACTATGGC-CCCAAGGACCAGG (hGM-CSFR) (832), TGGCTGATAGGCCTGATCGTGTAAGTCAAGGCT (hGM-CSFR) (627), or TGCTTCTAGATCGGCCCTAGTTAGCAAGCT (hGM-CSFR) (592). The PCR conditions for pCEP4-hβc 602, 627, and 592 were as described in our previous studies (5, 11, 12) using the primers BssHII-Xhol fragment of pCEP4-hGM-CSFR was replaced with the BssHII-Xhol fragments of the PCR products generated with 5' primer, TGGAGTGAGGCGCGCTCCTGGGACACCGAGTCGGTGCTG, and 3' primer, TGCTTCTAGATCGGCCCTAGTTAGCAAGCT (hGM-CSFR) (592). The PCR conditions for pCEP4-hβc 473, the wild-type hβc cDNA was introduced into WT19 cells in combination with the leader sequence to an SDS-polyacrylamide gel and detected by Western blotting.

MTS Cell Proliferation Assay—5,000 cells were incubated in 100 μl of RPMI 1640 containing 10% fetal bovine serum and various concentrations of hGM-CSF for 14 h at 37 °C in a humidified 5% CO2 atmosphere. 20 μl of freshly prepared combined 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt/phenazine methosulfate (MTS/PMS) solution (Promega, Madison, WI) was added to each well. After an additional 4 h of incubation at 37 °C, the conversion of MTS into the aqueous soluble formazan was measured at an absorbance of 490 nm.

Graft Fusion Protein and Affinity Purification—Glycine (gift from Dr. E. M. Blackwood, University of California, San Diego, La Jolla, CA) was previously described (15). Glycine was fused to the N terminus of recombinant hGM-CSF. As evaluated by Western blotting, the fusion protein was isolated from 2.9 ml of fresh cultures of WT19 cells treated with mGM-CSF 2 μM in PLC lysis buffer, and then sonicated in SDS-sample buffer, and heated at 95 °C for 5 min. The eluted proteins were applied to an SDSPolyacrylamide gel and detected by Western blotting.

RESULTS

Expression of Wild-type and Mutant hβc in WT19 Cells—To study the role of hβc in hGM-CSF-mediated differentiation, we constructed a series of cytoplasmic deletion and point mutants of the hβc subunit (Fig. 1A). These mutant hβc subunits were transfected into WT19 cells expressing the wild-type hGM-CSFR subunit (Fig. 1, B and C). The amino acid length of each hβc mutant represents the number of amino acids from the N terminus including the leader sequence. As evaluated by Western blotting and flow cytometry, the expression level of each mutant hβc subunit in these clonal cell lines (Fig. 2, A and B) was found to be similar. The mutations introduced into the hβc cytoplasmic domain did not affect the high affinity ligand binding as reported by others (16) (data not shown). At least three clones were isolated expressing each hβc mutant and were analyzed in the following experiments (see below).

Induction of Monocytic Differentiation through hβc in WT19 Cells—Parental WT19 cells growing in mIL-3 demonstrated a myeloblast morphology including rounded nuclei, fine chromatin, and basophilic cytoplasm. In response to mGM-CSF, the cells demonstrated monocytic characteristics: an indented nucleus with fine stranded appearance, increased cytoplasm containing a variable number of vacuoles, and larger total cell size. WT-19 cells treated with mGM-CSF demonstrated significantly increased surface expression of F4/80, a marker of monocyte differentiation (17). As previously reported (10), hGM-CSF...
treatment of WT19αβwt cells expressing hGM-CSFRα and the wild-type hβc subunits induced monocytic differentiation of WT19 cells, as measured by changes in morphology and the surface expression of F4/80 (Fig. 3, A and B).

The type I cytokine receptors share amino acid sequence homology in a membrane proximal region called the box 1 region (18, 19). The box 1 region contains conserved prolines that are essential for Jak2 activation by various cytokines (20). To test whether these prolines are necessary for hGM-CSF-induced differentiation, they (Pro-479 and -481) were mutated to alanines and this receptor (hβcPA) was expressed along with the wild-type hGM-CSFRα in WT19 cells. The addition of hGM-CSF to this cell line (WT19αβPA) failed to induce differentiation as measured by morphology and the lack of induction of F4/80 expression (Fig. 3, A and B), indicating that the prolines of the box 1 region are essential for hGM-CSF-mediated differentiation.

To determine which regions of hβc subunit are required for hGM-CSF-mediated cell differentiation and growth, additional truncation mutants were generated, and stable cell lines were isolated. Clear morphological differentiation was observed in WT19 cell lines expressing hβc subunits of 832, 796, or 681 amino acids in length when stimulated by hGM-CSF (Fig. 3A, Table I). In these cell lines, hGM-CSF induced the increase of F4/80 surface expression (Fig. 3B, Table I). In contrast, WT19 cells expressing hβc subunit containing 627 or fewer amino acids did not exhibit morphological differentiation or increased F4/80 expression in response to hGM-CSF (Fig. 3, Table I). These results suggest that the region between amino acids 627 and 681 of hβc is essential for hGM-CSF-induced cell differentiation of WT19 cells.

As phosphorylated tyrosines are important for the induction of cell growth and differentiation by several growth factor receptors (12, 21–23), the possibility that tyrosine residues of hβc681 were involved in the differentiation signal was examined next. Only one tyrosine (Tyr-628) is found between amino acids 627 and 681 of hβc subunit. To determine whether Tyr-628 is involved in differentiation signals, it was mutated to phenylalanine in the truncated hβc681. First, to examine if Tyr-628 of hβc is phosphorylated in response to hGM-CSF in WT19 cells, WT19αβ681 and WT19αβ681Y628F cells were stimulated with hGM-CSF, and the immunoprecipitated hβc

![Fig. 1. Schematic structure of the hβc mutants. A, the extracellular domains are abbreviated. Positions of the tyrosine residues, the conserved box 1 and 2, and extended box 2 regions are indicated. Amino acid residues are numbered from the first methionine of the signal peptide. B, flow cytometric analysis of GM-CSFRα expression on WT19 pEF-BOS-hGM-CSFRα cells. Cells were stained with either the anti-GM-CSFRα monoclonal antibody or the control antibody followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG. C, Western blotting analysis of hGM-CSFRα expression in WT19 pEF-BOS-hGM-CSFRα cells. Cell lysates (3 x 10⁶ cells/lane) were separated on a 7% SDS-PAGE gel and immunoblotted with the monoclonal anti-hGM-CSFRα antibody.](image-url)
was probed with either the anti-phosphotyrosine antibody or the anti-h\(b\)c antibody (Fig. 4). The anti-h\(b\)c immunoblot demonstrates that the expression levels of the two receptors in WT19 cells was similar, whereas the anti-phosphotyrosine immunoblot demonstrates that the hGM-CSF-mediated tyrosine phosphorylation of h\(b\)c681Y628F was 10% of that of h\(b\)c681. Therefore, Tyr-628 is the major tyrosine-phosphorylated site within the h\(b\)c681 subunit.

Addition of hGM-CSF to clonal cell lines expressing this mutant, h\(b\)c681Y628F, failed to induce monocytic differentiation in response to hGM-CSF (Fig. 3, A and B). In comparison, when this identical point mutation was introduced into the full-length h\(b\)c subunit, h\(b\)cY628F, and expressed in WT19 cells, the cells differentiated normally in response to hGM-CSF (Fig. 3 A and B). These findings demonstrated that although Tyr-628 plays an important role in hGM-CSF-mediated cell differentiation in the truncated 681 amino acid receptor, the C-terminal region of the wild-type h\(b\)c may transmit a similar differentiation signal.

Proliferation and Differentiation Signals Are Mediated by Different Regions of h\(b\)c—GM-CSF regulates both cell differentiation and proliferation of myeloid progenitor cells. To investigate whether differentiation and proliferation signals are generated by different or identical regions of the h\(b\)c subunit, the growth of each of the above mutants was examined at low concentrations (1–40 pg/ml) of hGM-CSF (Fig. 5). The growth response to hGM-CSF of WT19 cells containing h\(b\)c796 was similar to \(a\)\(b\)wt cells. Cells containing the h\(b\)c627, h\(b\)c681, and h\(b\)c681Y628F transfectants grew at a reduced rate when compared with \(a\)\(b\)wt cells incubated with comparable concentrations of hGM-CSF. Similar results were obtained with WT19

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**Fig. 2.** Expression of h\(b\)c in WT19 transfectants. A, Western blotting analysis of h\(b\)c mutants expression in WT19 \(a\)\(b\) transfectants. Cell lysates (3 × 10^6 cells/lane) were separated on a 7% SDS-PAGE gel and immunoblotted with the polyclonal anti-h\(b\)c antibody. B, flow cytometric analysis of h\(b\)c expression on WT19\(a\)\(b\) transfectants. Cells were stained with either the anti-h\(b\)c monoclonal antibody or the control antibody followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG.

**Fig. 3.** Monocytic differentiation of WT19 \(a\)\(b\) transfectants with hGM-CSF treatment. A, morphological changes of WT19 transfectants in response to hGM-CSF. WT19\(a\)\(b\) transfectants were maintained in mIL-3 alone (1 ng/ml) or mIL-3 + 10 ng/ml hGM-CSF. Wright staining was performed (original magnification × 400) on day 3. B, surface expression of the monocyte-specific F4/80 antigen on WT19 transfectants treated with hGM-CSF. WT19 transfectants were maintained in mIL-3 alone or mIL-3 + 10 ng/ml hGM-CSF for 3 days. The cell surface expression of monocytic specific F4/80 was analyzed by flow cytometry after staining with the monoclonal anti-F4/80 antibody and fluorescein isothiocyanate-labeled anti-rat Ig secondary antibody.
The role of ERKs in the induction of cell differentiation is controversial (23, 27–30). WT19 cells containing a C-terminal truncation mutant of hGM-CSFRα subunit and the wild-type hβc subunit, hGM-CSF were capable of differentiation, but not ERK activation, in response to hGM-CSF (10). To further examine the role of the ERK pathway in the induction of differentiation, WT19 cells were treated with PD98059, a specific inhibitor of (MEK), prior to the addition of mGM-CSF. Although PD98059 addition to mGM-CSF-treated WT19 cells caused a 3-fold decrease in the activity of immunoprecipitated ERKs to phosphorylate GST-myc in vitro (Fig. 7A), this compound had no effect on the hGM-CSF-mediated differentiation of WT19 cells, as evidenced by the induction of F4/80 and morphologic changes (Fig. 7, B and C). Although the possibility that a small amount of ERK activity persists after PD98059 treatment in vivo cannot be ruled out by this experiment, these results suggest that full activation of the ERK pathway is neither sufficient nor necessary for GM-CSF-induced differentiation of WT19 cells.

**Figure 4. Tyrosine phosphorylation of hβc681 and hβc681Y628F in response to hGM-CSF stimulation.** WT19αβ681 and WT19αβ681Y628F cells were cytokine-starved for 6 h and stimulated with 10 ng/ml of hGM-CSF for the indicated times. Cell extracts (1 × 10^7 cells/lane) were immunoprecipitated with the anti-hβc monoclonal antibody, separated by SDS-PAGE, and immunoblotted with the antiphosphotyrosine antibody (top panel) or the anti-hβc polyclonal antibody (bottom panel).

**Table I**

| Proliferation | Differentiation |
|---------------|-----------------|
| αβcwt         | ++              | +               | +               |
| αβ832         | ++              | +               | +               |
| αβ796         | +               | +               | +               |
| αβ681         | +               | +               | +               |
| αβ681Y628F    | +               | +               | +               |
| αβ627         | +               | +               | +               |
| αβ592         | +               | +               | +               |
| αβ559         | +               | +               | +               |
| αβ473         | +               | +               | +               |
| αβPA          | +               | +               | +               |
| αβY628F       | +               | +               | +               |

**Discussion**

By expressing truncated forms of hβc in WT19 cells, we demonstrated that hGM-CSF-induced morphological differentiation and the surface expression of F4/80 requires hβc subunits of 832, 796, or 681 amino acids in length (Fig. 3, Table I). In contrast, WT19 cells expressing hβc subunit containing 627 amino acids or less did not differentiate but were capable of growth in the presence of hGM-CSF (Fig. 3). Mutation of the two prolines in the box 1 region blocked both growth and differentiation. Thus, these results suggest that the signals generated by the box 1 region of the receptor are necessary for both cellular events.

**Similar results to those described above have been generated using M1 and WEHI-3 B D+ cells as a target cell population for transfection (11). In this study, wild-type hβc and hβc783, but not hβc541 or 461, could mediate hGM-CSF-induced morphological change and differentiation marker induction, whereas induction of cell differentiation by hβc626 required a 50- to 100-fold higher concentration of hGM-CSF (M1 cells). However, in the WT19 cell system, neither hβc627 nor hβc681Y628F could induce cell differentiation even at a very high concentration (1 μg/ml) of hGM-CSF (data not shown). The results derived from M1 cells suggest that the region between amino acids 627 and 783 is necessary for the full differentiation signal, whereas our results more narrowly delineate the crucial region for differentiation to lie between 628 and 681.

The results described here demonstrate that tyrosine 628 is
FIG. 5. MTS proliferation assay of WT19 transfectants and parental WT19 cells. Cells (5,000 cells/sample) were incubated in RPMI 1640 containing 10% fetal bovine serum and various concentrations of hGM-CSF for 14 h at 37 °C. After MTS/PMS solution was added, cells were incubated for 4 h at 37 °C. The conversion of MTS was measured by the amount of 490 nm absorbance. Error bars from triplicate experiments are shown.

FIG. 6. Activation of the ERK pathway by hGM-CSF in WT19 transfectants. Cells were cytokine-starved for 6 h and stimulated by 10 ng/ml of hGM-CSF for 10 min. A, tyrosine phosphorylation of SHP-2 in response to hGM-CSF in WT19 transfectants. Cell extracts were immunoprecipitated with anti-SHP-2 antibody, and the Western blot was probed with anti-phosphotyrosine antibody (top panel) or anti-SHP-2 antibody (bottom panel). B, hGM-CSF-dependent phosphorylation of Shc in WT19 transfectants. Cell extracts were immunoprecipitated with anti-Shc antibody, and the Western blot was probed with anti-phosphotyrosine antibody (top panel) or anti-Shc antibody (bottom panel). C, lysates from 3 × 10⁶ cells were separated on a 7.5% SDS-polyacrylamide gel and immunoblotted with the anti-phospho-ERK antibody (P-ERK, top panel) or the anti-ERK2 antibody (ERK, bottom panel).

FIG. 7. The effect of PD98059, a specific MEK inhibitor, on mGM-CSF-mediated differentiation of WT19 cells. A, immune complex kinase assay. WT19 cells were cytokine-starved for 6 h, treated with or without PD98059 (50 μM) for 30 min, followed by addition of 10 ng/ml of mGM-CSF for 10 min. ERK2 was immunoprecipitated from 1 × 10⁷ cells, and a kinase reaction was performed using GST-MycN262 as a substrate. The degree of activation is indicated. B and C, the effect of PD98059 on mGM-CSF-induced monocytic differentiation of WT19 cells. WT19 cells were maintained in mIL-3 alone (1 ng/ml), mIL-3 + 10 ng/ml hGM-CSF, or mIL-3 + 10 ng/ml hGM-CSF + 50 μM PD98059 for 3 days. The monocytic cell differentiation was measured by cell morphology (B), and the surface expression of monocytic specific F4/80 antigen was analyzed by flow cytometry (C).
in gp130, the IL-6 receptor signaling subunit involved in ERK pathway has been suggested to be essential for the neu-
and may depend on the cell type and the stimulus involved. The 
cell differentiation.

In the macrophage colony stimulating factor signaling, both Tyr-807 and Tyr-721 of macrophage colony stimulating factor receptor were shown to be necessary for monocyte differentiation and for the tyrosine phosphorylation of phospholipase C-γ (PLCγ) (12, 22). The involvement of PLCγ in M-CSF-mediated cell differentiation was further demonstrated by the inhibition of differentiation using U-73122, a compound that acts as a specific inhibitor of PLCγ (22). The surrounding amino acid sequence of the hbc Tyr-628 (YLCL) is homologous to the optimal binding site for the N-terminal SH2 domain of PLCγ in a phosphopeptide binding assay (32). However, PLCγ may not be involved in GM-CSF-induced differentiation as suggested by the following data. First, tyrosine phosphorylation of PLCγ was not induced by addition of hGM-CSF to WT19 cells (data not shown) or another myeloid cell line treated with this hormone (33), although M-CSF induced significant tyrosine phosphorylation of PLCγ. Second, U-73122 did not inhibit the differentiation of WT19 cells by mGM-CSF at 1 μM, a concentration 10 times higher than that capable of inhibiting the M-CSF-mediated differentiation (22) (data not shown).

Although the single tyrosine mutation in the 681 hbc containing cell line had significant biologic effects, this mutation had no significant effect on differentiation in the wild-type receptor. The wild-type hbc contains four tyrosines C-terminal to Tyr-628. One of them (Tyr-882) has a similar amino acid (YLCL) to Tyr-628. Two of them (Tyr-628 and Tyr-642) have a leucine at the +3 amino acid (YVEL and YCEL, respectively). The amino acid at +3 position of the tyrosine is important in dictating the binding affinity for many SH2 domain-containing proteins (32). It is possible that a common signaling molecule binds to a number of tyrosines in the hbc-signaling cell differentiation so that, in the wild-type receptor, mutation of a single tyrosine is not sufficient to abolish cell differentiation.

The role of ERKs in differentiation appears to be complex and may depend on the cell type and the stimulus involved. The ERK pathway has been suggested to be essential for the neutrophil outgrowth of PC12 cells (27, 34). Also, interleukin 6 (IL-6) can induce PC12 differentiation when the cells are pretreated with nerve growth factor (35). A mutation of a specific tyrosine in gp130, the IL-6 receptor signaling subunit involved in ERK activation, abolishes the induction of cell differentiation by IL-6 (23). Our results suggest that the ERK pathway may not be involved in hGM-CSF-mediated differentiation of WT19 cells. 1) Although Tyr-628 of hbc was shown to be a binding site for SHP-2 SH2 domains (36), it was not necessary for hGM-CSF-induced tyrosine phosphorylation of SHP-2 or ERK activation (Fig. 6) (36). 2) PD98059, a specific inhibitor of MEK, blocked activation, but this compound had no significant effect on differentiation. 3) The expression of a constitutively active mutant of MEK in WT19 cells did not induce differentiation.2 Thus, activation of ERK does not appear to be sufficient or necessary for hGM-CSF-induced differentiation of WT19 cells.

Activation of Stat proteins by several cytokines has been suggested to be involved in cell differentiation (37–42). In WT19 cells transfected with hGM-CSFRs and wild-type hbc, hGM-CSF induced tyrosine phosphorylation of both isoforms of Stat5, but not other Stat proteins (Stat1, Stat3, and Stat6 examined). Tyr-628 was not necessary for the hGM-CSF-mediated tyrosine phosphorylation of either Stat5a or 5b. In addition, both hbc628 and hbc681Y628F could induce tyrosine phosphorylation of Stat5a and 5b in response to hGM-CSF equivalent to that seen in WT19 cells containing the wild-type receptor (Fig. 8). These results suggest that Stat5 activation is not sufficient for the induction of cellular differentiation. In a recent report, using chimeric receptors of erythropoietin and IL-3, a delayed activation of Stat5 was suggested to be important for the erythroid differentiation induction of BaF3 cells (43). However, we did not see a delay in the time course of Stat5 activation in WT19 transfectants which differentiate in response to hGM-CSF in contrast to those cell lines which are capable of only growth (data not shown). Thus, none of the signals examined (STAT5 activation along with SHP-2 and Shc phosphorylation) are sufficient to modulate differentiation.

In conclusion, our data suggest that cell differentiation and cell growth induced by hGM-CSF are mediated by different regions of the hbc subunit. Attempts are underway to define the novel signals generated by amino acids 627–681 of the hbc which control the differentiated phenotype.

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