A Dominant Negative Granulocyte-Macrophage Colony-stimulating Factor Receptor α Chain Reveals the Multimeric Structure of the Receptor Complex*

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The receptor for the hemopoietic growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF) is composed of two chains, both of which belong to the superfamily of cytokine receptors. The α chain confers low affinity binding only, whereas the β chain (βc) confers high affinity binding when associated with α. Ectopic expression of both chains of the receptor in murine NIH-3T3 fibroblasts results in signal transduction, mitogenesis, and morphologic transformation. The cytoplasmic domain of the GM-CSF receptor α subunit (GMR-α) comprises 54 amino acids that have been shown to be important for signal transduction through the β chain. The present study was designed to address the possibility of receptor oligomerization and its functional implication. Cross-linking studies with 125I-GM-CSF on NIH-3T3 transfectants is consistent with the presence of α and βc dimers and of receptor oligomers. We have, therefore, generated an inert α chain through polymerase chain reaction-mediated truncation of 47 amino acids of the COOH-terminal domain of α (αt), and coexpressed αt, α, and βc in NIH-3T3. In cells in which αt and α are present in stoichiometric proportion within the GM-CSF-binding complex, we provide evidence that αt is dominant negative over wild type α on the basis of two different functional assays: cell proliferation and foci formation. Hence, our results suggest the requirement for at least two functional α chains for signal transduction. Together with the cross-linking studies, our data indicate that the functional GMR is an oligomer that contains at least two α chains.

Granulocyte-macrophage colony stimulating factor (GM-CSF) is a multifunctional growth factor (for review, see Ref. 1).

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§ The abbreviations used are: GM-CSF, granulocyte-macrophage colony-stimulating factor; GMR, GM-CSF receptor; βc, β common; IL, interleukin; gp, glycoprotein; PCR, polymerase chain reaction; αt, α subunit with truncated COOH-terminal domain; IMDM, Iscove's modified Dulbecco's medium; FCS, fetal calf serum; RT, reverse transcriptase; NGS, normal goat serum; MEM, minimal essential medium; Epo, erythropoietin; EpoR, erythropoietin receptor; w/t, wild type.

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has been shown to form hexamers in solution with IL-6R and gp130 in the proportion 2:2:2 (27). Furthermore, ectopic expression of β, with a point mutation in the transmembrane domain (V449E) has been shown to confer ligand-independent growth to the hemopoietic cell line FDC-P1 (29). By analogy with a similar mutation in neu, it is suggested that the V449E mutation triggers constitutive β, homodimerization and, by extrapolation, that wild type (wt) β, may also be able to form homodimers. There is, however, no direct evidence for a higher order of receptor association.

In the present study, affinity cross-linking indicates the presence of both α and β, dimers within the GMR complex. Our data also indicate that α can homodimerize in the presence of ligand, even when β, is absent. Using functional assays, we provide evidence that a GMR-α truncated in its cytoplasmic domain acts as a dominant negative mutant over wt GMR-α, suggesting higher order association and a functional role for the oligomerization of GMR-α.

MATERIALS AND METHODS

Construction of the COOH-terminal Truncated GMR-α—The cytoplasmic domain of GMR-α was deleted by PCR as follows. Wild type GMR-α cDNA, cloned in the plasmid vector pGEM7 (Promega, Madison, WI), served as a template for the PCR using two oligonucleotides, primer A (5′-GGATCCCATCGTATTTCAGGAATATTTCAGG-3′) (position 727–746, located in the extracellular domain of the receptor) and GMR-α primer (GGCTCTAGACTACTGTATCCTAAGGAACCTTTT) (position 1208–1188, covering the first 21 nucleotides of the intracellular domain, to which 12 mismatched nucleotides were added to create a stop codon and one XbaI site. The PCR fragment and vector with wt insert were digested with PstI and XbaI and ligated. The truncated insert (GMR-α) was then excised with XbaI and EcoRI and recloned into the expression vector pME18 (graciously provided by Dr. Toshio Kitamura, DNAX, Palo Alto, CA; 8, 16). The insert was sequenced to confirm its identity.

Plasmids, Cells, and Transfection—NIH-3T3 cells were graciously provided by Dr. A. Veillette (Cancer Center, McGill University, Montreal, Quebec) and were maintained in Iscove’s modified Dulbecco’s medium (IMDM, Life Technologies, Inc.) supplemented with 10% fetal calf serum (FCS, Life Technologies, Inc.) at 37°C in a fully humidified incubator containing 5% CO₂. Wild type β, cDNA (KH97) was cloned in the same expression vector as GMR-α cDNA but in the absence of the cassette conferring neomycin resistance. The plasmid conferring hygromycin resistance pCEP4 was from Invitrogen (San Diego).

The strategy is shown in Fig. 1. In a first step, stable transfected expressing either wt GMR-α or β, only or both wt GMR-α and β, contained within the pME18-neo expression vector were generated using G418 selection (500 μg/ml) for α, and hygromycin selection (500 μg/ml) for β, alone. The transfections were done by calcium phosphate precipitation at a molar ratio of 5:1 of KH97/pME18 or of KH97/pCEP4. Briefly, 6–15 μg of plasmid DNA was diluted in 100 μl of sterile H₂O and added to an equal volume of 4 × CaCl₂, pH 7.9 (2 mm Tris (BDH, Poole, UK), pH 8, 0.2 mm EDTA (BDH), pH 8, 500 mm CaCl₂ (BDH)). The mixture was then added slowly with air bubbles to 200 μl of 2 × HEPES-buffered saline, pH 7.1 (50 mm HEPES (Fluka Biochemica, Ronkonkoma, NY), 280 mm NaCl (BDH), 1.5 mm Na₂HPO₄/7H₂O (BDH)). After 30 min at room temperature, 400 μl was distributed evenly over a 60-mm culture dish of NIH-3T3 cells seeded at 200,000 cells/dish the day prior to transfection. The cells were incubated overnight at 37°C, the precipitate was removed, and the cells were fed with culture medium (IMDM + 10% FCS). The selection was applied 2 days after the transfection. Several independent clones were chosen after 14 days in selective medium on the basis of their binding properties and response to GM-CSF in a proliferative assay (16). The cells were expanded in the presence of 200 μg/ml G418 or 250 μg/ml hygromycin, respectively. Clones 13 (αβ,β,) and 9.2 (β,β,), only were retained for further transfections.

In a second step, the truncated GMR-α cDNA and the pCEP4 plasmid (Invitrogen) conferring hygromycin resistance were cotransfected into clone 13 at a molar ratio of 2:5:1, i.e., 12 μg of GMR-α cDNA and 6 μg of pCEP4. The cells were selected using 500 μg/ml hygromycin (clone 13). In parallel, GMR-α cDNA was also transfected into clone 9.2 (expressing wt β, and G418-resistant clones were selected in the presence of 500 μg/ml G418. Twenty-five to 30 independent clones from each transfection were screened for both GMR-α and α expression by reverse transcriptase (RT)-PCR for GM-CSF binding and GM-CSF-dependent cell proliferation. Five to six stable clones were retained from each transfection and characterized further.

RT-PCR—To select clones with variable levels of expression of wt and truncated GMR-α, total RNA was extracted, and 5 μg was subjected to an RT reaction using an oligonucleotide that covers the SV40 polyadenylation signal (PAD1) (GGCTTTATTTGAAATTTTGAATG) contained in the vector pME18, the murine ribosomal S16 ribosomal primer as an internal control, and the Moloney murine leukemia virus RT (18 units, Life Technologies, Inc.). The PCR (25 cycles; Ref. 30) was performed using 1 μl of RT product (from a total volume of 20 μl), Vent buffer (10 ×, New England Biolabs, Beverly, MA), 5 mm dNTP, 0.5 μg each of GMR-α, and F primers, 100 mm MgSO₄ (New England Biolabs), and 1 unit of Vent enzyme (New England Biolabs), with annealing temperatures of 58°C.

Binding Assay and Saturation Analysis—Purified recombinant GM-CSF was iodinated with the Bolton-Hunter reagent (DuPont NEN). Specific activity was determined by radioimmunoassay (13, 31) and confirmed independently by enzyme-linked immunosorbent assay (32). To cover concentrations up to 20 nm, iodination conditions were chosen to yield a moderately low specific activity (300–500 cpm/fmol). Cells were distributed in 24-well plates (Linbro, IZN Biomedicals, Costa Mesa, CA) at a concentration of 86,000 cells/well (for saturation analysis and binding assays on transiently transfected NIH-3T3) or confluent monolayers in 35-mm tissue culture dishes (Falcon, Becton Dickin-son, Lincoln Park, NJ) for screening. After overnight adherence, the cultures were washed once, and the binding reaction was initiated with the indicated concentrations of ¹²⁵I-GM-CSF in a total volume of 100 μl/well or 350 μl/35-mm tissue culture dish of bicarbonate-free IMDM supplemented with 1% bovine serum albumin. Where indicated, 100-fold excess cold GM-CSF was added to the binding reaction to determine the nonspecific binding. The reaction was allowed to proceed for 5 h at 4°C with rocking and was stopped by three rapid washes with ice-cold phosphate-buffered saline. Cells were collected by the addition of 100 μl of trypsin. Binding assays were done in duplicate for screening (in 35-mm tissue culture dishes) and in triplicate for all subsequent experiments, which were performed in 24-well plates. Saturation curves were analyzed with the program SCAFIT using a nonlinear curve fitting routine (13, 15, 31).

Immunoperoxidase Assay—NIH-3T3 cells were seeded at 5 × 10⁵ cells/60-mm tissue culture dishes and left to adhere overnight. They were transfected using the CaPO₄ method with 5 μg of pME18-neo vector containing the cDNA for GMR-α, or GMR-α wt. Total DNA concentration was brought up to 10 μg with pGEM4 as a carrier. Sixteen hours later, cells were washed and distributed into six wells.
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RESULTS

The Multimeric Nature of the GMR Complex—GM-CSF cross-linking studies were done with clone 13 expressing w/t α and β, and clone 18 expressing w/t α alone. Previous studies occasionally suggested the presence of high molecular mass complexes, in addition to the two major bands corresponding to α and β. To determine the size of these high molecular mass complexes, the cross-linked proteins were resolved by electrophoresis in a denaturing continuous 4–8% polyacrylamide gel. Under these conditions, there was a linear relationship between RF values and the molecular masses of protein standards ranging between 67 and 669 kDa (Fig. 2B). In clone 18, 125I-GM-CSF cross-linked to a single α chain, as for clone 13 (Fig. 2A). Furthermore, two additional bands were observed at 186 kDa, corresponding to an α dimer (α2) with a single molecule of ligand, as well as a slowly migrating complex of more than 800 kDa. All three bands were observed in clone 13 as well. However, data observed with clone 13 indicate that the α dimer is not favored in the presence of β. Several additional bands are observed with clone 13 and not clone 18. The most prominent at 160 kDa corresponds to a single molecule of β cross-linked to the ligand. The two bands of higher molecular masses correspond potentially to ligand β cross-linking (290 kDa) and to the ternary complex GMαβc (250 kDa) (Fig. 2). That the bands corresponding to GMαβc and GMαβcβc should be flatter than the neighboring bands may also be attributed to inefficient αβc cross-linking, as reported elsewhere (7, 13, 23, 36). Cross-linking of 125I-GM-CSF to all observed bands is competed by 125-fold excess cold GM-CSF, indicating their specificity. Our observations therefore suggest a higher order of association within the GMR complex.

Coexpression of COOH-terminal Truncated GMR-α (αt) and Wild Type β, in NIH-3T3 Cells—It has been shown previously that the cytoplasmic tail of the α subunit is important for signal transduction through βc (19). Given the importance of the COOH-terminal domain in signal transduction and our cross-linking studies, which revealed the presence of α dimers, we reasoned that an inert COOH-terminal truncated GMR-α could inhibit signal transduction through the w/t receptor, if the biologically active complex consists of at least two α chains. We therefore addressed the question of whether or not a fully truncated COOH-terminal α chain could act as a dominant negative receptor over w/t α.

To this end, we used three types of NIH-3T3 stable transfectants: clone 18 expressing only w/t α, clone 9.2 expressing only w/t βc, and clone 13 expressing both w/t α and βc. The level of βc, which is the limiting element for high affinity binding differs between clones 9.2 (lower level) and clone 13 (higher level) (data not shown). The effect of COOH-terminal truncation on ligand binding and signal transduction was first examined by transfecting GMR-αt into clone 9.2. Five subclones (9.2 variants) expressing high levels of both GMR-αt and w/t βc were selected. Binding assays performed at 200 pM GM-CSF confirmed that the introduction of αt in 9.2 restored ligand binding (Table 1). Selected clones were then tested for their proliferative response to GM-CSF in serum-free medium. The positive control, clone 13 expressing w/t GM-CSF and βc, was shown to respond well to GM-CSF in this assay (Table 1 and Ref. 16). In contrast, clone 9.2 and its variants showed no proliferation increase on exposure to saturating concentrations of GM-CSF (800 pM) (Table 1), confirming that αt was inert.

To verify that the truncation of the cytoplasmic domain of
GMR-α did not affect its capacity to be expressed at the cell surface, we compared the immunoreactivity of a monoclonal anti-GMR-α with NIH-3T3 cells transiently transfected with equal amounts of w/t α and αt. There was no significant difference between the two groups (data not shown). We also compared the capacity of w/t α and αt to associate with βc in order to form a high affinity complex. To this end, various concentrations (0.25–10 μg) of truncated or w/t GMR-α cDNA in pME18-neo (~6.5 kilobases) were transiently transfected in NIH-3T3 together with 5 μg of βc cDNA in pME18 (~6.0 kilobases) (Table II). Binding assays were then performed at 200 pm radioligand, a concentration that would be sufficient for binding to the high affinity complex (αβc) with minimal occupancy of the low affinity binding site (α alone). Increasing the α:βc ratio up to 1:1 resulted in increased 125I-GM-CSF binding (Table II). There was no further increase in binding when α was 2-fold higher than βc, suggesting that at 200 pm radioligand, most of the binding may be attributed to occupancy of the high affinity complex. More importantly, there was no significant difference between αt and w/t α in this assay.

Coexpression of αt, w/t α and βc.—We then proceeded to induce αt expression in clone 13, which expresses both w/t α and βc. Twenty-five clones were screened by RT-PCR for obvious variations in ratio of expression of αt versus w/t α. Six clones were selected with ratio of αt over w/t α which varied from less than 0.001:1 (13-T6) to 1.001:1 (13-T2) (Fig. 3). These clones were also subjected to binding and proliferation assays to determine their response to GM-CSF. Our data indicated that the binding of 125I-GM-CSF was not affected by αt expression, which was further confirmed by GM-CSF saturation analyses (Table III). For example, 13-T5 and the w/t clone 13 expressed both high and low affinity GM-CSF binding sites with Kd that were comparable. Similarly, the numbers of high and low affinity binding sites/cell were not significantly different between the two clones.

Reduced GM-CSF Responsiveness in Cells Coexpressing αt and α.—Cross-linking studies were performed to determine the ratio of αt over that of w/t α and βc at the protein levels (Fig. 4). There was a good overall concordance between the levels of mutant relative to w/t proteins and their relative mRNA levels determined by RT-PCR. Thus, clone 13-T6 does not exhibit detectable αt, whereas clones 13-T3 and 13-T5 express equal levels of each type. In contrast, clones 13-T1 and 13-T4 express predominantly αt. Furthermore, 13-T2 expresses very low levels of βc. In this experiment, cross-linked proteins were run on a single 7.5% polyacrylamide gel, and, under these conditions, high molecular mass complexes were not as well resolved as in a continuous 4–8% polyacrylamide gel (compare Fig. 2 with Fig. 4). Nonetheless, GM-CSF is efficiently cross-linked to βc and to both w/t α and αt, suggesting that both w/t αβc and αtβc complexes exist.

The effect of αt expression on GM-CSF responsiveness was assessed in two biological assays: thymidine uptake and morphologic change. GM-CSF induced a dose-dependent proliferation in serum-free medium in clone 13 (Fig. 5) and foci formation (data not shown). 13-T6, which does not express detectable αt, behaved similarly to the parental clone 13 in both assays (Figs. 5 and 6). In contrast, in clones 13-T3 and 13-T5, which express both mutant and w/t proteins in equal proportion, there was a sharp decrease in GM-CSF responsiveness in the thymidine incorporation assay and a significant shift in EC50 (Fig. 5 and Table III). Similarly, morphologic change induced by GM-CSF was also reduced drastically in 13-T3 and 13-T5 (Fig. 6). Finally, both cell proliferation and foci formation were minimal in 13-T4 (exhibiting mainly αt expression) in response to GM-CSF.

In summary, our observations indicate a drastic decrease in GM-CSF responsiveness in subclones expressing αt. Since 13-T3 and 13-T5 express both w/t α and αt in stoichiometric proportion, a mere sequestration of the ligand or βc could not explain the observed data. Rather, our observations indicate that a functional GM-CSF receptor comprises at least two α subunits, and they support the hypothesis of a higher order of

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**Table I**

| Clone | Bound GM-CSF | GM-CSF-dependent cell proliferation |
|-------|--------------|-------------------------------------|
|       | molecules/cell | fold increase |                         |
| 13 w/t (αβc) | 21,211 | 5.6 |
| 9.2 w/t (βc) | 0 | 0.87 |
| 9.2-T1 (αβc) | 1,922 | 1.04 |
| 9.2-T15 (αβc) | 2,289 | 0.90 |
| 9.2-T22 (αβc) | 2,449 | ND |
| 9.2-T30 (αβc) | 2,968 | 0.85 |
| 9.2-T33 (αβc) | 7,256 | 0.90 |

**Table II**

| Concentration αt | 125I-GM-CSF specific binding | | 125I-GM-CSF specific binding |
|-----------------|-----------------------------|------------------|-----------------------------|
|                 | αt/βc                        | αt/βc            |
|                 | cpm                         | cpm              |
| 0.25            | 467 ± 166                    | 459 ± 67         |
| 0.5             | 625 ± 66                     | 692 ± 64         |
| 1.0             | 1,334 ± 134                  | 1,485 ± 140      |
| 2.0             | 1,783 ± 202                  | 1,758 ± 100      |
| 5.0             | 1,613 ± 306                  | 2,152 ± 133      |
| 10.0            | 2,211 ± 235                 | 2,005 ± 476      |

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![Fig. 3. RT-PCR of NIH-3T3 transfectants. RNA was extracted from clone 13 and the various subclones coexpressing w/t GMR-α and αt (clones 13-T1-T6). Reverse transcription was performed using primer PAD1 and murine S16AS (internal control) as described under "Materials and Methods." One μl of the reactions was used for PCR, and 10 μl of these reactions was separated by electrophoresis on 1% agarose-TAE gel. Varying ratios of αt to αw was observed, whereas the internal control ribosomal MS16 was more constant.](image)
TABLE III

Binding characteristics and biologically active concentrations of GM-CSF (EC50)

| Binding constants | 13 w/t | 13-T5 |
|-------------------|--------|-------|
| Kd (nM)           |        |       |
| Kd high affinity  | 149 ± 31 | 117 ± 80 |
| Kd low affinity   | 20 ± 20  | 11 ± 6  |
| No. of sites of high affinity | 1.63 × 10^4 ± 4 × 10^4 | 1.11 × 10^6 ± 0.2 × 10^6 |
| No. of sites of low affinity   | 9.1 × 10^4 ± 6.3 × 10^4 | 14.1 × 10^4 ± 5.8 × 10^4 |

**Fig. 4.** 125I-GM-CSF cross-linking at the surface of clone 13 transfectants. GM-CSF binding assays were performed on confluent 60-mm culture dishes at a concentration of 2 μM radioligand for 4 h in the presence or absence of a 100-fold molar excess of cold GM-CSF (competitor) for clone 13 w/t and 13-T6; 125-fold excess for clone 13-T5; and 200-fold excess for clones 13-T1, 13-T2, and 13-T4. After removal of excess radioligand, bound GM-CSF was cross-linked to its receptor in the presence of 1 mM BS3. Electrophoresis was performed on a 7.5% SDS-polyacrylamide gel as described under “Materials and Methods.”

**Fig. 5.** Dose-dependent induction of cell proliferation by GM-CSF on clone 13 transfectants. Five independent clone 13 transfectants (25 × 10^4 cells) were deprived of serum through an overnight incubation in Opti-MEM medium. GM-CSF was added at the indicated concentrations for 48 h. Cell proliferation was evaluated by a thymidine incorporation assay as described under “Materials and Methods.” The curves passing through the data were obtained by nonlinear regression analysis with the program ALLFIT.

**DISCUSSION**

A Dominant Negative GMR-a—The present study provides evidence that a COOH-terminally truncated GMR-a can suppress the function of its w/t counterpart, even when expressed in equal amounts. That the COOH-terminal domain of the a chain might be important for signal transduction was inferred previously from the specificity of response triggered by IL-3R, IL-5R, and GMR, despite their sharing a common signal transducing β chain (for review, see Ref. 37). Indeed, COOH-terminal truncation results in a receptor complex that is no longer competent for signal transduction (11, 19, 38 and the present study). A soluble GMR was also previously shown to decrease the response of the cells to GM-CSF (39). The mechanism of inhibition was unclear, however, and could be the result of GM-CSF sequestration by the soluble α chain. Through quantitative cross-linking studies and coexpression of α and w/t α in stoichiometric proportion, we provide direct evidence that α acts as a dominant negative mutant of w/t α. Moreover, we also verified that α is not expressed preferentially at the cell surface, nor does it show increased association with β when compared with w/t α. Therefore, mere GM-CSF or β sequestration was ruled out by the fact that 13-T3 and 13-T5 in which equal amounts of mutant and w/t proteins were present in the GM-CSF binding complexes showed a drastically impaired response to GM-CSF compared with w/t clones. A dominant negative suppression can be inferred from the observations that in cells expressing equal levels of α and w/t α, there was more than a 75% decrease in cell proliferation in response to GM-CSF. A possible explanation may be the composition of the various complexes that are formed. Thus, 25% of the complex would be α_αβ_β_β_β_β and fully functional, whereas the remaining 75% would be inactive since 50% would be α_αβ_β_β_β_β and 25% α_αβ_β_β_β_β. Comparison between 13-T3 and 13-T5 with 13-T6 or parental shows such a drastic decrease in the amplitude of the response together with a significant shift in EC50 values, which were 8–20-fold higher.

Implications for the Structure of the Biologically Active GMR Complex—The negative effect of truncated GMR-a on the function of w/t GMR is better explained by the possibility of α homodimerization and the requirement for at least two normal α chains in a GMR complex that is competent for signal transduction. Moreover, recent reports (18, 27, 40, 41) provide evidence for an active β homodimer complex in BaF3 and FDC-P1 cell lines. Consistent with our observations, IL-6 has been shown to form a hexameric complex with IL-6R and gp130 in the ratio of 2:2:2 in solution phase binding assays (27). Interestingly, two distinct and differently oriented sites on IL-6 have been implicated in gp130 dimer formation, predicting that a similar mechanism may be operating for GM-CSF receptor binding.
assembly. Because of structural homologies between ligands and receptors, computer modeling and site-directed mutagenesis of GM-CSF may allow us to address this possibility directly.

Stoichiometry of the GMR Complex—A direct demonstration of the multimeric nature of the GMR complex was provided by our optimized cross-linking and binding studies with three types of NIH-3T3 GMR transfectants. Thus, clone 9.2 expresses a full-length $\beta_c$ but does not bind GM-CSF even at 100 nM radioligand (data not shown). Clone 18 ($\alpha$ alone) binds GM-CSF with low affinity only, whereas clone 13 ($\alpha\beta_c$) exhibits both high and low affinity binding. On NIH-3T3 cells expressing the $\alpha$ chain alone (clone 18), GM-CSF was shown to cross-link to an $\alpha$ dimer at a 1:2 ratio of GM to GMR-$\alpha$. The dimer configuration was not favored by the presence of $\beta_c$ in clone 13. More importantly, comparison of the binding characteristics as well as the cross-linking data between clone 18 ($\alpha$ alone) and clone 13 ($\alpha\beta_c$) indicate that both the ternary complex GM$\alpha\beta_c$ and possibly an oligomeric complex are present in cells coexpressing $\alpha$ and $\beta_c$ and constitute the high affinity binding site. The dominant negative effect of $\alpha$ over w/t $\alpha$ indicates that the oligomeric complex is the minimal structure required for delivering a proliferative signal into the cells.

It has been suggested previously that $\beta_c$ could homodimerize because of an activating point mutation analogous to the one observed in neu, known to confer ligand-independent activation. Our observations provide a direct demonstration for the presence of $\alpha$ and $\beta_c$ homodimers and further underscore the possibility of gain of function mutations that cause homodimerization as reported for tyrosine kinase receptors. A truncated EpoR was first thought to be competent for mitogenic signaling but not for suppression of apoptosis. It was later found that the BaF3-EpoR transfectants also expressed low levels of w/t EpoR and that the truncated EpoR had a dominant negative effect over that of w/t EpoR, probably because of dimerization (42). Although data are not available for Epo, there is recent evidence for IL-6 that the ligand can also form dimer or tetramer, as shown previously for Steel factor, M-CSF, and ligands for other tyrosine kinase receptors (43). Taken together, the results suggest that cytokine receptors may deliver mitogenic signals to the cells through receptor oligomerization. Since associated tyrosine kinases such as Jak-2, Yes, and Lyn have been implicated in signal transduction by GMR (38, 44), the observations are consistent with the view that receptor dimer and/or oligomerization brings together two or more tyrosine kinase molecules, resulting in their activation. Furthermore, our approach provides a more general strategy to designing dominant negative receptors, either as membrane-anchored molecules or as soluble receptors, and to address the functional importance of receptor oligomerization.

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FIG. 6. GM-CSF-dependent morphologic transformation of clone 13 transfectants. Clone 13 transfectants were maintained in IMDM supplemented with 10% FCS in the presence or absence of GM-CSF for 10 days, as described under “Materials and Methods.” The number of foci/dish was then scored with an inverted microscope.
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