The Structure and Dynamics of the Granulocyte Macrophage Colony-stimulating Factor Receptor Defined by the Ternary Complex Model*

(Received for publication, June 18, 1992, and in revised form, February 3, 1993)

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Myeloid cell lines and primary leukemic myeloblasts express two classes of granulocyte macrophage colony-stimulating factor (GM-CSF) binding sites of high ($K_d$ 20–50 nM) and low affinity ($K_d$ 5–10 nM). High affinity binding is caused by the association of two chains, p80α and p130β, whereas p80α alone confers low affinity binding only. Furthermore interleukin-3 (IL-3) competes for the binding of GM-CSF to its high affinity receptor (for review see Nicola, N. A., and Metcalf, D. (1991) Cell 67, 1–4). In the present study, we took advantage of the perturbation of GM-CSF binding equilibrium by IL-3 to take a quantitative approach to the structure and dynamics of the GM-CSF receptor complex. First, cross-linking studies were performed at two concentrations of radioligand. At 200 pM, a concentration sufficient for near saturation of the high affinity binding site R1, the association between p80α and p130β is stoichiometric, and the addition of IL-3 prevents the binding to both chains. At 5 nM, a concentration sufficient for half-occupancy of the low affinity binding site R2, IL-3 prevents cross-linking to the β chain only. Second, GM-CSF saturation curves were analyzed both at equilibrium and under conditions of perturbation of the equilibrium by IL-3. In the presence of IL-3, the interaction of GM-CSF with its receptor is converted from high to low affinity binding. Computer modeling of binding data with a ternary complex model involving GM-CSF, p80α, and p130β indicates that the model fits the data with accuracy and suggests that ligand binding stabilizes the interaction between p80α and p130β by 3 orders of magnitude. Third, membrane solubilization dissociates p80α and p130β whereas on ligand-stabilized preformed complexes, solubilization did not dissociate the two chains. Finally, upon addition of GM-CSF, there is an increase with time in the proportion of ligand bound to the high affinity receptor, at the expense of that bound to low affinity receptor, suggesting that stabilization of the ternary complex is a time-dependent process.

Hemopoietic cells express two classes of binding sites for the growth factor granulocyte macrophage colony-stimulating factor (GM-CSF)1 (1–7). The high affinity GM-CSF receptor (GM-CSF R) binds the ligand with a dissociation constant ($K_d$) of 20–50 pM, whereas the $K_d$ of the low affinity GM-CSF R is 5–8 nM. Our previous data indicated that the concentration of GM-CSF required for half-maximal stimulation of cell proliferation (EC50) is in the range of the $K_d$ of the high affinity GM-CSF receptor, suggesting that only the high affinity receptor is responsible for signal transduction across the membrane in hemopoietic cells (3). Cross-linking studies and gene cloning indicate that the high affinity GM-CSF R is a heterodimer: the α chain has a molecular mass of 80 kDa and the β chain a molecular mass of 130 kDa (4–7). The gene encoding the α chain confers low affinity GM-CSF binding only, when expressed in COS cells (6). Coexpression of the α and β chains restores high affinity binding (7) and signal transduction (8), consistent with our previous results suggesting that only the high affinity GM-CSF R mediates the biological response to the ligand (3).

We and others have shown that interleukin-3, another hemopoietic growth factor that elicits many of the biologic responses of GM-CSF in target cells, competes for the binding of GM-CSF to its high affinity receptor (9–14). Consistent with our binding results, cross-linking studies indicate that IL-3 competes for the binding of GM-CSF to the p130 component of the receptor only (15). Finally, coexpression of the GM-CSF α chain (GRα), IL-3R α chain (IL-3Rα), and the β chain in COS cells reconstituted the competition for binding between the two cytokines (14). In the present study, we took advantage of the competition by IL-3 to take a quantitative approach to analysis of the structure and dynamics of the GM-CSF receptor complex. First, cross-linking studies were performed at two concentrations of radioligand: a concentration sufficient for near saturation (200 pM) of the high affinity binding site (R1) with minimum occupancy of the low affinity binding site (R2) and a higher concentration that allows for saturation of R1 and half-occupancy of R2 (5 nM). Our data indicate that high affinity binding is caused by the stoichiometric and reversible association of p90 and p130. Second, GM-CSF saturation curves were performed in the absence of or in the presence of a 100-fold excess of unlabeled IL-3. In the presence of IL-3, the interaction of GM-CSF with its receptor is converted from high to low affinity binding. Analysis of the binding data with the ternary complex model

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* This work was supported in part by a grant from the Medical Research Council of Canada (to T. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: GM-CSF, granulocyte macrophage colony-stimulating factor; R, receptor; IL-3, interleukin-3; GRα, GM-CSF R α chain; IL-3Rα, IL-3R α chain; AML, acute myeloid leukemia.
GM-CSF Receptor

(16) suggests that ligand binding stabilizes the association between the α and β chains by 3 orders of magnitude.

MATERIALS AND METHODS

Source of Cells and Growth Factors—Purified human recombinant GM-CSF and IL-3 were produced as described previously (17, 18). The cell line TF-1 was maintained in culture in the presence of 240 pm GM-CSF, in Iscove’s modified Dulbecco’s medium (GIBCO) supplemented with 15% fetal calf serum (GIBCO) (19). The cells were subcultured every three days weekly at 2 × 10^6 cells/ml. Primary myeloblasts were obtained by Ficol-Hypaque separation of the blood cells of AML patients. Cells were cryopreserved until use (3). Sample AML 44, selected for binding studies, contained 100% myeloblasts.

GM-CSF Binding Assay—GM-CSF was iodinated with the Bolton-Hunter reagent (New England Nuclear). Specific activity was determined by radioimmunoassay (20) and confirmed independently by enzyme-linked immunosorbent assay (21). To cover concentrations up to 20 nM, iodination conditions were chosen to yield a moderately low specific activity (300–500 cpm/nmol).

To up-regulate the expression of GM-CSF receptor, TF-1 cells were incubated overnight in the presence of erythropoietin (5 units/ml) instead of GM-CSF. Prior to binding, the cells were harvested and washed twice. Binding assay was performed in triplicates at 4 °C with constant rotation, in 200 µl of bicarbonate-free Iscove’s modified Dulbecco’s medium supplemented with 10 mg/ml bovine serum albumin (Sigma). Cell numbers were typically 1–2.5 × 10^5/tube. ¹²⁵I-GM-CSF was added in the concentration range of 5 pm to 20 nM. The reaction was stopped by centrifugation through a mixture of silicone and oil as described previously (3). Nonspecific binding was determined in the presence of a 200-fold molar excess of unlabeled GM-CSF. GM-CSF concentration curves were analyzed by nonlinear regression, using the program SCAPIT (3, 11, 20) or by modeling with the ternary complex model HRXFIT (16).

GM-CSF Cross-linking—All steps were performed on ice or at 4 °C. After binding, the reaction was stopped by rapid centrifugation (2 min, 1,500 g, 4 °C), followed by one rapid washing step. Unless otherwise indicated, the cell pellet was resuspended in cold phosphate-buffered saline, and cross-linking was performed for 30 min at 4 °C in the presence of 1 mM BS3 (Pierce) (22). Under these conditions, dissociation of the ligand-receptor complex was minimal (see Fig. 5). The reaction was quenched by the addition of 10 mM Tris, 1 mM EDTA, 150 mM NaCl (22). The cells were centrifuged for 30 s and lysed in Tris buffer containing 1% Triton X-100 (Sigma) (5). Nuclei were removed by rapid centrifugation (10,000 × g, 30 s) and protein concentrations in the Triton X-100 soluble fractions determined according to Bradford (23). Proteins (100 µg/tube) were size separated on a denaturing 7.5% acrylamide gel. After drying, the gel was exposed for 24 h to Kodak XAR-5 film. The volume under each peak of radioactivity was integrated and expressed in arbitrary units. Data are corrected for nonspecific binding determined by GM-CSF binding to GMRα, the stability constants of the Ra·Rd complex in the absence (M) and in the presence (L) of GM-CSF, and finally the concentration of Ra and Rd subunits. Because the two concentrations of Ra and Rd should be considered in the membrane environment instead of the assay medium, only the ratio of L to M is reported and represents the stabilization by GM-CSF of the receptor subunits.

RESULTS

GM-CSF Cross-linking—Previous cross-linking studies with ¹²⁵I-GM-CSF gave variable results (4, 5, 9, 13). We therefore determined the optimal conditions for cross-linking. After the binding reaction and removal of excess ¹²⁵I-GM-CSF, different concentrations of the cross-linking agent BS3 were added to whole cells or to solubilized membrane proteins (data not shown). The efficiency of ligand-receptor cross-linking was dependent on the concentration of BS3 and was optimal at a final concentration of 1–5 mM. Solubilization of membrane proteins prior to the addition of BS3 did not change the pattern of proteins cross-linked to the radioligand. Because of the convenience of working with whole cells, subsequent cross-linking experiments were performed on whole cells at a final concentration of 1 mM of BS3. To study the stoichiometry of the GM-CSF receptor complex, we next determine whether the amount of GM-CSF cross-linked was proportional to the amount of GM-CSF-bound. ¹²⁵I-GM-CSF binding was performed at two concentrations of radioligand (Table I). An aliquot of the binding reaction was counted, whereas the rest of the cells were subjected to cross-linking and electrophoresis as described under "Materials and Methods." Data shown in Table I indicated that the amount of GM-CSF cross-linked to its receptor was proportional to the amount of ¹²⁵I-GM-CSF specifically bound at equilibrium.

In the cross-linking studies, we also took advantage of the perturbation of ¹²⁵I-GM-CSF binding by IL-3 to determine more accurately receptor stoichiometry. Our previous work suggested that the degree of inhibition of GM-CSF binding by IL-3 was variable among AML samples and was dependent on the relative proportion of high affinity IL-3 and GM-CSF receptors expressed on the cells. The presence of an excess of IL-3 receptors results in efficient competition, whereas diminished IL-3R expression results in decreased competition (11). While screening our AML samples, we found that in sample AML 44, IL-3 completely inhibited the binding of 200 pm GM-CSF. This sample was further analyzed by cross-linking studies (Fig. 1). GM-CSF could be cross-linked specifically to p80α and p130β at both concentrations of radioligand. As expected, the signal at 5 nm was much higher than that observed at 200 pm. Quantitation by scanning with the PhosphorImager indicated a nearly perfect stoichiometric association of the β chain with the α chain at 200 pm of radioligand (Fig. 1B). These data indicate that the high affinity GM-CSF-R is constituted by the stoichiometric association of α and β chains.

<table>
<thead>
<tr>
<th>Binding assay</th>
<th>Cross-linking</th>
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<tr>
<td>200 pm</td>
<td>1.65</td>
</tr>
<tr>
<td>5 nm</td>
<td>5.36</td>
</tr>
</tbody>
</table>

GM-CSF specifically bound

| Specific activity | 233,155 | 606,905 |

2 N. Aumont and T. Hoang, unpublished observation.
of the α chain with the β chain. Further, the data suggest that the low affinity receptor is unoccupied at this concentration of radioligand, providing that the high affinity complex is present. In contrast, at a concentration of radioligand sufficient for half-occupancy of the low affinity binding site, the radioactivity cross-linked to the α chain was twice that of the β chain, indicating association of the radioligand with both high and low affinity binding sites.

At 200 pM radioligand, the presence of a 200-fold molar excess of IL-3 prevented the cross-linking of GM-CSF to both chains. In contrast, IL-3 did not prevent GM-CSF binding to p80 (α chain) at the higher concentration of radioligand (Fig. 1). IL-3, nonetheless, induced a decrease in the amount of 125I-GM-CSF cross-linked to the α chain, similar to that observed on the β chain. These data suggest that the inhibition of GM-CSF binding by IL-3 is caused by sequestration of the β chain by IL-3 receptor, therefore preventing its association with GMα. It can be assumed from the inhibition experiments that at 5 nM radioligand, the residual signal on the α chain in the presence of an excess IL-3 (νβ = 211,281) represents GM-CSF binding to the low affinity receptor Rβ.

In the absence of any competitor, the signal detected on the β chain represents binding to the high affinity receptor Rα.

(Vβ = 190,066) and the radioactivity cross-linked to the α chain, total binding (Rα + Rβ, να = 431,030) respectively (Fig. 1B). Because να at 5 nM is approximately the sum of Vβ and να, it can again be deduced that the α chain and the β chain associated stoichiometrically to form the high affinity receptor Rα.

Binding Characteristics of GM-CSF R in the Presence of an Excess IL-3—We next verified the hypothesis that the transition from low affinity state to high affinity state may be caused by the reversible association of the two chains in the presence of GM-CSF. We reasoned that the perturbation of the equilibrium with IL-3 should result in a decrease of the high affinity state, without affecting the maximum binding capacity of the system. GM-CSF saturation curves were therefore performed in the absence or the presence of a 100-fold excess of cold IL-3 (see Fig. 3). For this experiment, we chose a model system that expresses high and low affinity GM-CSF binding sites in equal proportions. The only suitable cells that we have found are TF-1 cells (19). These cells express comparable numbers of high affinity and low affinity binding sites, whereas all other cell lines or freshly isolated leukemic cells express a proportion of low affinity binding sites exceeding that of the high affinity binding sites by at least 2 orders of magnitude (3, 11). Furthermore, binding was performed at 4 °C for 4 h to minimize internalization. Under these conditions, equilibrium binding was reached for all concentrations of radioligand which were higher than 200 pM and were found to be the most appropriate conditions to determine the maximum binding capacity of the system. Analyses of 125I-GM-CSF saturation curves performed in the presence or absence of IL-3, using TF-1 cells, are compatible with the presence of two classes of binding sites (Table I), as documented previously (3, 4, 10, 11). In the presence of IL-3 there was a 28-40% decrease in the number of high affinity GM-CSF binding sites (Table II). In contrast, the respective Kd values for Rα and Rβ remain relatively constant. Because binding was not at equilibrium for lower concentrations of radioligand, the equilibrium constant of the high affinity binding site may be slightly underestimated, hence the Kd for Rα was found to be two to three times the values reported previously by us (20–40 pm; 3, 31), whereas the Kd for Rβ was in the same range as reported previously (5–10 nM). Nonetheless, the IL-3 addition did not modify the Kd values of the GM-CSF R. Our data are, therefore, compatible with the view that IL-3 decreases the number of high affinity GM-CSF binding sites but does not modify the affinity of GM-CSF receptor for its ligand.

Binding with AML 44 was also performed at all concentrations of radioligand, in the absence or the presence of IL-3 as a competitor (Fig. 3). As observed in Fig. 3, the presence of IL-3 induced a decrease in GM-CSF binding at concentrations of radioligand which were below 10 nM. These data are consistent with the decrease in GM-CSF cross-linking to its

![Fig. 1. GM-CSF cross-linking to primary myeloblasts.](image)

**FIG. 1.** GM-CSF cross-linking to primary myeloblasts. GM-CSF binding to primary myeloblasts (sample AML 44) was performed at two concentrations of radioligand (200 pM and 5 nM), in the presence or absence of a 200-fold molar excess of either GM-CSF or IL-3 as indicated (competitor). After removal of excess radioligand, bound GM-CSF was cross-linked to its receptor in the presence of BS3 (1 mM). Quenching and electrophoresis were performed as described under "Materials and Methods." After a 1-week exposure, the radioactivity associated with each band was quantified through scanning with the PhosphorImager. At 200 pM radioligand, cross-linking efficiency for p80α and p130β was 5 and 4%, respectively. The gray scale shown here is 7.6 (lower limit) and 60 (upper limit) (panel A). The volume under each peak of radioactivity was integrated and shown here in arbitrary units (panel B). Data were not corrected for nonspecific binding because it was negligible. Data were typical of two independent experiments with AML 44.

![Fig. 2. Ternary complex model for growth factor receptors with two affinity states.](image)

**FIG. 2.** Ternary complex model for growth factor receptors with two affinity states. Symbols are as follows: GMα, p80α; Rβ, p130β. K represents the equilibrium constant of the ligand/low affinity receptor binding step. K' is that of the ligand/high affinity receptor complex. L is the stability constant of the transition step from low to high affinity state in the presence of the ligand, and M is in the absence of ligand (adapted from Ref. 16).
GM-CSF Receptor

Testing of the Ternary Complex Model—This possibility was further tested through computer modeling of the saturation curves using the ternary complex model (16). In this model, the initial binding step occurs between hormone (H) and receptor (R), to be followed by the reversible association of the low affinity state HR (hormone · receptor) to a membrane component X that results in the high affinity ternary complex HRX. The model also allows for the possibility of a free association RX on the membrane of the cells, in the absence of ligand. Thus, the initial ligand binding step occurs both with R and RX. In the present situation, H represents the ligand GM-CSF, R the α chain, and X the β chain. The model, summarized in Fig. 2, proved to fit the experimental data for both TF-1 cells (Table II) and primary myeloblasts (AML 44, Fig. 3 and Table III). For both cell types, the dissociation constant of the binding step, determined by modeling with the ternary complex, closely resembles the dissociation constant of the ligand for the low affinity GM-CSF receptor determined by regression analysis (Tables II and III). This value was not affected by the presence of IL-3. For both cell types, the affinity between the two chains was increased by 1,000-fold (L/M) in the presence of the ligand, suggesting that the ligand stabilizes the association between the two chains (Tables II and III). In TF-1 cells, where the inhibition of GM-CSF binding by IL-3 was only partial, the ratio of L/M could be compared in the presence and absence of IL-3 as a competitor. L/M was of the same order of magnitude, whether or not IL-3 was present in the binding assay, indicating that IL-3 does not destabilize the ternary complex (Table II). The presence of IL-3 does not alter the maximum GM-CSF binding capacity in both cell types (B_{max} = 150–200 pm for TF-1 and 80–90 pm for primary myeloblasts), whereas the concentration of the β chain available for association with GMα decreased significantly on addition of IL-3.

Comparison of Ligand-Receptor Cross-linking prior to or after Solubilization—The validity of the model of ligand-induced stabilization of the p80α/p130β association was further tested by cross-linking studies using TF-1 cells (Fig. 4). The binding and cross-linking of °°I-GM-CSF to whole cells prior to solubilization revealed the two bands of 80 and 130 kDa as above (Fig. 1). When the ternary complex was allowed to form on whole cells, followed by solubilization of membrane proteins in the absence of ligand and cross-linking in solution, both p80 and p130 were also cross-linked to GM-CSF (Fig. 4, lanes 2 and 3). However, when membrane proteins were solubilized prior to GM-CSF binding, the ligand-cross-linked specifically to a single species of membrane protein of 80 kDa. All other bands that were cross-linked to the iodinated ligand under these conditions were not displaced by an excess of unlabeled GM-CSF and were, therefore, nonspecific. These data indicate that solubilization did not dissociate the preformed ternary complex. In contrast, solubilization prior to ligand binding results in the dissociation of the two chains of GM-CSF receptor and prevents the formation of the ternary complex. Together with the modeling of GM-CSF saturation curves, our cross-linking data indicate that ligand binding stabilizes the p80α/p130β heterodimer, hence the displacement of the equilibrium toward high affinity binding.

Comparison of the Dissociation of GM-CSF at Various Times after Binding—The ternary complex model implies that the initial binding step does not discriminate between high and low affinity receptors and that stabilization of the ternary complex is a time-dependent process. This possibility was
GM-CSF binding to TF-1 cells: comparison of parameters estimated by modeling with the ternary complex model or by regression analysis of saturation curves

GM-CSF saturation curves, performed with TF-1 cells in the presence or in the absence of 100 × excess IL-3, were analyzed through curve fitting using the ternary complex model or by nonlinear regression analysis with a model for two independent classes of binding sites. Symbols are defined in Fig. 2. $K'_u$ and $K_u$ are the dissociation constants $(1/K)$ of the high and low affinity binding sites, respectively, and $R_o$, the maximum binding capacity of the system. Data are the mean of parameter estimates ± S.E. of two independent experiments with TF-1 cells.

### TABLE II

<table>
<thead>
<tr>
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<th>Ternary complex model</th>
<th>Two-site model</th>
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<tbody>
<tr>
<td></td>
<td>$-\text{IL-3}$</td>
<td>$+\text{IL-3}$</td>
</tr>
<tr>
<td>$K'_u$</td>
<td>NA*</td>
<td>NA</td>
</tr>
<tr>
<td>$K_u$</td>
<td>11 ± 12 nM</td>
<td>12 ± 1 nM</td>
</tr>
<tr>
<td>$L/M$</td>
<td>972 ± 322</td>
<td>1,455 ± 360</td>
</tr>
<tr>
<td>$R_o$ (mol./cell.)</td>
<td>3,904 ± 737</td>
<td>5,366 ± 1,100</td>
</tr>
<tr>
<td>$R_b^f$ (or $R_b$)</td>
<td>1,821 ± 184</td>
<td>912 ± 194</td>
</tr>
</tbody>
</table>

* NA, not applicable.

### TABLE III

GM-CSF binding to AML 44 myeloblasts: comparison of parameters estimated by modeling with the ternary complex model or by regression analysis of GM-CSF saturation curves

Data shown in Fig. 3 were analyzed through curve fitting using the ternary complex model or by nonlinear regression analysis with a model for two independent classes of sites. Symbols are as defined in Fig. 2 and Table II. Data are the estimates ± S.E. of the dissociation constants of the high ($K'_u$) and low ($K_u$) affinity GM-CSF binding site and of maximum binding capacity ($R_o$) of the system, derived by curve fitting with the two different models as indicated, using a nonlinear least squares curve fitting routine.

### DISCUSSION

In the present study, we show that the ternary complex model is compatible with the binding characteristics of GM-CSF-R. The validity of the model was confirmed by the high quality of fit to experimental data obtained through GM-CSF saturation analysis at equilibrium and under conditions in which the equilibrium was perturbed by the presence of IL-3. Our data suggest that a model based on the association of p80α, p130β, and GM-CSF is sufficient to explain the transition from low affinity binding to high affinity binding, without the need for additional components.

The cloning of the human genes encoding the α chains of GM-CSF-R (6) and of IL-3 R (7) as well as that of the β chain indicates that the two receptors share a common subunit, as we and others have postulated previously (6, 11). Cross-linking studies (4, 5) and gene cloning (6, 7) indicated that p80α binds GM-CSF with low affinity. Although p130β does not bind GM-CSF, its association with p80α is required for high affinity binding (5, 7). In the present work, two lines of evidence indicate that the association between the two chains p80α and p130β to form the high affinity complex is stoichiometric. First, at a concentration of radioligand sufficient for near saturation of the high affinity receptor R, without occupancy of the low affinity receptor R₂, the signal observed by cross-linking of the radioligand to the α chain was the same as that observed on the β chain, suggesting stoichiometry. Second, at 5 nM radioligand, a concentration approaching the $K_d$ of the low affinity GM-CSF-R, the presence of IL-3 should allow for occupancy of R₂ only. Stoichiometry would therefore predict that at the high concentration of radioligand, $ΔVβ = ΔV₀$ and $Vβ = V₀ - Vα$, consistent with the results observed in Fig. 1B. Therefore, both at equilibrium or upon perturbation of the equilibrium with IL-3, our data are com-

![Fig. 4. The preformed ternary complex is not dissociated by solubilization. GM-CSF binding (2 nM) was performed on whole cells (lanes 1-3) or on solubilized membrane proteins (lanes 4 and 5) under comparable conditions (in a total volume of 100 μL of sodium phosphate buffer). The binding reaction was allowed to proceed for 1 h at 4 °C. Unbound ligand was removed (lanes 1-3), and BS3 (1 mM) was added prior to (lane 1) or after solubilization of membrane proteins (lanes 2-5) for 30 min at 4 °C. The reaction was quenched prior to sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis. Two hundred ng of total proteins were loaded per lane. Nonspecific binding and cross-linking were determined in the presence of 200-fold molar excess of unlabeled GM-CSF in the binding step (lanes 3 and 5). The gel was dried and exposed for 10 days. All lanes come from the same gel and are shown here with a gray scale of 20-50 (lower and upper limits, respectively; lanes 1-3) and 20-150 (lanes 4 and 5). The gray scale was expanded for the last two lanes because unbound GM-CSF was not removed (cross-linking on solubilized membrane proteins), hence a higher amount of radioactivity was loaded per lane.](image-url)
pellet was diluted into 100 ml of phosphate-buffered saline, 0.1%
advantage of the perturbation of GM-CSF binding equilib-
control cells were shown are the mean of four independent
determination did not affect the maximum binding capacity
Previous work indicated that solubilization of membrane
states of the receptor are interchangeable. Modeling can, therefore, integrate the perturbation of the ternary complex by another ligand, or other agonists and antagonists.
Nicola (24) has shown previously that the kinetics of associa-
tion of GM-CSF to the high or low affinity binding site
is reversible since the presence of IL-3 converts high affinity
in previous studies, GM-CSF binding was determined after sol-
the ternary complex model applied here allows for the binding of GM-CSF to either GMαα (R2) or to the preformed complex GMααββ (R1). Further, our data indicate that the presence of ligand stabilizes the association between the two chains, p80α and p130β. Taken together, these results suggest that the initial binding step does not discriminate between high and low affinity states. Following ligand binding, the association between the two chains is stabilized, and the equilibrium is displaced toward the high affinity (slowly dissociating) complex.
Previous work indicated that solubilization of membrane
peptides resulted in a loss of high affinity GM-CSF binding sites and a conversion to low affinity binding sites (6, 24).

![Figure 5](image-url)  
**FIG. 5.** Time-dependent stabilization of the ternary com-
plex. GM-CSF binding (100 pm) was performed on TF-1 cells at 4 °C for 5 min, 30 min, and 4 h. Unbound GM-CSF was removed by centrifu-
gation through a cushion of fetal calf serum, and the cell

The high quality of fit of the data to the ternary complex model suggests that the association of the ligand with the two α and β chains is sufficient to convert low to high affinity binding. Second, our data also suggest that ligand binding stabilizes the association between the two chains by at least 1,000-fold. Further, the presence of IL-3 does not destabilize the ternary complex. Rather, IL-3 prevents high affinity GM-CSF binding through sequestration of p130β.

Computer modeling of GM-CSF saturation curves with the ternary complex model indicated that the presence of IL-3 did not affect the maximum binding capacity (Bmax) or the KD of the ligand-receptor complex. Estimation of the binding characteristics by nonlinear regression analysis with the program SCAFIT provided comparable results. However, linear regression (Scatchard transformation) or nonlinear regression analysis (SCAFIT) of binding data is based on equations describing the interaction of a single ligand to either one or several classes of binding sites that are not interchangeable. Such analysis may be of limited value when both IL-3 and GM-CSF are present in the binding reaction, i.e., when GM-CSF binding is perturbed by IL-3. In contrast, modeling as performed in the present study provides a more powerful approach because it relies on the premise that the two affinity
Acknowledgments—We thank Dr. Jun Pei Hu for help with the binding studies on AML 44, Dr. Pierre Haddad for suggestions on the manuscript, and F. De Coste for secretarial assistance.

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