Construction and Binding Kinetics of a Soluble Granulocyte-Macrophage Colony-stimulating Factor Receptor α-Chain-Fc Fusion Protein*

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) activity is mediated by a cellular receptor (GM-CSFR) that is comprised of an α-chain (GM-CSFRα), which specifically binds GM-CSF, and a β-chain (βc), shared with the interleukin-3 and interleukin-5 receptors. GM-CSFRα exists in both a transmembrane (tmGM-CSFRα) and a soluble form (sGM-CSFRα). We designed an sGM-CSFRα-Fc fusion protein to study GM-CSF interactions with the GM-CSFRα. The construct was prepared by fusing the coding region of the sGM-CSFRα with the CH2-CH3 regions of murine IgG2a. Purified sGM-CSFRα-Fc ran as a monomer of 60 kDa on reducing SDS-polyacrylamide gel electrophoresis but formed a trimer of 160–200 kDa under nonreducing conditions. The sGM-CSFRα-Fc bound specifically to GM-CSF as demonstrated by standard and competitive immunoassays, as well as by radioligand assay with 125I-GM-CSF. The sGM-CSFRα-Fc also inhibited GM-CSF-dependent cell growth and therein is a functional antagonist. Kinetics of sGM-CSFRα-Fc binding to GM-CSF were evaluated using an IAsys biosensor (Affinity Sensors, Paramus, NJ) with two assay systems. In the first, the sGM-CSFRα-Fc was bound to immobilized staphylococcal protein A on the biosensor surface, and binding kinetics of GM-CSF in solution were determined. This revealed a rapid $k_{on}$ of $2.43 \times 10^{7}$ M$^{-1}$s$^{-1}$. A second set of experiments was performed with GM-CSF immobilized to the sensor surface and the sGM-CSFRα-Fc in solution. The dissociation rate constant ($k_{off}$) for the sGM-CSFRα-Fc trimer from GM-CSF was $1.57 \times 10^{-3}$ s$^{-1}$, attributable to the higher avidity of binding in this assay. These data indicate rapid dissociation of GM-CSF from the sGM-CSFRα-Fc and suggest that in vivo, sGM-CSFRα may need to be present in the local environment of a responsive cell to exert its antagonist activity.

Granulocyte-macrophage colony-stimulating factor (GM-CSF)$^1$ plays an important role in myeloid differentiation and is involved in many inflammatory and immune processes. GM-CSF is a member of the four-helix bundle family of cytokines which also includes growth hormone, interleukin-2 (IL-2), IL-4, and IL-5. GM-CSF activity is mediated by specific cellular receptors (GM-CSFR), which belong to a supergene family (1–7). The GM-CSF consists of an α-chain (GM-CSFRα), specific for GM-CSF (4), and a β-chain (βc), which can also associate with IL-3 and IL-5 receptor α-chains (5). The GM-CSFRα expressed in the absence of βc binds GM-CSF but with a lower affinity than the heterodimeric receptor (8).

Naturally occurring soluble forms of cytokine receptors, including a soluble form of GM-CSFRα (sGM-CSFRα), have been described (9–11). sGM-CSFRα has been cloned from human placenta (11) and human choriocarcinoma cells (9) and has been detected in the supernatant of a human choriocarcinoma cell line (10). Previous work in our laboratory utilized polymerase chain reaction (PCR) to amplify and clone both the transmembrane and the soluble forms of the GM-CSFRα from a human GM-CSF-dependent myelomonocytic cell line (AML193). Supernatants from sGM-CSFRα-transfected cells, but not transmembrane GM-CSFRα (tmGM-CSFRα)-transfected cells, inhibited GM-CSF immunoprecipitation by neutralizing monomeric antibody (mAb) 128.213 and inhibited GM-CSF-dependent cellular proliferation. These experiments indicated that sGM-CSFRα binds GM-CSF and exhibits functional antagonist activity in vitro (12).

The GM-CSF antagonist activity of sGM-CSFRα could play a role in the regulation of biological responses to GM-CSF. Biological effects mediated by the sGM-CSFRα in vivo would be dependent on its binding characteristics. For example, if the sGM-CSFRα binds GM-CSF with high affinity, forming a stable complex, the sGM-CSFRα would be able to sequester GM-CSF away from responsive cells. In contrast, if the sGM-CSFRα binds with lower affinity, particularly with a rapid off-rate, sGM-CSFRα would need to be present in the local cellular environment where GM-CSF is present to exert its antagonist activity. Thus, the binding kinetics of the sGM-CSFRα will have a major impact on its in vivo functional role, and determining the kinetic properties of the protein would help determine the mechanistic nature of its biological role. Unfortunately, production and purification of sufficient sGM-CSFRα

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$^1$ The abbreviations used are: GM-CSF, granulocyte-macrophage colony-stimulating factor; GM-CSFR, GM-CSF receptor; sGM-CSF, soluble GM-CSF; tmGM-CSF, transmembrane GM-CSF; IL, interleukin; PCR, polymerase chain reaction; mAb, monoclonal antibody; SOE, splicing by overlap extension; bp, base pairs; DMEM, Dulbecco’s modified Eagle’s medium; ELISA, enzyme-linked immunosorbent assay; SPA, staphylococcal protein A; HRP, horseradish peroxidase; TMB, 3,3',5',5'-tetramethylbenzidine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

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for binding studies have been problematic because production by cell lines is limited, and simple purification methods are not available. Therefore, we prepared a DNA-construct fusing the sGM-CSFRa to the Fc region of mouse IgG2a. Here we describe the cloning, production, purification, binding, and biological activity of this sGM-CSFRa-Fc fusion protein, with preliminary analyses of binding kinetics.

**MATERIALS AND METHODS**

Cloning of sGM-CSFRa-Fc

sGM-CSFRa cDNA clones were available as described previously (12). sGM-CSFRa cDNA and the CH2-CH3 cDNA region of mouse anti-reovirus type 3 mAb 9BG5 (13) were amplified using primers containing restriction endonuclease sites for restriction enzymes BamHI and SalI for cloning the construct into the pBabe-puro vector and including sequences that allowed their use in the splicing by overlap extension (SOE) step for the preparation of the construct. sGM-CSFRa cDNA from clone 9 was PCR amplified using these primers (restriction sites underlined): sGM-CSFRa (BamHI), 5'-TGACCGGATCCATGCTCCTCTCGTGGTAAC; and sGM-CSFRa (SalI), 5'-CACCACCGTGGTCACTGGTGAGA.

The CH2-CH3 region (Fc region) of mouse anti-reovirus mAb 9BG5 was amplified from cDNA prepared from the hybridoma according to our prior protocols (12) with the following primers: Fe (SOE), 5'-TTCTCACAGATCAAAGACAAACGCACCTAACCTCTTGGGTGG; Fc (SalI), 3'-ATGCCGGATCCATTATACCCGGAGTCCGGGAGT.

These primers were used with cDNAs and Taq polymerase as described previously (14, 15) to amplify the sGM-CSFRa and Fc. The program of amplification was 94 °C for 1.5 min followed by 25 cycles at 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, with a final cycle at 72 °C for 5 min. The PCR products were stored at 4 °C until used. A 2% agarose gel (NuSieve, FMC BioProducts, Rockland, ME), extracted products, which were therefore purified on a 1% preparative low melt- agarose gel, ligation was performed following standard procedures (16, 19).

The product was transformed into Escherichia coli DH5α cells that were plated into LB plates; and minipreparations were made from colonies. Plasmid DNA from minipreps were digested with BamHI and SalI to identify the presence of the desired insert. After amplification of cells with the right insert, plasmid DNA was purified on a Qiagen column (Qiagen, Chatsworth, CA). The purified DNA was checked using two different pairs of restriction enzymes (BamHI/SalI and BamHI/XhoI). After alkaline phosphatase digestion of the amplified construct, the pBabe vector (17, 18) was digested with restriction enzymes BamHI and SalI. After alkaline phosphatase treatment of the pBabe purified on a 1% preparative low melting agarose gel (NuSieve, FMC BioProducts, Rockland, ME), extracted with phenol/chloroform (16), and used in the SOEing step.

The SOEing step consisted of two phases. In the first phase (94 °C for 1 min followed by five cycles at 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min) the two amplified cDNA products worked as primers for each other to obtain the construct. In the second phase (94 °C for 1 min followed by 25 cycles at 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, with a final cycle at 72 °C for 5 min. The PCR products were stored at 4 °C until used. A 2% agarose gel (NuSieve, FMC BioProducts, Rockland, ME), extracted products, which were therefore purified on a 1% preparative low melting agarose gel, ligation was performed following standard procedures (16, 19).

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**Transfection of PA317 Cells, Transfection into SP2/0 Cells**

PA317 cells (a retroviral packaging line (17, 18)) were transfected with the purified DNA using LipofectAMINE reagent (Life Technologies, Inc.) and the described protocol (21). Briefly, 2 × 10⁵ cells in 2 ml of DMEM (DMEM containing T-Gln, oxalate/pyruvate/insulin, penicillin/streptomycin, and 10% fetal calf serum) were seeded in each well of a six-well culture plate and grown until 80% confluence. 1.5 µg of DNA in 100 µl of serum-free DMEM was added to 12 µl of LipofectAMINE reagent diluted to 100 µl with serum-free DMEM. After gentle mixing and incubation at room temperature for 45 min to allow the DNA-liposome complex to form, the complex solution was diluted to 1 ml with serum-free DMEM and added to the rinsed adherent cells. Cells were kept in a 37 °C 5% CO2 incubator for 5 h and then rinsed, and serum-containing DMEM was added. 72 h after transfection, the cells were washed 1:10 into DMEM containing 5 µg/ml puromycin. Cells were grown until confluent, split twice, and grown for 1 week in medium without puromycin. The supernatant was then used to transduce SP2/0 cells.

3 × 10⁵ SP2/0 cells, grown in RPMI medium, containing T-Gln, sodium pyruvate, penicillin/streptomycin, 25 µM Hepes, and 15% fetal calf serum, was resuspended in 2 ml of medium with 0.5 µl of PA317 supernatant and 10 µl of Polybrene (0.4 µg/ml). After 3 h of incubation, 8 ml of RPMI medium was added; after 2 days of incubation, cells were grown in medium containing puromycin. SP2/0 cells were then cloned and the presence of inserts confirmed by PCR. An enzyme-linked immunosorbent assay (ELISA) (see below) was performed on PCR-positive clone supernatants to identify the clones producing the fusion protein.

**Purification and Characterization of sGM-CSFRa-Fc**

Clones that presented the highest binding to GM-CSF in two ELISAs were expanded, and the fusion protein from the supernatants was purified on a staphylococcal protein A (SPA) column. To 140 ml of filtered supernatant, glycine and NaCl were added to reach, respectively, 1.5 and 3 M final concentrations; the pH was adjusted to 8.0. After loading the column, the sample was washed with 10 mM boric acid, 3 M NaCl, pH 8.9, and eluted with 0.1 M sodium citrate, pH 3.5 (fractions collected into tubes containing 1 M Tris, pH 9.5). Elution fractions containing proteins were concentrated using Centrcelon (30 kDa cutoff) filtration systems (Amicon, Beverly, MA). The fusion protein was purified from the supernatant and eluted by SDS-polyacrylamide gel electrophoresis. An 8% acrylamide gel, 1 mm thick, was used in an electrophoresis system to analyze both oxidized and reduced forms of the fusion protein. 45 µg of purified sGM-CSFRa-Fc fusion protein purified from various cell clones was loaded into gels, run at 100 volts for ~1.5 h using 25 mM Tris, 250 mM glycine, 0.1% SDS, pH 7.5, and then stained with Coomassie Blue.

The purified 72-kDa S-200Hi gel filtration column (Pharmacia Biotech Inc.) was packed into a C-16/70 column (215 ml of resin) to analyze 500 µl of 0.676 mg/ml fusion protein preparation from clone 25, using 50 mM Tris, 150 mM NaCl, pH 7.5, buffer, 0.8 ml/min flow rate, 1 mm/min chart speed, and collecting 1.2-ml fractions.

**Western Blot Analysis**

Western blot analysis was performed using mAbs against GM-CSFR. 75 µg/well of both oxidized and reduced SPA-purified sGM-CSFRa-Fc fusion protein were loaded into three wells of each of two 8% polyacrylamide gels. After the electrophoretic run as reported previously, the gels were cut in thirds, each strip containing two wells, one with the sample and one with the high molecular mass markers, and transferred to Immobilon-P membranes (Millipore, Bedford, MA) for 1 h at room temperature, under stirring conditions, using 50 mM Tris, 150 mM NaCl, 0.1% Tween, pH 7.4, as the transfer buffer. The membranes were then blocked overnight at 4 °C with 5% non-fat dry milk in 50 mM Tris, 150 mM glycine, 0.05% Tween, pH 7.5 (5% NFDM in TTBs). The next morning they were washed three times for 10 min with TTBs, and the three pairs of oxidized and reduced membrane strips were incubated for 2 h at room temperature with one of the following monoclonal antibodies diluted in TTBs: (a) 7 µg/ml mouse IgG2a anti-human GM-CSFR (Pharmingen, San Diego) (this antibody binds GM-CSFR on the same site recognized by GM-CSF); (b) 7 µg/ml mouse IgM anti-human GM-CSFR (Pharmingen) (this antibody binds GM-CSFR on a site different from that recognized by GM-CSF); (c) no first antibody. The membranes were then washed three times for 2 min with 5% NFDM in TTBs and twice for 2 min with TTBs. Samples from a and b were then incubated for 2 h at room temperature, under shaking conditions, with a 1:40,000 dilution of biotin-goat-anti-mouse IgG (Fab-specific) (Sigma) in TTBs. After washing the membranes three times for 2 min with 5% NFDM in TTBs and twice for 2 min with 1 × TTBs, they were incubated for 2 h at room temperature, under shaking conditions with 0.5 µg/ml avidin-horseradish peroxidase (HRP, Pierce) in 1 × TTBs; samples c were incubated under the same conditions with goat anti-mouse IgG (H + L). HRP (Life Technologies, Inc.) The membranes were washed six times with 1 × TTBs, and the TMB substrate solution was added (Kirkegaard and Perry Laboratories, Gaithersburg, MD) until color development. The color reaction was then stopped by distilled water rinsing.

**ELISAs**

Clone supernatants were tested in three types of ELISAs. In the first type, ELISA plates (Dynatech Laboratories, Chantilly, VA) wells were
coated with 50 μl of 10 μg/ml GM-CSF (Sargamostim Leukine, Immunex Corporation, Seattle) in 0.1 M NaHCO₃ and after overnight incubation at 4 °C they were washed five times with 1 × PBS and blocked with 200 μl of 2% bovine serum albumin in 1 × PBS at 37 °C for 1 h. The plates were washed five times with 1 × PBS, and 50 μl of clone supernatants was added, incubated overnight at 4 °C, and washed seven times with cold 1 × PBS. 100 μl of 1/3,000 cold dilution in 1 × PBS of goat anti-mouse IgG (H+L) HRP was added per well and the plate kept overnight at 4 °C. After seven washes with cold 1 × PBS, 100 μl of 0.1 mg/ml substrate TMB dihydrochloride (Sigma) in 0.05 M phosphate citrate, 0.03% sodium perborate buffer, pH 5.0, was added per well. After color development at room temperature for 10 min, the enzymatic reaction was stopped with 20 μl of 2 N H₂SO₄ per well and the plate read at 450 nm.

In the second ELISA the wells were coated with 50 μl of 5 μg/ml SPA (Sigma) in 0.1 M NaHCO₃ in 0.1 M NaHCO₃ for 1 h at 37 °C, washed five times with 1 × phosphate-buffered saline with 0.1% Tween-20 (PBST), and blocked as reported previously. An ELISA plate was coated with SPA and blocked as reported previously. An amount (100 nM) of cold GM-CSF at room temperature for 1 h. The plate was then activated by a 10-min incubation with a mixture of 100 μl of 10 μg/ml GM-CSF or SPA in 10 mM sodium acetate, pH 5, was added to the cuvette, and after a response plateau was reached signifying completion of the reaction, the cuvette was washed out with PBST. The remaining activated groups were blocked by injection of 200 μl of 1 M ethanolamine, pH 8.5, for 2 min. After reequilibration with PBST, the cuvette was ready for the binding experiments. The cuvette matrix was regenerated to remove bound ligand using 200 μl of either 0.5 mM sodium carbonate, pH 11.0, or 10 mM HCl and 0.5 M NaCl and reequilibrated with PBST.

Immobilized SPA—A 200-μl solution containing 20 μg of sGM-CSFRo-Fc in PBST was added to the cuvette and allowed to bind for 6 min (phase 1). PBST was added for 5 min (phase 2). GM-CSF at various concentrations in PBS was then added and allowed to bind for 5 min (association phase, phase 3) for 5 min. PBST was then added for an additional 5 min (dissociation phase, phase 4). No regeneration was performed between runs in these assays.

Data Analysis

Data were collected automatically and analyzed subsequently using the Microsoft Excel program (Microsoft, Redmond, WA). Plots were fitted using Cricket Graph (Cricket Software, Malvern, PA). The sGM-CSFRo-Fc surface prepared by capturing sGM-CSFRo-Fc on immobilized SPA was corrected for a downward drift as dissociation of sGM-CSFRo-Fc from the SPA occurred along with GM-CSF binding to sGM-CSFRo-Fc. Accordingly, control experiments were run in which sGM-CSFRo-Fc was allowed to bind to SPA for 5 min (phase 1), the receptor-associated surface was washed for 5 min (phase 2), and then phosphate-buffered saline was added for an additional 5 min (to simulate the association phase of GM-CSF, phase 3) followed by a final 5-min wash (simulating the dissociation phase of GM-CSF from sGM-CSFRo-Fc, phase 4). In these experiments, the negative slope of the dissociation of GM-CSFRo-Fc from SPA decreased slightly after each of the washes (in phases 2, 3, and 4). Therefore, correction for sGM-CSFRo-Fc dissociation from SPA was performed separately for each phase. The slope of dissociation of sGM-CSFRo-Fc from SPA in the final 200 s of phase 2 was calculated for each experiment and used to normalize this portion of the sensorgrams. Similarly, the slope of the final 200 s of phase 4 was calculated and used to correct the dissociation phase of GM-CSF from sGM-CSFRo-Fc. A weighted average of the slopes from phases 2 and 4 was used to predict a slope for phase 3, and this was used to correct the association phase of GM-CSF to the sGM-CSFRo-Fc. Evaluation of these corrections on the mock experiments gave reasonable agreement with the observed data (data not shown). This triphasic correction was used for each experiment with immobilized SPA.

Kinetic analysis was performed as described previously (23). In the case of a bimolecular interaction of two species A and B whose association and dissociation are regulated, respectively, by kₐ and kₐoff for giving the final product AB,

\[
\text{d}[AB]\text{dt} = k_\text{a}[A][B] - k_\text{off}[AB].
\]  

(Eq. 1)

If B is immobilized on the cuvette, the response R is proportional to the amount of product AB, thus,

\[
\text{d}R\text{dt} = k_\text{a}[A][R_{\text{max}}] - k_\text{off}R,
\]  

(Eq. 2)

which rearranges to

\[
\frac{\text{d}R}{\text{dt}} = k_\text{a}[A][R_{\text{max}}] - (k_\text{a}[A] + k_\text{off})R
\]  

(Eq. 3)

where \(R_{\text{max}}\) is the maximum response. Therefore, a plot of \(\text{d}R/\text{dt} \text{ versus } R\) will have a slope of \(-k_\text{a}[A] + k_\text{off}\) and a plot of \(\text{d}R/\text{dt} \text{ versus } [A]\) will have a slope of \(-k_\text{off}\).

In the case of the dissociation phase, any free A from dissociation of
We had previously cloned a soluble form of the GM-CSFRα to a retroviral vector (12). We used this clone, which encodes for sGM-CSFRα, to prepare a fusion protein of the sGM-CSFRα with the Fc portion of murine IgG. The sGM-CSFRα-Fc construct (shown in Fig. 1) was obtained as follows. sGM-CSFRα cDNA from clone 9 and the CH2-CH3 cDNA region of mouse anti-reovirus mAb 9B5g (IgG2a) were PCR amplified using the primers described under “Materials and Methods.” An analytical 2% agarose gel indicated a band of ~1,100 bp and a band of ~650 bp, respectively, for sGM-CSFRα and CH2-CH3. After purification on a 1% low melting temperature agarose gel the two products were fused in a PCR-SOEing step, obtaining a construct of ~1,700 bp. The construct was digested and ligated into the BamHI and SalI site of the pBabe-puro vector. After transformation of DH5α cells, a colony with the right sized insert was selected, grown, and DNA purified on a QiaGen column. The construct was verified by restriction enzyme mapping and sequencing as described under “Materials and Methods.”

Transfection and Expression of the Fusion Protein

sGM-CSFRα-Fc in the pBabe vector was transfected into a retroviral packaging cell line, and the virus-containing medium was used to transduce SP2/0 cells. These SP2/0 cells were selected and subcloned as described under “Materials and Methods.” Clones were tested for the presence of the construct by reverse transcriptase PCR, with 9 of 96 clones expressing the sGM-CSFRα-Fc by this assay. Several subclones were isolated and grown to confluence; the selective media were removed and supernatants collected. These supernatants were screened for production of sGM-CSFRα-Fc by two different ELISAs as described under “Materials and Methods.” An example of one ELISA is shown in Fig. 2, comparing the binding ofclone supernatants with that of neutralizing anti-GM-CSF mAb 126.213 (24). In this example, the ELISA plate was coated with SPA to which the sGM-CSFRα-Fc was bound. Biotinylated GM-CSF was then added, and binding was detected by streptavidin-HRP conjugate. This assay shows significant binding of several clones to GM-CSF. Similar results were obtained in an assay in which the ELISA plate was coated with GM-CSF, then the sGM-CSFRα-Fc was bound and binding detected by anti-mouse Ig-HRP (data not shown). Clones that consistently displayed binding were chosen for further analysis.

RESULTS

Cloning of sGM-CSFRα-Fc

sGM-CSFRα-Fc was purified from ~140 ml of clone supernatant on an SPA column, as described under “Materials and Methods.” The partially purified sGM-CSFRα-Fc was analyzed in an 8% acrylamide gel (Fig. 3). In the gel run under oxidizing conditions there is a high molecular mass band of ~160–200 kDa, representing multimers of the fusion protein, whereas in the gel run under reducing conditions there are two main bands, at ~60 kDa and ~43 kDa (sGM-CSFRα-Fc monomer and uncharacterized contaminant, respectively). We scanned the gel using Image 1.41 (Wayne Rasband, NIMH, Bethesda, MD) and performed densitometry, allowing us to estimate that the sGM-CSFRα-Fc fusion protein represents ~50% of the total protein purified by the SPA column. Gel filtration chromatography (using a Sephacryl S-200HR column) was used to evaluate the molecular mass of the sGM-CSFRα-Fc multimer. The chromatogram indicated that the oxidized sGM-CSFRα-Fc runs at ~160 kDa (data not shown). When compared with the reducing SDS-polyacrylamide gel electrophoresis of the fusion protein, this suggests that the sGM-CSFRα-Fc forms trimers. We have seen the ~60–200-kDa form from several sGM-CSFRα-Fc preparations consistently and reduce to ~60 kDa upon reduction consistently.

Western blot analysis with anti-human-GM-CSF mAbs to detect the receptor portion of the fusion protein and anti-mouse-IgG to detect the Fc portion of the fusion protein indicated a high molecular mass band for the oxidized forms (sGM-CSFRα-Fc trimer) and a strong band at 60 kDa for the reduced form (sGM-CSFRα-Fc monomer) (Fig. 4).

Binding Analysis of sGM-CSFRα-Fc

The SPA-purified sGM-CSFRα-Fc was evaluated next for binding to GM-CSF and for bioactivity. A competitive ELISA assay (Fig. 5) was carried out to confirm that the sGM-CSFRα-Fc bound to native GM-CSF. An ELISA plate was coated with SPA followed by the sGM-CSFRα-Fc. We then added unlabeled GM-CSF at various dilutions, followed by biotinylated GM-CSF, with binding detected by an avidin-HRP conjugate. We saw increasing binding with increasing amounts of biotinylated GM-CSF (compare the A450 nm without competitor for 7, 70, and 700 pm biotinylated GM-CSF), and this was inhibited competitively by increasing amounts of unlabeled GM-CSF. This indicates that unmodified GM-CSF binds to our fusion protein.
A radiolabeled binding assay (Fig. 6) confirmed the binding of the sGM-CSFR\textsubscript{a}-Fc fusion protein to 125I-GM-CSF. In this experiment, increasing the amount of added fusion protein increased the binding 125I-GM-CSF, and this binding was blocked fully by excess cold GM-CSF. This confirms the binding of GM-CSF to the sGM-CSFR\textsubscript{a}-Fc fusion protein.

**Bioactivity of sGM-CSFR\textsubscript{a}-Fc**

We next evaluated the ability of the sGM-CSFR\textsubscript{a}-Fc to inhibit the biological activity of GM-CSF using a GM-CSF-dependent cell line. MO7E cells are a myelomonocytic cell line and are dependent on GM-CSF for growth. We evaluated the growth of MO7E cells in the presence of GM-CSF with or without added sGM-CSFR\textsubscript{a}-Fc using the MTT assay (see “Materials and Methods”). The presence of increasing amounts of sGM-CSFR\textsubscript{a}-Fc resulted in increasing inhibition of proliferation of the MO7E cells (Fig. 7). We found that 250 \(\mu\)g/ml sGM-CSFR\textsubscript{a}-Fc produced 75% inhibition in cellular proliferation. In contrast, the sGM-CSFR\textsubscript{a}-Fc had no effect on the growth of the IL-2-dependent cell line CTLL (data not shown). This indicates that the sGM-CSFR\textsubscript{a}-Fc is a specific biological antagonist of GM-CSF.

**Biosensor Analysis**

**GM-CSF Binding to sGM-CSFR\textsubscript{a}-Fc**—The sGM-CSFR\textsubscript{a}-Fc was evaluated for the kinetics of binding using a biosensor. Initially, the binding of sGM-CSFR\textsubscript{a}-Fc to immobilized SPA was analyzed to assure that the Fc portion of our receptor was functional. A typical sensorgram obtained is shown in Fig. 8A. After the addition of sGM-CSFR\textsubscript{a}-Fc, a bulk phase effect is seen initially (increase in the response from bulk phase refractive index) followed by an association phase. With washing, an initial rapid response decrease (likely from bulk phase refractive index decrease) becomes relatively exponential. Fig. 8B shows a typical \(dR/dt\) plot for the association phase. The initial rapid response shift (likely bulk phase effect) is shown in open circles, followed by a relatively linear association shown in filled circles. The plot of \(k_s\) of 2.51 \(\times\) 10\(^{-2}\)s for this concentration of sGM-CSFR\textsubscript{a}-Fc.

For these experiments, a standardized protocol was developed. sGM-CSFR\textsubscript{a}-Fc was allowed to bind to SPA for 6 min.
(phase 1), followed by a 5-min wash (phase 2). GM-CSF was then added and allowed to bind (association phase, phase 3) for 5 min, followed by a final dissociation phase of 5 min (phase 4). Preliminary experiments performed with phosphate-buffered saline added in place of GM-CSF at the initiation of phase 3 revealed that the downward slopes of phases 2, 3, and 4 increased sequentially. We therefore corrected for the downward slopes of these three phases separately, as noted under "Materials and Methods." The data from the final 200 s of phases 2 and 4 were used to correct these phases. An intermediate slope was calculated for phase 3 and used to correct this phase.

A typical sensorogram, with the predicted "base lines," is shown in Fig. 9A. The values for the predicted base lines were subtracted from the data points in the sensorgram and yielded the corrected sensorgram shown in Fig. 9B. A relatively rapid initial association phase is apparent, which is supplanted by a more gradual association phase. $dR/dt$ plots of these data were nonlinear (data not shown). Departure from the linearity expected for a single bimolecular interaction (Equation 3) could be caused by multiple modes of interaction with different affinities, cooperativity, or other complex models.

Analysis of the dissociation phase is shown in Fig. 9, C–F. The relative linearity of the plot of $\ln(R_1/R_n)$ versus time was seen for several concentrations of GM-CSF, including 400, 800, 1,600, and 3,200 nM. This linear relationship was maintained for at least the first 100 s of the dissociation phase. The calculated $k_{off}$ was remarkably stable for these different experiments, ranging from $2.32 \times 10^{-2}$ to $2.54 \times 10^{-2}$/s (average $2.43 \pm 0.12 \times 10^{-2}$/s).

**Immunized GM-CSF—**The binding of various concentrations of the sGM-CSFRα-Fc trimer to immobilized GM-CSF was measured using the conditions described under “Materials and Methods.” Fig. 10A shows an overlay of sensorgrams obtained with 75, 300, 600, and 900 nM sGM-CSFRα-Fc. After an association phase showing the binding of sGM-CSFRα-Fc to immobilized GM-CSF, a washing step with buffer alone was used to effect the dissociation phase.

The association phase of each sensorgram was analyzed by plotting the change in response over time ($dR/dt$) versus the response ($R$) according to Equation 3. The $dR/dt$ plots, shown in Fig. 10B, were nonlinear, with at least two phases apparent. This departure from linearity was similar to that seen in experiments with sGM-CSFRα-Fc bound to immobilized SPA, suggesting a complex process irrespective of the assay orientation.

![Figure 4: Western blot analysis of sGM-CSFRα-Fc](image)

![Figure 5: Binding of unmodified GM-CSF to purified sGM-CSFRα-Fc fusion protein](image)
The dissociation phase was analyzed by assuming that it represents the irreversible release of sGM-CSFRα-Fc and so should follow an exponential decay. Data were plotted as the ln(response at time zero of dissociation/response at time n) versus time according to Equation 5 (Fig. 10C). Again, nonlinearity was observed, but the initial ~140 s as quite linear, so rates were calculated for these time points as has been reported previously (25). The dissociation rate constant \( k_{off} \) was calculated as the slope of these plots. For this calculation, the highest concentration of sGM-CSFRα-Fc was used because this concentration gave the most accurate dissociation curve. The value of \( k_{off} \) was calculated as \( 1.57 \times 10^{-3} \text{s}^{-1} \). This indicates a slower off-rate for this experimental configuration than that shown in Fig. 9, C–F (see above), probably because of the multivalent nature of sGM-CSFRα-Fc.

**DISCUSSION**

In this paper we report the preparation and characterization of a fusion protein obtained by expression of a construct made from sGM-CSFRα and the Fc portion of a mouse antibody. The fusion protein is produced easily by transduced cells, recovered from their medium, and purified on an SPA column via the Fc portion. This protein should provide a useful means for the study of the interaction of GM-CSF or GM-CSF mimics with the GM-CSFRα.

After amplification and SOEing of the sGM-CSFRα cDNA and the CH2-CH3 region of mouse anti-reovirus mAb 9BG5 (IgG2a), a construct of the expected size was obtained and ligated into pBabe-puro vector (Fig. 1). This construct was used to produce recombinant retrovirus, which was used to transduce SP2/0 cells. After subcloning the sGM-CSFRα-Fc-transduced SP2/0 cells, supernatants were tested for their binding to biotinylated GM-CSF. For all supernatants we obtained low values in the ELISAs (Fig. 2 and data not shown), probably due in part to the low concentration of fusion protein in the supernatants and in part to the rapid off-rate of GM-CSF (see below). However, several positive clones were obtained which allowed more extensive analysis of purified sGM-CSFRα-Fc.

Denaturing polyacrylamide gel analysis of SPA-purified sGM-CSFRα-Fc under reducing and nonreducing conditions indicated a similar pattern of molecular masses for sGM-CSFRα-Fc purified from two different clones (Fig. 3). Moreover, both polyacrylamide gel electrophoresis under nonreducing conditions and gel filtration chromatography of the purified oxidized protein indicated that the fusion protein was present as a multimer, probably a trimer of ~160–200 kDa. SDS-polyacrylamide gel electrophoresis performed under reducing conditions indicated a ~60-kDa protein (Fig. 3), which represented ~50% of the total protein in the preparation. Also seen was a ~45 kDa band (which was absent in other preparations) and a smaller band of ~25 kDa which is seen at the dye front of the gel in Fig. 3. A gel run in the same conditions alongside fetal calf serum as a control shows bands of ~60 and ~25 kDa in the fetal calf serum (data not shown). Thus, it is likely that there is antibody contamination from the fetal calf serum used to grow our sGM-CSFRα-Fc-transduced SP2/0 cells. Bovine IgG under oxidizing conditions likely co-migrates with the multimeric sGM-CSFRα-Fc, with the bovine IgG heavy chain also co-migrating with the sGM-CSFRα-Fc under reducing conditions. Alternatively, the other bands seen on the reducing gel could represent proteins that covalently attach to the sGM-CSFRα-Fc. We think this is unlikely as the quantity of contaminating proteins varies in our different preparations. In addition, a two-dimensional gel run under reducing conditions showed a single band with an estimated pI of 6.7 (data not shown).

The nature of the ~60 kDa band was investigated by Western blot analysis, using antibodies against the GM-CSFRα and murine IgG. The experiment indicated that the ~60 kDa band is detected by two different anti-human GM-CSFR monoclonal antibodies, recognizing the receptor portion of the fusion protein, and by anti-mouse IgG, recognizing the Fc portion of the sGM-CSFRα-Fc fusion protein (Fig. 4). The other contaminating bands were not recognized by either reagent, making it unlikely that these represent breakdown products.
This biochemical and immunochemical analysis strongly suggests that the sGM-CSFRα-Fc exists as a trimer in the oxidized state. The reason for this is unclear. The fusion protein has a total of 16 cysteines: 12 from the sGM-CSFRα and 4 from the IgG2a Fc sequence. These are thought to participate in intramolecular disulfide bridges in native GM-CSFRα and murine IgG, respectively. However, some of these cysteines appear to form intermolecular disulfide bridges in the sGM-CSFRα-Fc. We are uncertain of the disulfide bonding pattern of the sGM-CSFRα-Fc which results in trimer formation. It is interesting to speculate that such trimers of the GM-CSFRα may exist naturally, possibly through noncovalent interactions. However, no data to support or refute this possibility are currently available. The trimers seen here show that trimer formation can occur without abrogating binding of the sGM-CSFRα-Fc to GM-CSF. We are uncertain if this is an artifact of the sGM-CSFRα-Fc construct or a reflection of a physiologic capability for receptor aggregation to occur.

The binding capacity of the fusion protein for GM-CSF was tested in several experiments. Competition of “cold” and biotinylated GM-CSF on an ELISA plate coated with sGM-CSFRα-Fc indicated that the fusion protein specifically binds to GM-CSF (Fig. 5). This binding was also confirmed by a radioligand binding assay, using [125I]-GM-CSF (Fig. 6), which again showed specific binding that was abrogated by cold GM-CSF. This indicates that the fusion protein possesses active binding site(s) for GM-CSF and that this binding is specific as it is inhibited competitively by unlabeled GM-CSF. It was also of interest to determine whether the sGM-CSFRα-Fc was active in a bioassay. Because the sGM-CSFRα-Fc binds GM-CSF, it would be expected to block binding of GM-CSF to cell surface receptors. This indeed appears to be the case, as the sGM-CSFRα-Fc also prevented proliferation of the GM-CSF-dependent cell line MO7E when present in the medium together with the growth factor (Fig. 7). Thus, the sGM-CSFRα-Fc is similar to sGM-CSFRα, as we described previously (12), in possessing biological antagonist activity.

The parameters regulating the association and dissociation between GM-CSF and sGM-CSFRα-Fc were evaluated in preliminary studies of the binding of GM-CSF to sGM-CSFRα-Fc in turn bound to immobilized SPA using an IAsys optical biosensor (Figs. 8 and 9). This was only possible because of the relatively linear dissociation of sGM-CSFRα-Fc from SPA, as shown in Fig. 8. The advantage of this experimental design is that GM-CSF exists as a monomer in solution, and a 1:1 interaction with a single receptor site is likely. This eliminates the problem posed by the trimeric nature of the sGM-CSFRα-Fc, which may contribute an avidity component to binding. The disadvantage is that a complicated correction for the dissociation of sGM-CSFRα-Fc from SPA was needed. In these experiments, association phase analysis revealed nonlinear plots of d[R]/dt versus [R] (data not shown). Although the reason for this departure from linearity is obscure, given this experimental configuration, it is unlikely to be the result of the trimer formation by sGM-CSFRα-Fc. This prevented formal koff calculations. We were able to obtain reproducible values for koff which averaged 2.43 × 10⁻⁹/s (Fig. 9, C–F). When used to solve for t¹/₂ in Equation 4 for the case of r = 1/2 Rn, the estimated half-time for receptor dissociation ranges from 27 to 30 s.

Similar analyses were carried out for immobilized GM-CSF with the sGM-CSFRα-Fc in solution. The association phase failed to follow the predicted theoretical model (23) as demon-

Fig. 8. Kinetics of sGM-CSFRα-Fc binding to SPA. Panel A, typical sensorgram obtained for binding of sGM-CSFRα-Fc to immobilized SPA. The relative response (in relative units, RU) is shown versus time for 20 μg of sGM-CSFRα-Fc added at 85 s. The increase in relative units indicates binding of sGM-CSFRα-Fc to SPA. The sGM-CSFRα-Fc was washed at 445 s, beginning the dissociation phase of the experiment. Panel B, association rate constant calculation. A plot of d[R]/dt versus [R] is shown as described under “Materials and Methods.” An early rapid association phase (probably the result of mass effect) is shown by open circles, with a later slower association phase indicated by filled circles. A curve fitted to the second phase is shown, which has a slope (kₐ) = 2.51 × 10⁻⁹/s. Panel C, dissociation rate constant calculation. The dissociation phase is replotted as ln(R/R₀) versus time. Data points are shown following a steep initial decline in relative units (see Fig. 9A), likely resulting from mass effect. The slope from the early data points (filled circles) was used to calculate a straight line with a slope corresponding to koff = 2.00 × 10⁻⁹/s.
stratified by the plots of both \( dR/dt \) versus \( R \) and \( k_a \) versus concentration. In the \( dR/dt \) versus \( R \) plot (Fig. 10B) we represented the first linear portion of the curves for 75, 300, and 600 nM concentrations, but we omitted from the plot the 900 nM concentration. This high sGM-CSFR\( \alpha \)-Fc concentration resulted in very rapid binding in the initial association phase (Fig. 10A). It was therefore impossible to obtain enough data points to draw a reliable \( dR/dt \) versus \( R \) plot for this concentration. Moreover, with such a rapid association rate, mass transfer becomes the rate-limiting factor in the initial stage of the binding. We therefore declined to calculate a formal \( k_{\text{on}} \) from these data.

For analysis of the dissociation phase, we chose to analyze only the plot for the highest concentration of sGM-CSFR\( \alpha \)-Fc, 900 nM, because this had the highest response/background ratio. This analysis did not fit the expected exponential decays shown by the curve of the plot in Fig. 10C. Nonlinearity in dissociation phase analysis has also been seen in the interaction of insulin-like growth factor and insulin-like growth factor-binding protein (26). In this case the effect was attributed to either long term maturation of the analyte-ligand complex or functional heterogeneity of the immobilized ligand. Other potential explanations include multiple sites with different affinities, cooperativity in binding, rebinding, and mass effects. Curve-fitting the data in Fig. 10C showed a fit to the sum of exponentials, and this is perhaps the result of differential binding characteristics of sites within the sGM-CSFR\( \alpha \)-Fc trimer, depending on the number of active receptor sites already involved in binding to immobilized GM-CSF. In the absence of a definite physical explanation the data were analyzed by taking the slope of the curve in the first 120 s in support of this method of analysis its simplicity and the consistency of the calculated \( k_{\text{off}} \) with the average value from experiments with different sGM-CSFR\( \alpha \)-Fc concentrations. The \( k_{\text{off}} \) value determined by this method is \( 1.57 \times 10^{-7}\text{s}^{-1} \). This is \( \sim 1 \) order of magnitude slower than for the reverse experimental configuration (see Fig. 9, C–F). This indicates that the trimer formation of sGM-CSFR\( \alpha \)-Fc slows the off-rate, likely because of an avidity effect.

Thus, our data indicate that the sGM-CSFR\( \alpha \)-Fc interaction with GM-CSF is characterized by a rapid dissociation phase, implying that the major energy of binding is contributed by a fast on-rate. These kinetics have relevance to the biological activity of the sGM-CSFR\( \alpha \). The ability of the sGM-CSFR\( \alpha \) to function as a biological antagonist is likely caused by competition for free GM-CSF with the transmembrane form of the receptor (tmGM-CSFRs) present on cells. Given the relatively fast off-rate of the sGM-CSFR\( \alpha \)-Fc, the antagonist activity of sGM-CSFR\( \alpha \) is then dependent on its continued presence, because once the sGM-CSFR\( \alpha \) diffuses away, the GM-CSF would dissociate and then be available for binding to the tmGM-CSFRs. As noted above, the \( t_{1/2} \) for dissociation is on the order of 27–30 s. Thus, for sGM-CSFR\( \alpha \) to sequester GM-CSF and remove it from the local environment of a responsive cell, diffusion away from the cell would have to be more rapid than this dissociation rate. Slower rates of diffusion would imply...
that the sGM-CSFRα needs to be present in the vicinity of the cell to act as a competitive inhibitor.

In terms of understanding the high affinity sites formed by tmGM-CSFRα and β subunits, these data would suggest that the contribution of β subunits is mostly to slow the off-rate, as the on-rate is already relatively rapid. This is supported by experiments reported by Gearing et al. (4), where binding of 125I-GM-CSF to low affinity sites on HL-60 cells was lost after a short (10-min) incubation in the absence of 125I-GM-CSF, but the high affinity sites remained. We would postulate, based on our data and theirs, that the primary role of the GM-CSFRα in the complex is to capture GM-CSF with a rapid association phase and that the β subunits primarily slow the dissociation phase. It is interesting to speculate on the role of the β-subunit (β subunit) in slowing the dissociation of GM-CSF from the heterodimeric receptor. Studies of GM-CSF mutants support a key role for Glu-21 in binding to the high affinity but not the low affinity receptor. However, direct evidence is lacking for such an interaction, and conformational changes or aggregation of the GM-CSFRα imparted by β subunit could also account for the slower off-rate seen. Clarification of this matter awaits direct binding data with GM-CSFRα, β subunit and GM-CSF.

When compared with data from other cytokine receptor interactions, the kinetics of binding for GM-CSF to the sGM-CSFRα-Fc are similar to those reported for other cytokines, such as IL-5 (25). The characteristics of rapid on-rates and somewhat slower, but still rapid, off-rates may be a general characteristic of cytokine-receptor interactions for four-helix bundle cytokines.

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REFERENCES

1. Cannistra, S. A., Grushke, P., Garlick, R., Miller, J., and Griffin, J. D. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 93–97
2. Chiba, S., Tojo, A., Kitamura, T., Urabe, A., Miyazono, K., and Takaku, F. (1990) Leukemia 4, 29–36
3. DiPersio, J. F., Hedvat, C., Ford, C. F., Golde, D. W., and Gasson, J. C. (1991) J. Biol. Chem. 266, 279–286
4. Gearing, D. P., King, J. A., Gough, N. M., and Nicola, N. A. (1989) EMBO J. 8, 3667–3676
5. Hayashida, K., Kitamura, T., German, D. M., Arai, K.-i., Yokota, T., and Miyajima, A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9655–9659
6. Onetto-Pothier, N., Aumont, N., Haman, A., Bigras, C., Wong, G. G., Clark, S. C., De Lean, A., and Hoang, T. (1990) Blood 75, 59–66
7. Park, L., Friend, D., Gillis, S., and Urdal, D. (1986) J. Biol. Chem. 261, 4177–4183
8. Shanafelt, A. B., and Kastelein, R. A. (1992) J. Biol. Chem. 267, 25466–25472
9. Raines, M. A., Liu, L., Quan, S. G., Joe, V., DiPersio, J. F., and Golde, D. W. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8203–8207
10. Sasaki, K., Chiba, S., Mano, H., Yazaki, Y., and Hirai, H. (1992) Biochem. Biophys. Res. Commun. 183, 252–257
11. Ashworth, A., and Kraft, A. (1990) Nucleic Acids Res. 18, 7178
12. Williams, W. V., VonFeldt, J. M., Rosenbaum, H., Ugen, K. E., and Weiner, D. B. (1992) Arthritis Rheum. 37, 1468–1478
13. Burstin, S., Spriggs, D., and Fields, B. (1982) Virology 117, 146–155
14. Williams, W. V., Fang, Q., Demarco, D., VonFeldt, J., Zurier, R. B., and...
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Weiner, D. B. (1992) J. Clin. Invest. 90, 326–333
15. Williams, W. V., Rosenbaum, H. R., and Weiner, D. B. (1992) PCR 2, 86–88
16. Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J., and Struhl, K. (1989) Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley-Interscience, New York
17. Morgenstern, J. P., and Land, H. (1990) Nucleic Acids Res. 18, 3587–3596
18. Morgenstern, J., and Hartmut, L. (1990) Nucleic Acids Res. 18, 1068
19. Sambrook, J., Fritsch, E., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY
20. Monfardini, C., Kieber-Emmons, T., VonFeldt, J. M., O’Malley, B., Rosenbaum, H., Godillett, A. P., Kaushansky, K., Brown, C. B., Voet, D., McCallus, D. E., Weiner, D. B., and Williams, W. V. (1995) J. Biol. Chem. 270, 6628–6638
21. Hawley-Nelson, P., Ciccacrone, V., Gebeyehu, V., Jessee, J., and Felgner, P. L. (1993) Focus 15.3, 73–80
22. Celgian, J. E., Krusiits, A. M., Margulis, D. H., Shevach, E. M., and Streber, W. (1990) Current Protocols in Immunology, Greene Publishing Associates and Wiley-Interscience, New York
23. Karlsson, R., Michaelsson, A., and Mattson, L. (1991) J. Immunol. Methods 145, 229–240
24. Brown, C. B., Hurt, C. E., Curtis, D. M., Bailey, M. C., and Kaushansky, K. (1990) J. Immunol. 144, 2184–2189
25. Morton, T., Bennett, D., Appelbaum, E., Cusimano, D., Johanson, K., Matico, R., Young, P., and Challen, I. (1994) J. Mol. Recognition 7, 47–55
26. Fagerstam, L. G., Frostell-Karlsson, A., Karlsson, R., Person, B., and Ronnberg, I. (1992) J. Chromatogr. 597, 397–410
27. Lopez, A. F., Shannon, M. F., Hercus, T., Nicola, N. A., Cambareri, B., Dottore, M., Layton, M. J., Eglinlon, L., and Vadas, M. A. (1992) EMBO J. 11, 909–916
28. Shanafelt, A. B., Miyajima, A., Kitamura, T., and Kastelein, R. A. (1991) EMBO J. 10, 4105–4112
29. Hercus, T. R., Bagley, C. J., Cambareri, B., Dottore, M., Woodcock, J. M., Vadas, M. A., Shannon, M. F., and Lopez, A. F. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5838–5842
30. Hercus, T. R., Cambareri, B., Dottore, M., Woodcock, J., Bagley, C. J., Vadas, M. A., Shannon, M. F., and Lopez, A. F. (1994) Blood 83, 3500–3508
31. Woodcock, J. M., Zacharakis, B., Plastinick, G., Bagley, C. J., Qiu, S., Hercus, T. R., Tavernier, J., and Lopez, A. F. (1994) EMBO J. 13, 5176–5185
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