Expression Cloning of a Human Granulocyte Colony-stimulating Factor Receptor: A Structural Mosaic of Hematopoietin Receptor, Immunoglobulin, and Fibronectin Domains

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Summary

We report the isolation from a placental library, of two cDNAs that can encode high affinity receptors for granulocyte colony-stimulating factor (G-CSF) when expressed in COS-7 cells. The cDNAs are predicted to encode integral membrane proteins of 759 and 812 amino acids in length. The predicted extracellular and membrane spanning sequences of the two clones are identical, as are the first 96 amino acids of their respective cytoplasmic regions. Different COOH termini of 34 or 87 residues are predicted for the two cDNAs, due apparently to alternate splicing. The receptor with the longer cytoplasmic domain is the closest human homologue of the murine G-CSF receptor recently described by Fukunaga et al. (Fukunaga, R., E. Ishizaka-Ikeda, Y. Seto, and S. Nagata. 1990. *Cell.* 61:341). A hybridization probe derived from the placental G-CSF receptor cDNA detects a ~3-kb transcript in RNAs isolated from placenta and a number of lymphoid and myeloid cells. The extracellular region of the G-CSF receptors is composed of four distinct types of structural domains, previously recognized in other cell surface proteins. In addition to the two domains of the HP receptor family-defining region (Patthy, L. 1990. *Cell.* 61:13) it incorporates one NH2-terminal Ig-like domain, and three additional repeats of fibronectin type III-like domains. The presence of both an NH2-terminal Ig-like domain and multiple membrane-proximal FN3-like domains suggests that the G-CSF receptor may be derived from an ancestral NCAM-like molecule and that the G-CSF receptor may function in some adhesion or recognition events at the cell surface in addition to the binding of G-CSF.

G ranulocyte colony-stimulating factor (G-CSF)[1] is a glycoprotein secreted by macrophages, fibroblasts, and endothelial cells originally identified by its ability to stimulate the survival, proliferation, and differentiation in vitro of predominantly neutrophilic granulocytes from bone marrow progenitors (1). The capacity of G-CSF to regulate in vivo granulopoiesis is supported by animal and clinical studies, which demonstrated a reversible rise in circulating neutrophil levels in response to administered recombinant G-CSF (2). G-CSF has pleiotropic effects on mature neutrophils, enhancing their survival and stimulating functional activation, including induction of neutrophil alkaline phosphatase (3) and high affinity IgA Fc receptors (4), priming for respiratory burst (5, 6), and increased chemotaxis (7). G-CSF effects have also been observed on hematopoietic cells that are not committed to the granulocyte lineage, for example, stimulation of the proliferation or monocytic differentiation in vitro of some myeloid leukemic cells (8–10) and, in synergy with other colony-stimulating factors, the proliferation in vitro of some multipotential hematopoietic precursors (11–13). A recent clinical study implicates G-CSF in the regulation of human erythropoiesis (14). G-CSF may also affect nonhematopoietic cells, since it appears to stimulate the proliferation and migration of endothelial cells (15), and the growth of cell lines derived from colon adenocarcinomas (16) and small cell lung carcinomas (17).

These diverse effects of G-CSF are mediated by the interaction of G-CSF and specific cell surface receptors. Initial binding studies with native murine G-CSF detected low numbers of receptors on responsive murine cell lines and human bone marrow cells of the neutrophilic lineage (18–20). Affinity crosslinking studies suggested a murine receptor mo-

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lecular weight (M_r) of ~150,000 (21). G-CSF muteins with improved stability have been shown to bind a single class of sites (K_d = 100–500 pM) on circulating neutrophils (22), U937 cells (23), placental membranes, and trophoblasts (24). Similar affinities have been measured for the binding of native G-CSF to a single class of sites on myeloid leukemic and small cell lung carcinoma cell lines (17). Although affinity crosslinking experiments detected human receptors of M_r ~150,000 on neutrophils, an additional crosslink to a protein of M_r ~120,000 could be detected on placental membranes, suggesting a more complex receptor composition (22, 24).

Here we report the isolation from a placental library of two cDNA clones that encode high affinity receptors for G-CSF when expressed in COS-7 cells. The two clones encode identical extracellular and transmembrane sequences, but differ in the COOH-terminal portion of their cytoplasmic regions, due to what appears to be alternate splicing. The predicted protein sequence of one clone suggests it is the strict human homologue of a recently cloned murine G-CSF receptor cDNA (25).

Materials and Methods

Human G-CSF Preparation. Human G-CSF (26) was expressed in yeast, using the α-factor secretion system (27), as a mutein in which Cys17 was replaced by serine and Arg22, by lysine. These alterations inhibit, respectively, the formation of disulfide-linked oligomers and inappropriate processing through destruction of a KexII recognition site. Alternatively, a fusion polypeptide of the same construct but incorporating a hydrophilic octapeptide at the NH_2 terminus to aid in purification (28) was also expressed. The biological activities of both purified forms were ~2 × 10^7 U/mg determined in a standard proliferation assay using the murine myeloid leukemic cell line DA-1. 1 U corresponds to that amount of G-CSF that gives half-maximal [^{3}H]TdR incorporation.

Radiolabeling of G-CSF. Purified human G-CSF was radiolabeled to a specific activity of 7 × 10^{14} cpm/mmol using a solid-phase chloramine-T analogue. 5 μg of purified G-CSF and 2 μCi $Na^{125}I$ in 150 μl PBS was placed in a 10 × 75 mm glass tube previously coated with 5 μg of Iodogen (Pierce Chemical Co., Rockford, IL) and incubated for 25 min, 4°C. Free and ligand-bound iodine were subsequently separated by gel filtration through a 1 ml column of Biogel P-6 (BioRad Laboratories, Richmond, CA) that had been blocked with BSA. Radiolabeled stocks were stored at 4°C in RPMI-1640 containing 2.5% BSA, 20 mM Hepes buffer, and 0.2% sodium azide, pH 7.2 (binding medium). The specific radioactivities of labeled G-CSF were based on determination of initial protein concentration by amino acid analysis, with correction from control experiments to determine protein recovery after iodination, in which an aliquot of G-CSF was mixed with ^{125}I-G-CSF and the iodination protocol repeated, with omission of $Na^{125}I$.

Cell Lines and Tissue Preparations. HL60, U937, C10, KG-1, HeLa, RAJI, MJ, and RPMI 1788 cell lines were maintained in RPMI 1640 (Gibco Laboratories, Grand Island, NY), 10% FCS, 2 mM glutamine, and 50 μM 2-ME at 37°C in a humidified atmosphere of 5% CO_2 in air. Peripheral blood granulocytes were isolated by sedimentation on discontinuous Ficoll Hypaque (Sigma Chemical Co., St. Louis, MO) gradients followed by red blood cell lysis in ammonium chloride. Placental membranes were isolated as follows. Fresh, full-term human placenta, obtained from Swedish

Hospital Medical Center (Seattle, WA) on ice, were washed six times with ice-cold PBS to extract excess blood, and the tissue, trimmed of amnion and chorion, was cut into small pieces. The pieces were transferred to one volume of Buffer A (30 mM Hepes, pH 7.4, containing 0.25 M sucrose, 1 mM PMSF, 1 μM pepstatin A, 10 μM leupeptin, 2 mM o-phenanthroline, and 0.02 U/ml aprotinin), homogenized for five 1-min intervals with a PT10/35 homogenizer (Brinkman, Westbury, NY) at setting 7 and centrifuged at 6,800 g for 30 min, 4°C. The supernatant was made 0.1 M in sodium chloride and 0.2 mM in magnesium chloride and centrifuged at 42,000 g for 40 min at 4°C. The resulting pellets were washed twice in Buffer A by resuspension and centrifugation as above. The final sedimented membranes were resuspended in 30 ml of Buffer A at a protein concentration of 10–20 mg/ml and stored at −70°C.

Binding Assays and Data Analysis. For equilibrium binding assays with native (placental membrane) G-CSF receptor, serial dilutions of ^{125}I-G-CSF in binding media were incubated with 300 μg membrane (protein) in 10 × 75 mm glass tubes in a total volume of 100 μl for 2 h, 4°C. Control experiments showed equilibration had been reached in this time. Bound ligand was measured by subsequent collection of membranes in the reaction mixture on glass microfiber filters (Whatman, Hillsboro, OR) using a vacuum filtration apparatus. Filters were washed three times with ice-cold PBS/BSA (1 mg/ml) before gamma counting. Nonspecific binding was determined for each data point with a control tube containing a 400-fold molar excess of unlabeled G-CSF. Free radiolabeled ligand for each data point was measured by counting an aliquot of ligand identically incubated in the absence of membranes, after subtraction of the corresponding bound counts. Binding curves were plotted in the Scatchard coordinate system, expressing bound ligand in units of fmole/milligram membrane protein.

For equilibrium binding assays with recombinant G-CSF receptor, COS-7 cells transfected with either the D-7 or 25-1 G-CSF receptor cDNA clone (COS-G-CSFr) were first diluted 10-fold with carrier cells (EL4-3$^+$ murine T cells) to prevent COS cell aggregation. EL4-3$^+$ and untransfected COS cells were both shown to lack receptors for human G-CSF. Serial dilutions of ^{125}I-G-CSF in binding media were incubated with cells (2 × 10^6 total cells/ml) for 2 h at 4°C in a total volume of 150 μl using 96-well microtiter plates. Free and bound ligand were separated by centrifugation of duplicate 60-μl aliquots of the reaction mixture in plastic tubes containing a phthalate oil mixture (29). The tubes were cut, and supernatant (free ligand) and pellets (bound ligand) were gamma counted. Nonspecific binding was determined by inclusion of a 200-fold molar excess of unlabeled G-CSF in the reaction mixture at one ligand dilution; the linearly extrapolated nonspecific binding was subtracted from each data point to generate specific binding. Binding parameters determined on adherent COS-G-CSF receptor cells were similar to those determined in the suspension assay.

Affinity Cross-linking. Adherent COS cells on 10-cm culture dishes transiently expressing the recombinant G-CSF receptor were incubated with ^{125}I-G-CSF (1 nM) in RPMI 1640 for 2 h at 4°C in the presence or absence of unlabeled G-CSF (1 μM). Cells were washed twice in ice-cold PBS and then crosslinked in situ with 0.1 mg/ml bis-(sulfosuccinimidyl) suberate (BS^3, Pierce Chemical Co.) in PBS at 25°C for 30 min. Cells were subsequently washed twice with PBS and then lysed with 0.5 ml of PBS/1% Triton containing protease inhibitors (2 mM PMSF, 10 μM pepstatin A, 10 μM leupeptin, 2 mM o-phenanthroline, 2 mM EGTA, 1.25 mM benzamidine, 0.5 mM EDTA, and 2 μg/ml soybean trypsin inhibitor). Lysates were scraped from plates, microfuged at 12,000 g for 10 min, and supernatants retained. Placental membranes (8 mg pro-

tein/ml) were incubated in 1.5-ml plastic microfuge tubes with 1 mM ^{125}I-G-CSF in a total volume of 100 μl PBS for 2 h at 4°C in the presence or absence of unlabeled G-CSF (1 μM). Membranes were then washed two times with ice cold PBS, resuspended in 100 μl of PBS, and incubated with BS^3 (0.1 mg/ml) for 30 min at 25°C. Membranes were washed twice, then lysed in 150 μl of PBS 1% Triton (with protease inhibitors) for 30 min at 4°C. Insoluble debris was removed by centrifugation for 30 min at 10,000 g, and the supernatant was retained.

SDS-PAGE. Samples, including methyl ^{14}C-labeled molecular weight markers (Bethesda Research Laboratories, Bethesda, MD), were boiled for 30 min in sample buffer (0.06 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-ME) and analyzed on an 8% SDS gel (30). After electrophoresis, gels were fixed in 25% isopropanol, 10% acetic acid, dried, and autoradiographed with Kodak X-Omat AR film at -70°C.

cDNA Library Construction and Screening. Total cell RNA was isolated from whole fresh placental tissue as described below and polyadenylated RNA prepared by chromatography on oligo(dT)-cellulose as described (31). Double-stranded, oligo(dT)-primed cDNA was prepared with a commercial kit (Amersham Corp., Arlington Heights, IL). The resulting cDNA was size fractionated by chromatography on Sephacryl S-1000 (Pharmacia Fine Chemicals, Piscataway, NJ) in 0.5 M sodium acetate. The excluded cDNA was cloned into the BglII site of the mammalian expression vector, pDC302 (32) by an adaptor method similar to that described by Haymerle et al. (33). Briefly, noncomplementary oligonucleotides of the sequence 5'-GATCTTGGAACGAGACGACCTGCT and 5'-AGCAGGTCGTCTCGTTCCAA synthesized on a DNA synthesizer (model 380A; Applied Biosystems, Foster City, CA) were annealed and ligated in separate reactions to either cDNA or BglII cut vector. Nonligated oligonucleotides were separated from cDNA or vector by chromatography over Sepharose CL-2B (Pharmacia Fine Chemicals) at 65°C in 10 mM Tris (pH 8.0), 0.1 mM EDTA. 5 ng of adaptored vector was ligated to adaptored cDNA in 10-μl reactions containing 50 mM sodium chloride, 50 mM Tris-HCl (pH 7.5), 10 mM magnesium chloride, 1 mM spermidine, 0.5 mM ATP, 0.1 U/μl T4 polynucleotide kinase and 0.4 U/μl T4 DNA ligase for 30 min at 37°C. Reactions were then desalted by drop dialysis on VSWP 013 filters (Millipore Corp., Bedford, MA) against distilled water for 40 min immediately before electroporation into *Escherichia coli* strain DH5 α as described (34). Transformants were obtained with an average cDNA insert size of 1.6 kb. Pools of 600 colonies were prepared and DNA minipreps of these were transfected into COS cells as described (35). After 3 d growth in DME/10% FCS, the cells were screened for ^{125}I-G-CSF binding by an in situ autoradiographic plate binding assay (36).

RNA Analysis. Total cellular RNAs were isolated by the guanidinium isothiocyanate–cesium chloride method and electrophoresed through formaldehyde agarose gels as described (31). RNA was transferred to nylon filters (Amersham) by capillary blotting and UV crosslinked using a Stratalinker (Stratagene, La Jolla, CA). Filters were probed with a ^{32}P-labeled antisense RNA prepared by T7 RNA polymerase transcription of a subclone of the D7 cDNA in pBlueSK (Stratagene). High stringency blot hybridization and washing conditions were as previously described (35).
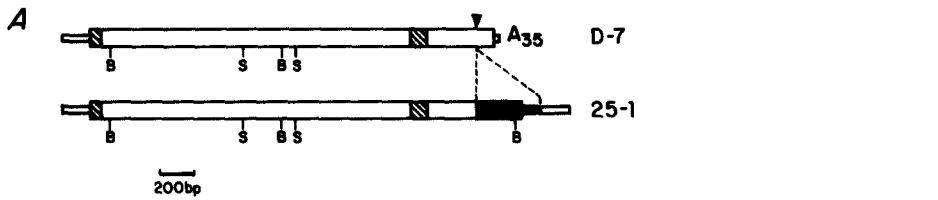
Sequence Analysis. Sequences were aligned using various computer programs (GAP; 37, 38) and the progressive alignment method of Feng and Doolittle (39) as well as by visual inspection. With the exception of the alignment between the human and murine G-CSF receptors, a consensus alignment was generated for all sequences, rather than optimizing the alignment between any given pair of sequences. Alignment scores were generated using the NBRF

program ALIGN using the MD data matrix with a bias of $+6$ and a gap penalty of 6. The prediction of residues involved in β-strands in immunoglobulin domain folding patterns used the turn and secondary structure prediction algorithms of Cohen et al. (40), the hydrophobic moment algorithm of Eisenberg et al. (41) as well as by inspection.

Results

Isolation of Human G-CSF Receptor cDNAs. Quantitative binding studies using radioiodinated G-CSF on a panel of human cell lines demonstrated low level expression of a single class of binding sites (N <1,000/cell, K_d ~1 nM; data not shown). G-CSF receptors with a similar affinity were detected on placental membranes (see below), but at a level of ~200 fmol/mg. Since binding of epidermal growth factor to A431 cell membranes at this level would correspond to a site number of ~10^6/cell (42) we concluded that G-CSF receptors were expressed at unusually high levels in placental tissue. A placental cDNA library was prepared in a mammalian expression vector and DNA from pools of ~600 transformants were transfected into COS cell that were then screened for ^{125}I-G-CSF binding by contact autoradiography (37). A positive clone, D7, was obtained after screening 20 pools and contained a 2.6-kb cDNA insert that was used as a hybridization probe to identify three additional related clones from the same library. Restriction digests and DNA sequencing showed that the cDNA clones fell into two classes: three were of the D7 type and one of a somewhat different form, 25-1, shown in Fig. 1. The 25-1 clone differs from the D7 clones only in its lack of a poly(A) tract and in the presence of a 419-bp internal sequence insert. This insert occurs between nt 2411 and 2412 of the D7 cDNA and appears to be derived from an unspliced intron since it contains splice donor and acceptor consensus sequences at the junctions with the D7 sequence.

DNA sequencing of these clones showed that the first ATG occurs in a context corresponding well to the Kozak consensus sequence (CCA/GCCATG; 43) and initiates a reading frame that terminates after 11 codons. The next potential initiation codon occurs 45 nt downstream, within an inferior Kozak context. This reading frame encodes proteins of 783 and 836 amino acids in the D7 and 25-1 cDNAs, respectively. Hydropathy analysis identified two major hydrophobic regions in the sequence (Fig. 1 D). The first, at the NH$_2$ terminus, is a presumed hydrophobic signal sequence of 24 residues; the second, between residues 604 and 629, is a presumed transmembrane domain that makes a single helical span (Fig. 1 B). Both forms of receptor are thus composed of an extracellular region of 603 amino acids and a transmembrane region of 26 amino acids, but differ in the predicted COOH-terminal portions of their cytoplasmic domains. The protein encoded by cDNA D7 has a cytoplasmic domain of 130 amino acids, while the unspliced intron sequence inserted in clone 25-1 after amino acid 725 predicts a cytoplasmic domain of 183 residues. The COOH-terminal amino acid sequence of D7 appears significantly more hydrophobic than that of 25-1 and contains one less Cys residue (Fig. 1). Both the D7 and 25-1 cytoplasmic sequences have high contents of proline (14.6 and

A D-7 ... 25-1

200bp

B

[Dense nucleotide and deduced amino acid sequence of clone D7 — panel B]

C

[Dense nucleotide and deduced amino acid sequence of the 3' end of clone 25-1 — panel C]

Figure 1. Human G-CSF receptor cDNAs. (*A*) Schematic representation and restriction map of G-CSF receptor cDNA clones D7 and 25-1. Restriction sites are indicated for BamHI (*B*) and SstI (*S*). The solid arrow marks the position in the D7 sequence at which the 25-1–specific insertion occurs. The insert sequence present only in the 25-1 clone is indicated as a filled bar, all other sequence is identical in the two clones. The deduced coding sequences are shown as as wide bars, noncoding sequences as narrow bars. The predicted signal transfer and transmembrane sequences are shown crosshatched. The 35-residue poly(A) tail of the D7 clone is shown as A35. (*B*) The nucleotide and deduced amino acid sequence of clone D7. The signal peptide cleavage site predicted by the probability weight matrix of von Heijne (75) is shown by an open arrow, and the predicted NH2 terminus of the mature protein is designated residue 1. The predicted membrane spanning sequence is indicated by heavy underline and the Trp-Ser motif by a light underline. Cysteine residues are boxed and potential N-linked glycosylation sites are indicated by asterisks. The position of the 25-1 intron insertion point is indicated by a solid arrow. (*C*) The nucleotide and deduced amino acid sequence of the 3' end of clone 25-1. Enumeration is continuous with B. Cysteine residues are boxed. Solid arrows indicate the junctions of the 419-bp insert with sequences common to both 25-1 and D7. (*D*) On facing page. Hydropathicity plot of the D7 and 25-1 receptor sequence according to the method of Kyte and Doolittle (76). The predicted signal and membrane-spanning sequences are indicated by arrows. These sequence data are available from EMBL/Genbank/DDJB under the accession numbers X55720 (clone D7) and X55721 (clone 25-1).

D
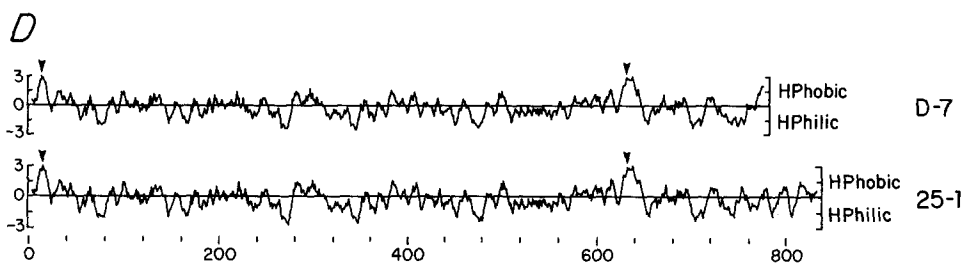
Figure 1 D.

13.1%, respectively) and serine (13 and 10.4%) a property noted for the cytoplasmic domains of many members of the hematopoietin (HP) receptor family (44). Neither the D7 nor 25-1 cytoplasmic regions contain sequences indicative of tyrosine kinase activity (45) but Ser760 of the 25-1 receptor represents a potential protein kinase C phosphorylation site (46). The predicted sequences of both G-CSF receptors con-

tain nine potential N-linked glycosylation sites (Fig. 1 *B*), all but one in the proposed extracellular region. A murine G-CSF receptor cDNA isolated from myeloid leukemia cell library (25) encodes a predicted mature protein of 812 amino acids, identical in length to that of 25-1, and its COOH-terminal sequence is homologous to that of 25-1 but to D7 only up to the position of the proposed splice site. The 25-1
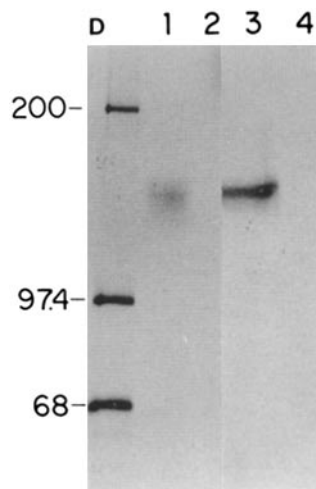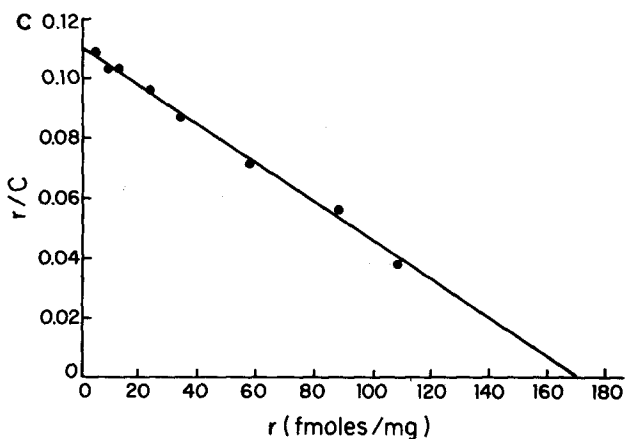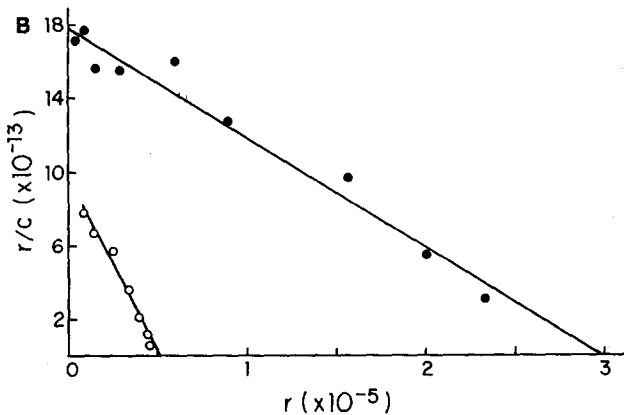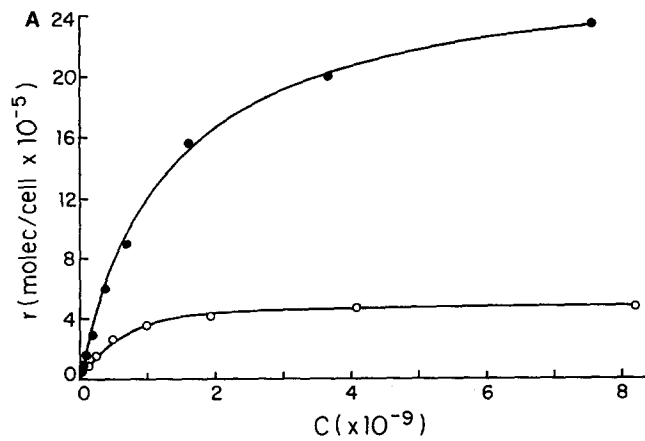
Figure 2. Comparison of recombinant and native G-CSF receptors. Binding assays described in Material and Methods. (*A*) Specific in situ binding of ^{125}I-G-CSF to COS cell–expressed recombinant G-CSF receptors 25-1 (O) and D7 (●) for 2 h at 4°C. Data plotted as bound (*r*) vs. free ligand (*C*). (*B*) Replotting *A* in Scatchard coordinate system, linear least squares analysis (30) yielded similar ligand affinities for D7 (K_d = 1.6 nM; n = 3.0 × 10⁵) and 25-1 (K_d = 0.53 nM; n = 5.2 × 10⁴); but ∿6× higher expression levels of the former. (*C*) Specific binding of ^{125}I-G-CSF to native (placental membrane) G-CSF receptors. Linear least squares fit to Scatchard plot give a K_d of

0.70 nM, similar to both recombinant receptors. (*D*) Affinity cross-linking of native (placental; lanes *1* and *2*) and recombinant (COS-expressed; lanes *3* and *4*) in the absence (*1* and *3*) or presence (lanes *2* and *4*) of a 200-fold molar excess of unlabeled G-CSF. The calculated receptor M_r, after subtraction of ligand molecular weight, is ∿150,000 in both cases. Cross-linking conditions described in Materials and Methods.

cDNA appears, therefore, to encode the strict human homologue of the murine receptor. At the protein level, these homologues are highly conserved in sequence (62% identity).

Comparison of Native and Recombinant G-CSF Receptors.
The ligand-binding and affinity cross-linking characteristics of the recombinant G-CSF receptors encoded by D7 and 25-1, expressed in COS cells, are compared with those of native (placental) receptors in Fig. 2. All three receptors display a single class of binding sites, with equilibrium dissociation constants of 1.6 nM (D7), 0.53 nM (25-1), and 0.67 nM (placental). Expression levels of the D7 clone, however, were approximately sixfold higher than 25-1 (298,000 v. 52,000 sites/cell). Affinity cross-linking studies of COS-expressed D7 and placental G-CSF receptors detected a single subunit in each case with an apparent M_r of ~150,000. As the calculated protein molecular mass of the recombinant receptors are 92 kD (25-1) and 86 kD (D7), the G-CSF receptor is estimated to contain ~35% carbohydrate by weight. Thus both native and recombinant receptors share similar characteristics.

Expression of G-CSF Receptor mRNA. An antisense RNA transcript of the entire D7 sequence was used to probe Northern blots of total cellular RNAs isolated from a variety of sources (Fig. 3). A hybridizing band of ~3 kb was detected in placental RNA samples (lane 4) and RNA isolated from human hematopoietic cells previously reported to express G-CSF receptors (17, 22, 23, 47), including the myelogenous leukemia cell line KG-1 (Fig. 3, lane 7), the promyelocytic cell line HL-60 (lane 1), the premonocytic cell line U937 (lane 5), bone marrow cells (lane 7), and peripheral blood granulocytes (lanes 2, 3), the latter containing particularly high levels, consistent with the prominent G-CSF responsiveness of this cell type. The KG-1 and peripheral blood granulocyte samples both show a minor additional hybridizing species at ~7 kb (lanes 2, 3, and 7), as did placental RNA upon longer exposure (data not shown). This species was not detectable in cytoplasmic placental RNA, suggesting it is a nuclear precursor (data not shown). The observed pattern of expression suggests that one or both of the G-CSF receptors cloned from the placental library also encode the receptors used by hematopoietic cells. Unexpectedly, we have also detected low levels of these transcripts in the HTLV-1-transformed T cell lines C-10 and MJ (lanes 6, 10) and the B lymphoblastoid cell lines RAJI and RPMI 1788 (lanes 9, 11), cells that are of lymphoid not myeloid lineage. The significance of this observation is unclear and requires further investigation. Under the stringent hybridization conditions used, no specific hybridization was seen with total RNAs isolated from HeLa cells (lane 8), dermal fibroblasts, brain, or COS cells (data not shown).

Domain Structure and Sequence Homology of the G-CSF Receptor. A computer search of several databases queried with the entire G-CSF receptor sequence revealed significant homology of the G-CSF receptor extracellular region to three distinct groups of sequences: (a) members of the Ig superfamily (48), (b) the extracellular regions of all members of the recently identified hematopoietin (HP) receptor family
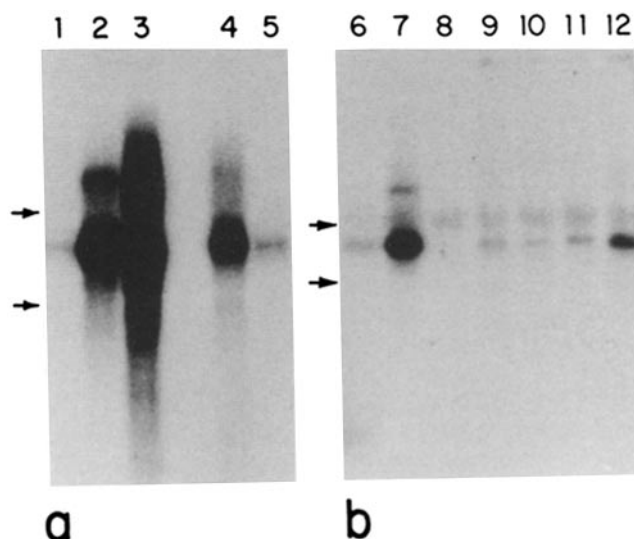
Figure 3. G-CSF receptor RNA analysis. Northern blots of human total cellular RNAs electrophoresed on formaldehyde agarose gels, hybridized with antisense D7 probe, and washed as described in Materials and Methods. The positions of the 18S and 28S ribosomal RNAs are indicated by arrows. All lanes contain 2.5 μg of total cellular RNA except lane 3, which has 0.5 μg of total RNA to avoid overexposure. The blots were exposed at minus 80° for different times. (A) 2-h exposure of a blot of RNA samples from the following sources: HL-60 (lane 1), peripheral blood granulocytes (lanes 2, 3), placenta (lane 4), U937 (lane 5). (B) 24-h exposure of a blot of RNAs isolated from the following sources: C-10 (lane 6), KG-1 (lane 7), HeLA (lane 8), RAJI (lane 9), MJ (lane 10), RPMI 1788 (lane 11), aspirated pelvic bone marrow (lane 12).

(44, 49–53) and (c) the type III homology units (FN3) of several vertebrate fibronectins (54) and neural cell adhesion molecules (NCAMs) (55, 56). Each of these homologies is localized to discrete regions of the extracellular portion of the G-CSF receptor. The NH₂-terminal 90 residues of the G-CSF receptor show statistically significant alignment scores (>3 SD) with several members of the Ig superfamily (data not shown). Fig. 4 A shows a consensus alignment of these NH₂-terminal residues with the NH₂-terminal sequences from the murine G-CSF receptor (25), human IL-6 receptor (57), and light chain domains of Ig NEW (58, 59). The G-CSF receptor contains an invariant Trp, two appropriately spaced Cys, and other residues in conserved positions that define Ig-like domains (48). Cys23 and Cys79 of the G-CSF receptor, therefore, are likely to form a disulfide loop characteristic of the Ig fold (60). This structural motif is further supported by the presence of sequences predicted to form the β strands characteristic of Ig domains (underlined Fig. 4 A).

The ~200 residue segment of the G-CSF receptor following the Ig-like moiety (Arg94 to Pro 299) shows strong homology to extracellular regions of all members of the HP receptor family (ALIGN scores Fig. 4 D) (44) and is shown aligned with the corresponding segments of the murine receptor and other selected family members in Fig. 4, B and C. This region contains the sequence features that define the

A

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            A                        B                          C              C'            D                    E            F            G
huGR-Ig  ..ECGHISVSAPIVHLGDP.....ITASCIIKQNCSHLDPEPCIIVRLGAE.LQ.EGGRQQ...HISDGTQES...........IITIPHLNHTQ.AFLSCCLNW..GNSLQIILDQVEL
muGR-Ig  LESCGHIEISPPVVRLGDP......VLASCTISPNCSKLDQQAKIIVRLQDEPIQ.EGDRQH....HIPDGTQES...........LITILPHLNYTQ.AFLECLVPW..EDSVQLLDQAEL
il6R-Ig  IAPRRCPAQEVARGVLTSLP..GDSVIITOPGVEPEDN....ATVHIVLRKP...AAGSHPS...FWAGMGRR...........IIIRSVQLHISGNMSCYRAG..RPAGTVHLLVDV
hNEW-VL  QSVLTQP.PSVSGAP.......GQRVIIISCTGSSSNIGAG..NHVKAY.QQLPGTAEKLLIFHNNARFSVSKSG.........SSATIAITGLQAEIEADYYCQSYDR.LRVFGGGTKLTVL
hNEW-CL  QPKAA...PSVTLFPPSSEELQANKAIIVCLISDFYPGA...VTVAWKADSS...HVKA.......GVETTTPSKQSNNKYAASSYISLTPEQWKSHRSMSCQVTHEGSTVEKTVAPTECS
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B

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huIL6R   HLLVDVPPEEPQ.LSCFRKPSLSNVVCEWGPRSTPSLTTKAVLLVRKF..QNSPAEDFQEHCQYSQ......ESQKFSCQLAVPEGDSSFYIVSMCVASSVGSKFSKTQTFQGCG
muIL3R1  EVTEEEETVPLKTLECYND.YTNRIICSWADTEDAQGLINMTLLYH......QLDKIQSVSCELSEKLMWSECPSSHRCVPRRCVIPYTRFSNGDNDYYSFQPDRDLGIQLMVPLAQ
muIL3R2  .DSQPGDKAQPQNLQCFFDG.IQSLHCSWEVWTQTTGSVSFGLFYRPSP......AAPEEKCSPVVKEPQASVYTRYRCSLPVPEPSAHSQYTVSVKHLEQGKFIMSYY
muEpoR   SKAALLASRGSEELLCFTQR.LEDLVCTWEEAASSGMDFNYSFSYQLEG......ESRKSCSLHQAPTVRGS.VRFWCSLPTADTSSFVPLELQVTEASGSPRYHRIIHINE
raPrlR   ....QSPPGKPEIHKCRSPDK.ETFTCWWNPGTDGGLPTNYSLTYSKEG......EKTTYECPDYK......TSGPNSCFFSKQYTSIWKIYIITVNATNQMGSSSSDPLYVDVTY

huGR     ..RAGYPPAIPHNLSCLMNLTTSSLICWEPGPETHLPTSFTLKSFKSRGNCQTQGDSILDCVPKD.......GQSHCCIPRKHLLLYQNMGIWVQAENALGTSMSPQLCLDPMD
muGR     ..HAGYPPASPSNLSCLMHLTTNSLVCQWEPGPETHLPTSFIILKSFRSRADCQYQGDTIPLCVAKK.......RQNNCSIPRKNLLLYQYMAIWVQAENMLGSSESPKLCLDPMD
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C

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huIL6R    ....ILQPDPPANITVTAVA.....RNPRWLSVTWQDPHS.....WNSSFYRLRFELRYRAERSKTFTTWMVKDLQHH......CVIHDAWSGLRHVVQLRAQE...EFGQGEWSEWSPEAMG
muIL3R1   ..HVQPPPPKDIHISPS........GDHFLLEWSVSLGDSQVSWLSS.KDIEFEVAYKRLQDSWEDASSLHTSNFQ....VNLEPKLFLPNSIYAARVRTRLSAGSSLSGRPSRWSPEVHW
muIL3R2   .HIQMEPPILNQTKNR.........DSYSLHWETQKIPK......YIDHTEQVQYKKKSESWKDSKTENLGRVN.....SMDLPQILEPDTSYCARVRVKPI..SDYDGIWSEWSNEYTW
muEpoR    ..VVLLDAPAGLLARR......AEEGSHVVLRWLPPPGAPM......TTHIRYEVDVSAGNRAGGTQRVEVLEG.....RTECVLSNLRGGTHYTFAVRARMAP..ESFGFWSAWSEPASLL
raPrlR    ..IVEPEPPRNLTLE...VKQLKDKKTYLWVKWSPPTITDVKTGWF...TMEYEIRLKPEEAEEWEIHFTGHQ.......TQFKVFDLYPGQKYLVQTRCK.....PDHGYWSRWSQESSVEM

huGR-1    ....VVKLEPPMLRTMDPSPEAA.PPQAGCLQLCWEPWQPGLHINQKCELRHKPQRG.......EASWALVGPLPLEAL....QYELCGILPATAYTLQIRCIRW...PLPGHWSDWSPSLELRT
muGR-1    ....VVKLEPPMLQALDIGPDVV.SHQPGCLWLSWKPWKPSEYMEQECELRYQPQLK.......GANWTLVFHLPSSKD....QFELCGILHQAPVYTLQMRCIRS...SLPGFWSPWSPGLQLRP

huGR-2    ......TERAPTVRLDTWW..RQRQLDPRTVQLFWKPVPLEEDSGRI......QGYVVSWRPSGQAGAIL.PLCNTTELSCTFHLPSEAQEVALVAYNSAGTSRPTPVVFSES
muGR-2    .......MKAPTIRLDTWCQKQLDPGTVSVQLFWKPTPLQEDSGQI......QGYLLSWNSPDHQGQDI.HLCNTTQLSCIFLLPSEAQNVTLVAYNKAGTSSPTTVVFLEN
huGR-3    .........RGPALTR.....LHAMARDPHSLWVGWEPPNPWP........QGYVIEWGLGPSASNSNKTWRMEQNGRATGFLLKENIRPFQLYEIIVTPLYQDTMGPSQHVYAYSQE
muGR-3    .........EGPAVTG.....LHAMAQDLNTIWVDWEAPSLLP........QGYLIEWEMSSPYNNSYLSWMIEPNGNITGILLKDNINPFQLYRITVAPLYPGIVGPPVNVYTFAGE
huGR-4    .........MAPSHAPE....LHLKHIGKTWAQLEWVPEPPELGKSP.....LTHYTIFWTNAQNQSFSAIL......NASSRGFVLHG.IEPASLYHIHLMAASQAGATNSTVLTLMTL
muGR-4    .........RAPPHAPA....LHLKHVGTTWAQLEWVPEAPRLGMIP.....LTHYTIFWADAGDHSFSVTL......NISLHDFVLKH.IEPASLYHYLMATSRAGTNSTGLTLRTL

muL1-1    ....VVGSPGPVPHLELSDRHLLKQ...SQVHLSWSPAEDHNSPI.......EKYDIEFEDKEMAPEKWFSLGKV...PGNQTSTTLK.ISPYVHYTFRVTAINKYGPGEPSPVS
muL1-2    ...VVTPEAAPEKNPVDVRGEGNETNN...MVITWKPLRWMDWN.....APQIQYRVQWRPQGKQETWRKQTVSD......PFLVVSN.TSTFVEYEIKVQAVNNQGKGPEPQVTIGY
muL1-3    ...SGEDYPQVSPELEDITIFN...SSTVLVRWRPVDLAQVK.....GHLKGYNVTYWWKGSQRKHSKRHIHKSHIVVPANTTSAI.ISGLREYSSYHVEVQAFNGRGL
muL1-4    ...ASEWTFSTPEGVPGHPEALHLECQSDTSLLLHWQPPLSHN......GVLTGYLLSYHPVEGESKEQLFFNL..SDPELRTHNLTN.LNPDLQYRFQLQATTQQGGPG
muL1-5    ...AIVREGGTMALFGKPDFGNISATAGENYSVVSWVPRKGQC.........NFREHIIFKALPEGKVSPDHQPQPQYVSYNQSSYTQWNLQPDTKYEIHLIKEKVLLHHLDVK

drNGL-2   .....TQPDVEFKNPDNVVGQGTEPNN...LVISWTPMPEIEHN......APNFHYYVSWKRDIPAAAWENNNIF....DWRQNNIVIADQPTFVKYLIKVVAINDRGESNVAAEEVVGYSGEDRPL

huFN-9    .....AVPPPTDLRFTNIGP.......DTMRVTWAPPPSI.........DLTNFLVRYSPVKNEEDVAELSI....SPSDNAVVLTN.ILPGTEYVVSVSSVYEQHESTPLRGRQKT
huFN-13   ......NIDRPKGLAFTDVDVD.......SIKIAWESPQGQ..........VSRYRVTYSSPEDGIHELFPAPD....GEEDTAELQG.LRPGSEYTVSVVALHDDMESQPLIGTQST
huFN-14   ......AIPAPTDLKFTQVTP.......TSLSAQWTPPNVQ.........LTGYRVRVTPKEKTGPMKEIN....LAPDSSSVVVSG.LMVATKYEVSVYALKDTLTSRPAQGVVTTL
huFN-15   ......ENVSPPRRARVTDATE......TTITISWRTKTET.........ITGFQVDAVPANGQTPIQ......RTIKPDVRSYTITG.LQPGTDYKIYLYTLNDNARSSPVVIDAST
huFN-16   ......AIDAPSNIRFLATTPN.......SLLVSWQPPRAR..........ITGYIIKYEKPGSPPRE....VVPRPRPGVTEATITG.IEPGTEYTIYVIALKNNQKSEPLIGRKK
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Align Scores

Sequence	hu GCSFR	hu IL7R	mu IL7R	hu IL4R	mu IL4R	hu IL2Rβ	hu IL6R	mu IL3R1	mu IL3R2	hu GMFR	mu EPOR	ra PRLR	rb PRLR	hu GRHR	rb GRHR
huGCSFR	-	5.8	3.9	4.9	5.3	5.5	5.6	5.7	6.2	6.0	6.2	10.7	11.4	5.6	6.3
huIL-7R	25.1	-	33.8	5.6	5.9	3.8	4.8	3.1	6.7	2.4	5.6	6.0	8.2	4.3	3.9
muIL-7R	22.9	67.2	-	5.6	4.1	4.8	4.6	4.0	5.7	3.1	6.9	5.5	7.1	3.6	4.5
huIL-4R	21.6	17.2	19.3	-	26.9	5.4	8.2	4.9	8.1	5.7	8.4	3.8	5.7	3.1	3.2
muIL-4R	20.7	17.0	19.1	50.8	-	4.7	6.1	4.9	7.3	4.8	5.7	4.6	5.5	1.7	1.8
huIL-2Rβ	22.0	18.7	21.1	18.7	21.2	-	5.4	3.4	8.0	7.3	7.3	4.8	4.3	4.4	2.6
huIL-6R	18.4	19.5	19.1	23.3	21.7	21.5	-	4.5	5.8	6.6	7.0	8.7	9.3	5.5	6.5
muIL-3R1	21.1	20.1	19.3	22.7	22.7	18.3	22.0	-	7.6	5.7	4.3	6.2	5.4	5.3	5.0
muIL-3R2	20.4	20.1	22.0	24.6	24.3	23.5	23.1	21.9	-	8.4	9.9	7.6	6.2	5.2	4.5
huGMCSFR	17.3	17.9	18.2	18.4	18.6	18.5	17.0	18.9	20.3	-	9.7	7.1	6.1	5.9	5.3
muEPOR	18.6	18.7	20.9	20.5	17.1	19.9	20.4	15.2	22.0	19.6	-	9.4	9.9	6.2	6.8
raPRLR	28.2	19.6	20.6	17.5	21.8	19.0	23.7	19.3	19.5	21.9	21.6	-	47.0	13.3	13.3
rbPRLR	27.4	25.5	20.6	21.1	20.8	21.9	25.8	18.3	16.5	20.9	23.7	76.9	-	14.4	14.5
huGRHR	18.1	22.4	18.7	20.3	20.0	19.5	20.2	22.4	23.8	15.1	18.0	33.3	35.4	-	51.0
rbGRHR	20.6	22.6	20.6	19.8	20.3	18.0	21.2	21.5	20.7	17.9	24.2	34.4	35.4	86.3	-

D

Percent Identity

Figure 4. Homology domains of the G-CSF receptor. (*A*) Alignment of the human and murine G-CSF receptor NH$_2$-terminal Ig-like domain with the human IL-6 receptor Ig-like domain and the sequences from the light chains of IgNEW (58, 59). Boldface type indicates residues conserved in Ig domains. Underlined residues indicate the actual residues involved in the β-strands of the Ig domain folding pattern in the case of NEW and predicted residues comprising the same strands for the receptor sequences. (*B*) Alignment of the human and murine G-CSF receptor HP receptor family-defining region cysteine-rich domains (62) with sequences from the same region of the receptors for IL-6, IL-3, erythropoietin, and prolactin (53, 57, 77, 78). The conserved Cys and Trp residues of the HP receptor family (44, 49–53) are indicated by asterisks and shading. Cys residues are in boldface type. (*C*) Alignment of the four fibronectin type III (FN3) domains of the human and murine G-CSF receptor extracellular region with the FN3-like domains of the HP receptor family defining regions (62) of the receptors for IL-6, IL-3, erythropoietin, and prolactin and with the five FN3 repeats of murine L1 NCAM (55) the second FN3 repeat of Drosophila neuroglian (63) and four type III repeats of human fibronectin (64). Asterisks and shading indicate the three residues conserved in all 16 repeats of human fibronectin. Shading indicates other residues conserved in some FN3 domains and the WSXWS motifs. Cys and Trp residues are in boldface type. (*D*) Scores generated by the NBRF ALIGN program (38) for the indicated sequences.

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HP receptor family; notably a Trp and four Cys residues (boxes, asterisks: Fig. 4 B) conforming to a conserved pattern (49–53) found in nearly all HP receptors (44) and a COOH-terminal WSXWS motif (50–53) which has proven to be a hallmark of the HP receptor family (44). The four Cys residues form two successive disulfide loops in the growth hormone receptor (61), and it is likely that a similar pairing occurs in the G-CSF receptor between Cys107 and 118, and Cys153 and 162. A recent sequence analysis of the HP receptor family-defining region (62) has suggested it can be resolved into two distinct elements each ~100 residues in length: one NH₂-terminal "cysteine-rich" region and a COOH-terminal "cysteine-poor" region, the latter homologous to fibronectin type III repeats. Although in the G-CSF receptor these two regions do not differ significantly in number of Cys residues (NH₂-terminal, 6; COOH-terminal, 5), the last 100 residues of the region do contain the sequence features characteristic of FN3 repeats, as shown by alignment with examples of FN3 repeats from human fibronectin (54) and two neural cell adhesion molecules, murine L1 (55) and Drosophila neuroglian (63) in Fig. 4 C. The FN3-like character of the region is demonstrated by the presence of three residues, Trp229, Leu274, and Tyr279 (asterisks), in the pattern which is the sole sequence feature absolutely conserved in all of the type III repeats of fibronectin (54, 64). Although this region of the G-CSF receptor exceeds the low Trp and Cys content characteristic of FN3 repeats (54) its designation as an FN3-like domain is further supported by statistically significant ALIGN scores when compared with 14 of the 16 type III repeats of human fibronectin (data not shown).

The ~300 residues of the G-CSF receptor bordered by the WSXWS motif and the transmembrane region also show significant homology to FN3 repeats of several vertebrate fibronectins and NCAMs, suggesting this region consists of three additional repeats of this element. These proposed FN3-like domains are shown aligned with the corresponding segments of the murine receptor and the examples of FN3 repeats in Fig. 4 C. These three FN3-like domains of the G-CSF receptor contain the conserved Trp and Tyr residues, described above, but only the last domain contains the hallmark Leu, a residue only partially conserved in NCAM FN3 repeats. These domains of the G-CSF receptor also contain a pair of aromatic residues common to the FN3-like domains of many NCAMs and fibronectins (boxed). The four proposed FN3-like domains of the G-CSF receptor each contain at least three Trp residues, and in this respect resemble the FN3 repeats of NCAMs rather than those of fibronectin. The proposed second, third, and fourth FN3 domains of the G-CSF receptor gave significant ALIGN scores respectively to 4, 13, and 15, of the 16 type III repeats of human fibronectin (data not shown).

Discussion

Here we report the isolation and characterization of two distinct types of G-CSF receptor cDNAs from a human placental library. The equilibrium ligand binding and cross-linking characteristics of the recombinant receptors are similar to those of native receptors on placental membranes (Fig. 2). The D7 and 25-1 cDNAs are predicted to encode integral membrane glycoproteins, 759 and 812 amino acids in mature length, respectively. The predicted molecular masses of these receptors, 86 and 92 kD, are substantially less than the ~150 kD inferred by affinity crosslinking, suggesting that some or all of the nine potential N-linked glycosylation sites contain carbohydrate. The two receptors share identical extracellular (603 aa) and transmembrane (26 aa) regions, as well as the first 96 residues in their cytoplasmic regions, but have alternate COOH-terminal sequences of 34 residues (D7) and 87 residues (25-1). The nucleotide sequences of the two cDNAs indicate that they are probably derived from alternatively processed transcripts of the same gene, since they differ only with respect to a 419-bp insert in the 25-1 cDNA that appears to be an unspliced intron. A recently reported murine G-CSF receptor cDNA (25) encodes a protein that shows strong homology to both placental G-CSF receptors up to the splice point in the cytoplasmic domain, after which the homology continues only in the 25-1 clone, indicating it is the strict human homologue of the reported murine receptor. It is unclear if the reported murine G-CSF receptor cDNA sequence has the splicing potential to encode an alternate COOH-terminus. Inspection of the murine sequence reveals a very similar (murine, CAG GTCCTC; human, CAG GTCCTT) potential splice donor sequence located within the same DQ/VLY peptide sequence as the human 25-1 cDNA. However, while there are potential splice acceptor sites in the murine sequence, translation of the sequences downstream in all three reading frames reveal no significant homology to the D7 type COOH terminus. Thus, generation of a D7-type cytoplasmic terminus in murine G-CSF receptors might be possible if an alternative splice acceptor site exists in 3' sequences of the murine gene.

Differential splicing results in the tissue-specific expression of transcripts encoding alternate cytoplasmic domains for at least two other cell surface proteins, rat liver prolactin (PRL) receptor (65) and chicken NCAM (66). The tissue specificity of expression of the potential G-CSF receptor isoforms remains to be determined at both the mRNA and protein level, but a preliminary analysis with specific oligonucleotide probes suggests human granulocytes express predominantly transcripts of the 25-1 type (data not shown). This raises the possibility that the D7 receptor is specifically expressed at higher levels in nonhematopoietic cells such as placenta, and suggests that the alternate cytoplasmic domains may confer functional differences to the two receptors. It has been proposed that the smaller form of the PRL receptor functions in ligand transport across epithelial barriers in liver rather than in signal transduction (65). By analogy the D7 isoform of the G-CSF receptor may serve to transport or sequester G-CSF in placental tissues. Alternatively, the two isoforms may differ in signal transduction properties, reflecting in part, the diverse biological effects of G-CSF. While the signal transduction mechanism of the G-CSF receptor is unclear, it is interesting that the 25-1 receptor, unlike D7, does contain one potential C kinase phosphorylation site (46). Signal transduction may also be effected through a distinct subunit with which the ligand-

G-CSF receptor complex interacts, as is found in the gp130–IL-6 receptor system (67). Conceivably, this subunit may be gp130 itself, and it is interesting, in this regard, that IL-6 and G-CSF show significant sequence homology (68), and their receptors share a similar domain composition (see below). Both G-CSF receptor cytoplasmic domains contain a high proportion of Pro and Ser residues, like those of many other HP receptor family members (44), the significance of which remains to be elucidated. The cytoplasmic sequence of these receptors may influence stability, cellular localization, or association with other membrane proteins.

The extracellular region of the G-CSF receptor consists of three distinct regions of homology to other cell surface proteins: (a) a ~90 residue NH2-terminal Ig-like region, (b) a ~200 residue HP receptor superfamily-defining region, and (c) ~300 residues of three tandem FN3-like repeats. Consistent with the proposal of Patthy (62), the COOH-terminal 100 residue segment of the HP receptor family-defining region of the G-CSF receptor appears to be an FN3-like domain, albeit one with an elevated Trp and Cys content that is unique among FN3 repeats. Since many protein domains are ~100 residues in length, it is likely that the NH2-terminal "cysteine-rich" or double-loop region of the HP receptor-defining region is, like the WSXWS-containing FN3 element, a discrete structural domain. Thus, the structure of the G-CSF receptor extracellular region can be resolved into the 6 domains of ~100 residues each shown schematically in Fig. 5. By this analysis, the G-CSF receptor appears to be a mosaic of four types of domains, two found associated only in HP receptors (double-loop and WSXWS-FN3) and two found together in NCAMs (NH2-terminal Ig and membrane-proximal FN3s). Given the likelihood that the Ig superfamily molecules of the immune system arose from NCAM-like ancestors (48), it is tempting to speculate that the G-CSF receptor retains the domain structure of an early intermediate in the evolution of the HP receptor superfamily from these same ancestors. Successive deletion of the terminal extracellular domains of a G-CSF receptor-like molecule could thus yield all known HP receptor domain structures; deletion of the three FN3 domains yields an IL-6 receptor-like structure, and further deletion of the Ig domain leads to the core domain structure of most HP receptors, which is duplicated in the case of the IL-3 receptor. Key to such a proposal is determination of the origin of the cysteine-rich or double-loop domain, thus far a unique domain feature found only in the HP receptors, but which, like the Ig and FN3 domains, may also have arisen from a domain in some NCAM-like ancestor.

The function of these structural domains in the G-CSF receptor is unclear. The HP receptor family-defining region, which comprises the entire extracellular region of many of
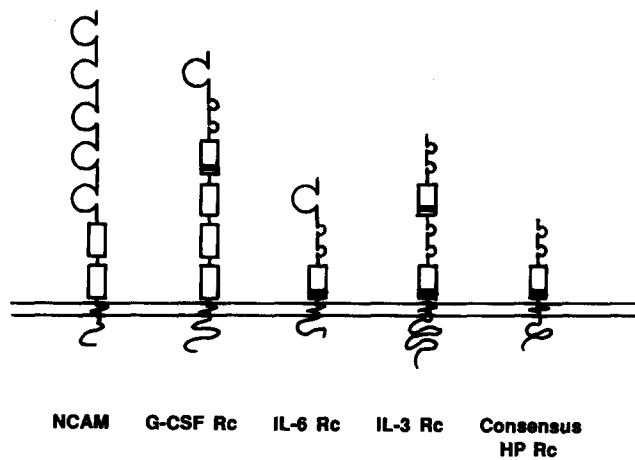

NCAM   G-CSF Rc   IL-6 Rc   IL-3 Rc   Consensus HP Rc

Figure 5. Schematic representation of the nominal structure of hematopoietin receptors and a typical neural cell adhesion molecule. Ig-like domains are shown as large loops. FN3 domains are shown as rectangular boxes. The cysteine-rich, double-loop domains of HP receptor family-defining regions are shown as a pair of small loops, to represent the disulfide pairing determined for human growth hormone receptor (61). The FN-3 like domain of HP receptor family-defining regions is designated as a rectangular box crossed by a heavy bar representing the WSXWS motif. Sequences represented are rat NCAM (reference 79 as drawn in 56), IL-6 receptor (57), and IL-3 receptor (53).

these receptors, presumably contains the ligand binding site of the G-CSF receptor. The Ig and extra FN3-like domains of the G-CSF receptor may confer additional activities to this receptor, similar to the complex functions recognized for these domains in other cell surface molecules. These generally appear to involve participation in some form of cell recognition or adhesion. Both types of domains are capable of homotypic and heterotypic interactions (48, 69) that might lead to receptor self-association or binding to other proteins. Specific functions have been attributed to some type III repeats of fibronectin, including the binding of cells and heparin (70, 71) and heparin binding activity is also a property of at least one neural cell adhesion molecule (72). The potential for simultaneous recognition of heparin and G-CSF by the G-CSF receptor would have interesting functional implications, especially in light of the affinity of the heparin component of extracellular matrix for CSFs (73, 74). The particular response of a cell to G-CSF could thus depend on its adherence or that of G-CSF to the extracellular matrix. Alternatively it could allow G-CSF to mediate or specify interactions between cells and matrix, thus, directing margination or chemotaxis. The cloning of the human G-CSF receptor will provide reagents useful in the further elucidation of the biological roles of G-CSF and may allow development of new diagnostic or therapeutic agents.

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