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Three different mRNAs encoding human granulocyte colony-stimulating factor receptor

(granulopoiesis/cytokine receptor/placenta/polymerase chain reaction/WSXWS motif)

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ABSTRACT **Three cDNAs for the human granulocyte colony-stimulating factor (G-CSF) receptor were isolated from the cDNA libraries of human U937 leukemia cells and placenta by using a murine G-CSF receptor cDNA as the probe. The human G-CSF receptor containing 813 amino acids had a marked homology (62.5%) with its murine counterpart and consisted of extracellular, transmembrane, and cytoplasmic domains. The WSXWS motif found in members of the newly identified growth factor receptor family was also present in the extracellular domain of the human G-CSF receptor. Expression of the cloned cDNA in monkey COS cells gave rise to a protein that could specifically bind G-CSF with a high affinity (K_d, 550 pM). Two other classes of the human G-CSF receptor were also identified, one of which had a deletion of the transmembrane domain and seemed to encode a secreted, soluble receptor. The third class of the G-CSF receptor contained a 27-amino acid insertion in the cytoplasmic domain and was highly expressed in placenta.**

The proliferation and differentiation of granulocytes and macrophages are regulated by a family of colony-stimulating factors (CSFs) (1). Granulocyte colony-stimulating factor (G-CSF) is known to specifically act on cells that are committed to the neutrophilic granulocyte lineage (2, 3). G-CSF is a glycoprotein of M_r 20,000–25,000 that is produced by macrophages stimulated with endotoxin (2, 3), and it plays an important role in granulopoiesis during the inflammatory process. In addition to colony-stimulating activity in a semisolid culture of bone marrow cells, G-CSF has the ability to induce differentiation of some myeloid leukemia cells into granulocytes and monocytes and can also support the proliferation of other myeloid leukemia cells (2, 3).

G-CSF exerts its biological effects through interaction with specific cell-surface receptors (4). Binding studies with radiolabeled G-CSF have indicated that the G-CSF receptor is expressed not only by progenitor and mature neutrophilic granulocytes (5–7) but also by nonhemopoietic cells, such as placental cells (8), endothelial cells (9), and various carcinoma cell lines (10, 11). The number of receptors is around 300–2000 per cell, and G-CSF binds to its receptor with a K_d of 100–500 pM (5–7). We have recently purified the murine G-CSF receptor from the mouse myeloid leukemia cell line NFS-60 and shown that it has a M_r of 100,000–130,000 (7). Furthermore, recent cloning of murine G-CSF receptor cDNA from mouse NFS-60 cells has revealed that it contains 812 amino acids and consists of the extracellular, transmembrane, and cytoplasmic domains (12).

Administration of recombinant G-CSF to patients suffering from neutropenia due to various causes has suggested that G-CSF is beneficial as an adjuvant in chemotherapy and in bone marrow transplantation (13). However, to more effectively apply G-CSF in the clinical setting, it is important to examine the structure and expression of the human G-CSF receptor, since some human myeloid leukemia cells (14) or various carcinoma cells can proliferate in response to G-CSF (10, 11).

In this report, we describe the molecular cloning and characterization of human G-CSF receptor cDNAs.* The human G-CSF receptor was found to be abundantly expressed in the human placenta and analysis of the cDNAs showed that there are at least two variants of the human G-CSF receptor.

MATERIALS AND METHODS

Isolation of Human G-CSF Receptor cDNA Clones. A U937 cDNA library containing cDNAs >2.5 kilobases (kb) was prepared as described (12) with the mammalian expression vector pEF-BOS (15). A total of 3.4×10^4 clones was screened by colony hybridization with a 2.5-kb *Hin*dIII/*Xba* I DNA fragment of murine G-CSF receptor cDNA used as a probe. Hybridization was carried out as described (16), except that the hybridization temperature was lowered to 28°C, and the filter was washed at 37°C in 150 mM NaCl/15 mM sodium citrate, pH 7.0/0.1% $NaDodSO_4$. A human placental cDNA library prepared in λgt11 (Clontech) was screened by plaque-hybridization as described above.

Construction of Expression Plasmids, Labeled G-CSF, and Binding Assays. To construct a full-length cDNA containing the insertion in the cytoplasmic domain, plasmid pHG11 was digested completely with *Nhe* I and partially with *Bst*XI (at nucleotide position 1425). The 1.38-kb *Bst*XI/*Nhe* I fragment was then ligated with the 6.9-kb *Bst*XI/*Nhe* I fragment of pHQ3 to yield pQW11.

Labeling of murine recombinant G-CSF, transfection of COS cells with mammalian expression plasmids containing human G-CSF receptor cDNA, and binding assays of ^{125}I-labeled G-CSF (^{125}I-G-CSF) to COS cells were carried out as described (7, 12).

Analysis of RNA by Polymerase Chain Reaction (PCR). Single-stranded cDNA synthesis and the PCR were carried out as described by Kawasaki (17). In brief, 2 μg of total or poly(A) RNA was used as a template for cDNA synthesis in 50 μl of reaction mixture with 0.5 μg of random hexamer and 80 units of avian myeloblastosis virus reverse transcriptase (17). An aliquot (5 μl) of the reaction mixture was diluted with 100 μl of PCR buffer (17) containing 50 pmol each of the upstream and downstream primers and was placed in a DNA thermal cycler (Perkin–Elmer/Cetus) preheated to 80°C. The reaction was started by adding 2.5 units of *Thermus aquaticus* DNA polymerase (*Taq* polymerase), and the conditions

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Abbreviations: CSF, colony-stimulating factor; G-CSF, granulocyte CSF; GM-CSF, granulocyte–macrophage CSF; PCR, polymerase chain reaction; IL, interleukin.
*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M38025, M38026, and M38027).

for the PCR were 1.5 min at 95°C, 1.5 min at 70°C, and 1.5 min at 72°C for 30 cycles.

General Procedures. Total RNA was prepared from various cell lines and from fresh human full-term placenta as described (18). Northern hybridization was carried out under stringent conditions (16) with the 3-kb *Xho* I DNA fragment of pHQ3.

For DNA sequencing analysis, the DNA fragment was subcloned in the pBluescript SK(+) vector (Stratagene), and a series of deletion plasmids was generated by using exonuclease III and mung bean nuclease (18). The sequencing reaction was performed by the dideoxynucleotide chain-termination method (18) using T7 DNA polymerase, deaza-dGTP, and deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate.

RESULTS

Isolation and Characterization of Human G-CSF Receptor cDNAs. Binding studies using radioactive G-CSF have suggested that human U937 and placental cells express the G-CSF receptor on their surfaces (6, 8). To isolate cDNA clones containing the sequence for the human G-CSF receptor, a U937 cDNA library was screened as described in *Materials and Methods*, and 5 positive clones were identified (pHQ1–pHQ5). A human placental cDNA library (1.5 × 10^6 clones) was also screened by plaque hybridization with murine G-CSF receptor cDNA. More than 100 clones gave positive signals, and the *Eco*RI DNA fragments of 6 positive clones were subcloned in pBluescript SK(+) (pHG series).

As shown in Fig. 1, the restriction enzyme mapping and DNA sequencing analysis of the 9 cDNA clones revealed that they could be divided into three classes. Most of the cDNA clones (6 clones) isolated from the U937 and placental cDNA libraries belonged to the first class, which is represented by the clones pHQ3 and pHG12. The plasmids of pHQ3 and pHG12 contained a large open reading frame that encoded a protein consisting of 836 amino acids (Fig. 2). Hydropathy analysis of the predicted protein sequence indicated that the N-terminal 23 amino acid residues corresponded to the signal sequence, while the following 604, 26, and 183 residues constituted the extracellular, transmembrane, and cytoplasmic domains, respectively. The mature human G-CSF receptor thus consists of 813 amino acids with a calculated M_r of 89,743. This M_r differs by 30,000–60,000 from that reported for the native human G-CSF receptor (8), which may be explained by N-glycosylation on some of the nine potential N-glycosylation sites in the extracellular domain of the receptor. The overall similarity of the human G-CSF receptor to the murine G-CSF receptor was 72% at the nucleotide sequence level and 62.5% at the amino acid sequence level. The WSXWS motif that is conserved in the members of the cytokine receptor family (19–21) can be found in the extracellular domain.

The second class of cDNA is represented by the clone pHQ2, which was derived from the U937 cDNA library. The nucleotide sequence of pHQ2 was identical to that of pHQ3, except that it lacked 88 nucleotides from positions 2034 to 2121, including the region encoding the transmembrane domain (Figs. 1 and 2). This deletion resulted in an altered translation reading frame that encoded an additional 150 amino acids after the deletion point. Thus, the polypeptide encoded by pHQ2 seems to be a secreted, soluble form of the G-CSF receptor, consisting of 748 amino acids with a calculated M_r of 82,707.

As shown in Figs. 1 and 2, two clones (pHG11 and pHG5) isolated from the placental cDNA library were distinguished from other clones by having an 81-base-pair (bp) insertion at nucleotide 2210, and these represent the third class of the G-CSF receptor. The insertion was in the cytoplasmic domain of the G-CSF receptor and it did not change the translational reading frame. The putative polypeptide encoded by this class of cDNA, therefore, is 27 amino acids larger (M_r 2957) than that encoded by the class I G-CSF receptor cDNA.

Binding Characteristics of the Cloned Human G-CSF Receptors. We examined the binding of ^{125}I-G-CSF to the recombinant human G-CSF receptor expressed in COS cells. As shown in Fig. 3, murine ^{125}I-G-CSF bound to COS cells transfected with pHQ3 in a saturating manner. Scatchard analysis of the specific binding revealed a single species of binding site, with an equilibrium dissociation constant of 550 pM and 3.4 × 10^4 receptors per cell. The dissociation constant for the binding of murine G-CSF to human G-CSF receptors expressed in COS cells was similar to that observed for the binding of murine G-CSF to murine G-CSF receptors expressed in COS cells (Fig. 3). Since the native human G-CSF receptor expressed in U937 cells can bind human G-CSF with an equilibrium dissociation constant of 420 pM (6), these results suggested that the polypeptide encoded by the cDNA in pHQ3 is sufficient to express the high-affinity receptor for human G-CSF.

To examine the binding properties of the third class of G-CSF receptors, a full-length cDNA was constructed by using the 5′ half of the pHQ3 cDNA and the 3′ half of the pHG11 cDNA, and it was inserted into the mammalian expression vector pEF-BOS (designated as pQW11). COS cells transfected with pQW11 could bind labeled G-CSF with a K_d similar to that observed for COS cells transfected with pHQ3 (Fig. 3), indicating that the 27-amino acid insertion in the cytoplasmic domain of the receptor has little effect on the binding of G-CSF to the receptor. When COS cells were transfected with the second class of cDNA (pHQ2), a low level of binding of ^{125}I-G-CSF was observed (Fig. 3*A*). As shown in Fig. 3*B*, Scatchard analysis of the specific binding revealed that there were 6 × 10^3 binding sites per cell with a K_d of 440 pM. These results suggested that the receptor encoded by pHQ2, which lacks the transmembrane domain, was probably secreted by the cells. However, we do not understand why a low but significant amount of the membrane-bound receptor was found in COS cells transfected with pHQ2.

Analysis of G-CSF Receptor Transcript. RNA from various human cell lines and from placental tissue was analyzed by Northern hybridization using radiolabeled human G-CSF

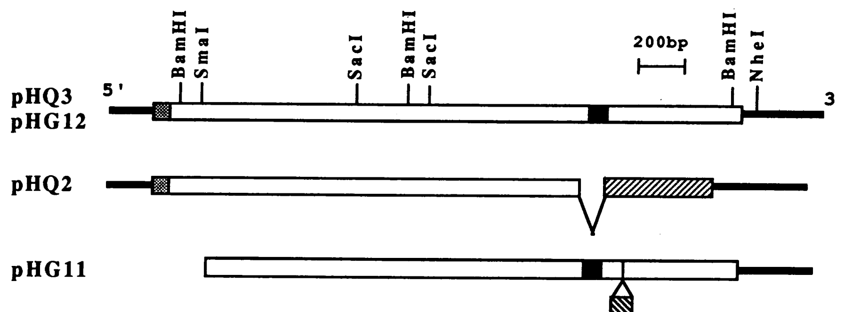

FIG. 1. Diagram and restriction map of the three human G-CSF receptor cDNAs. Boxes represent the open reading frames, of which the shaded and solid regions, respectively, indicate the signal sequence and the transmembrane domain for the G-CSF receptor. The hatched region in pHQ2 indicates where the amino acid sequence differs from those in the other clones as a result of an altered open reading frame. The hatched box in pHG11 shows the inserted sequence of 27 amino acids.

A

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     GAAGCTGGACTGCAGCTGGTTTCAGGAACTTCTCTTGACGAGAAGAGAGACCAAGGAGGCCAAGCAGGGGCTGGGCCAGAGGTGCCAACATGGGGAAACTGAGGCTCGGCTCGGAAAGGTGA

 123 AGTAACTTGTCCAAGATCACAAAGCTGGTGAACATCAAGTTGGTGCT ATG GCA AGG CTG GGA AAC TGC AGC CTG ACT TGG GCT GCC CTG ATC ATC CTG CTG CTC CCC GGA AGT CTG
 -23                                                 Met Ala Arg Leu Gly Asn Cys Ser Leu Thr Trp Ala Ala Leu Ile Ile Leu Leu Leu Pro Gly Ser Leu

 239 GAG GAG TGC GGG CAC ATC AGT GTC TCA GCC CCC ATC GTC CAC CTG GGG GAT CCC ATC ACA GCC TCC TGC ATC ATC AAG CAG AAC TGC AGC CAT CTG GAC CCG GAG
   1 Glu Glu Cys Gly His Ile Ser Val Ser Ala Pro Ile Val His Leu Gly Asp Pro Ile Thr Ala Ser Cys Ile Ile Lys Gln Asn Cys Ser His Leu Asp Pro Glu

 344 CCA CAG ATT CTG TGG AGA CTG GGA GCA GAG CTT CAG CCC GGG GGC AGG CAG CAG CGT CTG TCT GAT GGG ACC CAG GAA TCT ATC ATC ACC CTG CCC CAC CTC AAC
  36 Pro Gln Ile Leu Trp Arg Leu Gly Ala Glu Leu Gln Pro Gly Gly Arg Gln Gln Arg Leu Ser Asp Gly Thr Gln Glu Ser Ile Ile Thr Leu Pro His Leu Asn

 449 CAC ACT CAG GCC TTT CTC TCC TGC TGC CTG AAC TGG GGC AAC AGC CTG CAG ATC CTG GAC CAG GTT GAG CTG CGC GCA GGC TAC CCT CCA GCC ATA CCC CAC AAC
  71 His Thr Gln Ala Phe Leu Ser Cys Cys Leu Asn Trp Gly Asn Ser Leu Gln Ile Leu Asp Gln Val Glu Leu Arg Ala Gly Tyr Pro Pro Ala Ile Pro His Asn

 554 CTC TCC TGC CTC ATG AAC CTC ACA ACC AGC AGC CTC ATC TGC CAG TGG GAG CCA GGA CCT GAG ACC CAC CTA CCC ACC AGC TTC ACT CTG AAG AGT TTC AAG AGC
 106 Leu Ser Cys Leu Met Asn Leu Thr Thr Ser Ser Leu Ile Cys Gln Trp Glu Pro Gly Pro Glu Thr His Leu Pro Thr Ser Phe Thr Leu Lys Ser Phe Lys Ser

 659 CGG GGC AAC TGT CAG ACC CAA GGG GAC TCC ATC CTG GAC TGC GTG CCC AAG GAC GGG CAG AGC CAC TGC TGC ATC CCA CGC AAA CAC CTG CTG TTG TAC CAG AAT
 141 Arg Gly Asn Cys Gln Thr Gln Gly Asp Ser Ile Leu Asp Cys Val Pro Lys Asp Gly Gln Ser His Cys Cys Ile Pro Arg Lys His Leu Leu Leu Tyr Gln Asn

 764 ATG GGC ATC TGG GTG CAG GCA GAG AAT GCG CTG GGG ACC AGC ATG TCC CCA CAA CTG TGT CTT GAT CCC ATG GAT GTT GTG AAA CTG GAG CCC CCC ATG CTG CGG
 176 Met Gly Ile Trp Val Gln Ala Glu Asn Ala Leu Gly Thr Ser Met Ser Pro Gln Leu Cys Leu Asp Pro Met Asp Val Val Lys Leu Glu Pro Pro Met Leu Arg

 869 ACC ATG GAC CCC AGC CCT GAA GCG GCC CCT CCC CAG GCA GGC TGC CTA CAG CTG TGC TGG GAG CCA TGG CAG CCA GGC CTG CAC ATA AAT CAG AAG TGT GAG CTG
 211 Thr Met Asp Pro Ser Pro Glu Ala Ala Pro Pro Gln Ala Gly Cys Leu Gln Leu Cys Trp Glu Pro Trp Gln Pro Gly Leu His Ile Asn Gln Lys Cys Glu Leu

 974 CGC CAC AAG CCG CAG CGT GGA GAA GCC AGC TGG GCA CTG GTG GGC CCC CTC CCC TTG GAG GCC CTT CAG TAT GAG CTC TGC GGG CTC CTC CCA GCC ACG GCC TAC
 246 Arg His Lys Pro Gln Arg Gly Glu Ala Ser Trp Ala Leu Val Gly Pro Leu Pro Leu Glu Ala Leu Gln Tyr Glu Leu Cys Gly Leu Leu Pro Ala Thr Ala Tyr

1079 ACC CTG CAG ATA CGC TGC ATC CGC TGG CCC CTG CCT GGC CAC TGG AGC GAC TGG AGC CCC AGC CTG GAG CTG AGA ACT ACC GAA CGG GCC CCC ACT GTC AGA CTG
 281 Thr Leu Gln Ile Arg Cys Ile Arg Trp Pro Leu Pro Gly His Trp Ser Asp Trp Ser Pro Ser Leu Glu Leu Arg Thr Thr Glu Arg Ala Pro Thr Val Arg Leu

1184 GAC ACA TGG TGG CGG CAG AGG CAG CTG GAC CCC AGG ACA GTG CAG CTG TTC TGG AAG CCA GTG CCC CTG GAG GAA GAC AGC GGA CGG ATC CAA GGT TAT GTG GTT
 316 Asp Thr Trp Trp Arg Gln Arg Gln Leu Asp Pro Arg Thr Val Gln Leu Phe Trp Lys Pro Val Pro Leu Glu Glu Asp Ser Gly Arg Ile Gln Gly Tyr Val Val

1289 TCT TGG AGA CCC TCA GGC CAG GCT GGG GCC ATC CTG CCC CTC TGC AAC ACC ACA GAG CTC AGC TGC ACC TTC CAC CTG CCT TCA GAA GCC CAG GAG GTG GCC CTT
 351 Ser Trp Arg Pro Ser Gly Gln Ala Gly Ala Ile Leu Pro Leu Cys Asn Thr Thr Glu Leu Ser Cys Thr Phe His Leu Pro Ser Glu Ala Gln Glu Val Ala Leu

1394 GTG GCC TAT AAC TCA GCC GGG ACC TCT CGC CCC ACC CCG GTG GTC TTC TCA GAA AGC AGA GGC CCA GCT CTG ACC AGA CTC CAT GCC ATG GCC CGA GAC CCT CAC
 386 Val Ala Tyr Asn Ser Ala Gly Thr Ser Arg Pro Thr Pro Val Val Phe Ser Glu Ser Arg Gly Pro Ala Leu Thr Arg Leu His Ala Met Ala Arg Asp Pro His

1499 AGC CTC TGG GTA GGC TGG GAG CCC CCC AAT CCA TGG CCT CAG GGC TAT GTG ATT GAG TGG GGC CTG GGC CCC CCC AGC GCG AGC AAT AGC AAC AAG ACC TGG AGG
 421 Ser Leu Trp Val Gly Trp Glu Pro Pro Asn Pro Trp Pro Gln Gly Tyr Val Ile Glu Trp Gly Leu Gly Pro Pro Ser Ala Ser Asn Ser Asn Lys Thr Trp Arg

1604 ATG GAA CAG AAT GGG AGA GCC ACG GGG TTT CTG CTG AAG GAG AAC ATC AGG CCC TTT CAG CTC TAT GAG ATC ATC GTG ACT CCC TTG TAC CAG GAC ACC ATG GGA
 456 Met Glu Gln Asn Gly Arg Ala Thr Gly Phe Leu Leu Lys Glu Asn Ile Arg Pro Phe Gln Leu Tyr Glu Ile Ile Val Thr Pro Leu Tyr Gln Asp Thr Met Gly

1709 CCC TCC CAG CAT GTC TAT GCC TAC TCT CAA GAA ATG GCT CCC TCC CAT GCC CCA GAG CTG CAT CTA AAG CAC ATT GGC AAG ACC TGG GCA CAG CTG GAG TGG GTG
 491 Pro Ser Gln His Val Tyr Ala Tyr Ser Gln Glu Met Ala Pro Ser His Ala Pro Glu Leu His Leu Lys His Ile Gly Lys Thr Trp Ala Gln Leu Glu Trp Val

1814 CCT GAG CCC CCT GAG CTG GGG AAG AGC CCC CTT ACC CAC TAC ACC ATC TTC TGG ACC AAC GCT CAG AAC CAG TCC TTC TCC GCC ATC CTG AAT GCC TCC TCC CGT
 526 Pro Glu Pro Pro Glu Leu Gly Lys Ser Pro Leu Thr His Tyr Thr Ile Phe Trp Thr Asn Ala Gln Asn Gln Ser Phe Ser Ala Ile Leu Asn Ala Ser Ser Arg

1919 GGC TTT GTC CTC CAT GGC CTG GAG CCC GCC AGT CTG TAT CAC ATC CAC CTC ATG GCT GCC AGC CAG GCT GGG GCC ACC AAC AGT ACA GTC CTC ACC CTG ATG ACC
 561 Gly Phe Val Leu His Gly Leu Glu Pro Ala Ser Leu Tyr His Ile His Leu Met Ala Ala Ser Gln Ala Gly Ala Thr Asn Ser Thr Val Leu Thr Leu Met Thr

2024 TTG ACC CCA GAG GGG TCG GAG CTA CAC ATC ATC CTG GGC CTG TTC GGC CTC CTG CTG TTG CTC ACC TGC CTC TGT GGA ACT GCC TGG CTC TGT TGC AGC CCC AAC
 596 Leu Thr Pro Glu Gly Ser Glu Leu His Ile Ile Leu Gly Leu Phe Gly Leu Leu Leu Leu Leu Thr Cys Leu Cys Gly Thr Ala Trp Leu Cys Cys Ser Pro Asn

2129 AGG AAG AAT CCC CTC TGG CCA AGT GTC CCA GAC CCA GCT CAC AGC AGC CTG GGC TCC TGG GTG CCC ACA ATC ATG GAG GAG GAT GCC TTC CAG CTG CCC GGC CTT
 631 Arg Lys Asn Pro Leu Trp Pro Ser Val Pro Asp Pro Ala His Ser Ser Leu Gly Ser Trp Val Pro Thr Ile Met Glu Glu Asp Ala Phe Gln Leu Pro Gly Leu

2234 GGC ACG CCA CCC ATC ACC AAG CTC ACA GTG CTG GAG GAG GAT GAA AAG AAG CCG GTG CCC TGG GAG TCC CAT AAC AGC TCA GAG ACC TGT GGC CTC CCC ACT CTG
 666 Gly Thr Pro Pro Ile Thr Lys Leu Thr Val Leu Glu Glu Asp Glu Lys Lys Pro Val Pro Trp Glu Ser His Asn Ser Ser Glu Thr Cys Gly Leu Pro Thr Leu

2339 GTC CAG ACC TAT GTG CTC CAG GGG GAC CCA AGA GCA GTT TCC ACC CAG CCC CAA TCC CAG TCT GGC ACC AGC GAT CAG GTC CTT TAT GGG CAG CTG CTG GGC AGC
 701 Val Gln Thr Tyr Val Leu Gln Gly Asp Pro Arg Ala Val Ser Thr Gln Pro Gln Ser Gln Ser Gly Thr Ser Asp Gln Val Leu Tyr Gly Gln Leu Leu Gly Ser

2444 CCC ACA AGC CCA GGG CCA GGG CAC TAT CTC CGC TGT GAC TCC ACT CAG CCC CTC TTG GCG GGC CTC ACC CCC AGC CCC AAG TCC TAT GAG AAC CTC TGG TTC CAG
 736 Pro Thr Ser Pro Gly Pro Gly His Tyr Leu Arg Cys Asp Ser Thr Gln Pro Leu Leu Ala Gly Leu Thr Pro Ser Pro Lys Ser Tyr Glu Asn Leu Trp Phe Gln

2549 GCC AGC CCC TTG GGG ACC CTG GTA ACC CCA GCC CCA AGC CAG GAG GAC GAC TGT GTC TTT GGG CCA CTG CTC AAC TTC CCC CTC CTG CAG GGG ATC CGG GTC CAT
 771 Ala Ser Pro Leu Gly Thr Leu Val Thr Pro Ala Pro Ser Gln Glu Asp Asp Cys Val Phe Gly Pro Leu Leu Asn Phe Pro Leu Leu Gln Gly Ile Arg Val His

2654 GGG ATG GAG GCG CTG GGG AGC TTC TAG GGCTTCCTGGGGTTCCCTTCTTGGGCCTGCCTCTTAAAGGCCTGAGCTAGCTGGAGAAGAGGGGAGGGTCCATAAGCCCATGACTAAAAACTACCCCAGCCCA
 806 Gly Met Glu Ala Leu Gly Ser Phe End

2784 GGCTCTCACCATCTCCAGTCACCAGCATCTCCCTCTCCTCCCAATCTCCATAGGCTGGGCCTCCCAGGCGATCTGCATACTTTAAGGACCAGATCATGCTCCATCCAGCCCCACCCAATGGCCTTTTGTGCTTGTTTCC

2923 TATAACTTCAGTATTGTAAAC
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B

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2024 TTG ACC CCA GCC CCA ACA GGA AGA ATC CCC TCT GGC CAA GTG TCC CAG ACC CAG CTC ACA GCA GCC TGG GCT CCT GGG TGC CCA CAA TCA TGG AGG AGG ATG CCT
 596 Leu Thr Pro Ala Pro Thr Gly Arg Ile Pro Ser Gly Gln Val Ser Gln Thr Gln Leu Thr Ala Ala Trp Ala Pro Gly Cys Pro Gln Ser Trp Arg Arg Met Pro

2129 TCC AGC TGC CCG GCC TTG GCA CGC CAC CCA TCA CCA AGC TCA CAG TGC TGG AGG AGG ATG AAA AGA AGC CGG TGC CCT GGG AGT CCC ATA ACA GCT CAG AGA CCT
 631 Ser Ser Cys Pro Ala Leu Ala Arg His Pro Ser Pro Ser Ser Gln Cys Trp Arg Arg Met Lys Arg Ser Arg Cys Pro Gly Ser Pro Ile Thr Ala Gln Arg Pro

2234 GTG GCC TCC CCA CTC TGG TCC AGA CCT ATG TGC TCC AGG GGG ACC CAA GAG CAG TTT CCA CCC AGC CCC AAT CCC AGT CTG GCA CCA GCG ATC AGG TCC TTT ATG
 666 Val Ala Ser Pro Leu Trp Ser Arg Pro Met Cys Ser Arg Gly Thr Gln Glu Gln Phe Pro Pro Ser Pro Asn Pro Ser Leu Ala Pro Ala Ile Arg Ser Phe Met

2339 GGC AGC TGC TGG GCA GCC CCA CAA GCC CAG GGC CAG GGC ACT ATC TCC GCT GTG ACT CCA CTC AGC CCC TCT TGG CGG GCC TCA CCC CCA GCC CCA AGT CCT ATG
 701 Gly Ser Cys Trp Ala Ala Pro Gln Ala Gln Gly Gln Gly Thr Ile Ser Ala Val Thr Pro Leu Ser Pro Ser Trp Arg Ala Ser Pro Pro Ala Pro Ser Pro Met

2444 AGA ACC TCT GGT TCC AGG CCA GCC CCT TGG GGA CCC TGG TAA CCCCAGCCCCAAGCCAGGAGGACGACTGTGTCTTTGGGCCACTGC
 736 Arg Thr Ser Gly Ser Arg Pro Ala Pro Trp Gly Pro Trp End
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C

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2201 ATG GAG GAG [CTG CCC GGA CCC AGA CAG GGA CAG TGG CTG GGG CAG ACA TCT GAA ATG AGC CGT GCT CTC ACC CCA CAT CCT TGT GTG CAG] GAT GCC TTC
 655 Met Glu Glu  Leu Pro Gly Pro Arg Gln Gly Gln Trp Leu Gly Gln Thr Ser Glu Met Ser Arg Ala Leu Thr Pro His Pro Cys Val Gln  Asp Ala Phe
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FIG. 2. (*Legend appears at the bottom of the opposite page.*)

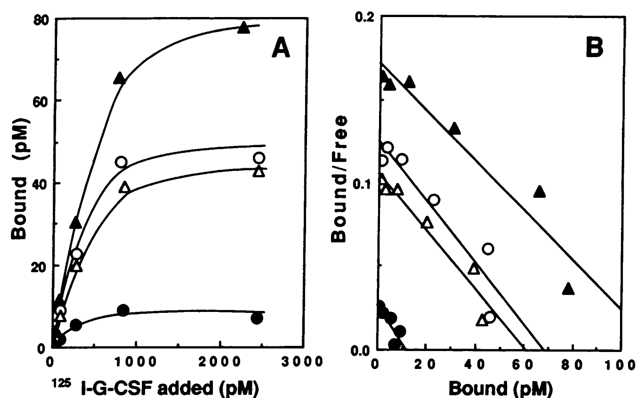

FIG. 3. Binding characteristics of recombinant human G-CSF receptors expressed by COS cells. COS cells transfected with murine G-CSF receptor cDNA (12) (▲) or human G-CSF receptor cDNAs [pHQ3 (○), pHQ2 (●), or pQW11 (△)] were incubated with various amounts of ^{125}I-G-CSF with or without excess unlabeled G-CSF. The specific binding of ^{125}I-G-CSF was determined after subtracting nonspecific binding. (*A*) Saturation binding of ^{125}I-G-CSF to COS cells. (*B*) Scatchard plot of G-CSF binding data.

receptor cDNA. As shown in Fig. 4*A*, a single band of 3.7 kb was observed in the RNAs from U937, KG-1, and placenta, but not in the RNAs from HL-60, FL, or CHU-2 cells. Unexpectedly, the signal detected in RNA from the placenta was >20 times stronger than that detected with RNA from either U937 or KG-1 cells. When the blot was rehybridized with ^{32}P-labeled human elongation factor 1α cDNA (22), the signals were almost similar (Fig. 4*B*). These results indicate that the placenta expresses G-CSF receptor mRNA more abundantly than myeloid leukemia cells.

As described above, we isolated three different human G-CSF receptor cDNAs from the U937 and placental cDNA libraries (Figs. 1 and 2). To examine which mRNA is expressed by these cells, two sets of forward and reverse primers were synthesized, and the specific cDNAs were amplified by the PCR method. Using the first set of oligonucleotides (nucleotides from positions 1790–1810 for the forward primer and 2179–2156 for the reverse primer), amplification of RNA from U937 or placental cells gave a major band (A1) of 390 bp, which corresponded to the class I human G-CSF receptor as represented by pHQ3 or pHG12. In addition, U937 mRNA gave a faint band (A2) of 302 bp, which seemed to correspond to the cDNA containing the deletion in the transmembrane domain (Fig. 5, lanes 1–3). On the other hand, when the second set of oligonucleotides (nucleotides from positions 2086–2105 and 2322–2303) was used as the specific primers, placental cDNA gave two bands of 318 bp (B1) and 237 bp (B2), which appeared to be derived from the class III and class I mRNAs, respectively (lanes 4–6). These assumptions were confirmed by the sequencing of the DNA fragments (A1, A2, B1, and B2) after subcloning in M13mp9 vector. Several other high molecular weight bands seen in Fig. 5 were not characterized. They may correspond to the cDNAs for unspliced RNAs or other variants of the receptor. From these results, it can be concluded that both U937 and placental cells express the class I G-CSF receptor. In addition, U937 cells express the soluble form of the G-CSF

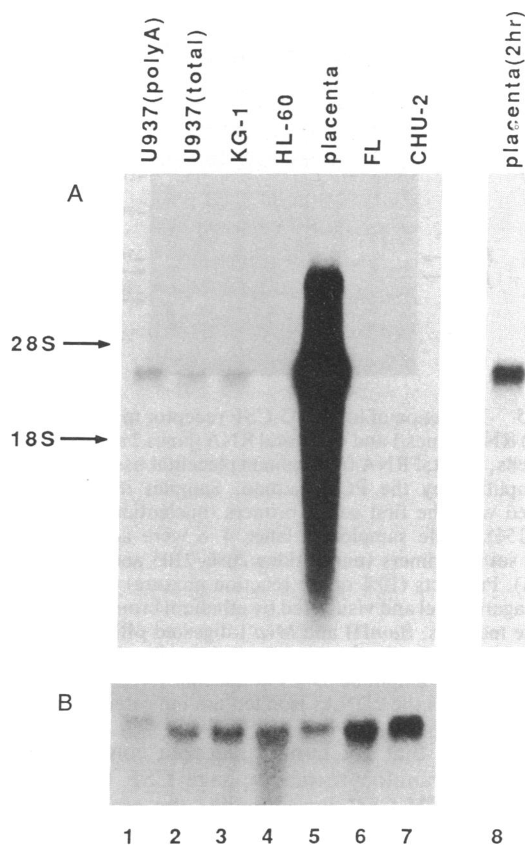

FIG. 4. Northern hybridization analysis of human G-CSF receptor mRNA. Twenty micrograms of total RNA (lanes 2–6) or 1 μg of poly(A) RNA (lanes 1 and 7) from various human cell lines and human placental tissue was used for Northern hybridization analysis. The DNA probes used were human G-CSF receptor cDNA (*A*) and human elongation factor 1α cDNA (*B*). In *A*, the filter was exposed to x-ray film for 40 hr except for lane 8, which was exposed for 2 hr. In *B*, the filter was exposed to x-ray film for 1 hr.

receptor, while the G-CSF receptor containing the insertion in the cytoplasmic domain is expressed at a significant level by placental cells.

DISCUSSION

In this study, we isolated cDNAs for the human G-CSF receptor by cross-hybridization with murine G-CSF receptor cDNA. The amino acid sequence of the human G-CSF receptor (Fig. 2) was highly homologous to that of the murine G-CSF receptor, in agreement with the observation that murine G-CSF could bind to the recombinant human and murine G-CSF receptors with almost identical dissociation constants (Fig. 3). In the murine G-CSF receptor, there are regions that have similarities to some members of the cytokine receptor family, chicken contactin, and the interleukin 4 (IL-4) receptor (12). The amino acid sequences of these regions are also highly conserved in the human G-CSF receptor and confirm that the G-CSF receptor is a member of the newly identified cytokine receptor family (19–21).

FIG. 2 (*on opposite page*). The nucleotide sequences and deduced amino acid sequences of the three human G-CSF receptor cDNAs. (*A*) Nucleotide sequence and predicted amino acid sequence of pHQ3. Numbering of the amino acids starts at Glu-1 of the putative mature G-CSF receptor. The signal sequence and the transmembrane domain are underlined with a solid bar. Potential N-glycosylation sites (Asn-Xaa-Thr/Ser) are boxed. The WSXWS motif conserved in the cytokine receptor family is underlined with thin double lines. Arrowheads indicate the ends of the deletion in pHQ2, while the thick arrows indicate the site of the insertion in pHG11 and pHG5. The oligonucleotide primers used for the PCR are indicated by the thin arrows. (*B*) Predicted amino acid sequence in pHQ2, which occurs following the 88-bp deletion (open arrowhead) of nucleotides 2034–2121 of pHQ3. (*C*) The nucleotide sequence and deduced amino acid sequence of the insertion present in pHG11 and pHG5. Insertion occurs following amino acid 657 in pHQ3, and the inserted sequence is bracketed.

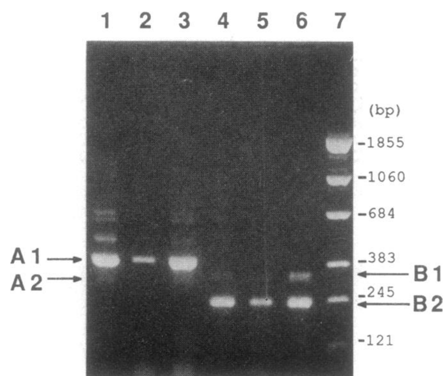

FIG. 5. Detection of human G-CSF receptor mRNA by the PCR. Poly(A) RNA (lanes 1 and 4) or total RNA (lanes 2 and 5) from human U937 cells, or total RNA from human placental tissue (lanes 3 and 6) was amplified by the PCR method. Samples in lanes 1–3 were amplified with the first set of primers (nucleotides 1790–1810 and 2179–2156), while samples in lanes 4–6 were amplified with the second set of primers (nucleotides 2086–2105 and 2322–2303) (see Fig. 2A). Products (10% of the reaction mixture) were analyzed on a 1.5% agarose gel and visualized by ethidium bromide fluorescence. For size markers, *Bam*HI and *Mva* I-digested pBR322 was electrophoresed in parallel, and sizes of the DNA fragments are given in base pairs. The amplified DNA fragments (A1, A2, B1, and B2) corresponding to the cDNAs isolated are indicated by arrows.

It is known that the human placenta constitutively produces both granulocyte–macrophage CSF (GM-CSF) and G-CSF (23). GM-CSF can stimulate the growth of the placenta, especially that of trophoblastic cells (24), and the human GM-CSF receptor was recently isolated from a placental cDNA library (20). Since the G-CSF receptor is very abundantly expressed in the placenta (Fig. 4), it is possible that G-CSF also has the ability to stimulate the growth and maturation of placental cells. About 20% of the G-CSF receptors expressed in the human placenta were receptors containing a 27-amino acid insertion in the cytoplasmic domain (Fig. 5). Previously, we have shown that the murine G-CSF receptor forms oligomers to produce a high-affinity binding site for G-CSF (7). Since the placental membrane binds G-CSF with a high affinity (8), the G-CSF receptor in the placenta may be a heterooligomer of the class I and class III G-CSF receptors. Whether the class I and class III receptors transduce the signals to the cells by a similar mechanism remains to be studied.

In human leukemia U937 cells, we have found the cDNA (pHQ2) and the transcript that encode the putative G-CSF receptor without a transmembrane domain (Figs. 1 and 5). Soluble forms of receptors for various cytokines and growth factors, such as interferon γ, IL-6 (25), IL-4 (26), and IL-7 (21), have been reported. The physiological roles of these soluble receptors are not fully understood. They may act as a sink for growth factors to carry out the feedback regulation of growth factors and cytokines. In this regard, it may be noteworthy that serum G-CSF levels show an inverse correlation with the neutrophil count in aplastic anemia (27). Patients with acute leukemia often suffer from neutropenia, and some circulating substances inhibiting granulopoiesis have been found in leukemia patients (28, 29). It is possible that one of these inhibitors is the soluble form of the G-CSF receptor produced by leukemia cells. In the human IL-6 system, the soluble IL-6 receptor can transduce signals to cells in association with the so-called gp130 protein (30). If such a molecule is also involved in the G-CSF system, the soluble G-CSF receptor may then also stimulate the growth or differentiation of cells. To clarify these points, it will be necessary to examine the G-CSF receptor transcripts in human primary leukemia cells by Northern hybridization and the PCR method and to study the functions of the soluble receptor.

This study identified at least three different mRNAs for the human G-CSF receptor (Figs. 1 and 2), which had nucleotide sequences that were identical except for specific deletions or insertions. Since the human genome seems to contain a single gene for the human G-CSF receptor (unpublished results), these different mRNAs are probably produced by alternative splicings from a single precursor RNA. Characterization of the human chromosomal gene for the G-CSF receptor will allow clarification of the mechanism that generates different mRNAs for the G-CSF receptor.

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