sensitivity of the radioimmunoprecipitation assay employed might be characteristic of the development of ARC or AIDS in certain individuals.

The possibility that antibodies to nonenvelope structures are effective in neutralizing HIV in vitro has been raised (*22*). Antibody to RT similarly might interfere with virion assembly or budding. Whether such a mechanism is responsible for the clinical correlations observed here is unknown. Most likely, IgGs with anti-RT capacity are surrogate markers for protective cellular immune responses or for other antibodies with reactivity against neutralizing envelope epitopes.

This latter area is controversial. Virus-neutralizing factors have been found in >50% of serum samples obtained from ARC and AIDS patients (*16*), and in a higher number of HIV-seropositive asymptomatic carriers (*23*). Clinical correlations in patients followed over time have been weak, however. The assays typically involve single HIV isolates as stock targets, although envelope variability is well documented among HIV isolates, even from a single individual (*24*). We attempted to link HIV neutralization in vitro with anti-RT activity, using IgG from ten of the asymptomatic carriers. We incubated 1×10^3 tissue culture infectious doses of stock $HIV_{HTLV\text{-}III_B}$ with 1 or 10 μg of IgG for 2 hours at 25°C. Phytohemagglutinin-activated human peripheral blood mononuclear cells (2×10^6) were then added in RPMI-1640 containing 10% fetal calf serum and 5% interleukin-2. Cultures were maintained for 18 hours at 37°C, the medium was changed, and HIV replication was assessed by RT determinations on days 7 and 14 after infection. Using ≥75% inhibition of enzyme activity as the criterion for neutralization, we found most samples effective at 10 μg, without regard to the patient's clinical status or the anti-RT capacity of the IgG.

In conclusion, circulating IgGs from certain individuals infected with HIV can block the catalytic activity of the viral polymerase in vitro. This effect is specific for the RT of HIV, although cross-reaction with HTLV-IV might be anticipated. RT-IgG binding, determined by radioimmunoprecipitation, did not correlate with RT suppression. Follow-up of a small cohort of asymptomatic HIV carriers revealed loss of this inhibitory capacity prior to development of clinical ARC or AIDS. If these data are reproduced in larger surveys of HIV-infected individuals, the assay could serve as a marker for disease progression, similar to the correlation of anti-RT activity with clinical status observed in other mammalian retroviral models.

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Human CSF-1: Molecular Cloning and Expression of 4-kb cDNA Encoding the Human Urinary Protein

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A 4-kilobase complementary DNA (cDNA) encoding human macrophage-specific colony-stimulating factor (CSF-1) was isolated. When introduced into mammalian cells, this cDNA directs the expression of CSF-1 that is structurally and functionally indistinguishable from the natural human urinary CSF-1. Direct structural analysis of both the recombinant CSF-1 and the purified human urinary protein revealed that these species contain a sequence of at least 40 amino acids at their carboxyl termini which are not found in the coding region of a 1.6-kilobase CSF-1 cDNA that was previously described. These results demonstrate that the human CSF-1 gene can be expressed to yield at least two different messenger RNA species that encode distinct but related forms of CSF-1.

THE PROLIFERATION OF HEMATOPOIETIC cells in culture requires the presence of one or more hematopoietic growth factors known as the colony-stimulating factors (CSFs) (*1*). Four subtypes of CSFs have been identified on the basis of hematopoietic cell lineages observed in colonies grown in the presence of the different factors. In the human system, the genes for all four of these subtypes have been cloned, thereby permitting analysis of the expression of these sequences in different cell types (*2–5*). In analyzing the expression of messenger RNAs (mRNAs) encoding

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human macrophage-specific CSF (CSF-1) we observed that the predominant transcript encoding this protein in several different cell types is 4 kb in size. Because the complementary DNA (cDNA) clone for CSF-1 (pcCSF-17) described earlier (*2*) was derived from an mRNA 1.5 to 2.0 kb in size, we isolated cDNA clones encoding the longer transcript to determine the basis for this apparent size difference. We report that the 4-kb mRNA encodes a larger CSF-1 precursor protein (61 kD) than does the 1.5- to 2.0-kb mRNA. The cDNA clone (p3ACSF-69) encoding this 61-kD polypeptide, when introduced into mammalian cells, directs high-level expression of a form of CSF-1 that is very similar in its properties to those reported for authentic human urinary CSF-1. Subsequent determination of the complete amino acid sequence of the urinary CSF-1 revealed that it can be encoded by the 4-kb but not by the 1.5- to 2.0-kb mRNA. In addition, determination of the amino acid sequences of the amino- and carboxyl-terminal tryptic peptides of purified recombinant human CSF-1 shows that these peptides are structurally indistinguishable from those of human urinary CSF-1. These results demonstrate that there are at least two different CSF-1 mRNAs encoding distinct but related forms of CSF-1.

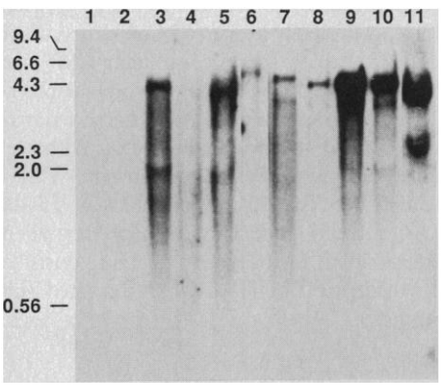


Fig. 1. Blot analysis of CSF-1 mRNA sequences from different cellular sources of CSF-1. Polyadenylated mRNA (5 μg) from each of the following sources was analyzed: (lane 1) CCRF-CEM cells (*26*), (lane 2) human peripheral blood lymphocytes (PBL), (lane 3) lectin-stimulated peripheral blood lymphocytes (*1*), (lane 7) Mia-Paca cells (*27*), (lane 8) human liver, (lane 9) TPA30-1 cells (*6*), (lane 10) human placenta, and (lane 11) murine W20 cells (*28*). Total RNA (30 μg) from the following sources was analyzed: (lane 4) unstimulated C10-MJ2 cells (*29*), (lane 5) lectin-stimulated C10-MJ2 cells (*29*), and (lane 6) murine L cells. The RNA blot analysis was performed as described previously (*3*), with the labeled cDNA insert fragment of p3ACSF-69 as probe (see legend to Fig. 2). The relative mobilities of the Hind III fragments of phage lambda DNA were as indicated. C10-MJ2 cells and PBLs were induced with phytohemagglutinin and phorbol myristate acetate, and RNAs were prepared as described (*3*). The RNAs from all of the other cell lines and tissues were prepared as described elsewhere (*30, 31*). The RNAs were fractionated by electrophoresis through a 1% agarose gel in the presence of formaldehyde. The size-fractionated RNA was transferred from the gel to nitrocellulose and the resulting filter was probed with the 4-kb insert of p3ACSF-69 labeled with ^{32}P by using the random oligonucleotide priming reaction as described (*32*). The relative mobilities of the different CSF-1 species were visualized by exposure of the filter to Kodak XAR film. Similar results were obtained with the synthetic oligonucleotide probes (*7*).

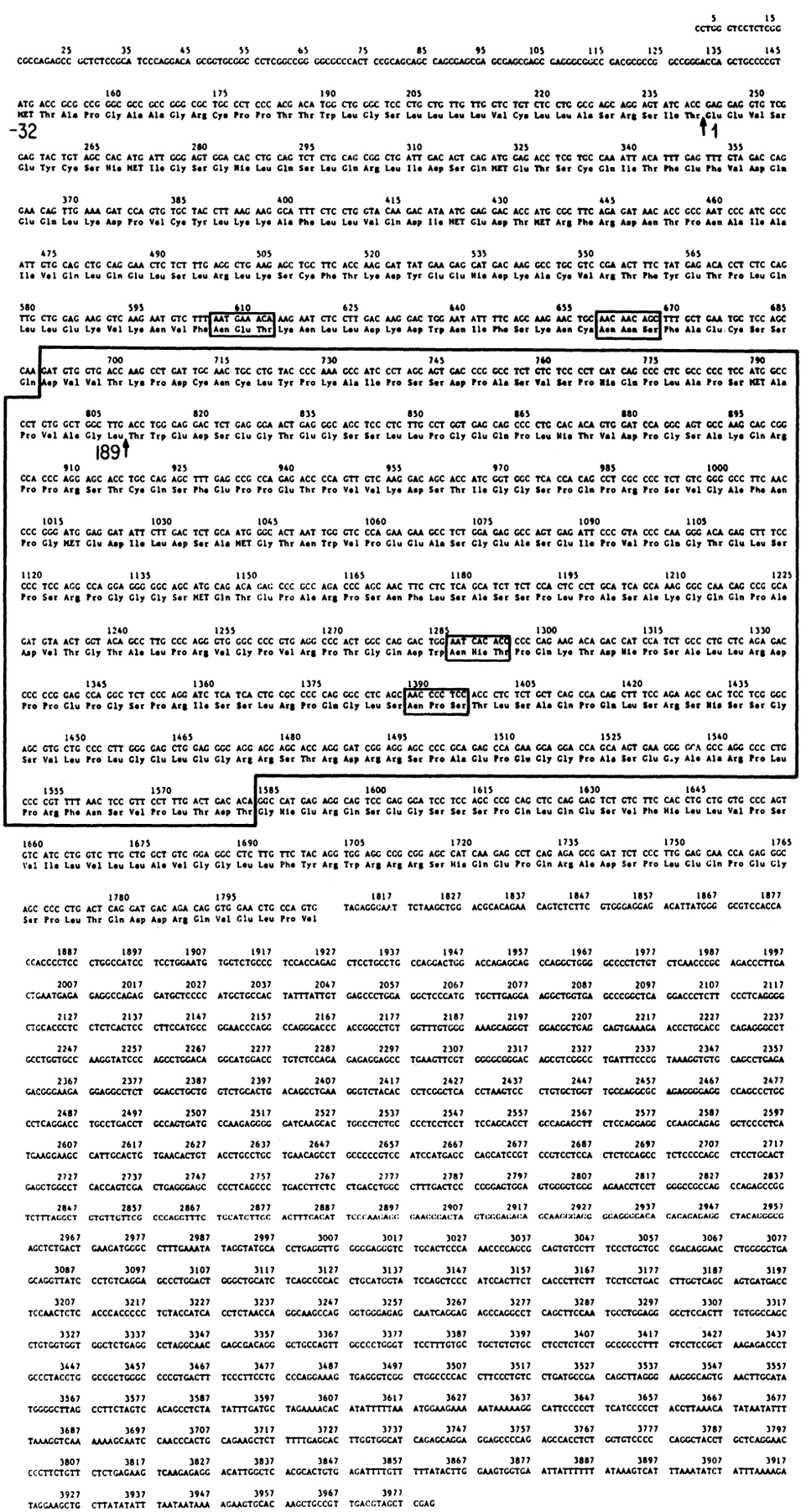
Analysis of the mRNA from several different cellular sources of CSF-1 revealed that the most abundant form of the mRNA for this factor in either human or murine cells is approximately 4 kb in size (Fig. 1). Other sizes of CSF-1–related sequences were also detected, most notably a 2-kb mRNA found in RNA preparations from murine stromal cells. These studies also revealed that an SV40-transformed trophoblast cell line (TPA30-1) (*6*) is a particularly abundant source of the 4-kb mRNA encoding CSF-1 (Fig. 1). We isolated CSF-1 cDNA clones corresponding to the 4-kb transcript found in TPA30-1 cells to determine the reason for the difference in size between the two species of CSF-1 mRNAs. These CSF-1 cDNA sequences were isolated from libraries of cDNA clones prepared from TPA30-1 mRNA (*7*) by use of the phage cloning vector λgt11 (*8*) or the plasmid cloning vector pXM (*9*). Numerous overlapping clones were identified by filter hybridization with synthetic oligonucleotides based on pcCSF-17, the previously reported nucleotide sequence encoding human CSF-1 (*7*). The nucleotide sequence of the 4-kb mRNA deduced from these cDNA clones is depicted in Fig. 2. The sequence contains a long open translational reading frame of 1662 nucleotides encoding a 554–amino acid polypeptide as well as 2200 nucleotides of 3′ noncoding sequence. The coding region of this 4-kb sequence differs from the coding region of pcCSF-17 by the insertion of 894 base pairs (indicated by the box in Fig. 2) between nucleotides 721 and 722 of the latter sequence. Because the 894-bp insertion preserves the reading frame, the resulting coding region of the 4-kb mRNA encodes a 61-kD CSF-1 precursor which is substantially larger than the 26-kD precursor predicted from the sequence of pcCSF-17. The nucleotide sequences of the two different cDNAs diverge immediately after their respective termination codons.

For initial studies of the expression of the CSF-1 polypeptide encoded by the 4-kb mRNA, two incomplete but overlapping cDNA clones were introduced into the mammalian cell expression vector pXM (*9*) in such a way that the complete sequence illustrated in Fig. 2 was regenerated. The resulting plasmid, designated p3ACSF-69, was introduced into monkey COS-1 (*10*) cells. The conditioned medium generated was capable of supporting the formation of murine macrophage colonies even at dilutions as high as 1:6000 (*11*) (Table 1). As noted by other investigators (*12–16*), human CSF-1 was much less effective in supporting the proliferation of human macrophage progenitors; a final dilution of 1:432 of the same COS-1 cell–conditioned medium yielded half the maximal number of human macrophage colonies achieved with saturation levels of CSF-1. The recombinant human CSF-1 expressed by COS-1 cells was completely inactivated by prior incubation with an excess of a rabbit antiserum to partially purified urinary CSF-1. Thus p3ACSF-69 can, in COS-1 cells, direct the high-level expression of biologically active CSF-1.

Table 1. Growth of macrophage colonies in the presence of recombinant human CSF-1 (*11*).

Dilution of CSF-1	Number of macrophage colonies in the presence of	
	No antibody	1:30 dilution of antiserum to CSF-1
	Murine macrophages	
1:50	20	21
1:250	20	7
1:1,250	12	1
1:6,250	7	0
1:31,250	2	0
	Human macrophages	
1:48	7	
1:144	8	
1:432	4	
1:1,296	1	

To investigate the structure of recombinant CSF-1, we used p3ACSF-69 to construct stable cell lines that constitutively express high levels of human CSF-1. This was accomplished by cotransformation of dihydrofolate reductase (DHFR)–deficient Chinese hamster ovary cells (CHO) with p3ACSF-69 and a plasmid that expresses DHFR [pADd26SVp(A)3] (*17*). One CSF-1–expressing cell line, CHO-3ACSF-69, which was selected for growth in 0.25 μ*M* methotrexate consistently generated conditioned medium that was active in supporting murine macrophage colony formation at a final dilution of 1:60,000. The CHO-3ACSF-69 cells were labeled with [^{35}S]methionine, and the labeled CSF-1 protein in the conditioned medium was precipitated by incubation with antiserum to human urinary CSF-1 (*18*). Analysis of these immunoprecipitates by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing conditions revealed that



the conditioned medium from the CSF-1–producing CHO cells contained two heterogeneous CSF-1 related species, one of apparent size 70 to 90 kD and the other greater than 150 kD (Fig. 3). The observed size heterogeneity of these CSF-1 species is characteristic of many glycoproteins and most likely reflects differential glycosylation of a common polypeptide. Analysis of the same samples after reduction revealed that the mobility of the 70- to 90-kD species of CSF-1 shifted to a position consistent with a molecular weight of 35 to 45 kD, whereas the relative mobility of the larger species (greater than 150 kD) appeared unaffected. This experiment demonstrated that at least two different forms of CSF-1 are expressed by the CHO-3ACSF-69 cells: a 70- to 90-kD protein (also known as CSF-69) comprising a disulfide-linked dimer of a 35- to 45-kD subunit and a much larger species of indeterminate structure that may represent an incompletely processed CSF-1 precursor. The 70- to 90-kD recombinant CSF-1 secreted by CHO-3ACSF-69 had displayed physical properties similar to those reported for human urinary CSF-1 (*13*). Although size estimates for human urinary CSF-1 range between 40 kD and 90 kD (*13–15, 19*) there is general agreement that all of these forms of the native protein consist of two identical but heavily glycosylated subunits.

Fig. 2. Nucleotide sequences of the 4-kb CSF-1 cDNA (3ACSF-69) and the predicted amino acid sequence of prepro–CSF-1. Although none of the clones that we isolated proved to be full length, one of them proved to be lacking only 54 nucleotides at the 5′ end; the sequence presented here was largely derived from this clone. The remaining sequence was obtained from several other overlapping clones. The nucleotide sequences of all of the clones were consistent with the one shown here. The position of cleavage of the signal peptide was determined by comparison with the amino-terminal sequence of human urinary CSF-1 peptide 14 (Table 2) (*2*) and is indicated by the arrow. The position of the identified amino acid nearest the carboxyl-terminal amino acid—namely, leucine—is indicated by the arrow at residue 189. The positions of potential sites for asparagine-linked glycosylation are indicated by the small boxes. The coding sequences present in 3ACSF-69 but not found in pcCSF-17 (*2*) are contained within the large open boxed area. DNA sequence analysis of two additional CSF-1 cDNA clones show that the nucleotide sequence at amino acid position 59 is GAC and thus codes for Asp, consistent with the CSF-1 genomic exon sequence (*2*). This suggests that the Tyr found at position 59 (TAC) in cDNA clone pcCSF-17 is a reverse transcriptase error as postulated by Kawasaki *et al.* (*2*). At nucleotide position 1678, we find that there is a C/T polymorphism. In two of three CSF-1 cDNA clones thus far sequenced, a C residue was found at this position; however, this change does not alter the amino acid sequence of CSF-1.

To determine how the subunit of human urinary CSF-1 is related to either the 61-kD precursor encoded by p3ACSF-69 or the 26-kD precursor encoded by pcCSF-17, we sequenced all of the major tryptic peptides of the purified human urinary hematopoietin. The human CSF-1, purified as described (*20*), comprised a 70- to 90-kD dimer of a 35- to 45-kD subunit as described previously (*13*). The sequences of all the major tryptic peptides determined with the gas phase microsequenator (*21*) are illustrated in Table 2. Alignment of these peptide sequences with the sequences predicted from p3ACSF-69 demonstrated that the sequence of the mature human urinary CSF-1 extended from residue 1 at least through residue 189 of the sequence of the 61-kD precursor encoded by this cDNA (Fig. 2). The leucine residue at position 189 was the last amino acid that could be positively identified in the sequence (peptide 16 in Table 2) but it is likely that the carboxyl terminus of mature CSF-1 lies beyond tryptophan at position 191, since peptides corresponding to this region of the precursor polypeptide show high absorbance at 280 nm. However, none of the peptides expected from the remaining 331 residues of the precursor protein could be found in the tryptic map of CSF-1, thus providing compelling evidence that the carboxyl terminus lies between residue 191 (tryptophan) and residue 218 (lysine). Several peptides (*7*, 8, 9, and 12 in Table 2) contained sequences that spanned the point of divergence between the coding regions of 3ACSF-69 and pcCSF-17 (nucleotide 722 in Fig. 2). Because the sequence of these peptides precisely matched the sequence of 3ACSF-69 and diverged from the sequence of pcCSF-17, we conclude that the human urinary CSF-1 that we purified was encoded by the 4-kb mRNA represented by 3ACSF-69 and could not be derived from the sequence of the 1.5-kb mRNA represented by pcCSF-17. In all, approximately 40 amino acids of the mature human urinary CSF-1 encoded by 3ACSF-69 are not found in the 26-kD precursor encoded by pcCSF-17. We cannot exclude the possibility, however, that other natural forms of CSF-1 that might correspond to the protein encoded by pcCSF-17 were lost during purification or that such forms of the hematopoietin might be found in other natural sources of CSF-1. A similar structural analysis of the 70- to 90-kD recombinant CSF secreted by CHO-3ACSF-69 demonstrated that this molecule has the same amino- and carboxyl-terminal sequences as the form of CSF-1 we purified from human urine (*22*).

Our results demonstrate that a major form of human urinary CSF-1 is encoded by a 4-kb mRNA, which is the predominant form of the CSF-1 mRNA in several human cellular sources of this factor. This mRNA encodes a 61-kD prepro–CSF-1 which is processed at the amino terminus by removal

Table 2. Sequences of all of the major tryptic peptides from human urinary CSF-1. The indicated sequences, in standard single letter code, were from the peptides in the order of their elution from a reversed-phase HPLC column (Vydac, C18). Residues in parentheses are tentative assignments while blanks indicate that no residue could be determined from that cycle of the sequenator. A portion of the purified CSF-1 was denatured in guanidine hydrochloride (6*M*), alkylated with iodoacetic acid, and desalted by reversed-phase HPLC (Brownlee RP300 column). The alkylated CSF-1 was digested with trypsin (Trypsin TPCK, Cooper Biomedical) at a concentration of 1% of total protein in 100 μl of 100 m*M* ammonium bicarbonate (*p*H 7.5), and the resulting peptides were fractionated by reversed-phase HPLC (Vydac C18) by use of a 0 to 90% gradient of acetonitrile in 0.1% trifluoroacetic acid. The sequence of each of the peptides contained within the 23 major peaks that eluted from this column was determined with the gas phase sequenator. The standard single letter code is: A, alanine; B, aspartic acid or asparagine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine; and Z, glutamic acid or glutamine. Dashes represent residues that are undetermined or ambiguous.

Peak no.	Sequences
1	FR---
2	DYEE(H)D--
	FR------
3	S-FTK
4	DYEEHDKA-VR
5	NVF(N)ETK
6	LK------
7	N-(N)NSFAE--(S)Q--V---
8	N-(N)NSFAE-SSQDVV-KPD-N-LY--
	AIP--DP--VP--P-A-S-------
9	EEVSEY----IG---LQ----
	N-(N)NSFAE-SSQDVV-KP---
	AIP-SDPA-VSP---LA---
10	EEVSEY-S(H)MIG---
11	EEVSEY-S(H)MIG---
12	EEVSEY-SHMIGS-HLQSLQ---
	AIPS-DP--V-P---LA(P)---
	N-(N)N-FAE---QD------
13	DPV-YL-
14	EE(V)SEY-SHMIGSGHLQSLQR--
15	AIP-SDPA-VSP-QPLA(MV)
16	AIP-SDPA-VSPHQPLAP-M-PV--L--
17	AIP--DPA-VSP-QPLA--MA-(V)-
18	NLLDKDWNIFSKN-(N)N-FAE-S---VV--
19	NLLDKDWNIFSK--
20	FRDNTPNAIAIVQLQELLSL(R)
	(D)N(T)PNAI--VQL(E)-(S)S--
21	TFYETPLQLLEK----------
	LIDSQMETS-QITFEFVDQ-(Q)--
	FR(D)N(T)(P)NAIAIVQLQELSL---
22	KAFLLVQDIM-D-M--
23	AFLLVQDIMEDTMR---

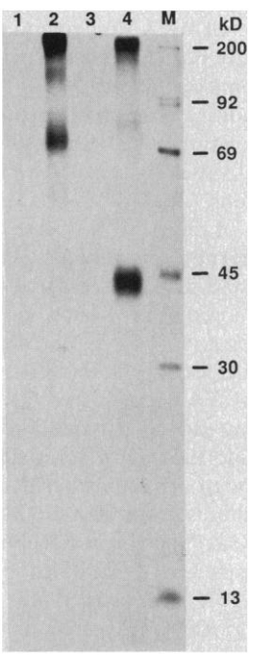


Fig. 3. Analysis of ^{35}S-labeled CSF-1 expressed by CHO-3ACSF-69 cells. The CSF-1–related peptides secreted by CHO-3ACSF-69 cells following labeling with [^{35}S]methionine were precipitated with antiserum to purified urinary CSF-1 (*33*) and fractionated by SDS-PAGE. Lanes 1 and 2 represent the CSF-1 polypeptides from the parent CHO cell line and CHO-3ACSF-69, respectively, analyzed under nonreducing conditions; lanes 3 and 4 represent the same samples following reduction with 2-mercaptoethanol. Lane M contains molecular weight standards.

of a 32-residue signal peptide and at the carboxyl terminus by removal of about 333 residues to yield a subunit of approximately 189 amino acids with a predicted molecular weight of 21 kD. This subunit retains two of the four potential sites for addition of asparagine-linked carbohydrate (Asn-X-Ser/Thr) (*23*) that are present in the sequence of the 61-kD CSF-1 precursor and 9 of the predicted 12 cysteine residues. We presume that *N*- and *O*-linked glycosylation of the 21-kD polypeptide accounts for most of the remaining mass of the 35- to 45-kD subunit of urinary CSF-1.

In comparison to the other CSFs, CSF-1 has a complex dimeric structure maintained by disulfide bridges. The finding that the polypeptide is synthesized as a large precursor molecule that is extensively processed to yield the mature protein raises questions regarding the mechanism of assembly of this hematopoietin. Our results demonstrate that CHO cells and monkey COS-1 cells are capable of efficiently secreting biologically active CSF-1 with a dimeric structure very similar to the 70- to 90-kD urinary protein. It will be interesting to determine the function, if any, of the 333 carboxyl-terminal amino acids of the CSF-1 precursor.

The expression of the CSF-1 gene in both human and murine cells is complex. Multiple species of mRNA ranging in size from 1 to 4 kb have been identified (*2, 16*). Because there is only one gene for CSF-1 (*2*), these species must result from differential processing of the primary CSF-1 transcript (*2, 16*). Our results, when compared with previous observations (*2*), now demonstrate that at least two of these mRNAs encode substantially different forms of prepro–CSF-1 polypeptides and that processing of these polypeptides results in related but different forms of mature CSF-1. Our data indicate that the largest, most abundant species of CSF-1 mRNA encodes the 61-kD prepro–CSF-1 and this is processed to a mature polypeptide subunit of approximately 189 residues with a molecular mass of 21 kD. This subunit is larger than the 14- to 17-kD deglycosylated polypeptide subunit of human urinary CSF-1 identified by Das and Stanley (*15*). The purified 45- to 60-kD human urinary CSF-1 used in their study was smaller than our urinary CSF-1 (70 to 90 kD), and it is possible that the smaller species might be encoded by another form of the CSF-1 mRNA such as is represented by pcCSF-17. However, the answer to this question will require the determination of the complete amino acid sequence of the 45- to 60-kD form of human urinary CSF-1 isolated by these authors.

CSF-1, like the other CSFs, has various different biological activities (*24*). In addition to its growth factor activity for macrophage progenitor cells, it is capable of stimulating the various biological activities of mature macrophages and the cells of continuous macrophage-like cell lines (*25*). The finding that the CSF-1 gene can express several different but related species of mature CSF-1 raises the possibility that different forms of the hematopoietin might mediate the different biological activities reported for natural CSF-1. The availability of two different forms of recombinant human CSF-1 will now permit us to begin to test this possibility.

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7. RNA was isolated from TPA30-1 cells and double-stranded cDNA was prepared as described previously (*5*). Half of this cDNA was used to prepare a plasmid cDNA library (35,000 clones) with the vector pXM as described (*5*). CSF-1–specific sequences were identified by colony hybridization with the use of synthetic oligonucleotides derived from the previously reported sequence (*2*): oligonucleotide 1643 had the sequence GCCCAGCCATGTCGTGGGAGGCAGCGCCCGGCGGCGCCGGCGCGGTCAT, which represents the antisense sequence for the first 17 codons of CSF-1; oligonucleotide 1646 had the sequence CACTGGCAGTTCCACCAGTCTGTCATCCTGAGTCAGGGGCTGCCCTCTGG, which represents the antisense sequence for the final 17 codons of CSF-1. These probes were hybridized to filters under standard hybridization conditions (*5*), but the temperature was lowered to 55°C to compensate for the length of the oligonucleotide. In screening the 35,000 clones with these probes, two cDNAs that hybridized with probe 1646 were identified, and none were found that hybridized with probe 1643. The longer of these two cDNAs (p3ACSF-3.5) was 3.7 kb in size and corresponded to the sequence of nucleotides 205 through 3981 above. The remaining cDNA was ligated with Eco RI adapters having the sequence

   AATTCCTCGAGAGCT
   GGAGCTCTCG

   and inserted into the phage vector λgt11 by standard methods (*31*); a library of 400,000 cDNA clones was screened with probe 1643 and the labeled insert from p3ACSF-3.5 (*30*). Four plaques that hybridized with both probes were identified. Because the Eco RI adapter contained the recognition sequence for the endonuclease Xho I, the inserts from these clones were readily excised by digestion with this enzyme and introduced into the Xho I site of pXM. One of these clones (p3ACSF-1.3), which contained the sequence from nucleotide 1 to 1332 above, was used to complete the missing sequence from p3ACSF-3.5. This was accomplished by joining the sequence from 1 to 478 from p3ACSF-1.3 (using the unique recognition site for Pvu II at 478) with the sequence from 478 to 3981 derived from p3ACSF-3.5. This DNA was subcloned into the expression vector pXM to yield the CSF-1 expression plasmid designated p3ACSF-69.
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11. Murine bone marrow colony assays were performed as described by Metcalf (*1*), using 2×10^4 murine (BALB/c) bone marrow cells in a 0.1-ml methocel culture. All samples were tested in triplicate and the average number (determined on day 7) is reported. The COS cell CSF-1 was prepared by transfection of COS-1 cells with p3ACSF-69 DNA as described elsewhere (*3*). In the absence of CSF-1 or in cultures supplemented with mock transfected COS-cell medium no macrophage colonies were obtained. Human marrow cultures were performed as described elsewhere (*3*). Antibody to CSF-1 was prepared by immunizing rabbits with partially purified urinary CSF-1 (K. Motoyoshi, unpublished).
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18. p3ACSF-69 and pADd26SVp(A)3 were cotransfected into DHFR-deficient CHO cells as described elsewhere (*17*). The initial transfectants were selected for growth in increasing concentrations of methotrexate. These cells (one 10-cm dish) as well as the parent CHO cells were labeled with 1 mCi of [^{35}S]methionine in 4 ml of minimal essential medium for 4 hours at 37°C. The resulting conditioned media samples were incubated with rabbit antiserum to purified urinary CSF-1. The antigen-antibody complexes were precipitated by adsorption to *Staphylococcus aureus* cells (Calbiochem). The complexes were solubilized in Laemmli loading gel buffer lacking reducing agent. The samples for lanes 3 and 4 in Fig. 3 were brought to 100 m*M* 2-mercaptoethanol and incubated at 37°C for 30 minutes. After electrophoresis (10% polyacrylamide gel) [U. K. Laemmli, *Nature (London)* **227**, 680 (1970)], the pattern of labeled proteins was visualized by fluorography (EnHance, New England Nuclear) with Kodak XAR film. The indicated molecular weight standards were the high molecular weight preparation from Bio-Rad.
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20. CSF-1 was isolated from human urine by use of a purification scheme of DEAE-cellulose chromatography, gel filtration (Sephacryl S-300), hydrophobic affinity chromatography (phenyl-Sepharose), high resolution gel filtration (TSK-G 3000 SW), and reversed-phase high-performance liquid chromatography (HPLC) (Vydac C18). The CSF-1 purified through the penultimate step had a specific activity of 2×10^7 unit/mg [one unit is the amount of CSF-1 that will stimulate the formation of one colony per 10^5 murine bone marrow cells plated in a 1-ml culture (*12*)]. The overall yield of activity through this step was 43%. The activity of the CSF-1 was lost during the fractionation by reversed-phase HPLC. However, SDS-PAGE analysis of proteins eluted from the column revealed a single species of protein, which shifted in apparent molecular weight upon reduction. This protein, when analyzed under nonreducing conditions, had an apparent molecular weight of 70 to 90 kD. Amino-terminal sequence analysis of this material confirmed that it was CSF-1.
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