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Multiple biological activities are expressed by a mouse interleukin 6 cDNA clone isolated from bone marrow stromal cells

(plasmacytoma/hepatocyte/growth factor)

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ABSTRACT **Interleukin 6 (IL-6) refers to the gene product that was characterized initially as β_2 interferon/26-kDa protein produced by human fibroblasts and later was found to be identical to B-cell stimulatory factor 2, hybridoma/plasmacytoma growth factor, and probably hepatocyte-stimulating factor. Using the human IL-6 cDNA as a probe, we have isolated functional cDNA clones from mouse bone marrow stromal cell cDNA libraries. Sequence analysis of the mouse cDNA insert revealed significant homology between the human and mouse IL-6 cDNA clones both at the level of nucleotide (65%) and deduced amino acid (41%) sequences. The NH_2-terminal sequence of the deduced protein is identical to a partial NH_2-terminal sequence determined previously for a hybridoma/plasmacytoma growth factor and a plasmacytoma growth factor isolated from mouse T cells and macrophages, respectively. The mRNA for mouse IL-6 is expressed in IL-1-treated stromal cells and in activated T-cell and macrophage cell lines. Supernatants from COS-7 monkey cells transfected with the cDNA clone have plasmacytoma growth factor, hepatocyte-stimulating factor, and colony-stimulating factor activities, as well as the ability to support the growth of a factor-dependent myeloid cell line, thus revealing an additional biological activity for IL-6.**

Human B-cell stimulatory factor 2 (BSF-2), a B-cell differentiation factor, was identical to β_2 interferon (IFN-β_2) and to a hybridoma/plasmacytoma growth factor (HPGF) (1–4; for a review, see refs. 5 and 6). Recently, it also has been shown that BSF-2 is functionally and immunologically related to hepatocyte-stimulating factor (HSF) derived from human monocytes (7, 8). These results, therefore, suggest a role for BSF-2 in the regulation of growth and differentiation of many cell types, and it seems appropriate that BSF-2 be renamed "interleukin-6" (IL-6) as suggested (2).

The molecular and biological aspects of IL-6 so far have been studied for the human molecule only, and it is not clear if similar observations would be true in other species. Recent reports show a plasmacytoma growth factor activity (PCT-GF) and a HSF activity in the conditioned medium of mouse macrophages (9, 10). Furthermore, the NH_2-terminal amino acid sequence determined for PCT-GF is essentially identical to that of a mouse HPGF (11, 12). However, this sequence shows no homology with that of human IL-6. Thus, it is of interest to identify the mouse homologue of human IL-6 and determine if it possesses multiple biological activities.

The production of certain hemopoietic growth factors, such as granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF), by bone marrow stromal cells can be induced by IL-1 (13, 14). Human fibroblasts up-regulate their production of these same growth factors as well as IL-6 under the same conditions (refs. 15 and 16; A. Troutt and F.L., unpublished result). Based on these functional similarities between stromal cells and fibroblasts, we thought it likely that IL-6 would be induced by IL-1 in mouse stromal cells. In this paper, we report the isolation and characterization of mouse IL-6 (mIL-6) cDNA clones from cDNA libraries constructed with mRNA from IL-1-induced bone marrow stromal cells. The DNA sequence of mIL-6‡ and its deduced amino acid sequence are also reported. These results suggest a possible role for IL-6 in the regulation of hemopoiesis in the bone marrow. Like the human homologue, recombinant mIL-6 produced in mammalian cells has PCT-GF, CSF, and HSF-like activities. In addition, a novel myeloid cell growth factor activity is also described here for mIL-6.

MATERIALS AND METHODS

Cell Lines and Isolation of mRNA. GY30, 30R, and ALC-5 cells are stromal cell clones derived from mouse bone marrow cultures (14, 17) and were induced for 7 hr with recombinant human IL-1 at ≈650 units/ml (provided by G. Zurawski, DNAX) without or with cycloheximide at 10 μg/ml. The IL-1 activity was determined by using a proliferation assay with the D10.G4.1 T cell clone (18). D9, a type I helper T-cell clone ($Ly1^+2^-$/9; ref. 19), was induced with Con A at 2 μg/ml for 7 hr. The mouse macrophage cell lines $P388D_1$ and J774A.1 were induced with lipopolysaccharide (LPS) (10 μg/ml) and phorbol 12-myristate 13-acetate (PMA) (500 ng/ml) for 24 hr. Rat H35 and FAZA cells and human HepG2 hepatoma cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal calf serum. FDC-P1, 32D/Cl.5, NFS-60, and DA3.15 are myeloid cell lines (14, 20, 21). MC/9, IC-2, and HT-2 cells are mast cells and helper T cells as described (14, 22).

Total cellular RNA was extracted by using the guanidinium isothiocyanate method (23), and poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography.

Construction and Screening of cDNA Libraries. cDNA libraries were constructed by the method of Okayama and Berg (24). Poly(A)$^+$ RNA from GY30 and 30R cells induced with IL-1 alone or with cycloheximide was used to make cDNA libraries by using the pcD-SRα plasmid vector (25). Based on the human IL-6 cDNA sequence from Hirano *et al.* (1), a full-length cDNA clone was isolated from a human

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Abbreviations: BSF-2, B-cell stimulatory factor 2; IFN-β_2, β_2 interferon; HPGF, hybridoma/plasmacytoma growth factor; HSF, hepatocyte-stimulating factor; PCT-GF, plasmacytoma growth factor; IL-1 through IL-6, interleukins 1–6; mIL-6, mouse IL-6; CSF, colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; Dex, dexamethasone; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (thiazolyl blue).

‡The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03783).

peripheral blood lymphocyte cDNA library (T. Yokota, personal communication).

DNA Sequence Analysis and S1 Nuclease Analysis. Nucleotide sequences were determined by the dideoxy chain-termination method (26) on supercoiled DNA templates with synthetic oligonucleotide primers (27).

S1 nuclease protection analysis was carried out as described (28). pcD-mIL-6 linearized with *Bgl* II was used as probe, and the protected hybrids were analyzed on 5% polyacrylamide/8 M urea gels.

Expression of cDNA Clones by Transfection and Bioassays. Plasmid DNA (5–10 μg) was transfected into 10^6 COS-7 monkey cells by using DEAE-dextran as described (29).

Proliferation of MOPC-315 plasmacytoma cells and factor-dependent hemopoietic cell lines was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; thiazolyl blue] colorimetric assay (30). CSF activity on mouse bone marrow cells was assayed as described (31), and cell morphology of the colonies was determined after staining with Wright–Giemsa stain. HSF activity was measured by using the hepatoma cell lines H35, FAZA, and HepG2. Hepatoma cells were incubated with 10% (vol/vol) COS supernatants in RPMI 1640 medium in the presence or absence of 0.1 μM dexamethasone (Dex; Sigma). Cell-conditioned media were collected, and the amount of secreted fibrinogen was determined by an indirect ELISA assay with a goat anti-rat fibrinogen antibody (Cappel Laboratories, Cochranville, PA) and an anti-goat immunoperoxidase conjugate (Jackson ImmunoResearch, West Chester, PA).

RESULTS

Isolation of mIL-6 cDNA. The production of hemopoietic growth factors such as G-CSF and GM-CSF in bone marrow stromal cells is inducible by IL-1 (13, 14), and the induction of GM-CSF mRNA production can be further augmented by an inhibitor of protein synthesis such as cycloheximide (C.M. and F.L., unpublished results). Therefore, we used mRNA isolated from stromal cell clone 30R induced with IL-1 and from clone GY30 induced with both IL-1 and cycloheximide to construct cDNA libraries. The cDNA libraries were constructed in an expression vector, pcD-SRα, which is able to efficiently express cDNA inserts in mammalian cells (25).

Recombinant clones containing mIL-6 were identified by positive hybridization under moderately stringent conditions to a ^{32}P-labeled 482-base-pair (bp) *Sau*3AI fragment isolated from a human IL-6 cDNA. Twenty-two positive clones were obtained by screening 120,000 recombinant clones from both libraries. pcD-mIL-6, isolated from the GY30 cDNA library, contained an insert 1.3 kilobases (kb) long and was chosen for further analysis.

Nucleotide and Amino Acid Sequences of mIL-6. Fig. 1 shows the DNA sequence of the mIL-6 cDNA and the amino acid sequence deduced from the single long open reading frame. The cDNA insert is 1104 bp long excluding the poly(A) tail. The consensus polyadenylylation signal sequence, AATAAA, is located at positions 1086–1091. The open reading frame of 632 bp (positions 49–681) could encode a primary translation product of 211 amino acids. In addition, a stretch of 21 amino acids (positions 24–44) matches completely with the reported NH_2-terminal sequences of mouse HPGF and PCT-GF, which start at adjacent amino acids (11, 12). This information suggests that the first 24 or 25 amino acids represent the signal peptide for mIL-6, and the mature protein has a final length of 184 or 185 amino acids with a predicted molecular weight of about 18,400. The length of the cDNA sequence and that of the predicted protein sequence are quite similar to those of the human IL-6 gene and its product, suggesting that pcD-mIL-6 is a full-length cDNA clone.

Computer analysis of mIL-6 and human IL-6 sequences reveals 65% homology at the nucleotide level and 41% homology at the amino acid level. As is evident in Fig. 1, there is a high degree of homology between the human and mouse sequences in the region of the signal peptides; however, the sequences of the NH_2-terminal ends of the mature proteins of the two species are quite distinct. This divergence is consistent with the distinct amino acid sequences obtained from purified human IL-6 and mouse HPGF/PCT-GF (1, 3, 4, 11, 12). Interestingly, no potential N-glycosylation sites are present in mIL-6 even though there are two sites in the human sequence. This finding is consistent with reports that human

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TTCTGCTCTG GAGCCCACCA AGAACGATAG TCAATTCCAG AAACCGCT ATG AAG TTC CTC TCT GCA AGA GAC TTC CAT CCA GTT GCC   87
                                                      MET Lys Phe Leu Ser Ala Arg Asp Phe His Pro Val Ala

TTC TTG GGA CTG ATG CTG GTG ACA ACC ACG GCC TTC CCT ACT TCA CAA GTC CGG AGA GGA GAC TTC ACA GAG GAT ACC ACT  168
Phe Leu Gly Leu MET Leu Val Thr Thr Thr Ala Phe Pro Thr Ser Gln Val Arg Arg Gly Asp Phe Thr Glu Asp Thr Thr

CCC AAC AGA CCT GTC TAT ACC ACT TCA CAA GTC GGA GGC TTA ATT ACA CAT GTT CTC TGG GAA ATC GTG GAA ATG AGA AAA  249
Pro Asn Arg Pro Val Tyr Thr Thr Ser Gln Val Gly Gly Leu Ile Thr His Val Leu Trp Glu Ile Val Glu MET Arg Lys

GAG TTG TGC AAT GGC AAT TCT GAT TGT ATG AAC AAC GAT GAT GCA CTT GCA GAA AAC AAT CTG AAA CTT CCA GAG ATA CAA  330
Glu Leu Cys* Asn Gly Asn Ser Asp Cys* MET Asn Asn Asp Asp Ala Leu Ala Glu Asn Asn Leu Lys Leu Pro Glu Ile Gln

AGA AAT GAT GGA TGC TAC CAA ACT GGA TAT AAT CAG GAA ATT TGC CTA TTG AAA ATT TCC TCT GGT CTT CTG GAG TAC CAT  411
Arg Asn Asp Gly Cys* Tyr Gln Thr Gly Tyr Asn Gln Glu Ile Cys* Leu Leu Lys Ile Ser Ser Gly Leu Leu Glu Tyr His

AGC TAC CTG GAG TAC ATG AAG AAC AAC TTA AAA GAT AAC AAG AAA GAC AAA GCC AGA GTC CTT CAG AGA GAT ACA GAA ACT  492
Ser Tyr Leu Glu Tyr MET Lys Asn Asn Leu Lys Asp Asn Lys Lys Asp Lys Ala Arg Val Leu Gln Arg Asp Thr Glu Thr

CTA ATT CAT ATC TTC AAC CAA GAG GTA AAA GAT TTA CAT AAA ATA GTC CTT CCT ACC CCA ATT TCC AAT GCT CTC CTA ACA  573
Leu Ile His Ile Phe Asn Gln Glu Val Lys Asp Leu His Lys Ile Val Leu Pro Thr Pro Ile Ser Asn Ala Leu Leu Thr

GAT AAG CTG GAG TCA CAG AAG GAG TGG CTA AGG ACC AAG ACC ATC CAA TTC ATC TTG AAA TCA CTT GAA GAA TTT CTA AAA  654
Asp Lys Leu Glu Ser Gln Lys Glu Trp Leu Arg Thr Lys Thr Ile Gln Phe Ile Leu Lys Ser Leu Glu Glu Phe Leu Lys

GTC ACT TTG AGA TCT ACT CGG CAA ACC TAG TGCGTTATGC CTAAGCATAT CAGTTTGTGG ACATTCCTCA CTGTGGTCAG          734
Val Thr Leu Arg Ser Thr Arg Gln Thr •

   AAAATATATC CTGTTGTCAG GTATCTGACT TATGTTGTTC TCTACGAAGA ACTGACAATA TGAATGTTGG GACACTATTT          814
   TAATTATTTT TAATTTATTG ATAATTTAAA TAAGTAAACT TTAAGTTAAT TTATGATTGA TATTTATTAT TTTTATGAAG          894
   TGTCACTTGA AATGTTATAT GTTATAGTTT TGAAATGATA ACCTAAAAAT CTATTTGATA TAAATATTCT GTTACCTAGC          974
   CAGATGGTTT CTTGGAATGT ATAAGTTTAC CTCAATGAAT TGCTAATTTA AATATGTTTT TAAAGAAATC TTTGTGATGT         1054
   ATTTTTATAA TGTTTAGACT GTCTTCAAAC AAATAAATTA TATTATATTT                                         1104
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FIG. 1. Nucleotide sequence and deduced amino acid sequence of mIL-6 cDNA. The nucleotide sequence begins with position 1 at the first nucleotide following the oligo(dG) segment. The amino acid sequence begins with the first in-phase ATG codon for the single long open reading frame. The arrows indicate the possible cleavage sites of the signal peptide and the beginning of the mature protein (11, 12). The presumed poly(A) addition signal sequence is boxed. Underlining indicates amino acid residues that are identical to the corresponding human IL-6 protein sequence, and asterisks indicate cysteine residues that are conserved among mouse and human IL-6 and mouse and human G-CSF.

but not mouse HSF is a glycoprotein (32) and suggests that N-glycosylation is not critical for biological activity.

mIL-6 Gene Expression in Different Cell Types. The expression of the mIL-6 gene in different cell types was examined by S1 nuclease analysis. GY30, 30R, and ALC-5 are clonal stromal cells derived from mouse bone marrow cultures (14, 17). mIL-6 gene expression was strongly induced by IL-1 in GY30 and 30R cells (Fig. 2, lanes 1, 2, 4, and 5), and this induction was not affected by cycloheximide (Fig. 2, lane 3). In contrast, ALC-5 cells expressed a low basal level of mIL-6 mRNA, which was unaffected by IL-1 (Fig. 2, lanes 6 and 7). D9, a helper T-cell line (19), only expressed significant amounts of mIL-6 mRNA upon stimulation with Con A (Fig. 2, lanes 8 and 9). For the two mouse macrophage cell lines examined, expression of mIL-6 mRNA was inducible by treatment with LPS and PMA.

It should be noted that in cells expressing mIL-6 mRNA, two additional bands slightly shorter than 650 bp were observed. This may represent overdigestion by the S1 nuclease, or, alternatively, multiple transcriptional start sites as demonstrated by Yasukawa *et al.* (33) for the human IL-6 gene.

Biological Activities of mIL-6. To characterize the biological activities of mIL-6, pcD-mIL-6 plasmid DNA was transfected into COS-7 monkey cells, and the cell supernatants were tested for PCT-GF, HSF, and hemopoietic cell growth factor activities.

Cells derived from *in vivo* plasmacytomas require exogenous factors for survival and proliferation in culture (34). Nordan and Potter (9) recently described a PCT-GF derived from mouse macrophages, and Van Damme *et al.* (3, 4) have demonstrated that human IL-6 possesses HPGF activity. As shown in Fig. 3, the growth of MOPC-315 plasmacytoma cells was stimulated significantly in response to supernatants from mIL-6- or human IL-6-transfected COS-7 cell cultures. These

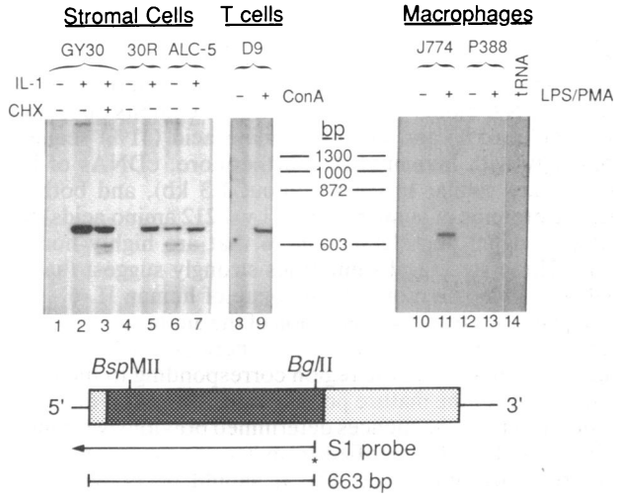

FIG. 2. S1 nuclease protection analysis of mIL-6 mRNA expression. RNA isolated from different cell types was hybridized to the mIL-6 probe, which was end-labeled at the *Bgl* II site (specific activity, 10^6 cpm/μg). S1 nuclease-resistant hybrids were fractionated by denaturing polyacrylamide gel electrophoresis. RNA in lanes: 1–3, from uninduced (lane 1), IL-1-induced (lane 2), or IL-1/cycloheximide (CHX)-induced GY30 cells; 4 and 5, from uninduced (lane 4) or IL-1-induced (lane 5) 30R cells; 6 and 7, from uninduced (lane 6) or IL-1-induced (lane 7) ALC-5 cells; 8 and 9, from uninduced (lane 8) or Con A-induced (lane 9) D9 cells; 10 and 11, from uninduced (lane 10) or LPS/PMA-induced (lane 10) J774A.1 cells; 12 and 13, from uninduced (lane 12) or LPS/PMA-induced (lane 13) P388D_1 cells; and 14, tRNA. Different amounts of RNA were used in this analysis: GY30 and 30R, 2 μg of poly(A)$^+$ RNA; ALC-5, 5 μg of poly(A)$^+$ RNA; D9, 1 μg of poly(A)$^+$ RNA; and P388D_1 and J774A.1, 50 μg of total RNA. In the diagram at the bottom, the expected size of the completely protected fragment is shown.

results strongly suggest that mIL-6 is functionally similar to human IL-6 in its PCT-GF activity.

In response to HSF, rat and human hepatoma cells can be induced to secrete acute-phase reactants such as fibrinogen and α_2-macroglobulin. The induction in rat cells is further enhanced by the presence of glucocorticoids such as Dex (32). Using a modified ELISA assay to detect fibrinogen, we found that the level of fibrinogen secreted by H35 cells was stimulated ≈2-fold in response to supernatants from mIL-6- or human IL-6-transfected cells, and this stimulation was augmented >5-fold by the presence of Dex (Fig. 4). Similar patterns of fibrinogen induction were observed with another rat hepatoma cell line, FAZA (data not shown). Interestingly, human HepG2 cells only responded to human IL-6 but not to mIL-6-containing cell supernatants (data not shown). This species-specific difference in responsiveness has been observed for HSF activity (10). Taken together, the HSF activity exhibited by mIL-6 and its enhancement by Dex in rat cells strongly suggest that mIL-6 is also a mouse HSF.

Several CSFs and lymphokines have been shown to be secreted by stromal cells (for review, see ref. 35) and may be important for regulating hemopoietic cell growth. The finding that mIL-6 is produced by bone marrow stromal cells led us to explore the potential of mIL-6 to support myeloid or lymphoid cell growth. For this purpose, a number of factor-dependent cell lines were used. NFS-60 was the only factor-dependent cell line examined that showed significant proliferation in the presence of mIL-6 (Fig. 5; summary in Table 1). Human IL-6 was also capable of supporting NFS-60 cell growth (data not shown). None of the T cells, mast cells, or other myeloid cell lines that were tested responded to mIL-6.

To examine the effects of mIL-6 on normal cells, hemopoietic progenitor cells from mouse bone marrow were exposed to mIL-6, which stimulated significant colony formation when incubated with bone marrow cells in methylcellulose (Table 2). Of the colonies formed in the presence of mIL-6, 20% were granulocytes, 13% were macrophages, and 67% were mixed granulocyte and macrophage colonies. The

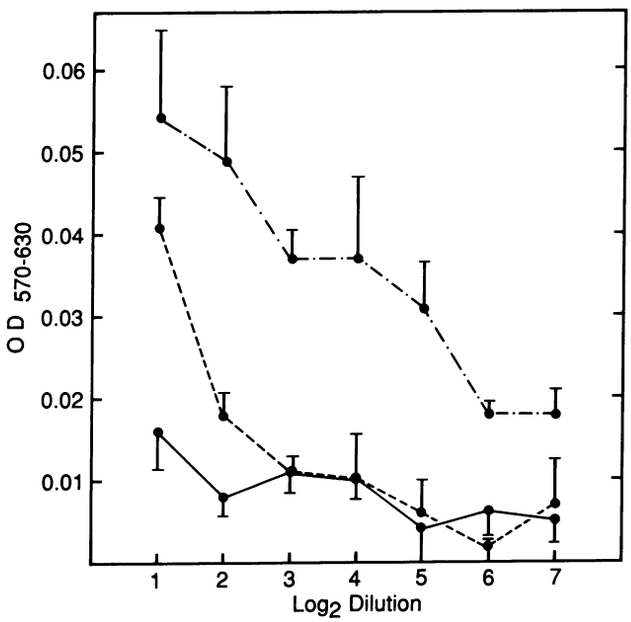

FIG. 3. PCT-GF activity of mIL-6: proliferation of plasmacytoma MOPC-315 cells. MOPC-315 (a murine plasmacytoma cell line) cells were incubated with serially diluted cell culture supernatants from mock-transfected (●—●), mIL-6-transfected (●-·-●), or human IL-6-transfected (●---●) COS-7 cells. After 3 days, viable cell number was determined by the colorimetric MTT assay (30). Starting dilution was 1:500. Data points are means ± SD of triplicate measurements.

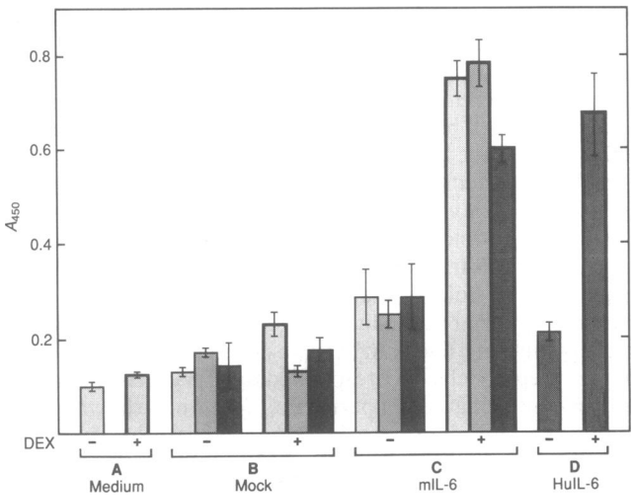

FIG. 4. HSF activity of mIL-6: induction of fibrinogen secretion. H35 cells were exposed to serum-free medium (bars A) or to medium containing 10% supernatant from mock transfection (bars B) or from COS-7 cells transfected with mIL-6 (bars C) and human IL-6 (bars D) in the presence or absence of 0.1 μM Dex as indicated. The cell media were collected 24 hr later, and the amount of secreted fibrinogen was determined by an ELISA assay with 2-2'-azinobis[(3-ethylbenzthiazoline) sulfonic acid] as substrate. Results obtained with COS-7 supernatants from three separate transfections are presented here as differently shaded bars. Each bar represents the mean of triplicate absorbance readings ± SD for H35-conditioned medium samples at 1:270 dilution, a dilution at which the response measured in the ELISA assay was linear.

mixed colonies contained predominantly granulocytes. These results indicate that mIL-6 is a CSF for normal bone marrow progenitor cells and stimulates both granulocyte and macrophage formation.

To confirm that the biological activities detected for mIL-6 are due to direct effects on the target cells, mIL-6-containing COS- supernatants were partially purified by hydroxyapatite or by reverse-phase column chromatography. In both cases, the four different biological activities described above were coeluted in the same fractions, indicating that the same moiety is responsible for all four activities (data not shown). Together, these results suggest that, in addition to being a PCT-GF and HSF as previously described, IL-6 is also a

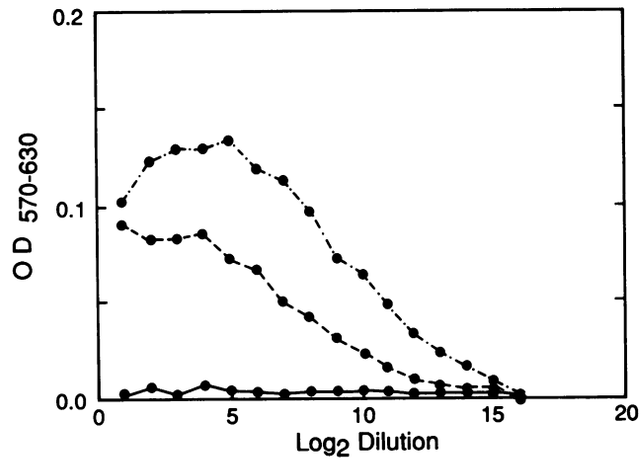

FIG. 5. mIL-6 supports the proliferation of NFS-60 cells. NFS-60 cells were incubated for 24 hr with serial dilutions of COS-7 cell supernatants containing mIL-3 (●-·-●), reversed-phase HPLC-purified mIL-6 (●---●), or mock-transfected COS-7 cell supernatant (●—●). Viable cell number was determined by the colorimetric MTT assay (30).

Table 1. Growth response of factor-dependent cells to mIL-6

Cells	Growth factor dependence	mIL-6 response*
Myeloid cells		
FDC-P1	IL-3	−
32D/Cl.5	IL-3	−
DA3.15	IL-3/GM-CSF	−
NFS-60	IL-3/G-CSF	+
Mast cells		
MC/9	IL-3/IL-4	−
IC2.9	IL-3/GM-CSF	−
T cells		
HT-2	IL-2/IL-4	−

*Different cell lines were plated in the presence of serial dilutions of COS-7 supernatants from mock transfection or from mIL-6-transfected cells. After incubation for 24 hr, cell proliferation was determined by an MTT colorimetric assay.

growth factor for a myeloid cell line and for normal bone marrow progenitor cells.

DISCUSSION

Multiple biological activities have been described for human IL-6. Depending on the target cells, IL-6 can act as a weak antiviral agent (IFN-β_2), a HPGF or PCT-GF, a BSF-2, a HSF, or a T-cell-activating factor (1–4, 7, 8, 36, 37). To date, PCT-GF, HSF, and T-cell-activating factor activities have been reported in mouse cells, but it was not clear if they were activities of multiple gene products or if they represented the mouse equivalent of human IL-6 (9, 10, 38). Recently, the amino-terminal sequence of mouse HPGF and PCT-GF was determined, but neither of them showed any obvious homology to human IL-6 (11, 12). Therefore, it appeared possible that mouse HPGF/PCT-GF and human IL-6 have overlapping biological activities but are products of two unrelated genes.

We describe here the isolation and characterization of a mIL-6 cDNA clone, pcD-mIL-6, which exhibits substantial nucleotide (65%) and deduced amino acid (41%) sequence homology with human IL-6. Furthermore, cDNAs of both species are similar in length (about 1.3 kb), and both can encode proteins of similar size (211 vs. 212 amino acids) with putative signal peptide sequences that are highly homologous. These structural similarities strongly suggest that this cDNA is indeed a mouse homologue of human IL-6.

Despite the strong conservation of the amino acid sequence between human IL-6 and mIL-6, there is considerable sequence divergence in the region corresponding to the first 30 amino acids of the mature polypeptide. However, this region is identical to the sequences determined previously for mouse HPGF and PCT-GF purified from T-cell or macrophage cell culture supernatants (11, 12). It should be noted that the NH_2-terminal residue was reported to be either at position 24 (phenylalanine, ref. 11) or 25 (proline, ref. 12) of the open

Table 2. mIL-6 stimulates colony formation by bone marrow cells

Stimulus*	Colonies†
Mock	2 ± 1
mIL-6	33 ± 7
mIL-3	196 ± 18

*COS-7 cell culture supernatants containing recombinant mIL-3 or mIL-6 were used at 100 unit/ml; 1 unit of activity corresponds to that dilution of supernatant at which the half-maximal response is obtained in the NFS-60 cell proliferation assay.

†Data represent the means ± SD for triplicate cultures of normal bone marrow cells plated at 1×10^5 cells each. The number of colonies was determined after 10 days of incubation.

reading frame of the cDNA. This difference may reflect heterogeneity in the NH_2 terminus of the mature protein. Expression of mIL-6 in mammalian cells demonstrates that it does support plasmacytoma cell proliferation *in vitro*. Thus, the functional identity and the amino acid sequence identity strongly suggest that pcD-IL-6 is a cDNA clone for PCT-GF.

It has been recently reported that human IL-6 and human monocyte-derived HSF are functionally and immunologically similar (7, 8). The HSF activity demonstrated here for mIL-6 further confirms the identity of mIL-6 as a mouse homologue of human IL-6.

Our results show that the expression of IL-6 was inducible in bone marrow stromal cells, helper T cells, and macrophage cells. This is consistent with and extends previous observations that human IL-6 mRNA is inducible in fibroblasts, an astrocytoma cell line, and a gliocytoma cell line (15, 16, 33). The inducibility of IL-6 expression by exogenous factors such as IL-1 and LPS may be important in the regulation of IL-6 function in these different cell types.

A novel myeloid cell growth activity for IL-6 is also described in the present report. The *in vitro* survival and proliferation of a factor-dependent myeloid cell line (NFS-60) was supported by the presence of mIL-6 or human IL-6. It has been noted that IL-6 and G-CSF, a myeloid cell growth factor, share some similarities in their nucleotide sequences and in the locations of cysteine residues in their amino acid sequences, suggesting at least a distant evolutionary relationship between the two genes (ref. 1 and this report). The response to IL-6 by NFS-60 cells, which also respond to IL-3 and G-CSF, suggests functional similarities between IL-6 and CSFs. Indeed, IL-6 has CSF activity upon normal bone marrow progenitor cells (ref. 39 and this report) and appears to be preferential in stimulating granulocyte and macrophage colony formation. Combined with the fact that IL-6 expression is inducible in bone marrow stromal cells, which are critical for hemopoiesis, it is likely that IL-6 is involved in the regulation of hemopoiesis in the bone marrow. In addition, independent studies have shown that IL-6 can enhance thymocyte proliferation (40). These results and the multiple activities described here for IL-6 demonstrate that IL-6 plays an important role in regulating the growth and differentiation of many cell types.

Note Added in Proof. After the submission of this manuscript, Van Snick *et al.* reported the cloning of murine HP-1 and its homology to human IL-6 (41).

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