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Leukemia inhibitory factor receptor is structurally related to the IL-6 signal transducer, gp130

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Leukemia inhibitory factor (LIF) is a cytokine with a broad range of activities that in many cases parallel those of interleukin-6 (IL-6) although LIF and IL-6 appear to be structurally unrelated. A cDNA clone encoding the human LIF receptor was isolated by expression screening of a human placental cDNA library. The LIF receptor is related to the gp130 'signal-transducing' component of the IL-6 receptor and to the G-CSF receptor, with the transmembrane and cytoplasmic regions of the LIF receptor and gp130 being most closely related. This relationship suggests a common signal transduction pathway for the two receptors and may help to explain similar biological effects of the two ligands. Murine cDNAs encoding soluble LIF receptors were isolated by cross-hybridization and share 70% amino acid sequence identity to the human sequence.

Key words: cDNA cloning/hematopoietin receptor/IL-6/LIF/placenta

Introduction

LIF is a glycoprotein growth and differentiation regulator that has pleiotropic activity in several adult and embryonic systems. In the hematopoietic system, LIF induces the differentiation of certain leukemic cells (Tomida *et al.*, 1984; Metcalf *et al.*, 1988; Maekawa and Metcalf, 1989) and the proliferation of hematopoietic stem cells (Fletcher *et al.*, 1990; Leary *et al.*, 1990), megakaryocyte progenitor cells (Metcalf *et al.*, 1990) and DA1 cells (Moreau *et al.*, 1988). LIF also has activity in bone remodelling (Abe *et al.*, 1986; Metcalf and Gearing, 1989a,b; Reid *et al.*, 1990), induction of the acute phase response in hepatocytes (Baumann and Wong, 1989), inhibition of adipogenesis (Mori *et al.*, 1989), regulation of nerve differentiation (Yamamori *et al.*, 1989; Murphy *et al.*, 1991) and inhibition of kidney epithelial cell differentiation (Tomida *et al.*, 1990). Furthermore, LIF is known to suppress embryonic stem cell differentiation (Smith *et al.*, 1988; Williams *et al.*, 1988).

Like LIF, IL-6 is a potent inflammatory mediator and is active in hepatocyte stimulation in the acute phase response, bone remodelling, neuronal differentiation, hematopoietic stem cell and megakaryocyte progenitor cell proliferation, and myeloid leukemic cell differentiation (reviewed in Hirano *et al.*, 1990), and may also have a role in the developing embryo (Murray *et al.*, 1990). In contrast to LIF, IL-6 induces the proliferation of renal mesangial cells and is strongly implicated in mesangial proliferative glomerulonephritis (Tomida *et al.*, 1990; Hirano *et al.*, 1990). Additionally, IL-6 is a potent T cell growth and differentiation factor, myeloma, plasmacytoma and hybridoma growth factor, and a B cell differentiation factor (Hirano *et al.*, 1990) but has no reported activity on adipogenesis. Two components of the IL-6 receptor have been characterized, a low affinity, binding subunit (Yamasaki *et al.*, 1988) and a non-binding, high affinity-converting, signal transducing subunit, gp130 (Taga *et al.*, 1989; Hibi *et al.*, 1990). The extracellular region of gp130 shares extensive homology to the granulocyte colony-stimulating factor (G-CSF) receptor (Fukunaga *et al.*, 1990a,b; Larsen *et al.*, 1990).

Molecular clones encoding murine and human LIF have been isolated (Gearing *et al.*, 1987; Gough *et al.*, 1988; Moreau *et al.*, 1988) and the recombinant protein tested in animal model systems (Metcalf and Gearing, 1989a,b; Metcalf *et al.*, 1990). LIF has been known under a variety of synonyms (Moreau *et al.*, 1988; Baumann and Wong, 1989; Lowe *et al.*, 1989; Mori *et al.*, 1989; Yamamori *et al.*, 1989) and is naturally produced by a wide range of hematopoietic, mesenchymal and endodermic cell types as both a conventionally secreted form (Gearing *et al.*, 1987; Moreau *et al.*, 1988), and as a matrix-associated form (Rathjen *et al.*, 1990).

LIF action is mediated following binding to specific cellular receptors that trigger differentiation-induction, differentiation-suppression, proliferation or activation depending on the cell type. Studies of the binding characteristics of LIF receptors on a wide range of both human and murine cell types responsive to LIF have generally revealed a dissociation constant K_d = 10-200 pM (Yamamoto-Yamaguchi *et al.*, 1986; Hilton *et al.*, 1988; Williams *et al.*, 1988; Rodan *et al.*, 1990; Tomida *et al.*, 1990), regardless of the function of LIF induced in the various cells. The number of receptors on such cells is relatively low (150-400 per cell). Furthermore, a more numerous, lower affinity LIF receptor has been reported on murine peritoneal macrophages (2000-6000 per cell, K_d = 1-3 nM; Hilton *et al.*, 1991), which also display high affinity receptors. The size of the LIF receptor on responsive cells has not yet been reported.

As a first step in understanding the diverse actions of LIF following binding to its receptor, we have isolated cDNA clones encoding the LIF receptor by expression screening of a cDNA library using radioiodinated LIF as a probe. In this paper, we describe the structure of human and murine LIF receptors and demonstrate the existence of both membrane bound and soluble forms. The structure of the human LIF receptor (HLIFR) offers new insight into a mechanism whereby LIF and IL-6 induce similar biological effects.

Results

Expression cloning of the human LIF receptor

Initial experiments determined that human placental membranes bound between 53 and 65 fmol [^{125}I]LIF per milligram of membrane protein (not shown). We therefore screened a human placental cDNA expression library (Larsen *et al.*, 1990) in COS-7 cells using ^{125}I-labeled recombinant human LIF ([^{125}I]hLIF) and a microscopic autoradiographic detection method (Gearing *et al.*, 1989a). A single cDNA clone (pHLIFR-65) that conferred LIF binding activity was isolated (Figure 1), and its insert used as a hybridization probe to isolate five other clones from the same library, one from an SKHep human hepatoma cDNA library and four from a human genomic library. The nucleotide sequence of the clones was determined and their structures are shown in Figure 1A. The cDNA insert in pHLIFR-65 encoded a single large open reading frame of 971 amino acid residues that had no in-frame translation termination signal at its 3′-end and instead, ended in a stretch of 15 A residues (beginning after nucleotide 3138 in Figure 1B) that were not preceded by a typical polyadenylation signal. The open reading frame was terminated by an in-frame translational stop codon following 15 additional amino acids encoded by the expression vector. Six additional clones, from both the placental library and a human SKHep hepatoma cDNA library, were isolated by hybridization to clone pHLIFR-65 and sequenced (Figure 1A). In each case the 3′ end of the cDNAs coincided with this stretch of A residues, but were otherwise identical in sequence with pHLIFR-65. Based on the assumption that these cDNAs were the result of oligo(dT) priming at an internal site in the HLIFR mRNA during construction of the libraries, a human genomic library was screened with both the insert of pHLIFR-65 and an oligonucleotide based on its 3′ sequence (nucleotide residues 3099–3115 in Figure 1B) and four hybridizing clones were isolated. A subclone derived from one of the genomic clones (HLIFR-gen1) contained sequence that extended the cDNA sequence through and beyond this A-rich stretch of nucleotides. The genomic sequence expanded the open reading frame by 111 amino acid residues until the first in-frame stop codon was encountered. The sequence of the open reading frame deduced by alignment of pHLIFR-65 cDNA and the 3′ genomic sequence is presented in Figure 1B.

In order to confirm that the genomic sequence used to complete the amino acid sequence of the HLIFR cytoplasmic domain was exonic we used a PCR-based approach to detect the contiguous sequence assembled in Figure 1B in human placental mRNA. First strand cDNA was prepared and used as a template in a PCR reaction primed with oligonucleotides that span two introns in the HLIFR gene (intron 1 of >700 bp @ nt 2770 and intron 2 of >900 bp @ nt 2848 in Figure 1B; D.P.Gearing and S.D.Gimpel, unpublished observations). The 5′ oligonucleotide (880, Figure 2) is predicted from the sequence of pHLIFR-65 and the 3′ oligonucleotides (969, 970, Figure 2) are predicted from the sequence of the genomic clone. Specific amplification products of the predicted size were detected following PCR with the cDNA and not with genomic DNA as template (Figure 2). Since no bands were detected in the genomic PCR products it is likely that the distance between the primers was too great for efficient PCR under the conditions used. The assembled sequence in Figure 1B therefore corresponds to the true sequence of the human LIFR cDNA.

The HLIFR preprotein (1097 amino acid residues) thus comprises a 44 amino acid residue signal sequence, a 789 residue extracellular domain, a 26 residue transmembrane domain, and a 238 residue cytoplasmic domain. The extracellular domain of the HLIFR has homology to members of the hematopoietin receptor family (Bazan, 1989; Cosman *et al.*, 1990) and consists of two hematopoietin receptor domains (defined from the first conserved Cys residue to the Trp-Ser-X-Trp-Ser motif) and three repeats of a fibronectin type III-like module (FN III). The membrane-proximal hematopoietin receptor domain has only the first two of the four conserved cysteine residues present in other such structures. There are 20 potential sites for N-linked glycosylation in the HLIFR, 19 of which occur in the extracellular domain. The transmembrane domain consists of a continuous stretch of non-polar residues with a single cysteine residue near the cytosolic face. The cytoplasmic domain is rich in serine and threonine (13%), proline (11%) and acidic (29%) residues, a feature shared by some of the other members of the hematopoietin receptor family such as the erythropoietin receptor (D'Andrea *et al.*, 1989), IL-2 receptor β-chain (Hatekeyama *et al.*, 1989) and G-CSF receptor (Fukunaga *et al.*, 1990a,b; Larsen *et al.*, 1990), and, like other members of the hematopoietin receptor family, appears to be devoid of any protein kinase, GTP binding, or other known motifs associated with signal transduction.

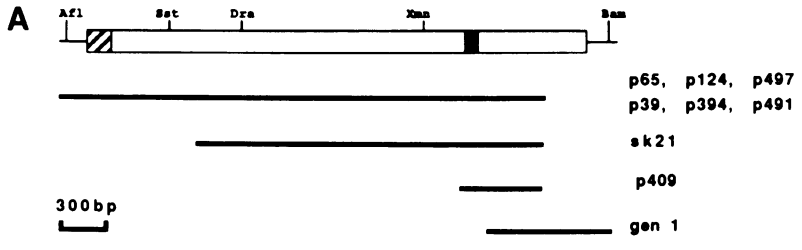
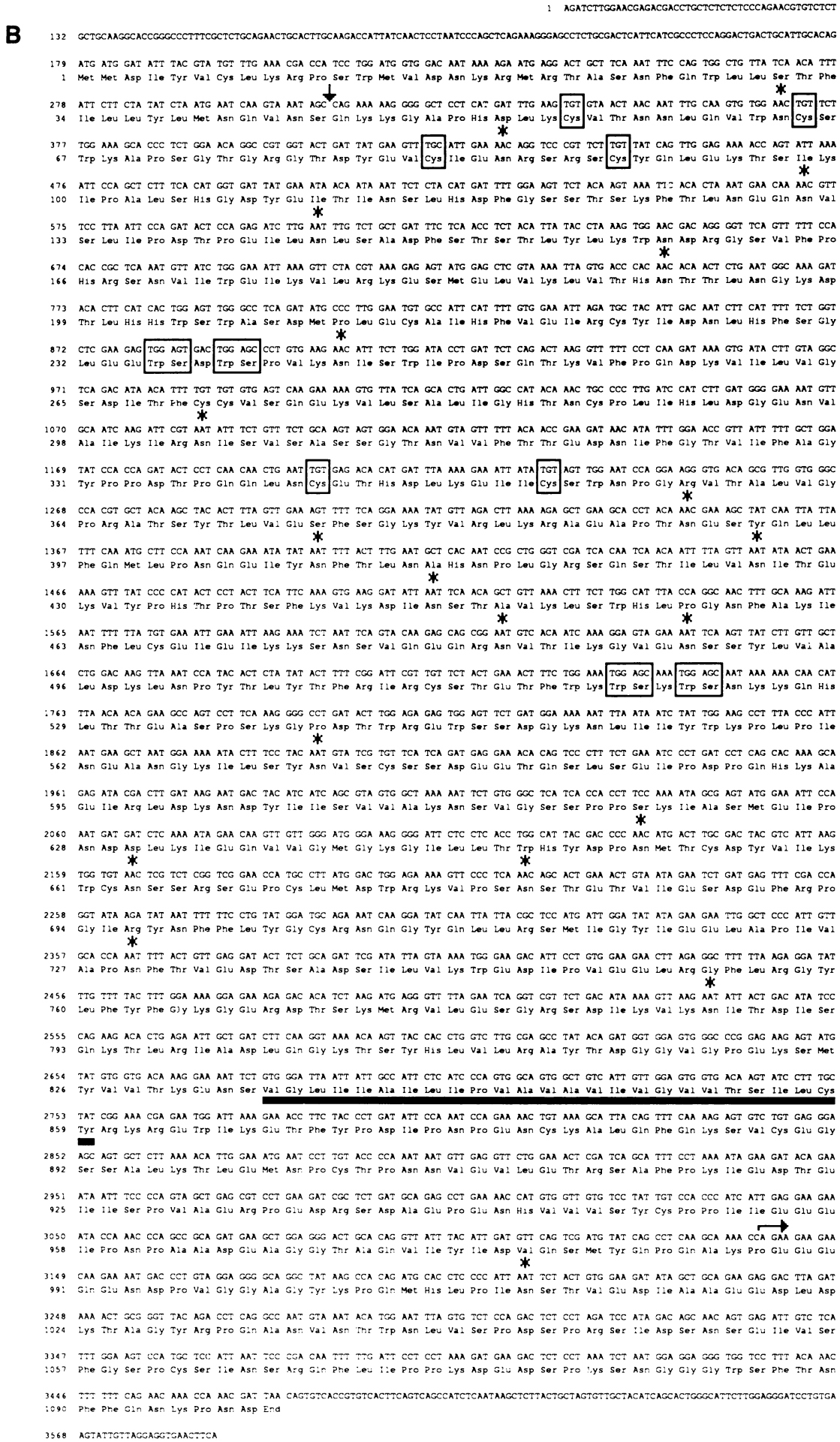


Fig. 1. Composite map and sequence of human LIF receptor clones. (**A**) Alignment of cDNA clones from the placental library (p) and the SKHep library (sk) with the region of the cloned genomic sequence (gen). The HLIFR open reading frame is shown boxed. The signal sequence is shown as a hatched box and the putative transmembrane domain is shown as a solid box. Some restriction endonuclease cleavage sites are shown; *Afl*II (Afl), *Sst*I (Sst), *Dra*I (Dra), *Xma*I (Xma), *Bam*HI (Bam). (**B**) Composite sequence derived from the cDNA and genomic clones shown in (A). The HLIFR open reading frame is shown beneath the nucleotide sequence. The predicted signal peptidase cleavage site is marked with a vertical arrow. The putative transmembrane domain is heavily underlined. Potential N-linked glycosylation sites are marked with asterisks. Hallmark residues associated with the hematopoietin family of receptors (Cosman *et al.*, 1990) are shown boxed. The horizontal arrow marks the point at which genomic sequence was used to derive the 3′ coding region of the HLIFR. All cDNA clones terminated with a stretch of A nucleotides at this point.

B


1 AGATCTTGGAACGAGACGACCTGCTCTCTCTCCCAGAACGTGTCTCT 131
132 GCTGCAAGGCACCGGGCCCTTTCGCTCTGCAGAACTGCACTTGCAAGACCATTATCAACTCCTAATCCCAGCTCAGAAAGGGAGCCTCTGCGACTCATTCATCGCCCTCCAGGACTGACTGCATTGCACAG 178
179 ATG ATG GAT ATT TAC GTA TGT TTG AAA CGA CCA TCC TGG ATG GTG GAC AAT AAA AGA ATG AGG ACT GCT TCA AAT TTC CAG TGG CTG TTA TCA ACA TTT 277
1 Met Met Asp Ile Tyr Val Cys Leu Lys Arg Pro Ser Trp Met Val Asp Asn Lys Arg Met Arg Thr Ala Ser Asn Phe Gln Trp Leu Leu Ser Thr Phe 33
278 ATT CTT CTA TAT CTA ATG AAT CAA GTA AAT AGC CAG AAA AAG GGG GCT CCT CAT GAT TTG AAG TGT GTA ACT AAC AAT TTG CAA GTG TGG AAC TGT TCT 376
34 Ile Leu Leu Tyr Leu Met Asn Gln Val Asn Ser Gln Lys Lys Gly Ala Pro His Asp Leu Lys Cys Val Thr Asn Asn Leu Gln Val Trp Asn Cys Ser 66
377 TGG AAA GCA CCC TCT GGA ACA GGC CGT GGT ACT GAT TAT GAA GTT TGC ATT GAA AAC AGG TCC CGT TCT TGT TAT CAG TTG GAG AAA ACC AGT ATT AAA 475
67 Trp Lys Ala Pro Ser Gly Thr Gly Arg Gly Thr Asp Tyr Glu Val Cys Ile Glu Asn Arg Ser Arg Ser Cys Tyr Gln Leu Glu Lys Thr Ser Ile Lys 99
476 ATT CCA GCT CTT TCA CAT GGT GAT TAT GAA ATA ACA ATA AAT TCT CTA CAT GAT TTT GGA AGT TCT ACA AGT AAA TTC ACA CTA AAT GAA CAA AAC GTT 574
100 Ile Pro Ala Leu Ser His Gly Asp Tyr Glu Ile Thr Ile Asn Ser Leu His Asp Phe Gly Ser Ser Thr Ser Lys Phe Thr Leu Asn Glu Gln Asn Val 132
575 TCC TTA ATT CCA GAT ACT CCA GAG ATC TTG AAT TTG TCT GCT GAT TTC TCA ACC TCT ACA TTA TAC CTA AAG TGG AAC GAC AGG GGT TCA GTT TTT CCA 673
133 Ser Leu Ile Pro Asp Thr Pro Glu Ile Leu Asn Leu Ser Ala Asp Phe Ser Thr Ser Thr Leu Tyr Leu Lys Trp Asn Asp Arg Gly Ser Val Phe Pro 165
674 CAC CGC TCA AAT GTT ATC TGG GAA ATT AAA GTT CTA CGT AAA GAG AGT ATG GAG CTC GTA AAA TTA GTG ACC CAC AAC ACA ACT CTG AAT GGC AAA GAT 772
166 His Arg Ser Asn Val Ile Trp Glu Ile Lys Val Leu Arg Lys Glu Ser Met Glu Leu Val Lys Leu Val Thr His Asn Thr Thr Leu Asn Gly Lys Asp 198
773 ACA CTT CAT CAC TGG AGT TGG GCC TCA GAT ATG CCC TTG GAA TGT GCC ATT CAT TTT GTG GAA ATT AGA TGC TAC ATT GAC AAT CTT CAT TTT TCT GGT 871
199 Thr Leu His His Trp Ser Trp Ala Ser Asp Met Pro Leu Glu Cys Ala Ile His Phe Val Glu Ile Arg Cys Tyr Ile Asp Asn Leu His Phe Ser Gly 231
872 CTC GAA GAG TGG AGT GAC TGG AGC CCT GTG AAG AAC ATT TCT TGG ATA CCT GAT TCT CAG ACT AAG GTT TTT CCT CAA GAT AAA GTG ATA CTT GTA GGC 970
232 Leu Glu Glu Trp Ser Asp Trp Ser Pro Val Lys Asn Ile Ser Trp Ile Pro Asp Ser Gln Thr Lys Val Phe Pro Gln Asp Lys Val Ile Leu Val Gly 264
971 TCA GAC ATA ACA TTT TGT TGT GTG AGT CAA GAA AAA GTG TTA TCA GCA CTG ATT GGC CAT ACA AAC TGC CCC TTG ATC CAT CTT GAT GGG GAA AAT GTT 1069
265 Ser Asp Ile Thr Phe Cys Cys Val Ser Gln Glu Lys Val Leu Ser Ala Leu Ile Gly His Thr Asn Cys Pro Leu Ile His Leu Asp Gly Glu Asn Val 297
1070 GCA ATC AAG ATT CGT AAT ATT TCT GTT TCT GCA AGT AGT GGA ACA AAT GTA GTT TTT ACA ACC GAA GAT AAC ATA TTT GGA ACC GTT ATT TTT GCT GGA 1168
298 Ala Ile Lys Ile Arg Asn Ile Ser Val Ser Ala Ser Ser Gly Thr Asn Val Val Phe Thr Thr Glu Asp Asn Ile Phe Gly Thr Val Ile Phe Ala Gly 330
1169 TAT CCA CCA GAT ACT CCT CAA CAA CTG AAT TGT GAG ACA CAT GAT TTA AAA GAA ATT ATA TGT AGT TGG AAT CCA GGA AGG GTG ACA GCG TTG GTG GGC 1267
331 Tyr Pro Pro Asp Thr Pro Gln Gln Leu Asn Cys Glu Thr His Asp Leu Lys Glu Ile Ile Cys Ser Trp Asn Pro Gly Arg Val Thr Ala Leu Val Gly 363
1268 CCA CGT GCT ACA AGC TAC ACT TTA GTT GAA AGT TTT TCA GGA AAA TAT GTT AGA CTT AAA AGA GCT GAA GCA CCT ACA AAC GAA AGC TAT CAA TTA TTA 1366
364 Pro Arg Ala Thr Ser Tyr Thr Leu Val Glu Ser Phe Ser Gly Lys Tyr Val Arg Leu Lys Arg Ala Glu Ala Pro Thr Asn Glu Ser Tyr Gln Leu Leu 396
1367 TTT CAA ATG CTT CCA AAT CAA GAA ATA TAT AAT TTT ACT TTG AAT GCT CAC AAT CCG CTG GGT CGA TCA CAA TCA ACA ATT TTA GTT AAT ATA ACT GAA 1465
397 Phe Gln Met Leu Pro Asn Gln Glu Ile Tyr Asn Phe Thr Leu Asn Ala His Asn Pro Leu Gly Arg Ser Gln Ser Thr Ile Leu Val Asn Ile Thr Glu 429
1466 AAA GTT TAT CCC CAT ACT CCT ACT TCA TTC AAA GTG AAG GAT ATT AAT TCA ACA GCT GTT AAA CTT TCT TGG CAT TTA CCA GGC AAC TTT GCA AAG ATT 1564
430 Lys Val Tyr Pro His Thr Pro Thr Ser Phe Lys Val Lys Asp Ile Asn Ser Thr Ala Val Lys Leu Ser Trp His Leu Pro Gly Asn Phe Ala Lys Ile 462
1565 AAT TTT TTA TGT GAA ATT GAA ATT AAG AAA TCT AAT TCA GTA CAA GAG CAG CGG AAT GTC ACA ATC AAA GGA GTA GAA AAT TCA AGT TAT CTT GTT GCT 1663
463 Asn Phe Leu Cys Glu Ile Glu Ile Lys Lys Ser Asn Ser Val Gln Glu Gln Arg Asn Val Thr Ile Lys Gly Val Glu Asn Ser Ser Tyr Leu Val Ala 495
1664 CTG GAC AAG TTA AAT CCA TAC ACT CTA TAT ACT TTT CGG ATT CGT TGT TCT ACT GAA ACT TTC TGG AAA TGG AGC AAA TGG AGC AAT AAA AAA CAA CAT 1762
496 Leu Asp Lys Leu Asn Pro Tyr Thr Leu Tyr Thr Phe Arg Ile Arg Cys Ser Thr Glu Thr Phe Trp Lys Trp Ser Lys Trp Ser Asn Lys Lys Gln His 528
1763 TTA ACA ACA GAA GCC AGT CCT TCA AAG GGG CCT GAT ACT TGG AGA GAG TGG AGT TCT GAT GGA AAA AAT TTA ATA ATC TAT TGG AAG CCT TTA CCC ATT 1861
529 Leu Thr Thr Glu Ala Ser Pro Ser Lys Gly Pro Asp Thr Trp Arg Glu Trp Ser Ser Asp Gly Lys Asn Leu Ile Ile Tyr Trp Lys Pro Leu Pro Ile 561
1862 AAT GAA GCT AAT GGA AAA ATA CTT TCC TAC AAT GTA TCG TGT TCA TCA GAT GAG GAA ACA CAG TCC CTT TCT GAA ATC CCT GAT CCT CAG CAC AAA GCA 1960
562 Asn Glu Ala Asn Gly Lys Ile Leu Ser Tyr Asn Val Ser Cys Ser Ser Asp Glu Glu Thr Gln Ser Leu Ser Glu Ile Pro Asp Pro Gln His Lys Ala 594
1961 GAG ATA CGA CTT GAT AAG AAT GAC TAC ATC ATC AGC GTA GTG GCT AAA AAT TCT GTG GGC TCA TCA CCA CCT TCC AAA ATA GCG AGT ATG GAA ATT CCA 2059
595 Glu Ile Arg Leu Asp Lys Asn Asp Tyr Ile Ile Ser Val Val Ala Lys Asn Ser Val Gly Ser Ser Pro Pro Ser Lys Ile Ala Ser Met Glu Ile Pro 627
2060 AAT GAT GAT CTC AAA ATA GAA CAA GTT GTT GGG ATG GGA AAG GGG ATT CTC CTC ACC TGG CAT TAC GAC CCC AAC ATG ACT TGC GAC TAC GTC ATT AAG 2158
628 Asn Asp Asp Leu Lys Ile Glu Gln Val Val Gly Met Gly Lys Gly Ile Leu Leu Thr Trp His Tyr Asp Pro Asn Met Thr Cys Asp Tyr Val Ile Lys 660
2159 TGG TGT AAC TCG TCT CGG TCG GAA CCA TGC CTT ATG GAC TGG AGA AAA GTT CCC TCA AAC AGC ACT GAA ACT GTA ATA GAA TCT GAT GAG TTT CGA CCA 2257
661 Trp Cys Asn Ser Ser Arg Ser Glu Pro Cys Leu Met Asp Trp Arg Lys Val Pro Ser Asn Ser Thr Glu Thr Val Ile Glu Ser Asp Glu Phe Arg Pro 693
2258 GGT ATA AGA TAT AAT TTT TTC CTG TAT GGA TGC AGA AAT CAA GGA TAT CAA TTA TTA CGC TCC ATG ATT GGA TAT ATA GAA GAA TTG GCT CCC ATT GTT 2356
694 Gly Ile Arg Tyr Asn Phe Phe Leu Tyr Gly Cys Arg Asn Gln Gly Tyr Gln Leu Leu Arg Ser Met Ile Gly Tyr Ile Glu Glu Leu Ala Pro Ile Val 726
2357 GCA CCA AAT TTT ACT GTT GAG GAT ACT TCT GCA GAT TCG ATA TTA GTA AAA TGG GAA GAC ATT CCT GTG GAA GAA CTT AGA GGC TTT TTA AGA GGA TAT 2455
727 Ala Pro Asn Phe Thr Val Glu Asp Thr Ser Ala Asp Ser Ile Leu Val Lys Trp Glu Asp Ile Pro Val Glu Glu Leu Arg Gly Phe Leu Arg Gly Tyr 759
2456 TTG TTT TAC TTT GGA AAA GGA GAA AGA GAC ACA TCT AAG ATG AGG GTT TTA GAA TCA GGT CGT TCT GAC ATA AAA GTT AAG AAT ATT ACT GAC ATA TCC 2554
760 Leu Phe Tyr Phe Gly Lys Gly Glu Arg Asp Thr Ser Lys Met Arg Val Leu Glu Ser Gly Arg Ser Asp Ile Lys Val Lys Asn Ile Thr Asp Ile Ser 792
2555 CAG AAG ACA CTG AGA ATT GCT GAT CTT CAA GGT AAA ACA AGT TAC CAC CTG GTC TTG CGA GCC TAT ACA GAT GGT GGA GTG GGC CCG GAG AAG AGT ATG 2653
793 Gln Lys Thr Leu Arg Ile Ala Asp Leu Gln Gly Lys Thr Ser Tyr His Leu Val Leu Arg Ala Tyr Thr Asp Gly Gly Val Gly Pro Glu Lys Ser Met 825
2654 TAT GTG GTG ACA AAG GAA AAT TCT GTG GGA TTA ATT ATT GCC ATT CTC ATC CCA GTG GCA GTG GCT GTC ATT GTT GGA GTG GTG ACA AGT ATC CTT TGC 2752
826 Tyr Val Val Thr Lys Glu Asn Ser Val Gly Leu Ile Ile Ala Ile Leu Ile Pro Val Ala Val Ala Val Ile Val Gly Val Val Thr Ser Ile Leu Cys 858
2753 TAT CGG AAA CGA GAA TGG ATT AAA GAA ACC TTC TAC CCT GAT ATT CCA AAT CCA GAA AAC TGT AAA GCA TTA CAG TTT CAA AAG AGT GTC TGT GAG GGA 2851
859 Tyr Arg Lys Arg Glu Trp Ile Lys Glu Thr Phe Tyr Pro Asp Ile Pro Asn Pro Glu Asn Cys Lys Ala Leu Gln Phe Gln Lys Ser Val Cys Glu Gly 891
2852 AGC AGT GCT CTT AAA ACA TTG GAA ATG AAT CCT TGT ACC CCA AAT AAT GTT GAG GTT CTG GAA ACT CGA TCA GCA TTT CCT AAA ATA GAA GAT ACA GAA 2950
892 Ser Ser Ala Leu Lys Thr Leu Glu Met Asn Pro Cys Thr Pro Asn Asn Val Glu Val Leu Glu Thr Arg Ser Ala Phe Pro Lys Ile Glu Asp Thr Glu 924
2951 ATA ATT TCC CCA GTA GCT GAG CGT CCT GAA GAT CGC TCT GAT GCA GAG CCT GAA AAC CAT GTG GTT GTG TCC TAT TGT CCA CCC ATC ATT GAG GAA GAA 3049
925 Ile Ile Ser Pro Val Ala Glu Arg Pro Glu Asp Arg Ser Asp Ala Glu Pro Glu Asn His Val Val Val Ser Tyr Cys Pro Pro Ile Ile Glu Glu Glu 957
3050 ATA CCA AAC CCA GCC GCA GAT GAA GCT GGA GGG ACT GCA CAG GTT ATT TAC ATT GAT GTT CAG TCG ATG TAT CAG CCT CAA GCA AAA CCA GAA GAA GAA 3148
958 Ile Pro Asn Pro Ala Ala Asp Glu Ala Gly Gly Thr Ala Gln Val Ile Tyr Ile Asp Val Gln Ser Met Tyr Gln Pro Gln Ala Lys Pro Glu Glu Glu 990
3149 CAA GAA AAT GAC CCT GTA GGA GGG GCA GGC TAT AAG CCA CAG ATG CAC CTC CCC ATT AAT TCT ACT GTG GAA GAT ATA GCT GCA GAA GAG GAC TTA GAT 3247
991 Gln Glu Asn Asp Pro Val Gly Gly Ala Gly Tyr Lys Pro Gln Met His Leu Pro Ile Asn Ser Thr Val Glu Asp Ile Ala Ala Glu Glu Asp Leu Asp 1023
3248 AAA ACT GCG GGT TAC AGA CCT CAG GCC AAT GTA AAT ACA TGG AAT TTA GTG TCT CCA GAC TCT CCT AGA TCC ATA GAC AGC AAC AGT GAG ATT GTC TCA 3346
1024 Lys Thr Ala Gly Tyr Arg Pro Gln Ala Asn Val Asn Thr Trp Asn Leu Val Ser Pro Asp Ser Pro Arg Ser Ile Asp Ser Asn Ser Glu Ile Val Ser 1056
3347 TTT GGA AGT CCA TGC TCC ATT AAT TCC GGA CAA TTT TTG ATT CCT CCT AAA GAT GAA GAC TCT CCT AAA TCT AAT GGA GGA GGG TGG TCC TTT ACA AAC 3445
1057 Phe Gly Ser Pro Cys Ser Ile Asn Ser Arg Gln Phe Leu Ile Pro Pro Lys Asp Glu Asp Ser Pro Lys Ser Asn Gly Gly Gly Trp Ser Phe Thr Asn 1089
3446 TTT TTT CAG AAC AAA CCA AAC GAT TAA CAGTGTCACCGTGTCACTTCAGTCAGCCATCTCAATAAGCTCTTACTGCTAGTGTTGCTACATCAGCACTGGGCATTCTTGGAGGGATCCTGTGA 3567
1090 Phe Phe Gln Asn Lys Pro Asn Asp End
3568 AGTATTGTTAGGAGGTGAACTTCA

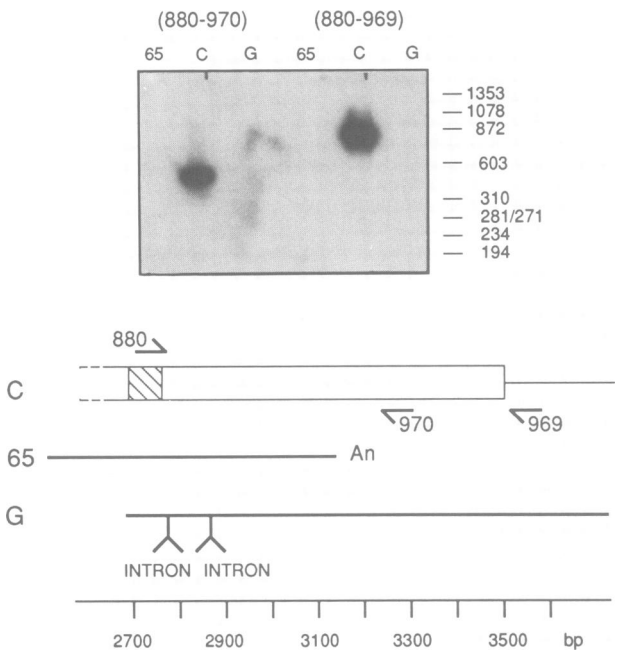


Fig. 2. Polymerase chain reaction amplification of the LIFR cytoplasmic domain from human placental cDNA. (**Upper**) Autoradiograph of PCR products transferred to nitrocellulose. Oligonucleotides used as primers are shown above the templates used in each reaction: 880, 970 and 969 refer to identification numbers of each oligonucleotide; 65, pHLIFR-65; C, cDNA prepared from human placental mRNA; G, human genomic DNA. The blot was probed with a 17mer oligonucleotide (nt 3099–3115 in Figure 1B) radiolabeled with [^{32}P]ATP and polynucleotide kinase (Sambrook *et al.*, 1989). Sizes of marker DNAs used (ϕX174/*Hae*III) are shown at right. (**Lower**) Explanatory diagram. The open box refers to the HLIFR coding region and the hatched box refers to the transmembrane domain. Symbols as upper panel except A_n, poly(A). The two introns are located at nt 2770 (>700 bp) and nt 2848 (>900 bp). Oligonucleotides 880, 970 and 969 have their 5′ ends at positions 2720, 3233 and 3529 respectively in Figure 1B. Amplification products of 513 bp (880–970) and 809 bp (880–969) were predicted from Figure 1B. Scale at bottom refers to Figure 1B.

Cloning of murine LIF receptor cDNAs

A murine cDNA library prepared from adult liver mRNA was hybridized to the insert from pHLIFR-65 and two clones were isolated and their nucleotide sequences were determined. The predicted amino acid sequence corresponding to the open reading frame of the longer clone (pMLIFR-3) is aligned with the human receptor in Figure 3. The sequences share 76% amino acid identity throughout the signal sequence, the two hematopoietin receptor domains and the first two FN III repeats, but the mouse cDNAs have a stop codon before the third FN III repeat. Thus these MLIFR clones would be predicted to encode a soluble LIFR. The MLIFR is most homologous to the HLIFR in the region of the FN III repeats. Strikingly, the membrane-distal hematopoietin receptor domain has none of the potential N-linked glycosylation sites found in the human receptor (Figure 3).

Characterization of LIF receptors expressed in COS-7 cells

Clone pHLIFR-65 was transfected into COS-7 cells and binding analysis of the expressed receptors with [^{125}I]HLIF was performed (Figure 4). A representative experiment

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  1 MMDIYVCLKRPSWMVDNKRMRTASNFQWLLSTFILLYLMNQVNSQKKGAPH  51
  1  -AA-SWWRQ---------S-MTP-LP----ALT--H-TMHA-GL-R-V#Q  49

 52 DLKCVTNNLQVWNCSWKAPSGTGRGTDYEVCIENRSRSCYQLEKTSIKIP 101
 50 ----T---MR--D-T-P--L-VSP--VKDI--KD-FH--HP--T-NV---  99

102 ALSHGDYEITINSLHDFGSSTSKFTLNEQNVSLIPDTPEILNLSADFSTS 151
100 ---P--H-V---Y-NG-Q###-------KD-----E-----D-----F--  146

152 TLYLKWNDRGSVFPHRSNVIWEIKVLRKESMELVKLVTHNTTLNGKDTLH 201
147 S-L--------AL--P--AT------QNPRT-P-A--LL--M-S----VQ 196
                                  ** **
202 HWSWASDMPLECAIHFVEIRCYIDNLHFSGLEEWSDWSPVKNISWIPDSQ 251
197 --N-T--L--Q--T-S-S--WH--SP----YK------L------RNTE  246

252 TKVFPQDKVILVGSDITFCCVSQEKVLSALIGHTNCPLIHLDGENVAIKI 301
247 -N-------V-A--NM-I--M-PT----GQ--N-LR-----Y-QT---H- 296

302 RNISVSASSGTNVVFTTEDNIFGTVIFAGYPPDTPQQLNCETHDLKEIIC 351
297 L--P--EN----II-I-D-DVY---V-------V--K-S---------- 346

352 SWNPGRVTALVGPRATSYTLVESFSGKYVRLKRAEAPTNESYQLLFQMLP 401
347 ------I-G-----N-E---F--I---SAVFH-I-GL---T-R-GV--H- 396

402 NQEIYNFTLNAHNPLGRSQSTILVNITEKVYPHTPTSFKVKDINSTAVKL 451
397 G---H----TGR----QA--AVVI-V--R-A--D---L--------V-TF 446

452 SWHLPGNFAKINFLCEIEIKKSNSVQEQRNVTIKGVENSSYLVALDKLNP 501
447 --Y-----T---L--Q---C-A--KK-V--A--R-A-D-T-H--V-----  496
                  ** **
502 YTLYTFRIRCSTETFWKWSKWSNKKQHLTTEASPSKGPDTWREWSSDGKN 551
497 --A----V---SK------R--DE-R------T----------------- 546

552 LIIYWKPLPINEANGKILSYNVSCSSDEETQSLSEIPDPQHKAEIRLDKN 601
547 --V----------------------LN-----VL--F----R---Q-S-- 596

602 DYIISVVAKNSVGSSPPSKIASMEIPNDDLKIEQVVGMGKGILLTWHYDP 651
597 --------R--A-----------------ITV--A--L-NR-F---RH-- 646

652 NMTCDYVIKWCNSSRSEPCLMDWRKVPSNSTETVIESDEFRPGIRYNFFL 701
647 --------------------L-----------------Q-Q--V----Y- 696

702 YGCRNQGYQLLRSMIGYIEELAPIVAPNFTVEDTSADSILVKWEDIPVEE 751
697 ---T----------I---V---EA* 719

752 LRGFLRGYLFYFGKGERDTSKMRVLESGRSDIKVKNITDISQKTLRIADL 801

802 QGKTSYHLVLRAYTDGGVGPEKSMYVVTKENSVGLIIAILIPVAVAVIVG 851

852 VVTSILCYRKREWIKETFYPDIPNPENCKALQFQKSVCEGSSALKTLEMN 901

902 PCTPNNVEVLETRSAFPKIEDTEIISPVAERPEDRSDAEPENHVVVSYCP 951

952 PIIEEEIPNPAADEAGGTAQVIYIDVQSMYQPQAKPEEEQENDPVGGAGY 1001

1002 KPQMHLPINSTVEDIAAEEDLDKTAGYRPQANVNTWNLVSPDSPRSIDSN 1051

1052 SEIVSFGSPCSINSRQFLIPPKDEDSPKSNGGGWSFTNFFQNKPND*    1097
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Fig. 3. Comparison of human and murine LIFR clones. The amino acid sequence encoded by two murine LIFR clones is presented under the sequence of the human receptor. The single letter amino acid code is used. Identities are marked with dashes. Gaps introduced to maximize the alignment are shown with hatch marks. The two hematopoietin receptor domains are boxed.

shown in Figure 4A illustrates that COS-7 cells display endogenous high and low affinity receptor subtypes (132 sites per cell, $K_a = 4.2\times10^{10}$ M^{-1}) and 2400 sites per cell, $K_a = 7.9\times10^{8}$ M^{-1}). This is in accordance with their derivation from kidney epithelium (Gluzman, 1981), a tissue that is known to be responsive to LIF (Tomida *et al.*, 1990). Following transfection with pHLIFR-65, a large increase of low affinity receptors was detected (74 200 sites per cell, $K_a = 1.1\times10^{9}$ M^{-1}; Figure 4B). A minor increase in the number of high affinity sites was also detected (460 sites per cell, $K_a = 1.5\times10^{11}$ M^{-1}). The [^{125}I]LIF binding to the HLIFR expressed in COS-7 cells was specifically competed against by excess unlabeled human LIF, but not murine LIF or a range of other cytokines including interleukins 1–8, melanoma growth stimulatory activity (MGSA/GRO), G-CSF, GM-CSF, mast cell growth factor and tumor necrosis factor (Figure 5).

COS-7 cells transfected with a control plasmid or with pHLIFR-65 were labeled with [^{35}S]methionine/cysteine and detergent extracts of these cells were prepared. The

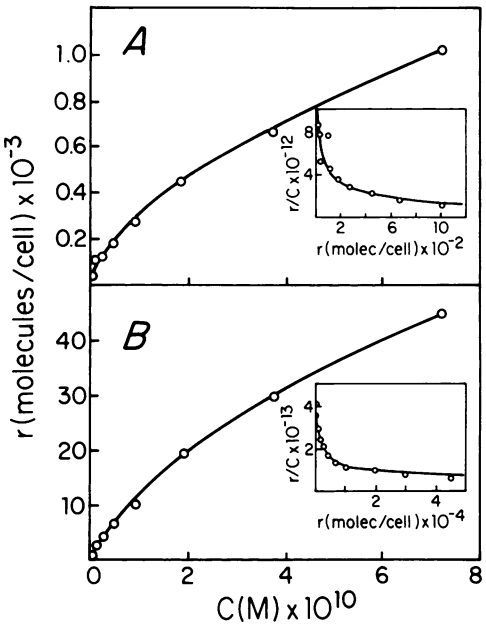

Fig. 4. Binding characteristics of human LIF receptors expressed in COS-7 cells. (**A**) Monolayers of COS-7 cells transfected with the control pDC302 expression vector, or (**B**) pHLIFR were incubated with various concentrations of [^{125}I]hLIF for 1 h at room temperature and assayed for binding as described in Materials and methods. The insets show Scatchard representations (Scatchard, 1949) of specific binding replotted in each panel.

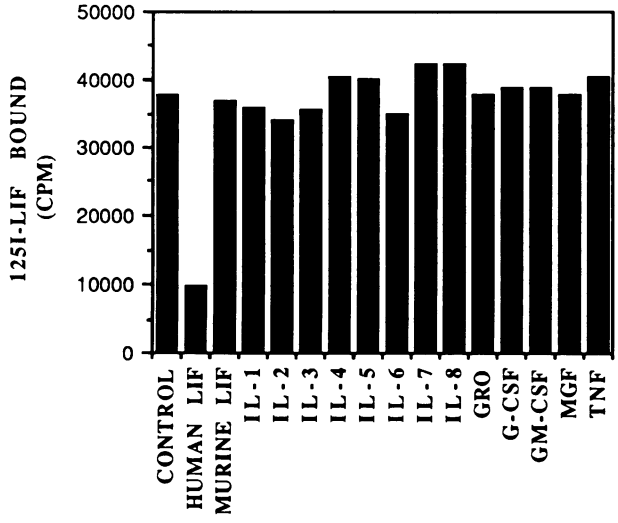

Fig. 5. Specificity of [^{125}I]hLIF binding to human LIF receptors. COS-7 cells were transfected in six-well plates (4×10^5 cells/well) with pHLIFR-65 as described in Materials and methods. Two days following transfection cells were incubated with [^{125}I]hLIF (1.25×10^{-9} M) in the absence or presence of competitor proteins (2.5×10^{-7} M; all human unless shown otherwise; see text for abbreviations shown). Cells were incubated with competitors for 2 h at room temperature and, following washing, the contents of the dish were harvested by trypsinization and counted. Results represent duplicate assays, and variation in [^{125}I]hLIF binding was $<10\%$ in each.

radiolabeled receptor was purified by affinity chromatography on LIF Hydrazide Affigel and analyzed by SDS−PAGE. As shown in Figure 6, COS-7 cells transfected with pHLIFR-65 expressed a cell-associated receptor of M_r 190 000. The binding to the affinity matrix of the M_r 190 000 protein was competed by excess LIF and

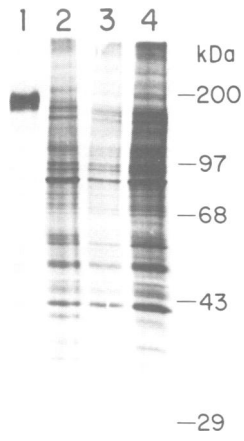

Fig. 6. Affinity purification of radiolabeled human LIF receptor. COS-7 cells transfected with pHLIFR (lanes 1 and 2) or pDC302 control expression vector (lanes 3 and 4) were labeled with [^{35}S]methionine/cysteine and cell lysates were prepared as described in Materials and methods. Cells lysates were incubated with LIF Hydrazide−Affigel in the absence (lanes 1 and 3) or the presence (lanes 2 and 4) of excess unbound LIF and analyzed by SDS−PAGE. Samples were normalized to equivalent radioactivity prior to loading.

also was absent from COS cells transfected with vector alone. We assume that the number of endogenous LIF receptors on COS-7 cells is too low to be distinguished in the experiment shown in Figure 6. The insert of pHLIFR-65 encodes a protein of mol. wt 111 374 (including 15 amino acids from vector sequences), so the difference between this size and the observed M_r of 190 000 is likely to be due to glycosylation at the numerous potential N-linked glycosylation sites, as has been shown for other receptors of this family (Gearing *et al.*, 1989; Fukunaga *et al.*, 1990; Larsen *et al.*, 1990; Hibi *et al.*, 1990).

The coding region of pMLIFR-3 was transferred to the mammalian expression vector pSMAG4 (D.Cerretti, unpublished) and transfected into COS-7 cells. Following [^{35}S]methionine/cysteine labeling and affinity purification, the murine LIFR was detected in both the soluble fraction and in the cell-associated fraction as a labeled protein of M_r 130 000 (Figure 7A). Indeed, most of the soluble LIFR produced during the 2 h labeling period was detected in the cell associated fraction. This may indicate slow processing of the soluble LIFR for secretion by the COS-7 cells or association of the soluble LIFR with the extracellular matrix, as has been shown for one version of the murine LIF ligand (Rathjen *et al.*, 1990). More detailed analysis of the kinetics of secretion will be needed to establish this point. Supernatants from COS-7 cells transfected with pMLIFR-3 were able to compete for binding of [^{125}I]hLIF in a receptor competition assay (Figure 7B). This further demonstrates that the murine LIFR clones encode a natural soluble form of the LIF receptor.

The ability of the soluble mLIFR to bind to a human LIF affinity column and compete the binding of [^{125}I]hLIF (Figure 7) confirms previous observations that human LIF can bind the murine receptor (Gough *et al.*, 1988; Moreau *et al.*, 1988) and contrasts with the lack of binding of murine LIF to the human receptor (Figure 5). The structural explanation for this apparent lack of species cross-reactivity is not clear since human and murine LIF are highly

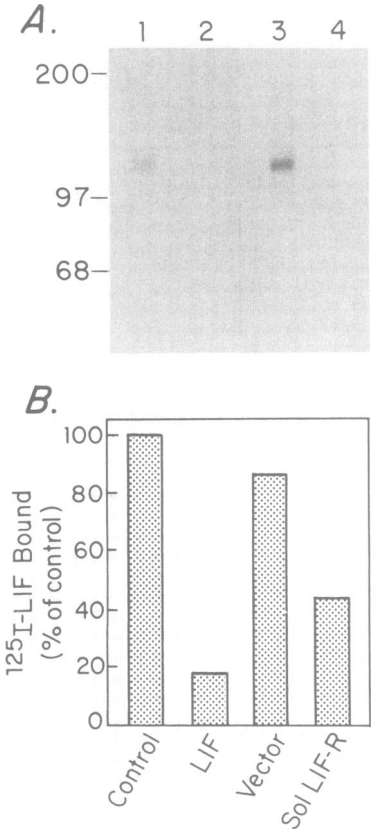

Fig. 7. Characterization of soluble murine LIF receptor. (**A**) COS-7 cells transfected with pMLIFR-3 were labeled with [^{35}S]methionine/cysteine, supernatants harvested and cell lysates prepared as described in Materials and methods. Cell supernatants (lanes 1 and 2) and lysates (lanes 3 and 4) were incubated with LIF Hydrazide–Affigel in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of unbound LIF and analyzed by SDS–PAGE. (**B**) Six-well plates of COS-7 cells expressing pHLIFR-65 were incubated with [^{125}I]hLIF in the absence or presence of 200-fold molar excess unlabeled hLIF, or in the presence of 0.5 ml of conditioned medium from COS-7 cells transfected with pDC302 (Vector) or pMLIFR-3 (Sol LIF-R) in a total volume of 1.0 ml of binding medium. Mixing experiments with the Sol LIF-R fraction indicated that this competition was titratable (not shown). Cells were incubated with [^{125}I]LIF and competitors for 2 h at room temperature, monolayers washed, trypsinized, and the contents of the dishes counted. Results represent duplicate samples and variation in [^{125}I]LIF binding was $<10\%$.

conserved molecules (78% sequence identity; Gough *et al.*, 1988).

Expression of LIF receptor RNA

The presence of human LIFR cDNA clones in libraries prepared from placenta and liver suggests that the LIFR mRNA is normally expressed in these tissues. LIFR expression in placenta is in agreement with our initial observation of binding to placental membranes (see above) and expression in liver is in agreement with a recent report of high numbers of LIF receptors on hepatocytes (Hilton *et al.*, 1991). In order to define the size of the full-length LIFR mRNA the insert of pHLIFR-65 was used to detect HLIFR transcripts in human placental RNA (Figure 8). Two major RNA species of ~6 kb and ~4.5 kb and a minor band of 5 kb were detected. These RNA species may represent alternately spliced transcripts, such as transcripts for membrane bound and soluble forms of the human LIF

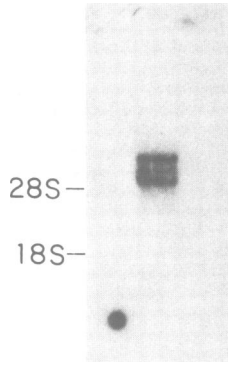

Fig. 8. Detection of multiple LIF receptor transcripts in human placental mRNA. Ribosomal RNA markers are indicated.

receptor, or transcripts utilizing different poly(A) addition signals.

Relationship of the human LIF receptor to the IL-6 receptor signal transducer, gp130

We compared the sequence of the HLIFR to other members of the hematopoietin family of receptors and found that from the Trp-Ser-X-Trp-Ser motif of the membrane-distal hematopoietin receptor domain to the C-terminus, the HLIFR has extensive homology to the entire sequence of the gp130 signal transducing subunit of the human IL-6 receptor (gp130) (Hibi *et al.*, 1990), and to a lesser extent, to the human G-CSF receptor (GCSFR) (Fukunaga *et al.*, 1990b; Larsen *et al.*, 1990) (ALIGN scores of 16.0 and 8.2 respectively, where a score of >3 is considered significant: Doolittle, 1981, Dayhoff *et al.*, 1983). Homology between the HLIFR and gp130 is greatest in the region of the transmembrane domains (residues 834–859 of HLIFR) (65% amino acid similarity; compared to gp130–GCSFR, 31%; HLIFR–GCSFR, 34%) (Figure 9A). Furthermore, the cytoplasmic domains of the HLIFR and gp130 are similar in length, show homology throughout and are longer than the same domain of the GCSFR. Alignment of the cytoplasmic regions of gp130 and the HLIFR shows that their reading frames terminate one amino acid apart from each other and are homologous to each other in the region of sequence derived from the genomic clone HLIFRgen1, providing further support that the sequence predicted from clone HLIFRgen1 (Figure 1) is indeed the true C-terminal sequence of the HLIFR. When compared with other hematopoietin receptors, the membrane-distal hematopoietin receptor domain of the HLIFR is more similar to the membrane-proximal domain of the LIF receptor (ALIGN score of 8.7) than to other members of the receptor family, suggesting that the two domains most likely derive from a gene duplication event within the LIF receptor locus itself.

Discussion

The structural similarity between the LIF, IL-6 and G-CSF receptors is intriguing in the light of their various, shared biological functions and may help to explain similarities in the biological roles of their ligands. IL-6 and G-CSF share some amino acid sequence homology including four conserved cysteine residues (Hirano *et al.*, 1986) and are

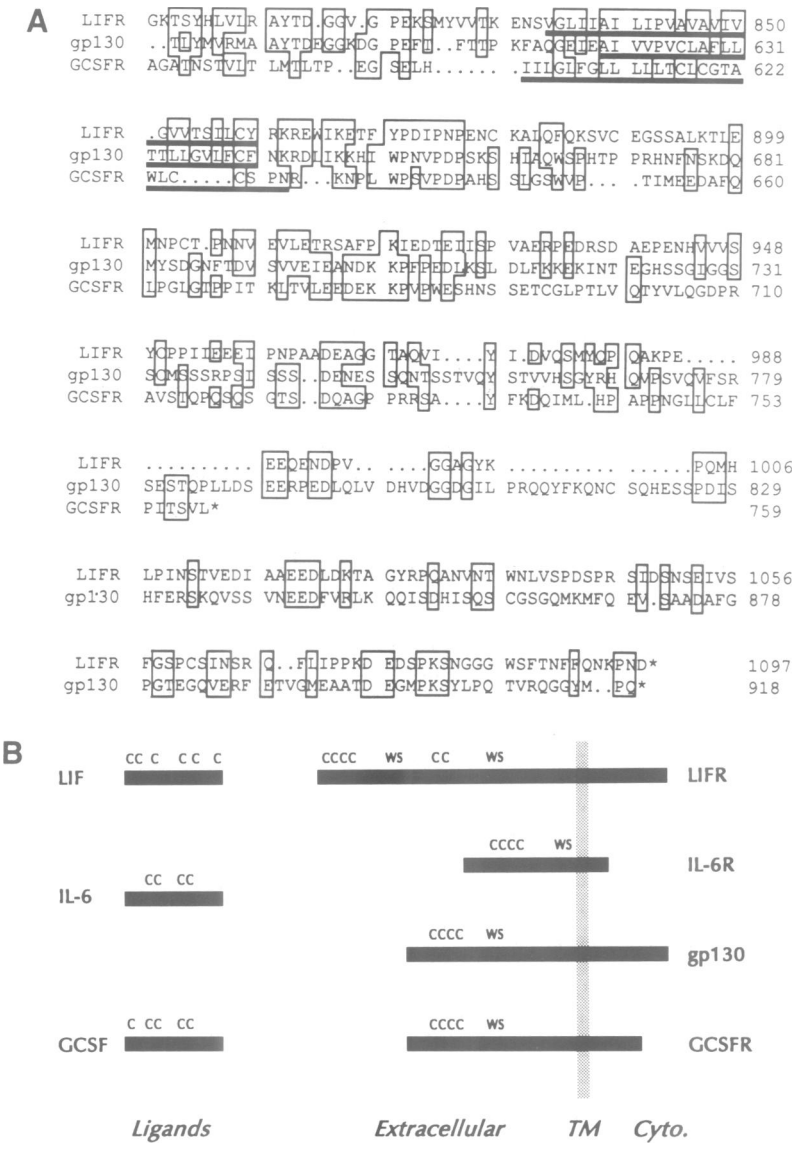

Fig. 9. (**A**) Comparison of the transmembrane and cytoplasmic domains of the HLIFR, gp130 and G-CSFR. Similar amino acid residues are shown boxed. Amino acids were grouped as follows; (K,R,H), (D,E,N,Q), (W,F,Y), (V,I,L,A,M), (P), (C), (S,T), (G). The transmembrane domains are shown underlined. Each receptor was compared using the ALIGN program, which computes the optimal alignment of two sequences and generates an alignment score (Dayhoff *et al.*, 1983; Doolittle, 1981). A score of >3.0 is considered statistically significant. ALIGN scores in this region for HULIFR: gp130, HULIFR: GCSFR and gp130: GCSFR were 6.3, 3.3 and 4.3 respectively. (**B**) Schematic comparison of LIF, IL-6 and G-CSF ligands and receptors. Cysteine residues (C) and Trp-Ser-X-Trp-Ser motifs (WS) are indicated.

encoded by genes with similar exon organization (Yasukawa *et al.*, 1987). However, neither the LIF polypeptide nor the structure of the LIF gene show significant homology to those of IL-6 or G-CSF (Gearing, 1989). Such relationships are especially interesting, and seemingly contradictory, in the context of the broader range of biological roles played by this group of factors, since LIF and IL-6 are active in many of the same biological systems and often share similar roles (as described above), while G-CSF is far more limited in its range of activities. G-CSF appears to be mainly restricted to promoting the survival, proliferation, and differentiation of predominantly neutrophilic granulocytes from bone marrow progenitors (Nicola, 1989) although it has also been shown to induce the differentiation of myeloid leukemic cell lines, the proliferation and migration of endothelial cells, and the growth of colonic and small cell lung carcinomas (Nicola and Metcalf, 1984; Berdel *et al.*, 1988; Bussolino *et al.*, 1989; Avalos *et al.*, 1990). Given the similarity in their biological roles, it now seems appropriate to think of the structural relationship between the receptors for LIF and IL-6 as the common thread between their biological activities (Figure 9). The extracellular domain of the G-CSF receptor is similar in size to gp130, which may reflect the similarity of structure of their ligands as well as indicating a close evolutionary relationship between these two receptor components. In contrast, the LIF receptor is more homologous in its transmembrane and cytoplasmic domains to gp130 than to the G-CSF receptor, suggesting that common signal transduction machinery might explain the biological functions they share. An alternative explanation for the similar biological functions of LIF and IL-6 might be an interaction of the cloned LIF receptor with gp130, analogous to that between the low affinity IL-6 receptor and gp130.

The extensive homology between the transmembrane and cytoplasmic domains of the HLIFR and the gp130 signal

transducer suggests that the cloned LIFR alone may be capable of direct signal transduction. However, the observation that predominantly low affinity LIF receptors increase in number following transfection of COS-7 cells with pHLIFR-65 (Figure 4) indicates that the cloned cDNA encodes a low affinity receptor. The presence of high affinity receptors on most LIF responsive cells might be explained by the existence of an associated high affinity converting subunit coexpressed with the low affinity receptor. Alternatively, the high affinity receptor may be independent of the low affinity receptor. High affinity LIF receptors would allow a particular cell to respond to lower levels of LIF than a lower affinity receptor, and would be desirable where LIF action is locally, and not systemically, mediated, e.g. in the blastocyst, the nervous system, and the bone marrow cavity (where LIF might simultaneously affect hematopoietic stem cells and osteoblasts).

The LIF receptors encoded by the cloned cDNAs have two copies of the extracellular hematopoietin receptor domain and as such are similar to the murine IL-3 receptor (Itoh *et al.*, 1990) and the human GM-CSF receptor β-subunit (Hayashida *et al.*, 1990). While these receptors do not have the triple FN III domains of the LIF, G-CSF and gp130 receptor subunits, it is interesting to note that there are ligand-binding components and affinity converting components in both groups of receptors; the IL-3R, LIFR and G-CSFR bind their ligands directly, whereas the GM-CSFR β-chain and gp130 complex with associated subunits to form higher affinity receptor sites. Such comparisons might also support the existence of another component in the high affinity LIF receptor complex. Structural comparisons indicate that the LIFR may be a composite structure resulting from a gene duplication of the hematopoietin receptor domain. This hypothesis would be enhanced through knowledge of the exon structure of the LIF receptor gene.

Both of the murine LIF receptor cDNAs described above encode a soluble form of the LIF receptor that is assumed to result from alternate splicing of LIFR transcripts. A homologous membrane-bound form of murine LIFR mRNA and soluble form of human LIFR mRNA are expected to exist, but our efforts towards cloning cDNA for these forms have so far been unsuccessful. Genomic Southern blots probed with a DNA fragment encoding the transmembrane and cytosolic domains of the human receptor indicate the existence of a cross-hybridizing species in murine genomic DNA (D.P.Gearing, unpublished) but we have so far been unable to isolate genomic clones encoding this region of the LIFR gene.

Naturally occurring soluble forms of other members of the hematopoietin receptor family, produced from alternatively spliced mRNAs, have been reported. These include the IL-4 receptor, IL-5 receptor, IL-7 receptor, G-CSF receptor, and GM-CSF receptor (Mosley *et al.*, 1989; Takaki *et al.*, 1990; Goodwin *et al.*, 1990; Fukunaga *et al.*, 1990b; Ashworth and Kraft, 1990). Such soluble receptors may act as natural cytokine antagonists. Recombinant soluble murine IL-4 receptor has been shown to be a potent antagonist of IL-4 activities *in vitro* (Maliszewski *et al.*, 1990) and *in vivo* (Fanslow *et al.*, 1991), and a soluble IL-1 receptor construct also has been shown to antagonize IL-1 mediated immune activation *in vivo* (Fanslow *et al.*, 1990). Alternatively, soluble receptors may have stimulatory effects. Recombinant soluble IL-6 receptor has been demonstrated to transduce a signal through membrane-bound gp130 in the presence of IL-6 (Taga *et al.*, 1989) and natural killer cell stimulatory factor (NKSF) is a dimeric molecule synthesized from cytokine-like and soluble hematopoietin receptor-like moieties (Gearing and Cosman, 1991). Thus it is possible that other soluble receptors, including the soluble LIFR, might also have stimulatory effects.

An immobilized, matrix-targeted form of the LIF ligand has recently been described (Rathjen *et al.*, 1990). This form of LIF is derived from alternative splicing of signal sequences from the same LIF gene that produces the secreted version of the ligand, allowing LIF to be localized in the vicinity of the producer cell without systemic release. It should now be possible to define the actions and interactions of the two forms of the LIF ligand with both the membrane-bound and soluble forms of the LIF receptor.

Animal model systems of LIF overproduction produce a wasting disease that has been likened to the cachexia induced by TNF (Metcalf and Gearing, 1989a; Metcalf *et al.*, 1990), and *in vitro* studies have indicated that both LIF and TNF produce this effect through inhibition of lipoprotein lipase (Mori *et al.*, 1989; Rosenblum and Donato, 1989). The LIF receptor bears no resemblance to the p60 or p80 TNF receptors, which belong to a separate family of receptors (Smith *et al.*, 1991), so this shared action of LIF and TNF, if mechanistically similar, would imply a convergence of post-receptor signaling pathways. A possible shared intracellular component between these two receptors is the stress protein hsp27 which has recently been implicated in the signal transduction pathways of LIF and TNF (Michishita *et al.*, 1991).

The availability of a cloned human LIF receptor subunit should help elucidate aspects of signal transduction induced by LIF in various cell types. It should also allow more efficient screening of agonists and antagonists of LIF action that may be clinically relevant. Moreover, soluble forms of this receptor may prove useful as LIF antagonists to evaluate the contribution of LIF to the pathology of various animal models of human disease.

Materials and methods

Recombinant cytokines

Recombinant human LIF (Gough *et al.*, 1988; Moreau *et al.*, 1988) was expressed in yeast utilizing the ADH2 promoter and the prepro-α-factor leader sequence as described (Price *et al.*, 1987). The cDNA for the mature coding region of LIF was fused in-frame to a synthetic oligonucleotide coding for the five C-terminal amino acids of the α-factor leader and an eight amino acid synthetic marker sequence (termed Flag). Purification of Flag human LIF (hLIF) was accomplished by immunoaffinity chromatography with a monoclonal antibody against the Flag sequence essentially as described by Hopp *et al.* (1988). Briefly, yeast culture supernatants were brought to physiological saline concentrations (0.15 M sodium chloride) and 1.0 mM calcium chloride ($CaCl_2$), and loaded on an anti-Flag Affigel column. Columns were washed with phosphate-buffered saline (PBS) containing 0.5 mM $CaCl_2$ and eluted with 0.1 M acetic acid, pH 3.0. Recombinant hLIF was characterized as a hyperglycosylated protein (M_r 100 000) that had a biological activity of $\sim 2\times10^7$ U/mg in a standard proliferation assay using the murine myeloid leukemic cell line DA-1 (Moreau *et al.*, 1988). Fifty units corresponded to the amount of LIF that gave half-maximal [^{3}H]thymidine incorporation. Recombinant murine LIF was expressed in *E.coli* as described (Gearing *et al.*, 1989b) and had an activity of $\sim 4\times10^7$ U/ml in the DA-1 assay.

Radiolabeling of LIF

Recombinant hLIF was radiolabeled to a specific activity of $0.5-1\times10^{16}$ c.p.m./mmol using the radioiodination reagent Enzymobead (Biorad) essentially as described (Park *et al.*, 1987). [^{125}I]hLIF was stored as a stock solution of 3×10^{-8} M in binding medium (RPMI 1640 medium, 2%

bovine serum albumin, 0.2% sodium azide and 20 mM HEPES, pH 7.2). Radioiodinated hLIF maintained >90% biological activity as measured by [^{3}H]thymidine incorporation on DA-1 cells.

Screening of the cDNA expression library

COS-7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal calf serum (FCS) at 37°C in a humidified atmosphere containing 10% CO_2 and passaged twice weekly. Subconfluent COS-7 monolayers on fibronectin-treated chamber slides (Labtek) were transfected by a chloroquine-mediated DEAE−dextran procedure with miniprep DNA derived from pooled transformants (2400 transformants per pool) of a human placental cDNA library (Larsen *et al.*, 1990). Following 2 days of culture, the slides were incubated with 1 ml of binding medium containing 1.25 nM [^{125}I]hLIF for 2 h at room temperature, then washed with PBS, fixed with PBS containing 3% glutaraldehyde, dried and dipped in liquid photographic emulsion as described (Gearing *et al.*, 1989a). After 1−4 days exposure, the slides were developed and inspected microscopically for evidence of silver grain accumulation over the COS-7 cells. Positive pools were partitioned until a single positive clone, pHLIFR-65, was isolated.

Hybridization and nucleotide sequencing

The entire cDNA insert of pHLIFR-65 was radiolabeled using a random priming kit (Stratagene) and used as a hybridization probe to isolate further human cDNAs from the placental expression library and a library prepared from SKHep cells (Goodwin *et al.*, 1989), and to isolate murine clones from a commercial liver cDNA library (Clontech), according to standard procedures (Sambrook *et al.*, 1989). Hybridization conditions were essentially as described (Goodwin *et al.*, 1989). Human clones were detected following high stringency washing conditions (0.2×SSC, 0.1% SDS at 65°C) and murine clones following moderate stringency washing conditions (2×SSC, 0.1% SDS at 65°C). DNA sequences were obtained using vector- and cDNA-derived oligonucleotide primers on denatured double-stranded templates following shotgun and directed subcloning according to standard procedures (Sambrook *et al.*, 1989).

First strand cDNA synthesis, the polymerase chain reaction and blotting from agarose gels were performed essentially as described (Gearing *et al.*, 1989a).

Resolution of RNA samples in agarose gels and transfer to nylon filters was done as described previously (Goodwin *et al.*, 1989). Blots were hybridized overnight with the entire insert of pHLIFR-65 that had been radiolabeled using a random priming kit (Stratagene), and washed using high stringency conditions (see above).

Binding experiments

For quantitative binding assays with adherent monolayers of COS-7 cells, cells were transfected as described above and seeded in Costar 6-well plates at a density of $\sim 5\times10^4$ pLIFR transfectants mixed with 5×10^5 carrier COS-7 cells transfected with vector alone. Cell monolayers were assayed for LIF binding 2 days later by incubation with various concentrations of [^{125}I]LIF for 2 h at room temperature. In all binding assays, non-specific binding of [^{125}I]LIF was assessed in the presence of 200-fold molar excess of unlabeled hLIF and subtracted prior to receptor affinity calculations. Scatchard analysis of binding isotherms (Scatchard, 1949) was carried out using the program RS/1 (Bolt, Beranek and Newman, Boston, MA)

The presence of soluble LIF receptors in COS-7 supernatants was measured by inhibition of [^{125}I]LIF binding to pHLIFR transfected COS-7 cells. Supernatants from control and soluble pMLIFR-3 transfected COS-7 cells were harvested in DMEM with 0.1% FCS three days post-transfection. [^{125}I]LIF binding was assessed as described above in the presence of 0.5 ml control or pMLIFR-3 conditioned media, or in the presence or absence of 200-fold molar excess unlabeled LIF.

Metabolic labeling and affinity purification

Approximately 10^6 COS-7 cells transfected with control plasmid, or human or murine LIFR expression plasmids were washed twice with methionine and cysteine free DMEM, and then incubated with 3.0 ml methionine and cysteine free DMEM containing 40 μCi/ml Tran^{35}S-label (ICN Biomedicals Inc., sp. act. 1008 Ci/mmol) for 2 h at 37°C. Cell supernatants were harvested and cells washed twice with ice-cold PBS. Cells were then lysed with PBS containing 1% Triton X-100 and 200 mM PMSF, EDTA, EGTA, 250 μM benzamidine, 400 μM *o*-phenanthroline, 2 μM leupeptin and pepstatin, and 0.1% BSA. Supernatants and cell lysates were subsequently spun in a microfuge for 10 min at 4°C. Equal quantities of cell lysates or supernatants (generally cell lysate or supernatant from $\sim 2.5\times10^5$ cells) were incubated with a slurry of LIF-Hydrazide Affigel (5% total volume; prepared according to manufacturer's recommendations; BioRad) in 0.01 M Tris-buffered saline, pH 7.4, 1% BSA, with or without 10 μg of human LIF, for 4 h at 4°C on a rocking platform. The LIF-Hydrazide Affigel beads were then pelleted, washed four times with 0.05 M Tris−HCl, pH 7.5, 0.15 M NaCl, 1.0% sodium deoxycholate, 0.1% SDS and prepared for SDS−PAGE analysis as previously described (Mosley *et al.*, 1989).

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