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Human monocyte chemoattractant protein-1 (MCP-1)

Full-length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE

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The purpose of this work was to analyze cDNA encoding human monocyte chemoattractant protein-1 (MCP-1), previously isolated from glioma cell line culture fluid. Screening of a cDNA library from total poly(A) RNA of glioma cell line U-105MG yielded a clone that coded for the entire MCP-1. Nucleotide sequence analysis and comparison with the amino acid sequence of purified MCP-1 showed that the cDNA clone comprises a 53-nucleotide 5'-non-coding region, an open reading frame coding for a 99-residue protein of which the last 76 residues correspond exactly to pure MCP-1, and a 389-nucleotide 3'-untranslated region. The hydrophobicity of the first 23 residues is typical of a signal peptide. Southern blot analysis of human and animal genomic DNA showed that there is a single MCP-1 gene, which is conserved in several primates. MCP-1 mRNA was induced in human peripheral blood mononuclear leukocytes (PBMNLs) by PHA, LPS and IL-1, but not by IL-2, TNF, or IFN-γ. Among proteins with similar sequences, the coding regions of MCP-1 and mouse JE show 68% identity. This suggests that MCP-1 is the human homologue of the mouse competence gene JE.

Inflammation; Chemotaxis; Macrophage infiltration

1. INTRODUCTION

Macrophages participate in the initial reaction to tissue injury, which includes not only antigen processing and presentation, but also secretion of factors that mediate systemic host defense responses and local inflammation [1]. They originate from blood monocytes, which leave the circulation in response to several signals that are thought to include chemoattractants produced at the inflammatory focus. Our objective has been to isolate and characterize monocyte chemoattractants in order to evaluate their significance as mediators of inflammation.

We recently purified to homogeneity two human monocyte chemoattractants from the culture fluid of a glioma cell line. Although these two attractants could be separated into two peaks by cation-exchange HPLC, their amino acid compositions were identical [2]. Likewise, two cation-exchange HPLC peaks of monocyte chemotactic activity, purified from culture fluid of PHA-stimulated human blood lymphocytes, were identical in amino acid composition to one another and to the glioma-

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Abbreviations: CSF, colony-stimulating factor; CTAP, connective tissue activating peptide; IFN, interferon; IL-1, interleukin-1; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; PBMNL, peripheral blood mononuclear leukocyte; PDGF, platelet-derived growth factor; PHA, phytohemagglutinin; TNF, tumor necrosis factor

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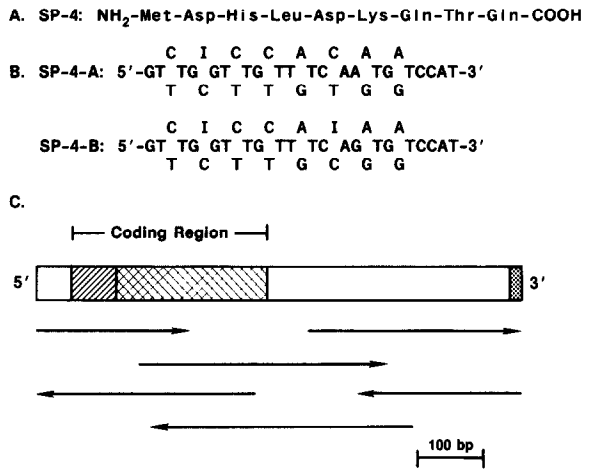

Fig.1. (A) Amino acid sequence of a portion of a peptide fragment of an *S. aureus* V8 protease digest of MCP-1. (B) Probes based on the above sequence. (C) Structural organization and sequencing strategy of human MCP-1 cDNA. Arrows show direction and extent of determined sequences. Cross-hatched area indicates the coding region for the mature form of MCP-1. Dotted region indicates poly(A).

derived proteins [3]. The complete amino acid sequence of one of the monocyte chemoattractants purified from glioma culture fluid was determined on a set of partial digests by a combination of Edman degradation and mass spectrometry. A single protein chain with a blocked N-terminus (pyroglutamic acid) and a total of 76 residues were identified [4]. In this and future communications, it will be named monocyte chemoattractant protein-1 (MCP-1). We now report the cloning of MCP-1 full-length cDNA, its hybridization to genomic DNA from other species, and detection of MCP-1 mRNA in normal cells stimulated by mediators of inflammation.

2. MATERIALS AND METHODS

Restriction enzymes, DNA modifying enzymes, and reagents for cDNA preparation were from Bethesda Research Laboratories (Bethesda, MD). DNA sequencing reagents were from US Biochemicals. Radiochemicals were from Amersham

```
                                                  -50       -40       -30       -20       -10        -1
                                                  CTAACCCAGAAACATCCAATTCTCAAACTGAAGCTCGCACTCTCGCCTCCAGC

                10        20        30        40        50        60
ATG AAA GTC TCT GCC GCC CTT CTG TGC CTG CTG CTC ATA GCA GCC ACC TTC ATT CCC CAA
Met Lys Val Ser Ala Ala Leu Leu Cys Leu Leu Leu Ile Ala Ala Thr Phe Ile Pro Gln

                70        80        90        100       110       120
GGG CTC GCT CAG CCA GAT GCA ATC AAT GCC CCA GTC ACC TGC TGT TAT AAC TTC ACC AAT
Gly Leu Ala Gln Pro Asp Ala Ile Asn Ala Pro Val Thr Cys Cys Tyr Asn Phe Thr Asn
           △
               130       140       150       160       170       180
AGG AAG ATC TCA GTG CAG AGG CTC GCG AGC TAT AGA AGA ATC ACC AGC AGC AAG TGT CCC
Arg Lys Ile Ser Val Gln Arg Leu Ala Ser Tyr Arg Arg Ile Thr Ser Ser Lys Cys Pro

               190       200       210       220       230       240
AAA GAA GCT GTG ATC TTC AAG ACC ATT GTG GCC AAG GAG ATC TGT GCT GAC CCC AAG CAG
Lys Glu Ala Val Ile Phe Lys Thr Ile Val Ala Lys Glu Ile Cys Ala Asp Pro Lys Gln

               250       260       270       280       290       300
AAG TGG GTT CAG GAT TCC ATG GAC CAC CTG GAC AAG CAA ACC CAA ACT CCG AAG ACT TGA
Lys Trp Val Gln Asp Ser Met Asp His Leu Asp Lys Gln Thr Gln Thr Pro Lys Thr

         310       320       330       340       350       360       370        38
ACACTCACTCCACAACCCAAGAATCTGCAGCTAACTTATTTTCCCCTAGCTTTCCCCAGACACCCTGTTTTATTTTATT

0        390       400       410       420       430       440       450        4
ATAATGAATTTTGTTTGTTGATGTGAAACATTATGCCTTAAGTAATGTTAATTCTTATTTAAGTTATTGATGTTTTAAG

60       470       480       490       500       510       520       530
TTTATCTTTCATGGTACTAGTGTTTTTTAGATACAGAGACTTGGGGAAATTGCTTTTCCTCTTGAACCACAGTTCTACC

540      550       560       570       580       590       600       610
CCTGGGATGTTTTGAGGGTCTTTGCAAGAATCATTAATACAAAGAATTTTTTTTAACATTCCAATGCATTGCTAAAATA

 620      630       640       650       660       670       680
TTATTGTGGAAATGAATATTTTGTAACTATTACACCAAATAAATATATTTTTGTACAAAAAAAAAAAAAA
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Fig.2. Nucleotide sequence of human MCP-1. Triangle: N-terminus of mature MCP-1. Dashed line: potential *N*-linked glycosylation site. Solid line: oligonucleotide probe sequence. Dotted line: polyadenylation signal [19].

or New England Nuclear. λ ZAP II vector was from Stratagene (La Jolla, CA). Cytokines were from Boehringer Mannheim.

Total RNA was isolated from glioma cell line U-105MG by the guanidinium isothiocyanate method; poly(A) RNA was isolated by oligo(dT)-cellulose chromatography [5]. cDNA synthesized by a modification of the method of Gubler and Hoffman [6] was used to prepare a library in λ ZAP II vector [7]. Oligodeoxynucleotides were synthesized by the phosphoramidite method [8] and purified by HPLC. Probes (fig.1B) were synthesized on the basis of the sequence of a peptide fragment (SP-4, fig.1A) generated by digestion of MCP-1 with *S. aureus* V8 protease [4]. Approx. 5×10^5 recombinant phage from the cDNA library were screened by high-density plaque hybridization [5,9] with a mixture of ^{32}P-labeled oligonucleotides SP-4-A and SP-4-B. Hybridization to nitrocellulose filters was carried out overnight at 45°C in a solution containing 6 × standard saline citrate (SSC), 5 × Denhardt's solution, 0.05% sodium pyrophosphate, 1% SDS, 100 μg/ml heat-denatured, sheared, salmon sperm DNA and 1×10^6 dpm/ml probe. Filters were washed once with 6 × SSC, 0.1% SDS at 45°C for 5 min, three times at 35°C for 30 min, and were dried and exposed overnight to XS-5 film (Kodak) with an intensifying screen at −80°C. Phagemids carried within λ ZAP II recombinants were rescued with helper phage [7]. cDNA inserts were subcloned into M13mp19 [10] and single strands were sequenced on field gradient gels [11] by the dideoxynucleoside triphosphate chain-termination method [12]. Sequence data were compiled and analyzed with computer assistance [13].

Human PBMNLs were stimulated with 2.5 μg/ml of PHA, 10 μg/ml LPS, or 100 U/ml of the following human recombinant LPS-free cytokines: IL-1β, IL-2, TNFα, IFN-γ. Northern blot analysis of poly(A) RNA was performed by the glyoxal-dimethyl sulfoxide method [5] in a 1% agarose gel with a probe of MCP-1 cDNA insert labeled with [α-^{32}P]CTP by random priming [14]. Filters were hybridized at 42°C overnight in 50% formamide, 1 M NaCl, 5 × Denhardt's solution, 1 mM EDTA, 0.1% sarkosyl, 100 μg/ml sheared-denatured salmon sperm DNA, 1×10^6 dpm/ml probe and 50 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid), pH 7. Filters were washed twice with 2 × SSC, 0.1% SDS at 37°C for 30 min and 0.1 × SSC, 0.1% SDS at 50°C for 30 min prior to autoradiographic exposure.

Southern blot analysis was performed as described [5] in a 1% agarose gel with 10 μg restriction-enzyme-cleaved DNA per lane. Hybridization was as described for library screening except that transfers were made to nylon filters, hybridization temperature was 65°C and the probe was ^{32}P-labeled MCP-1 cDNA. Filters were washed once in the hybridization solution used for library screening at 65°C for 1 h, then twice in 0.1 × SSC, 0.1% SDS at 48°C for 30 min.

3. RESULTS

A cDNA library was constructed with poly(A) RNA from the human glioma cell line (U-105MG) in cloning vector λ ZAP II. Approx. 5×10^5 recombinant phage were screened with the oligonucleotide probes shown in fig.1B. 48 positive signals on duplicate filters were obtained (~0.01% abundance). 15 clones were plaque purified and phagemid DNA was prepared. By preliminary nucleotide sequence analysis, at least three clones coded for MCP-1. The insert from the clone with the longest 5'-untranslated region was sequenced (figs 1C,2).

Based on the amino acid sequence of pure MCP-1 [4], the mature form of the protein starts with glutamine at residue 24 (nucleotide 70). The amino acid sequence deduced from nucleotides 70 to 297 is identical to the directly determined 76-residue sequence of pure MCP-1 [4]. The cDNA sequence contains an in-frame methionine triplet 69 nucleotides upstream from the triplet corresponding to the NH_2-terminus of MCP-1. Seven

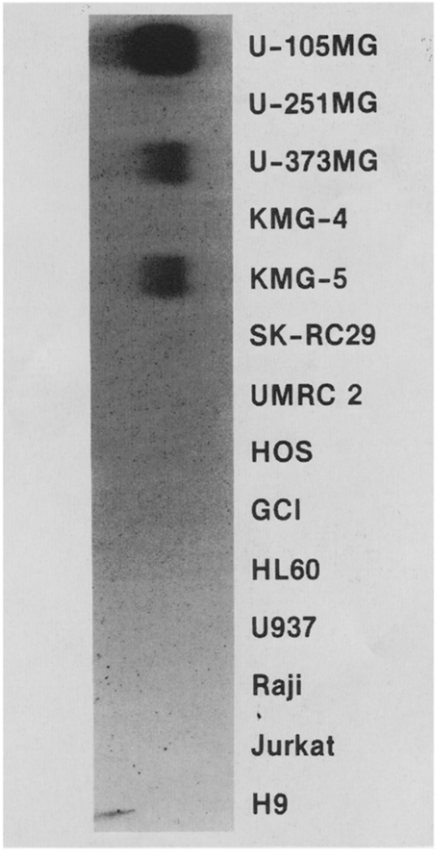

Fig.3. Expression of MCP-1 mRNA in tumor cell lines. 5 μg poly(A) mRNA from each cell line were used. The first 5 blots are from glioma lines. SK-RC29, UMRC 2: renal cell carcinomas. HOS: osteosarcoma. GCT: fibrous histiocytoma. HL60, U937, Raji, Jurkat, H9: leukemia or lymphoma cell lines.

of the 9 residues in the methionine triplet region, CCAGCATGA, match the sequence believed to be optimal for translation initiation [15]. The length and hydrophobic character of the deduced amino acid sequence from the methionine to the NH_2-terminus of MCP-1 are typical of a signal peptide [16]. There is a single consensus sequence for *N*-linked glycosylation targeting amino acid 38.

The A + T content of the 3′-untranslated region (66%) is not nearly as high as that found in some transiently expressed mRNAs [17]. Unlike a number of genes encoding proteins related to the inflammatory response [18], there is no 8-nucleotide sequence, TTATTTAT, in the 3′-untranslated region.

In a survey of 5 different glioma cell lines, we reported that all released chemotactic activity for human monocytes [20]. It was therefore of interest to probe these and other tumor cell lines for MCP-1 mRNA message. Fig.3 shows Northern blots with a cDNA probe for MCP-1. The high and low mRNA, respectively, of gliomas U-105MG and KMG-5 correlates with previously observed

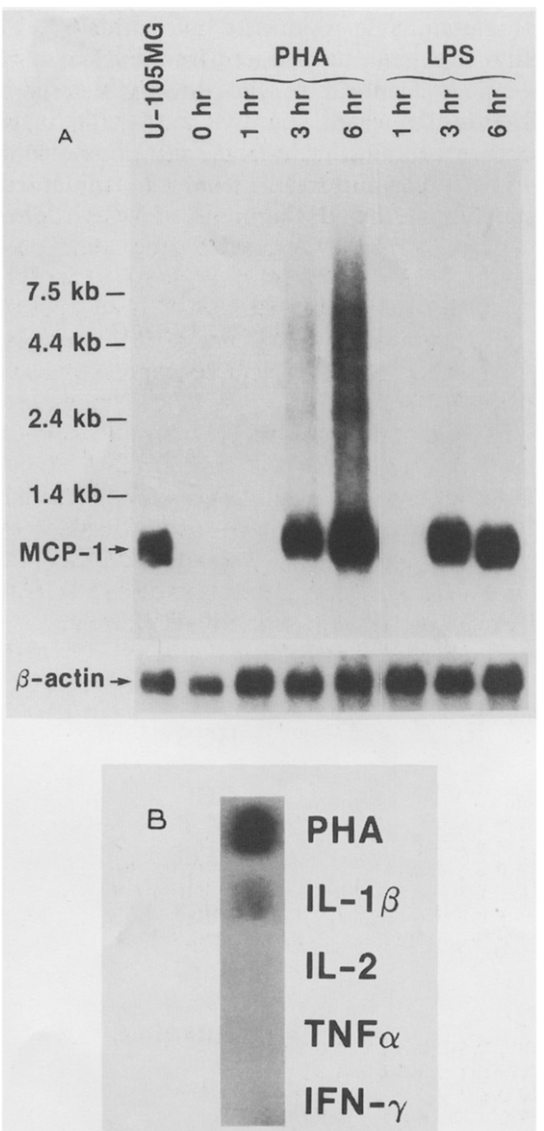

Fig.4. Induction of MCP-1 mRNA in human PBMNLs by mitogens or human recombinant cytokines. (A) PBMNLs were cultured with 2.5 μg/ml PHA or 10 μg/ml LPS, and mRNA was extracted at the indicated times. (B) Cells were cultured with 100 U/ml of each cytokine for 6 h; then mRNA was extracted.

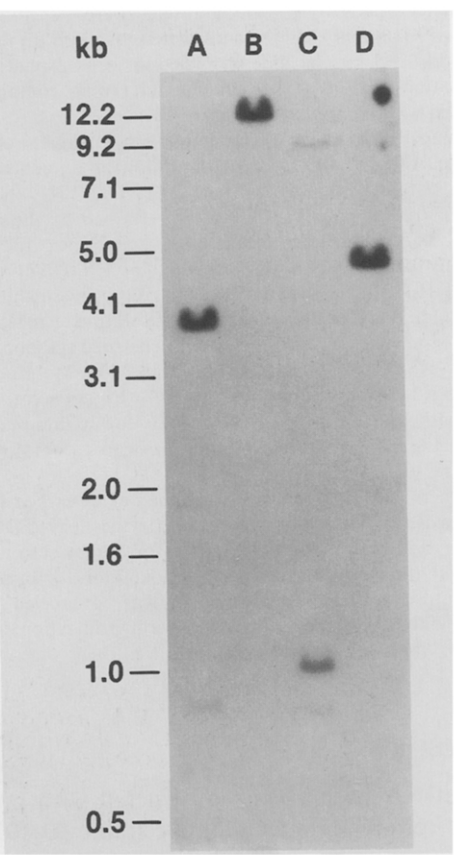

Fig.5. Southern blotting analysis of human genomic DNA digested with various endonucleases. (A) *Eco*RI, (B) *Bam*HI, (C) *Pst*I, (D) *Hin*dIII.

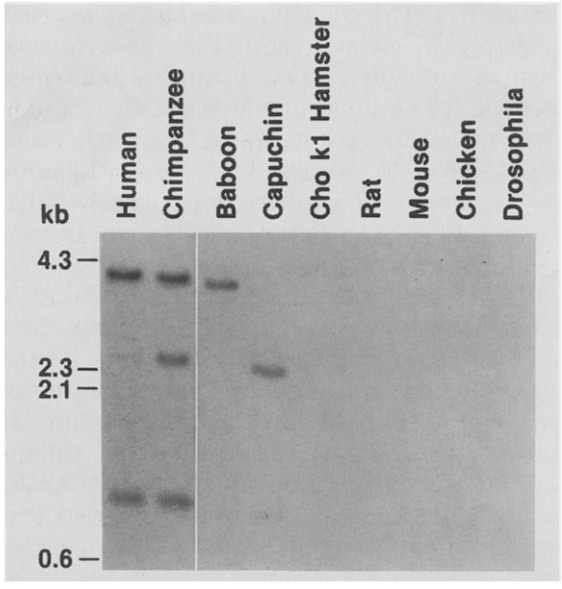

Fig.6. Hybridization of MCP-1 cDNA with genomic DNA from various species.

levels of chemotactic activity produced by these two lines [20]. MCP-1 mRNA was not detected in other human tumor cell lines.

Since PBMNL-derived MCP-1 was indistinguishable from glioma-derived MCP-1, we carried out Northern blot analyses of mRNA from PBMNLs stimulated with PHA. No mRNA was detected before stimulation, but high levels of mRNA were detected 3 and 6 h after addition of PHA (fig.4A). 10 μg/ml of LPS also induced high mRNA levels in these cells. IL-1β induced MCP-1 mRNA, though the level was less than for PHA (fig.4B). Induction of MCP-1 mRNA by IL-2, TNFα, or IFN-γ was not detected.

To identify genomic DNA fragments carrying the gene for MCP-1, human DNA restriction endonuclease digests were subjected to Southern blot analysis (fig.5). After *Bam*HI or *Hin*dIII digestion, a single band was seen. *Pst*I digestion gave 2 major bands, which is in agreement with the fact that there is a *Pst*I restriction site in the MCP-1 cDNA. The data show that there is a single MCP-1 gene. DNA from different species was digested with *Eco*RI and hybridized to the same probe (fig.6). Under conditions of high stringency, hybridization occurred with DNA of chimpanzee, baboon and capuchin, but not of other species.

Proteins with amino acid sequences similar to that of MCP-1 are shown in fig.7. Among the 5 proteins, 4 are known only by the nucleotide sequence of cDNAs cloned from mRNA that increased in response to mitogenic stimuli. All 5 have 4 half-cystine residues in the same location, and the first 2 half-cystines are adjacent. Sequence similarity to MCP-1 is greatest for mouse JE (42 of 76 residues). Comparison of the sequences of mouse JE and human MCP-1 cDNAs shows that 68% of the nucleotides of the coding region are matched.

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                          10                  20                  30
MCP-1     QPDAINAPVT  CC YNFTNRKISVQRLASYRRITSSKCPK  E
RANTES    SPYSSDT   TP CC FAYIARPLPRAHIKEY  FYTSGKCSN  P
pLD78     S  LAADTP  TA CC FSYTSRQIPQNFIADY  FETSSQCSK  P
TCA 3     S  KSMLTVSNS CC LNTLKKELPLKFIQCYRKMGSS  CPDPP
JE        QPDAVNAPLT  CC YSFTSKMIPMSRLESYKRITSSRCPK  E
MIP       APYGADTP  TA CC FSYSRK  IPRQFIVDY  FETSSLCSQ  P

          40                  50                  60                  70
MCP-1     AVIFKTIVAKEICADPKQKWVQDSMDHLDKQTQTPKT
RANTES    AVVFVTRKNRQVCANPEKKWVREYINSLEMS
pLD78     GVIFLTKRSRQVCADPSEEWVQKYVSDLELSA
TCA 3     AVVFRLNKGRESCASTNKTWVQ    NHLKKVNPC
JE        AVVFVTKLKREVCADPKKEWVQTYIKNLDRNQMRSEP---
MIP       GVIFLTKRNRQICADSKETWVQEYITDLELNA
```

Fig.7. Amino acid sequence of proteins similar to MCP-1. RANTES: deduced from cDNA cloned from antigen or mitogen-stimulated human T cell lines [21]. pLD78: from stimulated human tonsillar lymphocytes [22]. TCA 3: from ConA-stimulated mouse T cell line [23]. JE: from platelet-derived growth factor (PDGF)-stimulated mouse fibroblast line [24]. MIP: from culture fluid of LPS-stimulated mouse macrophage cell line [25,26].

4. DISCUSSION

In view of the fact that MCP-1s from glioma cells and mitogen-stimulated PBMNLs are indistinguishable, either glioma cells or stimulated PBMNLs could provide mRNA for cDNA library construction. We selected the glioma cell line, since it produced the attractant constitutively. The cDNA clone derived from the glioma cell library detected mRNA in both glioma cells and PHA-stimulated PBMNLs. This is consistent with the observation that the amino acid compositions of MCP-1s from the two sources are identical [2,3].

Although MCP-1 mRNA was detected in several glioma cell lines, no message was found in 9 cell lines representing other types of tumors. Thus, expression of the MCP-1 gene is not a property of all neoplastic cells.

The amino acid composition of a monocyte chemoattractant produced by aortic smooth muscle cells of the baboon [27] is identical to that of MCP-1 [3]. Hybridization of the MCP-1 cDNA probe with baboon DNA (fig.6) is added evidence for the relationship between MCP-1 and the smooth muscle product, and indicates that both lymphocytes and vascular smooth muscle cells can produce this attractant. Penetration of vascular endothelium by blood monocytes occurs in experimental atherosclerosis [28]. Since local production of MCP-1 could mediate accumulation of monocytes in the lesions, it will be of interest to determine by in situ hybridization if MCP-1 mRNA is detectable in endothelium or smooth muscle in the early stages of atherosclerosis.

In contrast to MCP-1, which does not attract neutrophils, a protein attractant for neutrophils has been described [29], which has sequence similarity to several host defense cytokines. Members of this group contain 4 half-cystine residues, the positions of which are identical. The first two cysteines are separated by a single amino acid (C-X-C). As shown in fig.7, MCP-1 is part of another group of proteins with 4 half-cystine residues. In contrast to the C-X-C group, the first 2 half-cystines are adjacent (C-C). The C-C group does not have sequence similarity to the C-X-C group. Like members of the C-X-C group, MIP and MCP-1 have inflammatory properties. The sequences of the other C-C proteins were deduced from mRNAs that increase in response to mitogenic stimuli; although they are without known function, they all have signal peptide sequences, suggesting that they are secreted products. The protein with the greatest sequence similarity to MCP-1 is mouse JE (68% nucleotide similarity in the coding sequence of the cDNAs). This high degree of similarity suggests that MCP-1 is the human homologue of mouse JE. If we assume identity of MCP-1 with the JE product, which is a cell-cycle competence protein, then many mitogenic and activation stimuli may cause secretion of MCP-1 by a wide variety of cells (table 1). Thus, the potential biological role of MCP-1 is not limited to recruitment of blood monocytes in cellular immune reactions [3]. The increased JE mRNA associated with LPS activation of macrophages [31] suggests that generation of activated macrophages is accompanied by a signal for accumulation of more effector cells at the site. Moreover, the generation of JE/MCP-1 mRNA by mitogenic stimuli emphasizes that both proliferation (for wound healing) and cell recruitment (for host defense) are part of the response to tissue injury.

Table 1

Cellular expression of MCP-1 or JE mRNA

Cell	Species	Stimulus	Reference
Lymphocyte	human	PHA, IL-1	This work, [30]
Macrophage	mouse	LPS, *Listeria*	[31]
Fibroblast	mouse	PDGF, IL-1, VSV, poly(I):poly(C)	[24,32]
Endothelium	rat	EGF	[33]
Smooth muscle	baboon	(−)	[27]

VSV, vesicular stomatitis virus; poly(I):poly(C), double-stranded RNA; EGF, epidermal growth factor

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