Cloning and Characterization of cDNAs for Murine Macrophage Inflammatory Protein 2 and its Human Homologues

By Patricia Tekamp-Olson, Carol Gallegos, Diane Bauer, Joyce McClain, Barbara Sherry,* Myriam Fabre,* Sander van Deventer,* and Anthony Cerami

From the Chiron Corporation, Emeryville, California 94608; and the Laboratory of Medical Biochemistry, Rockefeller University, New York, New York 10021

Summary

A cDNA clone of murine macrophage inflammatory protein 2 (MIP-2) has been isolated from a library prepared from lipopolysaccharide (LPS)-stimulated RAW 264.7 cells and the nucleotide sequence determined. This cDNA was used to clone cDNAs for human homologues of MIP-2 from a library prepared from phorbol myristate acetate-treated and LPS-stimulated U937 cells. Two homologues were isolated and sequenced. Human MIP-2α and MIP-2β are highly homologous to each other and to a previously isolated gene, human gro/melanoma growth-stimulating activity (MGSA). These three human genes, MIP-2α, MIP-2β, and gro/MGSA, constitute a sub-family within the cytokine family represented by platelet factor 4 and interleukin 8.

Materials and Methods

cDNA Library Construction. The isolation of poly(A)* RNA from Escherichia coli LPS-stimulated murine RAW 264.7 cells and the construction of a cDNA library have been described previously (22). The stimulation of the human monocytic-like cell line U937 (23), the isolation of total and poly(A)* RNA, and the construction of a cDNA library were performed as follows. U937 cells (American Type Culture Collection, Rockville, MD) were grown to confluence and stimulated to differentiate by the addition of PMA to a final concentration of 5 x 10^-8 M. After 24 h in the presence of PMA, LPS (LPSW, E. coli 0127:B8; Difco Laboratories Inc., Detroit, MI) was added to a final concentration of 1 ug/ml, and the cells were incubated for an additional 3 h at 37°C. Total RNA was prepared essentially as described (24). Poly(A)* RNA was prepared by single passage over oligo-dT-cellulose essentially as described (25). Double-stranded cDNA was prepared using a kit for cDNA synthesis (Pharmacia LKB Biotechnology, Inc., Pleasant Hill, CA) and cloned and packaged into λgt10.

Murine MIP-2 cDNA Isolation. A degenerate oligonucleotide probe corresponding to amino acids 9–14 of the NH2-terminal sequence of MIP-2 (2) was synthesized. This portion of the partial sequence was chosen because it was predicted to be in a highly conserved coding region and because of its lower codon degeneracy when compared with the other parts of the partial sequence. The resulting probe was a 128-fold degenerate pool of oligomers 17 nucleotides in length.

Duplicate nitrocellulose filters lifts of the plated RAW 264.7

Abbreviations used in this paper: GM, granulocyte/macrophage; MGSA, melanoma growth-stimulating activity; MIP-2, macrophage inflammatory protein 2.

Macrophages secrete a wide variety of proteins that mediate many aspects of inflammation (1). We recently described the purification and characterization of a novel monokine, murine macrophage inflammatory protein-2 (MIP-2),1 which was one of two heparin-binding proteins secreted from RAW 264.7 cells in response to endotoxin stimulation (2). Determination of the NH2-terminal sequence of purified MIP-2 and comparison with protein and DNA sequence databases indicated that it was a member of a rapidly expanding cytokine family whose members appear to modulate the inflammatory response and to have growth-promoting activities. Members of this family include human and hamster gro (3, 4); human, rat, and bovine platelet factor 4 (5–9); murine KC (10); rat CINC (11); chicken 9E3/CEF4 (12, 13); human platelet basic protein (14), the precursor of connective tissue-activating peptide and β-thromboglobulin; an IFN-γ-inducible protein, human γIP-10 (15); and IL-8, also known as 3–10C, MDCNP, NAF, and MONAP (16–20).

MIP-2 has been shown to elicit a localized inflammatory response when injected subcutaneously into the footpads of C3H/HeJ mice, to have potent chemotactic activity for human PMN, and to induce PMN degranulation of lysozyme but not of β-glucuronidase (2). In addition, MIP-2 has been shown to have CSF-dependent myelopoietic-enhancing activities for granulocyte/macrophage CFU (CFU-GM) (21). To further investigate the role of this cytokine at the molecular level, we describe here the cloning and sequencing of the cDNA for murine MIP-2, as well as the isolation and nucleotide sequencing of human cDNA homologues.
cDNA library (5 × 10⁶ plaques) were prehybridized at 42°C in 5× SSC, 2× Denhardt's, 50 mM sodium phosphate buffer, pH 6.5, 50% formamide, 0.2% SDS, and 0.25 mg/ml sonicated salmon sperm DNA, and then were hybridized overnight at 42°C in 5× SSC, 1× Denhardt's, 20 mM sodium phosphate buffer, pH 6.5, 50% formamide, 10% dextran sulfate, 0.1% SDS, 0.1 mg/ml sonicated salmon sperm DNA, and 5 × 10⁶ cpm/ml per degeneracy of 32P-ATP 5'-end-labeled synthetic oligonucleotide probe pool. After hybridization, the filters were washed using tetramethylammonium chloride (26). Plaques that were positive on duplicate filters were subjected to a second round of low density plating and screening. Positive phage clones were isolated from which DNA was prepared for further analysis.

Isolation of Human Homologues of Murine MIP-2. Plating of the U937 cDNA library, nitrocellulose filter prehybridization, and hybridization of the filters were performed as described above for the screening of the RAW 264.7 cDNA library. The probe DNA was a 186-bp Ball-BglII fragment isolated from the mu-MIP-2 cDNA. The BglII site was introduced in vitro mutagenesis using the mutagenic primer 5'-CAAAGATCTTGAACAGAGAG-3'. The Ball-BglII fragment encodes most of the mature mu-MIP-2 amino acid sequence, lacking those base pairs encoding the three NH2-terminal and eight COOH-terminal amino acids. This fragment was nick-translated, and ∼500,000 cpm/ml was included in the hybridization solution.

After hybridization, filters were subjected to three low stringency washes at room temperature for 30 min each in 2× SSC, 0.1% SDS. Plaques positive on duplicate filters were subjected to a second round of low density plating and screening. Positive phage clones were isolated from which DNA was prepared for further analysis.

Southern Analysis. Genomic DNA from RAW 264.7 cells was isolated as described by DiLella and Woo (27). Human genomic DNA and murine C3H/HeN genomic DNA were purchased from Clontech (Palo Alto, CA).

Genomic DNA was digested with restriction enzymes according to the supplier's specifications. Digested DNA was separated on 1% agarose gels and then transferred to Hybond nylon membranes (Amer sham Corp., Arlington Heights, IL). Filters were prehybridized and hybridized in 50 mM sodium phosphate buffer, pH 6.5, 5× SSC, 1 mM sodium pyrophosphate, 40% formamide, 10% dextran sulfate, 5× Denhardt’s solution, 0.1% SDS, and 100 µg/ml sonicated salmon sperm DNA. DNAs used for Southern analysis were the 1.1-kb mu-MIP-2 cDNA (clone mMIP-2-20a), the 0.98-kb hu-MIP-2β cDNA (clone hMIP-2-4a), and a 1.05-kb hu-MIP-2α cDNA (clone hMIP-2-5a). All cDNAs were labeled by random priming with 32P-CTP using a Multiprimer DNA Labeling System (Amersham Corp.). After prehybridization for 2–4 h at 37°C, labeled cDNA was added at 10⁶ cpm/ml. Hybridization was for 16–18 h at 37°C. Filters were rinsed at room temperature for 10 min in 2× SSC, 0.1% SDS, then washed three times at 65°C for 45 min each in 0.1 × SSC, 0.1% SDS. In some cases, hybridized probe was stripped from the blot by treatment for 45 min at 65°C in 0.5× SSC, 0.1% SDS, and 50% formamide to allow rehybridization.

DNA Sequence Analysis. cDNA inserts were subcloned into M13 phage vectors, and DNA sequencing was performed by the dideoxy chain termination method of Sanger et al. (28).

Results

Cloning of Murine and Human MIP-2 cDNAs. Screening of the cDNA library derived from poly(A)+ RNA from RAW 264.7 cells with a degenerate oligonucleotide pool specific for the NH2-terminal sequence of murine MIP-2 (2) resulted in the isolation of clone MIP-2-20a. Insert cDNA (∼1,100 bp) was isolated, cloned into M13, and the nucleotide sequence determined. The nucleotide sequence and predicted protein sequence are shown in Fig. 1. The predicted mature protein sequence starting at position 1 exactly matches the NH2-terminal peptide sequence determined previously for purified MIP-2 (2).

To isolate the human homologue(s) of murine MIP-2 cDNA, a fragment encoding most of the mature mu-MIP-2 protein was isolated and used to probe a U937 cDNA library prepared from poly(A)+ RNA of PMA-treated and LPS-stimulated cells. DNA from plaques positive on low stringency wash was isolated and subjected to restriction enzyme analysis, which suggested the presence of two classes
of clones. Insert cDNAs from representative clones of each class were subcloned into M13, and the nucleotide sequences were determined. The nucleotide sequence and predicted amino acid sequence of hu-MIP-2α are presented in Fig. 2. This sequence was confirmed on four independent clones and is representative of the more abundant of the two classes of human cDNA homologous to mu-MIP-2. The nucleotide sequence and predicted protein sequence of hu-MIP-2β, representative of a second class of human cDNAs homologous to mu-MIP-2, are shown in Fig. 3. This sequence was confirmed on two independent clones.

**Features of Murine and Human MIP-2 cDNAs.** The nucleotide sequences of mu-MIP-2, hu-MIP-2α, and hu-MIP-2β each encode a single open reading frame. The nucleotide sequence environment of the initiating ATG codon of mu-MIP-2 conforms to the consensus sequence shared by many mRNAs of higher eukaryotes (29, 30); those of human MIP-2α and MIP-2β lack the highly conserved purine at position -3 but possess many features of the consensus sequence, including C residues at positions -1, -2, and -4, and a G residue at position +4.

The 3' untranslated region of mu-MIP-2 includes the eukaryotic consensus polyadenylation signal AATAAA (31) at position +719-724 followed by a poly(A) string beginning at nucleotide +735. The consensus polyadenylation signal is present in the hu-MIP-2α cDNA at position +698-703 of the 3' untranslated region followed by a poly(A) beginning at nucleotide +716. No AATAAA polyadenylation signal was found in the 3' untranslated region of clones hMIP-2-4a or hMIP-2-7d of hu-MIP-2, which is most likely due to the fact that these clones have a truncated 3' untranslated region since no poly(A) string was present.

The consensus sequence TAATTTAT found in the 3' untranslated region of many cytokine genes (32) and implicated...
The start of the mature mu-MIP-2 protein in the predicted amino acid sequence. The assignment of the start position for hu-MIP-2α and hu-MIP-2β was based on alignment of these sequences with that of mu-MIP-2, as well as consensus rules for signal peptide cleavage sites (36–38). The predicted length of the mature peptide sequence for all three proteins is 73 amino acids. Murine MIP-2 is a basic protein, and the human MIP-2 polypeptides are basic as well, based on predicted isoelectric points of 9.9 and 9.7 for hu-MIP-2α and hu-MIP-2β, respectively. None of the three predicted polypeptides has a consensus signal for N-linked glycosylation.

Murine and Human MIP-2 Homologues. Use of the murine MIP-2 cDNA coding region to isolate a human homologue resulted in the identification of two candidate cDNAs, MIP-2α and MIP-2β. The percentage of nucleotide sequence identity among these three cDNAs, as well as the human gro/MGSA cDNA (3, 4), and mu-KC (10), is presented in Fig. 4A. The human gro/MGSA cDNA encodes a protein with MGSA (40); murine KC is a platelet-derived growth factor-inducible gene presumed to be the murine homologue of human gro/MGSA. Noteworthy is the high degree of nucleotide homology among the three human cDNAs, particularly between hu-gro and hu-MIP-2α. There is an even more striking degree of nucleotide sequence identity among the three human homologues in the coding region, as shown in Fig. 4B. The nucleotide sequence identity in the 3′ untranslated regions of the human MIP-2 homologues is considerably less than that observed in the coding regions, with the exception of these regions of hu-MIP-2α and hu-gro/MGSA (Fig. 4C). These two cDNAs show a high percentage of sequence homology throughout the 3′ untranslated region as well.

Homology comparisons and alignments of the predicted amino acid sequences of the precursor proteins of MIP-2 homo-
logues, including hu-MIP-2α, hu-MIP-2β, hu-gro, ha-gro, mu-MIP-2, and mu-KC, are presented in Fig. 5 A and B. The three human proteins are highly homologous (87-90%), but amino acid differences occur throughout the predicted sequences, particularly at the COOH termini of the mature protein sequences. Based on predicted amino acid homologies alone, it is not possible to assign hu-MIP-2α, hu-MIP-2β, or hu-gro/MGSA as the human homologue of mu-MIP-2 or mu-KC.

Figure 5. Amino acid homology and alignment of MIP-2 homologues and human IL-8. (A) Percentages of identity between the predicted amino acid sequences of MIP-2 homologues as well as human IL-8 (16) were determined with the ALIGN program (52). (B) These amino acid sequences were aligned with GENALIGN, a multiple sequence alignment program based on an algorithm developed by Needleman and Wunsch (53) and Sobel and Martinez (54). An amino acid that is not identical to a corresponding residue in adjacent homologues is designated by a lower case letter. Bold-type amino acid residues indicate the NH2-terminal amino acid(s) determined by sequencing of the isolated protein (2, 4, 17, 18, 55). The predicted protein sequence of hu-gro and ha-gro are from Anisowicz et al. (3); hu-IL-8 is from Schmid and Weissman (16). Sequences were verified against GenBank entries (release no. 62) whenever possible.

Southern Analysis. RAW 264.7 DNA was digested with each of three restriction enzymes, BamH1, EcoR1, and EcoRV, separated by agarose gel electrophoresis and probed with 32P-labeled mu-MIP-2 cDNA. The results, shown in Fig. 6 A, are consistent with mu-MIP-2 cDNA defining a single gene. The same results were obtained when mouse C3N/HeJ DNA was similarly analyzed (data not shown).

A Southern analysis of human genomic DNA was performed with hu-MIP-2α and hu-MIP-2β cDNA probes. Hy-
Figure 6. Southern analysis of genomic DNA with murine and human MIP-2 cDNAs. Genomic DNA, as indicated below, was digested with BamH1, B; EcoR1, E; or EcoRV, R. (A) Murine RAW 264.7 DNA hybridized with mu-MIP-2 cDNA. A blot of restricted human DNA was hybridized first with labeled hu-MIP-2, then the filter was stripped and rehybridized with labeled hu-MIP-2a cDNA (B).

Discussion

We have cloned the cDNA for murine MIP-2 by using a degenerate oligonucleotide probe pool corresponding to a portion of the NH2-terminal amino acid sequence determined on the purified protein. Murine MIP-2 purified from the conditioned medium of endotoxin-stimulated RAW 264.7 cells has diverse activities, including CSF-dependent myelopoesis enhancing activity for CFU-GM (21), elicitation of a localized inflammatory response after subcutaneous administration, and a potent chemotactic activity for human PMN (2). The latter activity is characteristic of human IL-8, also known as 3-10C, MDNCF, NAF, and MONAP (16–20). This functional equivalence suggested that mu-MIP-2 could be the murine homologue of hu-IL-8. However, given that the amino acid homology of mu-MIP-2 to hu-IL-8 is low relative to mu-MIP-2 homology to hu-MIP-2α, hu-MIP-2β, or hu-gro/MGSA (3, 4) (Fig. 5), it seems unlikely that mu-MIP-2 and hu-IL-8 are murine/human homologues. Redundancy of function among cytokines is not uncommon: cachectin/TNF-α and IL-1 have an overlapping activity profile (41). MIP-2 and IL-8 may be another example of this functional redundancy.

Based on nucleotide and proteins homologies, it is likely that MIP-2 and KC (10) are murine homologues of the human cytokines MIP-2α, MIP-2β, and gro/MGSA (3, 4). More specific homologue assignments are difficult to make based only on these data. Further studies of these factors may establish functional homologies that in conjunction with nucleotide and protein identities will allow accurate assignment of interspecies homologues. The identification of two murine factors homologous to three human factors also suggests the existence of an additional murine factor.

We have used the cDNA for murine MIP-2 to clone cDNAs for homologous human genes from the monocytic-like cell line U937, which was stimulated to differentiate by treatment with PMA, and then further stimulated by LPS treatment. We have cloned two cDNAs, hu-MIP-2α and hu-MIP-2β, which are murine homologues of the human cytokines MIP-2α, MIP-2β, and gro/MGSA (3, 4). More specific homologue assignments are difficult to make based only on these data. Further studies of these factors may establish functional homologies that in conjunction with nucleotide and protein identities will allow accurate assignment of interspecies homologues. The identification of two murine factors homologous to three human factors also suggests the existence of an additional murine factor.

We have used the cDNA for murine MIP-2 to clone cDNAs for homologous human genes from the monocytic-like cell line U937, which was stimulated to differentiate by treatment with PMA, and then further stimulated by LPS treatment. We have cloned two cDNAs, hu-MIP-2α and hu-MIP-2β. A noteworthy feature of these cDNAs is the high degree of both nucleotide and predicted amino acid sequence identity among these two cDNAs and the previously cloned cDNA hu-gro/MGSA (3, 4). The percentage of nucleotide sequence identity between hu-MIP-2α and hu-gro/MGSA is particularly striking, as it extends throughout the entire cDNA. The presence of both MIP-2α and MIP-2β in our U937 cDNA library prepared from poly(A)+ RNA from PMA- and LPS-stimulated cells prompted us to screen for gro/MGSA as well. Screening of 5 × 105 plaques from the amplified library with oligonucleotides specific for gro/MGSA and not MIP-2α/β gave no positive signals; in contrast, 56 MIP-2α-positive signals were detected. This suggests that gro/MGSA transcription is not induced in U937 cells stimulated by PMA and LPS.

The results of Southern analysis are consistent with hu-MIP-2β and hu-MIP-2α defining two distinct genes. It is not possible by Southern analysis with a hu-MIP-2α cDNA probe to unequivocally determine if hu-MIP-2α and hu-gro/MGSA define separate genes given the high degree of
nucleotide homology between these cDNAs. The complexity of the hybridization pattern with hu-MIP-2β cDNA, compared with that with hu-MIP-2β cDNA, especially to BamHI-digested genomic DNA, is consistent with the detection of more than one gene. The pattern we observe with hybridization of MIP-2α cDNA to EcoR1-restricted genomic DNA is similar to that reported by Richmond et al. (4) for MGSA cDNA. Differences between the two may reflect differences in the size of the cDNA used as probe and/or the completeness of digestion of genomic DNA.

The high degree of homology among these three cDNAs suggests that their genes may have arisen by duplication and predicts that these genes should be located near each other on the chromosome. Anisowicz et al. (42) have reported in situ hybridization of hu-gro cDNA to a single loci, chromosome band 4q21. Richmond et al. (4) have mapped hu-MGSA to region q13-q21 of chromosome 4 by Southern analysis of human/hamster hybrids using a 700-bp cDNA probe. Given the sequence homology among grolMGSA and MIP-2α/β genes, these experiments may not have distinguished among them. Thus, the detection of only a single chromosomal loci for grolMGSA is indirect evidence for close proximity of the three genes. Thus far, four members of the platelet factor 4 cytokine family, including platelet factor 4 (43), grolMGSA (4, 42), γIP-10 (44), and IL-8 (45), have been localized to this chromosomal region.

Previous studies using hu-gro/MGSA cDNA as a probe for gene expression have shown expression in various transformed cell lines, fibroblasts, epithelial cells, and endothelial cells (3, 4, 42, 46, 47). Expression in some of these different cell types was shown to be markedly induced by stimuli including serum, PMA, IL-1, TNF, LPS, and thrombin (3, 4, 42, 46, 47). IL-1-mediated induction of hu-gro in human fibroblasts has also been confirmed by NH2-terminal sequence analysis of the induced protein (47). The high homology of hu-gro, hu-MIP-2α, and hu-MIP-2β makes problematic the interpretation of the pattern of expression of any one gene from Northern data using cDNA probes. In fact, in several of the above studies, RNA species of two different sizes were noted. Northern analysis with specific oligonucleotide probes will be required to accurately determine the pattern of expression of these genes.

The degree of nucleotide and predicted amino acid homology among human MIP-2α, MIP-2β, and grolMGSA is particularly high compared with their homology with other human members of this cytokine family (Fig. 7). Interestingly enough, there is another example of highly homologous proteins within the platelet factor 4 family of cytokines. A genomic clone has been isolated that encodes a platelet factor 4 variant that is highly homologous to platelet factor 4; there is 85% amino acid identity in the predicted precursor protein and 96% amino acid identity in the mature protein (48).

The existence of these highly homologous human MIP-2α/β and grolMGSA peptides raises the question of their functional independence. Recent structural studies indicate that two members of this cytokine family, platelet factor 4 and IL-8, which have 31% amino acid identity (Fig. 7), share many structural features (49-51), including a COOH-terminal helix that has been postulated to be involved in receptor binding. It is interesting to note that one of the main regions of greatest amino acid variability among hu-MIP-2α, hu-MIP-2β, and hu-gro/MGSA is at their COOH termini. Further studies will be required to address the significance of this observation.
References

1. Nathan, C.F. 1987. Secretory products of macrophages. J. Clin. Invest. 79:319.

2. Wolpe, S.D., B. Sherry, D. Juers, G. Davatelis, R.W. Yurt, and A. Cerami. 1989. Identification and characterization of macrophage inflammatory protein 2. Proc. Natl. Acad. Sci. USA. 86:612.

3. Anisowicz, A., L. Bardwell, and R. Sagar. 1987. Constitutive overexpression of a growth-regulated gene in transformed Chinese hamster and human cells. Proc. Natl. Acad. Sci. USA. 84:7188.

4. Richmond, A., E. Balentien, H.G. Thomas, J. Spiess, R. Body, U. Francke, and R. Derynck. 1988. Molecular characterization and chromosomal mapping of melanoma growth stimulatory activity, a growth factor structurally related to α-thromboglobulin. EMBO (Eur. Mol. Biol. Organ.) J. 7:2025.

5. Deuel, T.F., P.S. Keim, M. Farmer, and R.L. Heinrikson. 1977. Amino acid sequence of human platelet factor 4. Proc. Natl. Acad. Sci. USA. 74:2256.

6. Hermodson, M., G. Schmer, and K. Kurachi. 1977. Isolation, crystallization, and primary amino acid sequence of human platelet factor 4. J. Biol. Chem. 252:6276.

7. Ciaglowski, R.E., J. Snow, and D.A. Walz. 1986. Isolation and amino acid sequence of bovine platelet factor 4. Arch. Biochem. Biophys. 250:249.

8. Poncz, M., S. Surrey, P. LaRocco, M.J. Weiss, E.F. Rappaport, T.M. Conway, and E. Schwartz. 1987. Cloning and characterization of platelet factor 4 cDNA derived from a human erythrocyte precursor cell line. Blood. 69:219.

9. Doi, T., S.M. Greenberg, and R.D. Rosenberg. 1987. Structure of the rat platelet factor 4 gene: a marker for megakaryocyte differentiation. Mol. Cell. Biol. 7:898.

10. Oquendo, P., J. Albertta, D. Wen, J.L. Graycar, R. Derynck, and C.D. Stiles. 1989. The platelet-derived growth factor-inducible KC gene encodes a secretory protein related to platelet α-granule proteins. J. Biol. Chem. 264:4133.

11. Watanabe, K., K. Konishi, M. Fujisaka, S. Kinoshita, and H. Nakagawa. 1989. The neutrophil chemotactic factor produced by the rat kidney epithelial cell line NRK-52E is a protein related to the KC/gro protein. J. Biol. Chem. 264:19559.

12. Sugano, S., M.Y. Stoeckle, and H. Hanafusa. 1987. Transformation by Rous sarcoma virus induces a novel gene with homology to a mitogenic platelet protein. Cell. 49:321.

13. Bedard, P.A., D. Akorta, D.L. Simmons, K.C. Lü, and R.L. Erikson. 1987. Constitutive expression of a gene encoding a polypeptide homologous to biologically active human platelet factor 4 and α-thromboglobulin-like protein in Rous sarcoma virus-transformed fibroblasts. Proc. Natl. Acad. Sci. USA. 84:6715.

14. Holt, J.C., M.E. Harris, A.M. Holt, E. Lange, A. Henschen, and S. Niewiarowski. 1986. Characterization of human platelet basic protein, a precursor form of low-affinity platelet factor 4 and α-thromboglobulin. Biochemistry. 25:1988.

15. Luster, A.D., J.C. Unkeless, and J.V. Ravetch. 1988. γ-Interferon transcriptionally regulates an early-response gene containing homology to platelet proteins. Nature (Lond.). 315:672.

16. Schmidt, J., and C. Weissmann. 1987. Induction of mRNA for a serine protease and a β-thromboglobulin-like protein in mitogen-stimulated human leukocytes. J. Immunol. 139:250.

17. Yoshimura, T., K. Matsushima, S. Tanaka, E.A. Robinson, E. Appella, J.J. Oppenheim, and E.J. Leonard. 1987. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. Proc. Natl. Acad. Sci. USA. 84:9233.

18. Wals, A., P. Peveri, H. Aschauer, and M. Baggiolini. 1987. Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. Biochem. Biophys. Res. Commun. 149:755.

19. Schroder, J.M., U. Mrowietz, E. Morita, and E. Christophers. 1987. Purification and partial biochemical characterization of a human monocyte-derived, neutrophil activating peptide that lacks interleukin 1 activity. J. Immunol. 139:3474.

20. Matsushima, K., M. Morishita, T. Yoshimura, S. Lavy, Y. Kobayashi, W. Lew, E. Appella, H.F. Kung, E.J. Leonard, and J.J. Oppenheim. 1988. Molecular cloning of human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J. Exp. Med. 167:1883.

21. Broxmeyer, H.E., B. Sherry, L. Lu, S. Cooper, C. Carow, S.D. Wolpe, and A. Cerami. 1989. Myelopoietic enhancing effects of murine macrophage inflammatory proteins 1 and 2 on colony formation in vitro by murine and human bone marrow granulocyte/macrophage progenitor cells. J. Exp. Med. 170:1583.

22. Davatelis, G., P. Tekamp-Olson, S.D. Wolpe, K. Hurscen, C. Luedeke, C. Gallegos, D. Coit, J. Merryweather, and A. Cerami. 1988. Cloning and characterization of a cDNA for murine macrophage inflammatory protein (MIP), a novel monokine with inflammatory and chemokinetic properties. J. Exp. Med. 167:1939.

23. Sundstrom, C., and K. Nilsson. 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int. J. Cancer. 17:565.

24. Cathala, G., J.F. Savoure, B. Mendez, B.L. West, M. Karin, J.A. Martial, and J.D. Baxter. 1983. A method for the isolation of intact, translationally active ribonucleic acid. DNA (NY). 2:329.

25. Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Laboratory, NY. 197-198.

26. Wood, W.I., J. Gitschier, L.A. Laskey, and R.M. Lawn. 1985. Base composition-independent hybridization in tetramethylammonium chloride: a method for oligonucleotide screening of highly complex gene libraries. Proc. Natl. Acad. Sci. USA. 82:1585.

27. DiLella, A.G., and S.L.C. Woo. 1987. Cloning large segments of genomic DNA using cosmid vectors. In Guide to Molecular Cloning Techniques. S.L. Berger and A.R. Kimmel, editors. Academic Press, Inc., Orlando, FL. 199-212.

28. Sanger, F., S. Nicklen, and R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA. 74:5463.

29. Kozak, M. 1986. Point mutations define a sequence flanking the ATG initiator codon that modulates translation by eu-caryotic ribosomes. Cell. 44:283.

30. Kozak, M. 1987. An analysis of 5' noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res. 15:8125.

31. Birnstiel, M.L., M. Busslinger, and K. Strub. 1985. Transcription termination and 3' processing: the end is in site. Cell. 699 vertebrate messenger RNAs. Nucleic Acids Res. 15:8125.

32. Caput, D., B. Buteler, K. Hartog, R. Thayer, S. Brown-Schmer, and J.J. Oppenheim. 1986. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. Proc. Natl. Acad. Sci. USA. 83:1670.

33. Shaw, G., and R. Kamen. 1986. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates
34. Kruys, V., M. Wathelet, P. Poupart, R. Contreras, W. Fiers, J. Content, and G. Huez. 1987. The 3' untranslated region of the human interferon-beta mRNA has an inhibitory effect on translation. Proc. Natl. Acad. Sci. USA. 84:6030.
35. Han, J., T. Brown, and B. Beutler. 1990. Endotoxin-responsive sequences control cachectin/tumor necrosis factor biosynthesis at the translational level. J. Exp. Med. 171:465.
36. Perlman, D., and H.O. Halvorson. 1983. A putative signal peptidase recognition site and sequence in eukaryotic and prokaryotic signal peptides. J. Mol. Biol. 167:391.
37. von Heijne, G. 1984. How signal sequences maintain cleavage specificity. J. Mol. Biol. 173:243.
38. von Heijne, G. 1986. A new method for predicting signal sequence cleavage sites. Nucleic Acids Res. 14:4683.
39. Cochran, B.H., A.C. Reffel, and C.D. Stiles. 1983. Molecular cloning of gene sequences regulated by platelet-derived growth factor. Cell. 33:939.
40. Richmond, A., and H.G. Thomas. 1986. Purification of melanoma growth stimulatory activity. J. Cell. Physiol. 129:375.
41. Manogue, K.R., and A. Cerami. 1988. Cachectin (tumor necrosis factor): a macrophage protein that induces a catabolic state and septic shock in infected animals. In Cellular and Molecular Aspects of Inflammation. G. Poste and S.T. Crooke, editors. Plenum Publishing Corporation, New York. 123-150.
42. Anisowicz, A., D. Zajchowski, G. Stemman, and R. Sagar. 1988. Functional diversity of gro gene expression in human fibroblasts and mammary epithelial cells. Proc. Natl. Acad. Sci. USA. 85:9645.
43. Griffin, C.A., B.S. Emanuel, P. LaRocco, E. Schwartz, and M. Poncz. 1987. Human platelet factor 4 gene is mapped to 4q12-q21. Cytoget. Cell Genet. 45:67.
44. Luster, A.D., S.C. Jhanwar, R.S.K. Chaganti, J.H. Kersey, and J.V. Ravetch. 1987. Interferon inducible gene maps to a chromosomal band associated with a (4;11) translocation in acute leukemia cells. Proc. Natl. Acad. Sci. USA. 84:2868.
45. Modi, W.S., M. Dean, H.N. Seuanez, N. Mukaida, K. Matsushita, and S.J. O'Brien. 1990. Monocyte-derived neutrophil chemotactic factor (MDNCF/IL-8) resides in a gene cluster along with several other members of the platelet factor 4 gene superfamily. Hum. Genet. 84:185.