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

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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-2 α and MIP-2 β are highly homologous to each other and to a previously isolated gene, human gro/melanoma growth-stimulating activity (MGSA). These three human genes, MIP-2 α , MIP-2 β , and gro/MGSA, constitute a sub-family within the cytokine family represented by platelet factor 4 and interleukin 8.

acrophages 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 γ IP-10 (15); and IL-8, also known as 3-10C, MDNCF, 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,

inflammatory protein 2.

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.

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 5×10^{-8} M. After 24 h in the presence of PMA, LPS (LPS W, *E. coli* 0127:B8; Difco Laboratories Inc., Detroit, MI) was added to a final concentration of $1 \mu g/ml$, 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 λ gt10.

Murine MIP-2 cDNA Isolation. A degenerate oligonucleotide probe pool corresponding to amino acids 9–14 of the NH_2 -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

¹ Abbreviations used in this paper: GM, granulocyte/macrophage; MGSA, melanoma growth-stimulating activity; MIP-2, macrophage

cDNA library (5 × 10⁵ 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 × 10⁴ 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 by in vitro mutagenesis using the mutagenic primer 5'-CAAAAGAT<u>CT</u>TGAACAAAG-3'. The Ball-BglII fragment encodes most of the mature mu-MIP-2 amino acid sequence, lacking those base pairs encoding the three NH₂terminal 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 $2 \times$ 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 (Amersham Corp., Arlington Heights, IL). Filters were prehybridized and hybridized in 50 mM sodium phosphate, pH 6.5, 5× SSC, 1 mM sodium pyrophosphate, 40% formamide, 10% dextran sulfate, 5× Denhardt's solution, 0.1% SDS, and 100 μ g/ml sonicated salmon sperm DNA. DNAs used for Southern analysis were the 1.1-kb mu-MIP-2 cDNA (clone mMIP-2-20a), the 0.98kb hu-MIP-2 β 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 10° cpm/ml. Hybridization was for 16-18 h at 37°C. Filters were rinsed at room temperature for 10 min in $2 \times$ SSC, 0.1% SDS, then washed three times at 65°C for 45 min each in 0.1 \times 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

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CCCT GTAC GTAC CGCT TGTT AACC CACA TGGC AGAA	CAAC CCTGA TTAA TTAAT TAAT TTAAT CAGT TTTC CAGC TTCA	AAA CGGAA ATGTC CCACI CCACI CCACI CCACI CCACI CCACI CCACI CCACI CCACI CCACI CCACI CCACI CCACI CCACI CCACI CCACI CCACA CGGAA ATGTC CCGAA ATGTC CCGAA ATGTC CCGAA ATGTC CCGAA ATGTC CCGAA ATGTC CCGAA ATGTC CCGAA ATGTC CCGAA ATGTC CCGAA ATGTC CCGAA ATGTC CCCACI C	GGC AGAACA HIOO GCCTC CGTAA CCATT CGTAA CCATT CATTCAA GAGAGA FAATTT CAATG	AAG CCAAA CGCTC GGCCCA ATATC ATTAAC ATTAAC ATTAAC ACTGG VAGGT WAGGT	GCT AGAGA GATCTO AGATO CTTAA AGTT TCTO GGGAT TTCA GAGT	AAC AAAGA GAGAQ TTGI AAAGQ YAAGQ TGTI GTGI YAACQ	TGA TGA TTATC/ TTATC/ TTATC/ GTCAC/ TTATC/ CCAC/ TTTGI	CCTC AAACT ACTTX STTTF STTTF SOO TTATA GGGGGC GGGGGC ATCAI CAAACC 600 GGACA	AAACP AAAACP ATTTA ATTAT ATTAT AATAT AATAT AATAT AATAT AATAT AATAT AATAT AATAT AATAT AATAT AATAT AATAT AATAT AAAACP AAACP AAAACP AAACP AAACP AAACP AAACP AAACP AAAACP AAAACP AAAACP AAAACP AAAACP AAAACP AAAACP AAAACP AAAACP AAAAAAAA	AGGAC AGCAC ATTT/ AGAT/ AGGAC AGGAC ATTGI ATGTA	GGAGC CCCGC ATCT/ ATTT/ AAAG1 AAGG1 ACTAC AAATP CAAGC ACAAC	GGAAG GGAAG TTTAI CGATA GGTAC GGTAC GGTAC CACCC CACCC	GGCTG GCCTG ITTTA 200 TTTTA CATT CATT S00 GCAGT 500 AGGA TTGA	GATC GATC TTTA GATT ATAA TTTA CACC CGGA GAGT TGCT GGCAC
CCCTI GTAC TTTTA CGCTI TGTTI AACCO CACAA TGGCC AGAAA	CCAAC CCTGA TTAA TTAAT TTAAT TTAAT TTAAT TTAAT TTAAT TTAAT TTAAT TTAAT	AAA ATGTC CGGAA TTATT CCACT TTATT CGAAA CGAAA CGAAA TGTT TGTT	GGC AGAAC 100 CCTC CATTC CATTC 400 AGAGA CATTT AANG	AAG CCCAAA CGCTC GGCCCA ATATC ATTAA ATTAA ATTAA ATTAA CAGGT AGGT	GCT AGAGA GATCTO CTTAA AGTT TTCTO GGGAT TTCA GAGT	AAC AAAGA GAGAQ TTGI AAAGQ YAAGQ TGTI GGGGI YAACQ	TGA TGA TTATC TTATC TTATC GAGG GGGTC TTGT TTGT	CCTC ARACTI STTTF STTTF STTTF SGGGG GGGGG GGGGG C SGGGGC SGACA	AAACA ATTTA ATTTA ATTA ATTA ATTA GTTG GTGGG GTGGG GTGGG TTTT TGTA ATTTT	AGGAC AGCAC ATTT// AGAT// AGGA// AGGA// AGGA// ATGI/ ATGI/ ATGI/	CCCGC CCCGC ATCT/ ATTT/ AAAG1 AAGG1 ACTAC AAATP CAAGC ACAAC CCTTC	GGAAG ATGTF + + TTTAT TTAT TGATF GGTAG GGCTAG GGCTAG GGCTAG CCCCCG	GCCTC GCCTC ITTTA 200 ITTTA CATT CATT ACATT S00 AGGA TTGA	GATC GATC GATC GATT ATAA TTTA CACC CGGA GAGT TGCT GCAC
CCCT GTAC TTTA CCCT TGTT AACC CACA AGAA GGAT	CCAAC CCTG# TTAAT TAATT TAATT CAGT TTTCC CAGC TTCCA	AAA CGGAA ATGTC CCCCT CCCCT CCCCT CCCCT CCCCT CCCCT CCCCT CCCCT CCCCT	GGC GGAAC 100 GCCTC CATTC GTAA TTCAA CCATT 400 GGAAG GAAGT CATTT CAATG	AAG CCAAA CGCTC GGCCCA ATATC ATTAA ATTAA AGTT ACTGG WAGGT GTTGT ATTGG	GCT AGAGA ETCTO AGATO TTTAA AGTT TTCTO GGGAI TTCCA GAGI	AAC AAAAGZ GAGAQ GTTGI AAAGQ YAAGQ TGTI GTGI AACC GTCA	TGA TGA TTATC TTATC TTATC GGGTC GGGTC TTTGT	CCTC ARACTI STTTF STTTF STTTF SGGGG GGGGG GGGGG GGGCA CTTTT	AAACA AAAACA ATTTA ATTAI AATAI ATAGI GTTGG GTGGGG TTTTI TTTTI TTTTI CCGTG	AGGAA AGCAA ATTTI AGTTI AGGATI AGGAA AGGAA AGGAA AGGAA ATGTI ATGTI ATGTI	CCCGC CCCGC ATCT/ ATTT/ AAAG1 AAGG1 ACTAC AAATP CAAGC CCTCC CCTTC CGAAG	GGAAG ATGTX + + AAAGA TTTAI CGATA CGATA CGATA CGATA CACCO CCCCCC CCCCCC CCCCCC CCCCCC CCCCCCC	GGCTG GCCTG ITTTA 200 ITTTA 200 ITTTA CATT S00 AGGA TTGA TTGA CACA	GATGT GATC TTTA GATT ATAA TTTA CACC CGGA GAGT TGCT GCAC
CCCT GTAC TTTA CGCT TGTT AACC CACA AGGAT AGTG	CAAC CCAAC CCTGA TTTAA TAATI TAATI TAATI CAGI TTTTC CAGC TTCA CCTT	AAA CGGAA ATGTC CCACI TTATT T CGAAA CATGG TTATT T TTATT T CGAAA CATGG TTATT	GGC GGAAC HIOO GCCTC CAATTC CGTAA TTCAA CGTAA TTCAA GAAGT CATTT CAATG CAATTT CAATG	AAG CCAAA CGCTC GCCCA TTATC TTATC TTATC CTGG CAGTG CAGGT GTTGT	GCT GGAGA GACTO GGATO CTTAA AGTT TTCTO GGGAT TTCCA GAGT TTCCA GAGT	AAC AAAAGA GAGAC GTTGI GGGGA AAAGC TGTI GTGI GTCA	TGA TGA TTATC TTATC TTATC GGGTC GGGTC CCAC2 TTGGT TTGGT TTGCC	CCTC AAAACJ GTTTF GTTTF -300 GGGGG GGGGG GGGGG -17 CAAAC -600 GGACA -17 TTTTT	ATTTA ATTTA ATTTA ATTTA ATATA ATAGI ATTGG AGTTG AGTTG AGTTG AGTTG AGTTG AGTTG AGTTG AGTTG AGTTG AGTTG AGTTG AGTTG AGTTG AGTA AGTTA A	AGGAC AGCAC ATTT// AGAT// AGGA// AGGAC// TTGI/ ATGI/ ATGI/ ATGI/ TCTI/	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	ATGTA ATGTA ATGTA ANAGA TTTAI CGATA GGTAC AGATG AGATG CATTT CCTCG	GCCTG GCCTG ITTTA 200 ITATC ITTTA CATT SOCAGT 500 AGGA ITTGA TTGA CACA	GATC GATC ATTTA GATT ATAA TTTA CACC CGGA GAGT TGCT GCAC TTTA
CCCCT GTAC TTTA CGCT TGTT AACC CACA AGAC GGAT AGTG TTTA	CCAAC CCTGA TTAA AATTI TAATI TTAATI CAGT TTTCC CAGC TTCCA CCTTI TTCCA	AAA CGGAA ATGTC CCACI CC	GGC GGC 100 GCCTC CATTG CATTG CATTG CATTG CATTG CATT CATT	AAG CCAAA CGCTCC GCCCCA ATATCC ATTAAC AGGT ACTGG AGGT ATTGG ATTGG TACA	GCT GGAGA GTCTCC GTTAA AAGTI TTCCC GGGAT TTCCA GAGI TTTCA	AAC AAAGA BAGAG BTTGI BGGGA WATTI GGGGA TGTI GTGI GTGI GTCA ACAA	TGA TGA TTATC TTATC TTATC GGCTC CACA TTGT TCCCC	CCTC ANAACJ GTTTF GTTTF GGTGJ GGGGGG ATCAT TAAAAC 600 GGACA TTTTT	ATTTA ATTTA ATTTA ATTA ATTA AATA AATA	AGGAC AGCAC ATTTY AGTTY AGGTY AGGATY AGGATY AGGAC ATGI ATGI ATGI ATGI ATGI	COLOR COLOR	ATGTA ATGTA ATGTA ATGTA ANAGA TTTAI CGATA GGTGGA CACCO CATTI	GCCTC GCCTC TTTTA 200 TTTTA CATT CATTC CATT CATCC CAGT TTGA TTGA	GATC GATC ITTTA GATT ATAA TTTTA CCACC CGGA GAGT TCCAC GCACC TTTA
CCCT GTAC TTTA CGCT TGTT AACC CACA AGAA AGGGT TTTA	CCAAC CCAAC CCTGA TTTAA TAATT TAATT TAATT CAGT TTTCA CCTT TTCA	AAA CGGAN ATGTO CCACT CCACT CCACT CCACT CCACT CCACT CCACT CCACT CCACT CCACT CCACT CCACT CCACT	GGC GGAAC 100 GCCTC CATTC GGAAA CCATTC 400 GGAGA CCATT CATT FAATG CATTT FAATG CATTT FAATG CATTT	AAG CCAAA CGCTC GCCCA TATC TATC TATC CTGG CCGG CC	GCT GGAGA GAGAGA GATC TTTAA AAGTI TTCTC GGGAI TTCCA GAGTI TTTCA GAGTI	AAC AAAGA GAGAC GTTGI AAAGC YATTI GGGGA GTGI GTCA ACAA	TGA TGA TTATC TTATC TTATC TTATC GGGTC GGGTC CCACF TTGT TTTGT TTCCC	CCTCI AAAACJ ACTTJ GTTTF -300 TTATF GGTGJ GGGGGC GGGGGC GGGGGC GGACA TTAAA	ATTTA ATTTA ATTTA ATTA ATTA ATTA ATTA	AGGAC AGCAC ATTT? AGAT? AGGT? AGGAC? ATGG? ATGG? ATGG? ATGG? ATGG? ATGG? ATGG? ATGG? ATGG? ATGG? ATGG? AGGAC?	GGAGC CCCCGC ATCT/ ATTT/ AAAG7 AAAG7 AAAG7 AAAG7 CAAGC CCTCC CGAAC CTTAA	GGAAAG ATGTA AAAAGA TTTAI TTTAI CGATA GGATA CGATAC CACCO CCCCC CCCCCC CCCCCC CCCCCCCCCC	GCCTC GCCTC GCCTC TTTTA 200 TTTTA CATT CATC CATT S00 AGGA TTGA TTAAG CACA	GATC GATC ITTA GATT AATAA TTTA CACC CGGA GAGT TTGCT GCAC TTTA AAAA
CCCC1 GTAC TTTA CGC1 TGTT AACC CACA AGGAT AGGAT AGGAT	CCAAC CCTGA TTAA TTAAT TTAAT TTAAT TTAAT CAGT TTCA CCTT TTCA	AAA ATGTC ATGTC CCGTG TTATT CCGTG CGTG C	GGC AGAAC +100 GCCTC CATTO CATTO CGTAA GGAGA GAAGT CATTT CAATTT CAATTT CAATTT CAATTT CAATTT CAATTT CAATTT CAATTTT CAATTTT	AAG CCAAA CGCTCC CGCCCA ATATC ATTAA CTGG CTGG	GCT AGAGA STCTO GATC CTTAA AGTT TCTC GGGAT TTCTC GGGAT TTCTC GGGAT	AAC AAAGA GAGAG STTGI AAAGG VATTI GGGGP VAAGG GTGI VAACC GGTCA	TGA TGA TTATC: TTATC: TTATC: GGGGTC: CCAC: TTGGTC: TGCC: TGCC: TGCC: TGCC:	CCTC ANACJ STTTJ STTTJ STTTJ STTTJ STTTJ STTTJ STTTJ SGGGG SGG SGG SGG SGG SGG SGG SG S	AAAACA AAAACA AATTTA AATAT AATAT ATAGI GTTGG GTTGGG TTTTT TTGTA ATTTT CCGTG CAAACG	AGGAC AGCAC ATTTY AGATH AGATH AGGTH AGGATH AGGACH AGGACH ATGT ATGTA ATGTA ATGTA	GGAGG CCCGG ATCTI ATTTI AAAGT AAAGT AAAGT CAAC CCTTC CCTTC CGAAC STTAA	GGAAAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	GCCTG GCCTG ATTTA 200 TTTTA CATT ATCC CATT S00 AGGA TTGA TTGA CACA AAAAA	GATC GATC TTTA GATT ATAA TTTA CACC CGGA GAGT TGCT GCAC TTTA AAAA

Figure 1. Nucleotide sequence and predicted protein sequence of murine MIP-2. The molecular weight for the mu-MIP-2 precursor is 10,623. The mature protein sequence, starting at position 1, has a molecular weight of 7,851. These sequence data have been submitted to the EMBL/GenBank Data Library under the accession number X53798.

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 (\sim 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 LPSstimulated cells. DNA from plaques positive on low stringency wash was isolated and subjected to restriction endonuclease analysis, which suggested the presence of two classes

Met Ala Arg Ala CTCTCCTCCTCGCACAGCCGCTCGAACCGCCTGCTGAGCCCC ATG GCC CGC GCC -30-20 Thr Leu Ser Ala Ala Pro Ser Asn Pro Arg Leu Leu Arg Val Ala ACG CTC TCC GCC GCC CCC AGC AAT CCC CGG CTC CTG CGG GTG GCG -10 Leu Leu Leu Leu Leu Val Ala Ala Ser Arg Arg Ala Ala Gly CTG CTG CTC CTG CTC CTG GTG GCC GCC AGC CGG CGC GCA GCA GGA 10 1 Ala Pro Leu Ala Thr Glu Leu Arg Cys Gln Cys Leu Gln Thr Leu GCG CCC CTG GCC ACT GAA CTG CGC TGC CAG TGC TTG CAG ACC CTG 30 20 Gln Gly Ile His Leu Lys Asn Ile Gln Ser Val Lys Val Lys Ser CAG GGA ATT CAC CTC AAG AAC ATC CAA AGT GTG AAG GTG AAG TCC 40 Pro Gly Pro His Cys Ala Gln Thr Glu Val Ile Ala Thr Leu Lys CCC GGA CCC CAC TGC GCC CAA ACC GAA GTC ATA GCC ACA CTC AAG 50 60 Asn Gly Gln Lys Ala Cys Leu Asn Pro Ala Ser Pro Met Val Lys AAT GGG CAG AAA GCT TGT CTC AAC CCC GCA TCG CCC ATG GTT AAG 70 73 Lys Ile Ile Glu Lys Met Leu Lys Asn Gly Lys Ser Asn AAA ATC ATC GAA AAG ATG CTG AAA AAT GGC AAA TCC AAC TGA CCAG . ٠ ٠ AAGGAAGGAGGAAGCTTATTGGTGGCTGTTCCTGAAGGAGGCCCTGCCCTTACAGGAACA +100 GAAGAGGAAAGAGAGACACAGCTGCAGAGGCCACCTGGATTGCGCCTAATGTGTTTGAGC • . . +200 **TTCTATGTTAATATTTTATGTGTAAAATAAGGTTATGATTGAATCTACTTGCACACTCTC** +300**TAATTTGAAGATAGAAGGTTTGCAGATATTCTCTAGTCATTTGTTAATATTTCTTCGTGA** +400٠ TGACATATCACATGTCAGCCACTGTGATAGAGGCTGAGGAATCCAAGAAAATGGCCAGTG AGATCAATGTGACGGCAGGGAAATGTATGTGTGTCTATTTTGTAACTGTAAAGATGAATG +500TCAGTTGTTATTTATTGAAAATGATTTCACAGTGTGTGGTCAACATTTCTCATGTTGAAGC +600 ٠ . ٠ **ACTGCCTTGTTTAATGTTAATTATGCAGTGTTTCCCTCTGTGTTAGAGCAGAGAGGGTTTC** +700 . . GATATTTATTGATGTTTTCACAAAGAACAGGAAAATAAAATATTTAAAAATAAAAAA

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Figure 2. Nucleotide sequence and predicted protein sequence of human MIP- 2α . The molecular weight for the hu-MIP- α precursor is 11,391; the molecular weight of the predicted mature protein is 7,894. The NH₂-terminal amino acid of the mature protein was determined based on alignment with mu-MIP-2 and the rules for signal sequence cleavage sites. The nucleotides in the 3' untranslated region from +690 through the poly(A)⁺ were determined from two cDNA clones isolated from an independently derived cDNA library using a hu-MIP- 2α -specific oligonucleotide probe. (M. Fabre, S. van Deventer, personal communication). These sequence data have been submitted to the EMBL/GenBank Data Library under the accession number X53799.

of clones. Insert cDNAs from representative clones of each class were subloned into M13, and the nucleotide sequences were determined. The nucleotide sequence and predicted amino acid sequence of hu-MIP-2 α 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-2 β , 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-2 α , and hu-MIP-2 β 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-2 α and

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MIP-2 β 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-2 α 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 β cDNA. This 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 TTATTTAT found in the 3' untranslated region of many cytokine genes (32) and implicated

стсо	;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;	GCTI	recco	ACGO	GTC1	IGCTO	GAGCO	ccc	-34 Met ATG	Ala GCC	His CAC	Ala GCC	-30 Thr ACG	Leu CTC
Ser TCC	Ala GCC	Ala GCC	Pro CCC	Ser AGC	Asn AAT	Pro CCC	Arg CGG	-20 Leu CTC	Leu CTG	Arg CGG	Val GTG	Ala GCG	Leu CTG	Leu CTG
Leu CTC	Leu CTG	Leu CTC	-10 Leu CTG	Val GTG	Ala GCC	Ala GCC	Ser AGC	Arg CGG	Arg CGC	Ala GCA	Ala GCA	Gly GGA	1 Ala GCG	Ser TCC
Val GTG	Val GTC	Thr ACT	Glu GAA	Leu CTG	Arg CGC	Cys TGC	10 Gln CAG	Cys TGC	Leu TTG	Gln CAG	Thr ACA	Leu CTG	Gln CAG	Gly GGA
Ile ATT	His CAC	20 Leu CTC	Lys AAG	Asn AAC	Ile ATC	Gln CAA	Ser AGT	Val GTG	Asn AAT	Val GTA	Arg AGG	30 Ser TCC	Pro CCC	Gly GGA
Pro CCC	His CAC	Cys TGC	Ala GCC	Gln CAA	Thr ACC	Glu GAA	40 Val GTC	Ile ATA	Ala GCC	Thr ACA	Leu CTC	Lys AAG	Asn AAT	Gly GGG
Lys AAG	Lys AAA	50 Ala GCT	Cys TGT	Leu CTC	Asn AAC	Pro CCC	Ala GCA	Ser TCC	Pro CCC	Met ATG	Val GTT	60 Gln CAG	Lys AAA	Ile ATC
Ile ATC	Glu GAA	Lys AAG	Ile ATA	Leu CTG	Asn AAC	Lys AAG	70 Gly GGG	Ser AGC	Thr ACC	73 Asn AAC	TGA	CAGO	GAGAG	GAAGT
AAGA	AGC	TATO		STATO	TATTO	GACAC	CTTCC	TGC	AGGG1	GGTC	CCTO	SCCC1	TACO	CAGAG
TGAC	TATI	TCTI	TACGA	GGG	TCT	CTT	ATTT#	TGT <i>I</i>	TTT#	.100 <i>2</i>	TGA	AGCI	TGT	ATTTT
AATA	TTTI	ACA1	GCTO	STTAT	TTA	AGAT	GTG	GTG	GTTI	CATO		ATA0	SCTC#	GTCC
TGA1	CAT	TAA1	rtggø Igtcø	GCCF	CCTI	GGTT	TTTAF AAATO	ATG1	GTCA	GGGG	ACTA	GAGO	GTGC	GGGGG
ATTO	AAAT	GCAP	GCAA	• •	STGG#	TCAC	+400 CTGT1 -	AGGO	• STAAG	GGAA	TGT	ATGT <i>I</i>	CAC	ТСТА
TTTI ATT <i>P</i>	TTAT + TGTO	ACTI 500 TTCA	ACA1	TTTA TTTA	AAAA ATGO	AAGA TGAA	ATGI	CAG1	TGT1	CATT	TTAT TTA1	GTC1	TATO	TCAC
GGGC	ATA	TGCC	TTGI	• •	TGTO	CATI	CTGC	CAGCO	• STTTC	TCT1	+ TCCC	-600 CTTGC	GAAAJ	GAGA
ATTI	ATCA	TTAC	TGTI	AC										

Figure 3. Nucleotide sequence and predicted protein sequence of human MIP-2 β . The molecular weight of the hu-MIP-2 β precursor is 11,345; the molecular weight of the predicted mature protein is 7,867. The NH₂-terminal amino acid of the mature protein was determined based on alignment with mu-MIP-2 and rules for signal sequence cleavage sites. These sequence data have been submitted to the EMBL/GenBank Data Library under the accession number X53800.

in mRNA stability (33) and efficiency of translation (34, 35) is present in multiple copies in all three cDNAs. This sequence is present at four positions, two overlapping, in the 3' untranslated region of mu-MIP-2 (positions +122, +126, +142, +146); and is present two times (positions +158 and +471) and five times, two overlapping (positions +148, +152, +156, +160, and +492) in the 3' untranslated regions of human MIP-2 β and MIP-2 α , respectively.

Features of the Predicted Amino Acid Sequence. The open reading frames of mu-MIP-2, hu-MIP-2 α , and hu-MIP-2 β predict polypeptides of 100, 107, and 107 amino acids, respectively. Secondary structural analysis of the predicted protein sequences of the three polypeptides indicates that the initial ~30 amino acids of each have characteristic features of a signal sequence (36-38). The NH₂-terminal amino acid sequence determined for secreted MIP-2 purified from the conditioned medium of LPS-stimulated RAW 264.7 cells (2) determined

hu-MIP-2β	hu-MIP-2α 57.2 70.0	hu- <i>gro</i> 56.5	mu- <i>KC</i> 46.4
	57.2 70.0	56.5	46.4
mu-MIP-2 54.1	70.0		
hu-MIP-2β		69.5	57.0
hu-MIP-2a		87.4	47.6
hu-gro			50.8
B Coding			
mu-MIP-2 71.3	71.3	70.4	75.6
hu-MIP-2β	94.4	90.7	67.9
hu-MIP-2a		93.5	69.4
hu-gro			67.3
C 3' Untranslated			
mu-MIP-2 48.1	53.1	53.4	47.3
hu-MIP-2β	59.5	60.9	55.7
hu-MIP-2α		86.2	48.8
hu-gro			48.5

Figure 4. Nucleotide sequence homology of MIP-2 homologues. Percentages of nucleotide sequence identity between mu-MIP-2, hu-MIP-2 α , hu-MIP-2 β , hu-gro/MGSA (3, 4), and mu-KC (10) in the cDNA (A), coding region (B), and 3' untranslated region (C) were determined with the ALIGN program (52).

the start of the mature mu-MIP-2 protein in the predicted amino acid sequence. The assignment of the start of mature peptide sequence for hu-MIP-2 α and hu-MIP-2 β 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-2 α and hu-MIP-2 β , 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-2 α and MIP-2 β . The percentage of nucleotide sequence identity among these three cDNAs, as well as the human gro/MGSA cDNA (3, 4) and the murine KC cDNA (10, 39), is presented in Fig. 4 A. The human gro/MGSA cDNA encodes a protein with MGSA (40); murine KC is a plateletderived 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-2 α . There is an even more striking degree of nucleotide sequence identity among the three human homologues in the coding region, as shown in Fig. 4 B. 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-2 α and hu-gro/MGSA (Fig. 4 C). 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-

	hu-MIP-2β	hu-MIP-2a	hu-gro	ha-gro	mu-KC
mu-MIP-2	59.8	57.9	57.9	68.3	63.0
hu-MIP-2β		87.9	86.9	61.1	55.1
hu-MIP-2a			89.7	64.8	58.9

hu-gro	59.3	55.1
ha-gro		80.2
mu-KC		

B

hu-gro/MGSA	1	MARAaLS	AAP	SNP	RLLR	VALL:		/AAg	RRAA	.G ≱ .s∖ ∣∣	ATEL	RCQCL	QT	LQGIH	pKNIQ	SVnV	KSPGPH
hu-MIP-2α	1	MARATLS	AAP	SNP	RLLF	VALL	LLLL	VAAS	RRAA	GAp	LATEL	RCOCL	QT	LOGIHI	LKNIQ	SVkv	KSPGPH
hu-MIP-2 B	1	MAhATLS	III AAP	SNPI	 RLLR	VALL:	LLLLN	 /AAS	 RRAA	∣ GAs\	 /vTEL	RCOCL	II OT	LOGIHI	LKNIQ	 SVnV	IIII rSPGPH
h (MGG)	-							1 1									
na-gro/MGSA	T	I IIII		Р	ЦЦ	ا ما ما		ATS	RLAT	GAPV	ANEL	KCOCT			IIII WNTO		
mu-KC	1	MIPATRS	LLCA			A :		ATS	RLAT	GAPi 	LANEL	RCQCL	QT 	MaGiHI	LKNIQ	SLKV	1PsGPH
mu-MIP-2	1	MaPpT c	rLLsA			Alv1	LLLL	ATn	hqAI	GAV	Asel	RCQCL	KT	lprvd	fKNIQ	SLsV	/tPpGPH
hu-IL-8	1	M tskla	vaLlA			À f	L is	aAlc	eg a v	lpr	BAKEI	RCQCi	.KT	yskpfh	pKfIk	eLr\	/iesGPH

hu-gro/MGSA	69	CAQTEVIATLKNGrKACLNPASPiVKKIIEKMLnsdKSN
hu-MIP-2α	69	CAQTEVIATLKNGqKACLNPASPMVKKIIEKMLknGKSN
hu-MIP-2 β	69	CAQTEVIATLKNGkKACLNPASPMVQKIIEKiLnKGstN
ha-gro	63	CTQTEVIATLKNGqEACLNPEAPMVQKIVQKML KsGirK
mu-KC	59	CTQTEVIATLKNGrEACLDPEAPLVQKIVQKML K GvpK
mu-MIP-2	62	CAQTEVIATLKgGqkvCLDPEAPLVQKIiQKiLnK Gkan
hu-IL-8	61	CAnTEiIvkLsdGrelCLDPkenwVQrvveKfLkraens

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 II-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.

logues, including hu-MIP-2 α , hu-MIP-2 β , hu-gro/MGSA, 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-2 α , hu-MIP-2 β , or hu-gro/MGSA as the human homologue of mu-MIP-2 or mu-KC.

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 ³²Plabeled 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).

hu-IL-8

36.3 32.7

33.6

34.5

38.2

41.4

A Southern analysis of human genomic DNA was performed with hu-MIP-2 α and hu-MIP-2 β 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 β cDNA (C), then the filter was stripped and rehybridized with labeled hu-MIP-2 α cDNA (B).

bridization and wash conditions were determined that made it possible to distinguish between hu-MIP-2 α and hu-MIP- 2β when probed with their respective cDNAs. These conditions are, however, unlikely to distinguish between hu-MIP- 2α - and hu-gro/MGSA-specific sequences. The results of Southern analyses of genomic human DNA restricted with BamH1, EcoR1, or EcoRV, and probed with hu-MIP-2 α or hu-MIP-2 β cDNA, are presented in Fig. 6 B and C, respectively. The patterns of hybridization obtained with each cDNA clearly differ. MIP-2\alpha cDNA hybridized strongly to EcoRV fragments of \sim 23 and \sim 1.1 kb, whereas MIP-2 β cDNA hybridized to a 7.0-kb EcoRV fragment. Similarly, hu-MIP-2 β cDNA hybridized to 1.8- and 3.3-kb (weakly) EcoR1 fragments, whereas hu-MIP-2 α hybridized strongly to 3.3- and an ~1.1-kb EcoR1 DNA fragments and weakly to 4.6- and 3.8-kb EcoR1 fragments. Finally, MIP-2 β cDNA hybridizes strongly to a 2.4-kb BamH1 fragment and very weakly to a 4.3-kb BamH1 fragment, whereas human MIP-2a cDNA hybridizes strongly to 20-, 2.0-, and 1.1-kb BamH1 fragments and less strongly to a 4.3-kb BamH1 fragment. The greater complexity of the hybridization pattern obtained with hu-MIP-2 α cDNA probe, especially from BamH1-digested DNA, relative to that obtained with hu-MIP-2 β cDNA probe, suggests that the former probe is detecting more than one gene, presumably hu-gro. Experiments are in progress with specific oligonucleotides to distinguish hu-gro and hu-MIP- 2α genes. We can conclude from the data shown here that hu-MIP-2 β and hu-MIP-2 α are two distinct genes.

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 myelopoietic 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-2 α , hu-MIP-2 β , 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-2 α , MIP-2 β , 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-2 α and hu-gro/MGSA is particularly striking, as it extends throughout the entire cDNA. The presence of both MIP-2 α and MIP-2 β in our U937 cDNA library prepared from poly(A)⁺ RNA from PMA- and LPSstimulated cells prompted us to screen for gro/MGSA as well. Screening of 5 \times 10⁵ plaques from the amplified library with oligonucleotides specific for gro/MGSA and not MIP- $2\alpha/\beta$ gave no positive signals; in contrast, 56 MIP-2 α -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-2 β and hu-MIP-2 α defining two distinct genes. It is not possible by Southern analysis with a hu-MIP-2 α cDNA probe to unequivocally determine if hu-MIP-2 α and hugro/MGSA define separate genes given the high degree of hu-MIP-2α hu-gro hu-IL-8 hu-pbp hu-pf4 hu-pf4v hu-γip10

hu-MIP-2β	87.9(107)	86.9(107)	38.9(95) 48.1(77)	43.0(93)	37.6(93)	26.1(69)
hu-MIP-2a		89.7(107)	40.0(95) 48.8(82)	43.6(94)	39.2(102)	30.6(72)
hu- <i>gro</i>			41.1(95) 51.2(82)	46.8(94)	42.2(102)	29.0(69)
hu-1L-8			46.4(60)	30.6(85)	25.5(98)	27.7(83)
hu-pbp				55.4(65)	48.6(70)	35.9(64)
hu-pf4					88.2(93)	35.5(62)
hu-pf4v						36.7(60)

nucleotide homology between these cDNAs. The complexity of the hybridization pattern with hu-MIP-2 α cDNA, compared with that with hu-MIP-2 β cDNA, especially to BamH1digested genomic DNA, is consistent with the detection of more than one gene. The pattern we observe with hybridization of MIP-2 α 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 gro/MGSA and MIP-2 α/β genes, these experiments may not have distinguished among them. Thus, the detection of only a single chromosomal loci for gro/MGSA 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), gro/MGSA (4, 42), γ IP-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, Figure 7. Amino acid homologies between members of the human platelet factor 4 cytokine family. Homologies were determined with the FASTP sequence alignment program (56). The numbers in parenthesis indicate the number of amino acids used in the calculation of the percent identity. Amino acid sequences for the indicated proteins were from the following references: hu-gro (3); hu-IL-8 (16), human platelet basic protein, hu-pbp, (14); human platelet factor 4, hu-pf4 (6); variant of human platelet factor 4, hu-pf4v (48); and IFN- γ -inducible protein, γ ip10 (15). When possible, sequences were verified against GenBank (release no. 62) or Dayhoff (release no. 22) entries.

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-2 α , and hu-MIP-2 β 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-2 α , MIP-2 β , and gro/MGSA 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- $2\alpha/\beta$ and gro/MGSA 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.

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