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Biochemistry

A macrophage mRNA selectively induced by γ-interferon encodes a member of the platelet factor 4 family of cytokines

(differential screening)

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ABSTRACT **In order to identify novel mediators synthesized in activated macrophages, a cDNA library was prepared from cultures of the mouse macrophage cell line RAW 264.7 that had been treated with lymphokine-rich conditioned medium from mitogen-stimulated mouse spleen cells. Differential plaque hybridization identified a cDNA, designated m119, that detected a 1.6-kilobase mRNA that accumulated in response to γ-interferon (IFN-γ) but not in response to other macrophage activators, including IFN-α, IFN-β, and lipopolysaccharide. The mRNA encoded a predicted protein of M_r 14,461 containing a 21-amino acid signal peptide. The primary structure of the predicted protein indicated that it is a member of a recently described family of cytokines related to platelet factor 4, including Gro/melanoma growth stimulatory activity and neutrophil-activating peptide/interleukin 8. The selective induction of the m119 mRNA by IFN-γ suggests that the predicted m119 protein mediates a macrophage activity regulated by IFN-γ. The m119 protein may be a cytokine that affects the growth, movement, or activation state of cells that participate in immune and inflammatory responses. It is proposed that the gene encoding this protein be called *mig*, for *m*onokine *i*nduced by *g*amma interferon.**

Activated macrophages exhibit a wide range of activities, including the presentation of antigen, the recruitment of inflammatory cells, the stimulation of cell growth, and the destruction of pathogens and tumor cells. Cytokines play a central role in macrophage physiology, both as macrophage activators (1) and as mediators of macrophage activities (2). The best characterized macrophage-activating cytokine is γ-interferon (IFN-γ), which is able to induce the expression of major histocompatibility complex class II antigens (3), prime macrophages for the release of reactive oxygen intermediates that are important for pathogen and tumor cell killing (4), and enhance the expression of the pleiotropic macrophage products tumor necrosis factor (TNF) and interleukin 1 (IL-1) (5). IFN-γ (type II IFN) and other macrophage activators such as lipopolysaccharide (LPS) and type I IFN (IFN-α and IFN-β) act by altering gene expression (6, 7). While the sets of genes induced by these factors overlap, genes have been identified that are activated preferentially by type II IFN (8, 9) or by type I IFN (6) or by LPS (7). As regards the IFNs, the molecular mechanisms whereby genes are differentially regulated by type I and type II IFNs are unknown.

The cytokines produced by activated macrophages include, in addition to extensively studied mediators such as TNF and IL-1, secreted proteins such as the members of the platelet factor 4 (PF4) family IP-10 (8), IL-8 (10), and macrophage inflammatory protein 2 (MIP-2) (11). Both IL-8 (10) and MIP-2 (11) have been shown to be chemoattractants for human neutrophils, and IL-8 has been found to modulate neutrophil adherence to endothelial cells (12).

It was presumed that lymphokine-treated macrophages would produce novel cytokines that would mediate the functions expressed by activated macrophages. Because of the wide involvement of macrophages in processes relevant to human health and disease, novel macrophage products (and/or inhibitors of these products) would be of potential therapeutic value. A cDNA library was prepared from lymphokine-stimulated RAW 264.7 cells (a mouse monocyte/macrophage cell line), and the library was screened by differential hybridization. This has led to the identification of a set of genes that are induced by IFN-γ. In this report I describe the analysis of the cDNA of an mRNA that is selectively induced by IFN-γ and that encodes a member of the PF4 family of cytokines.*

MATERIALS AND METHODS

Cell Culture, Spleen Cell Conditioned Medium (CM), Cytokines, and LPS. RAW 264.7 cells (13) were obtained from the American Type Culture Collection and grown in RPMI-1640 supplemented with 10% fetal bovine serum. Lymphokine-rich CM was prepared using concanavalin A (Con A)-stimulated spleen cells from male C57BL/6 mice according to the procedure of Marcucci *et al.* (14). IFN-γ was mouse recombinant protein with a specific activity $\geq 10^7$ units/mg (Amgen Biologicals) or 1.2×10^7 units/mg (generously provided by Genentech). The IFN-α and IFN-β were murine natural products with specific activities of 1.4×10^6 international reference units (IRU)/mg and 1.3×10^8 IRU/mg, respectively (Lee BioMolecular Laboratories, San Diego, CA). All other cytokines were obtained from Genzyme, Boston, MA. When cycloheximide (CHX) was used, it was added simultaneously with the activator at 10 μg/ml. When assayed for endotoxin, medium saved following treatment of the RAW 264.7 cells with each of the cytokines gave levels of <0.5 endotoxin unit/ml (chromogenic *Limulus* amoebocyte lysate test, Whittaker Bioproducts). LPS used to activate cells was from *Escherichia coli* 0127:B8 (Difco).

Construction and Screening of cDNA Library. Poly(A)$^+$ RNA was prepared from RAW 264.7 cells that had been exposed for 3 hr, in the presence of CHX at 10 μg/ml, to 20% CM from Con A-stimulated spleen cells. cDNA was synthesized (15) and a library was constructed in λgt10 (16). The library was screened by differential plaque hybridization (17) using a cDNA probe prepared from RAW cells stimulated as described immediately above and using a cDNA probe prepared from control RAW cells treated for 3 hr with identical concentrations of Con A and CHX but without spleen cell CM.

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Abbreviations: IFN, interferon; TNF, tumor necrosis factor; IL-*n*, interleukin *n*; LPS, lipopolysaccharide; PF4 platelet factor 4; MIP, macrophage inflammatory protein; MGSA, melanoma growth stimulatory activity; CHX, cycloheximide; CM, conditioned medium.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M34815).

Sequencing of cDNA and Genomic Clones. Overlapping deletions for sequencing of cDNA clones inserted into the pBluescript phagemid (Stratagene) were made using exonuclease III and additional reagents (Promega) according to the vendor's protocols. DNA sequencing was done by the dideoxy chain-termination method (18) with reagents from United States Biochemical.

RESULTS AND DISCUSSION

Isolation of cDNA Clone m119. A cDNA library in the λgt10 vector was prepared from RAW 264.7 cells treated with CM from Con A-stimulated mouse spleen cells. Screening by differential plaque hybridization led to the isolation of cDNA clones that identified 11 mRNA species that accumulated in the RAW 264.7 cells following exposure to the spleen cell CM. As shown in Fig. 1*A*, one cDNA clone, 1.2 kilobases (kb) long and designated m119, hybridized to a major mRNA species of approximately 1.6 kb that was induced in RAW cells by the CM from Con A-stimulated spleen cells but not detectable in control RAW cells even with long exposure of the autoradiograph. CHX did not inhibit expression of the m119 mRNA, demonstrating that new protein synthesis was

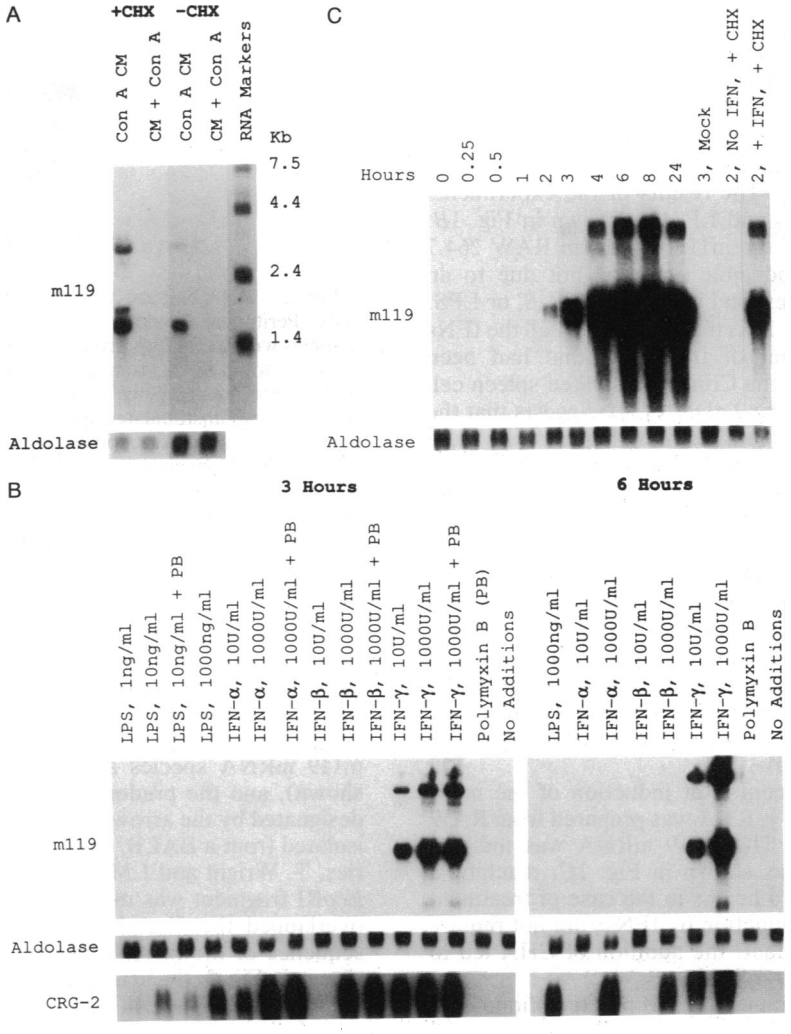

FIG. 1. RNA blot analysis of m119 mRNA in the RAW 264.7 cell line. All Northern analysis was done using total RNA (19) and formaldehyde/agarose denaturing gels as described (20). (*A*) m119 mRNA in RAW 264.7 cells treated with CM from Con A-stimulated spleen cells. Twenty micrograms of total RNA was loaded per lane and hybridized sequentially to ^{32}P-labeled m119 and aldolase A cDNA probes. The RAW 264.7 cells had been treated for 3 hr as follows (from left to right): 20% CM from Con A-stimulated spleen cells plus CHX (10 μg/ml); 20% CM from unstimulated spleen cells plus Con A (10 μg/ml) and CHX (10 μg/ml); 20% CM from Con A-stimulated spleen cells; 20% CM from unstimulated spleen cells plus Con A (10 μg/ml). In this and subsequent experiments, the ^{32}P-labeled m119 cDNA probe was prepared by the nick-translation or random primer method from a 1.2-kb cDNA clone, and the aldolase A probe was prepared from a 1.4-kb cDNA clone kindly provided by A. Levy, L. Sanders, and D. Nathans. The RNA markers were obtained from Bethesda Research Laboratories and were detected by hybridization to bacteriophage λ DNA probe. In all experiments, autoradiography was done using an intensifying screen at −70°C, unless otherwise noted. The m119 signals shown here are from a 12-hr exposure. The aldolase signals are from a 24-hr exposure at room temperature. The order of the lanes from the original blot has been changed for the figure. (*B*) m119 mRNA in RAW 264.7 cells treated with LPS and IFN-α, -β, and -γ. Total RNA was prepared from RAW 264.7 cells treated for 3 or 6 hr with the stimuli as noted and was analyzed as in *A*. Duplicate 3- and 6-hr blots were hybridized to a Crg-2 cDNA probe. While polymyxin B (PB, 5 μg/ml) significantly diminished the induction of Crg-2 mRNA by LPS, it had no effect on the induction of m119 mRNA by IFN-γ or on the induction of Crg-2 mRNA by the IFNs, suggesting that there were not trace amounts of contaminating LPS contributing to the IFN responses. The m119 signals are from a 4-day exposure, and aldolase and Crg-2 signals from exposures at room temperature for 24 and 33 hr, respectively. U, units. (*C*) Time course of m119 mRNA in RAW 264.7 cells in response to IFN-γ. RNA was prepared from RAW 264.7 cells harvested after treatment with IFN-γ (100 units/ml) for 0–24 hr, or after treatment with IFN-γ (100 units/ml) and CHX (10 μg/ml) or CHX alone for 2 hr, or at 3 hr after mock manipulation without the addition of IFN-γ or CHX and was analyzed as in *A*. The m119 and aldolase signals are from 5-day and 12-hr exposures, respectively. The order of the lanes from the original blot has been changed for the figure.

not required for induction. In addition to the 1.6-kb band, the m119 probe identified prominent inducible species of approximately 3.2 and 1.8 kb. The 1.8-kb band was seen only in RNA from cells stimulated in the presence of CHX. Preliminary evidence from analyses of multiple m119 cDNA clones and m119 genomic clones suggests that the 1.8-kb species is an alternatively spliced m119 mRNA. The 3.2-kb species is presumably a precursor of the 1.6-kb mRNA but has not been characterized. The level of aldolase A mRNA, shown as a control, was unaffected by exposure of RAW cells to CM from Con A-stimulated spleen cells.

Induction of m119 mRNA by Macrophage-Activating Factors. To identify the lymphokines in the spleen cell CM that may have been responsible for inducing the m119 mRNA, as well as to determine which other macrophage-activating factors were capable of enhancing expression of the m119 gene, total RNA was prepared from RAW cells treated with a variety of agents for 3 and 6 hr and the RNA was analyzed by Northern blot. Significant induction of the m119 mRNA was seen only with IFN-γ. The results of the experiments with IFN-α, IFN-β, IFN-γ and LPS are shown in Fig. 1*B*. The selective induction of the m119 mRNA in RAW 264.7 cells by IFN-γ was reproducible and was not due to an inability of RAW cells to respond to IFN-α, IFN-β, or LPS. For example, shown in Fig. 1*B* is the induction by all the IFNs and LPS of *crg-2*, another of the genes that had been identified as responsive to the Con A-stimulated spleen cell CM. The sequence of the Crg-2 cDNA (34) suggests that the Crg-2 protein may be the murine homologue of IP-10, a human protein characterized as preferentially induced by IFN-γ (8). In addition to the results with IFN-α, IFN-β, and LPS, treatment of RAW cells with recombinant murine IL-1α, recombinant murine IL-3, recombinant murine IL-4, recombinant murine granulocyte/macrophage colony-stimulating factor, recombinant human colony-stimulating factor 1, poly(I)·poly(C), the calcium ionophore A23187, phorbol 12-myristate 13-acetate, and the combination of A23187 and phorbol myristate acetate all failed to induce the m119 mRNA, nor was the m119 mRNA induced by serum (i.e., mitogen) stimulation of serum-starved BALB/c 3T3 fibroblasts (data not shown).

To determine the time course of induction of the m119 mRNA in response to IFN-γ, RNA was prepared from RAW cells treated for 0–24 hr. The m119 mRNA was induced rapidly and dramatically, as shown in Fig. 1*C*, reaching a maximum between 6 and 24 hr. As in the case of treatment with the spleen cell CM, induction by IFN-γ did not require new protein synthesis. In fact, the addition of CHX led to superinduction of the m119 mRNA.

While IFN-γ led to an immediate and marked stimulation of m119 gene expression, what is particularly striking is the degree of specificity of induction of the m119 gene by IFN-γ, exceptional even among those genes previously reported to be induced by IFN-γ preferentially (8, 9), which suggests a unique relationship between IFN-γ and m119, both in terms of IFN-γ-regulated gene expression and as regards the biological actions of IFN-γ.

Induction of m119 mRNA in Peritoneal Macrophages. To demonstrate the induction of m119 mRNA during the activation of normal macrophages, m119 gene expression was analyzed in the adherent population from starch-elicited peritoneal exudate cells obtained from both C3HeB/FeJ and BALB/cJ mice. As shown in Fig. 2, when these cells (>80% macrophages as determined by morphology) were exposed to CM from Con A-stimulated spleen cells, m119 mRNA was induced. Exposure of the C3HeB/FeJ cells to IFN-γ likewise led to the expression of the m119 gene. The electrophoretic mobilities of the m119 mRNA species from peritoneal cells as compared to RAW cells were identical.

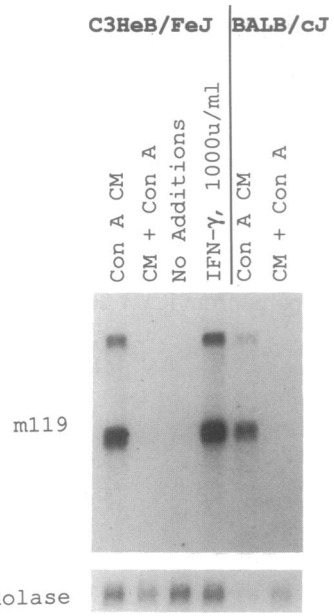

FIG. 2. RNA blot analysis of m119 mRNA in peritoneal exudate cells. Peritoneal exudate cells were obtained as described (21). Samples were as follows (from left to right). From C3HeB/FeJ mice: 1 μg of total RNA from cells exposed to 20% CM from Con A-stimulated spleen cells; 1 μg of total RNA from cells exposed to 20% CM from unstimulated spleen cells plus Con A (10 μg/ml); 1 μg of total RNA from cells exposed to medium without additions; 0.8 μg of total RNA from cells treated with IFN-γ [1000 units (u)/ml]; from BALB/cJ mice: 0.4 μg of total RNA from cells exposed to 20% CM from Con A-stimulated spleen cells; 0.4 μg of total RNA from cells exposed to 20% CM from unstimulated spleen cells plus Con A (10 μg/ml). The m119 and aldolase signals are from 2-week and 6-day exposures, respectively.

Sequence of m119 cDNAs and the m119 Predicted Protein. Portions of the original 1.2-kb m119 cDNA clones were used as probes to isolate a nearly full-length 1.4-kb cDNA, whose sequence is shown in Fig. 3. Primer extension using RNA from IFN-γ-stimulated RAW cells identified 5′ termini of m119 mRNA species at five adjacent positions (data not shown), and the predominant start site for transcription is designated by the arrow in Fig. 3. m119 genomic clones were isolated from a BALB/c mouse library (Clontech Laboratories, T. Wright and J.M.F., unpublished data), and a 1.7-kb *Eco*RI fragment was used to obtain genomic sequence that overlapped the 5′ end of the m119 cDNA, providing the sequence of the mRNA missing from the cDNA clone. As shown in Fig. 3, the genomic sequence contains an A+T-rich region (TAAATAAATAT) at positions −32 to −22 that includes several possible "TATA boxes." The m119 mRNA contains 1247 nucleotides exclusive of the poly(A) tail, with a single long open reading frame from the first AUG methionine codon beginning at nucleotide +58 to a termination codon UAA at nucleotides 436–438. This open reading frame encodes a predicted protein of 126 amino acids of relative molecular mass (M_r) 14,461. The sequence GCC that precedes the presumed initiation codon conforms to Kozak's consensus sequence (22). The 3′ end of the cDNA does not contain the typical AATAAA polyadenylylation sequence but does contain, 22 nucleotides 5′ of the poly(A) tail, the sequence AGTAAA, which has been reported to serve as a signal for polyadenylylation (23).

The N-terminal sequence of the predicted m119 protein has the characteristics of a signal peptide of a secreted or transmembrane protein, and the −3,−1 rule of von Heijne (24) predicts signal-peptide cleavage after Gly-21. m119 is unlikely to be a transmembrane protein because it lacks a second long hydrophobic sequence. There is a single pre-

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                TTTCCTAAATAAATATGATCCCCAAGAACATGCTCTCTAAAGACATTCTCG    14

    GACTTCACTCCAACACAGTGACTC
         CACTCCAACACAGTGACTCAATAGAACTCAGCTCTGCC ATG AAG TCC GCT GTT    72
 1                                              MET Lys Ser Ala Val

    CTT TTC CTT TTG GGC ATC ATC TTC CTG GAG CAG TGT GGA GTT CGA GGA|   120
 6  Leu Phe Leu Leu Gly Ile Ile Phe Leu Glu Gln Cys Gly Val Arg Gly

    ACC CTA GTG ATA AGG AAT GCA CGA TGC TCC TGC ATC AGC ACC AGC CGA   168
22  Thr Leu Val Ile Arg Asn Ala Arg Cys Ser Cys Ile Ser Thr Ser Arg

    GGC ACG ATC CAC TAC AAA TCC CTC AAA GAC CTC AAA CAG TTT GCC CCA   216
38  Gly Thr Ile His Tyr Lys Ser Leu Lys Asp Leu Lys Gln Phe Ala Pro

    AGC CCC AAT TGC AAC AAA ACT GAA ATC ATT GCT ACA CTG AAG AAC GGA   264
54  Ser Pro Asn Cys Asn Lys Thr Glu Ile Ile Ala Thr Leu Lys Asn Gly

    GAT CAA ACC TGC CTA GAT CCG GAC TCG GCA AAT GTG AAG AAG CTG ATG   312
70  Asp Gln Thr Cys Leu Asp Pro Asp Ser Ala Asn Val Lys Lys Leu Met

    AAA GAA TGG GAA AAG AAG ATC AAC CAA AAG AAA AAG CAA AAG AGG GGG   360
86  Lys Glu Trp Glu Lys Lys Ile Asn Gln Lys Lys Lys Gln Lys Arg Gly

    AAA AAA CAT CAA AAG AAC ATG AAA AAC AGA AAA CCC AAA ACA CCC CAA   408
102 Lys Lys His Gln Lys Asn Met Lys Asn Arg Lys Pro Lys Thr Pro Gln

    AGT CGT CGT CGT TCA AGG AAG ACT ACA TAA GAGACCATTACTTTACCAACAAG   461
118 Ser Arg Arg Arg Ser Arg Lys Thr Thr  *

    CACCCTGAATCTTAATGGGTTTTAGATTGTACTGAAAAGCCTTCCCTGGCAGAGCAGCCTTTA   524
    ATACATAGGCTTTTAATACATTAACTCAACTACAAAACATAAAGTGTTAATTTGAAATTATAA   587
    CTAACTTTAGGAAGTTAATTGCAAAACTCCAATAGTAACAATTGCTAGAGGCAAAAACTCTGT   650
    GTTCTACACAGCCAACAAAATTTCATCACGCCCTTGAGCCTAGTCGTGATAACATCAGATCTG   713
    GGCAAGTGTCCCTTTCCTTCATAGCTATCCAATGCACAACAGCTGTCTGGCTTCCAGAGCCAC   776
    ACATTTGGCAGCCTCCGGAGACTTCTGAGGCTCACGTCACCAAGTCCCAGGCCTGTCTGTTTG   839
    CTGGTGAGCTAGATAGACCTCACCAAGCTGGAGAGGCCCTCGGCAGCTGCATTTGGGTCAGCC   902
    TAGAGCCCCTGCACACATTGTGTCTCAGAGATGGTGCTAATGGTTTTGGGGTTCTACAGTGGA   965
    GACCACCAGAGTTGGCCTTCAGAACCTCCCACGTAGCTTTCGAGACCATGGGATTTCATTATT  1028
    AACTTGATCCCATCTTCAGAGCTTATTCTAAGTTTGCCTCTTCAATAAAACTCTCCTAGAAGG  1091
    TTGTGGCTGTAGCTTAGTGGCAGAACACTTGGTGTTGCAGGGACCAGGTCCTTCACTAACAGT  1154
    GCAAAAACTTAACCAATTTAAAGAACATTTTCTGGCTACTCAAATTCTCTTAAATTTATTCCT  1217
    GTTTCACAAGTAAACACTTCGCTGCTATCTA                                  1248
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FIG. 3. The cDNA sequence and inferred amino acid sequence of m119. Numbers at right margin refer to the last nucleotide in each line (nucleotide 1 is marked by the arrow); numbers at left correspond to the first amino acid in each line. The sequence from position −37 to +38 was obtained from genomic DNA by using an oligonucleotide primer complementary to m119 cDNA nucleotides 45–64, while sequence from position +20 to the poly(A) tail following nucleotide 1247 was obtained from cDNA. A TATA region at positions −32 to −22 is underlined. A vertical line marks the predicted cleavage site following Gly-21. The underlined Asn-58 is a predicted N-linked glycosylation site. The asterisk denotes the codon that ends the open reading frame. The possible poly(A)-addition signal AGTAAA is underlined. A_N represents the poly(A) tail. Several m119 cDNA clones were missing short stretches of nucleotides adjacent to the poly(A) tail as compared with the cDNA sequence shown here. None of the cDNA clones, however, contained sequence in this region additional to that shown here, and the sequence shown here was found in two independent cDNA clones. The cDNA sequence displayed was obtained from a single 1.4-kb m119 cDNA clone. The nucleotide sequence of the open reading frame was confirmed by sequencing a second independent m119 cDNA clone. Both strands of the 1.4-kb cDNA clone were sequenced in their entirety with the exception of the 31 nucleotides of the minus strand immediately adjacent to the poly(A) [poly(T)] tail, and the sequence of these 31 nucleotides from the minus strand was obtained from a second m119 cDNA clone.

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      MKSAVLFLLGIIFLEQCGVRGTLVIRNAR C S C ISTSRGTIHYKSLKDLKQFAPSPN C NKTEIIATL-KNGDQT C LDPDSANVKKLMKEWEKK-  m119
      MNQSAAVIFCLILLGLSGTQGIPLARTVR C N C IHIDDGPVRMRAIGKLEIIPASLS C PRVEIIATMKKNDEQR C LNPESKTIKNLMKAFSQK-  mCRG-2
      MNQTAILICCLIFLTLSGIQGVPLSRTVR C T C ISISNQPVNPRSLEKLEIIPASQF C PRVEIIATMKKKGEKR C LNPESKAIKNLLKAVSKE-  hIP-10
  MIPATRSLLCAALLLLATSRLATGAPIANELR C Q C LQTMAG-IHLKNIQSLKVLPSGPH C TQTEVIATL-KNGREA C LDPEAPLVQKIVQKMLKG-  mKC/Gro
-PSNPRLLRVALLLLLVAAGRRAAGASVATELR C Q C LQTLQG-IHPKNIQSVNVKSPGPH C AQTEVIATL-KNGRKA C LNPASPIVKKIIEKMLNS-  hGro/MGSA
 MTSKLAVALLAAFLISAALCEGAVLPRSAKELR C Q C IKTYSKPFHPKFIKELRVIESGPH C ANTEIIVKL-SDGREL C LDPKENWVQRVVEKFLKR-  hNAP/IL-8
            NLAKGKEESLDSDLYAELR C M C IKTTSG-IHPKNIQSLEVIGKGTH C NQVEVIATL-KDGRKI C LDPDAPRIKKIVQKKLAG-  hCTAP III
                     EAEEDGDLQ C L C VKT-TSQVRPRHITSLEVIKAGPH C PTAQLIATL-KNGRKI C LDLQAPLYKKIIKKLLES   hPF 4
MNGKLGAVLALLLVSAALSQGRTLVKMGNELR C Q C ISTHSKFIHPKSIQDVKLTPSGPH C KNVEIIATL-KDGREV C LDPTAPWVQLIVKALMAK-  c9E3
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HOMOLOGY TO m119

	TOTAL Values (%)	CYS WINDOW Values (%)
mCRG-2	31/98(32)	16/45(36)
hIP-10	32/98(33)	18/45(40)
mKC/Gro	31/94(33)	22/44(50)
hGro/MGSA	33/95(35)	21/44(48)
hNAP/IL-8	27/95(28)	17/44(39)
hCTAP III	24/86(28)	18/44(41)
hPF4	18/71(25)	13/44(30)
mMIP-2	4/32(13)	3/22(14)
c9E3	34/103(33)	22/44(50)

FIG. 4. Amino acid sequences of m119 and closely related members of the PF4 family. KC is a platelet-derived growth factor-induced protein and is the mouse homologue of the human Gro/MGSA (melanoma growth stimulatory activity) (26). Connective tissue-activating protein (CTAP III) is the precursor of β-thromboglobulin (27), and is stored in the α granules of human platelets. 9E3 is a chicken protein induced following transformation with Rous sarcoma virus (28). The other proteins are described in the text. Sequences were aligned according to the four conserved cysteine residues. Residues shared between m119 and another family member are shaded. Dashes terminate truncated sequences. Shown below the sequences are the numbers and percents of identical residues found in comparisons between m119 and each of the members of the family, both over their total lengths as far as comparable (Total) and within the most highly related region bounded by the conserved cysteines (Cys window) according to Oquendo *et al.* (26). The lowercase prefixes refer to the species of origin: m, mouse; h, human; and c, chicken. References for the sequences are as follows: mCRG-2 (34); hIP-10 (8); mKC (26); hGro/MGSA (29); hNAP (neutrophil-activating peptide)/IL-8 (10); hCTAP III (27); hPF4 (30); c9E3 (28).

dicted site for N-linked glycosylation at Asn-58. Of additional note is an extremely basic C-terminal sequence.

Relationship of m119 to Members of the PF4 Family. Comparisons of the m119 protein sequence to those in the National Biomedical Research Foundation library (June 1990) indicated that m119 is a new member of a family of proteins with ancestral relationships to PF4, a platelet granule protein (25). A comparison of the m119 predicted amino acid sequence with the sequences of the most closely related members of the family is shown in Fig. 4. The sequence comparisons suggest that although related to these other members of the family, m119 is neither identical to nor the mouse homologue of any of those described. It is proposed that this new member of the PF4 gene family be called *mig*, for *m*onokine *i*nduced by *g*amma interferon.

The members of the PF4 family are low molecular weight factors secreted by cells including fibroblasts, macrophages, and endothelial cells in response to a variety of stimuli such as growth factors, interferons, viral transformation, and bacterial products. Biological activities for some members of the family have been identified and include autocrine growth stimulation of a human melanoma line by Gro/MGSA (31), regulation of neutrophil–endothelial cell adhesion by IL-8 (12), angiostatic activity by PF4 (32), and chemotactic activity by PF4 (25), IL-8 (10), MIP-2 (11), and β-thromboglobulin (33). These cytokines are likely to be involved in the control of immune and inflammatory responses, tissue injury, growth, and repair. The selective responsiveness of the *mig* gene in macrophages to IFN-γ suggests that the Mig protein may have a role in those effects on macrophages specific to IFN-γ, such as the priming of macrophages for the release of reactive oxygen intermediates (4). If *mig* is selectively induced by IFN-γ in cells other than macrophages, the Mig protein would be a candidate to mediate additional IFN-γ-specific activities such as the induction of major histocompatibility complex class II antigens (3). Determination of the cell types in which *mig* can be induced and purification of the Mig protein with identification of the tissues to which it binds will permit a directed investigation into the biological roles of *mig*.

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