CHEMOATTRACTANT-INDUCED ACTIVATION OF *c-fos* GENE EXPRESSION IN HUMAN MONOCYTES

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Normal macrophage function is dependent upon the rapid accumulation and differentiation of blood-borne monocytes at sites of inflammation in response to chemoattractants (1). Phagocytic leukocytes express specific receptors for various chemoattractants and occupancy of these receptors by agonists leads to chemotaxis, O_2^- production, and lysosomal enzyme secretion (1–6). The initial cellular responses stimulated by chemoattractant receptors on polymorphonuclear leukocytes (PMNs)[1] or monocytes are mediated by a pertussis toxin/choleratoxin–sensitive guanine nucleotide regulatory (G_c) protein (7–14) that activates a membrane-associated phospholipase C to cleave phosphatidylinositide 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (8–14). IP_3 mobilizes intracellular calcium, while diacylglycerol activates protein kinase C (reviewed in references 15–16). Stimulation of this kinase in phagocytes appears to trigger O_2^- production and lysosomal enzyme secretion, since PMA and synthetic diacylglycerols that activate protein kinase C also initiate these functions (17–21). The mechanism for the differentiation of monocytes to macrophages at inflammatory sites is poorly defined. Biologically however, inflammatory macrophages have higher levels of microbicidal, tumoricidal, and phagocytic activities than do blood monocytes (22, 23).

In other types of cells, activation of protein kinase C by receptor occupancy or PMA is frequently associated with alterations in the regulation of gene expression (24–26). Among these genes, protooncogenes c-*myc* and c-*fos* are of particular interest. The expression of both genes are transiently induced when resting cells proliferate in response to mitogens and growth factors (27–30). However, during the differentiation of monomyelocytes to macrophages, the expression of c-*myc* gene decreases and c-*fos* gene increases (26, 31). Elevation of c-*fos* mRNA levels was also observed when pheochromocytoma cells (PC12) were induced to differentiate to sympathetic neuron-like cells (32). In addition, in-

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[1] *Abbreviations used in this paper:* CH, cycloheximide; diC_8, sn-1,2-dioctanoylglycerol; G protein, guanine nucleotide regulatory protein; G_c, pertussis toxin/cholera toxin–sensitive G protein; HHBSS, Hepes-buffered Hank's balanced salt solution; IP_3, inositol 1,4,5-triphosphate; PAF, platelet-activating factor; PIP_2, phosphatidylinositide 4,5-bisphosphate; PMN, polymorphonuclear leukocytes.

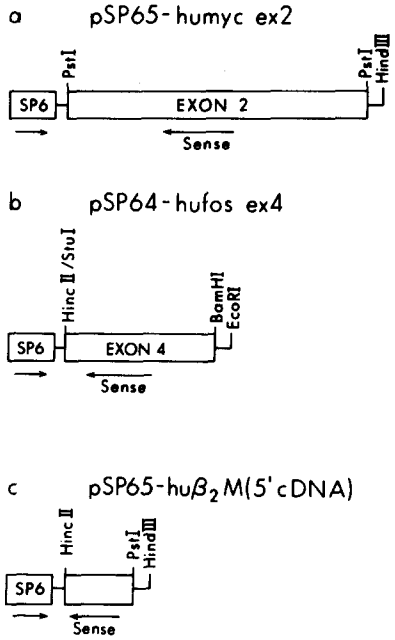

FIGURE 1. Structures of SP6 plasmids used in this study. For details, see Materials and Methods.

creased cellular c-*fos* mRNA levels causes F9 teratocarcinoma stem cells to differentiate (33). These observations suggest that these two genes may be involved in the regulation of cellular differentiation as well as division.

Little is known, however, about the regulation of expression of these genes in normal cells. In this study, we show that challenge of nonproliferating, normal human monocytes with chemoattractants modulates the expression of the c-*fos* and c-*myc* genes through a pertussis toxin–sensitive G protein.

Materials and Methods

Reagents. FMLP, platelet-activating factor (PAF), PMA, actinomycin D, and cycloheximide (CH) were obtained from Sigma Chemical Co. (St. Louis, MO). Recombinant human IFN-γ was a generous gift of Genentech, Inc. (South San Francisco, CA), and partially purified human colony-stimulating factor was purchased from Genzyme (Boston, MA).

Preparation of Human Monocytes. Heparinized venous blood from healthy human donors was sedimented with 3% dextran for 20 min at room temperature to remove most of the red cells. Mononuclear cells were then obtained from the leukocyte-rich plasma by Ficoll-Hypaque gradient centrifugation (34). These cells were washed three times in Hepes-buffered Hank's balanced salt solution (HHBSS); were divided into 75-mm^2 tissue culture flasks in 10 ml RPMI 1640 plus 5% heat-inactivated, pooled human type AB serum; and then were incubated for 1 h at 37°C under 5% CO_2. Nonadherent lymphocytes were removed by aspiration and adherent monocytes were washed four times with HHBSS.

Isolation of Total RNA from Human Adherent Monocytes. Each flask of adherent monocytes was incubated at 37°C with 10 ml HHBSS containing 0.5% BSA plus either the indicated amount of stimulant contained in DMSO or the same amount of DMSO alone. Cells were lysed in situ with guanidine isothiocyanate solution and total RNA was isolated after centrifuging through a CsCl cushion (35). RNA was quantified by optical density at 260 nM.

RNase Protection and Northern Blot Assays. Three plasmids, each carrying a portion of human c-*myc*, c-*fos* or β_2-microglobulin genes, were used to generate single-stranded ^{32}P-labeled RNA probes complementary to mRNA (Fig. 1). Plasmid pSP65-humyc ex2 (Pst

I) containing the Pst I fragment of exon 2 of human c-*myc* gene at Pst I site of plasmid pSP65 was linearized with restriction endonuclease Hind III before use (36). Plasmid pSP65-huβ_2M (5′ cDNA) containing an insertion of a 144-bp Pst I–Hinc II fragment of the β_2-microglobulin cDNA clone at Hinc II and Pst I sites of pSP65 was linearized with Hind III before use (37). To construct plasmid pSP64-hufos ex4, the 0.75-kb Acc I fragment derived from exon 4 of human c-*fos* gene was digested with mung bean nuclease to remove protruding 5′ ends and then ligated to Bam HI linkers (38, 39). This fragment was digested with enzymes Bam HI and Stu I and the 5′ fragment was inserted between the Bam HI and Hinc II sites of pSP64. This plasmid was linearized with Bam HI before use. Single-stranded ^{32}P-labeled RNA probes were generated by in vitro transcription of linearized plasmids with SP6 polymerase and ^{32}P-UTP as described by Melton et al. (40). Full-length, labeled RNA transcripts were purified after electrophoresis of reaction mixtures on 4% polyacrylamide/8.3 M urea gels.

To perform RNase protection assays, 2 μg of total cellular RNA was ethanol precipitated in the presence of 10^5 cpm of each antisense RNA probe of c-*myc*, c-*fos*, and β-2 microglobulin. Each sample was then dissolved in a 10-μl hybridization mix (75% formamide, 20 mM Tris, pH 7.0, 1 mM EDTA, 0.4 M NaCl) to hybridize at 60°C for 16–24 h. Excess unhybridized, single-stranded RNA probes were digested in 150 μl of RNase digestion buffer (0.3 M NaCl, 10 mM Tris, pH 7.5, 5 mM EDTA) containing 200 U RNase T1 and 1.2 μg RNase A at 37°C for 1 h. RNases were removed by the addition of 10 μl of 10% SDS, and 5 μl of proteinase K (10 mg/ml), and were incubated at 37°C for 15 min, followed by phenol/chloroform extraction and RNA precipitation with ethanol. Samples were finally dissolved in 8 μl formamide loading buffer (39), separated on 4% polyacrylamide/8.3 M urea gel, and visualized by autoradiography. The sizes of protected RNA fragments from human c-*myc*, c-*fos*, and β_2-microglobulin probes are roughly 420, 230, and 140 bases long, respectively.

Northern transfer was performed as described (41). Cytoplasmic RNA was denatured in 50% DMSO, 15% glyoxal, 8.3 mM sodium phosphate buffer, pH 7.0, at 50°C for 1 h. The glyoxylated RNA was separated on 1.2% agarose gel in 10 mM sodium phosphate buffer, pH 7.0, and then transferred to nitrocellulose paper overnight with 20× SSC (1× SSC: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.4). Northern blot filters were hybridized with nick-translated, ^{32}P-labeled c-*fos* DNA insert of plasmid pSP64-hufos ex4 (42).

Results

Chemoattractants Activate c-fos Gene Expression in Human Monocytes. Preliminary experiments revealed two difficulties in studying gene expression in human monocytes: (*a*) The quantity of total monocyte RNA that could be isolated from 1 U of blood is quite limited (60–120 μg); (*b*) The levels of c-*myc* and c-*fos* RNA varied from donor to donor. To solve these problems, we routinely collected 1 U of blood from each of two donors for each experiment. Monocytes prepared from each donor were treated independently, then the RNAs were isolated and pooled at each time point. The highly sensitive RNase protection assay was used to measure the levels of RNAs except where indicated. Total monocyte RNAs were hybridized at the same time with three complementary RNA probes, and the protected RNA fragments, corresponding to the levels of c-*myc*, c-*fos*, or β_2-microglobulin RNA, were then separated on the denaturing gel due to the size difference.

As shown in Fig. 2, the level of β_2-microglobulin RNA was relatively unchanged during the course of cellular manipulation and was therefore used as an internal control to monitor the input of total RNAs. Both chemoattractants, FMLP and PAF, induced the progressive increase of c-*fos* RNA by 6–15-fold over those of buffer control monocytes at 30 min after treatment, and then decreased to

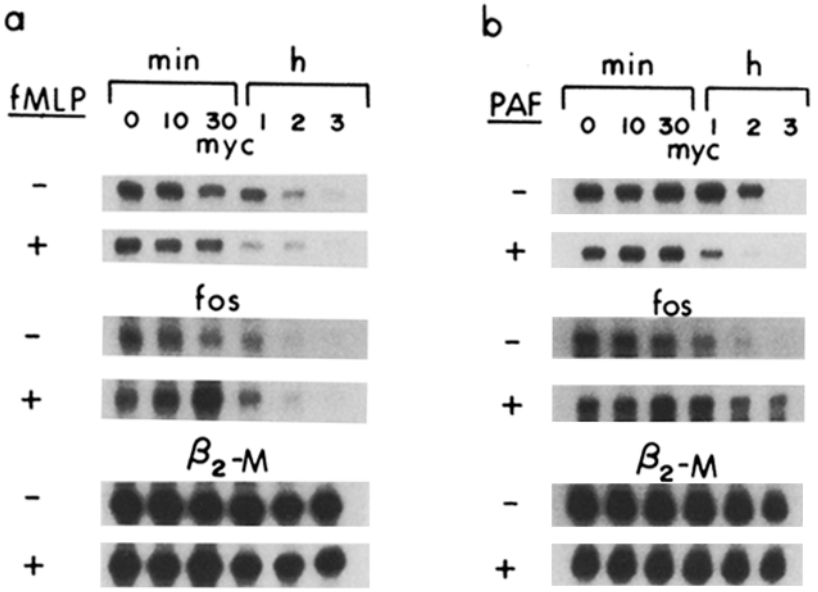

FIGURE 2. Kinetics of c-*myc*, c-*fos*, and β_2-microglobulin (β_2-*M*) gene expression in human monocytes in response to chemoattractants. Total cellular RNA was isolated from monocytes after treatment with 10^{-6} M FMLP (*a*), or 10^{-6} M PAF (*b*) for the indicated time intervals. Specific transcripts were detected by RNase protection assays using 2 μg total RNA. (− and +) The absence or presence of chemoattractants, respectively.

slightly above control levels by 4 h. Interestingly, levels of c-*myc* RNA decreased by 1 h after exposure to FMLP, and the decrease was even more profound when monocytes were exposed to PAF. This decrease was reproduced in three independent experiments (data not shown).

Recombinant human IFN-γ (20 U/ml) and partially purified human colony-stimulating factor (10 U/ml) were also tested, and they produced no effects on the RNA levels of these three genes during the incubation times studied (up to 4 h) (data not shown).

Protein Kinase C Activators Activate c-fos Gene Expression in Human Monocytes. Chemoattractant receptor occupancy in monocytes induces the hydrolysis of PIP_2 to generate the protein kinase C activator diacylglycerol and the intracellular calcium mobilizer IP_3 (8). We therefore studied the effects of agents that mimic these functions to see which secondary signal is involved in the c-*fos* gene activation. Fig. 3 shows that c-*fos* gene induction kinetics was similar in cells treated with PMA or sn-1,2-dioctanoylglycerol (diC_8) compared to those exposed to FMLP or PAF. The calcium mobilizer ionomycin (10^{-7} M) had no effect on the expression of the c-*fos*, although the ionophore effectively increased the intracellular calcium concentration and induced a respiratory burst by human monocytes (data not shown). Levels of c-*myc* and β_2-microglobulin RNAs remained unchanged upon exposure to either the protein kinase C activators or ionophore.

Inhibition of Protein Synthesis Elevates the Levels of Both c-fos and c-myc RNA in Human Monocytes. Studies on BALB/c 3T3 fibroblasts and U937 human mon-

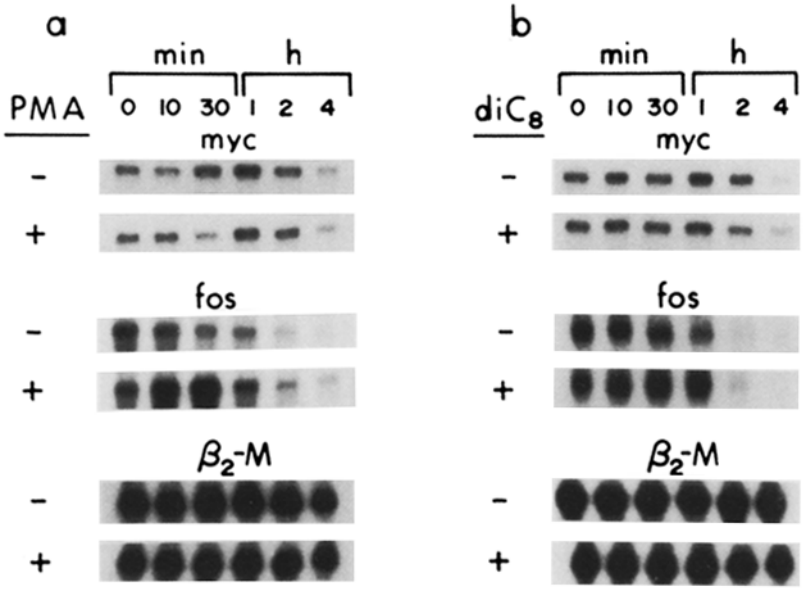

FIGURE 3. Kinetics of c-*myc*, c-*fos*, and β_2-microglobulin (β_2-*M*) gene expression in human monocytes treated with protein kinase C activators. Total cellular RNA (2 μg) isolated from monocytes that had been treated with (*a*) 100 ng/ml PMA or (*b*) 10^{-5} M diC_8 for the times indicated was used in RNase protection assays. (− and +) The absence or presence of the stimulants, respectively.

ocytic cells have shown that the protein synthesis inhibitor CH plus mitogen or PMA superinduces the expression of c-*myc* and c-*fos* gene, respectively, and treatment of the cells with CH alone leads to the accumulation of these RNAs (24, 26). The effect of CH on the RNA levels of these three genes in human monocytes was determined (Fig. 4). Both c-*myc* and c-*fos* RNA gradually increased to 3- and 15–20-fold, respectively, over the control at 1 h after treatment with CH. Superinduction of only c-*fos* RNA was observed when monocytes were treated with both CH and FMLP (or PMA). The level of c-*fos* RNA in CH plus FMLP–treated cells reached about fivefold over those treated with CH alone, or 75–100-fold over the level of untreated cells at 1 h after treatment, and then decreased. This superinduction was even more dramatic when cells were treated with both CH and PMA. The level of c-*fos* RNA was still increasing at 4 h after treatment and reached >400-fold over the level of control. There was no superinduction observed on the level of c-*myc* RNA and CH had no effect on the expression of the β_2-microglobulin gene.

Increase of c-fos RNA Levels in Stimulated Monocytes Is Due to mRNA Accumulation. Northern blot analysis of RNAs obtained at selected incubation time points was performed to determine whether the increase of c-*fos* RNA observed in the protection assays was due to accumulation of mRNAs or nuclear transcripts. Fig. 5 shows similar magnitudes of inductions of mature 2.2-kb c-*fos* mRNA over the control levels to those determined by RNase protection assays in cells treated with various stimuli. Furthermore, no accumulation of higher molecular weight c-*fos* mRNA precursors was detected.

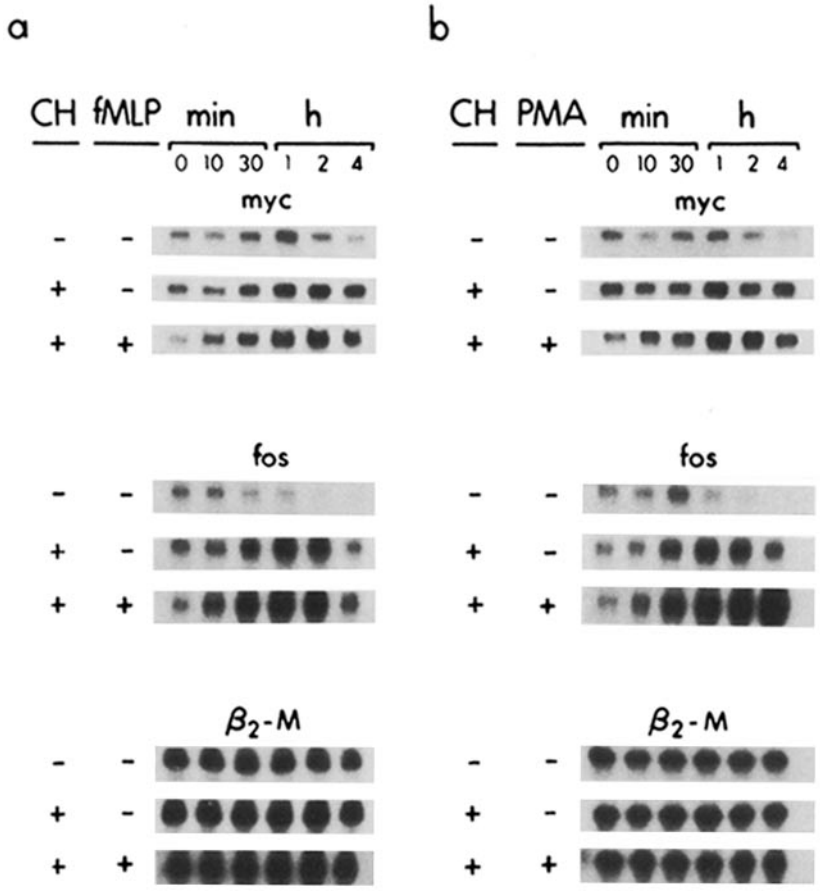

FIGURE 4. Effect of protein synthesis inhibition on the expression of c-*myc*, c-*fos*, and β_2-microglobulin (β_2-*M*) genes in human monocytes. Adherent human monocytes were incubated with CH (10 μg/ml) alone or in the presence of 10^{-6} M FMLP (*a*) or 100 ng/ml PMA (*b*). Total RNA (2 μg) isolated at the times indicated were used in the RNase protection assay. (− and +) The absence or presence of stimulants, respectively.

Activation of c-fos Gene Expression by FMLP Is Mediated through a Pertussis Toxin–sensitive G Protein. Previous studies (7–13) have shown that chemoattractant receptors stimulate PIP_2 hydrolysis via a membrane associated phospholipase C which is activated through a pertussis toxin–sensitive G protein. Fig. 6 shows that in vitro preincubation of monocytes with pertussis toxin for 4 h completely inhibited the elevation of c-*fos* RNA levels in response to FMLP. The amount of pertussis toxin used and the incubation time chosen were those previously determined to produce maximum G protein ribosylation (7, 10). Inhibition of gene activation in pertussis toxin–treated cells did not result from nonspecific toxicity since additional experiments demonstrated the normal induction of c-*fos* gene expression in pertussis toxin–treated cells when activated by PMA. Enhancement of c-*fos* gene expression by PMA or FMLP was normal in cells incubated for 4 h in the ribosylation buffers minus pertussis toxin.

Increased Expression of c-fos RNA in Human Monocytes by FMLP or PMA Results

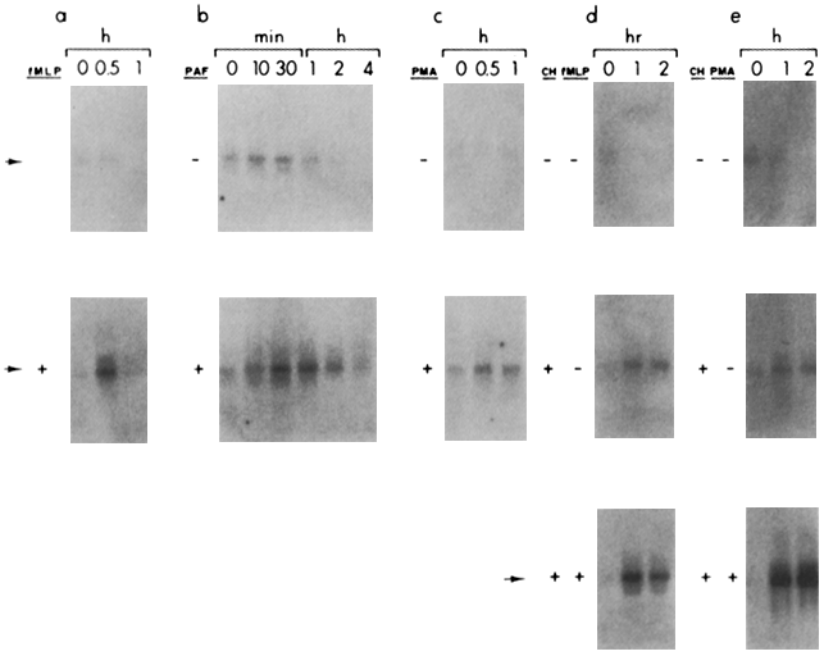

FIGURE 5. Northern blot analysis of c-*fos* transcripts. Total RNA (6 μg) isolated from control monocytes or monocytes treated with (*a*) 10^{-6} M FMLP, (*b*) 10^{-6} M PMA, (*c*) 100 ng/ml PMA, (*d*) CH (10 μg/ml) alone or CH (10 μg/ml) plus 10^{-6} FMLP, (*e*) CH (10 μg/ml) alone or CH (10 μg/ml) plus 100 ng/ml PMA at the times indicated were separated on agarose gel and then transferred to nitrocellulose paper. c-*fos*-specific transcripts were detected by hybridization to ^{32}P-labeled Bam HI–Hind III c-*fos* fragment of plasmid pSP64-hufos ex4. The arrow marks the position of matured 2.2-kb c-*fos* mRNA. (− and +) The absence or presence of stimulant, respectively.

from Increased Stability and Transcription. We investigated whether c-*fos* mRNA accumulation in treated monocytes resulted from transcriptional activation or stabilization of the messages. Attempts to measure the rate of transcription in human monocytes by nuclear run-off assays were unsuccessful, perhaps due to the abundance of proteases in monocytes, which leads to lysis of their nuclei during conventional isolation procedures. We therefore measured the stability of c-*fos*, c-*myc*, and β_2-microglobulin RNA by examining the levels of these RNAs in cells treated with various stimuli in the presence of actinomycin D to block the *de novo* RNA transcription. Preliminary experiments revealed that treatment with actinomycin D (20 μg/ml) inhibited 95% of the incorporation of α-[^{3}H]UTP into RNA in saponin (50 μg/ml)-permeabilized monocytes (data not shown). As shown in Fig. 7, *a* and *b*, the levels of c-*fos* and c-*myc* RNAs declined rapidly with a half-life of ~30 min in cells treated with actinomycin D alone. Additional treatment of cells with CH stabilized both c-*myc* and c-*fos* RNAs and extended their half-lives to ~2 h. The half-life of c-*fos* RNA in FMLP or PMA plus actinomycin D–treated cells was also slightly extended to ~1 h. Treatment of cells with either FMLP or PMA had no effect on the stability of c-*myc* RNA (Fig. 7 *b*).

The stability of c-*fos* RNA in actinomycin D–treated cells exposed to both

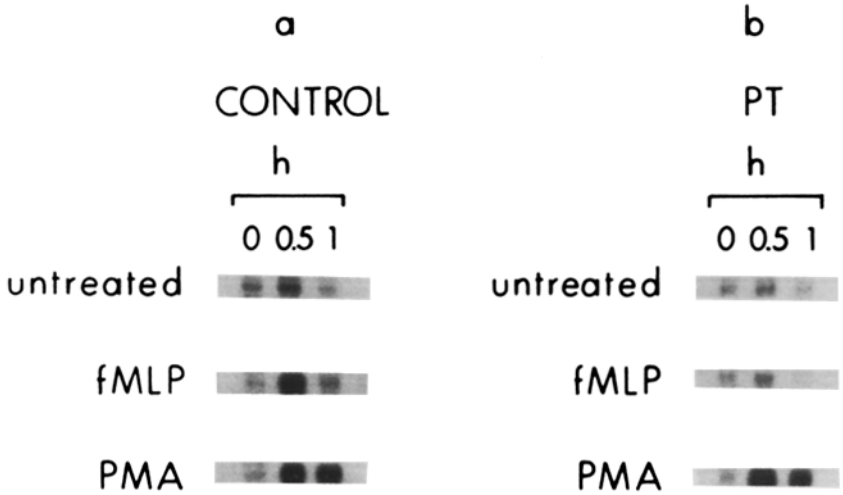

FIGURE 6. Inhibition of FMLP-induced c-*fos* gene expression in monocytes by pertussis toxin. Human monocytes were preincubated in HHBSS plus 0.5% BSA in the absence (*a*) or presence (*b*) of pertussis toxin (0.5 μg/ml) at 37°C, 5% CO_2 for 4 h. The cells were then washed with HHBSS and treated with FMLP (5×10^{-7} M) or PMA (100 ng/ml). Total cellular RNA (1 μg) isolated at the times indicated was used to detect c-*fos*-specific transcripts by RNase protection assays.

CH and FMLP (or PMA) was also determined. Fig. 7, *c* and *d*, show that CH alone greatly increased the stability of c-*fos* RNA and incubation at the same time with fMet-Leu-Phe or PMA slightly enhanced c-*fos* RNA stability.

The comparison of the ratios of c-*fos* RNA levels in cells treated with stimuli to cells without stimuli but in the absence or presence of actinomycin D allows the assessment of the role of transcriptional activation in the augmentation of the RNA level. For example, the ratio of c-*fos* RNA in FMLP- or PMA-treated cells without actinomycin D was 2–10-fold higher than that in cells treated with FMLP or PMA plus actinomycin D 1 h after treatment. This suggests that the activation of transcription may be an important factor in the elevation of c-*fos* RNA level in cells treated with chemoattractant or PMA (Fig. 7 *b*).

The relative transcriptional activities of c-*myc*, c-*fos*, and β_2-microglobulin genes in untreated human monocytes was also assessed. The levels of c-*myc* RNAs decreased more dramatically than those of c-*fos* RNA in actinomycin D–treated cells compared with control cells (Fig. 7). This suggests that the c-*myc* gene is more actively transcribed than the c-*fos* gene in unstimulated human monocytes. In contrast, the levels of β_2-microglobulin RNA remained unchanged for 2 h in the presence or absence of actinomycin D (data not shown), which implies that the transcription rate of this gene is very low and the half-life of this RNA is at least >2 h in human monocytes.

Discussion

Occupancy of chemoattractant receptors on phagocytes by chemoattractants initiates cellular responses that are essential for normal human function and wound healing (1, 22, 23). Recently, substantial progress has been made toward understanding the biochemical mechanisms of the signal transduction through

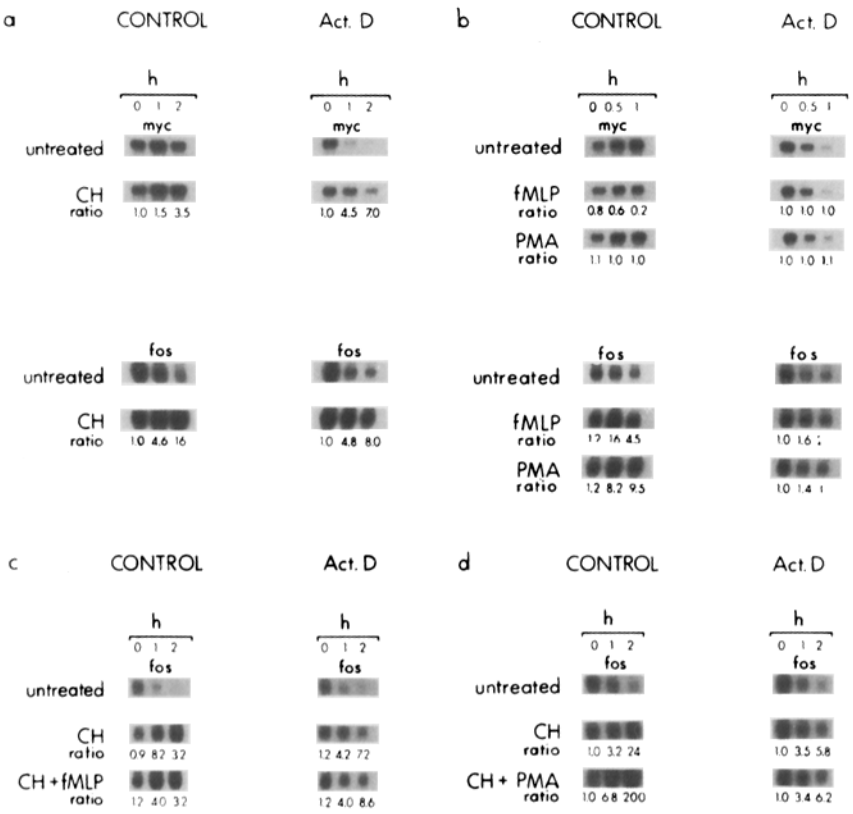

FIGURE 7. Effect of various stimuli on the stabilities of c-*myc* and c-*fos* RNAs in human monocytes. Adherant human monocytes were untreated or treated with (*a*) CH (10 μg/ml), (*b*) FMLP (10^{-6} M) or PMA (100 ng/ml), (*c*) CH (10 μg/ml) alone or CH (10 μg/ml) plus FMLP (10^{-6} M) or PMA (100 ng/ml) in the absence (indicated as control) or presence of actinomycin D (20 μg/ml) for the indicated times. The abundance of c-*myc* and c-*fos* RNA was determined by RNase protection assays using total cellular RNA (2 μg) isolated at the indicated times. The ratios between the densities of protected RNA probes in treated cells over those of untreated cells are indicated under each time point. (*b*) To enhance the detail, the autoradiographs of protected c-*myc* RNA probes in the right panels were exposed longer.

chemoattractant receptors. Apparently, phagocytes use the products of polyphosphoinositide hydrolysis as intracellular secondary messengers during cellular activation, since blockage of phospholipase C activation by inactivation of a pertussis toxin–sensitive G protein abrogates all cellular responses to chemoattractants (7–13). In addition, chemicals that mimic the activities of diacylglycerol and IP_3 also stimulate many similar cellular activities (17–21). Here we examined whether occupancy of chemoattractant receptors also regulates the expression of certain genes. These studies demonstrate that all agonists that can activate protein kinase C in monocytes elevated c-*fos* mRNA levels, suggesting a role for protein kinase C in controlling gene expression in these cells. Additionally, the chemoattractants FMLP and PAF decreased the level of c-*myc* RNA at 1 h after treatment, a phenomenon not observed during activation of cells with PMA, diacylglycerol, or calcium ionophore. This observation implies that there are additional intracellular signal(s) generated by chemoattractant receptor occu-

pancy that are capable of regulating gene expression. The persistence of superinduction by FMLP vs. PMA in CH-treated cells was also very different. Whereas FMLP plus CH was associated with a gradual decrease of c-*fos* RNA after 1 h, the level of c-*fos* RNA continued to rise even after 4 h of exposure to PMA plus CH. This observation further illustrates the differences in gene expression initiated by chemoattractant receptors vs. PMA. However, the induction kinetics of the c-*fos* gene were similar in FMLP- and PMA-treated cells. Thus, different signals appear to downregulate the level of c-*fos* RNA after chemoattractant receptor occupancy vs. direct protein kinase C activation, as the negative regulatory mechanism induced by the former but not the latter is resistant to inhibition of protein synthesis.

The biological significance of c-*fos* gene activation in human monocytes is not clear. Presumably, the pattern of gene expression in response to protein kinase C activation in various cells is associated with distinct modulatory signals. For example, in PMA-treated rat pituitary cells, there is induction in the expression of the prolactin gene but not the c-*fos* gene (27). In various fibroblasts, both c-*fos* and c-*myc* genes are activated by PMA as well as by mitogens that stimulate phosphoinositide hydrolysis. Additionally, the expression of c-*myc* is decreased and c-*fos* is increased when the human monomyelocytic cell lines HL60 or U937 are induced to differentiate to macrophages (26). This latter modulation pattern of gene expression resembles that observed during chemoattractant receptor occupancy of human monocytes. Thus, chemoattractants appear to initiate differentiation signals in monocytes at the genetic level. This process may be an initial mechanism for the rapid stimulation of monocyte-to-macrophage differentiation at sites of inflammation. However, recent studies (43) have shown that transient high-level expression of the c-*fos* gene in U937 cells induced by serum or diacylglycerol is insufficient to commit the cells to differentiate to macrophages. Moreover, these is no c-*fos* gene activation during the differentiation of certain HL-60 variants. These results, in neoplastic cell lines, imply that c-*fos* gene activation may not be absolutely required for the differentiation of monomyelocytes to macrophages, and there are response(s) other than induction of c-*fos* gene involved in this differentiation (43). Alternatively, augmentation of c-*fos* gene expression may be a sufficient signal for differentiation of nonneoplastic monocytes or may serve as a priming signal for cells to acquire functional competence to further stimulation by other signals. To determine the precise role of c-*fos* gene expression in monocytes it will be necessary to selectively augment or block the activity of this gene by transfecting cells with a functional c-*fos* gene or by generating c-*fos* antisense RNA (44), respectively.

The measurement of gene activation in the presence or absence of actinomycin D revealed that chemoattractants and protein kinase C activators regulate the level of c-*fos* RNA by altering both the rate of transcription and message stability. If there were no transcriptional activation in cells treated with the various stimuli and the accumulation of c-*fos* or c-*myc* RNA resulted only from the increased stability of these RNAs, the magnitude of increase of c-*fos* or c-*myc* RNA should have been the same in the presence or absence of actinomycin D. On the other hand, if there were transcriptional activation in treated cells, the magnitude of induction of c-*fos* or c-*myc* RNA by various stimuli would be higher in cells

without actinomycin D, since the RNase protection assay measures the level of both nuclear precursors and cytoplasmic RNAs. As shown in Fig. 7 *a*, the ratio of increase of c-*fos* RNA in cells treated with CH alone was higher at 2 h after treatment compared with that of cells treated with both CH and actinomycin D. In Fig. 7 *b*, the difference of ratios of c-*fos* RNA level in cells treated with FMLP or PMA in the absence of actinomycin D compared with those stimulated in the presence of actinomycin D was even more dramatic. These results suggest that the effect of FMLP, PMA, or CH on the accumulation of c-*fos* RNA results from both transcriptional activation and stabilization of messages. In FMLP- or PMA-treated cells, transcriptional activation appears to contribute more to the elevation of the c-*fos* RNA level, while stabilization plays a more critical role in CH-treated cells. Furthermore, it also implies that the superinduction of c-*fos* RNA in both CH and FMLP- (or PMA-) treated cells results from the additive effect of both transcriptional modulation and message stabilization. This conclusion is further supported by the observation that CH plus FMLP (or PMA) did not superinduce the stability of c-*fos* RNA since in the presence of actinomycin D the half-life of message was not prolonged (Fig. 7, *c* and *d*). Thus, the increase of c-*fos* RNA during superinduction must in part come from transcriptional activation by FMLP or PMA.

The molecular mechanisms by which chemoattractants decrease the levels of c-*myc* RNA can also be assessed from the actinomycin D experiments. As shown in Fig. 7 *b*, the ratios of c-*myc* RNA levels in cells treated with FMLP gradually decrease compared with those of untreated cells. This downregulation of c-*myc* RNA level was not observed in cells treated with FMLP in the presence of actinomycin D. These observations suggest that the effect of FMLP on decreasing c-*myc* RNA levels may result from inactivation of c-*fos* gene transcription.

The molecular signal(s) that were generated in monocytes by FMLP or PMA to activate the transcription of c-*fos* gene is not known. However, recent studies (45–47) have identified a *trans*-acting factor in nuclear extracts that binds to the c-*fos* enhancer sequence within the c-*fos* promoter. The enhancer-binding activity of this factor was induced by epidermal-growth-factor treatment of A431 epidermal carcinoma cells, and the rate of c-*fos* gene transcription is increased concomitantly. This observation suggests that the nuclear factor may play a role in regulation of the c-*fos* gene transcription. Whether a similar nuclear *trans*-acting factor(s) is present in monocytes that can be modulated by the chemoattractant FMLP (i.e., via activation of protein kianse C or other kinases) to regulate the expression of c-*fos* or c-*myc* genes remains to be investigated.

We were surprised that the magnitude of increase of c-*myc* RNA in cells treated with both CH and actinomycin D was slightly higher than those treated with CH alone. This suggests that the effect of CH on c-*myc* RNA accumulation is primarily enhancement of message stability. At this point, we have no explanation for the further stabilizing effects of actinomycin D on c-*myc* RNA.

Interestingly, treatment of monocytes with FMLP or PMA selectively stabilized c-*fos* RNA but not c-*myc* RNA. These results resemble the recent observations (48) that PMA selectively stabilizes granulocyte/monocyte colony-stimulating factor mRNA in T cells. It is possible that protein kinase C inactivates certain sequence-specific ribonucleases in the cells. Alternatively, activation of protein

kinase C may compartmentalize c-*fos* RNA to certain intracellular pools that are less accessible to ribonucleases.

The data presented in this paper indicate that c-*fos* and c-*myc* gene expression is finely regulated by chemoattractants in human monocytes. These cells must rapidly differentiate to macrophages to function normally and do not divide under ordinary circumstances. Thus, in addition to producing signals that trigger the immediate response of monocytes to inflammatory stimuli (i.e., chemotaxis, activation of the respiratory burst), chemoattractant receptors also modulate the expression of genes capable of affecting cellular differentiation.

Summary

Human monocytes use the products of phosphoinositide hydrolysis (1,2-diacylglycerol and inositol 1,4,5-triphosphate) as second messengers to trigger rapid cellular activation during the occupancy of chemoattractant receptors. The effect of chemoattractants on modulation of gene expression in monocytes was examined in this study. The chemoattractants FMLP and platelet-activating factor induced the progressive increase of c-*fos* RNA to 6–15-fold over those of control within 30 min after treatment. Similar kinetics of c-*fos* gene activation was also observed when cells were treated with PMA or sn-1,2-dioctanoylglycerol, but not with the calcium mobilizer ionomycin, suggesting a role for protein kinase C in gene regulation by chemoattractant receptors. Activation of c-*fos* gene expression by FMLP is mediated through a pertussis toxin–sensitive G protein, since pertussis toxin treatment of the cells blocked the induction of the c-*fos* gene by FMLP but not PMA. The level of c-*myc* RNA was slightly decreased after 1 h of treatment with chemoattractants, but not with PMA or diacylglycerol. This implies that chemoattractant receptor occupancy generates signals beyond protein kinase C activation that are capable of selectively downregulating monocyte gene expression. The effect of FMLP and PMA on the accumulation of c-*fos* RNA appears to result from altering both the rate of transcription and message stability. These observations indicate that signals generated through chemoattractant receptor occupancy may regulate monocyte function at the genetic level.

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