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Induction of c-*fos* proto-oncogene by a chemotactic peptide in human peripheral granulocytes

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The chemotactic peptide, fMet-Leu-Phe (fMLP), induced proto-oncogene c-*fos* mRNA in purified human peripheral granulocytes. The induction was transient, and was inhibited by pertussis toxin or by an inhibitor of protein kinase C. These results suggest that activation of a guanine nucleotide-binding protein and of protein kinase C is involved in c-*fos* induction in granulocytes.

c-*fos* proto-oncogene; Oncogene; Chemotactic peptide; Granulocyte

1. INTRODUCTION

Transcription of the proto-oncogene c-*fos* is known to be activated transiently by various growth factors and tumor promoters [1–12], and has been suggested to be involved in the control of cell growth. Transcription of c-*fos* is also induced during cellular differentiation [10,11] and by cAMP [13,14]. Most inducers of c-*fos* reported so far are all related to growth factors or mitogens. Activation of protein kinase C plays a key role as a second messenger for the signal of growth factors [15], and as we and others previously reported [10,16], c-*fos* induction depends on the activation of protein kinase C.

It was thus of interest to investigate whether the activators of protein kinase C induce c-*fos* gene in the absence of cell growth or differentiation. For this purpose, we used freshly isolated human peripheral granulocytes which are terminally differentiated and will not divide further in vitro. The chemotactic peptide, fMet-Leu-Phe (fMLP), is supposed to activate protein kinase C through the activation of cytoplasmic guanine nucleotide-binding protein and phospholipase C [17]. Here, we examined the effect of fMLP on c-*fos* gene expression in human peripheral granulocytes.

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2. MATERIALS AND METHODS

Peripheral granulocytes were isolated from normal human volunteers by the use of Ficoll-Conray. Fresh whole blood was collected in heparin-containing syringes. 1 vol. phosphate-buffered saline and 0.5 vol. of 4.5% dextran (in saline) were added, and the mixture was allowed to stand at room temperature for 30 min. The upper layer above the red blood cell layer was collected and spun at 800 rpm for 10 min. The pellet was suspended in Hanks' solution and layered on Ficoll-Conray solution (d = 1.077). After centrifugation at 1500 rpm for 30 min, the lower layer was collected, and contaminated red blood cells were eliminated by addition of 1 vol. distilled water. After 30 s, the osmolarity was adjusted by adding 10% NaCl. Granulocytes were collected by centrifugation at 800 rpm for 10 min, washed with Hanks' solution twice and suspended in the same solution.

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Cells were preincubated in a CO_2 incubator (37°C, 5% CO_2/95% air) for 2 h. fMLP and inhibitors were added, and cells were incubated further in a CO_2 incubator for the indicated times.

Total RNA was extracted by the guanidinium-hot phenol method, and run on a formaldehyde-containing agarose gel [18]. After transfer to nitrocellulose filters, RNA was hybridized with v-*fos* or β-actin probes as in [16].

3. RESULTS AND DISCUSSION

The purity of isolated granulocytes was nearly 95% as judged by microscopic observation of specimens stained using the May-Grünwald method. The observation described below is, therefore, most likely manifested in granulocytes, not by contaminated lymphocytes or macrophages.

Isolated granulocytes were incubated with 50 nM fMLP for 0–120 min or with various doses of fMLP for 30 min, and total RNA was extracted. Constant amounts of RNA were run on an agarose gel containing formaldehyde, transferred to membranes and hybridized with ^{32}P-v-*fos* probe. The increase in c-*fos* mRNA level evoked by fMLP was found to be dose- and time-dependent (figs 1,2), and was observed at levels as low as 2 nM fMLP. The induction was very rapid and was maximum within 15 min after addition of fMLP. The level of accumulated c-*fos* mRNA declined thereafter, and essentially no c-*fos* mRNA was detected 120 min after the addition of fMLP.

Kreipe et al. reported that the c-*fos* mRNA level was high in purified human granulocytes [24]. We noted that the endogenous level of c-*fos* mRNA differed significantly among different preparations of granulocytes, even when the cells were prepared from the same donor. However, the level decreased to become undetectable within 120 min of preincubation, fMLP being added after the preincubation in all the experiments.

The chemotactic peptide, fMLP, is supposed to bind to a specific receptor, and to transduce its signal via guanine-binding proteins which may then interact with phospholipase in membranes [19–21]. Such activation may subsequently induce phosphatidylinositol turnover, resulting in the activation of protein kinase C [15]. To investigate the involvement of such processes in activation of the

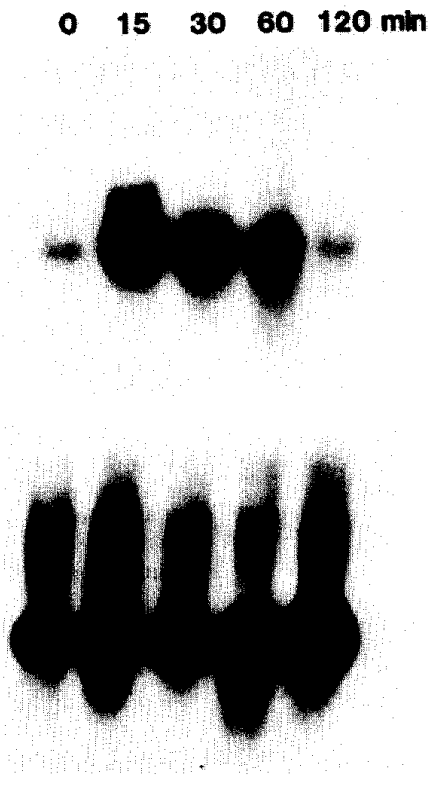

Fig.1. Time course of c-*fos* induction. Isolated granulocytes were incubated in the presence of 50 nM fMLP for the indicated times. Total RNA was extracted, and 15 μg were run on an agarose gel containing formaldehyde. After transfer to filters, filters were hybridized with ^{32}P-labeled v-*fos* or β-actin probe as described in the text. After washing, filters were exposed to X-ray films in the presence of an intensifying screen for 2 days, and developed.

c-*fos* gene, the effects of several inhibitors on the putative processes of induction were examined.

Pertussis toxin is known to inhibit fMLP-induced responses of granulocytes by inactivation of guanine-binding protein [17]. Fig.3 shows the effect of pertussis toxin on the accumulation of c-*fos* mRNA induced by fMLP. Cells were pretreated with 0.2 or 1 μg/ml of pertussis toxin and fMLP was added. RNA was extracted after 30 min and analyzed as described above. The results clearly indicate that the induction of c-*fos* was inhibited by pertussis toxin.

We then examined the effect of inhibitors of protein kinase on the induction. The isoquinoline derivative, 1-(5-isoquinolinesulfonyl)-2-methyl-

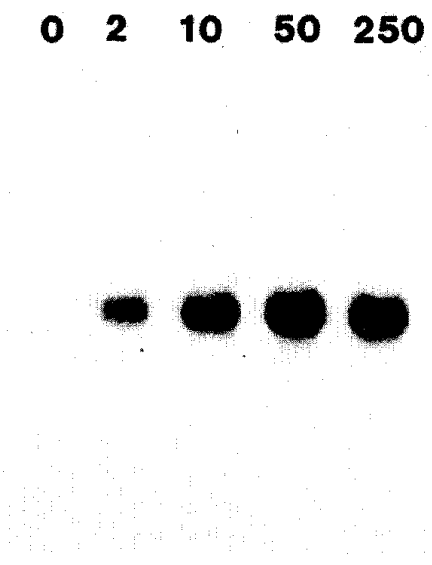

Fig.2. Induction of c-*fos*. Cells were incubated with 0–250 nM fMLP for 30 min. RNA was extracted and hybridized with v-*fos* probe.

piperazine (H7), is known to inhibit protein kinase C specifically, whereas *N*-(2-aminoethyl)-5-isoquinolinesulfonamide (H9) inhibits cyclic nucleotide-dependent protein kinases [22]. As shown in fig.4, H7 inhibited c-*fos* induction almost completely, whereas H9 showed only a marginal effect. The calmodulin antagonist, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W7) also inhibited induction. These results indicate that activation of protein kinase C is necessary for the induction of c-*fos* by fMLP.

The proto-oncogene c-*fos* is suggested to be involved in growth regulation and differentiation [1–11]. Peripheral granulocytes are, however, terminally differentiated and they neither grow nor differentiate further. The present results have shown that the c-*fos* proto-oncogene is induced in granulocytes possibly through the signal transduction system involved in the usual response of cells to chemotactic peptide, suggesting some physiological functions of c-*fos* induction in granulocytes.

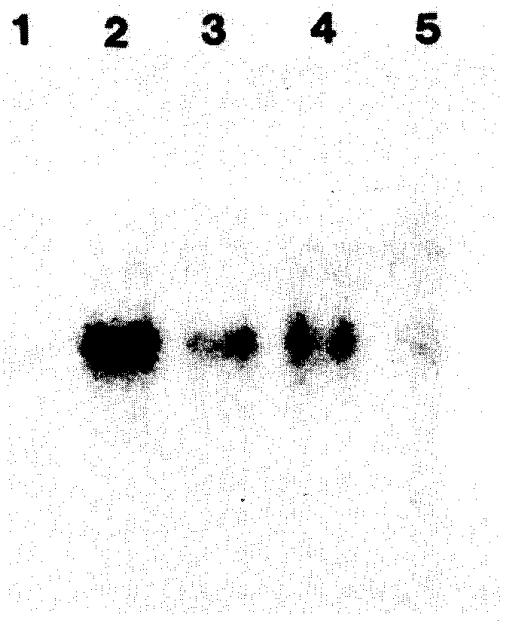

Fig.3. Induction of c-*fos* induction by pertussis toxin. Cells were preincubated with 0.2 (lane 3) or 1 μg/ml (lanes 4,5) pertussis toxin for 2 h, and 50 nM fMLP was added (lanes 2–4). After incubation for 30 min, RNA was extracted and probed with v-*fos*.

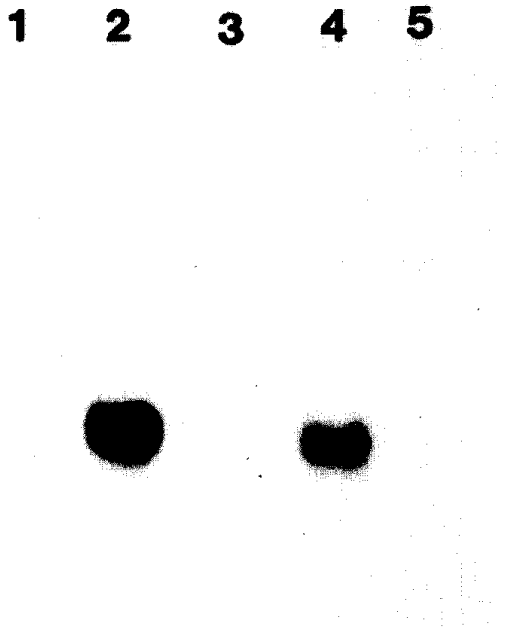

Fig.4. Inhibition of c-*fos* induction by inhibitors of protein kinase C. H7 (lane 3), H9 (lane 4) or W7 (lane 5) was placed in the medium at 50 μM and fMLP (50 nM, lanes 2–5) was added. RNA was extracted after 30 min incubation and probed with v-*fos*.

We recently described evidence that superoxide is produced in cultured cells stimulated with mitogens [23]. The signals which induce c-*fos* activation seem to give rise to superoxide production at the same time. Production of superoxide is regarded as a kind of stress for cells, and it may be possible that c-*fos* expression is required under such conditions of cellular stress.

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