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Accumulation of ornithine decarboxylase mRNA accompanies activation of human and mouse monocytes/macrophages

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Accumulation of ornithine decarboxylase (ODC) mRNA was investigated in human monocytes and mouse peritoneal macrophages. Treatment of both populations of mononuclear phagocytes with bacterial lipopolysaccharide induced a marked and rapid increase in the accumulation of the ODC gene transcript. A similar phenomenon, albeit less pronounced, was also observed following treatment of human monocytes with human recombinant interferon-γ. These results suggest a role for ODC, and therefore polyamines, in the regulation of mononuclear phagocyte functions.

Ornithine decarboxylase; Macrophage activation; Interferon-γ; Lipopolysaccharide

1. INTRODUCTION

Ornithine decarboxylase (ODC) is an initial, and rate-limiting enzyme in the biosynthesis of polyamines - putrescine, spermidine and spermine - which are implicated as regulatory molecules in a variety of metabolic processes. The intracellular availability of ODS is controlled at multiple levels, including transcription, translation, protein stability and endogenous inhibition of the enzymatic activity [1,2].

Accumulation of ODC mRNA has been shown to be associated with the stimulation of a plethora of physiological responses, leading to the suggestion that the ODC gene can belong to a group of immediate-early genes, like c-fos, c-jun and c-myc proto-oncogenes, and may be involved in a general process of cell activation [3]. The very rapid induction of expression of these genes has been repeatedly reported in different phenomena involving reprogramming of a genome, and it has been proposed that protein products of the aforementioned genes can regulate cellular ability to progress to a new phenotype [3]. This hypothesis derives mostly from studies performed on stimulation of cell growth and differentiation. In order to further approach this issue we analyzed ODC gene expression in mononuclear phagocytes which are terminally differentiated, non-proliferating cells which, however, can be induced to marked phenotypic changes by factors present in sites of inflammation [4].

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In this paper we show that bacterial lipopolysaccharide (LPS), a powerful activator of macrophage effector functions [4] induces ODC mRNA accumulation both in human monocytes and mouse peritoneal macrophages. We also show that recombinant human interferon-γ (IFN-γ) induced ODC mRNA accumulation in human monocytes. These observations indicate that accumulation of ODC mRNA is a marker of macrophage activation.

2. MATERIALS AND METHODS

2.1. *Cells and culture conditions*

Fresh human buffy coats were obtained from a blood bank, and after two-fold dilution with Ca^{2+}-, Mg^{2+}-free phosphate-buffered saline (PBS) containing 2.5 mM EDTA, were layered over Ficoll-Hypaque gradients. After 40 min of centrifugation at $400 \times g$, the mononuclear cells were collected, washed twice with PBS and resuspended in Iscove's medium supplemented with 2 mM glutamine, 50 μg/ml gentamycin and 10% fetal bovine serum. The cells were plated in tissue culture flasks at $1\text{-}2 \times 10^6$ cells per cm^2 of the vessel surface and after 3-4 hours of incubation at 37°C in 5% CO_2, the non-adherent cells were washed out with PBS. Adherent monocytes were cultivated overnight and then treated for different times as indicated, with either 50 ng/ml bacterial lipopolysaccharide (LPS from *E. coli*, serotype 026:B6, Sigma) or 50 U/ml human recombinant interferon-γ (IFN-γ, batch 26 AMGen, Amersham). The material for the single gene expression experiment (all experiments were done at least twice) was taken from 5-6 different individuals. Their monocytes were divided into 5 portions and cultured separately (one portion for a time-point).

Mouse macrophages were obtained from the peritoneal cavity after 4 days from the injection of 3% thioglycollate. The cells were resuspended in Dulbecco's modified Minimal Essential Medium supplemented with glutamine, gentamycin and fetal bovine serum (see above). After 3 h of cultivation the non-adherent cells were removed by washing with PBS and the cells were stimulated with LPS as described above for human monocytes.

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2.2. *Nothern blot analysis*

Monocytes and macrophage monolayers were washed twice with 0.9% NaCl and lysed with 4 M guanidine isothiocyanate. RNA was purified according to the procedure described by Chomczynski and Sacchi [5]. Fifteen μg of total RNA were denatured in the presence of 0.5 μg of ethidium bromide and electrophoresed in 1.2% agarose, 2.2 M formaldehyde. Then the gels were capillary blotted onto nitrocellulose. Prehybridization and hybridization were performed at 42°C in a mixture containing 50% formamide, 5×SSC, 5×Denhardt's solution, 20 mM sodium phosphate buffer, pH 6.5, and 100 μg/ml denatured salmon sperm DNA/ml. In the case of hybridization, the mixture was supplemented with 10% dextran sulfate and the denatured probe. Human ODC probe [6] was labelled by the random primer method to a mean specific activity of $0.5–1.3\times10^9$ cpm/μg DNA. Membranes were hybridized for 18–24 h and then washed twice for 5 min at room temperature with a mixture of 2×SSC and 0.2% SDS, and 3 times for 30 min at 52°C with a mixture of 0.1×SSC and 0.1% SDS. Membranes were exposed for 24–72 h to Kodak X-Omat X-ray films at −70°C with intensifying screens.

The ODC mRNA levels (2.6 kb band) were compared densitometrically with the aid of an LKB laser densitometer after normalization according to densitometry scanned pictures of 18 S rRNA visible on nitrocellulose after blotting due to the staining with ethidium bromide. The ODC mRNA levels have been expressed relatively to the level detected in non-stimulated cells. This latter level was taken as 1.

3. RESULTS AND DISCUSSION

LPS has been repeatedly reported to modulate mononuclear phagocyte functions like the capability to exert cytolytic activity for tumor cells, to produce toxic oxygen molecules and to release arachidonic acid [4,7–10]. The molecular bases of this phenomenon of 'activation' of macrophage functions are poorly understood [4]. Evidence has been, however, presented that LPS causes a marked increase in accumulation of c-fos and c-myc mRNA [11]. Although a causal relationship between expression of these early genes and macrophage activation has not yet been established there are indications supporting the hypothesis that early genes' products play a regulatory role in the development of an activated phenotype (reviewed in [3,4]).

As shown in Figs 1 and 3, LPS causes an accumulation also of ODC mRNA both in human monocytes and mouse peritoneal macrophages. As previously reported for c-fos and c-myc [12], ODC mRNA accumulation was very rapid and transient. Either in human monocytes and mouse peritoneal macrophages ODC mRNA levels peaked at about 30 min and then declined, returning to control levels within 24 h. Accumulation of ODC mRNA was observed with doses of LPS (50 ng/ml) which were shown to be optimal for activation of oxygen metabolism and arachidonic acid release [7–10] as well as for c-fos expression [11].

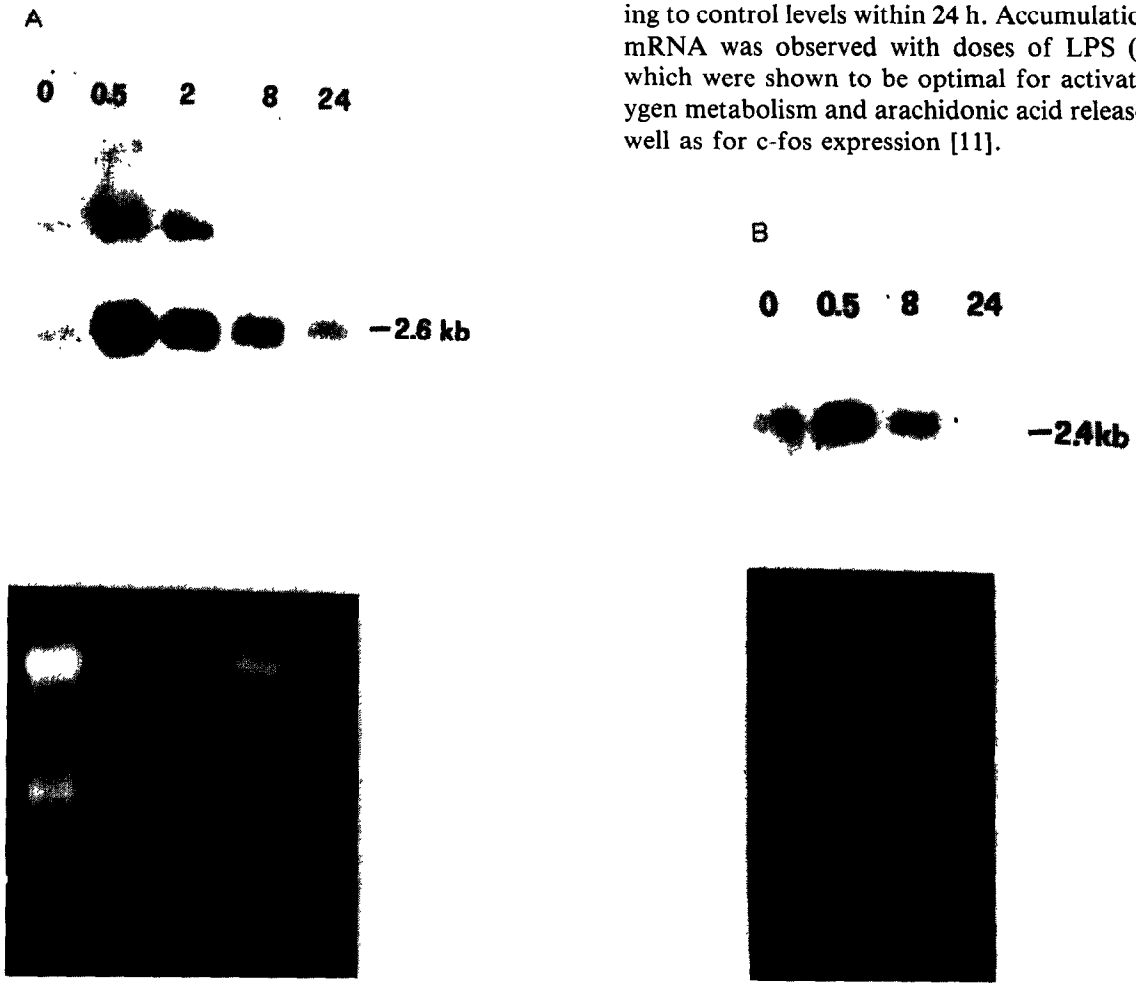

Fig. 1. Effects of LPS on accumulation of ODC mRNA in human monocytes (A) and mouse peritoneal macrophages (B). (Top panel) ODC mRNA level. (Bottom panel) Control rRNA ethidium bromide bands indicating the amount of RNA in each lane.

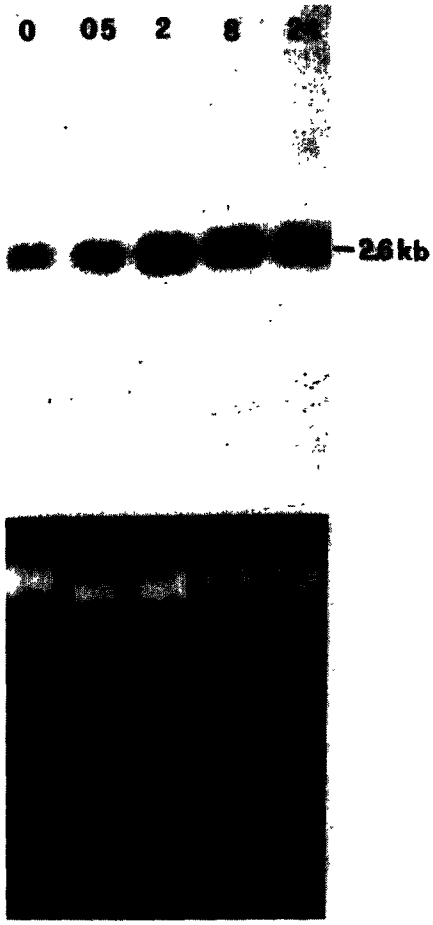

Fig. 2. Effect of IFN-γ on accumulation of ODC mRNA in human monocytes. Top and bottom panel as in Fig. 1.

To our knowledge, these results are the first so far presented on the expression of ODC gene in human monocytes. Previous studies demonstrated that LPS enhances ODC gene expression in a mouse macrophage cell line [12]. The time-course of ODC mRNA accumulation in this macrophage cell line was the same as that observed with human monocytes, but required doses of LPS 20–200 times higher.

To obtain further indication that accumulation of ODC mRNA accompanies activation of macrophage functions, we performed studies with human monocytes activated by IFN-γ. Treatment of one-day monocyte cultures with 50 U/ml of IFN-γ for 24 h caused a doubling of their capability to produce superoxide anion in response to phorbol myristate acetate (not shown). As shown in Figs 2 and 3, IFN-γ enhanced accumulation of ODC mRNA in a time-dependent fashion. Induction of ODC mRNA was less marked than with LPS, peaked at about 2 h and then declined to control levels within 24 h.

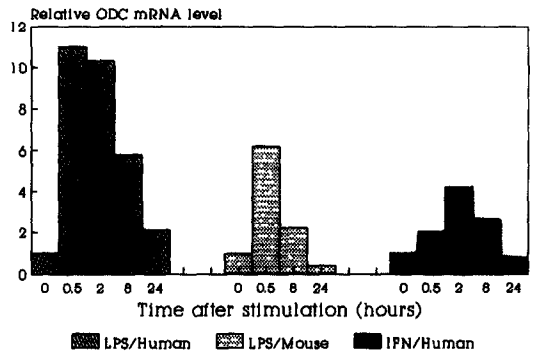

Fig. 3. The relative abundance of ODC mRNA levels in activated macrophages. (For details see section 2.)

Interestingly, we have observed two mRNA bands hybridizing to the ODC probe on blots containing human RNA. The main band is 2.6 kb and it corresponds in size to human ODC message one of us has previously described [6]. The other message - 5.2 kb - has not been reported before. It may represent an ODC mRNA precursor, as its level follows the level of the lower band. Please note that blots containing RNA from the LPS-treated cells this 5.2 kb band is clearly visible in lanes showing high level of 2.6 kb band, while in IFN-γ-treated material the 5.2 kb band became clearly visible only after overexposure of the blots.

The results presented in this paper provide further support to the hypothesis [3] that the gene encoding ODC is an activation-related, similarly as c-fos, c-jun and c-myc protooncogenes. They also raise the question of the role of ODC and the metabolism of polyamines in activation of mononuclear phagocyte functions.

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