IMMUNOCYTOCHEMICAL ANALYSIS OF INTERFERON-1β PRODUCTION BY HUMAN MONOCYTES

A. S. Simbirtsev, V. G. Konusova, and S. A. Ketlinskii

UDC 612.112.95.015.2.017.1.086

Key words: interleukin 1β; human monocytes.

Interleukin-1β (IL-1β) is a cytokine with molecular weight of about 18 kilodaltons, which is synthesized mainly by monocytes/macrophages in response to their activation during development of immune and inflammatory reactions in vivo. Production of IL-1 has hitherto been studied by analysis of its content in L-conditioned culture medium, in cell lysates, and also by assessment of the level of expression of IL-1 genes on the basis of accumulation of specific mRNA in the cells. Meanwhile, it has been shown to be possible in principle to analyze the IL-1 concentration directly in the cytoplasm of stimulated monocytes, by using immunocytochemical methods [3, 5], which means that its production can be studied at the cellular level.

By using a method of immunocytochemical detection we have studied the dynamics of IL-1β production by human peripheral blood monocytes.

EXPERIMENTAL METHOD

Mononuclears were obtained from the peripheral blood of healthy human blood donors by fractionation of a Ficoll—Pack ("Pharmacia") gradient and cultured in Eagle's medium with the addition of a 1% heat-inactivated human group IV(AB) serum in a concentration of $5 \cdot 10^6$ cells in 1 ml at 37°C in an atmosphere with 5% CO_2 for 48 h in 24-well plates ("Coster," USA). To obtain monocytes the mononuclears were cultured for 2 h under the above conditions with the addition of 10% serum, after which cells which had not adhered to the plastic surface were removed by washing with medium. Adherent cells consisted of monocytes, as shown by morphological analysis with staining by Wright's method and histochemical staining for nonspecific esterase. Fractions of mononuclears and monocytes were cultured under the conditions described above. As inducers of IL-1 synthesis we used phytohemagglutinin (PHA, from "Serva," West Germany) 20 μg/ml; prodigiosan (Moskhimfarmpreparaty) 2.5 μg/ml; lipopolysaccharide (LPS) from *E. coli* ("Sigma," USA) 10 μg/ml; phorbol myristate acetate (PMA) ("Serva," West Germany) 1 ng/ml.

After the end of culture 90-93% of the cells remained viable. At different times after activation the mononuclears were removed by means of a cell harvester ("Costar," USA) and films were prepared on slides, dried, and fixed with 96% ethanol for 10 min. Adherent monocytes were washed with physiological saline and fixed with 2% paraformaldehyde for 30 min at room temperature, or they were dried and fixed with ethanol for 10 min.

The immunohistochemical investigation was conducted by the indirect immunofluorescence method, using polyclonal antibodies to IL-1β, and in the 2nd stage, using activated immunoglobulins, labeled with fluorescein isothiocyanate ("Sigma," USA). To obtain antibodies to IL-1, rabbits were immunized intradermally with 200 μg of human recombinant IL-1β [2] in Freund's complete adjuvant. Reimmunization with 100 μg IL-1β in incomplete adjuvant was carried out 3 weeks later, and the immune serum was obtained after a further 10 days. As the control, pre-immune rabbit serum was used. The microscopic investigation was carried out on a LYUMAM I-3 luminescence microscope (LOMO, Leningrad).

All-Union Research Institute of Highly Pure Biological Preparations, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. D. Belyakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 278-280, September, 1991. Original article submitted December 17, 1990.

 0007-4888/91/0009-1288$12.50

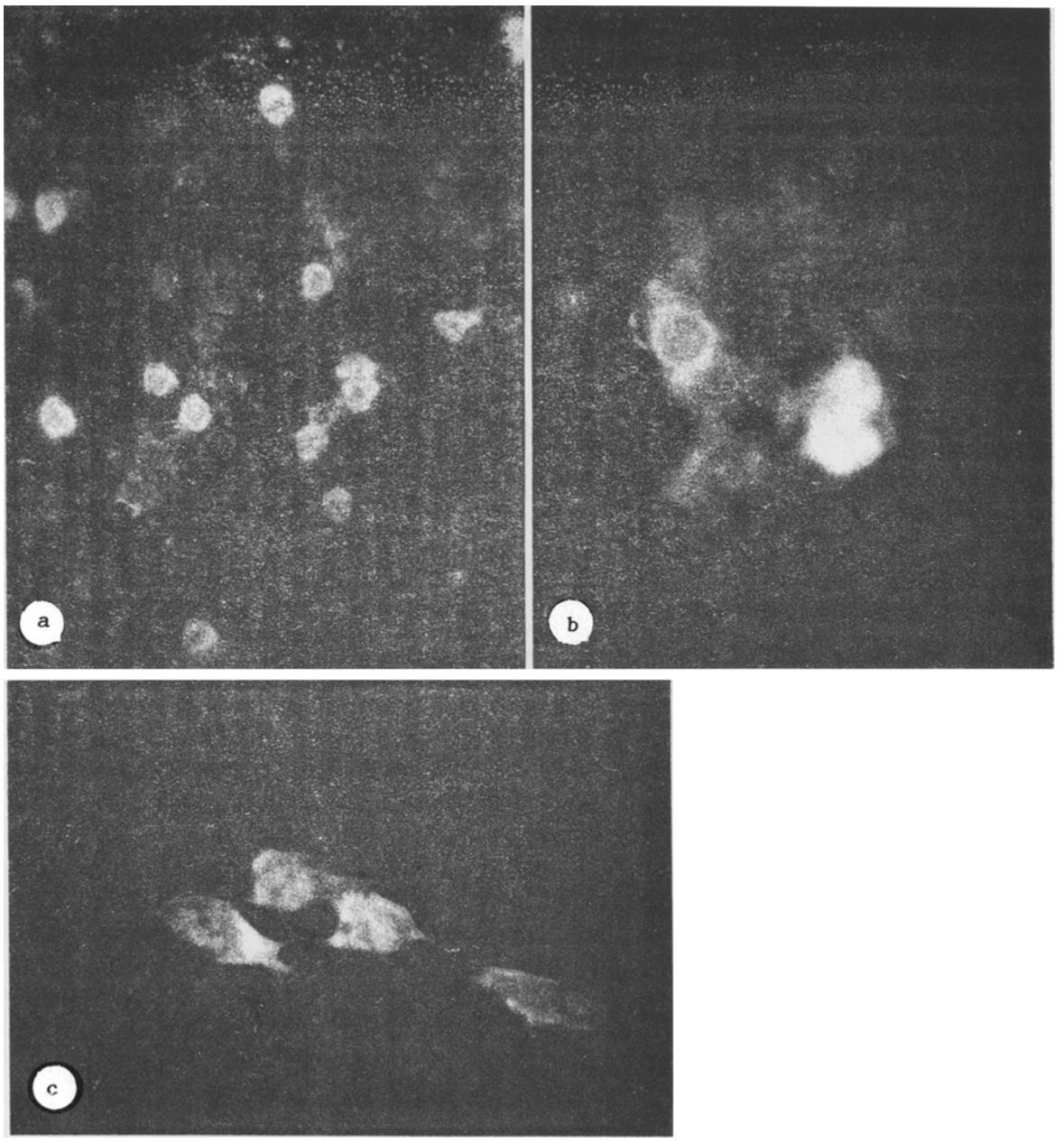

Fig. 1. Immunocytochemical localization of IL-1β in mononuclears stimulated by LPS (a) and PHA (b), and in human peripheral blood monocytes (c) (indirect immunofluorescence. Ocular 10, objective 40, water immersion).

The biological activity of IL-1 was determined in the commitogenic test on mouse thymocytes [7] with modifications [1]. Units of biological activity of IL-1 were calculated by using a preparation of human recombinant IL-1β with specific activity of 10^8 U/mg protein as the standard [2].

EXPERIMENTAL RESULTS

The results of the immunocytochemical investigation showed that unstimulated mononuclears isolated from healthy human peripheral blood contained no IL-1β. Solitary IL-1β-positive cells with a weak labeling intensity appeared 2 h after addition of inducers to the culture. Later the intensity of fluorescence, characterizing the IL-1β content in the cytoplasm of the cells, increased and this was accompanied by a parallel increase in the number of labeled cells. The content of IL-1β

TABLE 1. Changes in Content of Secretory IL-1 in Culture Medium of Human Blood Mononuclears at Different Times after Stimulation by Prodigiosan (M ± m, n = 3)

Time after stimulation, h	IL-1 concentration, U/ml
0	0
2	<1
6	186±33
10	628±71
18	1059±93
24	940±98
48	907±45

reached a maximum 10-24 h after stimulation (Fig. 1a). In this period IL-1β was found on average in 28% of cells, corresponding to the average number of monocytes among the mononuclear fraction. The number of cells containing IL-1β later decreased, and after 48 h it could be found only in single cells in very small quantities.

A high level of cytoplasmic IL-1β was found when inducers such as LPS, prodigiosan, PHA, and PMA were used; the dynamics of appearance of IL-1β when all the above-mentioned inducers were used was similar in character. Maximal production was observed under the influence of LPS and the LPS-containing preparation prodigiosan, which were the most appropriate inducers also of secretory IL-1, which was detectable in culture medium conditioned by mononuclears [1]. In four of the nine donors tested, intensive production of IL-1β was observed by mononuclears cultured without inducers, although immediately after their separation from blood these cells did not contain IL-1β. This fact can evidently be explained by the individual sensitivity of the donors' cells, in some of which IL-1 production may take place as a result of manipulations connected with the isolation and culture of the mononuclears in vitro.

Stimulation of blood mononuclears by PHA is known to lead to intensive agglutination of the cells and to the formation of cell conglomerates. Analysis of IL-1β-producing cells under these conditions of activation showed that IL-1β producers are present in the composition of cell conglomerates (Fig. 1b). This may perhaps lead to elevation of the level of local concentration of this cytokine and to the creation of optimal conditions for activation of the lymphocytes in the composition of the conglomerates.

To study IL-1β production by monocytes the fraction of cells adherent to the surface was used; the cells were cultured in the presence of LPS and prodigiosan. Like the results obtained with stimulation of the mononuclear fraction, production reached a peak 24 h after activation, when IL-1β was found in the cytoplasm of about 90% of monocytes with very small fluctuations in different donors (Fig. 1c). Thus, IL-1β is synthesized by the majority of human peripheral blood monocytes, although it is not found in individual cells if the activation method is used. This conclusion is in agreement with results of a study of IL-1 by human monocytes published previously. In response to stimulation by LPS, mRNA of IL-1β was found in 81% of monocytes [4], and it was discovered immunocytochemically in 93% of monocytes [3].

Judging by the data given above, blood monocytes have no "depot" of previously synthesized IL-1β, which can be used for rapid secretion in response to activation of the cells. Immunocytochemical analysis confirmed the inducible character of its synthesis. In fact, it was shown previously that mRNA of IL-1β appears in monocytes after 1-2 h and its peak content is observed 6 h after activation of the cells [6], whereas we found cytoplasmic IL-1β after 2 h. The results also agree with those of a study of the content of biologically active material in cell lysates, in which its peak concentration occurred after 12-24 h [8], when the highest level of IL-1β also was observed in the cell cytoplasm. Finally, determination of biological activity of secretory IL-1, most of which consists of IL-1β, in the culture medium of cells used for immunocytochemical analysis of cytoplasmic IL-1β, showed that its content gradually increased to reach a maximum after 18 h, and it remained at a high level for 2 days in culture (Table 1). Meanwhile, 48 h after induction, when the culture medium still contained a high level of IL-1β, its concentration in the cell cytoplasm fell significantly, indicating the evident cessation of its synthesis and secretion. Comparison of the data on the kinetics of accumulation of intracellular and secretory IL-1β shows that its peak concentration in the cell cytoplasm precedes the appearance of its peak concentration in the culture medium.

By immunocytochemical analysis it was thus possible to estimate production of IL-1β at the cellular level and to study the temporal parameters of its synthesis by human blood monocytes.

LITERATURE CITED

1. S. A. Ketlinskii, V. G. Konusov, A. S. Simbirtsev, et al., Byull. Éksp. Biol. Med., No. 11, 581 (1988).
2. S. V. Kotenko, M. T. Bulenkov, V. P. Veiko, et al., Dokl. Akad. Nauk SSSR, **309**, No. 4, 1005 (1989).
3. E. K. Bayne, E. A. Rupp, G. Limjuco, et al., J. Exp. Med., **163**, No. 5, 1267 (1986).
4. J.-F. Bernandin, K. Yamauchi, M. D. Wewers, et al., J. Immunol., **140**, No. 11, 3822 (1988).
5. P. J. Conlon, K. H. Grabstein, A. Alpert, et al., J. Immunol., **139**, No. 1, 98 (1987).
6. K. Matsushima, M. Taguchi, E. J. Kovacs, et al., J. Immunol., **136**, No. 8, 2883 (1986).
7. S. Mizel and D. Mizel, J. Immunol., **126**, No. 3, 836 (1981).
8. R. C. Newton, J. Leukemia Biol., **39**, No. 3, 299 (1986).

EFFECT OF IMMUNOMODULATORS ON MACROPHAGAL 5′-NUCLEOTIDASE ACTIVITY AND BLOOD CORTISOL LEVEL IN INBRED MICE

G. B. Kirillicheva, V. V. Mit'kin, M. S. Solov'eva, M. A. Tumanyan, Yu. V. Ezepchuk, and G. T. Sukhikh

UDC 615.275.4.015.4:[612.112.95.015.1:152.277].08

KEY WORDS: immunomodulators; 5′-nucleotidase; cortisol; macrophages; interlinear differences.

In recent years, a close connection has been found between the neuroendocrine and macrophagal systems. Macrophages play an active part in the regulation of hormones, including glucocorticoids. In turn, glucocorticoids occupy a special place among the hormones controlling the mononuclear phagocyte system [4, 5, 7]. However, the concrete mechanisms and particular features of interaction between macrophages and glucocorticoids, especially under the influence of immunomodulators, have not been adequately studied.

Accordingly, an investigation was carried out with the aim of studying the effect of immunomodulators of bacterial origin on the level of activity of the ecto-5′-nucleotidase (EC 3.1.3.5) of the peritoneal exudate macrophages (BEM) and the blood cortisol level in mice of different lines.

EXPERIMENTAL METHOD

Experiments were carried out in the winter on male mice aged 3 months, weighing 16-18 g, and belonging to the CBA, C57BL/6, and BALB/c lines and (CBA·C57BL/6)F_1 hybrids. Preparations of bacterial origin were used as immunomodulators: the polysaccharide salmosan, obtained from the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR (Director, Professor M. A. Tumanyan) from *Salmonella typhiabd.*, and the protein product staphylococcal enterotoxin A, produced by Ufa Research Institute of Vaccines and Sera. Salmosan

N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. All-Union Mental Health Research Center, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 280-282, September, 1991. Original article submitted March 29, 1991.