EVALUATION OF EXPRESSION OF INTERLEUKIN-1β mRNA UNDER NORMAL AND PATHOLOGICAL CONDITIONS

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One of the most important mediators of the immune response is interleukin-1 (IL-1). This cytokine is produced by activated macrophages/monocytes, and also by certain other cells [4]. IL-1 has a broad spectrum of biological activity and it plays an essential role in the development of the protective reactions of the body, such as acute-phase reactions, inflammation, and formation of the immune response [4].

Meanwhile IL-1 evidently is involved in the development of certain pathological forms. Abnormal expression of IL-1 has been described in rheumatoid arthritis [3], atherosclerosis [9], and endometriosis [5]. The role of IL-1 as a pathogenic factor has been discussed in diabetes [8]. Abnormal expression of this cytokine also is possible in pollinosis [1], an allergic disease developing under the influence of plant pollen.

The aim of this investigation was to study expression of the mRNA of IL-1 in peripheral blood cells of pollinosis patients. Pollinosis is a seasonal disease, whose course can vary depending on the climatic conditions of the region. Groups of patients and healthy controls for the investigations were accordingly set up in Novosibirsk and in Krasnodar.

EXPERIMENTAL METHOD

Altogether 64 patients sensitized to the pollen of trees and of plants of the Compositae family, and 32 healthy blood donors from Novosibirsk, and also 34 patients sensitized to ragweed pollen and 10 healthy blood donors from Krasnodar were investigated.

Monocytes and neutrophils were isolated from heparinized peripheral blood of the patients and controls, taken at the same time and under the same conditions. The blood was centrifuged in a Ficoll—Verografin density gradient (ρ = 1.078 and 1.114 mg/ml) for 40 min at 1000*g*. Interphase cells were washed with buffered physiological saline containing 0.5% fetal calf serum, and then with medium 199. Adherent cells were separated from nonadherent by incubation for 1 h on plastic at 37°C ($5 \cdot 10^6$ cells in 1 ml were transferred to a Petri dish 40 mm in diameter).

The isolated cells (monocytes and neutrophils) were transferred into 24-well cultural plates at the rate of $2 \cdot 10^6$ cells in medium RPMI-1640 per well. Neutrophils were cultured for 16-18 h and monocytes for 20-24 h in a humid atmosphere containing 5% CO_2. To stimulate IL-1 production, lipopolysaccharide of *E. coli* was added to the culture medium (25 μg/ml).

After culture the cells were collected and RNA isolated [7]. The isolated RNA was denatured by heating for 15 min at 60°C in medium consisting of 50% formamide, 2.2 M formaldehyde, 5 mM EDTA, 10 mM NaH_2PO_4, pH 7.0). Samples of denatured RNA were applied to nitrocellulose membranes in 10 SSC.

After application of the samples the membranes were heated for 1 h under a vacuum at 80°C.

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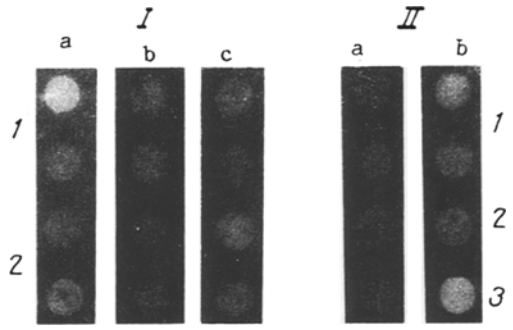

Fig. 1. Dot-hybridization of α-^{32}P-labeled probe to IL-1β with cytoplasmic RNA from peripheral blood cells of pollinosis patients in exacerbation stage. I) Novosibirsk, II) Krasnodar. RNA isolated from 10^6 unfractionated cells of leukocyte suspension (a) or of neutrophils (b), or of $0.5 \cdot 10^6$ monocytes (c) was applied at each point. 1) Healthy blood donors, 2) patients with pollinosis, 3) patients suffering with pollinosis for a long time and tolerating specific immunotherapy badly.

Prehybridization was carried out at 37°C (6 SSC, 0.5% sodium dodecylsulfate – SDS, 0.1% polyvinylpyrrolidone, 0.01% Ficoll, 0.1% bovine serum albumin) for 2-6 h Hybridization continued for 36 h in (6 SSC, 43% formamide, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin) at 37°C. As probes, α-^{32}P-labeled replicative form of M13-IL-1β, containing a fragment of human preinterleukin-1β [2], were used. After hybridization the filters were washed 3 times at 60°C in (2 SSC, 0.1% SDS), and then in (0.1 SSC, 0.1% SDS).

Autoradiography was carried out with the aid of RM-v x-ray film.

EXPERIMENTAL RESULTS

Analysis of RNA from peripheral blood mononuclear cells of patients with pollinosis in the exacerbation stage and of healthy blood donors revealed a high level of expression of IL-1 mRNA in monocytes, neutrophils, and unfractionated cells of the leukocyte suspension.

The IL-1 mRNA level in the monocytes fluctuated considerably, and no significant differences could be found between patients and controls relative to this test.

The scatter of IL-1 mRNA content in the neutrophils was less marked. During analysis of RNA from neutrophils and from unfractionated cells of the leukocyte suspension, in most cases no difference could be found between patients and healthy blood donors in groups from either Novosibirsk or Krasnodar.

The exception consisted of several patients suffering for a long time from allergy to ragweed pollen and having obtained poor results from specific immunotherapy In these patients we observed an increased IL-1β mRNA content in the neutrophils (Fig. 1).

The change in state of the cells in pathology may reflect the ability of the cells to respond to activation. We therefore estimated the content of IL-1 RNA in cells treated with lipopolysaccharide (LPS) also, a classical stimulator of IL-1 production [6].

The content of IL-1 RNA in LPS-activated monocytes of patients of the Krasnodar group did not differ from that in the control blood donors.

As regards neutrophils, in our culture system we observed two peaks of IL-1β mRNA content in cells activated by LPS. The first peak occurred at 4 h, the second 16 h after addition of LPS. During culture without the activator, a single peak of the IL-1β mRNA content was observed after 16 h.

Analysis of RNA from activated neutrophils showed that in patients of the Novosibirsk group, the content of IL-1β mRNA in response to treatment with LPS during the period of exacerbation of the disease was much lower than in the case of the control donors (Fig. 2).

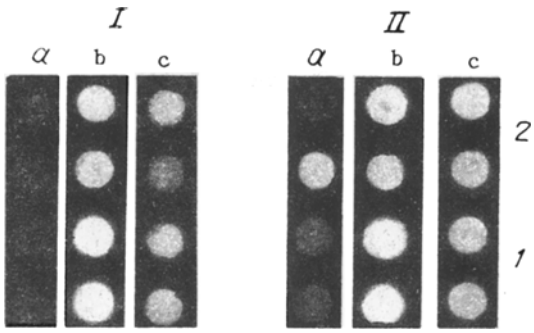

Fig. 2. Dot-hybridization of α-^{32}P-labeled probe to IL-1β with cytoplasmic RNA from neutrophils ($2 \cdot 10^6$ cells) of pollinosis patients in the exacerbation stage: a) freshly isolated cells, b) neutrophils activated by LPS, c) neutrophils cultured on plastic. Remainder of legend as to Fig. 1.

In patients of the Krasnodar group sensitized to ragweed pollen, the difference from the control donors was less marked. The exception once again was patients ill for a long time and responding poorly to treatment – a depressed response to activation of the neutrophils by LPS was observed in these subjects (Fig. 2).

In the Novosibirsk group we found no difference in the state of the patients cells during remission of the disease or in the healthy controls.

Incidentally, the difference in the state of the patients' and donors' cells could be recorded not only after activation of neutrophils by LPS, but also after a simpler procedure, namely culture on plastic. Adherence of the cells to plastic simulates adhesion of the neutrophils to endothelial cells, preceding migration of the neutrophils toward an inflammatory focus, and for that reason it is a perfectly adequate stimulus for cells of this type.

It follows from the results described above that neutrophils from pollinosis patients in the period of exacerbation lose their ability to respond adequately to activation.

This probably depends on the functional state of the cells, a change of which can explain the reduced ability of the neutrophils to synthesize IL-1β mRNA.

It is an interesting fact that in patients with pollinosis who respond poorly to treatment, the IL-1β mRNA content in the inactivated neutrophils was increased. Further activation of these cells by culture on plastic and treatment with LPS did not lead to any significant increase, as in the donors, in the IL-1β mRNA content. This confirms our conclusion of a change in functional capacity of the neutrophils of pollinosis patients.

Cellular RNA obtained from neutrophils of pollinosis patients and healthy controls also was tested by means of a probe specific for the mRNA of tumor necrosis factor α [10]. No differences in the content of this RNA could be found in the cells of the patients and controls. Functional disturbances of cells in allergy evidently do not extend to the system controlling expression of TNF-α.

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