ONCOLOGY

Effect of Tumor Necrosis Factor-α on the *In Vitro* Synthesis of Interleukin-8 by Human Monocytes and Lymphocytes

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The addition of recombinant tumor necrosis factor or monoclonal antibodies to this factor into human monocyte culture does not change the synthesis of interleukin-8. By contrast, tumor necrosis factor induces the synthesis of interleukin-8 in human lymphocytes.

Key Words: *interleukin-8; tumor necrosis factor; monocytes; lymphocytes; monoclonal antibodies*

Tumor necrosis factor-α (TNF) plays the key role in the development of inflammatory reaction due to its ability to induce the synthesis of mediators of inflammation in cells with TNF receptors. It was shown that the synthesis of interleukin-8 (IL-8), the main mediator of chemotaxis and neutrophil activation in inflammation, is also TNF-dependent [10]. TNF may induce the IL-8 synthesis in various human cells participating in the inflammatory reaction: endotheliocytes [13], fibroblasts and keratinocytes [9], astrocytes [6], and peripheral blood mononuclears [5]. Local production of IL-8 by cells in a focus of inflammation is thought to be triggered by TNF, i.e., is secondary to it [10]. In the present work we investigated the synthesis of IL-8 mRNA and IL-8 production in cultures of human monocytes and lymphocytes in the presence of recombinant TNF (rTNF) and monoclonal anti-TNF antibodies.

MATERIALS AND METHODS

Mononuclear cells were isolated from freshly collected heparinized donor blood on a single-step Ficoll/Hypaque gradient at 4°C [2] and fractionated into monocytes and lymphocytes on an intermittent Pharmacia Percoll gradient at 4°C [4,14]. The lymphocyte fraction was purified from adhering cells by plastic adhesion for 1 h at 37°C. Monocytes (0.5 or 2×10^6/ml) and lymphocytes (2×10^6/ml) were incubated in RPMI-1640 medium (Amimed) supplemented with 1% fetal calf serum (Amimed), 100 U/ml penicillin, and 100 μg/ml streptomycin in 50-mm plastic Petri dishes (Flow Lab.) at 37°C in an atmosphere with 5% CO_2. Formalin-treated suspension of *Staphylococcus aureus Cowan I* (SAC) (final concentration of bacterial sediment 0.001%, v/v) was used for activation of monocytes and lymphocytes [7]. In some experiments, lymphocytes were activated with 30 μg/ml phytohemagglutinin-P (PHA, Serva). High-affinity neutralizing monoclonal antibodies 10F to human TNF were used to neutralize TNF in cell culture. Reference rTNF (100 U/ml, one unit is equal to 25 pg) from the National Institute of Biological Standards and Control (London) was used for cell stimulation. The production of IL-8 in monocyte and lymphocyte culture was assessed in an enzyme immunoassay with monoclonal and polyclonal antibodies to IL-8 [8].

The cytokine mRNA was analyzed by Northern hybridization with DNA probes for IL-8, TNF, and

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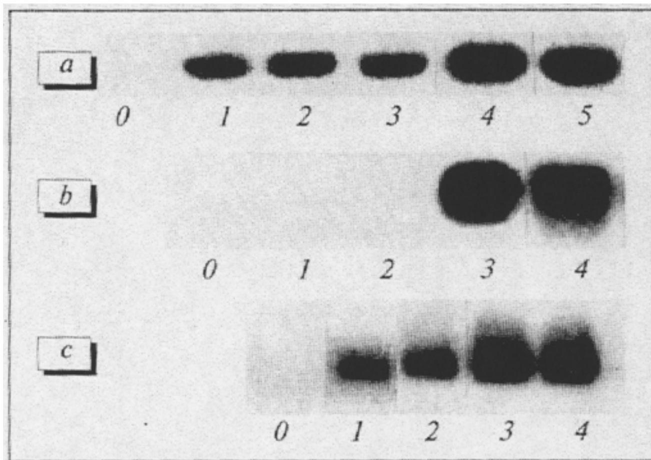

Fig. 1. Effect of monoclonal antibodies to tumor necrosis factor (TNF) and recombinant TNF (rTNF) on the synthesis of the interleukin-8 mRNA (*a*), TNF-α (*b*), and interleukin-1β (*c*) in human monocytes. *a*) *0* - freshly isolated monocytes. Monocytes incubated for 4 h: no stimulation (*1*), in the presence of 100 U rTNF (*2*), anti-TNF monoclonal antibodies (*3*), SAC (*4*), SAC and anti-TNF monoclonal antibodies (*5*). *b, c*) *0* - freshly isolated monocytes. Monocytes incubated for 4 h: no stimulation (*1*), in the presence of 100 U rTNF (*2*), SAC (*3*), SAC and anti-TNF monoclonal antibodies (*4*).

and interleukin-1β (IL-1) as described previously [11]. The total RNA was isolated from the cells as described elsewhere [2] after cell lysis with 4 M guanidine isothiocyanate (Fluka). After spectrophotometric determination of the RNA concentration, 10 μg of total RNA was fractionated in 1.2% agarose gel with 2.2 M formaldehyde. The quality of the specimens and the degree of RNA degradation were assessed after staining with ethidium bromide. After electrophoresis, RNA was osmotically transferred onto a Hybond N nylon membrane (Amersham) as described elsewhere [12]. The DNA probes were labeled with α-^{32}P d-ATP by primer elongation with Klenov's fragment of *E. coli* DNA polymerase 1 to attain the specific activity of about 10^9 decays/μg [12]. Hybridization with labeled probes and membrane washing were performed as previously [11]. The results were analyzed using Student's *t* test.

RESULTS

Figure 1 shows that human monocytes isolated under nonadhesive conditions on a Percoll gradient did not contain mRNA of IL-8, TNF, or IL-1. After a 4-h adhesion on plastic without any additional stimulation, accumulation of IL-8 and IL-1 mRNA was observed in the cells, but no TNF mRNA appeared in adhesive monocytes (Fig. 1). The addition of rTNF did not stimulate the synthesis of the IL-8 mRNA in a culture of adhesive intact monocytes. Neutralizing anti-TNF antibodies did not reduce the synthesis of IL-8 mRNA in SAC-stimulated monocytes (Fig. 1) nor did the antibodies to TNF and rTNF change the synthesis of the IL-1 mRNA (Fig. 1).

Figure 2 shows that the adhesion-induced production of IL-8 was not changed by TNF. Anti-TNF neutralizing antibodies did not reduce the synthesis of IL-8 in SAC-stimulated monocytes. The addition of exogenous TNF to SAC-activated monocytes did not enhance the production of IL-8 (data not shown).

These findings, which indicate that IL-8 production by monocytes is TNF-independent, are inconsistent with the effect of TNF on the IL-8 produc-

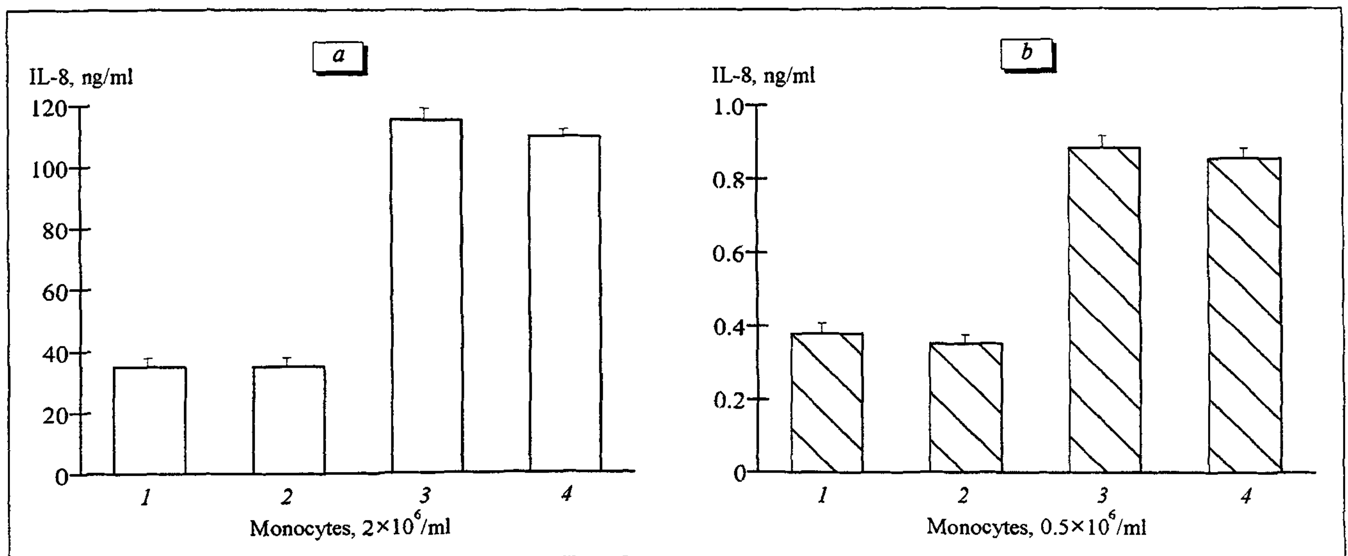

Fig. 2. Effect of recombinant tumor necrosis factor (rTNF) and anti-TNF neutralizing antibodies on the production of interleukin-8 (IL-8) in human monocyte culture after 20-h (*a*) and 4-h (*b*) incubation (*n*=3). Monocytes incubated without stimulation (*1*), in the presence of 100 U rTNF (*2*), SAC (*3*), SAC and anti-TNF monoclonal antibodies (*4*). Previously described enzyme immunoassay [1] detected from 30 to 140 pg of TNF/ml in a culture of unstimulated adhesive monocytes.

tion in a culture of nonfractionated peripheral blood mononuclear cells [5]. In order to clear up this issue we studied the synthesis of IL-8 in a culture of human lymphocytes in the presence of rTNF and anti-TNF neutralizing antibodies. The addition of TNF to unstimulated lymphocytes induced production of the IL-8 mRNA (Fig. 3, *b*) and IL-8 (Fig. 3, *a*). The level of IL-8 mRNA (Fig. 3, *b*) and production of IL-8 induced by SAC (Fig. 3, *a*) or PHA (data not shown) declined in the presence of anti-TNF monoclonal antibodies.

These observations indicate that the IL-8 production in human lymphocytes can be induced by exogenous TNF. On the other hand, endogenous induction of IL-8 synthesis by SAC or PHA is partially dependent on TNF. Hence, the relationship between the IL-8 production by mononuclear leukocytes and TNF [5] is due to the influence of TNF on lymphocytes, which represent the bulk of nonfractionated mononuclears.

In contrast to the generally accepted concept that the IL-8 production in human monocytes is TNF-dependent, our findings indicate that IL-8 is synthesized as a result of direct reaction of monocytes to stimulation. It can be suggested that during the development of inflammatory reaction *in vivo* IL-8 acts as a primary "signal" factor released from monocytes/macrophages directly under the action of an inflammatory agent on these cells.

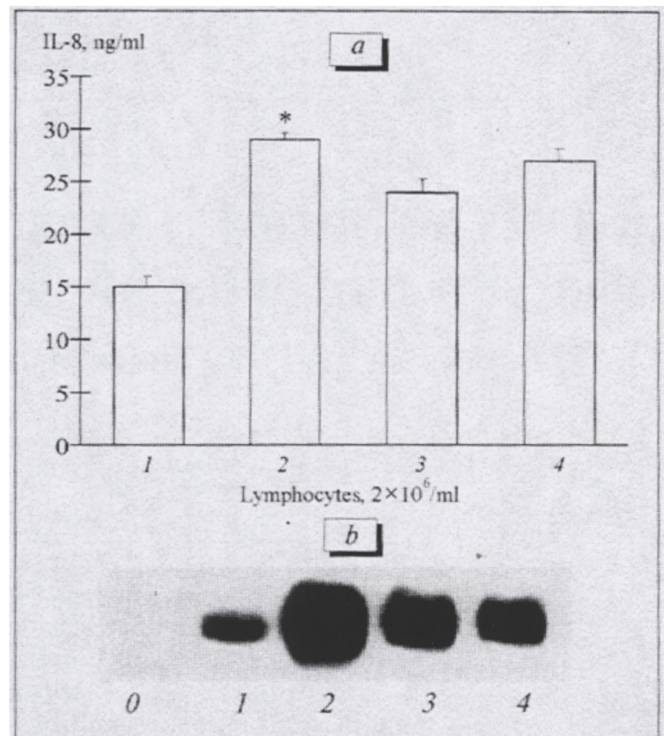

Fig. 3. Effect of recombinant tumor necrosis factor (rTNF) and anti-TNF neutralizing antibodies on the production of interleukin-8 mRNA (IL-8, *b*) and IL-8 (*a*) in human lymphocyte culture (n=3). *0*) freshly isolated lymphocytes. Lymphocytes incubated for 20 h: no stimulation (*1*), in the presence of 100 U of rTNF (2), SAC (*3*), SAC and anti-TNF monoclonal antibodies (*4*). *p<0.05.

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