Cow Milk Angiogenin Induces Cytokine Production in Human Blood Leukocytes

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Angiogenin isolated from cow milk induces the production of cytokines IL-1β, IL-6, and TNF-α in human leukocytes; the level of production of each cytokine depends on the concentration of the preparation, and the dynamics of production depends on the time from the beginning of induction. Simultaneous treatment with angiogenin and phytohemagglutinin had an additive effect on the production of cytokines, the time of this effect manifestation being individual for each cytokine.

Key Words: *angiogenin; cytokines; IL-1β; IL-6; TNF-α*

Angiogenin, a new representative of the pancreatic ribonuclease superfamily [1] discovered in 1985, is expressed by many animal cells [9]. It was found in the liver, placenta, epithelial cells, fibroblasts, blood cells, serum, female and cow milk [3,12,13]. This polypeptide (14.5-20 kDa) is highly homologous to pancreatic RNase A by amino acid composition, but differs from this enzyme by enzymatic and biological activity. Angiogenin is a unique ribonuclease. Its activity towards typical substrates is by several orders of magnitude lower than activity of pancreatic RNase, but it exhibits a unique property: cleavage of ribosomal RNA to very long fragments (100-400 nucleotides).

Angiogenin is an active inductor of vessel growth. Recent studies indicate that its functions are essential for the maintenance of homeostasis in living organisms [4]. Changes in its plasma concentration is associated with many pathological processes, and hence, this parameter can be used as a diagnostic test in clinical practice [10,12]. There are good grounds to suggest that angiogenin is involved in the mechanisms regulating the functions of the immune system [1,2]. However, studies in this direction are just beginning and the reports are scanty. The effect of angiogenin on the development and functioning of the cytokine network is unknown. It is however known that cytokines produced by immunocompetent and somatic cells mediate the immune processes [6,7].

We investigated the capacity of angiogenin to induce cytokine production in human leukocytes.

MATERIALS AND METHODS

Angiogenin (AG) was isolated from cow milk by chromatography on cation exchangers CM-cellulose-52 (Serva) and CM-Toyopearl-650S (Tosoh) with subsequent purification by hydrophobic chromatography on Butyl-Toyopearl-650S (Tosoh) (method was developed at our laboratory) [5]. The content of AG in chromatographic fractions was estimated by binding to placental RNase inhibitor. The molecular weight and homogeneity of the preparation were evaluated by electrophoresis in 15% polyacrylamide gel with sodium dodecyl sulfate as described previously [11]. A set of standard proteins for electrophoresis (Pharmacia) served as markers.

Leukocytes were isolated from donor blood by centrifugation in Ficoll-verograffin gradient according to manufacturer's instruction. The count of leukocytes was 1.5×10^6/ml culture medium (RPMI-1640, 10%

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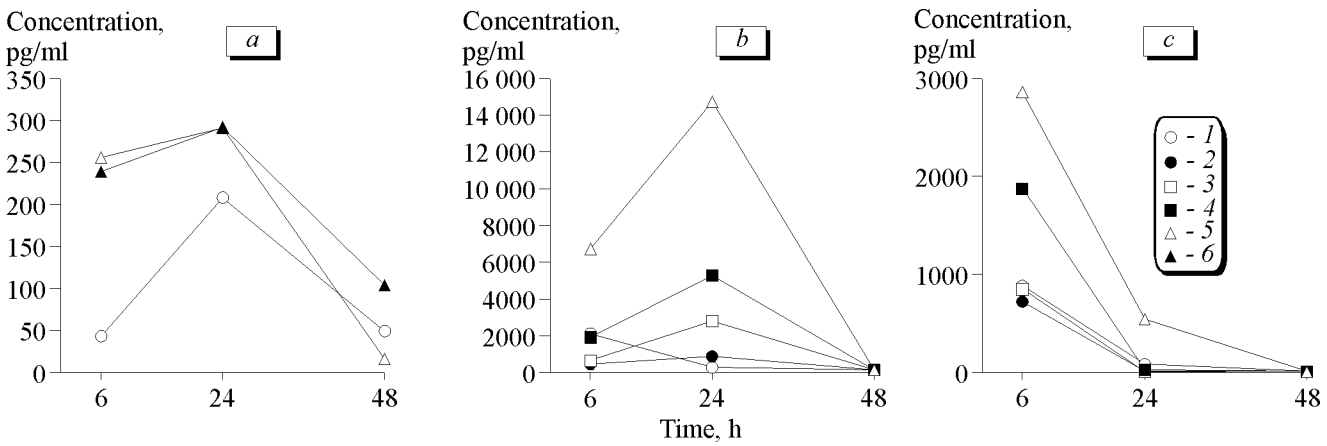

Fig. 1. Cytokine production by donor blood leukocytes after angiogenin induction. *a*) IL-1β; *b*) IL-6; *c*) TNF-α. *1*) control; *2*) 0.5 pg/ml; *3*) 2 pg/ml; *4*) 10 pg/ml; *5*) 50 pg/ml; *6*) 200 pg/ml.

fetal calf serum, 2 mM L-glutamine, and 50 μg/ml gentamicin). AG in different concentrations was added to leukocyte suspension and cultured for 48 h at 37°C. The medium was removed after 6, 24, and 48 h, the leukocytes were resuspended in a fresh portion of the same medium without AG. PHA was added to leukocyte suspension separately or simultaneously with AG. When used simultaneously, PHA and AG were used in optimal concentrations (15 and 50 μg/ml, respectively). AG alone was used in concentrations *a priori* surpassing the optimum (200 μg/ml). Cytokines in the samples were measured by enzyme immunoassay using commercial test systems (Proteinovyi Kontur).

RESULTS

The protein isolated from milk was homogenous. Electrophoregram showed only one protein band corresponding ~14 kDa, which is typical of angiogenin-1. Protein bound to placental RNase inhibitor competing with pancreatic ribonuclease A in accordance with the kinetic parameters of competitive reaction known for bovine AG [8].

AG induced production of the studied cytokines (IL-1β, IL-6, and TNF-α) in leukocytes (Fig. 1). The level of produced cytokines depended on the concentration of the preparation and the duration of leukocyte contact with the inductor. The production of IL-6 was observed at concentrations starting from 2.0 μg/ml, that of TNF-α starting from 10 μg/ml; the optimum concentration of AG for the production of all cytokines was 50 μg/ml. The maximum production of TNF-α was observed 6 h after the start of induction, that of IL-1β and IL-6 after 24 h of induction. Thus, AG induced cytokine production in human leukocytes.

After 6-h incubation of leukocytes with AG and PHA production of IL-1β and IL-6 attained the level observed after induction with these preparations alone

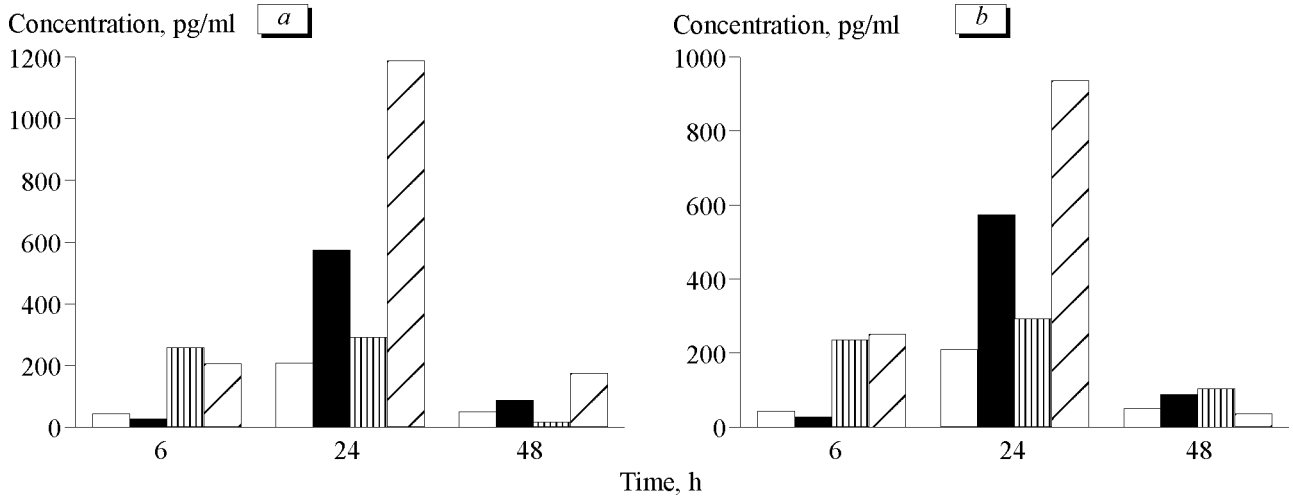

Fig. 2. Production of IL-1β by blood leukocytes after induction with angiogenin (AG, *a*) 50 μg/ml; *b*) 200 μg/ml) and phytohemagglutinin (PHA). Here and in Fig. 3: light bars: control; dark bars: PHA; vertical cross-hatching: AG; oblique cross-hatching: PHA+AG.

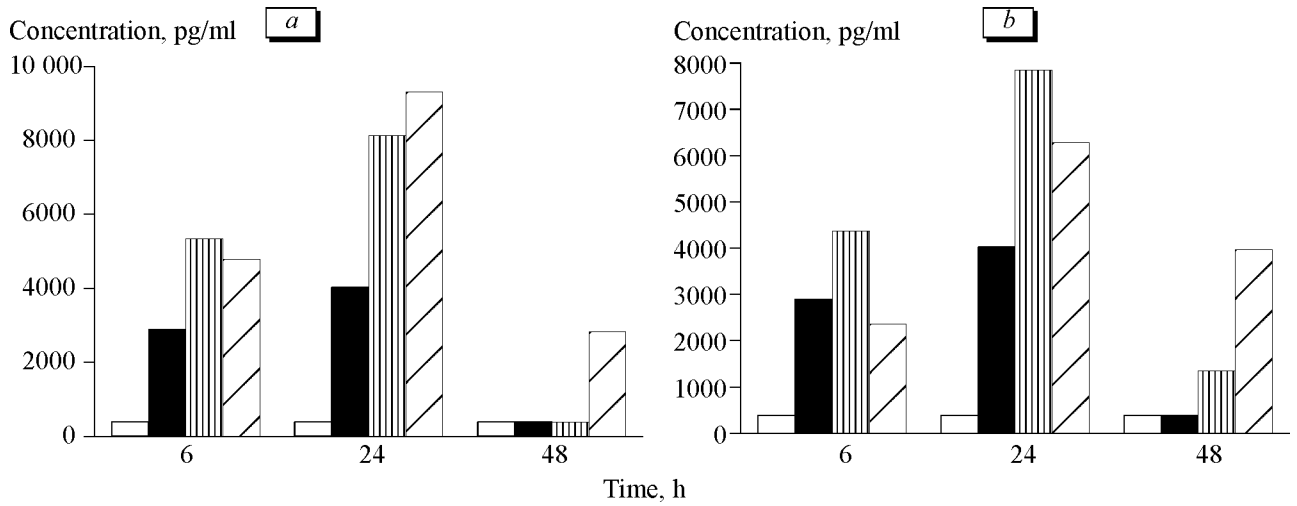

Fig. 3. Production of IL-6 by blood leukocytes after induction with angiogenin (AG, *a*) 50 μg/ml; *b*) 200 μg/ml) and phytohemagglutinin (PHA).

(Figs. 2, 3). However, 24 h after addition of both inductors IL-1β production markedly increased, and after 48 the production of IL-6 increased in comparison with induction with AG and PHA alone. The additive effect of the inductors manifested at AG concentrations of 50 and 200 μg/ml. Addition of the second inductor (PHA) into the medium at the peak of the effect of AG in the studied concentrations (Fig. 1) did not increase cytokine production. This can be due to the fact that the capacity of each cell, including blood leukocytes, to production of induced proteins is limited. The additive effect of AG and PHA attests to the differences in the molecular mechanisms of cell activation by these agents. This phenomenon can be explained with consideration for the mechanisms of the cytokine inductor interactions with cell receptors. The detected capacity of AG to induce the production of cytokines IL-1β, IL-6, and TNF-α in blood leukocytes indicates its involvement in the creation of the cytokine network and the functioning of the immune system. These cytokines are produced by blood cells (monocytes/macrophages, Th1, Th2), and therefore we conclude that these cell populations are induced (activated) by AG.

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