Antigenic Response in the Presence of High Levels of Angiogenin

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Immunomodulating effects of human recombinant angiogenin (vascular growth factor) are studied in CBA mice. It is demonstrated that the effects depend on dose, route of administration, and nature of immune responses.

Key Words: *immunogenesis; immunomodulation; human recombinant angiogenin; CBA mice*

Angiogenin (AGG), a growth factor identified in human adenocarcinoma [9], can be employed for immunocorrection. This agent has a structure similar to that of placental ribonucleases and exhibits ribonuclease activity [13]. Expression of the AGG gene and its mRNA were demonstrated in normal and tumor cells [11,15], AGG was detected in cells and blood serum [12,14], and high- and low-affinity AGG receptors were identified on endothelial cells [6,8]. It was reported that AGG modulates functions of endothelial cells [7,10]. Localization of AGG near immunocompetent cells suggests that AGG possesses immunomodulating activity. This suggestion was confirmed by immunostimulating effect of AGG [3].

In the present study were examined the immunomodulating effect of AGG in relation to dose, route of administration, and nature of immune reaction.

MATERIALS AND METHODS

Experiments were performed on CBA and BALB/c mice aged 3-3.5 months (n=314, not less than 7 animals in each group). Sheep erythrocytes (SE) were used as an antigen. They were injected intraperitoneally in a concentration of 5×10^6-5×10^7 cells per animal with or without AGG. Splenocyte suspension was prepared 3, 4, 5, 7, and 10 days after the first immunization and 7 days after the second. After a 15-min incubation with an equal volume of 3% SE suspension at 37°C, rosette-forming (antigen-binding) cells were counted under a phase-contrast microscope (×1000). Humoral reaction was assessed by blood hemagglutinin level on days 4, 8, 11, and 15 after the first immunization (5×10^6 SE or SE+ AGG per mouse) and on days 3, 4, 5, 6, and 7 after the second immunization. The interval between the first and second immunization was 15 days. Angiogenin emulsion (0.2 ml) prepared by mixing incomplete Freund's adjuvant and AGG in normal saline (1:1) was injected subcutaneously 40 min before SE. Control mice were injected with an equal volume of incomplete Freund's adjuvant. In the delayed hypersensitivity test the mice were injected intravenously with 5×10^5 SE/mouse. After 4 days, the antigen was injected in a dose of 10^8 cells/mouse in the hind paw (normal saline was injected in the other paw). After 24, 48, and 72 h the reaction was assessed by the volume of edema which was determined by measuring the volume of water displaced after immersion of the hind limb.

Human recombinant AGG was produced by technology developed at the Novosibirsk Institute of Bioorganic Chemistry (Siberian Division of Russian Academy of Sciences) and Institute of Therapy (Siberian Division of Russian Academy of Medical Sciences) [4].

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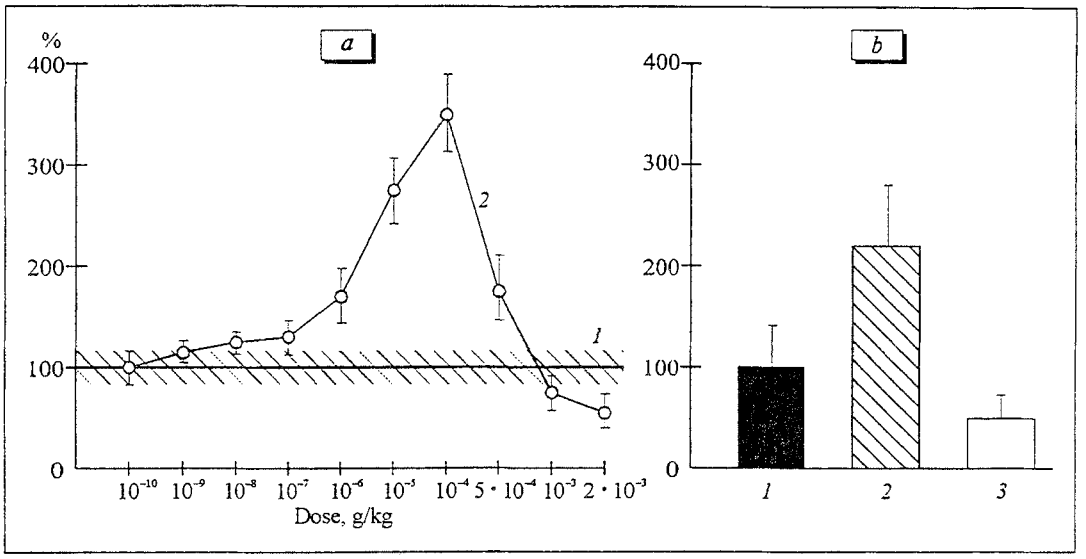

Fig. 1. Dose-dependent effects of angiogenin (AGG). *a*) dose—response curve (*2*) characterizing rosette formation in CBA mice injected with SE+AGG (g/kg), percent of the control mice injected with SE (10^7 per mouse, *1*); *b*) sensitivity of spontaneous rosette formation to different doses of AGG. *1*) control, 100%; *2*) SE+AGG in a dose of 10^{-5} (*2*) or 10^{-3} (*3*) g/liter.

The results were analyzed by Student's and Wilcoxon's tests. The values in figures are the means with 95% reliability intervals.

RESULTS

The relationship between the intensity of immune response and the AGG dose was assessed by rosette formation. Angiogenin acted as a bimodal immunoregulator: in low doses it increased and in high doses decreased the number of rosette-forming cells (Fig. 1, *a*). This reflects the situation when opposite effects are due to different affinity of receptors to a ligand [1]. Presumably, similar processes occurred in our study, since any regulation is realized via cell receptors, as was demonstrated in experiments with AGG and endothelium [6]. This suggestion is confirmed by a high rate of AGG interaction with the plasma membrane [7] and sensitivity of the "early rosettes" to AGG [2,3], indicating that the period of reception, interpretation, and realization of immunomodulating signal is extremely short (several seconds).

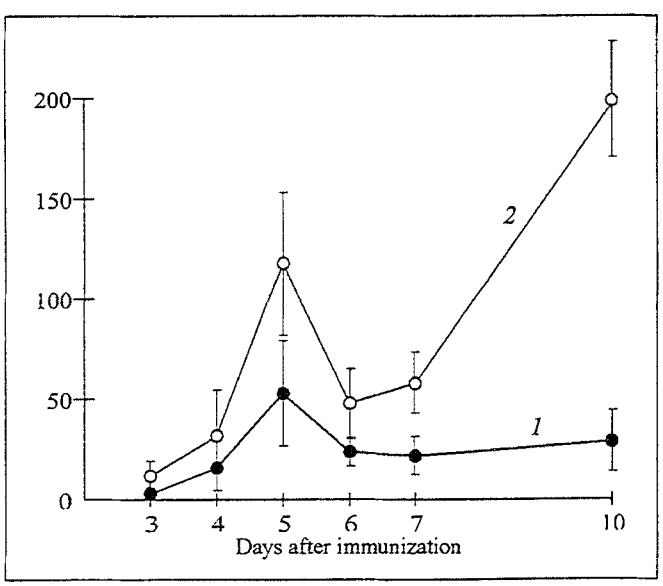

Fig. 2. Rosette formation after immunization of CBA mice with SE (10^7 per mouse, control, 100%, *1*) or SE+10^{-5} g/kg AGG (2). Ordinate: number of rosette-forming cells per 1000 cells.

Spontaneous rosette formation between SE and mouse splenocytes *in vitro* is highly sensitive to AGG. In low concentration AGG stimulated this reaction and inhibited it in high concentration (Fig. 1, *b*). This fact is very important, since rosette-forming cells determine the immune response [5], i.e., AGG acts as an immunomodulator.

We then examined the effects of suboptimal (stimulating) doses of AGG on rosette formation. Angiogenin stimulated this reaction throughout the entire experiment (Fig. 2). In contrast to the control, two peaks of the reaction were observed in AGG-treated mice. The first peak coincided with the maximum synthesis of IgM and maximum level of rosette-forming cells that bind SE via proteins with S-S bridges (IgM and IgM-like molecules). The second peak coincides with an increase in IgG production and in the number of rosettes formed via proteins which, similar to IgG, contain no S-S bridges.

In order to potentiate the effect of AGG on humoral immunity, which has a low-sensitivity to

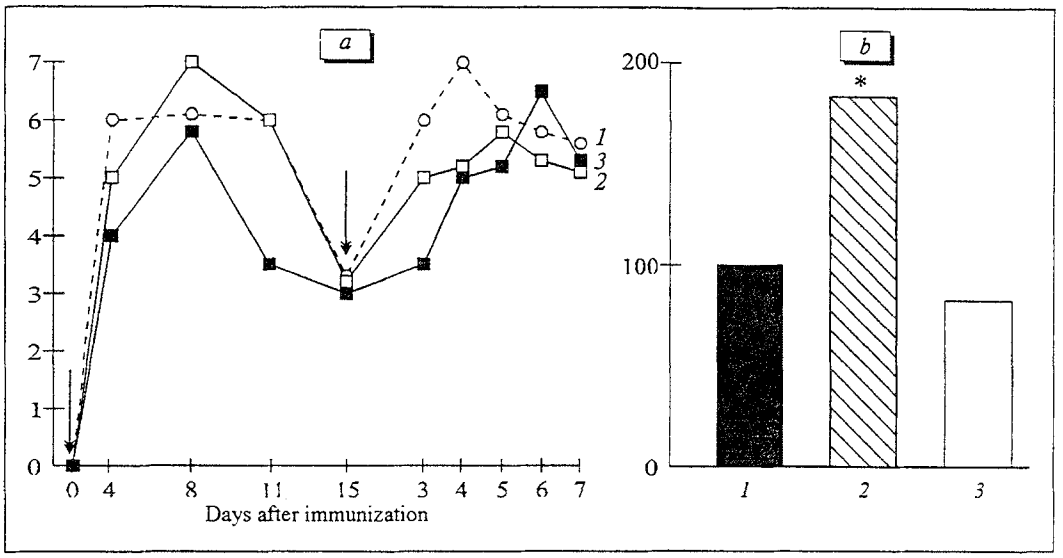

Fig. 3. Effects of AGG injected in incomplete Freund's adjuvant. *a*) hemagglutinin production (lg_2) after the first and second immunization (arrows) of BALB/c mice injected with SE (5 | 10^6 per mouse) and incomplete Freund's adjuvant (subcutaneously, control, *1*) or with SE+AGG in a dose of 10^{-6} g/kg (*2*) or 10^{-3} g/kg (*3*) in the adjuvant; *b*) rosette formation on day 7 after repeated immunization (the same groups, control 100%).

this agent [3], we prolonged the action of AGG with the aid of Freund's adjuvant. Low doses of AGG produced a more pronounced effect on secondary immune response than on primary. This effect was then reversed to inhibition, which may be due to the administration of AGG in adjuvant. High doses of AGG inhibited both primary and secondary immune responses (Fig. 3, *a*). However, on day 7 after repeated immunization, AGG in a low dose increased the number of rosette-forming cells 2-fold and had no effect in a high dose (Fig. 3, *b*).

The effects of AGG on humoral response suggest that immunological memory is sensitive to AGG. Inhibition of the reaction by low AGG doses administered in adjuvant may be indicative of chemoattractant properties of AGG. It can be assumed that gradual resorption of the adjuvant results in formation of a focus with high level of AGG which attracted a certain cell population. This population does not participate in the realization of the immunostimulating effect of AGG, thus swaying the balance toward the cell population suppressing humoral reaction. This assumption agrees with the results of experiments in which 0.02 or 2.0 μg/mouse AGG was injected together with the challenging dose of SE, after which delayed hypersensitivity was assessed. A 2-fold increase in the volume of edema at the early stage of delayed hypersensitivity (24 h) in the presence of AGG (low dose) may be due to the chemoattractant function of this agent. However, other mechanisms cannot be ruled out.

It can be hypothesized that in AGG-dependent tumors [9] AGG may act as an immunomodulator that blocks antitumor immunity. This mechanism is confirmed by the following facts: at certain concentrations AGG has no effect on a given immune reaction (Fig. 1, *a*), cell populations responsible for rosette formation, humoral immunity, and delayed hypersensitivity have different sensitivities to AGG, similar situations are observed upon the growth of AGG-dependent tumors and administration of AGG in Freund's adjuvant, more pronounced edema is formed in the presence of AGG in the focus of the delayed hypersensitivity reaction, and AGG produces a dose-dependent, bilateral, direct effect on primary antigenic activation of cells *in vitro*.

Our results indicate that AGG acts as an immunomodulator. Further investigations are necessary to elucidate the mechanisms of AGGergic immunomodulation and its coupling to other functions of this growth factor.

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