Immunotropic Effects of Potentiated Antibodies to Human Interferon-γ

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We studied immunotropic properties of antibodies to human interferon-γ in homeopathic doses. The effects of these antibodies on the ratio between the counts of T helper ($CD4^+$) and T suppressor cells ($CD8^+$), production of interleukin-1 and interleukin-2, and functional activity of natural killer cells were evaluated. Potentiated antibodies slightly decreased the number of $CD8^+$ cells in the spleen of experimental animals. After the addition to cultured human mononuclear cells the preparation produced a moderate comitogenic effect, stimulated proliferation of T and B lymphocytes, increased spontaneous and induced production of interleukin-1, and enhanced functional activity of natural killer cells (under certain conditions).

Keywords: *ultralow doses; antibodies to human interferon-γ; lymphocyte proliferation; cytokine production; activity of natural killer cells*

The effects of compounds in ultralow doses are associated with local concentration of the active substance and presence of high-efficiency systems for signal amplification [2]. Our previous studies showed that homeopathically potentiated substances produce various biological reactions in the macroorganism [4,5]. These data suggest that antibodies to immunoregulatory molecules in ultralow doses would produce the immune response. Here we studied the immunomodulatory effects of potentiated antibodies to human interferon-γ (PAB-IFN) *in vivo* and *in vitro*.

MATERIALS AND METHODS

Experiments were performed on 50 male CBA mice weighing 18-20 g and obtained from the Collection of the Laboratory of Experimental Biomedical Modeling (Institute of Pharmacology). The mixture of PAB-IFN (0.2 ml, equivalent concentration 10^{-24} wt %) was administered perorally for 10 days. Control mice did not receive PAB-IFN.

The animals were killed by cervical dislocation 1 day after the first, fourth, seventh, or tenth treatment with preparations. To obtain the suspension of splenocytes, the tissue was minced by pressing through a sieve and centrifuged at 500*g* for 5 min. Then the suspension was resuspended in RPMI medium containing 5% fetal bovine serum.

Lymphocytes were isolated from the suspension of splenocytes in a Ficoll-Paque gradient (density 1.077 g/cm^3). The number and ratio between $CD4^+$ and $CD8^+$ cells was estimated by immunofluorescence technique after administration of the preparation for 1, 4, 7, and 10 days. We used monoclonal antibodies conjugated to fluorescein 5(6)-isothiocyanate and reacting with mouse CD4 and CD8a antigens (Biosource International).

In vitro experiments were performed with the peripheral blood from 10 healthy volunteers (22-36 years). Proliferative activity of T and B lymphocytes was determined in the reaction of blast transformation reflecting their functional activity [3]. Phytohemagglutinin (PHA) and pokeweed mitogen were used to activate T and B lymphocytes, respectively. Radioactivity was measured on a Mark-III beta-counter (USA). Each sample was assayed in 3 wells. Average radioactivity was calculated. We determined the number of pulses over 1 min and evaluated the index of stimulation (IS). IS was calculated as the ratio between radioactivity in wells with and without the mitogen (experiment and control, respectively).

The effects of PAB-IFN on proliferative activity of T and B lymphocytes were evaluated in reactions of induced and spontaneous blast transformation. In

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the reaction of induced blast transformation we mixed 50 ml mononuclear cells, 50 ml mitogen in the suboptimal concentration, and 50 ml diluted preparation in a final concentration of 50 ml/ml (experimental wells); control wells contained 50 ml mononuclear cells, 50 ml mitogen, and 50 ml complete growth medium (CGM). In the reaction of spontaneous blast-transformation we mixed 50 ml mononuclear cells, 50 ml diluted preparation, and 50 ml CGM (experimental wells); control wells contained 50 ml mononuclear cells and 100 ml CGM. The influence of PAB-IFN on spontaneous and induced blast transformation of lymphocytes was determined by IS.

Supernatants of cultured mononuclear cells obtained after 1-day culturing *in vitro* served as the source of interleuin-1 (IL-1) and IL-2 [3]. Experiments were performed with 4 types of supernatants: 1) from cells stimulated with lipopolysaccharide (LPS, 75, mg/ml) and PHA (20 mg/ml) in the presence of PAB-IFN (50 μl/ml) to induce production of IL-1 and IL-2, respectively; 2) from cells stimulated with LPS or PHA (control for stimulation); 3) from cells cultured in the presence of PAB-IFN; and 4) from cells cultured in CGM (control). Production of IL-1 was determined as described elsewhere [6]. Thymocytes from intact C3H mice were washed 3 times in CGM and counted in a Goryaev chamber. Their concentration was brought to 5×10^6 cells per 1 ml CGM. Thymocytes (100 ml), test supernatants (100 ml), and PHA in a submitogenic dose of 2 mg/ml were placed in experimental wells of a 96-well round-bottom plate. Thymocytes (100 ml) and CGM (100 ml) were added to control wells. Culturing was performed at 37° and 5% CO_2 for 72 h. [^{3}H]-Thymidine (1 mCi) was added to each well 16 h before the end of incubation. After incubation lymphocytes were transferred to a glass-fiber filter. Radioactivity was measured on a Mark-III scintillation counter. The intensity of IL-1 and IL-2 production was expressed in IS.

IL-2 content was measured by the biological method using PHA-stimulated blasts [3]. We determined the ability of PAB-IFN to increase and induce IL-2 synthesis.

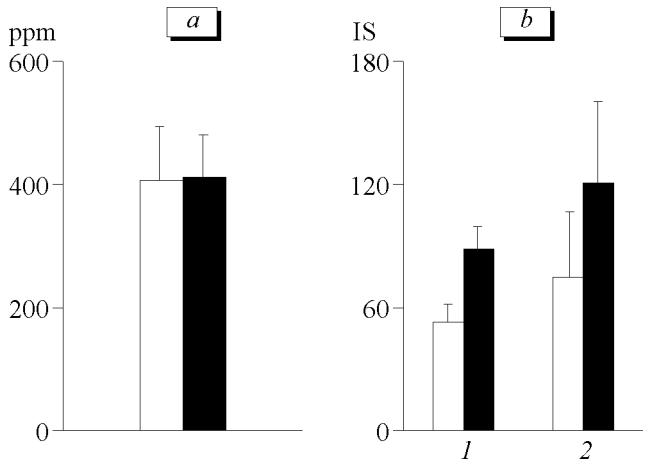

Fig. 1. Effect of potentiated antibodies to interferon-γ on spontaneous (*a*) and induced blast transformation of lymphocytes (*b*). Control (light bars) and treatment (dark bars). Stimulation with phytohemagglutinin (*1*) and pokeweed mitogen (*2*). IS: index of stimulation.

Functional activity of natural killer cells was estimated radiometrically by their ability to lyse myeloblastic K-562 cells in the cytotoxic reaction [3]. The index of cytotoxicity (IC) was calculated by the formula:

$$IC=[1-(A-B)]\times100/(C-B),$$

where A is radioactivity of target cells in the presence of effector cells, B is residual radioactivity after treatment of target cells with triton X-100 (maximum yield), and C is radioactivity of target cells in the absence of effector cells.

We compared IC for cultured mononuclear cells incubated in the presence and absence of PAB-IFN.

The results were analyzed by Student's t test. Initially we estimated whether the data are characterized by the normal distribution.

RESULTS

PAB-IFN produced no significant changes in the population of CD4$^+$ cells from experimental mice compared to the control (Table 1). However, on days 4 and

TABLE 1. Effect of PAB-IFN on the Count and Ratio between CD4$^+$ and CD8$^+$ Lymphocytes in the Spleen of CBA/CaLac Mice (ppm, $X\pm m$)

Parameter	Control	Days			
		1	4	7	10
CD4$^+$ cells	39.5±0.8	40.0±0.8	41.5±1.2	38.0±1.6	40.5±1.7
CD8$^+$ cells	20.0±0.8	21.0±0.9	18.0±0.5*	18.5±1.5	18.5±0.4*
CD4$^+$/CD8$^+$	2.0±0.1	1.7±0.1	2.3±0.1*	2.1±0.3	2.2±0.1

Note. *p<0.05 compared to the control.

TABLE 2. Effect of PAB-IFN on Production of IL-1 and IL-2 (IS, $\bar{X}\pm m$)

Experimental conditions	IL-1	IL-2
No stimulation		
control	2.70±0.84	1.1±0.1
PAB-IFN	2.70±0.84	1.1±0.1
Stimulation with mitogens		
control	11.52±3.23	1.44±0.15
PAB-IFN	20.90±3.12	1.36±0.15

TABLE 3. Effect of PAB-IFN on Functional Activity of Natural Killer Cells (IC, $\bar{X}\pm m$)

Target cell/effector cell ratio	Control	PAB-IFN
1:25	63.63±2.68	71.68±1.93*
1:10	59.06±11.53	59.08±12.03

Note. *p=0.036 compared to the control.

10 this preparation reduced the count of $CD8^+$ lymphocytes compared to that in intact animals. A decrease in the number of $CD8^+$ cells contributed to an increase in the T-helper/T-suppressor ratio after administration of PAB-IFN for 4, 7, and 10 days. On day 4 these differences were statistically significant (Table 1).

The addition of PAB-IFN to cultured mononuclear cells with the T or B cell mitogen promoted the increase in IS. However, these changes were statistically insignificant due to a considerable scatter in the data. The preparation did not modulate spontaneous blast transformation of lymphocytes (Fig. 1).

After the addition of PAB-IFN and LPS the intensity of IL-1 production by cultured mononuclear cells was greater than that observed in the presence of only LPS (Table 2). Incubation of mononuclear cells with PAB-IFN also stimulated production of IL-1. In cultures incubated with PAB-IFN the content of IL-1 was higher than in supernatants of cells cultured in CGM, but lower than that observed after the addition of PAB-IFN and mitogen (Table 2).

IL-2 is one of the major cytokines in the immune system that plays an important immunoregulatory role. IL-2 possesses the ability to stimulate proliferation of lymphoid cells. PAB-IFN had no effect on IL-2 production in the culture of peripheral blood mononuclear cells (Table 2).

After the addition to cultured mononuclear cells PAB-IFN improved functional activity of natural killer cells, which was manifested in the increase in IC at the 1:25 target cell/effector cell ratio. This effect was not observed at the 1:10 target cell/effector cell ratio (Table 3).

Our results indicate that PAB-IFN in specified doses given perorally to mice or *in vitro* added to the culture of peripheral blood mononuclear cells from healthy donors produce a strong immunotropic effect. PAB-IFN slightly decreased the count of $CD8^+$ cells (T suppressors) in the spleen of experimental animals. PAB-IFN added to the culture of human mononuclear cells with mitogens produced a moderate comitogenic effect, stimulated proliferation of T and B lymphocytes, and activated spontaneous and induced production of IL-1. Under certain conditions the preparation enhanced functional activity of natural killer cells.

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