Ultralow Doses of Anti-Idiotypic Antibodies to Human γ-Interferon: Immunotropic Properties

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We studied the effects of ultralow doses of anti-idiotypic antibodies to human γ-interferon on humoral immune response in experimental animals immunized with a thymus-dependent antigen, delayed-type hypersensitivity, and phagocytic activity of neutrophils in the peritoneal exudate. This preparation stimulated the humoral immune response and activated phagocytosis, but had no effect on functional activity of T cells.

Key Words: *ultralow doses; anti-idiotypic antibodies to human γ-interferon; humoral and cellular immune response*

Modern science did not find adequate explanation for biological activity of substances in ultralow concentrations obtained using homeopathic potentiation technique. Homeopathic preparations are efficient in the therapy of various diseases. Previous studies showed that ultralow doses of antibodies to endogenous regulators (*e.g.*, γ-interferon, γ-IFN) [4,6] modulate functional activity of these agents [1,4-7].

Here we studied immunotropic properties of ultralow doses of anti-idiotypic antibodies to human γ-IFN.

MATERIALS AND METHODS

Experiments were performed on 92 CBA/CaLac mice (63 males and 29 females) weighing 16-18 g and obtained from the Laboratory of Biological Models (Institute of Pharmacology). Female mice were used in studying phagocytosis. Other experiments were performed on males.

A mixture of homeopathic dilutions of anti-idiotypic antibodies to human γ-IFN (PAB-IFN, C12+C30+C200, 0.2 ml, equivalent concentration 10^{-24} wt %) was administered perorally for 10 days. Control mice received potentiated distilled water (PDW, C12+C30+C200) according to the same schedule. Baseline parameters were recorded in intact male and female mice.

For evaluation of the humoral immune response the mice were immunized with sheep erythrocytes (SE) in minimum doses [3]. SE were injected intraperitoneally in a single dose of 5×10^6 (0.2 ml) afetr 10-day treatment with PAB-IFN or PDW. The total count of peripheral blood leukocytes, mass indexes and cellularity of the thymus and spleen, relative and absolute contents of antibody-producing cells (APC) in the spleen [8], and plasma antibody titer (reaction of hemagglutination) were measured routinely on day 4 after immunization [2].

For modeling delayed-type hypersensitivity (DTH) [3] the animals were subcutaneously sensitized with SE in a dose of 10^7 cells/mouse (0.1 ml) after 10-day treatment with PAB-IFN or PDW. The challenge dose of SE (10^8 cells/20 ml) was injected into the hindlimb pad 5 days after sensitization. An equivalent volume of 0.9% NaCl was injected into the contralateral limb. The DTH response was evaluated in each animal 24 h after treatment. The index of this reaction was calculated as the difference between the weights of limbs in treated and control mice expressed in percents of the control.

Phagocytic activity of neutrophils in the peritoneal exudate was determined by their ability to phagocytize 1-day-old culture of *S. aureus* strain 209 (Department of Microbiology, Siberian State Medical University) 24 h after 10-day treatment with PAB-IFN or PDW. The concentration of microbial suspension was 10^8 cells/ml. We estimated the percent of neutrophils phagocytizing microbes (phagocytic index, PI) and average number of staphylococci captured by one phagocyte (phagocytic number, PN) [3].

The data were analyzed by Student's *t* test (Statgraphics 3.0) after verification of their normal distribution.

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TABLE 1. Effect of PAB-IFN on the Humoral Immune Response in CBA/CaLac Mice (*M*±*m*)

Parameter		Intact	Immunization with SE		
			control	+PDW	+PAB-IFN
Total leukocyte count		12.95±0.70	10.00±0.57*	7.45±0.48*	10.10±0.74*°
Total cellularity	thymus	107.75±9.10	67.20±16.20	95.00±6.39	88.20±11.22
	spleen	155.50±2.53	110.20±10.94*	139.60±3.00*	123.60±13.53
Mass index	thymus	1.75±0.13	1.46±0.09	2.30±0.24	1.80±0.18
	spleen	3.70±0.09	3.03±0.19*	3.09±0.25	3.33±0.20
APC, 10^6		4.18±0.31	6.42±0.63*	4.99±0.39*	4.19±0.57+++
%		2.70±0.24	5.84±0.13*	3.56±0.21*	3.36±0.16*+
Antibody titer, $\log_2$		0	9.00±0.37	6.20±1.24	4.25±0.63++

Note. Here and in Table 2: *significant differences from intact mice; +p=0.000002, ++p=0.0001, and +++p=0.03 compared to the control; °p=0.02 compared to PDW.

TABLE 2. Effect of PAB-IFN on Phagocytic Activity of Peritoneal Exudate Neutrophils from CBA/CaLac Mice (*M*±*m*)

Parameter	Intact	PDW	PAB-IFN
Phagocytic index, %	23.60±3.84	11.40±2.14*	51.20±3.98*++
Phagocytic number	4.06±0.31	6.18±0.80	7.42±0.45*

RESULTS

Immunization with SE without and after 10-day treatment with PAB-IFN or PDW decreased peripheral blood leukocyte count (Table 1).

The weight and cellularity of the thymus increased in mice receiving PDW, while PAB-IFN had no effect on these parameters (Table 1).

Immunization after the course administration of PDW increased the cellularity of the spleen compared to the control. PAB-IFN did not affect the state of the spleen.

As differentiated from studies of immunotropic activity in PAB-IFN [4], in our experiments CBA mice developed active humoral immune response to administration of minimum doses of SE. The relative and absolute count of APC in the spleen considerably increased in control mice (compared to the baseline level, Table 1). Plasma titer of specific hemagglutinins in these animals varied from 1:256 to 1:512.

Immunization with SE after repeated treatment with PAB-IFN also significantly increased the count of APC (compared to the baseline level). PAB-IFN did not potentiate the immune response. In mice receiving PAB-IFN the relative and absolute count of APC and antibody titers were much lower than in control animals (Table 1). Immunization of mice receiving PDW produced similar changes (Table 1).

Neither PAB-IFN nor PDW modulated the DTH response: the indexes of this reaction were 28.86±2.44 and 23.83±1.84%, respectively (*vs.* 26.10±2.14% in the control).

Repeated treatment with PAD-IFN markedly stimulated phagocytosis (Table 2). PDW did not stimulate phagocytic activity of neutrophils, but even decreased the ratio of cells capable of phagocytizing staphylococci (compared to intact animals). Phagocytic number was similar in mice treated with PDW and PAB-IFN (Table 2). The existence of targeted pharmacological (immunotropic) activity in PDW is of considerable interest.

Thus, studies of immunotropic properties of anti-idiotypic antibodies to human γ-IFN showed that peroral administration of this preparation to CBA/CaLac mice in selected doses under these experimental conditions does not stimulate the humoral immune response during immunization with thymus-dependent antigen and has no effect on functional activity of T cells in the DTH response after sensitization with SE. PAB-IFN markedly increase phagocytic activity of peritoneal exudate neutrophils by increasing the ratio of active phagocytes.

Since immunotropic activity of PAB-IFN is realized via induction of endogenous γ-IFN [7], potentiated anti-idiotypic antibodies to γ-IFN do not stimulate production of endogenous γ-IFN.

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