# Dose-Dependent Effects and Specificity of Action of Antibodies to Endogenous Regulators in Ultralow Doses

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We studied the effects of antibodies against interferon-γ, erythropoietin, and tumor necrosis factor-α in ultralow doses on the production of endogenous interferon-γ. Course peroral treatment with potentiated antibodies to interferon-γ in various dilutions produced similar changes in experimental animals. The treatment stimulated spontaneous production of endogenous interferon-γ by mouse lymphocytes. It should be emphasized that antibodies to other cytokines in ultralow doses did not stimulate interferon-γ production. These data illustrate the specificity of action of antibodies to endogenous regulators in ultralow doses.

**Key Words:** *ultralow doses; antibodies against interferon-γ, erythropoietin, and tumor necrosis factor-α; interferon-γ*

The possibility of pharmacological treatment with biologically active substances in low and ultralow doses (ULD) was studied for more than 50 years [1,2,4,5]. Potentiated solutions of substances possess pharmacological activity. The method of potentiation includes subsequent repeated dilution and treatment of the initial solution. Pharmacological activity was not detected in non-potentiated highly diluted solutions [9,10]. Study of potentiated substances on neurobiological and other experimental models showed that not only bioactive substances in ULD, but also potentiated antibodies (AB) to a endogenous regulator (antigen) can be used to achieve the specific pharmacological effect [7,8]. *In vivo* activity of potentiated AB can be mediated by the regulatory system of natural AB [6]. The mechanisms for action of potentiated AB are poorly understood. The specificity of action and dose-dependent effects of these substances remain unknown.

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Here we studied the effects of potentiated AB against interferon-γ (IFN-γ), erythropoietin, and human tumor necrosis factor-α (TNF-α) on the production of endogenous IFN-γ.

## MATERIALS AND METHODS

Experiments were performed on 342 male CBA/CaLac mice weighing 18-20 g. Certified animals were obtained from the Laboratory of Experimental Biological Modeling (Institute of Pharmacology).

We used polyclonal AB against human IFN-γ (IgG fraction), erythropoietin, and human TNF-α. AB in ULD were potentiated by the standard pharmacological method of homeopathy (centesimal dilution scale of S. Hanneman). AB against IFN-γ were administered in three ULD: (I) mixture of dilutions (C12+C30+C50), equivalent concentration $10^{-30}$ M; (II) dilution C3, equivalent concentration $10^{-12}$ M; and (III) dilution C12, equivalent concentration $10^{-30}$ M. AB to erythropoietin and TNF-α in ULD were administered in the mixture of dilutions (C12+C30+C50). The test solutions (0.2 ml) were given perorally for 10 days. Control animals re-

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ceived an equivalent volume of the solvent (distilled water). The reference group included intact mice.

We studied the effect of preparations on INF-γ production by lymphocytes from experimental animals. The mice were killed by cervical dislocation under ether anesthesia after administration of AB for 1-7 and 10 days. Lymphocytes were isolated from the suspension of mouse spleen cells on a Ficoll-Pack gradient (density 1.077) [3]. Lymphocytes were washed 2 times with medium 199 (State Research Center Vektor) containing 5% fetal bovine serum (FBS, Bioclot). The count of viable lymphocytes was brought to $2\times10^6$ cells/ml. The cells were incubated in complete nutrient medium containing 90% RPMI-1640 medium (GUP IPVE), 10% FBS (Bioclot) inactivated by preheating (56°C, 30 min), 2 mM L-glutamine (Sigma), 10 mM HEPES (Flow), 40 mg/liter gentamicin (Serva), and 25 μM 2-mercaptoethanol (Sigma) at 37°C, 5% $CO_2$, and 100% humidity for 1 day. Conditioned sections were collected after incubation and stored at -50°C for no more than 1 month.

IFN-γ concentration in culture supernatants was measured by enzyme immunoassay with Amersham Pharmacia Biotech kits using a Uniplan AIFR-01 enzyme immunoreaction analyzer (Pikon).

The results were analyzed using STATISTICA 5.0 software. The type of data distribution was predetermined. Statistical analysis included Student's *t* test.

## RESULTS

Spontaneous production of IFN-γ by mouse lymphocytes increased on days 2-6 of peroral treatment with the mixture of dilutions of AB against IFN-γ in ULD (C12+C30+C50). The intensity of IFN-γ production by lymphocytes from animals receiving the mixture of dilutions of AB against IFN-γ (I) was maximum on day 4. In this period IFN-γ content in culture supernatants surpassed the initial and control levels by more than 8 and 100 times, respectively (Table 1). On days 4-6 and 10, IFN-γ production by lymphocytes from mice receiving AB against IFN-γ in ULD (I) was higher than in animals of the reference group.

IFN-γ production by lymphocytes increased on days 3-5 and 10 of treatment with AB against IFN-γ in dilution C3 (II, compared to control animals). On days 4-5 and 10, IFN-γ production by lymphocytes from mice receiving AB against IFN-γ (II) was much more intensive compared to that in animals of the reference group.

AB against IFN-γ in dilution C12 (III) most significantly stimulated production of IFN-γ. This effect was observed for a long time. IFN-γ production in mice significantly surpassed that in animals of the control (days 2-10) and reference groups (days 2, 4-5, 7, and 10).

AB against erythropoietin in ULD insignificantly stimulated IFN-γ production (compared to control mice with low secretion of the cytokine). It should be emphasized that in these animals IFN-γ production was below the initial level (except for day 5).

We revealed no significant increase in the intensity of IFN-γ production in mice receiving AB against TNF-α in ULD. On days 2-3 and 5-6, the intensity of IFN-γ production in these mice was higher than in control animals. It was probably related to close relationship between secretion of IFN-γ and TNF-α [11,12].

Our results suggest that peroral treatment with potentiated AB to IFN-γ in different dilutions (equivalent concentrations $10^{-12}$ and $10^{-30}$ M) produces simi-

**TABLE 1.** Effects of AB against IFN-γ in ULD (I), Low Doses (II), and ULD (III), AB against Erythropoietin in ULD, and AB against TNF-α in ULD on IFN-γ Production by Lymphocytes from CBA/CaLac Mice (*X*±*m*)

| Days | IFN-γ content in culture supernatants, pg/ml | | | | | |
|---|---|---|---|---|---|---|
| | control | mixture of dilutions (I) | dilution C3 (II) | dilution C12 (III) | AB against erythropoietin in ULD | AB against TNF-α in ULD |
| Baseline level | 12.935±1.080 | | | | | |
| 1 | 9.28±0.86$^+$ | 2.01±0.51$^{*+}$ | 5.12±1.09$^{*+}$ | 2.00±0.35$^{*+}$ | 0.76±0.24$^{*+}$ | 1.23±0.29$^{*+}$ |
| 2 | 0.76±0.38$^+$ | 4.82±0.88$^{*+}$ | 0.21±0.12$^+$ | 118.97±19.18$^{*+}$ | 2.85±0.60$^{*+}$ | 5.30±1.01$^{*+}$ |
| 3 | 0$^+$ | 3.36±0.63$^{*+}$ | 0.46±0.14$^{*+}$ | 2.77±0.47$^{*+}$ | 1.83±0.62$^{*+}$ | 1.15±0.33$^{*+}$ |
| 4 | 1.12±0.22$^+$ | 114.48±19.60$^{*+}$ | 67.81±12.97$^{*+}$ | 185.83±25.15$^{*+}$ | 7.76±1.92$^{*+}$ | 0.68±0.17$^+$ |
| 5 | 4.24±0.74$^+$ | 31.86±5.03$^{*+}$ | 57.43±17.33$^{*+}$ | 41.35±7.31$^{*+}$ | 13.69±1.38* | 16.45±3.91* |
| 6 | 4.5±0.8$^+$ | 33.60±5.67$^{*+}$ | 5.25±1.02$^+$ | 18.19±2.40* | 7.69±1.91$^+$ | 16.34±1.56* |
| 7 | 14.50±1.91 | 1.10±0.28$^{*+}$ | 6.49±1.59$^{*+}$ | 36.45±5.92$^{*+}$ | 0$^{*+}$ | 0.21±0.12$^{*+}$ |
| 10 | 25.46±3.83$^+$ | 25.58±2.61$^+$ | 49.52±5.39$^{*+}$ | 146.34±10.23$^{*+}$ | 0.25±0.12$^{*+}$ | 1.75±0.45$^{*+}$ |

**Note.** $p<0.05$: *compared to the control; $^+$compared to baseline.

lar changes in experimental animals. Potentiated AB to these cytokines stimulate production of endogenous IFN-γ by mouse lymphocytes. Potentiated AB to other cytokines (erythropoietin and TNF-α) do not increase the intensity of IFN-γ production. Activity of AB in ULD is probably related to the technology of their treatment. These data illustrate the specificity of action of potentiated AB to endogenous regulators.

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