Selective Cytokine-Inducing Effects of Low Dose Echinacea

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Echinacea purpurea is a widely used plant immunomodulator with a selective immunomodulatory effect depending on the dilution of the initial preparation. In low doses, it causes selective induction of pro- and anti-inflammatory cytokines. The results recommend this preparation in a wide range of concentrations for adequate correction of the immune system work aimed at restoring the Th1/Th2 balance in various diseases.

Key Words: *Echinacea purpurea; cytokine-inducing activity*

The use of drugs selectively modulating the immune system, specifically, cytokine production, is a promising trend of immunotherapy. This approach provides an adequate immune response in each clinical situation. Cytokines secreted by stimulated immunocompetent cells trigger biochemical cascades in target cells, largely determining the course of the process. Acute inflammatory diseases, including infections, are associated with stimulation of T-helpers (Th) from the Th1 immunity component and increase of IL-1β, IL-6, and TNF-α levels. These proinflammatory cytokines stimulate chemotaxis and phagocytosis, promote an increase of vascular wall permeability, cytotoxic and bactericidal activities of macrophages and neutrophils in the focus of inflammation. Th2 cells and IL-4, IL-5, IL-13 cytokines produced by them play an important role in the development of allergic diseases [1]. Imbalance of the Th1 and Th2 cytokine-producing activities is essential for the development of autoimmune states, chronic transformation and progress of diseases [4]. Interleukin-10, characterized by potential immunosuppressive and anti-inflammatory effects, is involved in the immune response switch-over from Th1 to Th2. Hence, the regulation of activities of cells expressing different cytokines can be regarded as an effective method for correction of a disease course and promote the treatment efficiency. Therefore, evaluation of the cytokine inducing activity of the immunomodulator, underlying the scientifically based choice of treatment protocols for this or that clinical situation, is an important problem.

Echinacea purpurea (EP) preparations are the most widely used immunomodulators of plant origin. They stimulate activity of macrophages [7], synthesis of some cytokines *in vitro* [5] and *in vivo* [6]. Clinical trials confirmed the efficiency of EP preparations in inflammatory diseases, including those of infectious nature [2]. However, the therapeutic efficiency of different EP preparations varies greatly [3].

We studied the cytokine-inducing profiles of EP tincture in a wide range of concentrations.

MATERIALS AND METHODS

Mononuclear leukocytes (MNL) were isolated from heparin-stabilized (25 U/ml) peripheral blood of 15 donors by centrifugation at 400*g* (30 min) on a single-step Ficoll gradient (PanEco, 1.077 g/cm^3). Mononuclear leukocytes forming an interphase ring were collected with a pipette and washed 3 times in RPMI-1640 (PanEco). The cells were cultured in RPMI-1640 with 10% FCS (HyClone), 2 mM glutamine (PanEco), and streptomycin with penicillin (PanEco) 5000 U/ml each. The concentration of MNL in the medium was 10^6 cells/ml.

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A series of 10-fold dilutions (D) of EP tincture (Herbamed AG) in RPMI-1640 was prepared. Suspension of MNL for incubation with the test drug was pipetted (180 μl) in 96-well plates (Nung) and 20 μl test drug was added to each well. Saline (20 μl) containing ethanol in a concentration adequate to that in the tincture was added to the control wells. The active concentration range was D1-D17. Incubation was carried out for 48 h at 4.5% CO_2 and 37°C.

Cytokine-inducing activity was evaluated by measuring the concentrations of IL-10, IL-8, IL-1β, TNF-α, IL-6, and IL-4 cytokines in culture medium by EIA (Vector-Best).

The data were statistically processed using Statistica 6.0 software (StatSoft). The figures present only the cytokine concentrations differing significantly ($p<0.05$) from the control after incubation of cells with the studied preparation.

RESULTS

Echinacea tincture in the studied concentrations exhibited no appreciable cytotoxic effect on human blood MNL, but changed significantly the production of many cytokines by human immunocompetent cells. The tincture in a wide range of concentrations caused a significant increase of the production of pleiotropic IL-6 cytokine and IL-8 chemokine in comparison with the control (Fig. 1). The maximum release of IL-8 (3.5 times higher than in the control) was observed after incubation with D10-D11. The greatest increase of IL6 concentration in the medium (2.5-3 times *vs.* control) was caused by incubation of MNL with EP in concentrations of D6 and D14. The former peak (D6) was characterized by a gradual increase of the cytokine level with reduction of the tincture concentration.

The secretion of IL-1β proinflammatory cytokine by MNL increased significantly in response to high (D1, D2) and some low (D10 and D15) concentrations of EP, the maximum increment (in the presence of D10) reaching 540% of control (Fig. 2). The effect of EP on the production of another proinflammatory cytokine, TNF-α, was dose-dependent. The tincture concentrations of D9-D12 suppressed, while D4 and D15 stimulated this cytokine production. Its levels were 140-150% higher (D4 and D15) or 100% lower (D10) *vs.* control.

Only some of EP concentrations (D2-D4, D10, D13) stimulated the production of anti-inflammatory cytokines IL-10 and IL-4 (Fig. 3). Stimulation of IL-10 production predominated in the presence of EP in D2-D3 and D10 concentrations. This cytokine concentration in the well with the tincture surpassed the control level 2-3-fold, while the concentration of IL-4 increased only 1.5-1.9 times in comparison with the control. The increase in the production of IL-10 and IL-4 in the presence of D13 concentration was similar (180-190% of control).

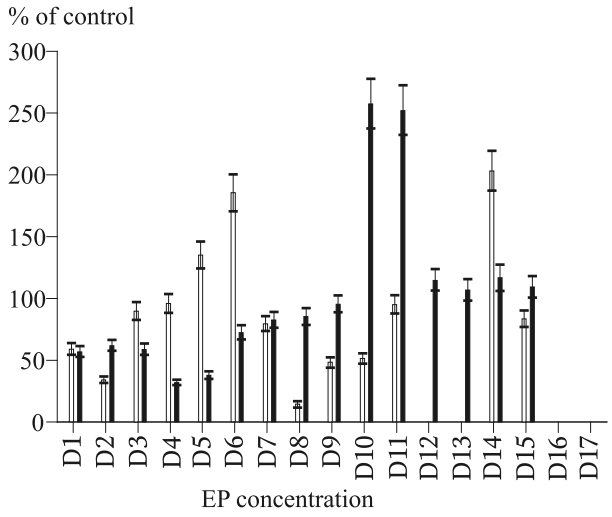

Fig. 1. Concentrations of IL-6 (light bars) and IL-8 (dark bars) in culture medium after MNL incubation with EP tincture. Here and in Figs. 2, 3: ordinate: cytokine concentration.

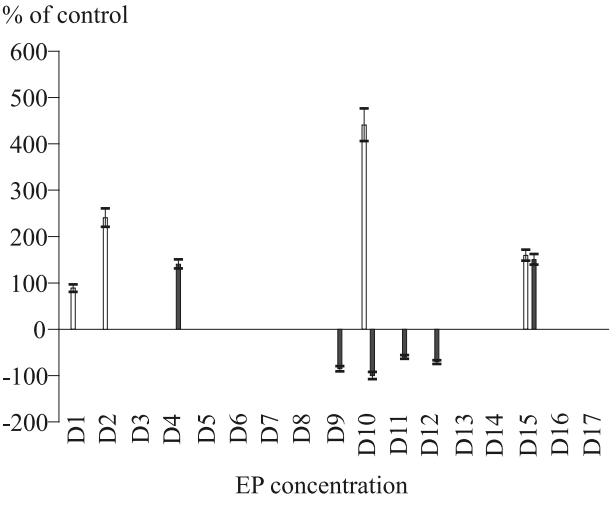

Fig. 2. Concentrations of IL-1β (light bars) and TNF-α (dark bars) in culture medium after MNL incubation with EP tincture.

Hence, the results indicate a selective dose-dependent immunomodulatory effect of EP tincture. By these data we can distinguish several most interesting (from clinical viewpoint) concentration ranges, at which the tincture exhibited certain profiles of cytokine-inducing activity.

The Th1 and Th2 cellular immunity components were stimulated under the effect of EP in D2-D4 concentrations. The concentration of anti-inflammatory IL-10 cytokine in that case was sufficiently high to prevent the probable development of hyperergic reactions caused by proinflammatory IL-1β, TNF-α, IL-6, and IL-8 (increase of vascular permeability, adhesion,

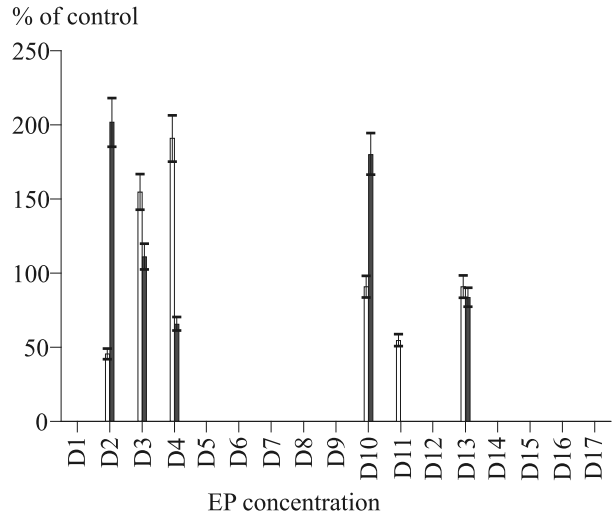

Fig. 3. Concentrations of IL-4 (light bars) and IL-10 (dark bars) in culture medium after MNL incubation with EP tincture.

etc.). This gave grounds to expect that these concentrations would be effective at the initial stages of inflammatory diseases, including infectious ones.

Echinacea tincture in D10 concentration also stimulated Th1 and Th2 cells, but the stimulatory effect on the production of proinflammatory IL-1β and IL-8 cytokines was more pronounced. Incubation with EP in a concentration of D15 led to selective stimulation of only Th1 immunity (release of IL-1β, IL-6, IL-8, and TNF-α). This profile of activity could be useful in cases when cellular immunity was to be stimulated (for example, in chronic diseases of infectious etiology). An opposite effect, predominant selective stimulation of anti-inflammatory IL-10 and IL-4 cytokines production, was observed after incubation with EP tincture in D13 concentration.

A moderate stimulatory effect exclusively on the production of pleiotropic IL-6 cytokine and IL-8 chemokine was observed after incubation of cells with EP in D5-D8 concentrations. These cytokines stimulated chemotaxis and phagocytosis, a fact clinically useful in inflammatory or infectious diseases liable to chronic transformation. Similar cytokine-inducing activity of EP was observed at its concentrations of D9, D11-D12, but the production of TNF-α was slightly inhibited in this case.

Hence, EP tincture in low concentrations induced selective production of pro- and anti-inflammatory cytokines. This recommends this drug in a wide range of concentrations for adequate correction of the immune system work aimed at amendment of the Th1/Th2 imbalance in various diseases.

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