Effects of Recombinant IL-4δ2 on Human Peripheral Blood Mononuclears

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In human cells, expression of IL-4 gene involves alternative mRNA splicing. IL-4δ2 splice variant is an antagonist of full-length IL-4 protein and blocks its effect on the functional activity of immunocompetent cells. The effect of recombinant IL-4δ2 on cytokine-producing activity of human peripheral blood mononuclear cells is shown for the first time.

Key Words: *alternative splicing; interleukin-4; recombinant protein*

IL-4 is one of the main immune response mediators. Alternative splicing of IL-4 mRNA yields a splice variant with deletion of exon 2: IL-4δ2 [1, 10]. The expression of IL-4δ2 mRNA is tissue-specific and was detected in human peripheral blood mononuclear cells (MNC), amygdala, bronchoalveolar lavage fluid, lungs, intestine, and thymus. The level of IL-4δ2 mRNA is changed in some diseases [2-7].

Recombinant IL-4δ2 protein (rIL-4δ2) binds to IL-4 receptors on cells with lower affinity than the full-length IL-4 protein and exhibits characteristics of IL-4 antagonist (blocks its effect on the functions of T and B cells and monocytes) [2,3]. For example, IL-4δ2 abolishes the effects of IL-4 on T cell proliferation, IgE synthesis and increase of its expression by CD23 B cells, as well as expression of cyclooxygenase and prostaglandin secretion by monocytes. Presumably, IL-4δ2 binds to IL-4 receptor without inducing signal transfer into the cell.

We evaluated biological activity of rIL-4δ2 and its effects on the expression of cytokine genes.

MATERIALS AND METHODS

Human rIL-4 (R&D Systems) and rIL-4δ2, a kind gift from Dr. L. R. Ptitsyn (Genetics Center) [8], were used.

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Blood samples from 20 donors were analyzed: proliferative activity of cells and intensity of IgE synthesis were evaluated in 14 samples, cytokine-synthesizing activity in 20 samples. Human peripheral blood MNC were isolated routinely in Ficoll-urografin density gradient. The cells were cultured in RPMI-1640 with 10% FCS, 2 mM L-glutamine, 100 mg/liter ampicillin, and 50 mg/liter gentamicin. The cells were incubated in 24- and 96-well plates at 37°C and 5% CO_2.

IgE in conditioned media of 14-day cultures were detected by IgE-IFA-Best-strip (Vector Company).

Proliferative activity of MNC was evaluated by the standard method by ^{3}H-thymidine incorporation in 72-h cultures.

Conditioned media of 48-h cultures were used for measuring cytokine content by the electrochemiluminescent method using an ORIGEN Analyzer (IGEN Inc.) [9].

The results were processed using analysis of dispersions and multiple comparison tests.

RESULTS

rIL-4δ2 effectively inhibited the stimulating effect of rIL-4 on proliferative activity of both intact cells and cells stimulated with concanavalin A or LPS. Addition of rIL-4δ2 alone to the culture in a concentration of up to 500 ng/ml did not modulate cell

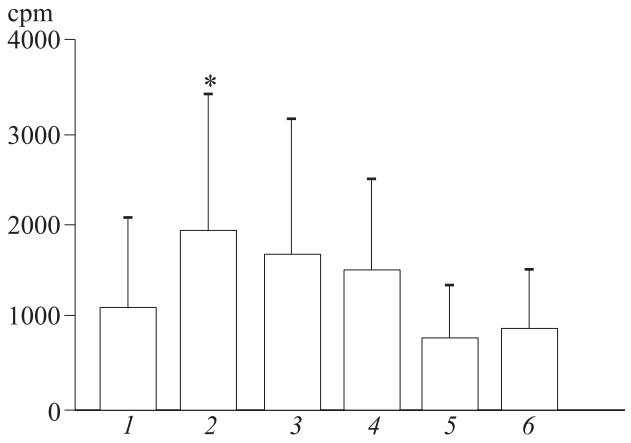

Fig. 1. Effect of rIL-4 and rIL-4δ2 on MNC proliferation. *1*) intact cells; *2*) 5 ng/ml rIL-4; *3*) 5 ng/ml rIL-4+100 ng/ml rIL-4δ2; *4*) 5 ng/ml rIL-4+500 ng/ml rIL-4δ2; *5*) 100 ng/ml rIL-4δ2; *6*) 500 ng/ml rIL-4δ2. Here and in Figs 2, 3: **p*<0.01 compared to cultures without recombinant cytokines.

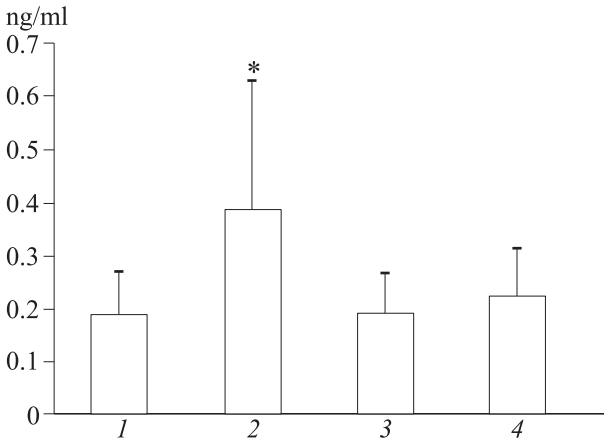

Fig. 2. Effect of rIL-4 and rIL-4δ2 on IgE synthesis. *1*) intact cells; *2*) 5 ng/ml rIL-4; *3*) 5 ng/ml rIL-4+500 ng/ml rIL-4δ2; *4*) 500 ng/ml rIL-4δ2.

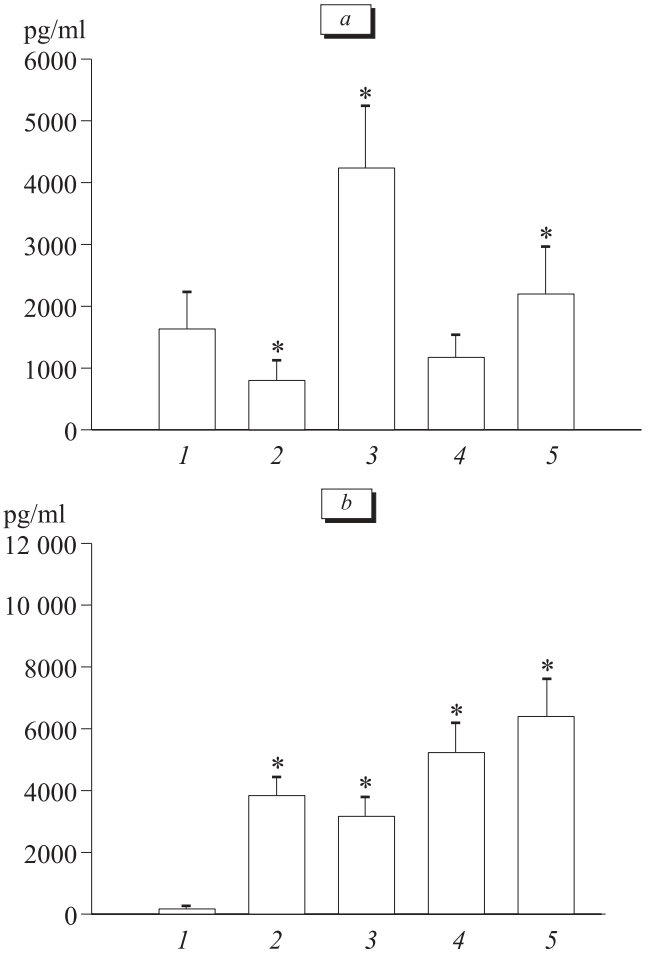

Fig. 3. Effect of rIL-4 and rIL-4δ2 on the cytokine-producing activity of MNC. *a*) effect on the production of IL-6; *b*) effect on the production of IL-4. *1*) intact cells; *2*) 5 ng/ml rIL-4; *3*) 500 ng/ml rIL-4δ2; *4*) 5 ng/ml rIL-4+100 ng/ml rIL-4δ2; *5*) 5 ng/ml rIL-4+500 ng/ml rIL-4δ2.

proliferation (Fig. 1). rIL-4δ2 inhibited the effect of IL-4 on IgE production in MNC cultures (Fig. 2). Our results are in line with published data and confirm the effects of IL-4 antagonist exhibited by rIL-4δ2 towards proliferative activity of T cells and synthesis of IgE by B cells [2,3]. We detected no appreciable effect of rIL-4d2 on the production of IL-1β, IL-2, IL-10, and IFN-γ. The level of IL-6 synthesis in MNC culture decreased significantly in the presence of rIL-4, while rIL-4δ2 abolished this inhibitory effect in a dose-dependent manner, which confirms the hypothesis that the only (main) function of IL-4δ2 isoform towards the immune system cells is the function of IL-4 antagonist [2]. However, rIL-4δ2 exhibited a pronounced stimulatory effect on IL-6 secretion (Fig. 3, *a*).

rIL-4δ2 (alone and in combination with rIL-4) effectively stimulated IL-4 synthesis by MNC. The full-length and alternative variants of recombinant protein added to the culture together potentiated the stimulatory effects of each other on the synthesis of IL-4 in a dose-dependent manner (Fig. 3, *b*).

Hence, rIL-4δ2 is characterized by opposite effects on the functions of human MNC. It effectively inhibits IL-4 effect on cell proliferation, IgE synthesis, and IL-6 production in human MNC cultures, but exhibits effects of its own on the expression of IL-6 and IL-4. rIL-4δ2 and rIL-4 stimulate the proliferation of fibroblasts and collagen synthesis by them [5]. These opposite effects can be due to different mechanisms of signal transduction from IL-4 and IL-4δ2, mediated by different receptor complexes.

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