Effect of Recombinant Interleukin-4 on Immunoglobulin Synthesis in a Culture of Mononuclear Cells Isolated from Human Peripheral Blood

L. K. Chelidze, Yu. S. Lebedin,* E. N. Tsytsikov, V. I. Pivnyuk, and R. G. Vasilov

UDC 612-112.-94

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 118, № 10, pp. 426-428, October, 1994
Original article submitted August 19, 1994

The effect of recombinant interleukin-4 on the synthesis of immunoglobulins is studied in a culture of mononuclear cells isolated from peripheral blood of healthy donors and patients with hay fever. It is shown that interleukin-4 stimulates production of IgE in cells of healthy donors, but not of hay fever patients, and does not affect the synthesis of other isotypes in either group. At the same time, increased cell proliferation under the influence of interleukin-4 was observed in cultured cells of both healthy donors and patients.

Key Words: *interleukin-4; IgE; hay fever*

Interleukin-4 (IL-4) formerly referred to as B cell stimulating factor, is a protein with a molecular weight of around 20 kD which is produced by activated T cells and mast cells [4]. This protein has a pleiotropic effect on different cell types, including T and B cells, monocytes, and mast cells. Culture medium conditioned by human T cells secreting IL-4 induces production of IgE by lymphocytes isolated from peripheral blood of healthy donors. Production of IL-4 is abolished by anti-IL-4 antibodies [3,5,7]. IL-4-induced synthesis of IgE in human peripheral blood mononuclear cells depends on the interaction between secondary cytokines produced during culturing. It has been shown that recombinant IL-4 (rIL-4)-induced synthesis of IgE is optimal in the presence of T cells, monocytes, IL-5, and IL-6 [9], although the role of IL-5 in the regulation of IgE synthesis remains unclear. We studied the effect of IL-4 on immunoglobulin (Ig) production by mononuclears isolated from peripheral blood of healthy donors and patients with hay fever.

Laboratory of Molecular Immunology, Institute of Biotechnology; *Institute of Pulmonology, Ministry of Health, Moscow. (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Recombinant IL-4 with a specific activity of 3×10^6 U/ml was kindly supplied by L. R. Ptitsin (Institute of Biotechnology, Moscow). Mononuclear cells were isolated from peripheral blood of four healthy donors (laboratory personnel, serum IgE content 50 U/ml, without signs of atopia) and four patients with hay fever during the winter, one week after a course of specific immunotherapy (blood IgE content 80-260 U/ml) by centrifugation on a Ficoll-Paque (Pharmacia) gradient. The cells were cultured as described [1] in 24-well flat-bottom plates (Cat. № 3524, Costar) at a concentration of 1 mln. cells/ml in Iskov's modified Dulbecco's medium (Gibco) supplemented with human insulin and transferrin, bovine serum albumin, fats (Gibco), and 10% inactivated fetal calf serum (Flow). Culturing was carried out in duplicates at 37°C in an atmosphere containing 5% CO_2. IL-4 was added to the culture medium to final concentrations of 0, 2, 10, 50, and

0007-4888/94/0010-1126$12.50

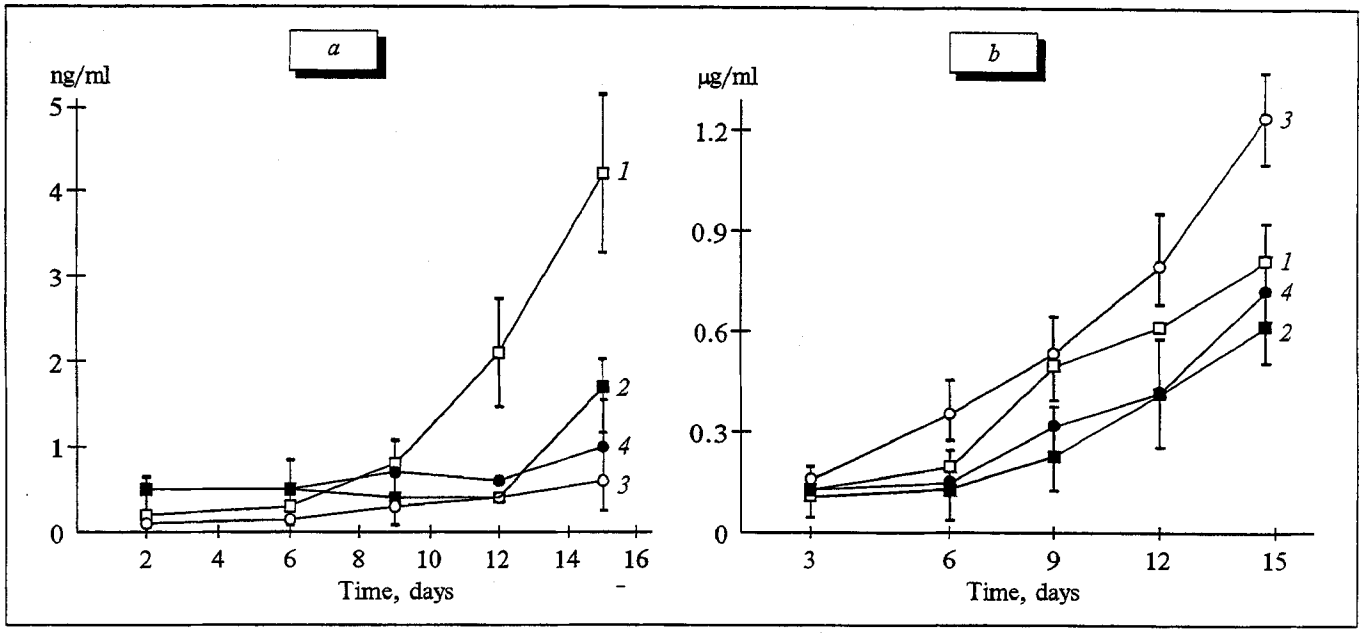

Fig. 1. Concentration of IgE (*a*) and IgG (*b*) in culture medium conditioned by peripheral blood mononuclear cells. *1*, *2*) culturing in the presence of 50 ng/ml rIL-4; *3*, *4*) culturing without rIL-4; *1*, *3*) healthy donors; *2*, *4*) patients with hay fever.

250 ng/ml. Culture medium was collected every third day during a 15-day period, centrifuged to separate cell detritus, and frozen at -20°C prior to analysis. Determination of IgE in supernatant was performed by solid-phase two-center immunoenzyme assay described elsewhere [8]. The same method was used to determine IgG, IgA, and IgM. Goat antibodies to IgG, IgA, or IgM (Sigma), respectively, were employed as binding antibodies, and sheep biotinilated antibodies to human Ig (Amersham) and horseradish peroxidase-streptavidin were used as developing agents. Cell proliferation was assessed by incorporation of ^{3}H-thymidine on day 15 of culturing.

RESULTS

Studies of the regulation of IgE synthesis in humans were for a long time hampered by difficulties in the choice of a culture system providing for synthesis of detectable amounts of IgE. Highly sensitive and specific methods have to be used to detect picogram amounts of IgE. Comparable amounts of preformed IgE at the initial stages of synthesis call for complex controlling systems.

Our test system [8] with a sensitivity of 100 pg/ml allows for the evaluation of IgE synthesis by peripheral blood mononuclear cells *in vitro*.

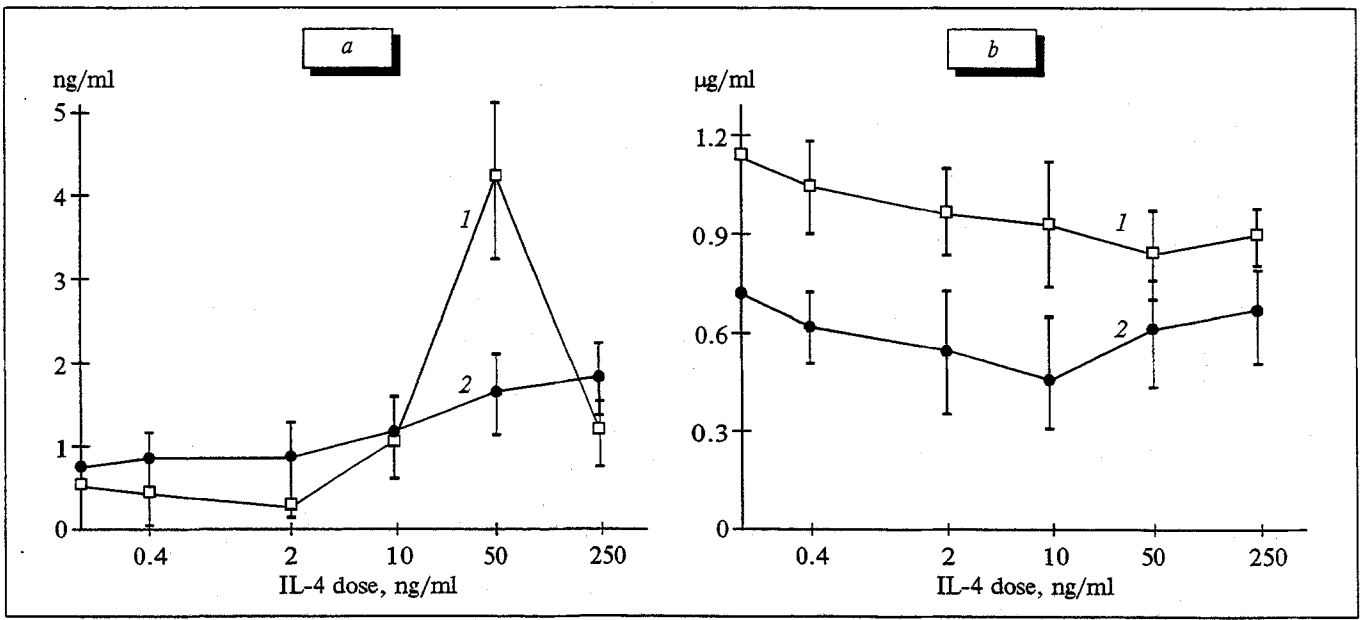

Fig. 2. Concentration of IgE (*a*) and IgG (*b*) in culture medium conditioned by human peripheral blood mononuclear cells as a function of the dose of rIL-4 (15th day of culturing). Here and in Fig. 3: *1*) healthy donors; *2*) patients with hay fever.

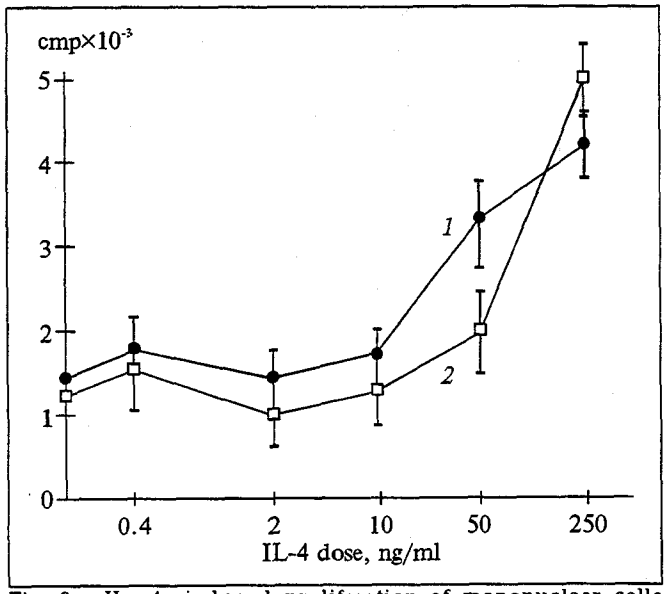

Fig. 3. IL-4-induced proliferation of mononuclear cells isolated from peripheral blood of healthy donors and patients with hay fever measured as incorporation of ^{3}H-thymidine on the 15th day of culturing.

We studied the *in vitro* time- and dose-dependence of IgE synthesis by mononuclear leukocytes from peripheral blood of healthy donors and patients with hay fever in the stage of remission. Prior to determination, supernatants (conditioned culture medium) were diluted 1:20 for IgG and IgA, 1:10 for IgM, and 1:2 for IgE.

Concentrations of Ig in blood of healthy donors and hay fever patients were determined every 3 days during a 15-day period. Within this time the IgG concentration increased from 0.1 to 2-5 ng/ml. The presence of 2-250 ng/ml IL-4 in the culture medium had no appreciable effect on IgG production. At the same time, there were no visible differences in the nature of IgG synthesis by the two groups (Figs. 1, *b*; 2, *b*). The concentrations of IgA and IgM during the period of culturing increased from 0.1 to 2-5 and from 0.1 to 0.5-1.0 ng/ml, respectively. The nature of their synthesis and its dependence on IL-4 were similar to those for IgG.

With IgE, the presence of IL-4 in the culture medium was absolutely essential for the production of detectable amounts of IgE by cells isolated from patients' and donors' blood. The IgE response varied from donor to donor; IgE was detected on day 9 of culturing (Fig. 1, *a*,) and its concentration was 1-8.5 ng/ml by the 15th day.

In healthy donors, the maximum stimulatory effect was observed at 50 ng/ml IL-4 (concentration range 10-250 ng/ml) (Fig. 2, *a*); an increase in the IL-4 concentration to 250 ng/ml weakened this effect. By contrast, in patients with hay fever the effect of IL-4 was less heterogeneous, and the "overdosage" phenomenon was absent. There was no correlation between spontaneous or IL-4-induced synthesis of IgE and other Ig isotypes.

The dependence of ^{3}H-thymidine incorporation on the IL-4 dose was similar in the two groups (Fig. 3).

In contrast to the observations of some researchers [3], we noted no increase in the IL-4-dependent IgE response *in vitro* either in healthy donors or in patients, which is consistent with the results reported by Claasen *et al.* [2]. Since the correlation between the intensity of IgE synthesis and cell proliferation was stronger in patients than in healthy donors, we assumed that in patients IL-4 acts predominantly as a proliferative signal, while in healthy persons its influence is more complex. It should be noted that we studied blood from patients after specific immunotherapy, which could have altered the ability of their cells to synthesize IgE *in vitro*.

There is controversy over the isotype-specific regulation of IgE synthesis by IL-4. It has been was reported that IL-4 stimulates synthesis of other Ig isotypes [6]. Our findings confirm the strict isotype-specific regulation of IgE synthesis by IL-4, the optimal concentration of IL-4 being 50 ng/ml. A further increase in the IL-4 concentration to 250 ng/ml lowered this effect (Fig. 2, *a*). Similar results were obtained by others [9,10].

Thus, we have demonstrated *in vitro* a specific IL-4-dependent synthesis of IgE (but not of other Ig isotypes) by mononuclear cells isolated from peripheral blood of healthy donors and patients with hay fever, as well as differences in the nature of IgE synthesis.

REFERENCES

1. J. L. Claasen, A. D. Levine, and R. H. Buckley, *J. Immunol. Meth.*, **126**, 213-222 (1990).
2. J. L. Claasen, A. D. Levine, and R. H. Buckley, *Pharmacia Allergy Research Foundation Award Book*, Uppsala (1989), pp. 19-23.
3. G. F. Del Prete, E. Maggi, P. Parronchi, *et al.*, *J. Immunol.*, **140**, 4193-4198 (1988).
4. M. Howard, J. Farrar, M. Hilfiker, *et al.*, *J. Exp. Med.*, **155**, 914-921 (1982).
5. H. H. Jabara, S. J. Ackerman, D. Vercelli, *et al.*, *J. Clin. Immunol.*, **8**, 437-445 (1988).
6. M. Lundgren, U. Persson, P. Larsson, *et al.*, *Europ. J. Immunol.*, **19**, 1311-1315 (1989).
7. J. Pene, F. Rousset, F. Briere, *et al.*, *J. Immunol.*, **141**, 1218-1225 (1988).
8. R. G. Vasilov and E. N. Tsitsikov, *Immunol. Lett.*, **26**, 283-284 (1990).
9. D. Vercelli, H. H. Jabara, K.-I. Arai, and R. S. Geha, *J. Exp. Med.*, **169**, 1295-1307 (1989).
10. X. D. Yang, A. L. De Weck, and B. D. Stadler, *Europ. J. Immunol.*, **18**, 1699-1704 (1988).