NEW BIOMEDICAL TECHNOLOGIES

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The Editorial Board of *Byulleten' Eksperimental'noi Biologii i Meditsiny* is launching a new section, entitled *"New Biomedical Technologies,"* in the hope that it will promote broader publication of papers in this important area of modern medical science. Contributions are most welcome.

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Relationship between the Expression of Surface Markers and Synthesis of Immunoglobulin E by Peripheral Blood Mononuclear cells under the Influence of Recombinant Interleukin-4

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The relationship between interleukin-4-induced synthesis of immunoglobulin E (IgE) by peripheral blood mononuclear cells of healthy donors and the expression of T- and B-cell surface differentiating markers is studied. It is demonstrated that the high level of IgE response on day 10 of culturing correlates with a higher level of expression of the T-cell activation markers HLA-DR and CD25 and a low level of expression of the low-affinity IgE receptor CD23 on the surface of B cells. Activation of T cells is interleukin-4-independent, since it occurs to an equal degree when these cells are cultured without interleukin-4.

Key Words: *interleukin-4, surface antigens, immunoglobulin E*

Interleukin-4 (IL-4) was originally described as a B-cell stimulatory factor [5]. It is known that supernatants of human T-cell clones secreting IL-4 induce biosynthesis of IgE by lymphocytes isolated from peripheral blood of healthy donors. This ability of IL-4 is completely inhibited by anti-IgE antibodies [4,6,10]. In addition, IL-4 can induce or stimulate the expression of a number of B-cell surface molecules [2,8,11].

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Both the lymphokines and cell-to-cell contacts are necessary for the activation and differentiation of B cells [13]. It has recently been demonstrated that the interaction between CD40, a B cell surface antigen, and its ligand (counter-receptor) on the plasma membrane of T cells plays a crucial role in contact activation of B cells [15].

We studied the relationship between IgE synthesis which was induced by recombinant IL-4 (rIL-4) in mononuclear cells isolated from the blood of healthy donors and expression of the surface differentiating markers of T and B cells.

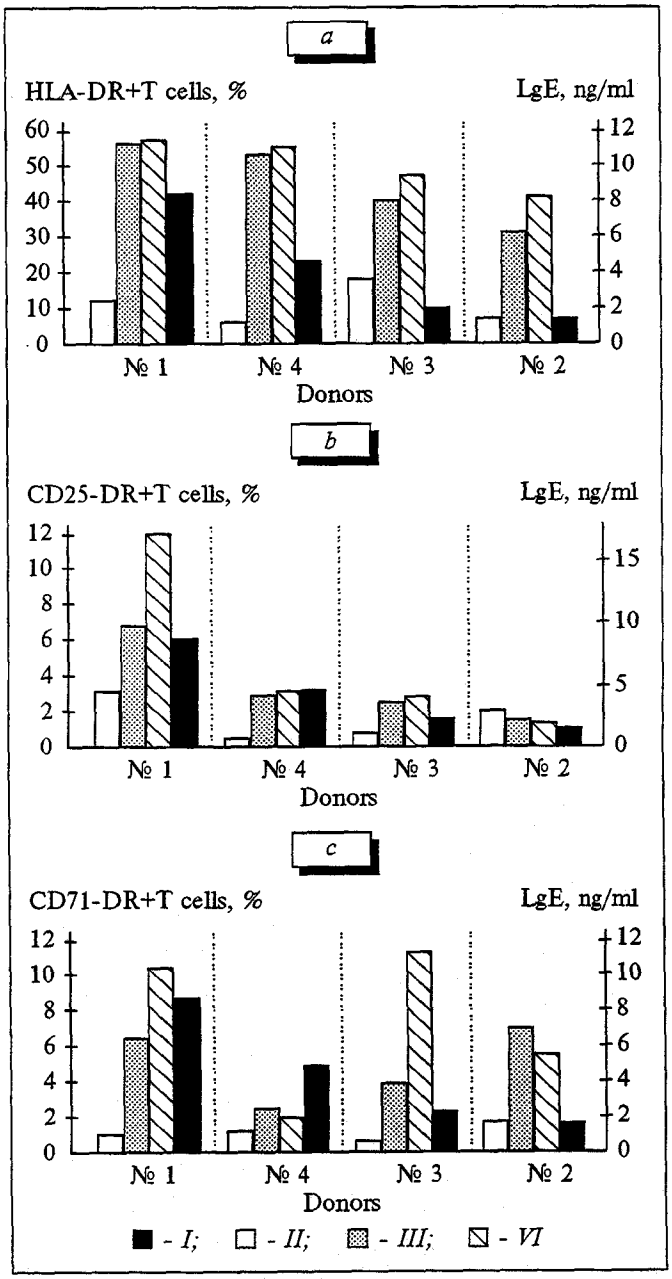

Fig. 1. Relationship between phenotypic changes in T cells and IgE synthesis in a culture of mononuclear cells isolated from peripheral blood of healthy donors. Level of IL-4-induced IgE synthesis (*I*). The percentage of T cells expressing HLA-DR (*a*), CD25 (*b*), or CD71 (*c*) was determined prior to culturing (*II*) or on day 10 of culturing without (*III*) and with (*IV*) 50 ng/ml IL-4.

MATERIALS AND METHODS

Recombinant IL-4 (specific activity 3×10^6 U/ml) was kindly supplied by Dr. L. R. Ptitsyn (Institute of Biotechnology, Moscow). Mononuclear cells were isolated from peripheral blood of four healthy donors (laboratory personnel, IgE serum content 50 U/ml, no signs of atopia) and of four patients with hay fever during the winter, one week after a course of specific immunotherapy (blood IgE content 80-260 U/ml) by the method described in the paper of L. K. Chelidze *et al.* published in this issue. Determination of IgE in supernatants was performed by solid-phase two-center immunoenzyme assay [12].

Expression of surface antigens in intact cells after 10 days of culturing with and without IL-4 (50 ng/ml) was studied using a FACScan flow cytofluorimeter (Becton Dickinson). Monoclonal antibodies to CD3, CD4, CD8, CD19, CD20, CD23, CD25, and CD71 (Becton Dickinson) and anti-CD23 (clone HD50) antibodies (a generous gift of Dr. G. Moldenhauer, Heidelberg, Germany) were used. When necessary, the antibodies were employed as conjugates with FITC, phytoerythrin (PE), or biotin. In the case of biotinilated antibodies streptavidin-PE (Calbiochem) served as the second agent. The fluorescence parameters were recorded using a logarithmic scale after setting up a window for falling and scattering light in order to separate living and dead cells.

RESULTS

The effect of IL-4 on the synthesis of Ig by mononuclear cells isolated from healthy donor blood is described in the paper of Chelidze *et al.* Specific rIL-4-dependent (in contrast to other Ig) synthesis of IgE was demonstrated *in vitro* in a culture of mononuclear cells isolated from peripheral blood of healthy donors. It was reported that the effect of IL-4 on IgE synthesis varies from donor to donor against the background of a generally high IgE concentration.

Variability of IgE synthesis by cells of healthy donors has also been shown by other researchers [9]. It was suggested that the intensity of the IgE response to IL-4 can be determined by the activation of B and T cells *in vivo* [9]. Heterogeneity of the cell population (particularly in the percent-

TABLE 1. Phenotypic Changes in Healthy Donor B cells 10 Days after Culturing without (−) and with (+) 50 ng/ml IL−4

Surface marker	IL-4	№ of Donor			
		1	2	3	4
B cells	−	0.29	0.82	0.54	1.19
($CD20^+$)	+	0.59	0.81	0.95	0.71
$CD23^+$	−	0.51	0.80	0.94	1.19
B−cells	+	0.39	0.55	0.89	1.54
$CD71^+$	−	0.47	0.64	3.00	2.83
B−cells	+	1.08	1.27	5.38	3.80

Note. The IgE concentration (ng/ml) is 8.5 in group 1, 4.6 in group 2, 2.5 in group 3, and 1.0 in group 4.

age of B cells) may be another cause of this phenomenon. Prior to culturing, we revealed no differences in the composition of peripheral blood mononuclear cells from strongly and weakly responding donors. There was no relationship between the composition of the subpopulations of mononuclear cells freshly isolated from peripheral blood of healthy donors and the IL-4-induced IgE response *in vitro* (results not shown).

B cells were determined as CD20-carrying cells; their percentage ranged from 10.5 to 27.8. The percentage of CD5-positive cells in the B-cell population varied from 4.8 to 10.4, the percentage of $CD71^+$ cells from 3.5 to 12.4, and that of $CD23^+$ cells from 17 to 56.

In order to assess phenotypic changes we calculated the following coefficient:

$$\frac{\%\text{ of positive cells on day 10 of culturing}}{\%\text{ of positive cells in freshly prepared suspension of peripheral blood mononuclear cells}}.$$

A study of changes in the phenotype of B cells upon spontaneous culturing and culturing in the presence of IL-4 (50 ng/ml, optimum for IgE synthesis) showed that IL-4 has no effect on the composition of B-cell subpopulations, with the exception of a slight increase in the number of CD71- and CD5-positive cells. As is well known, IL-4 markedly enhances the expression of CD23 (a low-affinity IgE receptor), which is determined at the RNA level after just 4 h of culturing [1].

The maximum expression of surface markers was observed 2 or 3 days after the addition of exogenous IL-4 [3]. However, on day 10 of culturing the expression of CD23 was not increased (Table 1), which may be due to autolysis of CD23 molecules [7]. Interestingly, on day 10 of culturing the percentage of CD23-carrying B cells negatively correlated with the IgE response: the highest percentage of such cells was recorded in weakly responding donors. These findings agree with published data to the effect that surface CD23 can participate in regulating the synthesis of its ligand according to the negative feedback principle [3].

T cells were phenotyped by the T-cell marker CD3, the subpopulation markers CD4 and CD8, and the activation markers HLA-DR, CD71, and CD25. It was found that during culturing the number of T cells carrying the activation markers HLA-DR and CD25 (the IL-2 receptor) increases, this strongly correlating with the level of the IgE response (Fig. 1 *a, b*). However, this potentiation of T-cell activation did not result from the presence of IL-4 in the culture medium, since incubation without rIL-4 led to a similar increase in the expression of these markers (Fig. 1, *a, b*).

The slight increase in the expression of the surface marker CD71 (transferrin receptor) did not correlate with the presence of IL-4 or with the level of the IgE response (Fig. 1, *c*).

Freshly isolated T cells carried small amounts of CD23 (Fig. 2, *a*). A small subpopulation of T cells expressed CD23 while in spontaneous or IL-4-induced culture. This expression was not associated with IgE synthesis, since it was the same or lower on T cells treated with IL-4 compared with spontaneous culture (Fig. 2, *a*).

Our results indicate that activation of T cells is necessary for efficient synthesis of IgE induced by IL-4. Presumably, this is due to the increased surface expression of the ligand CD40 upon activation of T cells [15]. However, this assumption requires experimental confirmation. The degree of activation varied from donor to donor and did not depend on the presence of IL-4 in the culture medium. We assume this variation to be a consequence of different effects of some components of the culture medium on cells of individual donors.

In addition, the different intensity of the IgE response negatively correlates with the "late" expression of CD23 on the surface of B cells, which is consistent with the inhibitory effect of membrane-bound CD23 on induction of IgE synthesis [14].

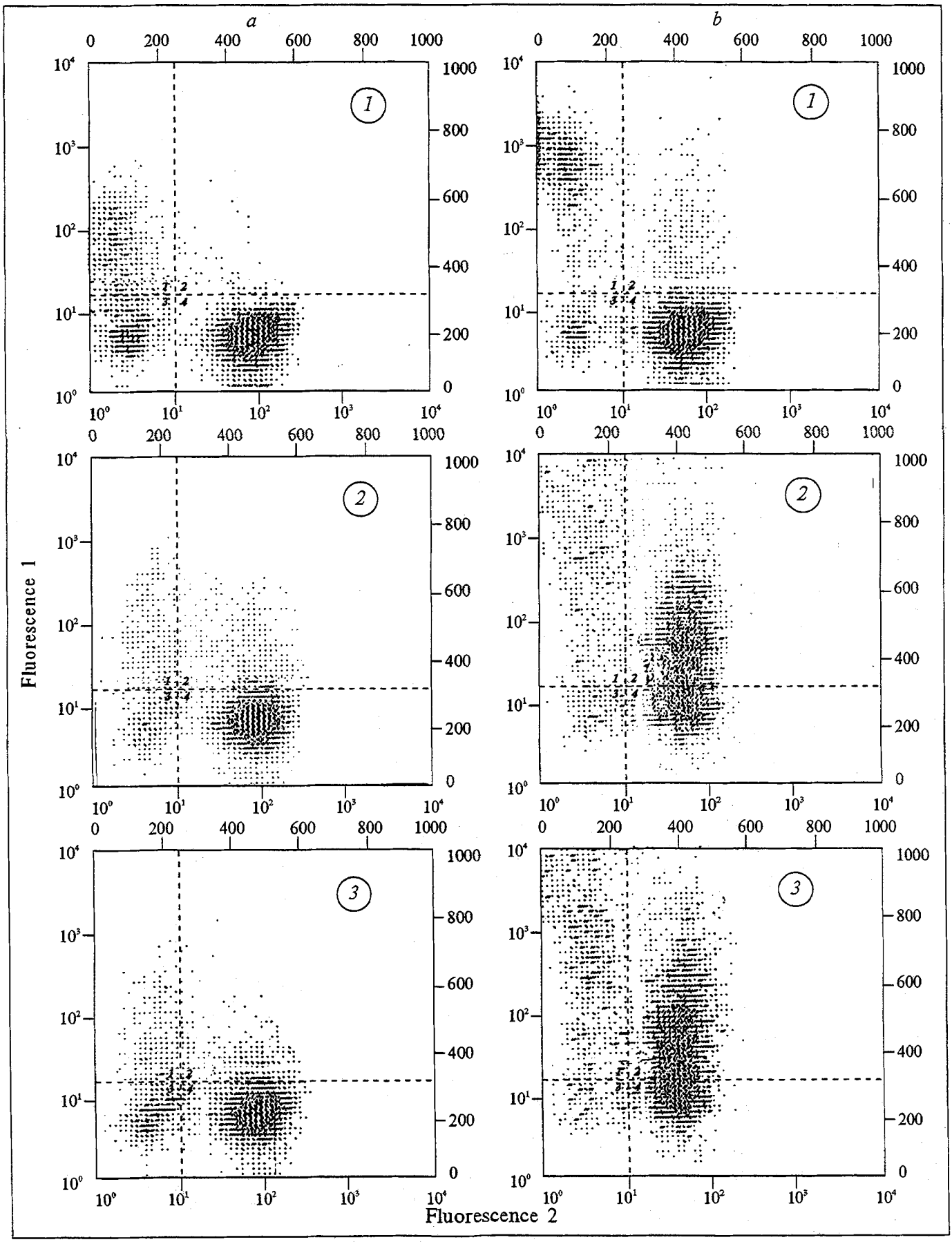
a
b
1
2
3
Fluorescence 1
Fluorescence 2

Fig. 2. Effect of a 10-day incubation in Iskov's modified Dulbecco medium on the phenotype of T cells. *1*) freshly prepared suspension of mononuclear cells; *2*) incubation without IL-4; *3*) incubation in the presence of 50 ng/ml Il-4. Fluorescence 1: CD3-FITC; Fluorescence 2: *a*) CD23-PE (Leu 20, Becton Dickinson); *b*) HLA-DR-PE.

Our results show that although IL-4 plays a crucial role in the induction of IgE biosynthesis, the intensity of the IgE response is determined by both the degree of T-cell activation and some other factors that are IL-4-independent.

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