Dynamics of the Expression of Activation Marker Complex During Polyclonal Activation of Human Peripheral Blood CD4+ and CD8+ Lymphocytes *In Vitro*

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A study of the expression of early and late activation markers on the surface of regulatory subsets of human peripheral blood T lymphocytes revealed marked differences between patients with bronchopulmonary pathology and healthy donors in the dynamics of CD25, CD71, and HLA-DR expression. The results are of significance for the evaluation of both the activation state of the cell and its functional potential in the realization of the immune response.

Key Words: *activation markers; dynamics; CD4+ and CD8+ lymphocytes*

One of the features distinguishing activated T lymphocytes from resting T cells is the appearance of various membrane glycoprotein markers that are synthesized de novo in the course of cell activation. Some of the markers, such as receptors of insulin, transferrin (CD71), interleukin-2 (CD25), CD69, 4F2, and EA-1, appear at the early stages preceding DNA synthesis, while other antigens, including HLA-DR, T10, Ta1, VLA1, and TLiSA1, appear later [3,8,9].

The content of activated lymphocytes in human peripheral blood has been studied in depth. Earlier we showed a significant increase in the number of activated lymphocytes belonging to the regulatory subsets (CD4, CD8) in patients with infectious-atopic bronchial asthma and tuberculosis as compared to healthy donors [1,2]. However the potential of such lymphocytes to be activated *in vitro* under conditions of a functional load, such as an antigen or mitogen, remains poorly understood.

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The goal of the present work was to study the dynamics of the early (CD25, CD71) and late (HLA-DR) activation markers on the surface of CD4+ and CD8+ cells using the model of phytohemagglutinin (PHA)-induced lymphocyte proliferation.

MATERIALS AND METHODS

Mononuclear cells (MNC) of human peripheral venous blood were tested. Blood was taken aseptically from the ulnar vein, in the morning, on an empty stomach. The control group consisted of 51 clinically healthy donors, 32 men and 19 women, aged 17 to 30 years. The group of patients included cases of infectious-atopic asthma at the stage of exacerbation and cases of primarily diagnosed tuberculosis. Eighteen patients with bronchial asthma (7 men and 11 women) aged 25 to 55 years were examined. The patients were under hospital treatment at the Institute of Immunology, Russian Ministry of Public Health. Sensitization to streptococcal and staphylococcal antigens was noted in the anamnesis. We also examined 35 patients with

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tuberculosis who underwent hospital treatment at the Department of Tuberculosis, Russian State Medical University. This group included 20 men and 15 women aged 23 to 45 years. The immunological testing was performed before the start of specific treatment.

MNC were isolated after Boyum [7]. The concentration of MNC was adjusted to 1 mln/ml. The culture medium was RPMI-1640 (Institute of Poliomyelitis and Viral Encephalitis, Russian Academy of Medical Sciences, Moscow) supplemented with 10% fetal calf serum (Flow Lab.), 2 mM L-glutamine (Flow Lab.), and 50 μg/ml gentamicin (Pharmachim). To 1 ml of MNC suspension (1 mln cells) a solution of PHA-P (Difco) was added to a final concentration of 5 μg/ml. The cells were cultured in the presence of PHA in glass tubes at 37°C for 24, 48, and 72 h.

Before immunophenotyping the MNC from experimental and control (without PHA) cultures were centrifuged on a Ficoll-Verographin gradient in order to discard inviable cells. The immunophenotyping was performed using direct double-stain immunofluorescence [10]. The phenotype of T lymphocytes was determined using the corresponding monoclonal antibodies (MAb) conjugated with phycoerythrin (PE) or fluorescein isothiocyanate (FITC) (MAb, Becton Dickinson). Five thousand viable cells were analyzed in each sample. Cell viability was at least 95% as judged from propidium iodide staining. The cells were analyzed in FAC-SCAN flow cytofluorimeter (Beckton Dickinson). The differences between mean values were assessed using Student's *t* test.

RESULTS

The dynamics of CD25 expression on CD4+ and CD8+ lymphocytes in the examined groups is presented in Fig. 1. The content of both CD4+CD25+ and CD8+CD25+ cells progressively increased during culturing in the presence of PHA, reaching the maximum at 72 h. As is seen from Fig. 2, the shape of the curve reflecting the dynamics of CD71 expression on CD4+ and CD8+ cells resembles the dynamics of CD25 expression. However, the number of CD4+CD71+ and CD8+CD71+ cells was considerably lower than that of cells expressing CD25. This may be due to the fact that in the course of activation there a threshold level of CD25 molecule expression is reached, which triggers the expression of the CD71 marker [6]. Another important fact that came out in the experiments is that the pattern (shape of the curve) of the dynamics of CD25 and CD71 markers on

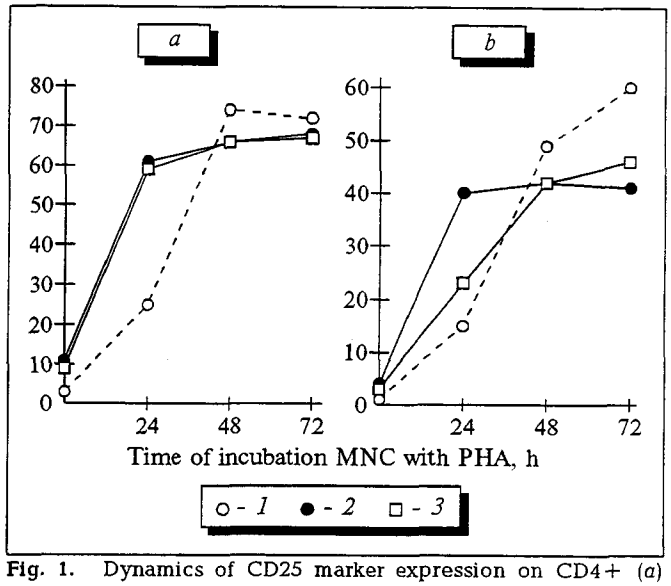

Fig. 1. Dynamics of CD25 marker expression on CD4+ (*a*) and CD8+ (*b*) cells in the course of treatment with PHA. Ordinate: percentage of CD4+CD25+ and CD8+CD25+ lymphocytes, respectively. Here and in Fig. 2: *1*) healthy donors; *2*) tuberculosis patients; *3*) asthma patients.

both CD4+ and CD8+ was fundamentally similar. This led us to conclude that the activation mechanisms in CD4+ and CD8+ lymphocyte subpopulations induced by culturing with a polyclonal mitogen are basically identical.

The dynamics of CD25 marker expression on CD4+ and CD8+ lymphocytes from patients and healthy donors substantially differed: after 24 h of culturing a reliable increase in the content of CD4+CD25+ and CD8+CD25+ lymphocytes was recorded in all groups of patients (Fig. 1); at 48 h the number of CD25+ cells was equal to that in the control group; however, after 72 h of culturing with PHA the number of CD8+CD25+ cells in the patient group remaind at the same level, while a progressive increase of this parameter was observed in the donor lymphocytes. In contrast, the number of CD4+CD25+ cells at this time of culturing was similar in both groups.

Lymphocytes of patients and donors also differed in the dynamics of CD71 expression (Fig. 2). After 24 h of culturing in the presence of

TABLE 1. MAb used in Lymphocyte Phenotyping

Name	Differentiation cluster	Specificity
Anti-LEU-3a	CD4	T helpers/inducers
Anti-LEU-2a	CD8	T suppressors/killers
Anti-IL-2R	CD25	Interleukin-2 receptor
Anti-TFR	CD71	Transferrin receptor
Anti-HLA-DR		HLA-DR antigens

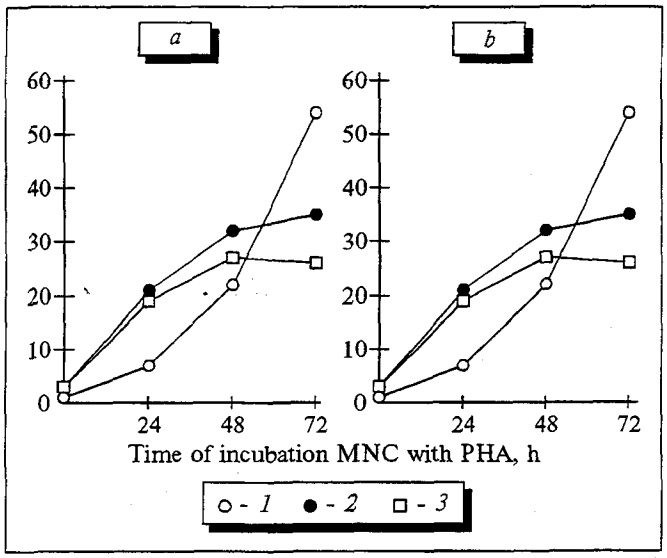

Fig. 2. Dynamics of CD71 marker expression on CD4+ (*a*) and CD8+ (*b*) cells in the course of treatment with PHA. Ordinate: percentage of CD4+CD71+ and CD8+CD71+ lymphocytes, respectively.

PHA the numbers of both CD4+CD71+ and CD8+CD71+ cells in the patient population considerably exceeded the analogous indexes in the donor population. At 48 h the number of activated cells population was similar in both populations. After 72 h of culturing with PHA the number of activated lymphocytes in the patient population remained at the same level, while the content of CD4+CD71+ and CD8+CD71+ cells in the donor population at that time substantially increased and reached the maximum level.

Concerning the expression of the HLA-DR molecule, we found a significant increase in HLA-DR+ content in the untreated cells in the patients' peripheral blood. Therefore, we considered the dynamics of increment in CD4+DR+ and CD8+DR+ resulting from PHA treatment as the most informative test. The value of the increase was defined as the difference between the experimental (PHA treatment) and initial DR+ content. The dynamics of CD4+DR+ and CD8+DR+ cell increment in the patients and healthy donors proved to be drastically different (Table 2). The maximum increase in the content of these cells was observed after 24-48 h of culturing in the presence of PHA; however, at 72 h the increment of CD4+ as well as of CD8+ cells bearing HLA-DR was reliably lower than that recorded in the donor population.

Since an increase in the content of lymphocytes expressing CD25, CD71, and HLA-DRwas recorded in the early periods of culturing in all groups of patients, it is logical to assume that the CD4+ and CD8+ subsets of patients' T cells possess a lowered threshold of generation and the spontaneous level of interleukin-2 production by the native cells is sufficient for their proliferation [4]. These lymphocytes may be memory cells, and hence one could speculate about a preliminary state of functional activation of CD4+ and CD8+ cells *in vivo* in patients with bronchopulmonary diseases [11]. At the same time, the expression of CD25, CD71, and HLA-DR markers on the CD4+ and CD8+ cells of patients recorded at 72 h of culturing in the presence of PHA failed to attain the indices of donor cells; moreover, the patterns of marker expression differed significantly. The disturbance of polyclonal activation of CD4+ cells was demonstrated only regarding CD71 marker expression, while in the case of CD8+ cells the alteration could be seen concerning both CD71 and CD25 marker expression. Therefore, we assumed that the changes of the CD8+ cell functional potential were of a secondary nature as compared to CD4+ cells.

Summing up the data, we may conclude that the analysis of the expression of activation markers on CD4+ and CD8+ lymphocytes is of great importance for evaluating the activation state of a given cell and makes it possible to judge its functional potential in the realization of the immune response.

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TABLE 2. Dynamics of Increase of CD4+DR+ and CD8+DR+ Lymphocytes in the Course of Culturing in the Presence of PHA

Differentiation cluster	Period, h	Donors (n=13)	Tuberculosis patients (n=15)	Asthma patients (n=11)
CD4+DR+	24	3.6±0.4	22.4±3.8	17.0±1.5
	48	10.0±2.4	34.6±4.9	29.9±5.2
	72	42.3±3.4	23.2±2.3	24.0±3.6
CD8+DR+	24	5.6±1.0	20.0±3.4	21.9±5.6
	48	13.4±2.4	25.5±4.2	25.0±4.3
	72	36.0±4.8	14.8±3.2	13.2±4.0

Note. The figures represent differences between the percentage of marker-bearing cells following 24, 48, and 72 h of treatment with PHA and the initial percentage of cells of similar phenotype in the peripheral blood of the same individual. All indices in the patient group reliably (p<0.05) differ from those in the control group (healthy donors). n – the number of individuals tested.

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MICROBIOLOGY AND IMMUNOLOGY

Variants of Secondary Immune Response in CBA and C57Bl/6 Mice

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The manifestation of a secondary immune response to intraperitoneal and subcutaneous injection of sheep red cells in various doses was studied in CBA and C57Bl/6 mice. The parameters under study were the delayed-type hypersensitivity reaction and antibody production assessed from the levels of antibody-producing cells of classes M and G in the lymph node and spleen. The manifestation and correlations between delayed-type hypersensitivity and antibody production were found to depend on the route of antigen administration and its first immunizing dose, interval between the two immunizations, and genetic control of the immune response.

Key Words: *antibody production; delayed-type hypersensitivity; secondary immune response*

Different types of immune response in CBA and C57Bl/6 mice are determined by the priority of development of antibody production (ABP) or delayed-type hypersensitivity (DTH) in response to suboptimal doses of sheep red cells (SRC) in the local immune response and to suboptimal and optimal doses thereof in the generalized response [1]. These differences increase for immunization 7 days before with SRC doses subthreshold for ABP but optimal for the DTH reaction [4]. At the same time, the data reflect only one variant of secondary response.

The purpose of this research was to evaluate different variants of secondary immune response of CBA and C57Bl/6 mice to SRC, namely, to use various doses of SRC for primary immunization at different intervals between the two immunizations.

MATERIALS AND METHODS

Four hundred CBA and C57Bl/6 mice weighing 18 to 22 g were used. Primary immunization

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