CHANGES IN DETECTABILITY OF HLA ANTIGENS ON LYMPHOCYTE SUBPOPULATIONS UNDER THE INFLUENCE OF MITOGENS AND CORTICOSTEROIDS

A. Yu. Volgin and L. A. Pevnitskii

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The writers showed previously [1] that the sensitivity of human lymphocytes to the specific action of HLA antisera depends not only on the properties of the sera, but also on the functional state of the lymphocytes themselves. Culture of lymphocytes *in vitro* and, in particular, their stimulation by mitogens, was found to increase the number of reacting antisera revealing a definite HLA antigen. Treatment of the cells with active metabolites of cyclophosphamide prevented this effect.

In the investigation described below changes in the detectability of HLA antigens of the A, B, and DR series on T and B-cell populations, with particular reference to the effect of mitogens and corticosteroids, were studied.

EXPERIMENTAL METHOD

Lymphocytes were isolated in a Ficoll-Verografin gradient and cultured for 72 h [1]. Phytohemagglutinin (PHA) in a dose of 10 μg/ml, and pokeweed mitogen (PWM), activator of B lymphocytes, in a dose of 30 μg/ml, were used as mitogens. Dexamethasone (DM), a synthetic analog of the corticosteroids, was added to the culture in a dose of 5×10^{-7} mole/ml. The lymphocytes were separated into populations enriched with T and B cells respectively on a Ficoll-Verografin gradient, using the formation of spontaneous rosettes by T cells with sheep's red blood cells treated with papain [6]. The content of T and B cells was determined with the aid of anti-T- and anti-B-sera. Populations enriched with B cells contained on average 80% of B cells, and those enriched with T cells contained 94% of T cells. Histocompatibility antigens were determined by the standard lymphocytotoxic test [8], using kits of typing sera for the identification of three antigens of the HLA-A and HLA-B series and eight antigens of the HLA-DR series. The degrees of detectability of the various HLA antigens was assessed by the number of antisera giving cytotoxic reactions with cells carrying this antigen, and the degree of cytotoxicity of the sera, expressed in plus signs. If there were 25% of dead cells the intensity of the reaction was described as +, 25-50% as ++, 50-75% as +++, and 75-100% as ++++. Since the cytotoxicity of antisera reacting with a given antigen differed from the beginning, an index of mean cytotoxicity was calculated as the quotient obtained by dividing the total number of plus signs expressing the intensity of reactions of the test sera by the number of antisera giving these reactions. To determine differences in the detectability of the HLA antigens, the mean cytotoxicity of each serum in the reaction with the given antigen was compared before culture of the cells and after they had been exposed to various factors. The significance of differences in the parameters obtained was determined by the criterion of signs [2].

EXPERIMENTAL RESULTS

Data on the detectability of HLA antigens of the A and B series on subpopulations of immunocompetent cells are given in Table 1. Even before culture the T cells reacted with a larger number of antisera and with higher intensity than B cells. After culture this rule still held good. For instance, before culture one HLA-A antigen was detected on the T cells on average by 1.7 sera, compared with 2.8 after culture. The corresponding number for B cells was 0.8 serum before culture and 1.4 sera after culture. Differences in the detectability of

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TABLE 1. Effects of Culture, Mitogenic Stimulation, and DM on Detectability of HLA Antigens on Lymphocyte Subpopulations

Cells	Before culture		After culture		DM		PHA		PHA + DM		PWM		PWM + DM	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Lymphocytes before fractionation	1,1	0,5	1,8	1,4	0,2	0,5	2,5	1,8	0,5	0,2	2,9	1,5	—	—
T cells	1,7	1,5	2,8	2,2	1,0	1,2	3,1	2,5	1,5	1,5	2,6	2,2	0.8	1,0
B cells	0,8	0,5	1,4	1,0	0,8	0	1,6	1,0	0,5	1,0	1,5	1,2	0	0,5

Legend. Mean number of antisera effecting one HLA-A antigen (A) and one HLA-B antigen (B) shown.

TABLE 2. Effect of Mitogens and Corticosteroids on Proliferation of Immunocompetent Cells

Mitogen	Lymphocytes	Lymphocytes + DM	T cells	T cells + DM	B cells	B cells + DM
Control	1,0	1,05	1,0	0,99	1,0	1,5
PHA	3,6	1,4	9,5	3,2	1,3	1,3
PWM	2,6	2,5	4,8	3,7	2,1	2,2

Legend. Values of IS shown: ratio of incorporation of ^{3}H-thymidine by cells after stimulation to its incorporation by cells without stimulation.

TABLE 3. Reaction of HLA Antisera with T Cells after Culture and Treatment with PHA

Anti-HLA-A-sera	T cells before culture	T cells after culture	T cells + PHA
Anti-A1	—	+++	+++
Anti-A1	+	+++	++++
Anti-A1 + A9	++++	++++	+++
Anti-A9	+	++++	++++
Anti-A23	—	+++	++++
Anti-A24	—	++	++++
Anti-A2	—	—	++
Anti-A3	—	—	++

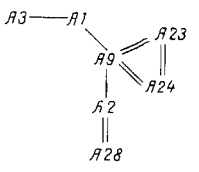

Fig. 1. Scheme of cross reactions in system of HLA antigens of series A [4]. Single line indicates weak reaction, double line strong reaction.

antigens on the T and B cells were significant ($P < 0.01$). The same tendency also was observed in relation to cross-reacting sera.

Data on the effect of mitogens on detectability of HLA antigens on T and B cells are also given in Table 1. They show that the increase in the number of reacting antisera during stimulation of the cells by mitogens took place chiefly on account of the T-cell population. The increase in the number of reactions of antisera with B cells was not statistically significant. Cross-reacting sera also reacted more strongly with T than with B cells.

The increase in the number of antisera reacting with T cells coincided with the greater proliferative activity of these cells under the influence of mitogens compared with B cells (Table 2). For instance, whereas the index of stimulation (IS) of T cells under the influence

of PHA was 9.5 and PWM 4.8, stimulation of B cells was observed only in response to PWM (IS = 2.1).

An example of the increased resolving power of the cytotoxic test during cell culture is given by the data in Table 3. Before culture it was impossible to identify the cell phenotype as regards HLA antigens sufficiently accurately. The reaction of the antisera which was observed could also have happened had HLA-A9 and HLA-A1 been present either singly or together (Fig. 1). On titration of T cells after culture, it could be confidently stated that antigen HLA-A9 was present on the cells, for cross reactions of antisera with HLA-A23 and HLA-24 antigens were given only by serum against antigen HLA-A9; the presence of HLA-A1 antigen can also be postulated because virtually all anti-HLA-A1 sera reacted completely with T cells, and the cross reactions between HLA-A1 and HLA-A9 were not as intensive (Fig. 1). Typing the T cells after their stimulation by PHA directly proved the presence of HLA-A1 antigen, for antiserum against HLA-A3 could give cross reactions with HLA-A1 but not with HLA-A9.

Treatment of lymphocytes with DM in a dose of 5×10^{-7} mole/ml did not kill the cells but considerably reduced the ability of lymphocytes and T cells to proliferate under the influence of mitogens (Table 2). Simultaneously with this, treatment with DM completely abolished the effect of an increase in the number of HLA antisera reacting with all cell populations both during culture and on addition of mitogens to the cells (Table 1).

Treatment of subpopulations of cells by mitogens modified detectability not only of antigens of the HLA-A and HLA-B series, but also of the HLA-DR series. One HLA-DR antigen was detected on B cells isolated from peripheral blood on average by 2.7 sera. Practically no HLA-DR antigens were detected on the T cells. Treatment of B cells with PWM reduced ($P < 0.01$) the number of reacting antisera from 2.7 to 1.0 per HLA-DR antigen. The T cell population stimulated by PHA, on the other hand, acquired the ability to react with anti-HLA-DR-sera: one HLA-DR-antigen was detected by 3.3 antisera ($P < 0.01$).

The data obtained on detectability of DR antigens on T cells agree with the results of investigations which showed the appearance of DR antigens on stimulated T cells [5, 9]. The reduction in detectability of DR antigens on B cells stimulated by PWM may perhaps be due to the fact that B cells, under the influence of PWM, pass through several stages of differentiation in the direction of plasma cells. Such a mechanism has been described by various workers [6], and plasma cells may lose their ability to express antigens [4] such as HLA-DR it is more difficult to interpret the fact that B cells reacted less strongly in the cytotoxic test than T cells, for several workers have shown [3, 7] that specific adsorption of antisera on B cells is two to three times higher than on T cells. Ability of specific binding of anti-HLA-antibodies with surface HLA antigens may perhaps not be directly dependent on the cytotoxic effect of these antibodies. A similar relationship was noted by other workers [7].

The results of these experiments confirm data obtained previously [1], and it can be concluded that although changes in expression of HLA antigens affect both T and B cells the main contribution to this phenomenon is made by T cells. Corticosteroids can also affect the detectability of HLA antigens. Changes in expression of histocompatibility antigens are thus active in character and depend on the populations of immunocompetent cells studied, their functional activity, and the degree of their differentiation.

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