LYMPHOCYTE RECEPTORS FOR TROPHOBLASTIC β_1-GLYCOPROTEIN

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Many investigators have recently shown that trophoblastic β_1-glycoprotein (TBG) has an immunosuppressive action. It has been found, for instance, that TBG inhibits proliferative activity of lymphocytes in response to stimulation by mitogens and by allogeneic cells, potentiates the action of concanavalin A inducing suppressor cells in culture, reduces the number of E- and EAC-rosette-forming cells, and inhibits production of macrophage migration inhibiting factor during uncomplicated pregnancy [1, 3, 4]. The immunosuppressive properties of TBG are thus no longer in question, but the mechanisms mediating its action have not been studied.

The aim of this investigation was to test the hypothesis that TBG interacts with surface structures of T lymphocytes. For this purpose the rosette formation technique was modified in order to determine receptors for TBG on the surface of T lymphocytes.

EXPERIMENTAL METHOD

Tests were carried out at intervals on women aged 20-30 years during uncomplicated pregnancy. The control group consisted of clinically healthy nonpregnant women of the same age. A dried, purified preparation of TBG was generously provided by the Problem Laboratory for Immunochemistry of Malignant and Embryonic Tissues, N. I. Pirogov Second Moscow Medical Institute, directed by Professor Yu. S. Tatarinov (the purity of the preparation was 80-90%).

A cell fraction enriched with T lymphocytes was obtained by the usual method by treating lymphocytes, isolated in a Ficoll—Verografin density gradient (d = 1.077) with a mixture of papain and cysteine [2]. The purity of the isolated fraction was 97-98%.

To prepare an erythrocytic diagnostic serum, a 20% suspension of bovine erythrocytes was mixed with an equal volume of TBG solution in a concentration of 60 μg/ml and incubated at 37°C for 1 h. After incubation 1 ml of fresh mouse serum in a dilution of 1:10 was added.

To 0.1 ml of the cell fraction enriched with T lymphocytes in a concentration of 2×10^6, 0.1 ml of a 0.5% suspension of erythrocytic diagnostic serum was added. The technique of the rosette formation test thereafter was the same as usual. The rosettes formed were conventionally described as TBG-RFC.

The next series of experiments was carried out to study the relative and absolute numbers of T suppressors, i.e., of lymphocytes carrying receptors for the Fc fragment of IgG on their surface (T_γ lymphocytes), by the method of EA-rosette-formation with boving erythrocytes, sensitized with rabbit antibodies against IgG [2]. The T_γ cells were isolated by subsequent precipitation in a Ficoll-Verografin density gradient and the number of TBG-RFC in this cell population was determined. The results were subjected to statistical analysis by Student's T test.

EXPERIMENTAL RESULTS

Rosettes clearly distinguishable under the light microscope were formed in preparations of T lymphocytes with TBG-treated bovine erythrocytes. No rosettes were seen to be formed in the control without addition of TBG.

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TABLE 1. Number of TBG-RFC in Uncomplicated Pregnancy

Trimester of pregnancy	TBG-RFC		T-RFC	
	%	abs.	%	abs.
I	50,2±5,3	300,4±23,1	50,3±2,1	598,4±31,2
II	30,3±4,1	183,1±14,4	50,8±2,3	604,3±52,7
III	12,1±3,0	72,6±6,8	62,5±3,2	599,6±38,4
Nonpregnant women	7,1±1,8	49,3±5,0	55,7±3,1	694,0±75,4

TABLE 2. Number of TBG-RFC in T_γ Lymphocyte Population in Uncomplicated Pregnancy

Trimester of pregnancy	Tγ-TBG-RFC		Tγ-lymphocytes	
	%	abs.	%	abs.
I	12,7±1,2	76,0±5,9	35,9±1,5	214,5±13,9
II	19,6±3,1	118,4±20,0	44,6±3,4	269,5±23,6
III	23,8±3,7	142,7±11,4	46,6±5,6	279,4±35,6
Nonpregnant women	1,3±0,8	8,7±2,0	16,3±2,0	113,1±17,8

The relative and absolute numbers of TBG-RFC changed depending on the time of pregnancy (Table 1). The maximal number of TBG-RFC was observed in the first 3 months of pregnancy (I trimester). The number of TBG-RFC in the peripheral blood in I trimester of pregnancy was 99.7% of the total number of T lymphocytes, i.e., virtually all the T lymphocytes carried receptors for TBG on their surface. In the II trimester the proportion of TBG-RFC was significantly less, namely 59.6%, and in III it was only 19.4%, which corresponded to the number of TBG-RFC in nonpregnant women (12.8%). In all cases the differences in the number of TBG-RFC between the trimesters was statistically significant. The number of TBF-RFC in the III trimester was close to the control and did not differ significantly from their number in nonpregnant women. The modification of the rosette formation test devised by the writers for determination of receptors for TBG on the surface of T lymphocytes is thus capable of revealing relative and absolute numbers of TBG-RFC in the peripheral blood of pregnant women.

The fact will be noted that the highest content of TBG in the peripheral blood of pregnant women was not observed until the end of the III trimester (230-240 µg/ml) [5], whereas, according to our data, the largest number of T lymphocytes with receptors for TBG was found in the I trimester. These results point to the existence of negative correlation between the content of TBG and the number of T lymphocytes with receptors for TBG in the peripheral blood of pregnant women. The writers showed previously [3] that the highest suppressive activity of TBG is found in lymphocytes taken in the I trimester of pregnancy. This fact suggested that the point of application of the action of TBG is to be found in TBG-RFC, and its effect is mediated by T_γ lymphocytes. Accordingly, the next step in the work was to determine the percentage of TBG-RFC among the population of T_γ cells. It was shown previously on a model of IgG production that T_γ lymphocytes play the role of suppressors in the body [7, 8]. Data on the absolute and relative content of T_γ lymphocytes and TBG-RFC in the T_γ lymphocyte population are given in Table 2. With an increase in the duration of pregnancy there is an increase in the number of T_γ lymphocytes and TBG-RFC among the population of cells carrying receptors for the Fc fragment of IgG: 35.3% in the I trimester, 43.9% in the II trimester, and 51.1% in the III trimester. The number of TBG-RFC in nonpregnant women in the T_γ lymphocyte population was minimal, namely 1.3 ± 0.8%. In other words, at the beginning of pregnancy only one-third of the T suppressors have receptors for TBG, but by the end of pregnancy these receptors are carried by half of all T_γ cells.

The discovery of TBG-receptors on lymphocytes, including on suppressors (T_γ lymphocytes) and the changes in the number of these lymphocytes during normal pregnancy raises a number of questions for further study on the role of these receptors in realization of the immunosuppressive action of TBG.

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INHIBITION OF FORMATION OF CELLS SECRETING ANTIBODIES AND ANTIGEN-DEPENDENT NONSPECIFIC IMMUNOGLOBULINS IN MICE TREATED WITH ISOLOGOUS ANTIERYTHROCYTIC IMMUNOGLOBULINS

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It was shown previously that treatment of animals with syngeneic immunoglobulins (Ig), conjugated with cellulose (cel) and containing antibodies against sheep's red blood cells (SRBC), leads to inhibition of specific reactivity: On immunization of such animals with SRBC the number of antibody-forming cells (AFC) in their spleens was found to be an order of magnitude less than in animals receiving the antigen alone [2]. Anti-idiotypic antibodies inhibiting antibody production against SRBC were found in the serum of animals areactive to SRBC [3].

Experiments to study induction of tolerance with the aid of antigen showed that not only are AFC not formed in tolerant animals in response to injection of homologous antigen, but there is no increase likewise in the number of cells producing antigen-dependent nonspecific Ig (NIGFC) [4, 5].

The aim of this investigation was to study how reactivity to SRBC, induced by injection of idiotype-positive syngeneic Ig into mice (Ig-anti-SRBC) affects the formation of NIGFC.

EXPERIMENTAL METHOD

(CBA × C57BL/6)F_1 mice were obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. Normal serum Ig (norm Ig) and Ig-anti-SRBC were conjugated with oxidized cellulose (norm Ig-cel and Ig-anti-SRBC-cel respectively) [2]. The authors are grateful to Professor A. E. Gurvich for providing the oxidized cellulose.

Areactivity to SRBC was induced in the mice by subcutaneous injection of Ig-anti-SRBC-cel at two points, followed 1 month later by injection of Ig-anti-SRBC in a dose of 0.2 mg protein per mouse. Normal mice, and also mice receiving norm Ig-cel, followed by norm Ig at two points, subcutaneously at the same time (0.2 mg per mouse in each case), served as the control. The animals were given an injection of 5×10^8 SRBC, 2×10^8 hens' red blood cells (HRBC), or Eagle's medium, 7 days later. On the 4th day the total number of cells forming Ig (IgFC) [10] and the number of 19S AFC [9] was determined in the spleens of individual animals. The number of NIGFC was calculated as the difference between the number of IgFC and the number of AFC per 10^6 living cells. The results are presented as arithmetic means with standard error.

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