COMPARISON OF THE IMMUNOSUPPRESSIVE PROPERTIES OF TROPHOBLASTIC β_1-GLYCOPROTEIN AND PLACENTAL α_2-MICROGLOBULIN IN VITRO

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The placenta is considered to play the principal role in the prevention of rejection of a genetically foreign fetus in mammals. In recent years the immunoregulatory properties of various substances produced by the placenta have therefore been extensively investigated [4, 15]. The present writers have isolated and highly purified two new proteins specific for the human placenta: trophoblastic β_1-glycoprotein (TBG) and placental α_2-microglobulin (PAMG-2) [6, 7, 10, 13]. TBG depresses antibody formation against sheep's red blood cells (SRBC) in mice, suppresses the proliferative activity of lymphocytes in the mixed lymphocyte (MLC) test and in the blast-transformation reaction (BTR) to mitogens [3], but has no effect on production of macrophage migration inhibition factor [5]. The immunoregulatory properties of PAMG-2 have not been studied.

The aim of this investigation was to continue the study of the mechanism of the immunosuppressive action of TBG and to compare it with that of PAMG-2 in experiments in vitro.

EXPERIMENTAL METHOD

Preparations of TBG and PAMG-2 with a purity of 90%, sterilized by passage through filters (pore diameter 0.22 μ, Millipore Corporation, USA) were kept at −20°C until required for use in the experiments. Male C57BL/6($H\text{-}2^b$) and BALB/c($H\text{-}2^d$) mice and (CBA × C57BL/6) $F_1(H\text{-}2^k \times H\text{-}2^b)$ hybrids were obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, and used in the experiments at the age of 2-4 months. The MLC test was carried out by the method in [1]. Lymph node cells from C57BL/6 mice were used as the responding cells and spleen cells (SC) from F_1 hybrids, irradiated in a dose of 22.5 Gy, as stimulating cells. In the experiments of series I, responding cells, stimulating cells, and various doses of TBG and PAMG-2 were mixed simultaneously in wells of 96-well flat-bottomed microplates (Linbro, England), maintaining a constant reaction volume. In series II the test proteins were added in a final concentration of 120 μg/ml 2, 6, 18, 24, 48, and 95 h after addition of the cell mixture. Medium RPMI-1640 (Flow Laboratories, England), frozen and thawed simultaneously with the proteins, was poured into the control wells. The index of inhibition (I_i) of DNA synthesis was determined by the equation $I_i = \frac{A \cdot B}{A} \cdot 100\%$, where A and B denote incorporation of ^{3}H-thymidine in the control and experimental cultures respectively. In series III, after culture for 112 h the contents of the wells were mixed with a 0.2% solution of trypan blue and the viability of the lymphocytes was determined. The effect of the proteins on viability and proliferative activity of other types of cells was not studied.

In series IV the BTR of lymphocytes was carried out by the method in [12]. For this purpose 100 μl of spleen cells of F_1 hybrids in a concentration of $5 \cdot 10^6$ cells/ml was cultured in microplates in the presence of different concentrations of phytohemagglutinin (PHA, from Wellcome, England) or pokeweed mitogen (PM; Grand Island Biological Company, USA).

TBG or PAMG-2 was added to the experimental wells in a concentration of 120 μg/ml, and thawed medium RPMI-1640 was added to the control wells. The volumes of the mitogens and proteins were 50 μl of each. In all the series three parallel experiments were carried out for each sample. The experiments were repeated 3-8 times. The results were subjected to statistical analysis by Student's test.

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TABLE 1. Suppressor Effect of TBG and PAMG-2 on BTR of Lymphocytes from F_1 Hybrids (M ± m)

Dilution of mitogen	Incorporation of ^{3}H-thymidine, cpm addition to reaction of		
	medium	TBG (120 μg/ml)	PAMG-2 (120 μg/ml
Medium	87±4	—	—
PHA			
1:8**	15 351±431	28±4*	40±23*
1:16	6 498±202	49±23*	45±8*
1:32	2 087±245	109±77*	41±31*
1:64	721±179	242±122	95±19
1:128	325±14	110±41*	141±85
Medium	411±53	—	—
PM			
1:2***	8 640±1 093	228±262*	1 724±89*
1:4	10 666±2 138	11±7*	5 560±451
1:8	13 058±1 665	9±4*	3 622±582*
1:16	12 511±1 896	21±7*	4 752±158
1:32	12 853±1 896	42±20*	6 859±960

Legend. *$p < 0.01$, **dose of mitogen 12.5 μg per well, ***dose of mitogen 20 μg per well.

EXPERIMENTAL RESULTS

In concentrations of 5-10 μg/ml both proteins potentiated, in half of the cases, and inhibited, in the other half, proliferation of lymphocytes in response to stimulation by alloantigens. With an increase in concentration of the proteins significant suppression of lymphocyte proliferation developed, and was more marked and showed a distinct dose effect in the case of TBG. In the experiments of series II the suppressor effect was maintained at about the same level on addition of the test proteins to the reaction for up to 48 h. I_i for TBG varied from 72.2 to 84.6% and for PAMG-2 from 23.9 to 41.4% ($p < 0.01$). If the substances were added 95 h after the beginning of the reaction, i.e., 1 h before addition of ^{3}H-thymidine, differences between the experiment and control were not significant ($p > 0.05$). In the concentrations tested neither TBG nor PAMG-2 had any cytotoxic action: after the end of culture the number of living cells was the same as in the control, namely 30-35%.

It will be clear from Table 1 that TBG and PAMG-2 depressed the proliferative response of mouse lymphocytes to stimulation by PHA about equally effectively. The suppressor effect of stimulation with PM was more marked in the case of TBG. It was noted that the lymphocytes could be divided into those with high and low sensitivity to the mitogens [2]. It has been suggested that different subpopulations of T-cells respond to low and high doses of mitogens [14]. Our results may perhaps be evidence that the two placental proteins can depress proliferation of either lymphocyte subpopulation.

The results of these experiments thus indicate that TBG and PAMG-2, in below physiological concentrations, possess immunosuppressive properties. TBG is synthesized in the outer layer of the chorionic villi, and is then secreted into the blood stream, where its average concentration is 290 μg/ml [9, 11]. According to our data, TBG depresses proliferation of mouse lymphocytes, including those which have started to proliferate. Similar data have been obtained with human lymphocytes proliferating in the BTR [3]. It has been noted that data concerning suppression of BTR in response to PHA or to concanavalin (con A) must be treated with caution, because TBG contains a binding site with con A and PHA [13]. Since TBG does not prevent incorporation of ^{3}H-thymidine into DNA of lymphocytes when added to the MLC reaction 1 h before the isotope and does not have a cytotoxic action on the cells, it probably influences the lymphocytes directly, by blocking DNA synthesis.

PAMG-2 predominates in placental extract, where its concentration reaches 400 μg/ml [8]. This protein has a similar action to TBG, but weaker, on proliferation of lymphocytes stimulated by alloantigens or mitogens. TBG evidently limits lymphocyte proliferation in the maternal blood, whereas PAMG-2 performs a similar function in the placenta.

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RECEPTORS FOR SECRETORY COMPONENT ON HUMAN THYMIC LYMPHOCYTES: STIMULATION OF THEIR EXPRESSION BY ADENOSINE, THEOPHYLLINE, AND THYMOCYTE SUPERNATANT

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Hetero-organic antigens characteristic of certain highly specialized tissues of the body are known to be represented in the thymus. The myoid cells of the thymus have been shown to contain antigens common with those of mouse tissues [6], and cells of the epithelial reticulum contain antigens common with epithelial tissues of epidermal type [1, 7, 8]. Cells synthesizing lactoferrin also have been found in the thymus, and a secretory component has been discovered in the membranous structures of the gland [2, 3]. These substances are known to be components of material excreted by the epithelium of several organs of ectodermal and entodermal origin (the salivary, lacrimal, and mammary glands, epithelium of the intestine and respiratory tract, etc.). It has been shown that receptors for lactoferrin are present on lymphocytes of the thymus, and their expression is stimulated by adenosine and theophylline, which raise the intracellular cAMP concentration, and also by thymocyte supernatant. Levamisole, which facilitates intracellular accumulation of cGMP, does not affect the ability of thymocytes to express these receptors [4].

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