COMPARATIVE ANALYSIS OF PREGNANCY-SPECIFIC PROTEINS IN THE REGULATION OF SUPPRESSOR T CELL FUNCTION DURING GESTATION

V. P. Chernyshov, V. L. Valetskii,
and T. V. Radysh

UDC 612.017.1.06:612.63

KEY WORDS: pregnancy-specific proteins; trophoblast products; con A-induced suppressor T cells; immunoregulation.

An important role in immunoregulation during pregnancy is played by pregnancy-specific proteins (PSP) and, in particular, by trophoblastic β_1-glycoprotein (TBG, SP1) and pregnancy-associated α_2-glycoprotein (α_2-GP, SP3), which possess marked immunoregulating properties [7-10]. It is interesting to note that physiological concentrations of TBG in vitro potentiated induction of suppressor T cells by concanavalin A (con A) [2, 3]. It is therefore considered that the suppressor effect of TBG may be due to its ability to stimulate suppressor cell function. Data on the presence of receptors for TBG on suppressor T cells [5] suggest that the regulatory effect of TBG is mediated through these structures. During pregnancy changes take place in the functional state of suppressor T cells, but there is no evidence in support of possible correlation between these changes and the accumulation of PSP.

The aim of this investigation was to study correlation between the TBG and α_2-GP concentrations and functional activity of con A-induced suppressor T cells during gestation.

EXPERIMENTAL METHOD

Altogether 63 women were tested at different stages of normal gestation. The TBG concentration was determined by low-voltage rocket immunoelectrophoresis [1], using monospecific antiserum against TBG obtained by the method in [6], the concentration of α_2-GP by low-voltage rocket immunoelectrophoresis [1] using monospecific antiserum against α_2-GP, obtained by the method in [4], and the PSP concentration in samples of blood sera preserved at −20°C.

The functional state of the suppressor T cells was determined in lymphocytes isolated by centrifugation in a Ficoll-Verografin density gradient (d = 1.077 g/cm^3). The lymphocytes were cultured for 48 h in sterile REAC-31 tubes ("Sorin Biomedica," France), containing (4-6) × 10^6 cells in 2 ml of complete medium RPMI-1640 ("Serva," West Germany) with con A ("Sigma," USA), in a concentration of 60 μg/ml, and without con A. At the end of incubation the cells were treated with mitomycin C ("Sigma") in a concentration of 40 μg/ml for 40 min at 37°C, after which they were washed 4 times with cold medium 199. The preincubated lymphocytes were added to a culture of freshly isolated donor's lymphocytes and cultured in 96-well plastic panels ("Medpolimer," Leningrad) in the presence of PHA-P ("Difco," USA) in a concentration of 1 μl/ml for 72 h in an atmosphere with 5% CO_2. The work with the cells was done under sterile conditions of laminar flow. Proliferation of the cells in the cultures was assessed by incorporation of ^{3}H-thymidine (All-Union "Izotop" Combine), added 4 h before the end of incubation. The index of con A-induced immunoregulation was calculated by the formula:

$$\text{Index} = \left[1 - \frac{\text{Level of incorporation of label in experimental cultures (in cpm)}}{\text{level of incorporation of label in control cultures (in cpm)}}\right] \times 100\%.$$

The results were subjected to statistical analysis by Student's t test and determination of the coefficient of correlation.

Laboratory of Immunology, Kiev Research Institute of Pediatrics, Obstetrics, and Gynecology, Ministry of Health of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR E. M. Luk'yanova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 8, pp. 229-231, August, 1989. Original article submitted July 13, 1988.

0007-4888/89/0008-1177$12.50

TABLE 1. Functional Activity of Con A-Induced Suppressor T Cells and PSP Concentration during Normal Gestation (M ± m)

Period of pregnancy, weeks	Index of induced suppression, %	TBG concentration in blood serum, g/liter	α_2-GP concentration in blood serum, g/liter
8—16	52,5±5,7 (11)	0,028±0,004	0,28±0,03
17—28	60,3±3,6* (30)	0,067±0,005**	0,58±0,04**
29—36	57,9±5,3* (13)	0,131±0,014***	1,14±0,06***
Control (non-pregnant women)	35,8±1,8 (4)	—	—

Legend. Number of cases given in parentheses. *$p < 0.05$ compared with control, **$p < 0.01$, ***$p < 0.001$ compared with parameters on 8th-12th week of pregnancy.

EXPERIMENTAL RESULTS

As Table 1 shows, functional activity of con A-induced suppressor T cells was higher in pregnant women than in women who were not pregnant, and the difference became statistically significant after 17-36 weeks ($p < 0.05$). Consequently, the physiological course of pregnancy is accompanied by an increase in functional activity of the suppressor T cells, and this is evidently an important factor protecting the fetus against maternal immunologic aggression at 8-36 weeks.

The concentration of the PSP (TBG and α_2-GP) rose progressively up to a maximum after 29-36 weeks.

Correlation analysis revealed certain relationships between the parameters. It must be emphasized that unlike in studies in vitro, the results were obtained for conditions in vivo by correlation analysis, allowing estimation of the degree of dependence of the test parameters on the level of a single individual.

Positive correlation was found between activity of suppressor T cells and the α_2-GP concentration at 8-36 weeks ($r = 0.3$); between 29 and 36 weeks, moreover, the strength of correlation increased significantly ($r = 0.87$). Thus functional activity of con A-induced suppressor T cells may correlate with the increase in the α_2-GP concentration and, in particular, between 29 and 36 weeks, when α_2-GP accumulation in the peripheral blood reaches a maximum.

Positive correlation was found between suppressor T cell activity and the progressively rising TBG concentration between the 8th and 28th weeks of pregnancy ($r = 0.44$). Consequently, functional activity of con A-induced suppressor T cells may correlate with the rise in the TBG concentration during the first two trimesters of the gestation process. Thus in vivo, correlation was found between the functional activity of con A-induced suppressor T cells and accumulation of two pregnancy-specific proteins in the peripheral blood. The progressive accumulation of these proteins is evidently essential for the formation of a suppressor bias in the state of the immunoregulatory system, starting with the early period of pregnancy.

A selective stimulating action of α_2-GP on suppressor cell function can be postulated on the basis of the correlation thus revealed. The over-all suppressive action of α_2-GP on lymphocyte proliferation, discovered previously in vitro [10], can evidently be explained in this way. On the basis of data in [2, 3] and the results of correlation analysis, the writers suggest that the increase in the level of functional activity of con A-induced suppressor T cells at 8-28 weeks of pregnancy is connected with TBG accumulation.

Thus high activity of suppressor T cells in the first two trimesters of the gestation process may be due to a combined rise of the levels of two pregnancy-specific proteins (TBG and α_2-GP), but between 29 and 36 weeks, it is due to α_2-GP alone. The normal gestation process in man is therefore characterized by controlled immunosuppression, manifested as an increase in the level of functional activity of the suppressor T cells, which may be the result of the regulatory influence of PSP in different periods. The correlations discovered allow determination of their physiological role as natural, endogenous immunoregulators. This linking of the changes may be evidence of the existence of a system of immunoregulatory mechanisms responsible for the reproductive strategy, and participation of PSP in that strategy.

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CORRECTION OF DISTURBANCES OF IMMUNITY ARISING AFTER HEAD INJURY

A. P. Romodanov, N. I. Lisyanyi, and L. V. Kurganova

UDC 616.714+616.831]-001-092:612.017.1]-085.275.4-092.9

KEY WORDS: head injury; immunostimulating humoral factor; antibody-forming cell; delayed-type hypersensitivity; correction

Head injuries, like any other type of trauma, act as extremely strong stress stimuli and are accompanied by the release of hormones, neuropeptides, catecholamines, and other biologically active substances into the circulation, giving rise to cellular depopulation of the lymphoid organs and forming a secondary immunodeficiency syndrome [1, 3, 4, 9, 10]. Meanwhile it has been shown that immune disturbances arising in such cases in the posttraumatic period can be largely corrected by the use of immunostimulators such as T-activin, myelopide or, which is particularly important, small doses of serum from traumatized animals [8]. In this connection it is important to study the immunostimulating action of the humoral factor isolated from the blood serum of animals subjected to craniocerebral trauma (CCT) on the development of posttraumatic immunodeficiency.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-190 g. A closed head injury was inflicted by means of a spring-operated hammer in the left fronto-parietal region of the head in rats [8]. The animals were immunized with sheep's red blood cells (SRBC) in a dose of 5×10^8 1 h after CCT and on the 1st, 3rd, 5th, and 7th days. The number of antibody-forming cells (AFC) in the spleen was determined on the 5th day by the method in [12]. A local delayed-type hypersensitivity reaction (DTHR) was induced by the method in [2], by sensitizing the animals 1 h after CCT.

A typical index was used as indicator of post-stress changes in the immune system, namely the ratio of the weight of the thymus to the animal's body weight [1, 3]. The immunostimulating humoral factor (IHF) was isolated by the writers' own method, by acetic acid extraction with selective fractionation on ultrafiltration membranes [5]. IHF was injected intramuscularly into the experimental animals in a dose of 1-10 mg/kg body weight depending on the experimental conditions.

Laboratory of Neuroimmunology, Research Institute of Neurosurgery, Ministry of Health of the Ukrainian SSR, Kiev.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 8, pp. 231-233, August, 1989. Original article submitted August 5, 1988.