# STUDY OF TROPHOBLASTIC β-GLOBULIN IN THE LEUKOCYTE ADHESION INHIBITION TEST DURING PREGNANCY

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It is generally considered that one mechanism of the development of immunologic tolerance of the pregnant woman to the genetically xenogenous fetus is the synthesis of specific immunosuppressive substances such as chorionic gonadotrophin, α-fetoprotein, placental lactogen, etc. [1]. Recently, particular attention in this respect has been paid to the specific protein of pregnancy identified in 1970 by Tatarinov and Masyukevich [3], namely trophoblastic β-globulin (TGB). For instance, the immunosuppressive effect of TBG preparations has been demonstrated on blast transformation of lymphocytes in mixed culture and of lymphocytes stimulated by phytohemagglutinin (PHA) and concanavalin A (Con A) [6, 8]. However, the problem of to what extent the action of TBG on immunologic reactions is specific, and on what phase of the immune response it produces its effect, has not yet been studied.

The object of the present investigation was to study the effect of TBG on the phase of recognition of specific antigens by sensitized lymphocytes, which is accompanied by the liberation of so-called lymphokines. The presence of lymphokines can be tested by making use of the adhesive properties of leukocytes in the leukocyte adhesion to glass inhibition test (LAIT) [7].

## EXPERIMENTAL METHOD

Halliday's LAIT technique [7], as modified by Frolova and Govallo [4], was used to study the effect of TBG on immunologic reactions *in vitro*.

Leukocytes were isolated from 5 ml of heparinized venous blood of pregnant women or women of the control group and suspended in medium No. 199 with the addition of 10% calf serum. The concentration of leukocytes was $20 \times 10^6$ cells/ml. A saline extract from a mixture of spleens and thymus glands of aborted fetuses at different periods of development, with a protein concentration of about 1%, was used as the specific antigenic material. Immediately before the experiments the antigenic material was diluted with medium No. 199 in the ratio of 1:5. The TBG preparation, obtained by a combination of the methods of salt precipitation and adsorption chromatography, with a purity of about 90%, was used in final concentrations of 1 and 10 mg%, approximately the same as its physiological concentration in the blood serum of pregnant women in the 1st and 3rd trimesters respectively. Before the experiments equal volumes (0.05 ml) of le-kocyte suspension, antigenic extract, medium No. 199, calf serum, and the TBG preparation or serum from a pregnant woman were mixed and the mixture was incubated at 37°C for 30 min. In control experiments the extract of fetal antigens was excluded from the composition of the incubation mixture.

## EXPERIMENTAL RESULTS

The results obtained by the LAIT (Table 1) showed that TBG preparations in physiological concentrations had no appreciable effect on the adhesive properties of leukocytes of pregnant women. No statistically significant changes were found ($P > 0.05$) in the LAIT when different concentrations of TBG were used, i.e., the TBG had neither immunosuppressive nor stimulating effect. The same picture was observed when sensitized lymphocytes of pregnant women in the 1st and 3rd trimesters were tested. Meanwhile blood serum from pregnant women in the 1st trimester gave a statistically significant ($P < 0.05$) blocking effect, and largely restored the percentage of adherent cells (Table 1). The same tendency was observed when

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TABLE 1. Effect of TBG on Adhesive Properties of Leukocytes in LAIT (in % of cells adherent to glass, M ± m)

| Group tested | Number of individual tests | With fetal antigens | With fetal antigens + pregnant women's serum (32 weeks) | With fetal antigens + TBG (1 mg %) | With fetal antigens + TBG (10 mg %) | Without fetal antigens |
|---|---|---|---|---|---|---|
| Pregnant women | | | | | | |
| 6—12 weeks | 5 | 27,4±2,0 | 39,4±3,5 | 27,6±1,5 | 28,2±2,1 | 48,0±1,3 |
| 32—38 weeks | 5 | 30,2±2,2 | 36,6±3,1 | 28,6±1,9 | 33,4±2,2 | 47,2±4,1 |
| Nonpregnant women | 6 | 45,1±2,5 | — | — | — | 44,7±2,3 |

sera from pregnant women in the 3rd trimester were added, but the differences in this case were no longer statistically significant ($P > 0.05$). In control tests with blood from nonpregnant women the percentage of adherent leukocytes was virtually unchanged after addition of the fetal antigens, evidence of the absence of specific sensitization of the lymphocytes of these women.

The results indicate that TBG has no appreciable immunosuppressive effect on the phase of recognition of fetal antigens by sensitized lymphocytes of pregnant women, the phase which is accompanied by liberation of lymphokine, which sharply reduces the ability of polymorphs to adhere to the surface of glass. It is considered that the LAIT is an improved version of the leukocyte migration inhibition test, which is based on the effect of a special lymphocytic mediator, the migration inhibiting factor (MIF) [1]. More recently, however, it has been stated in the literature [2] that the lymphokine responsible for LAIT is not identical with MIF. In this connection the present results showing that TBG has no effect on LAIT do not rule out the possibility of its immunosuppressive action on the immunologic recognition phase as such, but they are evidence simply that TBG does not play a part in the liberation by sensitized lymphocytes of a factor inhibiting the adhesive powers of leukocytes. It must also be noted that whole blood sera from pregnant women as a rule have a blocking action on LAIT, although as the end of pregnancy approaches this is replaced by a stimulating action [1]. In contrast to this, the serum TBG level in pregnant women rises steadily until delivery [3].

The writers showed previously [5] that TBG can bind with PHA and Con A, i.e., with classical mitogens used to determine the immunosuppressive properties of various substances in the blast transformation of lymphocytes test in tissue culture. This fact calls for definite caution to be exercised when drawing conclusions on the presence of immunosuppresive properties in TBG if their discovery is based on tests of blast transformation of lymphocytes under the influence of PHA and Con A.

The question of whether or not TBG has immunosuppressive properties thus requires further study with a wider range of techniques and with consideration of some of the nonspecific reactions of this protein of pregnancy.

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