EFFECT OF TROPHOBLASTIC β_1-GLYCOPROTEIN (TBG) ON FUNCTIONAL ACTIVITY OF VARIOUS CELL LINES

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Trophoblastic β_1-glycoprotein (TBG) is one of the principal proteins of pregnancy (placental polypeptides), produced by cells of the syncytiotrophoblast [9]. This polypeptide is a marker protein not only of pregnancy, but also of several neoplasms and, in particular, of trophoblastic tumors [3]. Measurement of the serum TBG level is used in the early diagnosis of pregnancy [11], for monitoring the dynamics of fetal development [10], for predicting termination of pregnancy [10], and for screening and monitoring malignant neoplasms [8]. Despite available data on interaction of TBG in vivo with various biologically active substances [6] and its action on cells of the immune system [1, 4], the true biological function of TBG remains unexplained.

The aim of this investigation was to assess the action of TBG on functional activity of cell lines of lymphoid and nonlymphoid nature.

EXPERIMENTAL METHOD

A preparation of TBG was obtained from the gamma-globulin fraction of retroplacental serum, using the following combination of chromatographic methods: adsorption chromatography on hydroxyapatite, hydrophobic chromatography on Sephadex G-150 [2]. The protein preparation thus obtained was analyzed by disk electrophoresis in 7% polyacrylamide gel in the presence of sodium dodecylsulfate and immunoblotting on nitrocellulose filters (indirect mode of analysis) using affinity-purified rabbit polyclonal antibodies against TBG and antispecific peroxidase conjugate of goat antibodies against rabbit Ig (Fig. 1). The following human cell lines were used as target cells to assess the action of TBG: CEM line of T-lymphoma, Turkat and MOLT-4 lines of T-cell leukemia, K-562 line of erythromyeloleucosis, I M-9 for B-cell leukemia, EB-2 for B-cell lymphoma, carcinoma of the body of the uterus, M-HeLa-76 for carcinoma of the cervix of the uterus, choriocarcinoma, and peripheral blood lymphocytes (PBL). PBL ($\times 10^5$ cells per well) were stimulated with con A (5 μg/ml) for 72 h. DNA synthesis was estimated as incorporation of ^{3}H-thymidine, which was added 4 h before the end of culture.

EXPERIMENTAL RESULTS

The results of the study of the effect of TBG on proliferation of the following cell lines are given in Fig. 1a: CEM, K-562, Turkat, MOLT-4, I M-9, and EB-2, cultured in the presence of TBG for 48 h. These cell lines reacted differently to TBG. With TBG in a concentration above 50 μg/ml (corresponding to physiological concentration during pregnancy) proliferation of these cells was suppressed. With lower concentration of TBG the proliferative activity most cell lines returned to the control level (without TBG), but proliferation of Turkat and K-562 cells was increased by 1.7-2.2 times, and only with a further fall of the TBG concentration in the culture did it return to the control level. TBG in concentrations with an activating effect on prolif-

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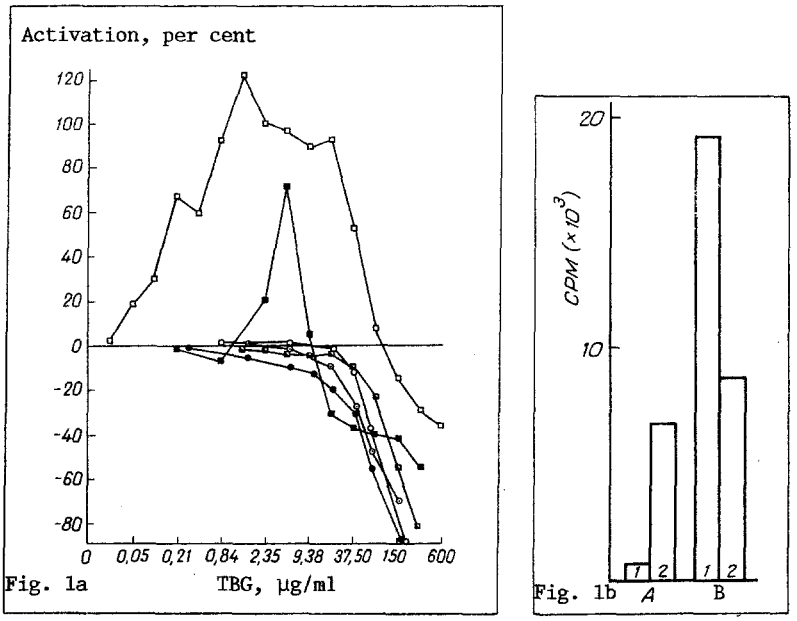

Fig. 1. a) Effect of TBG on proliferation of lymphoid and leukemic human cell lines. □) Iurkat, ■) K-562, ○) EB-2, ◙) CEM, ●) MOL T-4, ⊙) 1M-9. b) Modulation by TBG (14 μg/ml) of spontaneous (A) and con A-dependent (B) proliferation of human peripheral blood lymphocytes. 1) Absence, 2) presence of TBG.

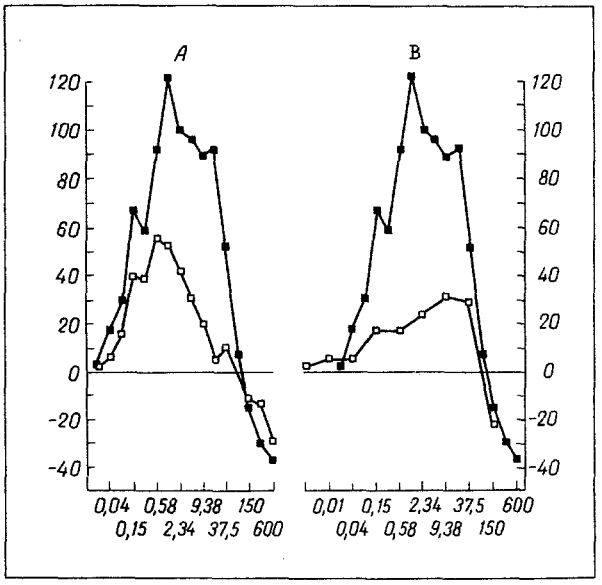

Fig. 2. Effect of TBG on proliferation of Turkat cells depending on incubation time (A) and number of cells (B). A: □) 24 h of incubation; ■) 48 h of incubation; B: ■) 10^4 cells/well; □) $5 \cdot 10^4$ cells/well. Abscissa, TBG concentration in μg/ml. Ordinate, percentage activation of proliferation.

erative activity of K-562 and Turkat cells caused a sharp increase in the intensity of spontaneous proliferation of PBL, and suppressed con A-induced proliferation of these same cells (Fig. 1b). However, data on inhibition by TBG of con A-induced proliferation do not permit the unequivocal interpretation of this result, for inactivation of con A in the composition of a soluble TBG — con A complex [5] and independent blockade of the action of con A as a result of reception by TBG-cells also are possible. In the next series of experiments we studied the effect of TBG on cell proliferation depending on the duration of

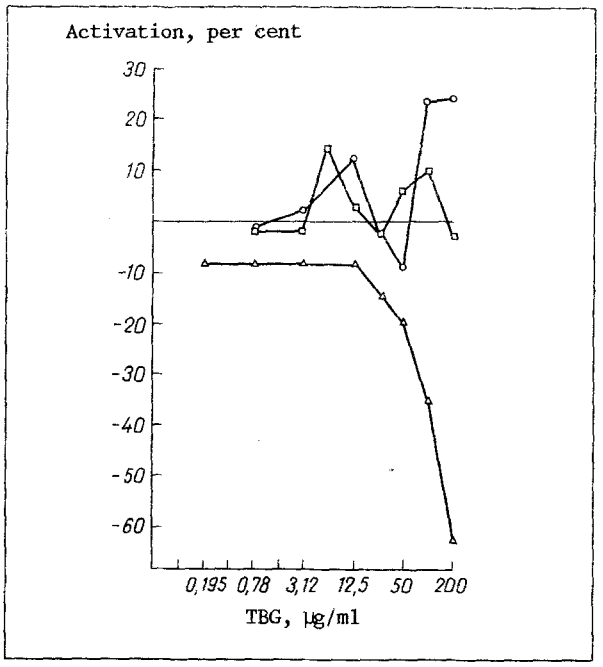

Fig. 3. Effect of TBG on proliferative activity of cell lines obtained from tumors of genital tract. △) M-Hela, ○) carcinoma of body of uterus, □) choriocarcinoma.

incubation (Fig. 2a) or on the number of target cells (Fig. 2b). The effect was more marked after culture for 48 h than for 24 h, and when a smaller number of cells was used. The quantity of TBG inducing maximal cell proliferation increased proportionally to the concentration of these cells, possible evidence of the existence of receptors for TBG. Since the previous experiments were conducted on cells of lymphoid origin, and since inhibtion of proliferative activity was a characteristic feature of the use of high TBG concentrations, we decided to assess the action of TBG on cells of nonlymphoid nature. To study this problem we investigated the effect of TBG on proliferative activity of cell lines obtained from various tumors of the genital tract M-HeLa (carcinoma of the cervic uteri), carcinoma of the body of the uterus, and choriocarcinoma. The results (Fig. 3) show that proliferative activity of M-HeLa cells was inhibited in the presence of high concentrations of TBG (>50 μg/ml), but proliferation of cells of carcinoma of the body of the uterus and choriocarcinoma did not change significantly over the whole range of concentrations of TBG. These results are evidence of the selectivity of action of TBG. Cells of both B and T lymphoid origin and also certain tumor cells of nonlymphoid nature are vulnerable to the action of TBG. All these are features of different cells populations. Experiments are currently in progress to study the mechanisms of action of TBG on cells.

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