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Influences of Clotting Factors (Thrombin, Factor XIII) and of Fibronectin on the Growth of Tumor Cells and Leukemic Cells in vitro

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Summary. Thrombin, factor XIII and fibronectin were incubated with cultures of mouse sarcoma cells, human cervix carcinoma cells (HeLa cells) and cells of an acute lymphoblastic leukemia. Thrombin induced a significant increase of ^{3}H-thymidine uptake into cells with a 1,5- to 2-fold increase of cell count. The cells of an acute lymphoblastic leukemia showed a similar response to the influence of thrombin. Factor XIII in tumor cells merely induced an increase of ^{3}H-thymidine uptake, the cell count remained constant. The cells of an acute lymphoblastic leukemia showed under the influence of factor XIII a significant increase of cell count and thymidine uptake. HeLa cell growth was optimal at low fibronectin concentrations. Fibronectin concentrations of 1 mg/ml to 3 mg/ml inhibited HeLa and mouse sarcoma cell growth.

Key words: Thrombin – Factor XIII – Fibronectin – Tumor cell proliferation – Leukemic cell proliferation

The present study was carried out in order to further clarify the influence of purified clotting factors (factor XIII, thrombin) as well as fibronectin on the growth of tumor cells and leukemic cells in vitro. Previous experiments have indicated that tumor cell thrombosis and malignant growth are closely related phenomena [2]. As thromboplastin-like material is released by tumor cells the formation of thrombin, the conversion of fibrinogen to fibrin and the activation of factor XIII is initiated. Although data are available on the significance of coagulation phenomena during the course of malignancies little is known on the direct influence of factor XIII and thrombin as well as fibronectin on the growth of tumor cells and of leukemic cells in vitro.

All experiments were performed in vitro using tumor cells from different origin: human cervix carcinoma cells (HeLa cells) cat. no. H 3002 (BCK Biocult-Chemie,

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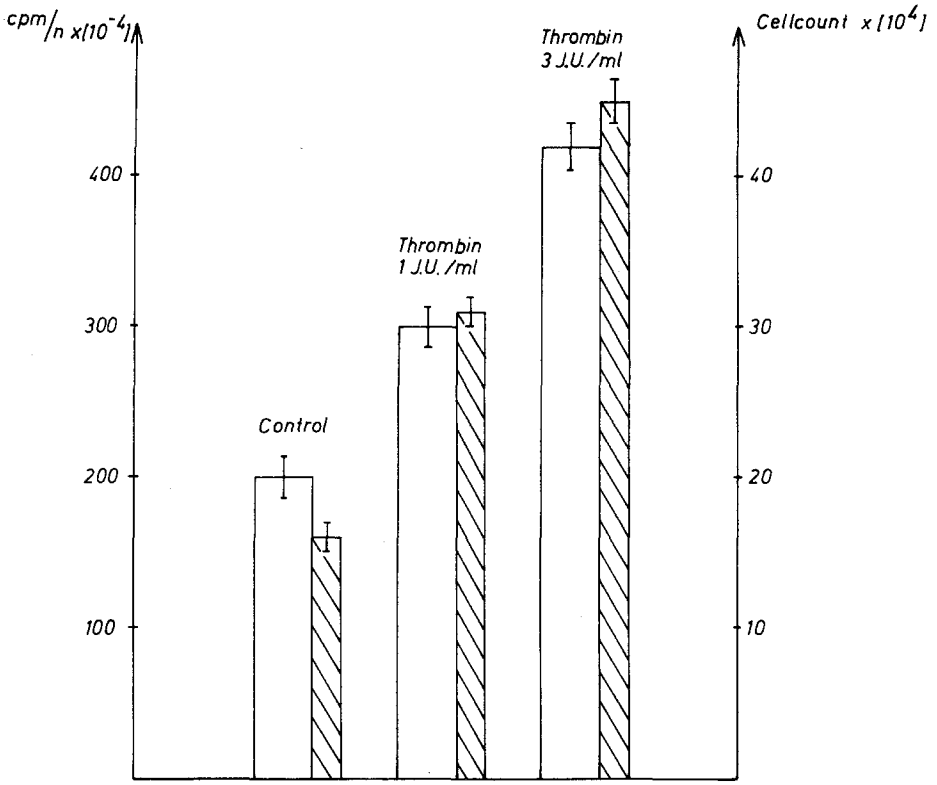

Fig. 1. Influence of a 12 h thrombin-incubation on the proliferation of cells (24 h after reseeding) of an acute lymphoblastic leukemia. White columns = cell counts, striate columns = thymidine uptake per cell. Column at the left: control sample. Column in the middle: leukemic cells stimulated by 1 international unit (I.U.)/ml thrombin. Column at the right: leukemic cells stimulated by 3 I.U./ml thrombin. The differences were significant at the 1%-level ($p < 0.01$) (statistical evaluation according to Sachs [5]. The graph shows in the middle of each column the standard deviation of the mean values

Karlsruhe, FRG) and mouse sarcoma cells, cat. no. H 4015 (BCK Biocult-Chemie, Karlsruhe, FRG). The cells of an acute lymphoblastic leukemia [6] were kindly donated by Prof. Müller-Ruchholtz, Institute of Immunology, University of Kiel. Thrombin, factor XIII and fibronectin were highly purified preparations from Behringwerke AG (Marburg/Lahn) (kindly donated by Prof. Heimburger). The cell cultures were incubated for 12 h with the investigated clotting factors. In all investigations control cultures were assayed. Methodological details are described by Bruhn and Pohl [1].

The following results were obtained: thrombin induced a 1.5- to 2-fold increase in the cell number of mouse sarcoma and HeLa cells (from 3×10^4/ml to 5×10^4/ml), the ^{3}H-thymidine uptake was increased 3- to 6-fold (from 600 cpm/n $\times 10^{-4}$ to 2400 cpm/n $\times 10^{-4}$) ($p < 0.01$). The optimum stimulating effect of thrombin was observed at concentrations of 10 units per ml in secondary cultures 24 h after reseeding of the cells. Cells of an acute lymphoblastic leukemia were also stimulated signifi-

cantly by thrombin (Fig. 1). Factor XIII induced about a 3-fold increase in ^{3}H-thymidine uptake in mouse sarcoma cells and 2.5-fold increase in HeLa cells at an optimum stimulating concentration of 1.0 unit factor XIII per ml ($p < 0.01$). The cell counts, however, appeared not to be influenced by these factor XIII concentrations and remained constant. The cells of an acute lymphoblastic leukemia showed under the influence of factor XIII (1.0 unit/ml) a significant 4-fold increase of cell count and 2.5-fold of thymidine uptake when the cells were incubated for 12 h with factor XIII. With fibronectin the cell number decreased when higher concentrations (1.0–3.0 mg/ml) were used (from 9×10^4/ml to 6×10^4/ml) ($p < 0.01$). ^{3}H-thymidine uptake of mouse sarcoma and of HeLa cells was also reduced under the influence of fibronectin (from 470 cpm/n $\times 10^{-4}$ to 170 cpm/n $\times 10^{-4}$) ($p < 0.01$). HeLa cells grown in the presence of low concentrations of fibronectin (0.03 mg/ml) showed an improvement and increase of ^{3}H-thymidine uptake.

In summary the mitogenic effect of thrombin on tumor cells as demonstrated by previous investigators [8] is also seen with HeLa cells and mouse sarcoma cells and with cells of an acute lymphoblastic leukemia in our experiments. However, it is shown that higher concentrations of thrombin (10 international units per ml) are needed to stimulate malignant cells in comparison to fibroblasts (1.0 I.U./ml). One of the possible reasons may be a reduced receptor function or a loss of receptors in tumor cells. Since there was no increase in cell counts the factor XIII-induced increase in ^{3}H-thymidine uptake of sarcoma cells appeared not to be followed by mitosis. Therefore the existence of a block in the postsynthetic phase of the cell cycle (G 2-block) could be discussed. The cells of an acute lymphoblastic leukemia, however, showed under the influence of factor XIII not only an increase of thymidine uptake but also of the cell count which was significant in comparison to the controls ($p < 0.01$). Recently Maxfield et al. [3] emphasized the role of factor XIII or a glutaminase-like enzyme for the clustering and subsequent internalization of membrane receptors. Since factor XIII exerts its effect via specific receptors a supportive effect of factor XIII on DNA metabolism of tumor cells may be present. Similar results were recently obtained by Rasche [4] with bone marrow stem cells.

Fibronectin was shown to normalize the morphology of transformed malignant cells [7]. In the present study the inhibition of ^{3}H-thymidine uptake of normal fibroblasts grown at physiological fibronectin concentrations [1] could be reproduced with mouse sarcoma and HeLa cells at higher (nonphysiological) fibronectin concentrations (1.0–3.0 mg/ml).

From the in vitro-data presented here some influences of thrombin, factor XIII and fibronectin on the growth of tumor cells and leukemic cells have to be considered. However, these influences only have the character of conditions that come from the environments. The malignant transformation and proliferation itself follows its own rules that are independent of changes of the "protein atmosphere."

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