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AN ACQUIRED HEPARIN-LIKE ANTICOAGULANT IN AN 8 MONTH OLD WITH MONOBLASTIC LEUKEMIA. JB Bussel, PG Steinherz, and DR Miller. Department of Pediatric, Memorial Sloan-Kettering Cancer Center, New York, N.Y., U.S.A.

Well documented heparin-like anticoagulants have been reported in association with malignancy only in 3 adults with multiple myeloma. We report an 8 month old white male with acute monoblastic leukemia with an initial WBC of 358,000/ul who was treated with adriamycin and ARA-C, developed a severe coagulopathy, and died 3 days later of presumed pulmonary and/or CNS hemorrhage. Liver and renal functions were normal and lysozyme was 112 mg/dl. The coagulation data were as follows:

day	PT secs	PTT secs	Thrombin time secs	Reptilase time secs	fibrinogen mg/dl	fibrin split products mg/dl
1	20	61	117	18.4	112	0
2	26	94	>120	21	88	10

The factor V level was 11% and 2%, the factor VIII level was 69% and 85%, and the platelet count was approximately 50,000/µl both days. The thrombin time with mixing was 59 secs and after adsorption with "hepasorb" the thrombin time was 33 secs. Coagulation parameters did not change despite the infusion of fresh frozen plasma, proplex, hemofil, and platelets.

Additional studies revealed:

1) Progressive shortening of the thrombin time when increasing amounts of protamine and PF4 were added to patient's plasma.

2) Patient's urine prolonged the thrombin time of normal plasma. This effect was neutralized by protamine and PF4. Urine from another patient with AMOL in relapse did not prolong the thrombin time of normal plasma.

3) Mucopolysaccharides were detected in the patient's urine by the Berry spot test.

The data suggest that the major coagulation abnormality in this patient was a circulating heparin-like anticoagulant. This is the first case report of an endogenous heparin-like anticoagulant in leukemia.

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PROCOAGULANT (TISSUE FACTOR) ACTIVITY IS HIGHER IN CELLS FROM MURINE SARCOMA SUBLINES WITH LOWER METASTATIC POTENTIAL M. Colucci, R. Giavazzi, G. Alessandri, N. Semeraro, A. Mantovani and M.B. Donati. Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy

It has been suggested that cancer cell procoagulant activity influences metastasis formation by promoting fibrin deposition around tumors. We have investigated the procoagulant activity of various tumor cell sublines with different metastatic capacity. These were derived from spontaneous lung nodules of mFS6, a benzopyrene-induced sarcoma in C57Bl/6 mice. After one in vivo passage by s.c. implantation, the resulting tumor was cultured once in vitro till confluence; cells were then harvested from plastic bottles by trypsin treatment, and washed extensively after trypsin neutralization. Tumor cell procoagulant activity was measured by a one-stage clotting assay using autologous plasma. All the cells tested possessed thromboplastin-like activity since they shortened the recalcification time of normal and factor VIII-deficient plasma to a similar extent but had no activity on factor VII-deficient plasma.

They were, however, heterogeneous as regards the degree of procoagulant activity; the two cell lines with virtually no metastatic capacity showed 6-8 times higher procoagulant activity than the cells from the parent line; in contrast, the procoagulant activity of the two sublines with higher metastatic capacity did not differ significantly from that of the parent line.

These findings support the hypothesis that fibrin is part of a defence reaction against cancer cell invasiveness.

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TISSUE FACTOR-LIKE ACTIVITY OF THE HUMAN MONOCYTIC TUMOR CELL LINE U937. D. Hudig, S.I. Rapaport and S.P. Bajaj. University of California, San Diego, California.

Cells of the human monocytic cell line U937, derived from a patient with histiocytic lymphoma (Sundstrom and Nilsson, Int. J. Cancer 17:565, 1976) have procoagulant activity similar to that of activated peripheral blood monocytes, although about 10-fold more U937 cells than monocytes are required for equivalent activity. Procoagulant activity of the cells is Ca^{2+} dependent and is not demonstrable in factor VII deficient or factor X deficient plasma. Culture with E. coli 0127:B8 lipopolysaccharide increases the procoagulant activity of washed U937 cells two-fold. Exposure of U937 cells to lymphokines from normal lymphocytes does not induce further coagulant activity. The slope of the log/log plot of cells vs. clotting time parallels that of human brain thromboplastin. Other cell lines of myeloid or lymphoid origin, e.g., K562 cells, WI-L2 cells, do not have procoagulant activity. Thus, U937 cells have constitutive factor VII-dependent coagulant activity similar to the tissue factor activity induced by activation of normal monocytes.

In further experiments, U937 cells were incubated with purified human factor VII in the presence or absence of Ca^{2+} and then repeatedly washed. When subsamples of the cells were then added to recalcified factor VII deficient plasma in the absence of added tissue factor, the following clotting times were obtained: for cells incubated with factor VII in the presence of Ca^{2+},45"; for cells incubated with factor VII in the absence of Ca^{2+}, 150". These data suggest that U937 cells can bind factor VII in a reaction requiring Ca^{2+}, which then enables the cells to express their tissue factor-like activity in factor VII deficient plasma.

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CANCER PROCOAGULANT: A NOVEL VITAMIN K-DEPENDENT ACTIVITY? M.B.Donati, F. Delaini, M. Colucci, G. De Bellis Vitti, D. Locati, A. Poggi and N. Semeraro. Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy

Cells from some experimental tumors have been found to possess a peculiar procoagulant activity, which directly activates coagulation factor X, independently of both the intrinsic and extrinsic clotting pathways. We report here that, in the Lewis Lung Carcinoma (3LL) model, such a cell procoagulant is inhibited by vitamin K-deficiency (either dietary or pharmacologically induced).

Warfarin was given chronically to mice in drinking water from day 7 after i.m. tumor implantation at a schedule which maintained the prothrombin complex activity (thrombotest) at less than 5% of control values. Vitamin K-deficiency was also induced by feeding the mice with an appropriate diet at a schedule which gave a similar degree of plasma anticoagulation. At day 18 after tumor implantation macrophage-free 3LL cell suspensions obtained by spilling of necrosis-free tumor fragments were tested for their specific clot-promoting capacity by a one-stage recalcification time of factor VII-deficient plasma. 3LL cells from either group of vitamin K-deficient animals gave significantly longer clotting times than controls (84 $\pm$ 4 and 82 $\pm$ 3 sec versus 42 $\pm$ 2 sec, $p < 0.001$, $n = 15$ for each of the 3 groups) corresponding to 18-25% of the activity of controls.

This effect was completely reversed by adding vit. K to drinking water of vitamin K-deficient animals. Administration of autologous prothrombin complex concentrate, sufficient to completely correct the thrombotest values for several hours before tumor cell harvest, did not modify the effect induced by vitamin K-deficiency on 3LL cells. This data suggests that cancer procoagulant may represent a new vitamin K-dependent activity; vitamin K-deficiency would thus display not only a plasmatic but also a "cellular" anticoagulant effect. The latter could be relevant to the reported antimetastatic effect of vitamin K-deficiency.