Time 16.00 cont.

0838 CANCER CELL-ENDOTHELIAL REACTIONS: THE MICROINJURY HYPOTHESIS AND LOCALIZED THROMBOSIS IN THE FORMATION OF MICROMETASTASES

B.A. Warren,* Department of Pathology, University of Western Ontario, London, Canada.

The two important cancer cell-endothelial reactions are the entrance of tumour cells into the blood stream and the exit of tumour cells from vessels. (i) In transplantable melanoma transplants in the hamster cheek pouch, tumour cells were found to migrate actively through the endothelial cell junctions and attenuated endothelium of "giant capillaries" at the edge of the tumours as early as 3 days after transplantation. (ii) The outcome of multiple episodes of microembolism by tumour cell emboli is dependent, in part, upon the structure of the specific microcirculatory units involved, the state of their endothelial lining and the size and deformability of the tumour cell emboli themselves. The Kawaguchi-Nakamura microinjury hypothesis suggests that the waves of emboli reach a specific microcirculatory bed and result in damage at certain sites in the unit due to prolonged temporary blockage of flow through the unit. This depends upon the presence of tumour emboli of a certain size and stiffness. The damage of the unit causes localized thrombus formation. Subsequent flow over the damaged area brings fresh emboli into contact with the injured region which is then favourably altered for the attachment and initial fixation of the circulating tumour cell emboli. In experiments with Walker 256 carcinoma, mouse thymoma and Hela cells these tumour cells were found to attach more firmly to damaged vessel wall than to regions of intact endothelium.

0839 IN VITRO MECHANISM OF PLATELET AGGREGATION (PA) BY PURIFIED PLASMA MEMBRANE VESICLES (PMV) SHED BY MOUSE 15091A TUMOR CELLS

G.J. Gasic*, J.L. Catalfamo, T.B. Gasic, and N. Avdalovic, Dept. of Pathology, University of Pennsylvania Sch. of Med. and Wistar Inst., Philadelphia, Pennsylvania, U.S.A.

Cell transformation leads to increased blebbing of the plasma membrane and release of PMV with capacity for PA in the presence of heparin or hirudin (G.J. Stewart et al.). This PA is preceded by a lag of 2 or more min. To investigate whether vesicle binding is a prerequisite for PA, ^{125}I-labelled vesicles were added to rat or mouse platelet rich plasma in the aggregometer, and radioactivity was measured in pellet and supernate fractions of samples taken at different time intervals. Most of the radioactivity was found with pelleted platelets, this association reached a maximal plateau at the mid point of the lag period. After ^{125}I-labelled vesicles interacted with platelets, characteristic polypeptide bands present in autoradiograms of SDS-PAGE of ^{125}I-labelled vesicles alone were also present in SDS solubilized pellet fractions. Binding of radioactivity by platelets depended on a plasma cofactor(s) other than fibronogen since gel filtered platelets failed to bind ^{125}I-labelled vesicles when fibronogen was added but did so in the presence of plasma; plasma was also required for aggregation. Isolation of the active fraction by step ammonium sulfate (AS) fractionation of plasma or its adsorption by barium citrate followed by AS elution, suggests that it may be a clotting factor(s) or a unique protein(s) that cofractionates with the prothrombin complex. In addition to its role in binding, this protein(s), when activated by vesicles or the platelet-vesicle complex, might also participate in PA.

0840 REQUIREMENT OF A PLASMA FACTOR FOR PLATELET AGGREGATING MATERIAL (PAM) EXTRACTED FROM TUMOR CELLS

E. Pearlstein and S. Karpatkin*, Depts. of Pathology & Medicine, NYU Med. Schl., NY, USA

Platelets are required for experimental tumor metastases and several lines of tumor cells aggregate platelets. We have extracted a sedimentable sialo-lipo-protein from SV40 transformed Balb C3T3 fibroblasts which is capable of aggregating heparinized rabbit or human platelet rich plasma (PRP) at 2.5 μg/ml via the release reaction; whereas a similar extract obtained from non-transformed 3T3 cells barely has activity at 40 μg/ml. PAM activity is not inhibited by collagenase (310 units/ml) but is inhibited by neuraminidase (2 mg/ml), trypsin (1 μg/ml) or boiled phospholipase A (0.1 μg/ml). Platelet aggregation is preceded by a 1 minute (avg.) lag period in PRP, and can be inhibited by EDTA (5mM), indomethacin (0.05 mM), adenosine (0.1mM) and dibutyrl cyclic AMP (0.1mM). Gel filtered (washed) platelets do not aggregate with PAM. However, reconstitution with 5% plasma results in typical PAM-induced platelet aggregation preceded by a lag period. PAM binds to platelets. When PAM is coupled to sepharose beads treated with cyanogen bromide, platelets adhere to these beads. PAM-sepharose beads aggregate PRP, as well as washed platelets in the presence of a plasma factor, following a lag period; sepharose beads alone or sepharose beads coupled to albumin or fibrinectin have no effect. The lag period can be abolished by prior incubation of plasma with PAM for 5 min at 37°C. The plasma factor is labile at 56°C for 30 min. However, the PAM-plasma product is stable to heating at 56°C for 30 min. These data suggest that the plasma factor may be a labile enzyme which con-