

ABSENCE OF ROLE FOR PLATELET-DERIVED ADP IN TUMOR CELL-INDUCED PLATELET AGGREGATION. Eva Bastida, Antonio Ordinas and G. A. Jamieson. Hospital Clínico y Provincial, Universidad de Barcelona, Spain, & American Red Cross, Bethesda, MD, USA.

Interaction with platelets is thought to be a major factor in the metastatic dissemination of human tumors. Previous studies using non-human lines have suggested that aggregation induced by tumor cells is dependent on the release of platelet-derived ADP. We have re-examined this phenomenon in heparinized platelet rich plasma with two human tumor cell lines: Hut 20 derived from a large cell carcinoma of the lung and U87MG derived from a glioblastoma. U87MG caused a single irreversible wave of aggregation simultaneously with the onset of platelet secretion and was inhibited by heparin and hirudin but not by apyrase. In contrast the Hut 20 line gave an initial reversible wave followed by a second irreversible wave which then led to secretion. Aggregation was unaffected by heparin or hirudin but was inhibited by apyrase. Blockage of the cyclooxygenase pathway and platelet secretion by aspirin did not affect the first wave of Hut 20-induced aggregation but gave moderate reductions in the second wave (~50%) and with U87MG (~25%). With both cell lines, aggregation was completely blocked by protease inhibitors, and by removal of  $Ca^{++}$  and did not occur with gel-filtered platelets. These results suggest that platelet aggregation by the Hut 20 line is induced by ADP released from the tumor cell themselves while aggregation induced by the U87MG line is dependent on the development of a procoagulant activity of the tumor cell surface.

## 1360

REDUCTION OF TUMOR METASTASIS BY COBRA VENOM FACTOR (CVF) VIA INHIBITION OF PLATELET AGGREGATION (PA). G.J. & T.B. Gasic. Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

Tumor cells or plasma membrane vesicles shed by these cells can induce PA *in vitro*. To induce PA, vesicles first bind to platelets and then, subsequently, promote platelet release or plasma generation of a platelet aggregating material. The first step of this pathway is mediated by complement. If, for example, plasma is depleted of the complement component C3 by CVF neither binding nor PA occurs.

Blood-borne tumor cells can also aggregate platelets and this activity appears to facilitate their establishment as metastases. Since CVF at lower doses prevents thrombocytopenia and death of mice injected with tumor vesicles, we investigated the effect of CVF on lung colonies produced by *in vivo* inoculation of syngeneic tumor cells (T241 and B16). CVF (1-4 units) was given *ip* at 0, 4, 10, and 24 hr and tumor cells ( $5 \times 10^4$  and  $2 \times 10^4$ ) inoculated *iv* 4 hr after the last injection of CVF. Two weeks later mice were killed and lung tumors counted. As shown by the Table lung tumors were significantly reduced in mice receiving the lowest doses of CVF but increased in the group treated with the highest doses.

TUMOR	CVF/24HR	AVG. NO. OF LUNG TUMORS AS % OF CONTROLS
T241	4 units	44
T241	8 units	81
T241	16 units	132
B16	4 units	71

These results are probably due to different effects of different doses of CVF as inhibitor of PA and suppressor of immune responses, the lowest doses being more beneficial because of their greater antiplatelet and lower immune suppressing activity.

## 1359

INTERACTION OF PLATELETS AND TUMOR CELLS: DIFFERENTIATION BETWEEN NINE HUMAN TUMOR CELL LINES BASED ON AGGREGATION AND EFFECTS OF APYRASE, HIRUDIN AND PHOSPHOLIPASE D. G. A. Jamieson, Eva Bastida and Antonio Ordinas. American Red Cross Blood Services Laboratories, Bethesda, MD 20014; Hospital Clínico y Provincial, Universidad de Barcelona, Spain.

Aggregation mechanisms have been examined in a homologous system using heparinized human platelet-rich plasma with cell lines derived from human tumors. The A549 (epithelial lung carcinoma) and Hut<sub>23</sub> (adenocarcinoma) did not aggregate platelets at  $10^6$  cells/ml. Other cell lines could not be classified by aggregation pattern alone since all gave biphasic or quasibiphasic patterns. HT 29 (adenocarcinoma), SKBR3 (adenocarcinoma), HT 144 (melanoma) and Hut 20 (large cell lung carcinoma) were inhibited by apyrase and phospholipase D but not by hirudin: aggregation induced by this group is probably primarily dependent on ADP. Aggregation by SKNMC (mesothelioma) and Hut 28 (mesothelioma) was inhibited by hirudin and phospholipase D but not by apyrase. Aggregation by this second group probably involves activation of the clotting system in the early stages but can be differentiated from a similar mechanism with U87MG since the latter is not inhibited by phospholipase D. Phospholipase C had no effect on any cell line and phospholipase A<sub>2</sub> inhibited all cell lines, as did its hydrolytic product, lysolecithin. Platelet aggregating material (PAM) could not be isolated by urea extraction of any of these human tumor cells. These results suggest that various inhibitors are necessary to allow classification of mechanisms of tumor cell-induced platelet aggregation.

## 1361

HYPERAGGREGABILITY OF PLATELETS IN CANCER, ESPECIALLY IN REFERENCE TO GENESIS OF DIC. H. Yamazaki, Y. Yahara, T. Motomiya, K. Tanoue, I. Isohisa, S.M. Jung and Y. Onozawa. Tokyo Metropolitan Institute of Medical Science and Tokyo Metropolitan Komagome Hospital, Tokyo

To clarify the role of platelets in the genesis of DIC in cancer, platelets of cancer patients with and without DIC were examined. Patients studied were 29 cases with cancer in stomach, 17 in lung, 7 in pancreas, 6 in liver (hepatoma), 6 in throat, nose and jaw, 2 in the gall bladder and biliary duct, 2 in uterus and 1 each in the small bowel, rectum and prostate, and 1 each with osteosarcoma, mesothelioma and choriocarcinoma. All patients were in stage 3 or 4. 105 healthy controls were also studied. They were evaluated on a scale of coagulation abnormalities, one point was given for each of the following criteria full-filled, and the score (0 to 4) was used. 1. Platelet count  $<150 \times 10^3/\mu\text{l}$ . 2. Prothrombin time prolonged more than 1 sec over control and/or activated partial thromboplastin time prolonged more than 10 sec over control. 3. Fibrinogen  $<250$  mg/dl (mean fibrinogen value of the cancer patients minus 1 SD). 4. FDP  $>20$   $\mu\text{g/ml}$ . The patients were distributed with 27 % for score 0, 38 % for 1, 20 % for 2, 7 % for 3 and 8 % for 4. Degrees of abnormality in groups with scores of 3 and 4 were significant when compared to scores 0 and 1, but score 2 was not clearly distinguishable. Platelet mode volume in score 4 was smaller than the other groups. Platelet aggregation by adrenaline and ADP decreased in score 3 and 4, while it increased significantly in score 0 and 1 respectively ( $P < 0.01 - 0.05$ ). The mean value of plasma  $\beta$ -TG in the cancer patients as a whole ( $44 \pm 24$  ng/ml) was significantly higher than that of control ( $22 \pm 13$  ng/ml) ( $P < 0.01$ ). PF4 showed the same tendency. During the time course of the disease, hyperaggregability of platelets associated with increases in  $\beta$ -TG and PF4 was observed before an appearance of DIC syndrome in several cases. The results suggest the existence of hyperfunction of platelets in cancer patients and the possibility of triggering mechanism of such activated platelets in the genesis of DIC in cancer.