Platelet Interaction With a Pancreatic Ascites Tumor

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The mechanism leading to the hypercoagulability in pancreatic carcinoma is unclear. The rapid progress of the disease after its diagnosis and the inaccessibility of the tumor make studies on the mechanism difficult in man. With the successful induction of this malignancy and conversion of it into an ascites tumor in Syrian golden hamsters, interactions between isolated tumor cells and individual hemostatic components can be investigated. In this paper, studies on *in vitro* tumor cell–platelet interactions and some hemostatic changes in hamsters following intravenous injection of isolated tumor cells are described. Freshly isolated tumor cells and tumor-cell sonicates, but not those that had been kept at 4 or −70 C overnight, induced comparable aggregation of human platelets in both heparinized and citrated platelet-rich plasmas (hPRP and cPRP). The aggregation was not followed by clot formation; a specific synthetic thrombin inhibitor had no effect on the aggregation in either hPRP or cPRP. Washed and gel-filtered platelets, even in the presence of 5% of citrated or heparinized platelet-poor plasma (cPPP or hPPP) failed to be aggregated by tumor cells. Tumor-cell–induced platelet aggregation was accompanied by thromboxane formation and serotonin release, both of which were several orders of magnitude greater in cPPP than in hPRP. Aspirin, apyrase, and PGI_2 all inhibited tumor-cell–induced platelet aggregation in both PRPs, but the inhibition by aspirin was minimal. Intravenous infusion of isolated tumor cells into normal hamsters resulted in a 50% reduction of platelet count and a 20–30% decline in antithrombin III and fibrinogen. Platelet aggregates and fibrin strands were seen in the lungs of these animals. (Am J Pathol 1986, 122: 160–168)

THE INTERACTIONS between platelets and various types of human and experimental tumors have been studied by several groups of investigators.[1–9] Some tumor cell lines activate homologous and heterologous platelets *in vitro*; others do not. Several mechanisms, including promotion of thrombin formation from plasma procoagulants, release of adenosine diphosphate (ADP) from platelets, and involvement of complement and tumor cell surface vesicles, are known to underlie the activation of platelets by tumor cell lines.[10–13] Regardless of which mechanism is responsible, the activation of platelets by most tumor cell lines occurs only in heparinized and not in citrated platelet-rich plasma. It has been found that tumors that induce platelet aggregation *in vitro* seem to have a greater propensity for metastatic spread.[14–17] Intravenous injection of those tumors that are capable of activating platelets *in vitro* often leads to thrombocytopenia and deposition of platelets in the lungs of experimental animals.[14,15] Platelet–tumor-cell interactions *in vivo* are thought to be an important facet of the metastatic process.[18–22] Therefore, it is necessary to elucidate the mechanism underlying the interactions between platelets and a given type of tumor if the metastatic process of that tumor is to be better understood.

Pancreatic cancer (usually ductal adenocarcinoma) is a highly metastatic tumor with profound effects on the hemostatic system.[23] The mechanisms underlying these effects have not been closely studied, partly because of the rapid progress of the disease after its diagnosis in man and also because of the technical difficulties in obtaining these tumors for study. The successful induction of a transplantable pancreatic ductal adenocarcinoma and its conversion into an ascites tumor in experimental animals in recent years[24,25] have provided an opportunity for close examination. In this paper, we report the interactions between an experimental pancreatic ductal ascites tumor and platelets and the observation of some hemostatic changes following an intravenous injection of isolated tumor cells in hamsters.

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Materials and Methods

Ascites Tumor

Pancreatic ductal adenocarcinoma was originally induced in the Syrian golden hamsters by repeated injection of N-nitroso-bis(2-oxopropyl)amine and converted to an ascites tumor.[24,25] The tumor was carried by intraperitoneal injection of 0.25 ml of ascitic fluid (~0.25 $\times$ 10^6 tumor cells) into weanling Syrian golden hamsters. After 7–10 days, approximately 10 ml of ascitic fluid was withdrawn and centrifuged at 80*g* for 4 minutes. Tumor cells, now in the lower two-fifths of the tube, were washed with phosphate-buffered saline (PBS) for further removal of contaminating hematopoietic cells. Isolation of single tumor cells was achieved by the method of Becich and Reddy[26] with some modifications. First, the tumor cells were subjected to two 15-minute incubations with collagenase (150 units/ml; Worthington Biochemicals, Freehold, NJ) in Krebs–Ringer buffer (pH 7.6). Following this, the cells were exposed to EDTA (2 mM) in calcium/magnesium-free Krebs–Ringer buffer three times for 5 minutes each and washed three more times with PBS. The preparation was then filtered through a 37-μ nylon mesh for removal of undissociated tumor cell clumps. The filtered cells were examined for purity and viability (trypan blue exclusion) under a phase-contrast microscope. All tumor cell preparations were >95% pure and >90% viable. The cell count was adjusted to about 5 $\times$ 10^6/ml and used in platelet aggregation studies. For some preparations, a portion of it was fixed for transmission electron microscopy,[27] and another was sonically disrupted.

Hamster Blood

In preliminary studies, 2.5–3 ml of blood, anticoagulated with 1/10 volume of buffered sodium citrate or sodium heparin (5 U/ml blood), was removed from the inferior vena cava of normal hamsters anesthetized with sodium pentobarbital (15 mg/kg, Butler Co., Columbus, Ohio). Blood samples were used within one-half hour.

Human Platelets

Platelets were prepared from blood donated by healthy laboratory personnel not exposed to platelet-inhibiting agents in the preceding 10 days. Anticoagulation was achieved with either sodium heparin (5 U/ml blood) or 1/10 volume of 0.1 M buffered sodium citrate. Platelet-rich and platelet-poor plasmas (PRP and PPP) were prepared by differential centrifugation. Platelet counts in PRP ranged from 2.9 to 5.6 $\times$ 10^8/ml. In separate experiments, platelets were either washed and resuspended in an artificial medium according to Rossi[28] or gel-filtered.[29] Platelet counts in these samples were 3.5 to 4.5 $\times$ 10^8/ml. In the presence of $CaCl_2$, washed platelets responded to thrombin, collagen, and arachidonate; gel-filtered platelets responded also to (ADP) when fibrinogen or plasma was included.

Chemicals

ADP, apyrase, and acetylsalicylic acid were purchased from Sigma Chemical Company (St. Louis, Mo). Prostaglandin I_2 was kindly provided by Dr. John Pike of the Upjohn Company (Kalamazoo, Mich). MCI-9038, (2R-4R)-4-methyl-1-[Nα- (3-methyl-1,2,3,4-tetrahydro-8-quinoline sulfonyl)-L-arginyl]-2-piperidene carboxylic acid monohydrate, a specific inhibitor of thrombin,[30,31] was kindly provided by Mitsubishi Chemical Industries (Tokyo). Collagen was prepared from the skin of a human cadaver according to Green et al.[32]

Platelet Aggregation Studies

Hamster Blood

An aliquot of 1.5 ml of hamster blood was placed in a 5-ml plastic beaker and mixed with 0.2 ml of tumor cells or PBS. The mixture was stirred on a stirring plate (~800 rpm) for 5 minutes. Then, an aliquot of 0.5 ml of the mixture was withdrawn to a tube containing 2 ml of 0.077% EDTA, and another aliquot to a tube containing EDTA plus 4% formalin. After proper handling of the samples according to the method of Wu and Hoak[33] for circulating platelet aggregates, platelets in the PRP fraction were counted by an ELT-8000 cell counter (Ortho Diagnostics, Westwood, Mass). If tumor cells were to cause hamster's platelets to aggregate, the aggregates would be fixed in EDTA–formalin and disperse in EDTA. As a result, the platelet count in the PRP of the former would be less than that in the latter. Therefore, when a ratio of <0.8 between platelet count in the EDTA–formalin sample and that in the EDTA sample is attained, it is indicative of presence of platelet aggregates in the EDTA–formalin sample. A drop of the remaining 0.5 ml of blood was used for a blood smear, and the rest of the blood was macroscopically examined 60 minutes later for the presence of fibrin clots. The blood smear was stained with Wright–Giemsa stain and examined under a light microscope.

Human Platelets

Platelet aggregation was measured photometrically with Chrono-log platelet aggregometers (Chrono-log Corp., Havertown, Pa) as previously described.[27] To

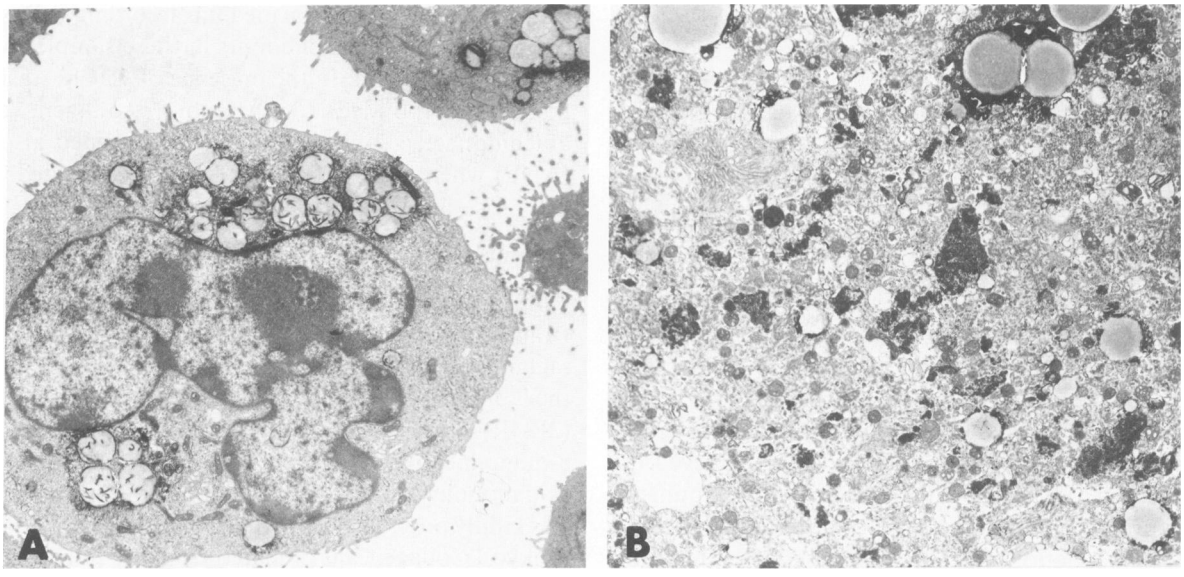

Figure 1—Electron micrographs of isolated pancreatic ductal ascites tumor cells (**A**) and tumor-cell sonicates (**B**). Numerous cytoplasmic projections and lipid bodies are seen in many tumor cells. Subcellular organelles and membranous structures constitute the bulk of the sonicated tumors. (×3750)

0.35 ml of citrated or heparinized platelet-rich plasma (cPRP or hPRP) was added 0.05 ml of tumor cell suspension or tumor cell sonicate; the aggregation reaction was recorded for 5 minutes. For washed and gel-filtered platelets, 0.02 ml $CaCl_2$ (final concentration, 1 mM) and 0.02 ml cPPP or hPPP were included. Collagen (1/6400 of stock) and PBS were used in place of tumor cells as positive and negative controls.

Thromboxane Formation and ^{14}C-Serotonin Release

Thromboxane formation was assayed in the supernatant of tumor-cell-aggregated human platelet samples (n = 4) with a radioimmunoassay (RIA) method. RIA kits for thromboxane B_2 were purchased from New England Nuclear, Boston. After 4 minutes of an aggregation reaction, the cuvette was placed on ice and centrifuged at 4 C. The supernatant was stored at −70 C until assay. ^{14}C-serotonin release was studied with the method of Jerushalmy and Zucker.[34] Platelets in citrated and heparinized plasma (n = 8) were labeled with radioactive serotonin (57 mCi/mM; Amersham Co, Arlington Heights, Ill) and then exposed to tumor cells; separate aliquots of 4 of the 8 samples were also exposed to collagen (1/6400 dilution). Radioactivity in the supernatant was determined.

Ultrastructure of Tumor-Cell-Aggregated Platelets

At ½, 2, and 4 minutes following the addition of tumor cells to cPRP or hPRP, a volume of 3% cacodylate-buffered glutaraldehyde (pH 7.5) was introduced into the platelet aggregation cuvettes and the fixed samples were prepared for transmission electron microscopy.

Potentiation of Tumor-Cell-Induced Aggregation by ADP and Collagen

In these experiments, hPRP or cPRP was first mixed with a subaggregatory strength of ADP (final concentration, 0.25–0.50 μM) or collagen (1/12,800 or 1/25,600 dilutions); then a subaggregatory strength of tumor-cell suspension was added (1–2 × 10^6 cells/ml).

Inhibition of Tumor-Cell-Induced Platelet Aggregation

Aspirin (final concentration, 0.2 to 1.0 mM), apyrase (0.125 to 1.0 mg/ml), PGI_2 (5–25 ng/ml), or MCI-9038 (5–100 μM) was mixed with cPRP or hPRP for 30–60 seconds, prior to the addition of tumor cells. The effect of these compounds on tumor-cell-induced aggregation was recorded.

Infusion of Tumor Cells

The effect of intravenous infusion of tumor cells was studied in normal hamsters. The animals were anesthetized with sodium pentobarbital (intraperitoneal, 15 mg/kg; Butler Co, Columbus, Ohio). The abdomen was opened by a midline incision, and the inferior vena cava was exposed and cannulated. A small volume of blood (0.5 ml) was taken from the cannula for baseline platelet count, fibrinogen, and Antithrombin III levels. Then an aliquot of 0.25 ml of tumor-cell suspensions con-

taining 1.5–6.0 × 10^6 tumor cells was injected into the cannula, which was immediately flushed with 0.2 ml PBS. At 2, 5, 10, 30, and 60 minutes after infusion, a drop of blood was obtained from the cannula for a platelet count; a larger volume of blood was obtained from some animals at 5 and 60 minutes for fibrinogen and Antithrombin III determinations. Fibrinogen levels were assayed by a thrombin clotting method.[35] Antithrobmin III was determined by a fluorogenic substrate technique with the aid of the American Dade Protopath system.[36] At the end of 60 minutes, 10 ml of glutaraldehyde was slowly infused into the cannula. Fragments of the lungs were removed and prepared for transmission electron microscopy. PBS, instead of tumor-cell suspensions, was used in control animals.

Results

Tumor Cells and Cell Sonicates

Isolated tumor cells retained their ultrastructural integrity and growth potential when injected into the intraperitoneal cavity of hamsters. Individual cells with numerous cytoplasmic projections were observed by electron microscopy. The cytoplasm contained the usual complement of organelles with an increased number of lipid droplets noted in some (Figure 1A). Almost all tumor cells were disrupted by sonication; only occasionally was an undisrupted cell encountered. Cell debris and vesicular and membranous structures constituted the bulk of the sonicated sample (Figure 1B).

Platelet Aggregation in Hamster Blood

Upon the addition of tumor cells to hamster whole blood, the ratio of platelet count between EDTA-formalin and EDTA samples was 0.5 for citrated and 0.3 for heparinized samples (n = 2), respectively. The ratio was 0.9–1.1 in similar samples added with PBS. Large platelet aggregates—some were surrounded by tumor cells and others not—were seen on the smears of blood stirred with tumor cells. Tumor cells were not found in the middle of platelet aggregates. No platelet aggregate of any size was seen in the smears of blood stirred with PBS. No fibrin clots were found in any of the blood samples 60 minutes after the addition of tumor cells or PBS.

Aggregation of Human Platelets

Freshly isolated tumor cells induced comparable platelet aggregation in hPRP and cPRP (Figure 2). Clot formation was not observed in either. The potency of platelet-aggregating activity of tumor cells varied from

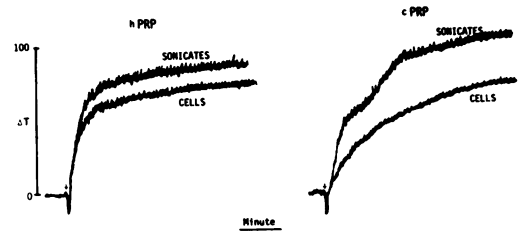

Figure 2—Aggregation of platelets, depicted by increase in light transmission, in heparinized and citrated human platelet-rich plasmas (hPRP and cPRP) induced by hamster pancreatic ductal ascites tumor cells or tumor-cell sonicates.

preparation to preparation. For a given tumor cell preparation and a given PRP, the aggregation time as well as the extent of aggregation were related to the tumor cell count. Tumor cells <10^6/ml failed to elicit aggregation in either hPRP or cPRP. A lag period was inconspicuous in some PRPs when the aggregation was induced by tumor cells >5 × 10^6/ml. Tumor cell sonicates were also capable of inducing aggregation in both PRPs; the aggregation was usually more vigorous than that induced by intact tumor cells from which the sonicates were made (Figure 2). Neither the last wash of tumor cells nor the supernatant of the sonicates (100,000*g*, 30 minutes) induced platelet aggregation. The platelet-aggregating activity of tumor cells and sonicates disappeared after they were kept at 4 C overnight or at −70 C for various periods of time from 24 hours to 3 weeks.

Washed platelets were not aggregated by freshly prepared tumor cells, even in the presence of 1 mM $CaCl_2$ and 5% of cPPP or hPPP. Gel-filtered platelets were aggregated following the addition of tumor cells or PBS in the presence of 5% cPPP and 1 mM Ca^{2+}. In these cases, the aggregation time was long and the aggregation was immediately followed by clotting. The aggregation of gel-filtered platelets by tumor cells or PBS was inhibited by MCI-9038. Neither platelet aggregation nor plasma clotting was observed in gel-filtered platelet samples when hPPP was substituted for cPPP.

Thromboxane B_2 (TxB_2) and Serotonin Release

Tumor-cell–induced aggregation in human PRPs was accompanied by thromboxane formation and release of prelabeled serotonin. The quantities of thromboxane formed and serotonin released were different in cPRP and hPRP. Sixfold more TxB_2 was found in cPRP samples than in hPRP samples, in spite of a comparable degree of aggregation in both PRPs (Table 1). The release of ^{14}C-serotonin from platelets challenged by tumor cells varied considerably among individual experiments. Overall, there was threefold more radioactivity in the supernatant of cPRP than in hPRP exposed

Table 1—Extent of Aggregation (Percent Light Transmission) and Thromboxane Formation of Platelets in cPRP and hPRP Following Exposure to Pancreatic Ascites Tumor*

	% Aggregation	TxB_2 (ng/ml)
cPRP	63.0 ± 2.2	76.0 ± 10.3
hPRP	68.0 ± 2.9	12.7 ± 2.2

* Mean ± SEM; n = 4.

to the same tumor cells and aggregated to the same degree. The percentage of ^{14}C-serotonin uptake by platelets in cPRP and hPRP and the release of radioactivity from platelets in these two samples following exposure to collagen were practically identical (Table 2).

Ultrastructure of Tumor-Cell-Aggregated Platelets

Loosely aggregated platelets, some of them apparently adhering to tumor cells, were frequently encountered in samples fixed at ½ minute (Figure 3A). In these instances, ultrastructural changes of platelets were largely confined to shape changes without degranulation. On the other hand, large and tightly packed platelet aggregates, with many of the individual platelets degranulated, were seen in samples fixed at 2 and 4 minutes (Figure 3B). Tumor cells were not found among aggregated platelets; only occasionally was a tumor cell seen near an aggregate. These ultrastructural changes were observed in both cPRP and hPRP samples. Fibrin strands were never seen in any of the 4 cPRP and hPRP samples fixed at any time. It is worth noting that tumor cells stirred in human PRPs were more electronlucent than those not stirred in PRPs.

Additive Effect of Tumor Cells and Other Aggregants

Subaggregatory strengths of tumor cells and ADP or collagen caused platelet aggregation in an additive, rather than synergistic, fashion.

Inhibition of Platelet Aggregation

Aspirin, apyrase, and PGI_2 inhibited platelet aggregation induced by tumor cells. Each of these compounds caused comparable inhibition in cPRP and hPRP. The manner of inhibition by these agents was different. Aspirin caused only minimal inhibition, whereas apyrase prevented the secondary phase of aggregation. PGI_2 of >10 ng/ml nearly completely inhibited the aggregation. Figure 4 is a composite of the effects of inhibitors on tumor cell induced platelet aggregation.

MCI-9038 had no effect on tumor cell-induced aggregation; it annihilated the aggregation induced by thrombin (Figure 5).

Infusion of Tumor Cells

Infusion of tumor cells resulted in a decline in platelet count, fibrinogen and antithrombin III (At-III) (Table 3). By the end of 1 hour, a 50% decline in platelet count and a 20–30% reduction in fibrinogen and At-III was recorded. These changes were not observed in animals given injections of the vehicle.

Electron-microscopic examination of the lungs of experimental animals revealed the presence of platelet aggregates in some capillary lumens. These aggregates were composed of tightly packed platelets, some of them degranulated. Fibrin strands were invariably found in or near the platelet aggregates (Figure 6). However, tumor cells were not found to be trapped within the platelet aggregates. Neither platelet aggregates nor fibrin strands were observed in control specimens.

Discussion

The isolated, collagenase–EDTA dissociated pancreatic ascites tumor cells of the hamster induced platelet aggregation in both heparinized and citrated hamster blood and in heparinized and citrated human platelet-rich plasmas (hPRP and cPRP). The tumor cells also caused reduction of platelet count, fibrinogen, and At-III in normal animals. Because of the limitation in the quantity of blood that could be obtained from hamsters, human platelets were used for the studies on tumor-cell–platelet interactions. Human platelet aggregation induced by these tumor cells is rather unique in several respects. First, the tumor cells are capable of activating platelets in heparinized and citrated PRP samples. Most other tumor cells are only

Table 2—Percent Uptake of ^{14}C-Serotonin, and Percent Aggregation and Release of Radioactivity of Platelets in cPRP and hPRP Induced by Tumor Cells and Collagen*

	% Uptake serotonin (n = 8)	Tumor cells (n = 8)		Collagen (n = 4)	
		% Aggregation	% Release	% Aggregation	% Release
cPRP	77.3 ± 0.8	45.5 ± 6.2	15.4 ± 5.2	57.7 ± 13.2	27.2 ± 5.2
hPRP	78.6 ± 0.6	43.0 ± 2.9	4.6 ± 1.6	63.3 ± 9.6	32.2 ± 7.5

* Mean ± SEM.

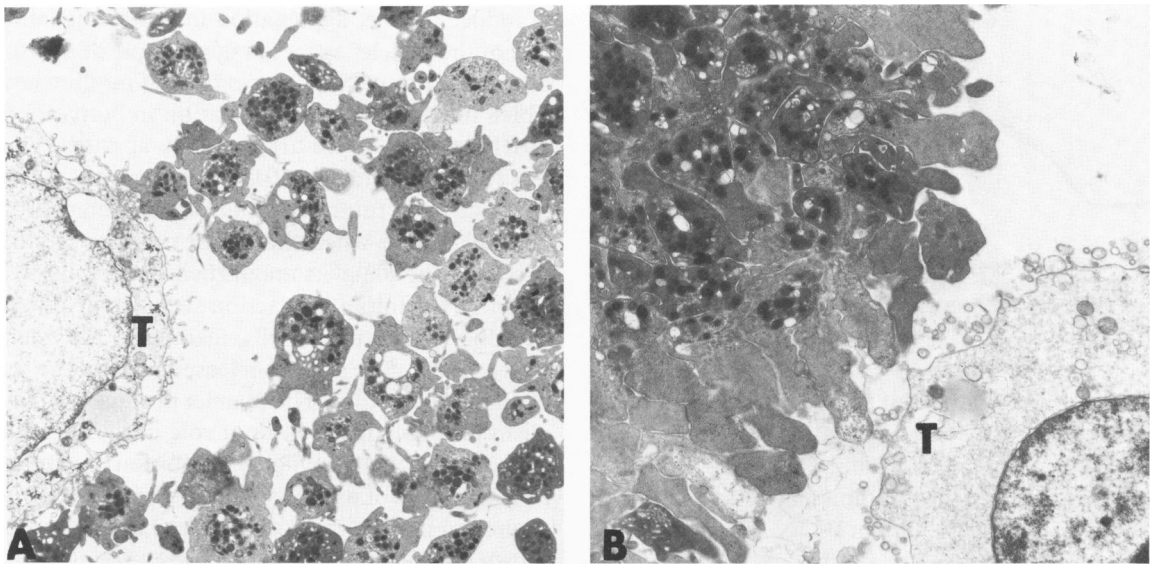

Figure 3—Ultrastructure of platelets in cPRP aggregated by tumor cells. Sample was fixed at ½ minutes (**A**) or 4 minutes (**B**) after the introduction of tumor cells. Loosely aggregated platelets, some of them apparently adhering to the tumor cell (*T*), are seen in the former. Large and tightly packed platelet aggregates are observed in the latter. However, tumor cells are not found among aggregated platelets; only occasionally was a tumor cell seen near an aggregate. Similar ultrastructural changes are seen in hPRP samples. (**A**, ×3600; **B**, ×4850)

able to do so in hPRP.[1-9] Recently, the virally transformed murine fibroblasts (SV3T3)[8] and the crude extracts of a rat fibrosarcoma (MC_{28})[37] have been found to cause comparable aggregation of human platelets in cPRP and hPRP; the mouse hepatoma 134 induced platelet aggregation in mouse cPRP and washed mouse platelets.[12] Secondly, in spite of a comparable degree of aggregation of human platelets in cPRP and hPRP, some chemical changes in platelets were at least quantitatively different. Thromboxane formation and serotonin release were prominent features in cPRP but were far less so in hPRP. It has been reported that, by virtue of lowering the Ca^{2+} concentration, sodium citrate is instrumental for serotonin release induced by ADP.[38,39] It is not known whether citrate would have a similar effect on thromboxane formation. Regardless of the mechanism leading to a differential release of serotonin and activation of prostaglandin metabolism, release of ADP from platelets appears to be a common feature in both PRPs, as evidenced by a similar inhibition of aggregation by apyrase in both PRPs. The ADP must be exclusively derived from platelets rather than tumor cells, because the supernatant of sonicated tumor cells, which should contain tumor cell ADP, failed to aggregate platelets. Thrombin is known to be responsible for platelet aggregation induced by several tumor cell lines.[10-12] However, this does not appear to be the case in the pancreatic ascites tumor-induced platelet aggregation. A synthetic thrombin inhibitor had no effect on ascites tumor-cell-induced aggregation, and no fibrin was observed in any of the aggregated platelet samples. Neither was fibrin present in heparinized and citrated hamster blood 60 minutes after exposure to tumor cells.

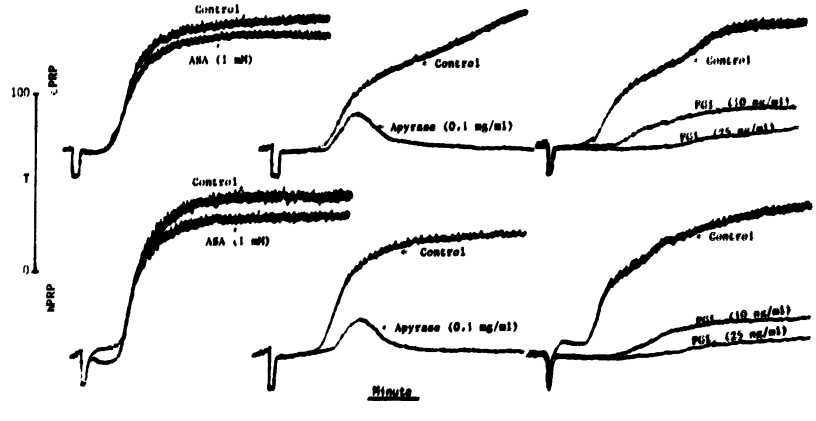

Figure 4—Inhibition of tumor-cell-induced platelet aggregation in cPRP and hPRP by aspirin, apyrase, and PGI_2. These platelet inhibitors caused similar degrees of inhibition of cPRP and hPRP samples.

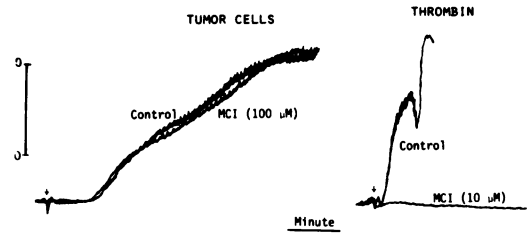

Figure 5—Absence of effect of tumor-cell-induced platelet aggregation (in cPRP) by a high concentration of a synthetic thrombin inhibitor (MCI), whereas one-tenth the concentration of the inhibitor completely prevented thrombin (0.25 μ/ml)-induced aggregation.

Ultrastructural studies of human PRP samples confirmed extensive platelet aggregation in both citrated and heparinized specimens. The relative electron lucency of tumor cells in these samples, as compared with tumor cells not stirred in human PRPs, may simply be a result of damage caused by stirring, or of partial lysis mediated by complement activation in a heterologous system. Because the supernatant of sonicated tumor cells did not cause platelet aggregation, a role of tumor cell lysis and release of internal contents in the induction of platelet aggregation is doubtful.

The inability of washed and gel-filtered platelets to respond to freshly isolated tumor cells and the inability of stored tumor cells to induce platelet aggregation in freshly prepared PRPs, merit some discussion. In the former instances, either a large quantity of plasma factors, more than what is needed for ADP-induced aggregation, is required for the ascites tumor-induced aggregation, or something on the platelets with which it is essential for tumor cells to interact has been lost in the washing and gel-filtration processes. The aggregation of gel-filtered platelets in the presence of cPPP and Ca^{2+}, which is always followed by clotting, is undoubtedly due to the generation of thrombin from cPPP. The thrombin generation requires no contribution or participation of tumor cells. Similar aggregation-clotting patterns were observed in gel-filtered platelet samples containing cPPP and Ca^{2+} when tumor cells were replaced with PBS. Gel-filtered platelets have been known to contain elevated procoagulant activities.[40] The inability of stored tumor cells and tumor cell sonicates to induce platelet aggregation indicates the labile nature of the platelet aggregating principle of the isolated pancreatic tumor cells. The extract of rat fibrosarcoma which induces platelet aggregation in both cPRP and hPRP remains active after storing at room temperature for 3 days and at −40 C for 1½ months.[37] Overall, the pancreatic tumor cell and platelet interaction can only take place when the tumor cells are freshly isolated and the platelets are in their native plasma. Apparently, the initial interaction seems to involve platelet adhesion to tumor cells, leading to the release of platelet ADP. The platelet-released ADP in turn causes platelet aggregation. The platelet protaglandin metabolism probably plays a minor role in the aggregation, as suggested by the negligible inhibition of aggregation by a high dose of aspirin. It should be pointed out that studies on other tumor-cell-induced aggregations have shown a positive correlation between the ability of the tumor cells to induce platelet aggregation and their ability to stimulate thromboxane formation.[41]

In the present study, subaggregative strengths of tumor cells and ADP or collagen caused platelet aggregation in an additive rather than a synergistic manner, as has been described for other tumor cell lines.[37] Release of ADP from parenchymal tissue and exposure of collagen could conceivably occur during an invasion process. Under those circumstances, the likelihood of tumor-cell-platelet interaction would be increased. Aspirin had only a negligible effect on pancreatic tumor-cell-induced aggregation. If tumor-cell-platelet interaction does play a pivotal role in tumor metastasis, the effect of aspirin on the spread of the pancreatic tumor may be limited. PGI_2 has been shown to be a potent inhibitor of platelet aggregation induced by several rodent tumor cell lines.[42] Infusion of PGI_2 into mice has been reported to result in a dose-dependent decrease in lung tumor colony formation in animals given injections of B16a tumor cells.[43,44] More recently, PGI_2 has been found ineffective in preventing the development of pulmonary tumor metastasis after an intravenous injection of CT26, Lewis lung, or B16a tumor cells in mice.[45] Our finding of inhibition of pancreatic tumor-induced aggregation by this prostanoid simply reiter-

Table 3—Changes in Platelet Counts, Fibrinogen, and Antithrombin III (At-III) Levels in Hamsters Given PBS or Tumor Cells*

Minutes after infusion	Platelets (× $10^3/\mu l$)		Fibrinogen (mg/dl)		At-III (%)	
	Control (n = 2)	Exp (n = 6)	Control (n = 2)	Exp (n = 4)	Control (n = 2)	Exp (n = 16)
0	555	655.8 ± 63.4	165	142.5 ± 21.3	95	87.3 ± 3.5
2	593	653.2 ± 43.1	–	–	–	–
5	688	479.2 ± 22.7	–	110.0 ± 20.0	–	–
10	613	448.0 ± 37.2	–	–	–	–
30	707	405.8 ± 33.1	–	–	–	–
60	556	336.0 ± 32.6	150	105.0 ± 15.2	95	70.5 ± 3.2

* Average values for control and mean ± SEM for experimental animals are given.

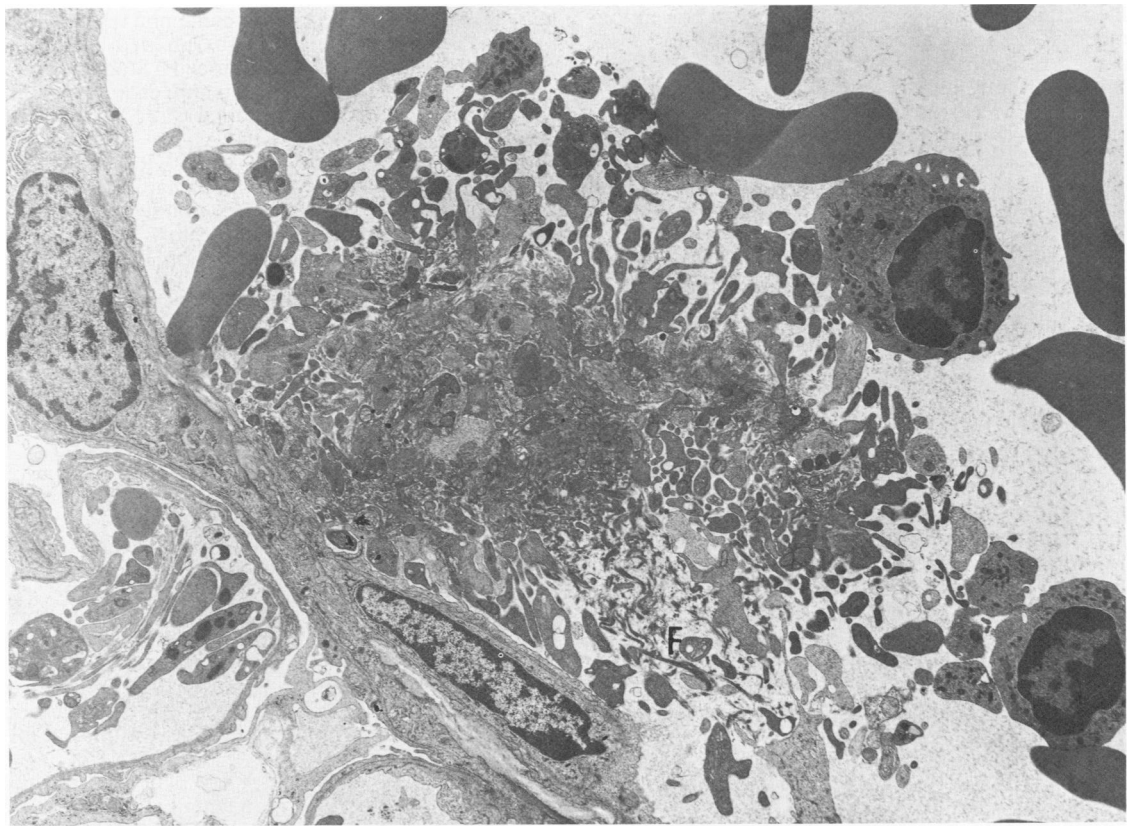

Figure 6—Electron micrographs of lungs of normal hamsters fixed at 1 hour after an intravenous injection of isolated tumor cells. Platelet aggregates intermingled with fibrin (*F*) are present in the former. (×5100)

ates the importance of intraplatelet cyclic adenosine monophosphate in platelet aggregation initiated by any agent.

Thrombocytopenia and concentration of radiolabeled platelets in the lungs of rats following an intravenous injection of Walker 256 tumor cells were described over a decade ago.[14,15] Our findings of decreased platelet count in the peripheral blood and deposition of platelet aggregates in the lungs of hamsters given injections of isolated pancreatic ascites tumor cells are consistent with those earlier observations. We further demonstrate a decline in fibrinogen and At-III, parallel to but smaller in degree than the thrombocytopenia. Although fibrin strands had never been observed in tumor-cell–aggregated platelet samples *in vitro*, they were invariably present in the platelet aggregates in the lung microvasculature. Presumably, fibrin formation is an indirect result of platelet activation in an *in vivo* system where no exogenous anticoagulant is introduced. The possibility that the tumor cells may contain procoagulants requires further exploration. If our findings can be extrapolated to human conditions, then platelet activation by pancreatic tumor cells disseminating via the vascular tree could be a major mechanism underlying the hypercoagulability so frequently seen in pancreatic cancer patients.

References

1. Gasic GJ, Koch PAG, Hsu B, Gasic TB, Niewiarowski S: Thrombogenic activity of mouse and human tumors: Effects on platelets, coagulation and fibrinolysis, and possible significance for metastasis. Z Krebsforsch 1976, 86:263–277
2. Gasic GJ, Gasic TB, Jiminez SA: Platelet aggregating material in mouse tumor cells: Removal and regeneration. Lab Invest 1977, 36:413–419
3. Pearlstein E, Cooper LB, Karpatkin S: Extraction and characterization of a platelet-aggregating material from SV40-transformed mouse 3T3 fibroblasts. J Lab Clin Med 1979, 93:332–344
4. Gasic GJ, Boettiger D, Catalfamo JL, Gasic TB, Stewart GJ: Aggregation of platelets and cell membrane vesiculation by rat cells transformed *in vitro* by Rous sarcoma virus. Cancer Res 1978, 38:2950–2955
5. Hara Y, Steiner M, Baldini MG: Characterization of the platelet-aggregating activity of tumor cells. Cancer Res 1980, 40:1217–1222
6. Karpatkin S, Smerling A, Pearlstein E: Plasma requirement for the aggregation of rabbit platelets by an aggregating material derived from SV40-transformed 3T3 fibroblasts. J Lab Clin Med 1980, 96:994–1001
7. Pearlstein, E, Ambrogio C, Gasic G, Karpatkin S: Inhibition of the platelet aggregating activity of two human adenocarcinomas of the colon and an anaplastic murine tumor with specific thrombin inhibitor, dansylarginine-N-(3-ethyl-1,5-pentanediyl) amide. Cancer Res 1981, 41:4535–4539
8. Lerner WA, Pearlstein E, Ambrogio C, Karpatkin S: A new mechanism for tumor-induced platelet aggregation: Comparison with mechanisms shared by other tumors

with possible pharmacologic strategy toward prevention of metastasis. Int J Cancer 1983, 31:463–469
9. Pearlstein E, Ambrogio C, Karpatkin S: Effect of antiplatelet antibody on the development of pulmonary metastasis following injection of CT26 colon adenocarcinoma, Lewis lung carcinoma, and B16 amelanotic melanoma tumor cells into mice. Cancer Res 1984, 44: 3884–3887
10. Bastida E, Ordinas A, Jamieson GA: Differing platelet aggregating effects by two tumor cell lines: Absence of role for platelet-derived ADP. Am J Hematol 1981, 11:367–378
11. Bastida E, Ordinas A, Giardina SC, Jamieson GA: Differentiation of platelet-aggregating effects of human tumor cell lines based on inhibition studies with apyrase, hirudin, and phospholipase. Cancer Res 1982, 42: 4348–4352
12. Tohgo A, Tanaka N, Ashida S, Ogawa H: Platelet aggregating activities of metastasing tumor cells: II. Variety of aggregation mechanisms. Invasion and Metastasis 1984, 4:134–145
13. Cavanaugh PG, Sloanne BF, Bajkowski AS, Taylor JD, Honn KV: Purification and characterization of platelet aggregating activity from tumor cells: Copurification with procoagulant activity. Thromb Res 1985, 37:309–326
14. Hilgard P: The role of blood platelets in experimental metastasis. Br J Cancer 1973, 28:429–435
15. Gasic G, Gasic T, Galanti N, Johnson T, Murphy S: Platelet-tumor cell interaction in mice: The role of platelets in the spread of malignant disease. Int J Cancer 1973, 11:704–708
16. Gastpar H: Platelet-tumor cell interaction in metastatic formation: A possible therapeutic approach to metastasis prophylaxis. J Med 1977, 8:103–113
17. Pearlstein E, Salk PL, Yogeeswaran G, Karpatkin S: Correlation between spontaneous metastatic potential, platelet aggregating activity of cell surface extracts, and cell surface sialylation in 10 metastatic-variant derives of a rat renal sarcoma cell line. Proc Natl Acad Sci USA 1980, 77:4336–4339
18. Karpatkin S, Pearlstein E: Role of platelets in tumor cell metastases. Ann Int Med 1981, 95:636–641
19. Tanaka N, Ashida S, Tohgo A, Ogawa H: Platelet aggregating activities of metastasing tumor cells. Invasion and Metastasis 1981, 2:289–298
20. Mehta P: Potential role of platelets in the pathogenesis of tumor metastasis. Blood 1984, 63:55–63
21. Al-Mondhiry H: Tumor interaction with hemostasis: The rationale for the use of platelet inhibitors and anticoagulants in the treatment of cancer. Am J Hematol 1984, 16:193–202
22. Markus G: The role of hemostasis and fibrinolysis in the metastatic spread of cancer. Semin Thromb Hemost 1984, 10:61–70
23. Sack G, Levin J, Bell WR: Trousseau's syndrome and other manifestations of chronic disseminated coagulopathy in patients with neoplasm: Clinical, pathophysiologic and therapeutic features. Medicine 1977, 56:1–37
24. Scarpelli DG, Rao MS: Transplantable ductal adenocarcinoma of the Syrian hamster pancreas. Cancer Res 1979, 39:452–458
25. Reddy JK, Scarpelli DG, Rao MA: Experimental pancreatic carcinogenesis, Advances in Medical Oncology, Research and Education. Vol 9. Edited by N Thatcher. New York, Pergamon Press, 1979, pp 99–107
26. Becich MJ, Reddy JK: Separation and characterization of neoplastic cell subpopulations of a transplantable rat pancreatic acinar carcinoma. Cancer Res 1982, 42: 3429–3740
27. Ts'ao C, Wirman JA, Ruder EA: Altered *in vitro* function of platelets prepared by the Haemonetics blood processor. J Lab Clin Med 1975, 86:315–325
28. Rossi EC: The effect of albumin upon the loss of enzymes from washed platelets. J Lab Clin Med 1972, 79:240–246
29. Lages B, Scrutton MC, Holmsen H: Studies on gel-filtered human platelets: isolation and characterization in a medium containing no added Ca^{++}, Mg^{++} or K^{+}. J Lab Clin Med 1975, 85:811–825
30. Okamoto S, Hijikata A, Kikumoto R, Hara H, Ninomiya K, Maruyama A, Sugano M, Tamao Y: Potent inhibition of thrombin by the newly synthesized arginine derivatives No. 805: The importance of stereostructure of its hydrophobic carboxamide portion. Biochem Biophys Res Commun 1981, 101:440–446
31. Green D, Ts'ao C, Reynolds N, Kahn D, Kohl H, Cohen I: *In vitro* studies of a new synthetic thrombin inhibitor. Thromb Res 1985, 37:145–153
32. Green D, Dunne B, Schmid FR, Rossi EC, Louis G: A study of the variable response of human platelets to collagen: Relation to aspirin-induced inhibition of aggregation. Am J Clin Pathol 1973, 60:920–923
33. Wu KK, Hoak JC: A new method for quantitative detection of platelet aggregates in patients with arterial insufficiency. Lancet 1974, 2:924–926
34. Jerushalmy Z, Zucker MB: Some effects of fibrinogen degradation products (FDP) on blood platelets. Thromb Diath Haemorrh 1966, 15:413–419
35. Clauss A: Rapid physiological coagulation method for the determination of fibrinogen. Acta Haematol 1957, 17:237–246
36. Jarrett MP, Green D, Ts'ao C: Relation between antithrombin III and clinical and serologic parameters in systemic lupus erythematosus. J Clin Pathol 1983, 36:357–360
37. Mohanty D, Hilgard P: A new platelet aggregating material (PAM) in an experimentally induced rat fibrosarcoma. Thromb Haemost 1984, 51:192–195
38. Mustard JF, Perry DW, Rinlough-Rathbone RL, Packham MA: Factors responsible for ADP-induced release of human platelets. Am J Pathol 1975, 228:1757–1765
39. Macfarlane DE, Walsh PN, Mills DCB, Holmsen H, Day HJ: The role of thrombin in ADP-induced platelet aggregation and release: A critical evaluation. Br J Haematol 1975, 30:457–463
40. Tangen O, Lestrup EB, Berman HJ: Characterization of clotting factors associated with blood platelets after gel filtration. Thromb Diath Haemorrh 1973, 30:289–298
41. Grignani G, Pacchiarini L, Almasio P, Pagliarino M, Gamba G: Activation of platelet prostaglandin biosynthesis pathway during neoplastic cell-induced platelet aggregation. Thromb Res 1984, 34:147–157
42. Menter DG, Onoda JM, Taylor JD, Honn KV: Effects of prostacyclin on tumor cell-induced platelet aggregation. Cancer Res 1984, 44:450–456
43. Honn KV: Inhibition of tumor cell metastasis by modulation of the vascular prostacyclin/thromboxane A_2 system. Clin Exp Metastasis 1983, 1:103–114
44. Honn KV, Cicone B, Skoff A: Prostacyclin: A potent antimetastatic agent. Science 1981, 212:1270–1272
45. Karpatkin S, Ambrogio C, Pearlstein E: Lack of effect of *in vivo* prostacyclin on the development of pulmonary metastasis in mice following intravenous injection of CT26 colon carcinoma, Lewis lung carcinoma, or B16 amelanotic melanoma cells. Cancer Res 1984, 44: 3880–3883

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