Activation of blood coagulation in cancer: Trousseau's syndrome revisited

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Three types of evidence exist that support an important relationship between blood coagulation and tumor homeostasis: clinical, histologic, and pharmacologic. Clinical and laboratory evidence focuses on the well known propensity of patients with certain forms of cancer to develop thromboembolic disease (TED) and/or disseminated intravascular coagulation (DIC), commonly observed following rapid tumor lysis or surgical manipulation. The postmortem observations of platelet and fibrin thrombi in vessels draining tumors and the immunochemical demonstration of fibrin surrounding tumor cells have provided incontrovertible histologic evidence for an association between growing tumors and the end-products of blood coagulation, e.g., platelet aggregates and fibrin. The specificity of these reactions, however, remains uncertain. The positive results of pharmacologic intervention with anticoagulant drugs and/or antiplatelet agents in animal tumor models and in human cancer have also supported the notion that a "hypercoagulable" state associated with cancer is disadvantageous to the host. Thus, local or systemic activation of blood coagulation can be produced by tumor products and favors tumor spread, while interruption of blood coagulation reactions, in general, favors the host and impairs tumor metastasis. The effect of blood coagulation on the growth of the primary tumor is less well defined, with some studies suggesting a beneficial effect and others demonstrating an inhibitory effect on the tumor growth.

We will summarize briefly the studies that have provided the three types of evidence described, and we will detail the concepts regarding the pathogenesis of these abnormalities of blood coagulation. We will then assess the potential pathways for activation of blood coagulation by tumor cells, emphasizing the induction of platelet aggregation and activation of blood coagulation. The potential for host monocytes (or macrophages) to participate in the coagulation sequence in response to tumor-associated stimuli will also be explored, and therapeutic strategies based on these concepts will be discussed.

Clinical and Laboratory Evidence
Thromboembolic Disease

The relationship between neoplastic disease and thromboembolic disorders has been recognized since 1865, when Armand Trousseau first reported a high incidence of venous thrombosis in a series of patients with gastric carcinoma. In the ensuing century, a number of clinical and postmortem studies appeared describing arterial and venous thrombosis, migratory thrombophlebitis, pulmonary embolism, nonbacterial thrombotic endocarditis, and, paradoxically, bleeding in association with a wide variety of malignant tumors. The overall incidence of clinical TED in patients with cancer has been reported to vary between 1% and 11%. The incidence of TED in postmortem studies of cancer patients is considerably higher. In one prospective study, Ambrus and associates reported that thrombosis and/or bleeding was the second most common cause of death in hospitalized cancer patients. Although patients with mucin-secreting tumors of the gastrointestinal tract have long been known to be prone to thromboembolic complications, other tumor types are also associated with an increased risk of TED. Table 1 lists the estimated frequency with which carcinomas arising in different sites have been reported to be associated with clinical TED. Although pancreatic carcinoma has been associated historically with the greatest risk of TED, other tumor types are also associated with an increased risk of TED. The total number of cases of TED is now higher in patients with carcinoma of the lung simply because of the greater prevalence of that tumor. Thus, the association of TED with specific tumor types may change in time as a function of many variables, including tumor prevalence, chemotherapy, and improved noninvasive techniques used for the diagnosis of TED. Indeed, a recent report detailed a series of 433 patients with breast cancer treated with chemotherapy, in whom the inci-
Abnormalities of Blood Coagulation

This increased incidence of TED has led several authors to examine various aspects of the coagulation system in patients with cancer. Abnormalities of routine tests of blood coagulation have been reported to occur in as many as 92% of patients with cancer. In a study of 108 patients with cancer, Sun and colleagues described 88 patients who had abnormalities of at least 5 coagulation tests. The most common clotting abnormalities in cancer patients are elevated levels of fibrin/fibrinogen degradation products (FDP), thrombocytosis, and hyperfibrinogenemia. Other common abnormalities have included prolongation of the prothrombin time or the thrombin time and elevation of the levels of specific coagulation factors. These abnormalities have been said to be consistent with the presence in cancer patients of "overcompensated intravascular coagulation with fibrinolysis" (ICF). In this situation, it is theorized that low-grade intravascular coagulation with accelerated clotting factor utilization is accompanied by increased synthetic rates for fibrinogen, clotting factors, and platelets, resulting in actual increases in their levels in the circulation. None of these test results, however, reflects the true kinetics of blood coagulation factors or platelets in such patients, and thus, they provide only limited information. Direct or indirect evidence supporting the presence of overcompensated ICF in these patients does exist. An increased rate of fibrinogen turnover, an increase in plasma levels of fibrinogen/fibrin-related antigen, and an increase in plasma levels of fibrinopeptide-A (FPA) have been observed in virtually all patients with acute leukemia and solid tumors. The increased FPA levels correlate with fibrinogen turnover rates in cancer patients, providing further evidence that fibrin generation occurs at an increased rate in such patients. Indeed, subclinical activation of blood coagulation may be a reflection of the interdependence of tumor growth and fibrin generation. We examined this potential relationship by studying FPA levels in a group of 50 patients with cancer. Fibrinopeptide-A levels were elevated above the normal range in 60% of the patients at the time of presentation. Serial studies demonstrated an upward trend of FPA levels that appeared to parallel progression of clinical disease. Persistent elevation of FPA levels in individual patients suggested treatment failure and a poor prognosis.

It should be noted that in spite of this evidence for the common occurrence of low-grade ICF in cancer patients, the occurrence of overt DIC, characterized by consumption of platelets and clotting factors with resultant bleeding complications, is uncommon. The unusually high incidence of DIC in patients with acute promyelocytic leukemia, particularly following cytotoxic therapy, is atypical and has been attributed to the release of thromboplastin from the malignant promyelocytes. In patients with other types of cancer, fulminant DIC is observed principally in patients who have mucin-secreting adenocarcinomas or predisposing conditions, such as gram-negative sepsis or liver impairment. Thus, although subclinical DIC is common in cancer, DIC of clinical significance occurs only in 9%–15% of patients with cancer, including those in whom cytotoxic therapy may be of importance in the production of coagulation dysfunction.

*Fibrinopeptide-A (FPA) is a 16-amino acid peptide cleaved from the A chain of fibrinogen by thrombin. Since the plasma half-disappearance time for FPA is less than 4 min, plasma levels of the peptide reflect ongoing coagulation, and therefore, the presence of intravascular thrombin (or other, as yet unknown, enzymes capable of generating the peptide). Plasma levels of FPA, therefore, provide a kinetic measure of fibrinogen cleavage.*
Quantitative and Qualitative Abnormalities of Platelets in Patients With Cancer

Thrombocytopenia, abnormal platelet function, and evidence for in vivo activation of platelets have all been reported to occur with increased frequency in patients with cancer. Although thrombocytopenia has been described in as many as 27% of patients with cancer, more conservative estimates, which exclude the effects of chemotherapy and radiation, vary from 4% in patients with inoperable lung cancer to 11% in a large series of patients with a variety of tumors. While the pathogenesis of thrombocytopenia in these patients is uncertain, several studies have documented either increased platelet destruction or evidence for in vivo platelet activation and release. In view of the ability of tumor cells to induce thrombocytopenia in experimental animals and platelet aggregation in vivo and in vitro, it is tempting to postulate that thrombocytopenia is also a manifestation of tumor-induced DIC. Thrombocytosis, which occurs much more frequently in untreated patients with cancer (30%–60%), may also be explained by the existence of low-grade DIC, thrombopoiesis, and overcompensation. Indeed, thrombopoietic activity has been recovered from the serum of patients with cancer and thrombocytosis, suggesting the existence of an aberrancy in the usual relationship between platelet count and the production of thrombopoietin in some individuals with cancer.

Qualitative abnormalities of platelet function have been described in cancer patients and have been attributed to the presence of elevated levels of fibrinogen degradation products. However, no evidence can be found that supports a relationship between abnormalities of platelet function and elevation of circulating levels of FDP. Moreover, since prospective studies of platelet function in untreated cancer patients have not been performed, it is difficult to determine the true incidence of primary thrombocytopenia (excluding the effects of chemotherapy on platelet function). A more detailed consideration of the platelet response to tumor cells and their products appears in a subsequent section and in several recent reviews.

HISTOLOGIC EVIDENCE

In 1878, Billroth first reported his autopsy observations that human tumor cells were found frequently in association with thrombi. He went on to suggest that metastases occur when a portion of the tumor–thrombus complex breaks off and forms a tumor embolus. In the first half of this century, others confirmed the appearance of thrombi in association with viable tumor metastases and primary tumor deposits using conventional histologic sections and light microscopy. More recent immunochemical and ultrastructural studies have confirmed the presence of fibrin deposition in and around both animal and human tumors.

In one animal model, the Walker 256 mammary adenocarcinoma in rats, lung tissue was excised following the intravenous injection of tumor cells. Examination of the lung using immunoperoxidase stains revealed that small amounts of fibrin accumulated within 30 sec following tumor cell injection. Electron micrographs demonstrated evidence of crosslinked fibrin in the tissue within 5 min. The fibrin increased in concentration rapidly, peaking by 1 hr and disappearing within 9 hr. In another model system, Dvorak and associates examined the early events following intraperitoneal injection of the strain-specific TA3-St mammary adenocarcinoma in mice. Within 3 hr of tumor injection, the peritoneal cavity contained large numbers of neutrophils and tumor cells, with smaller numbers of normal macrophages. After 12 hr, macrophages were increased in number and electron microscopy showed evidence of "macrophage activation." After 24 hr, no viable tumor cells remained, and the peritoneal exudate was dominated by aggregates of macrophages and lymphocytes, with an abundant fibrin deposit located on the surface of the macrophages. Similar results have been observed following implantation of either the line 1 or the highly malignant line 10 bile duct carcinomas in guinea pigs. A gelatinous material rich in fibrin was observed shortly after tumor implantation in the subcutaneous spaces (Fig. 1). Initially, the gel was relatively acellular, but after several days, an infiltrate of neutrophils and monocytes was observed. In the line 1 tumors, an exuberant inflammatory reaction led eventually to replacement of the fibrin gel by connective tissue and, subsequently, tumor rejection. In the line 10 tumors, the fibrin gel developed to a lesser extent, the inflammatory response was less dramatic, organization did not occur, and tumor growth was not limited. Similar histologic evidence for the association of platelet thrombi deposited in proximity to growing tumor cells has been detailed and supports an important role for platelets (and platelet products) in the process of tumor growth.

These observations have led to the postulate that the interaction of tumor cells, platelets, and perhaps, inflammatory cells leads to the generation of this peritumor "fibrin gel," which is critical to the pathogenesis of tumor growth and metastasis formation. While many investigators have suggested that fibrin acts as a "glue," facilitating tumor...
cell adhesion to the endothelium, others have maintained that tumor cells adhere independently to the endothelium, produce microinjury and secondary platelet adhesion with fibrin deposition.59,60,63,64 Even in the absence of endothelial injury, however, sequestration of fibrinogen and 51Cr-labeled platelets can be found at the sites of metastasis of some animal tumors.57 The relative importance of the contribution to fibrin formation of each of the components of the hemostatic system remains the subject of intense speculation in several recent reviews.63-88 Thus, in spite of strong histologic evidence for the association between tumor cells, inflammatory cells, platelets, and fibrin thrombi, the pathophysiology and precise sequence of events remain uncertain.

PHARMACOLOGIC EVIDENCE—EFFECTS OF ANTICOAGULANTS

Antiplatelet Agents

Support for the use of antiplatelet drugs and agents designed to produce thrombocytopenia in the treatment of cancer derives from the experimental and clinical observations reviewed above. Antiplatelet antibodies, capable of inducing thrombocytopenia, can reduce significantly the formation of lung implants following the intravenous infusion of TA3 tumor cells in mice.69 The protective effect can be reversed with platelet transfusions. Similar results have been reported in other animal models, including the 20 methylcholanthrene-induced fibrosarcoma in rats.70 Drugs that impair platelet function have also proved successful in the treatment of cancer in experimental animals (Table 2). The cyclo-oxygenase inhibitors, aspirin62,71,73 and indomethacin,74,75 are both capable of acting as antimetastatic agents, as are the phosphodiesterase inhibitors, dipyridamole,76 RA-233,76 and pentoxifylline.77 Pretreatment of mice with the potent platelet function inhibitor prostacyclin (PGI2), for example, has produced a striking reduction in the number of lung implants following injection of B16a melanoma cells into syngeneic mice. Liver and spleen “metastases” were totally prevented in recipient animals.78,79 Aspirin and indomethacin have been effective even when given to animals 1 or 2 wk following tumor cell inoculation.73,75 However, not all experimental tumors respond to all platelet function inhibitors. Negative results have been reported with the use of aspirin for the treatment of the V2 carcinoma in rabbits80 and the use of aspirin, RA-233, bencyclan, or cyproheptadine for the treatment of Lewis lung carci-
noma (3LL) in mice. Moreover, results obtained using models such as these, in which “metastases” are induced by intravenous injection, may not prove relevant to the treatment or prevention of spontaneous metastasis formation. Finally, since thromboxane inhibitors and prostacyclin can modulate directly the growth of tumor cells in vitro, the effects of these mediators on platelet function may have little to do with their antitumor properties.

Trials of these drugs in human subjects have been limited. Only preliminary data from nonrandomized and/or uncontrolled studies have been published. In a group of 38 patients with tumors of the head and neck, RA 233 reduced the recurrence rate and frequency of metastasis formation when compared with historic controls. The results of the animal studies previously reviewed and preliminary data from humans have stimulated the organization of additional studies in patients with cancer. Several randomized controlled studies of antplatelet agents in the treatment of various human tumors are now in progress. No data are yet available from these cooperative trials.

**Anticoagulants**

Administration of high doses of an antibody to fibrin fragment E has been used successfully in the induction of complete regression of an experimental hepatoma (line 10) in guinea pigs. Although antibody was not injected until either 6 or 16 days after the animals were inoculated intradermally with tumor cells, no evidence of tumor was found in the treated animals when biopsies of the injection sites were examined at day 35. All treated animals were alive and without visible tumor at 180 days, while all of the control animals, who received normal rabbit IgG, were dead of tumor progression by day 90. None of 8 immunized animals who were rechallenged with line 10 cells developed tumors at the site of injection. Although the mechanism of action of the antibody used in this intriguing study is not at all clear, the authors suggested that the antitumor effect observed was “presumably a consequence of its interaction with the disulfide knot region of fibrinogen, fibrin monomer, or fibrin polymer at the tumor site.” Indeed, other experimental approaches to the inhibition of fibrin deposition or polymerization have been successful. The timing of the therapeutic intervention, however, may be critical, depending on whether the end-point is the arrest of growth of primary tumors as opposed to the prevention of metastasis formation. For example, use of the thrombin-like enzyme batroxobin to defibrinate mice 11 days after implantation of 3LL tumor cells resulted in a decrease in pulmonary metastases with no change in the size of the primary tumor. Defibrination of the animals prior to tumor implantation, however, produced an increase in pulmonary metastases with no change in the size of the primary tumor. These results led the authors to suggest that formation of peritumor fibrin in the early phases of tumor growth may be beneficial, preventing the egress of tumor cells from the primary tumor to distant sites. In contrast, fibrin formation at metastatic sites may be harmful, favoring implantation.

The inhibitory effects of anticoagulant drugs on various properties of tumor cells have been recognized for at least 30 yr, since Strauss and Saphir first reported the effects of the vitamin K antagonist dicoumarol on circulating carcinoma cells in rabbits. Coumarin derivatives can inhibit tumor cell locomotion, metabolism, lung colony formation, and the development of spontaneous metastases in various experimental tumor systems. The results of these animal
studies have been reviewed recently. It would appear that the effects of coumarin drugs in the treatment of cancer are mediated by their ability to interfere with the utilization of vitamin K, since administration of vitamin K promptly reverses the anticoagulant effect.9, 26 Experimentation with the vitamin K-deficient animal model indicates that warfarin administration in vivo reduces the in vitro expression of procoagulant activity by human monocytes9, 10 and 3LL tumor cells recovered from C57BL/Rij mice.9 Moreover, treatment of mice with a human prothrombin complex concentrate, which reversed the plasma anticoagulant effect of warfarin, failed to reverse the protection from metastases enjoyed by warfarin-treated animals.9 In an attempt to find an alternative mechanism for the effect of warfarin, Maat turned to the macrophage as a potentially important target cell, active in both cell-mediated coagulation and tumor cell killing. Inhibition of macrophage function in C57BL/Rij mice, accomplished by the administration of either carrageenan or silica, was effective in abolishing the protective effect of warfarin.9 These data suggest that the antimitotic effect of warfarin may be mediated, in part, by selective effects of warfarin on monocyte-macrophage function. Further details of this alternative hypothesis are described below. It should be noted, however, that other inhibitors of blood coagulation have antimetastatic properties in experimental tumor models. Potent tumor growth suppression has been observed following the use of heparin,99 other sulfated polysaccharides,102 and fibrinolytic agents,103 all of which act presumably by impairing fibrin deposition or accelerating fibrinolysis.

Human studies of defibrinating agents, anticoagulants, or fibrinolytic drugs in cancer have, until recently, been limited to uncontrolled nonrandomized trials in heterogeneous groups of patients.104 However, the results of the Veterans Administration Cooperative Study on the use of warfarin in the treatment of small cell carcinoma of the lung (SCCL), the first randomized controlled study of this agent as an anticancer drug, were recently published.105 The median survival of patients who received warfarin in addition to standard chemotherapy (50 wk) was significantly greater than the median survival of subjects who received chemotherapy alone (26 wk, p = 0.026). The median length of time to evidence of tumor progression was also increased significantly in the warfarin group (p = 0.03). Although the results of this study are consistent with the hypothesis that warfarin interferes with local fibrin formation and tumor growth, no consistent relationship was demonstrated between the degree of hypoprothrombinemia and therapeutic effect.106 Thus, it is possible that the beneficial effects of warfarin might have been related to other properties of the drug. In addition, the small size of the study (50 patients) and the negative results of another randomized study of warfarin treatment of SCCL107 indicate the need for caution in the interpretation of the results of the VA Cooperative study. Further studies of warfarin in the treatment of larger numbers of patients with SCCL are now in progress, and results should be forthcoming.

**PATHOGENESIS**

**Specificity and Source of Fibrinopeptide-A in Cancer**

Several authors have now established a semiquantitative relationship between the extent of tumor growth and the subclinical activation of blood coagulation in patients with acute leukemia22, 24 and solid tumors.22, 24, 26 As mentioned above, fibrinopeptide-A (FPA) levels appear to reflect clinical responses in cancer patients,26 suggesting a direct relationship between tumor growth and thrombin generation. Peuscher and colleagues demonstrated elevated FPA levels in 93 of 98 patients with metastatic cancer (95%), as opposed to 3 of 11 with limited primary disease only (27%) and 1 of 11 with tumors in remission (9%).25 We studied patients with solid tumors, including individuals with both limited and disseminated cancer, and found that 26 of 43 subjects had elevated FPA levels at the time of initial presentation (60%).26 In our study of patients with acute leukemia, 15 of 17 subjects presented with elevated plasma levels of the peptide (88%).24 In both groups of patients, tumor regression was associated with a reduction in FPA levels. Relapse of acute leukemia was associated with abrupt increases in the generation of FPA in most individuals. Elevated FPA levels, however, often preceded clinical evidence for relapse or disease progression (solid tumors) by as much as 2 mo.24, 26 Failure of successive FPA levels to drop within 4 mo following the initiation of chemotherapy predicted a poor survival in 11 patients with solid tumors in whom consecutive datum points were available (p = 0.02).27 Thus, in the absence of complicating factors that can elevate FPA levels, such as infection, thromboembolic disease, or recent intravenous administration of chemotherapy, activation of blood coagulation in patients with cancer, evidenced by persistently elevated FPA levels, suggests
recurrent or continued tumor growth. While other evidence of activated blood coagulation may be demonstrated in isolated cases, it should be emphasized that no consistent abnormalities of routine tests of coagulation have been found in any of these patient groups.24,26,27

The pathogenesis of abnormalities of blood coagulation has been the subject of intense speculation and investigation for many years. Recent studies of FPA levels in patients with thromboembolic disease (TED) following intravenous heparin have provided some insight into the source of plasma FPA in cancer and inflammatory disorders. Yudelman and colleagues noted that patients who developed TED as a complication of cancer or inflammatory disorders failed to normalize their plasma FPA levels in response to the intravenous administration of heparin, in contrast to subjects with uncomplicated TED.108 Similar data have been reported by others25,26 and support the older clinical observation that such patients may prove refractory to conventional anticoagulant therapy.24 These observations suggest the following possibilities: (1) a heparin-resistant enzyme (rather than thrombin) is responsible for the cleavage of intravascular fibrinogen in cancer patients; (2) thrombin generation occurring within the vasculature in such patients is less susceptible to inactivation by heparin; and (3) thrombin generation occurs in the extravascular compartment with leakage of FPA into the circulation.

Alternative enzymes for the cleavage of fibrinogen clearly exist and could be released by tumor cells109 or reactive leukocytes110,111 in the circulation of cancer patients. Neoplastic tissue and body fluids from patients with cancer contain a variety of such proteases that have been implicated by others in the processes of invasion and metastasis.109 For example, considerable experimental evidence supports the potential importance of tumor-associated plasminogen activator(s) in the pathogenesis of tumor growth.109 Since plasmin, the end product of the proteolytic reaction induced by these activators, is capable of cleaving a peptide from fibrinogen (Naa 1–23) that can cross-react in the FPA radioimmunoassay,112,113 some concern has arisen regarding the specificity of the observation of elevated plasma FPA levels in cancer. However, the extraction procedure used in the preparation of samples for the FPA assay has been shown previously to eliminate this larger cross-reacting peptide.114 Moreover, Yudelman and Greenberg108 were able to detect elevated FPA levels in cancer patients utilizing an antiserum that reacts poorly with peptides of fibrinogen larger than FPA.111,112 In addition, no consistent relationship could be demonstrated between circulating levels of α2-antiplasmin–plasmin complexes and FPA in patients with cancer.115 Thus, it seems unlikely that a larger fibrinogen fragment is being detected in the FPA assay. We are left with the intriguing possibility, however, of the existence of a unique heparin-resistant protease capable of cleaving the same arginyl(16)–glycine(17) bond as does thrombin. At this time, no direct evidence exists for the presence of such a protease in the plasma of cancer patients.

Intravascular generation of thrombin (e.g., DIC) may occur in some patients with cancer and be accompanied by a reduction in antithrombin-III levels.116 Relative heparin resistance might be observed under such circumstances and might account for the failure of plasma FPA levels to drop in response to heparin. However, antithrombin-III levels are generally within the normal range in patients with cancer, even in the presence of widespread hepatic metastases,117 and Yudelman and Greenberg were unable to identify a difference in antithrombin-III levels in cancer patients with heparin-resistant FPA as compared to a control group of patients with uncomplicated TED.108

Leakage of FPA into the circulation from extravascular sites has been demonstrated in an experimental model, although less than 3% of the total peptide injected into an inflammatory site was recovered in the vasculature.118 An extravascular source of FPA in patients with cancer would be compatible with the common histologic observation of peritumor fibrin deposition and with the failure of plasma FPA levels to respond to the administration of intravenous heparin. It may be that heparin, which is a large charged polymer, is unable to diffuse readily from the circulation and thus has little effect on extravascular thrombin generation. In support of this notion is the observation that sodium warfarin, a small nonpolar molecule that can diffuse out of the vasculature and act at the tumor site or can impair coagulation by decreasing the synthesis of functional clotting factors, is effective in reducing plasma FPA levels in the majority of cancer patients into the normal range.119 In a small series of patients, 12 of 18 (67%) developed normal FPA levels following therapeutic anticoagulation with coumadin. The results of these preliminary studies support the third hypothesis for the pathogenesis of elevated plasma FPA levels in patients with cancer and are compatible with the concept that tumor cells and/or associated extravascular host cells participate in the activation of coagulation. It is likely, however, that a complex interaction of both intravascular and extravascular events leads, ultimately, to local fibrin deposition in and about tumors and results in the activation of plasma coagulation.

We will now review evidence implicating several cellular pathways in these tests. Figure 2 illustrates
several proposed mechanisms for the activation of blood coagulation in patients with neoplastic disorders. These include direct interaction between tumor cells and platelets, production of procoagulants (such as tissue factor or cancer procoagulant-A) by tumor cells, and the production of procoagulants (tissue factor or prothrombin activators) by activated monocytes/macrophages. The latter mechanism may result from monocyte stimulation by tumor products (antigens, proteases, etc.) or indirectly following activation of other components of the immune system (such as T lymphocytes). These mechanisms will be elaborated upon below. While the available data are supportive of the schema illustrated in Fig. 2, the pathogenesis of the activation of blood coagulation and fibrin deposition in cancer is far from clear, and other cell-mediated or plasma protein-derived pathways may be critical to this process.

**Tumor Cell–Platelet Interactions**

Since 1903, when Schmidt made the first observation of platelet aggregates surrounding cancer cells in human pulmonary arterioles,\(^1\) it has been presumed that neoplastic cells possessed some special property that rendered them capable of inducing platelet adhesion and aggregation. Although the prevailing theory held that platelet adherence to tumor cells and subsequent aggregation around tumor cells was mediated indirectly by the activation of fluid phase blood coagulation, recent evidence suggests that tumors contain one or more cell surface glycoproteins that can aggregate platelets directly.\(^2\) Gasic and colleagues were the first to observe a rough correlation between the capacity of experimental tumors to produce thrombocytopenia in vivo and their ability to induce platelet aggregation in vitro.\(^3\) The distribution of metastatic foci in vivo also appeared to reflect the platelet-aggregating activity of tumors—limited pulmonary metastases characterized those tumors with prominent activity, while a widespread distribution of metastases was associated with those tumors possessing little or no platelet-aggregating activity.\(^4\) Initial experiments with disrupted tissue from various murine and human\(^5\) tumors demonstrated platelet-aggregating activity (PAA) in heparinized platelet-rich plasma only (citrate and inhibitory). Substantial variation in PAA was recorded among the various tumors studied leading to speculation that the PAA might be a result of contamination of some of the whole tissue extracts with connective tissue collagen. Failure of collagenase treatment of the extracts to obliterate the activity and subsequent studies of collagenase-treated PAA isolated from tumor cell lines more rigorously excluded the possibility that collagen contamination of the tumor cell preparations might have been responsible for the initial observations.\(^6\)

Gasic and colleagues were able to remove the PAA from two murine tumor cell lines with dilute trypsin\(^7\) and recovered the PAA in vesicles shed by the tumor cells.\(^8\) Vesicles with PAA could be found in the sedimentable fraction of supernates of these cultures even in the absence of trypsin, suggesting that PAA was being shed routinely by some tumor cells in
<table>
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<th>Classification</th>
<th>Source of Procoagulant</th>
<th>Sedimentable</th>
<th>DFP-Sensitive</th>
<th>FVII-Dependent</th>
<th>FX-Dependent</th>
<th>Warfarin-Sensitive and/or Vitamin-K-Dependent</th>
<th>Other</th>
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<td>Bind calcium and FVII; neutralized by heterologous antibody to TF</td>
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<td>Cells have some macrophage characteristics</td>
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<td>?</td>
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<td>&quot;Macrophage-depleted&quot; cultures; PCA inversely related to metastatic potential?</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Dosage human plasma less than homologous plasma (factor dependent?)</td>
</tr>
<tr>
<td></td>
<td>TA3-3 cells‡</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>Probably TF, but some PCA in Vili-deficient plasma</td>
<td>172</td>
</tr>
</tbody>
</table>

* Squamous cell carcinoma derived from a transplantable virus-induced papilloma.
† Short-term cultures of peripheral blood cells from patients with chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL).
‡ Human adenocarcinoma cell lines.
§ Anaplastic mouse carcinoma cell line.
$Cell line derived from a rat ascites hepatoma.
$§ Homogenates of plasma cell tumors from BALB/c mice x A/J, NIf, ICAF, I.
|| Short-term cultures of plasma cell tumors from BALB/c mice x A/J, NIf, ICAF, I.
†† Short-term cultures of cells derived from an ascites variant of an indoluble bile duct carcinoma in Sewell-Wright, inbred, strain 2 guinea pigs.
‡‡ Short-term cultures of cells derived from a mouse breast carcinoma and harvested from the ascites of A/J mice.
** Cell lines of human bladder cancer (KU1), gastric cancer (MKN1), and lung cancer (GGS6).
Platelet-aggregating activity of the trypsin-treated intact cells was recovered spontaneously during culture, independent of cell division. Since publication of the results of these initial experiments, several laboratories have reported similar findings and have isolated PAA from both murine and human tumor cell lines. Although the inability to recover PAA from detergent-solubilized tumor cell membranes has prevented the purification of PAA, preliminary data on enzyme sensitivity, cofactor requirements, and cell type of origin suggested the existence of at least two types of PAA.

Platelet-aggregating activity recovered from shed vesicles or extracts of virus-induced tumor cell membranes appeared to be trypsin, phospholipase, and neuraminidase-sensitive, required activation by a heat-labile, cobra venom factor-sensitive, plasma cofactor (presumably an alternative pathway complement), and required a heat-stable, nondialyzable, plasma cofactor for expression of its activity. Gel-filtered platelets or washed platelets failed to aggregate in response to vesicle-associated PAA. Platelet-poor plasma or a fraction of plasma precipitable with 50% saturated ammonium sulfate restored PAA. This PAA has been shown to be resistant to inhibition by heparin, hirudin, and the thrombin-specific inhibitor, dapsylarginine-N-(3-ethyl-1, 5-pentanediyl) amide (DAPA).

In contrast, PAA from two spontaneous human adenocarcinoma lines (HCT-8 and LOVO) and one anaplastic murine cell line (HUT-20) have been shown to be insensitive to trypsin and neuraminidase and act directly on platelets without the need for complement components. The PAA from these tumors was directly correlated with procoagulant activity (PCA) of the cells and was inhibited by DAPA at approximately 10^-6 M. Although the PCA of these cells was shown to be factor-VII-dependent, and this is most likely tissue factor, complete characterization has not been accomplished. It is conceivable that this second PAA, which has the properties of the enzyme thrombin and may be generated directly on the surface of tumor cells, is a product of the generation of tumor PCA with or without the need for plasma substrates.

Other properties of PAA of various tumor cell lines, including susceptibility to treatment with phospholipases, dependency on either platelet cycloxygenase-mediated reactions or the von Willebrand factor, and requirements for divalent cations, are less clear and must await solubilization and complete purification of the responsible protein(s).

Tumor Cell–Blood Coagulation Interactions

The first evidence that a relationship might exist between the in vitro clot-promoting properties of a tumor and in vivo thrombogenesis was published by Lawrence and colleagues 30 yr ago. Suspension of rabbit V, carcinoma cells, which produced very short prothrombin times when substituted for rabbit brain thromboplastin in vitro, were equally effective in inducing lethal thrombotic events in vivo (when administered intravenously to rabbits). Of importance, both heparin and dicoumarol treatment were protective of the animals, suggesting that one or more tumor-associated PCA were acting via traditional coagulation pathways. The protective effect of the vitamin K antagonist dicoumarol was dose dependent. The mortality rate observed in the group of animals with prothrombin times (PT) in the control range was 56%, while in those animals with PT of 14–19 sec, it was 33%, and in those animals with PT of greater than 20 sec, it was 0%. Although no physical or chemical characteristics of the PCA were described in this early study, the property of ultimate importance, i.e., in vivo thrombogenicity, was thus demonstrated to be dicoumarol-sensitive and/or dependent for expression on factors II, VII, IX, and/or X. Subsequent studies of different types of cancer cells have established the presence of at least 3 types of PCA, the properties of which are summarized in Table 3.

Factor-X Activators

Recent studies of the rabbit V, carcinoma PCA, prepared from tissue extracts, have revealed that it is most likely a single chain cysteine protease of mol wt 68,000 with an isoelectric point of 4.8–4.9. Unlike tissue factor, or thromboplastin, to which it has been compared, the rabbit V, PCA has been shown to activate bovine factor X directly in the absence of factor VII and can be inactivated by diisopropylfluorophosphate (DFP). Protease activity of this material can be regenerated in the presence of 5 mM dithiothreitol (DTT), and the PCA can be purified by affinity chromatography on p-chloromercurial benzoate-agarose and elution with DTT—characteristics of a cysteine protease rather than a serine protease. A similar DFP-sensitive, VII-independent, X-dependent PCA has been found in tissue extracts of the following human cancers; breast, colon, vagina, kidney, and lung. This PCA has not been found in extracts of adjacent normal tissue, but has been described in transformed hamster fibroblasts, mouse Lewis lung carcinoma (3LL) cells (free of macrophages), B16 mouse melanoma cells, mouse Ehrlich ascites carcinoma cells, and mouse...
JW sarcoma cells.\(^4\) Pineo and colleagues described the partial purification of a direct activator of factor X obtained from either normal human bronchial and ovarian cyst mucus or from mucin-producing adenocarcinoma tissue.\(^{11,14}\) Indeed, 9 of 10 patients with ovarian cyst mucus or from mucin-producing adenocarcinoma cells\(^3\) Pineo and colleagues described activator(s) from both the V\(_2\) carcinoma and 3LL cells suggestive of DIC.\(^{14,33}\) It is of interest that the factor-X activator(s) from both the V\(_2\) carcinoma and 3LL cells is (are) sensitive to vitamin K depletion and/or coumarin compounds.\(^{91-94,98,132,138-142}\)

**Tissue Factor (TF) Like Procoagulants**

Although the thromboplastic properties of leukemic white blood cells were described as early as 1954,\(^{144}\) the preliminary identification of tissue factor (TF) in buffy coat preparations of cells from patients with progranulocytic leukemia did not occur until 1973, when Gralnick and Abrell applied specific criteria to the PCA in those cell preparations.\(^{30}\) Further support came in a subsequent study in which a line of identity between human brain TF and a sonicate of peripheral blood cells from a patient with progranulocytic leukemia was identified by an antibody to brain TF.\(^{31}\)

In addition, this antibody neutralized the PCA of the white blood cell sonicate.\(^{31}\) Tissue factor is a potent PCA that has the following properties: it is factor-VII-dependent, factor-X-dependent, sedimentable, lipophilic, and is a membrane protein without discernible protease activity (e.g., DFP-resistant). TF has now been described in human and rat progranulocytic leukemia cells,\(^{30,31,145,146}\) human chronic myeloid and lymphoid leukemia cells,\(^{147}\) and in cells derived from a human histiocytic lymphoma\(^{148,149}\) from a human osteogenic sarcoma,\(^{150}\) a mouse sarcoma, a human adenocarcinoma,\(^{159}\) an anaplastic mouse carcinoma\(^{159,160}\) and a rat ascites hepatoma.\(^{152}\) It is important to emphasize that, while these studies of isolated cells in tissue culture are supportive of the postulate that tumor cells themselves are the source of TF, with rare exception,\(^{151}\) no experiments designed to eliminate contamination with monocyte-macrophage cells have been performed. Indeed, many of the tumor cell lines studied have characteristics of monocyte-macrophage cells de novo\(^{148-150}\) or develop TF activity only when induced in vitro to acquire the morphological and cytochemical characteristics of macrophages.\(^{146}\) In view of the controversy as to the existence of TF in cells of granulocytic origin\(^{153}\) and the capability of cells of monocyte origin to generate TF activity in short-term tissue culture,\(^{65}\) it is possible that a small percentage of monocyte-macrophage cells, contaminating cultures of tumor cells, may contribute to the observed PCA.

**Miscellaneous Tumor-Associated Procoagulants**

Procoagulant activity of an uncertain specificity has been described in cell cultures of tissue extracts derived from a variety of animal\(^{132,135,134-136}\) and human\(^{157-156}\) tumors. In retrospect, many of these extracts probably contained the cysteine-protease, factor-X activator\(^{132,134}\) implicated by more recent studies of the same tumors.\(^{133,134,136}\) However, the PCA from some of these tumor extracts has proved partially resistant to DFP (human kidney adenocarcinoma, human thigh liposarcoma) or factor-VII-dependent (human metastatic squamous cell carcinoma, human abdominal wall liposarcoma), properties more compatible with TF activity. Since the PCA described in older studies was neither isolated from a homogeneous cell population nor purified to homogeneity, it is impossible to exclude the potential contribution of stromal elements and monocyte-macrophages to TF-like PCA present in some of these extracts. Indeed, recent evidence suggests that macrophages isolated from either malignant ascites or solid tumors in experimental animals contain increased TF activity (Donati MB: personal communication).

A second problem in interpretation of PCA studies of tumor cell extracts and tumor cell lines deals with species specificity. Measurements of TF activity in cells from one species using substrate plasmas from another species may produce confusing results. For example, we and others “rediscovered” recently the very old observation that guinea pig TF activity cannot be measured effectively unless the substrate plasma used is derived from guinea pigs.\(^{156,162,163}\) It remains difficult, therefore, to determine the specificity of PCA described in many studies of animal tumors. Dvorak and colleagues have analyzed PCA in cells derived from both a guinea pig bile duct carcinoma and a mouse breast carcinoma\(^{53,155,156}\) Both PCA were sedimentable at 100,000 g and DFP-resistant.\(^{156}\) The PCA of the line 10 tumor was said to be factor-II-, factor-V-, factor-X-, and calcium-dependent, but able to clot guinea pig plasma, which is “normally deficient in factor VII” and human factor-VII-deficient plasma.\(^{156}\) The authors interpreted these results to be compatible with the presence of TF, but expressed appropriate concern about the confusing substrate dependency pattern. This study illustrates many of the problems. For example, guinea pig plasma appears to be relatively deficient in factor VII when human factor-VII-deficient plasma and rabbit brain thromboplastin are utilized in the assay system\(^{164}\) or when chromogenic
substrates and heterologous cofactors are utilized (Dvorak HF: personal communication). Based on preliminary data in our laboratory, it is likely that complex formation between tissue factor and factor VII and the subsequent interaction of that complex with factor X are both highly species-specific reactions. Thus, it may prove difficult to evaluate substrate specificity in future studies, since species-specific deficient substrates for all model systems will not be available. The development of monoclonal antibodies to human PCA, however, may provide important corroborative evidence regarding the specificity of tumor-associated PCA.

**Tumor Cell–Monocyte Interactions**

In addition to the substantial evidence that monocytes and macrophages are critical to both the afferent and efferent limbs of the immune response to tumor antigens, it is now clear that monocyte-macrophages are also capable of activating blood coagulation in response to a variety of stimuli, both immune specific and nonspecific.

Recent evidence from our laboratory indicates that peripheral blood monocytes from human subjects with cancer express increased TF activity when grown in tissue culture, as compared to cells from control human subjects. Furthermore, as illustrated in Fig. 3, a strong correlation was observed (in patients with lung cancer) between circulating FPA levels and unstimulated mononuclear cell TF activity generated in vitro. Indeed, in this study of 35 consecutive patients with lung cancer, all patients with TF generation greater than $10 \times 10^{-5}$ U/cell had FPA levels above the upper limit of normal (2.0 ng/ml). No such relationship was demonstrable in control normal subjects. While correlation does not prove causation, these data suggest that some patients with “preactivated” monocytes, e.g., those whose cells are able to express TF in vitro without exogenous stimulation, also have an increased rate of fibrin generation in vivo. "Preactivation" of peripheral blood monocytes in these patients may occur in at least 3 ways. As suggested by the construct in Fig. 2, activation of peripheral blood monocytes (or tissue macrophages) may occur in patients with cancer due either to stimulation by tumor-specific tumor antigens, immune complexes, or tumor-associated proteases. Although several of these “immune stimuli” may interact directly with the monocyte, the T cell has been shown to play a central role in regulating monocyte procoagulant generation. The regulation of monocyte procoagulant activity is illustrated in Fig. 2 and discussed below.

An increased level of circulating immune complexes has been found in 30.7% of patients with bronchogenic cancer.
carcinoma and in up to 75% of patients with giant cell bone tumors. Preliminary evidence also suggests that such complexes contain tumor-associated antigens. TF in peripheral blood monocytes or macrophages can be activated as part of a specific immune response to antigens, such as tuberculin or bovine serum albumin, or in response to the binding of immune complexes, such as antiovalbumin-ovalbumin or antialbumin-human serum albumin.

The regulation of monocyte or macrophage procoagulant activation appears to be quite complex. Amplification of the response by T cells, lymphokines, lipoproteins, and trace serum components has been described. Moreover, single cells stimulated to express PCA can be shown to generate fibrin on their surface, and this process is linked to immune-specific inhibition of macrophage migration. These observations suggest that a series of linked reactions can occur resulting in impaired macrophage mobility, perhaps in response to antigen recognition. No evidence for a proteolytic sequence exposing TF activity in monocytes, macrophages, or tumor cells has been demonstrated. However, TF in other cell membranes can be "stripped" from the cell surface by proteases and released in an active form. It is possible, therefore, that tumor cell proteases might provide an additional stimulus to the generation of PCA.

Although the role of monocyte (or macrophage) PCA in the pathogenesis of cancer is uncertain, a similar set of linked reactions may occur in the host response to tumor growth. Indeed, macrophages isolated from the rabbit V carcinoma contain increased TF activity as compared to macrophages from the peritoneum of a control rabbit (Donati MB: personal communication). In the mouse sarcoma cell line mFSb, metastatic potential appears to vary inversely with the TF activity of the tumor cells (Table 3). Finally, stimulation of local tumor growth following subcutaneous injection of lethally irradiated tumor cells along with viable cells appears to relate to the thrombogenicity of the necrotic cells. It is tempting, therefore, to conclude that TF and other tumor or monocyte-macrophage PCA are of fundamental importance in tumor growth and metastasis formation. To date, however, the evidence is entirely indirect. Similarly, the beneficial effects of warfarin therapy on

![Graph showing the effect of warfarin anticoagulation on FPA levels and cell TF generation in cancer patients.](image)

**Fig. 4.** The effect of warfarin anticoagulation on FPA levels and cell TF generation in cancer patients. (A) FPA levels were compared in 18 subjects before and after achievement of stable anticoagulation with warfarin (prothrombin time ≥ 1.5 times pretreatment value). A significant reduction in FPA levels (p < 0.005) was demonstrated using nonparametric analysis (Mann-Whitney test). (B) Mixed mononuclear cells (32.5% ± 2.7% monocytes) were tested for TF activity without exogenous stimulation (unstimulated) and following exposure to lipopolysaccharide (LPS-stimulated) in 12 subjects before and after achievement of stable anticoagulation. Only the reduction observed in the stimulated cultures was statistically significant using Student's t test for paired data.
survival of patients with SCCL cannot be related necessarily to the ability of warfarin to reduce both FPA levels (Fig. 4A) and circulating monocyte TF activity (Fig. 4B), as is illustrated for a separate set of patients with lung cancer. However, since monocyte (macrophage) TF and (one or more of) the factor-X activator(s) of the rabbit V, carcinoma and the 3LL mouse carcinoma are warfarin-sensitive in vivo, a unifying hypothesis could place TF and tumor-associated proteases with PCA at the center of an activation sequence in which the product, local fibrin deposition, influences tumor cell growth and movement. No direct evidence for cause and effect yet exists, however, and the reduction in FPA levels and monocyte TF activity may be secondary to an effect of warfarin on tumor cell metabolism independent from its effects on vitamin K utilization. Further studies are needed in this interesting area of research.

In summary, we have reviewed the clinical, histologic, pharmacologic, and pathophysiologic evidence supporting the existence of a close relationship between abnormalities of blood coagulation and the biology of cancer. It seems clear that cancer cells can exert a profound effect on hemostatic function and, conversely, blood coagulation reactions can impact on tumor homeostasis. The selection of appropriate strategies for the treatment of cancer in the future, based on interference with platelet function or soluble clotting reactions, will require an improved understanding of the physicochemical interactions of specific types of tumors with these reactions.

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