Interactions of cancer cells with the microvasculature during metastasis[1, 2]

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ABSTRACT

Metastasis of cancer via the bloodstream is a major factor in the diagnosis, treatment, and prognosis of patients with cancer. Key events in hematogenous metastasis occur in the microvasculature. This is a brief, selective review of some interactions involving cancer cells and the microvasculature in pathological sequence, specifically: *1*) intravasation of cancer cells; *2*) the arrest of circulating cancer in the microvasculature; *3*) cancer cell trauma associated with arrest; *4*) microvascular trauma; *5*) the inflammatory and *6*) coagulative responses associated with arrest; and *7*) the fate of arrested cancer cells. The evidence shows that in addition to providing routes for cancer cell dissemination and arrest sites for cancer cell emboli, the microvasculature, through a series of complex interactions with cancer cells, controls the efficiency of and acts as a rate regulator for the metastatic process. — WEISS, L.; ORR, F. W.; HONN, K. V. Interactions of cancer cells with the microvasculature during metastasis. *FASEB J.* 2: 12-21; 1988.

Key Words: metastasis • invasion • microcirculation • endothelium • inflammation • platelets • coagulation

METASTASIS IS A MAJOR CONSIDERATION in the staging, treatment, and prognosis of most solid cancers (1).

The bloodstream is the most common pathway for dissemination of cancer cells, which usually enter it on the venous side and gain access to the microvasculature, either via larger vessels or by direct invasion.

Hematogenous metastases arise from cancer cells that arrest in the microvasculature and survive the trauma, which occurs coincident with arrest. Both the cancer cells and the microvasculature are damaged during arrest. The subsequent inflammatory and coagulative responses, together with interactions resulting in angiogenesis, play an important part in the pathophysiology of metastasis formation. We briefly review some of the interactions of the microvasculature with cancer cells that appear to play a pivotal role in determining the efficiency of the subsequent phases of the metastatic process.

INTRAVASATION OF CANCER CELLS

A few comments will be made on intravasation of cancer cells, although the more general topic of invasion (1, 2) is outside the scope of this review.

Direct entry into blood vessels is usually confined to smaller veins and microvasculature. Large veins may also be invaded, but arterial invasion occurs rarely. Correlations have been established between tissue invasion, metastasis, and the ability of cancer cells to release collagenase IV, which degrades collagen IV (3), a dominant component of basement membranes. Cancer cells may intravasate by passive shedding processes and/or active movement.

The generalization that intravasation always requires basement membrane breakdown by collagenolysis is incorrect. In some tumors (i.e., sarcomas), blood is carried in vascular clefts, which are lined by cancer cells, rather than in vascular endothelium. In these tumors, shedding of cancer cells directly into the circulating blood within the clefts does not require prior degradation of basement membranes. However, the shedding process would be aided by the action of proteolytic enzymes (4). In some anatomic sites, the normal basement membranes may be very thin and virtually devoid of collagen bundles. For example, in the lung, the combined thickness of the alveolar epithelium, basement membrane, and capillary endothelium is only 100 μm. In addition, the neovasculature of tumors tends to be fenestrated and leaky. Therefore, intravasation into these vessels may require much less degradation than

[1]Based in part on a Satellite Workshop of the Microcirculation Society, "Interaction of Circulating Cancer and Non-cancer Cells with the Microvasculature," held on March 27, 1987 at the Department of Physiology, Armed Services University of Health Sciences, Bethesda, MD, and also supported by the International Institute for Microcirculation and the American Hoechst Corporation.

[2]Partially supported by grants PDT-273 (to L. W.) and BC-524 (to K. V. H.) from the American Cancer Society, Inc.; the National Cancer Institute of Canada (to F. W. O.); and R01-CA29997 from the National Institutes of Health (to K. V. H.).

0892-6638/88/0002-0012/$01.50.

intravasation into normal vessels. Cancer cells may also enter the bloodstream via lymphaticovenous connections, after entry into the lymphatic saccules or lymphatic capillaries. Neither of these structures has basement membranes.

Finally, it should be emphasized that interactions of cancer cells with the microvasculature and surrounding tissues evoke inflammatory responses that attract leukocytes and macrophages. These cells, together with endothelial cells and fibroblasts, can provide noncancerous sources of enzymes that could enhance both the intravasation and extravasation of cancer cells.

ARREST OF CIRCULATING CANCER CELLS

Attachment of circulating cancer cells to the vessel wall is a prerequisite for their entry into the extravascular space.

Biophysics of arrest

After introduction into the vasculature, rigid particles are arrested in blood vessels having diameters smaller than the particles themselves. Single, circulating cells can deform to some extent to avoid or reduce entrapment in the microvasculature, as observed in the case of erythrocytes (diameter ~ 7 μm) passing through capillaries (diameter ~ 5 μm). On the one hand, some cell types such as macrophages are exceptionally deformable and can therefore apparently avoid mechanical entrapment by compensating for their larger diameters with their rheologic properties. On the other hand, cancer cells, which in general are less deformable than macrophages, apparently cannot rheologically compensate for their greater diameters, and tend to be trapped soon after entry into microvascular beds.

The sequence of events after entry of cancer cells into the microvasculature and their subsequent arrest has been analyzed in terms of electrostatics, electrodynamics, and fluid dynamics (1, 5). Entry into capillaries requires a spherical to cylindrical transformation by the cancer cells, and passage along them is facilitated by the presence of plasma acting as a lubricant between the cancer cell surface and vessel wall. During transit, the tendency to return to a spherical shape is expected to drive out the plasma lubricant. The cancer and endothelial cell surfaces would then rapidly come into contact and arrest will occur. Arrest times of approximately 1 and 2 s have been calculated for cancer cells in the myocardial and pulmonary capillaries, respectively (5, 6).

Among other complexities in this situation are the relative deformabilities of the microvessel walls and the cancer cells, which determine the extent of their perturbation by hydrodynamic fields generated by the circulating cancer cells. This will be discussed below in connection with cancer cell trauma.

Adhesive macromolecules

Kinetic studies of experimental metastasis have demonstrated initial contact between tumor cells and the endothelial plasma membrane, followed by attachment to the underlying basal lamina. As discussed later, platelet and fibrin thrombi have been found at points of tumor cell attachment to the vascular endothelium or exposed basal lamine, which suggests their involvement in the initial phases of the attachment process. In vitro studies indicate that cells attach more readily to the basal lamina than to the endothelium. However, there is also evidence that variation in the surface properties of endothelial cells from different target organs may partially account for differences in the binding of circulating cells, which leads to organ preference in metastatic localization (7).

Many components of basement membranes have the ability to act directly as cellular binding sites, e.g., type IV collagen (8), laminin, fibronectin, and heparin sulfate proteoglycan. A molecular feature that seems common to several of these attachment molecules is the amino acid sequence arginylglyclaspartylserine (9). Receptors for laminin and fibronectin have been identified in the plasma membranes of malignant cells, and there are functional domains in the laminin and fibronectin molecules that regulate the adhesion and motility of metastatic melanoma cells (2). Based on the evidence that the combined i.v. injection of laminin and tumor cells results in an increase in lung colony formation, laminin is presumed to have a significant influence on the metastasis of circulating cells. Blocking the laminin receptor on melanoma cells with laminin fragments or i.v. injection of antibodies to laminin at the same time as the tumor cells leads to a reduction in the number of metastatic nodules formed after i.v. injection (10). Other glycoproteins are also likely to be involved in regulating this and other phases of metastasis. For example, direct correlations have been reported between the metastatic potential of several tumor lines and the degree of sialylation and expression of specific branched cell surface oligosaccharides by a glycoprotein designated gp130 (11). In addition, molecular interactions between various attachment molecules are postulated to be of potential importance when one or more of the molecules is produced endogenously by the cells, e.g., production of laminin, permitting attachment to type IV collagen, proteoglycans, nidogen (8), or thrombospondin, a high-molecular-weight glycoprotein released from the α granules of platelets during activation. The roles of platelets in arrest are discussed under the section Coagulative Response Associated with Arrest.

Interpretation of adhesion experiments

In any discussion of the various components of adhesion of cancer cells to vessel walls, it is important to recognize differences between the various techniques used to study adhesion inasmuch as interpretations are technique-dependent. Many direct experiments on adhesion, particularly those designed to explore the role of adhesive macromolecules, have been made in vitro when cancer cells are usually allowed to sediment under the influence of gravity onto endothelial cell and/or stromal constituent monolayers. Although hy-

drodynamic factors are also thought to play a role in determining the rates of cancer cell adhesion in these in vitro systems, there are obvious physical differences between them and the in vivo situation, where moving cancer cells are forced along capillaries by blood-pressure gradients. It may well be that in vivo cancer cell arrest results predominantly from mechanical impaction, whereas in vitro adhesion is predominantly caused by adhesion macromolecules.

Thus, for example, the in vivo observation that pulmonary tumor colony formation is reduced in mice previously injected with monoclonal antibodies against laminin (12) does not prove that vessel wall laminin is involved in initial cell adhesion to vascular subendothelial collagen IV. It is well documented that laminin affects other cell interactions involving cell migration, postarrest adhesion, differentiation, and possibly the release of enzymes involved in stromal degradation and invasion.

CANCER CELL TRAUMA ASSOCIATED WITH ARREST

It has been known for many years that most nonhematopoietic cancer cells detected in the bloodstream are dead (1).

Rapid killing

Bioassays of the lungs, liver, myocardium, and skeletal muscle of mice after they had received tail-vein, portal-vein or left-ventricular injections of cancer cells indicate that the majority of these cells are killed in the microcirculation of these organs within seconds. It has been suggested that mechanical trauma is a major cause of rapid intravascular cancer cell death. This occurs very shortly before or after cell arrest (13), and is thought to be a major contributory factor to metastatic inefficiency (1).

Pioneering observations on mechanical aspects of intravascular damage to circulating cancer cells were made by Sato and Suzuki (14). These authors correlated cell survival with cell deformability, and later with cell survival after passing through polycarbonate filters. Some mechanisms for mechanical trauma have been advanced based on experimental data and detailed theoretical considerations relating to membrane rupture (13). In this approach, a physically unrestrained, circulating cancer cell is assumed to have an equilibrium configuration with a pleated surface (Fig. 1). Under small deformation pressures, reversible unpleating occurs with no change in cell surface area and no increase in membrane tension. As deformation pressures increase, the unpleated surface is stretched, and its tension rises above a critical level at which membrane rupture occurs. It is well documented that bending of membranes (as required for unpleating) requires orders of magnitude less energy than stretching them. In some noncancer cells and artificial systems, when membrane tension exceeds 4–10 ergs/cm^2, and the expansion exceeds 2–3% of total membrane area, membrane

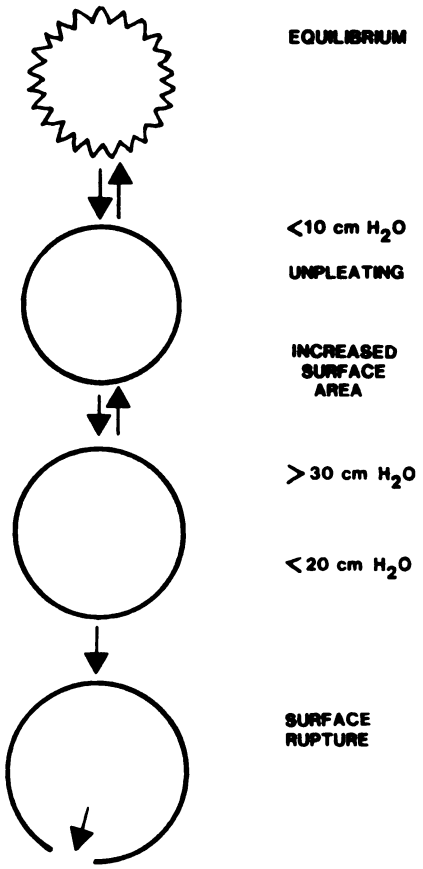

Figure 1. Model of cancer cell subjected to increasing pressures in progressing from the equilibrium state with a pleated surface to an unpleated condition at increased volume (not shown). There is first no change and later minimal increase in surface area. Additional increases in pressure lead to irreversible increases in membrane area, with lethal rupture. From ref 13. Reprinted with permission from L. Weiss, The hemodynamic destruction of circulating cancer cells, © 1987, Pergamon Press.

rupture occurs (13). As described in this simplistic model, surface pleating has a protective function. A complication in this approach is the unknown role of the cytoskeleton in relation to deformation.

Part or all of the sequence of membrane unpleating, stretching, and rupture could take place when cells enter capillaries from larger vessels (Fig. 2); when capillary geometry converts the cancer cell into an extended sausage shape; when an arrested cancer cell blocks a capillary so that the capillary blood-pressure differential is then applied between the free ends of the arrested cancer cell; and when external tissue pressures act on stretched cancer cells.

According to the above hypothesis, the myocardial microcirculation should be an effective site for geometric damage because under the influence of myocardial pressure, the capillaries virtually close. Therefore, after entering a 5-μm myocardial capillary during diastole, a 15-μm-diameter cancer cell having an unpleated surface area of approximately 1400 μm^2 would be squeezed into a 0.5-μm-diameter capillary during systole to develop an approximate 20-fold increase in surface area, unless membrane rupture intervened. If cancer cell arrest took place during diastole, hemodynamic

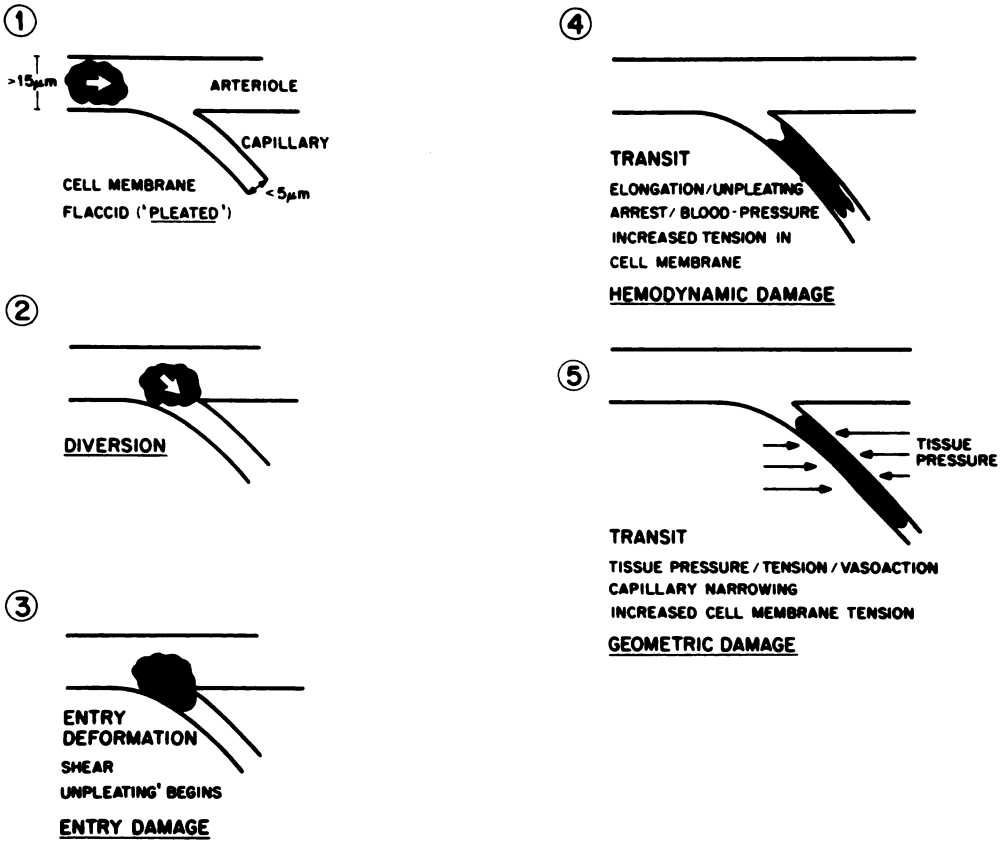

Figure 2. The changes outlined in Fig. 1 are related to cancer cell damage suffered on entry, arrest, and transit in the microvasculature.

damage would occur. These considerations lead to the prediction that the myocardium is an unfavorable soil for metastasizing cancer cells, a fact that is consistent with autopsy (6) and experimental (13) data.

From a knowledge of measured pulmonary alveolar tensions and structure and cancer cell deformability, it is possible to calculate the contributions of mechanical trauma to the observed rapid killing of cancer cells in the lung capillaries. Thus, when capillary wall tension is low at the end of expiration, it is expected that cancer cells will enter and travel along the capillaries without damage, because the vessels will be deformed by the cells and the hydrodynamic field surrounding them. During normal inspiration when the alveoli are stretched, the increased tension within the capillary walls will compress the cells and inflict both hemodynamic and geometric damage, which in at least some cases may be lethal to the cancer cells. In deep inspiration, when very substantial increases in capillary wall tensions occur, cancer cells already within the capillaries are expected to develop membrane tensions greatly exceeding the critical values for rupture (5).

By the use of a nuclepore membrane filtration technique, attempts were made to determine whether survival of L1210 and Ehrlich ascites cancer cells from mechanically initiated filtration trauma was a random event or whether it was a manifestation of subpopulations of cells with inherited resistance to mechanical trauma. Studies of the patterns of cell survival after repeated filtration, and comparisons of the filtration resistance of clonal derivatives of filtration survivors with the original populations, failed to demonstrate that survival was a heritable event (15).

Slow killing

Arrest of cancer cells shares many features with microembolism in general, including elicitation of the inflammatory response. The traumatic effects of inflammatory and associated processes on arrested cancer cells are discussed below (see the next three sections).

MICROVASCULAR TRAUMA

Effects of vascular damage on metastasis

It has been postulated that endothelial damage facilitates cancer cell arrest by causing exposure of the basement membrane. Agents that damage the endothelium can enhance the colonization of i.v. injected tumor cells or spontaneous metastasis. These agents include commonly employed antineoplastic drugs, high concentrations of oxygen, and X-irradiation (16). Direct evidence that vessel wall injury facilitates tumor arrest and metastasis comes from experiments with mice in which dose-dependent, pulmonary endothelial damage was induced by i.v. bleomycin or by exposure to 90% oxygen for 2–4 days. Endothelial injury was demonstrated by morphology and by an increased content of protein

or i.v. injected ^{125}I-labeled albumin in lung lavage fluids. There was a 3- to 36-fold increase in the arrest of [^{131}I]iododeoxyuridine-labeled cancer cells injected i.v. during periods of endothelial injury. Subsequently, more tumors developed in mice with endothelial cell damage. There were direct correlations between the extent of endothelial injury and the numbers of metastatic tumors. The importance of the endothelium as a target for tumor cell arrest was emphasized by the observation that no increase in tumor arrest or metastasis was found after endothelial repair occurred even in the presence of pulmonary fibrosis (16, 17).

Effects of metastasis on vascular integrity

Morphological and biochemical data suggest that there is damage to the basement membrane as cancer cells enter the circulation from the primary tumor. Histological studies have demonstrated correlations between dissolution of basement membrane constituents and tumor malignancy. Other studies provide evidence that the production of proteases by tumor cells facilitates their ability to degrade basement membrane constituents in vitro, an observation that may relate to both intravasation and extravasation. Enzymes of particular interest include type IV collagenase, cathepsin B, and plasminogen activator, which in addition to its role in fibrinolysis, activates collagenase (18, 19). However, the role of these phenomena in in vivo metastasis needs to be defined.

INFLAMMATORY RESPONSES ASSOCIATED WITH TUMOR CELL ARREST THAT AFFECT METASTASIS

Cancer cell arrest within the microvasculature may be influenced by the acute inflammatory response mediated by neutrophil polymorphs. Therefore, although macrophages, lymphocytes, natural killer, and other cells are involved in the later steps of the metastatic process, we will focus on neutrophil polymorphs.

On the one hand, the inflammatory process kills many cancer cells and contributes to metastatic inefficiency; on the other hand, inflammation appears to promote invasion by the survivors. The underlying mechanism is that agents killing cancer cells also damage normal tissues.

Metastasis inhibition

Polymorphonuclear neutrophils (PMNs) are likely to be an effective component of the cytolytic host cell response to circulating tumor cells; PMN-released reactive oxygen radicals are toxic to some tumor cells (20). Thus, the increase in pulmonary retention of i.v. injected tumor cells after depletion of neutrophils or after treatment with superoxide dismutase has been interpreted as evidence that PMN-derived superoxide anion plays an important role in the destruction of arrested tumor cells (21). Further evidence for destruction of intravascular arrested tumor cells by neutrophils has come from ultrastructural studies of the fate of i.v. injected cancer cells in mice (22).

Tail-vein injection of 4×10^4 Lewis lung carcinoma (3LL) cells into C57BL-6J mice results in the formation of few (range 0–7) lung colonies 21 days later. In contrast, the injection of similar numbers of B16a melanoma cells resulted in a significantly larger number (range 50–80) of lung tumor colonies. Ultrastructural examination of the lungs was made at various times after tumor cell injection, and the numbers of arrested tumor cells found in close proximity (i.e., membrane-membrane interaction) with PMNs were recorded. Ten minutes after injection, 20% of the arrested 3LL carcinoma tumor cells were found to be associated with PMNs and 95% were associated at 4 h (Fig. 3). When a comparable number of B16a cells were injected into mice, less than 10% of the arrested tumor cells were found in association with neutrophils (K. V. Honn, unpublished observation). These results suggest a role for circulating PMNs in the destruction of arrested 3LL cells, and the absence of such a role for arrested B16a tumor cells. This suggestion was supported by experiments in which mice given the IgG fraction of rabbit antimouse neutrophil antiserum 1 h before injection of 2.5×10^4 or 4×10^4 3LL cells developed an (antiserum) dose-dependent increase in the number of pulmonary tumor colonies at 21 days (Fig. 4). Ultrastructural observations of lungs removed from animals pretreated with antineutrophil antiserum confirmed a decreased association of PMNs with arrested 3LL cells.

Metastasis promotion

Systemic effects

It is interesting to note that in some experiments that involve the intravascular stimulation of neutrophils and/or an increase in their number (23), neutrophils appear to have a positive effect (i.e., increase) on tumor cell metastasis. Conversely, in some host/tumor systems, depletion of the normal circulating pool of neutrophils results in a negative effect (i.e., decrease) on metastasis (17, 21). One possible explanation of these results is that the general or nonspecific activation of circulating neutrophils may result in some endothelial cell damage with concomitant exposure of and/or damage to the subendothelial matrix.

This hypothesis has been tested on a lung metastasis model. Leukocyte activation is followed by diffuse sequestration of neutrophils into the pulmonary capillaries and injury to the pulmonary endothelium with subsequent lung dysfunction. Rats were given i.v. injections of cobra venom factor resulting in complement activation, rapid sequestration of neutrophils in the lung, and endothelial damage. When radiolabeled cancer cells were injected i.v. (during the period of endothelial injury), there was a threefold increase in pulmonary retention of tumor cells after 24 h when compared with untreated controls. This increased tumor cell retention was associated with more pulmonary tumor colonies 2 wk later. This enhancement was prevented by pretreatment of the rats with catalase or

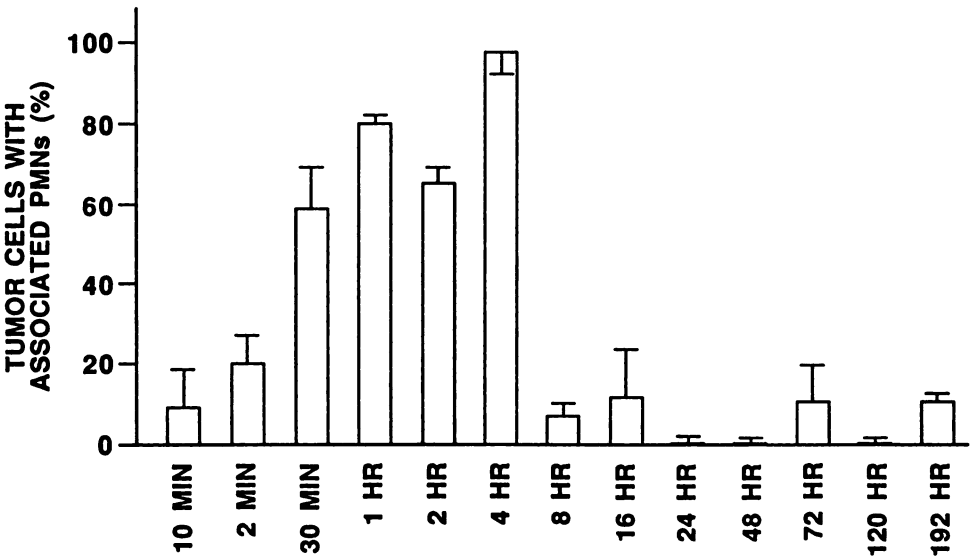

Figure 3. Polymorphonuclear leukocyte association to Lewis lung carcinoma cells in vivo. After i.v. injection, 3LL cells were found in association with host polymorphonuclear leukocytes. The association of Lewis lung cells with host PMNs was observed at the earliest point (10 min) and increased steadily during the first 4 h of observation. Thereafter, the association of tumor cells with PMNs was dramatically decreased and remained at minimal levels throughout the experimental period (192 h).

by antineutrophil antiserum. Therefore, in this model the effects on metastasis may well be related to the generation by the neutrophils of reactive oxygen species, especially H_2O_2 (17).

Local effects

The arrest and metastasis of circulating tumor cells can also be promoted at locally inflamed sites. In models of peritonitis and experimental pneumonitis, it has been demonstrated that acute inflammatory reactions are associated with the local generation of chemoattractants for tumor cells. These chemoattractants are most likely a result of digestion of the C5a component of complement by leukocyte-derived lysosomal enzymes. It has been suggested that inflammatory processes could promote metastasis through the local generation of growth factors, although in general, direct experimental evidence is lacking. It has been repeatedly observed that local or systemic infusion of vasopermeability factors does not alter the metastasis of experimental tumors injected into the circulation (24).

A large number of agents are now recognized to have chemoattractant properties for tumor cells. These include constituents of the vascular basement membrane such as fibronectin, laminin, and collagen-derived peptides. This suggests a means by which attachment of the tumor cell to the basement membrane could stimulate cell motility (2, 10, 24). The mechanisms involved in tumor chemotaxis seem to be analogous to receptor-ligand interactions in leukocytes, involving activation of intermediate metabolites (cyclic nucleotides and arachidonic acid). The end responses of chemoattractant stimulation involve not only cell migration, but also increased cell adhesion and enzyme release (type IV collagenase and plasminogen activator). Recent experiments provide evidence that oxygen radical generation, which may be important if, as in leukocytes, this is a means for inducing endothelial damage (25).

Studies of interactions between the endothelium and circulating leukocytes or platelets indicate that endothelial cells carry interactive sites for a variety of stimuli such as interleukin 1 and tumor necrosis factor. Perturbation of the endothelium is followed by endothelial

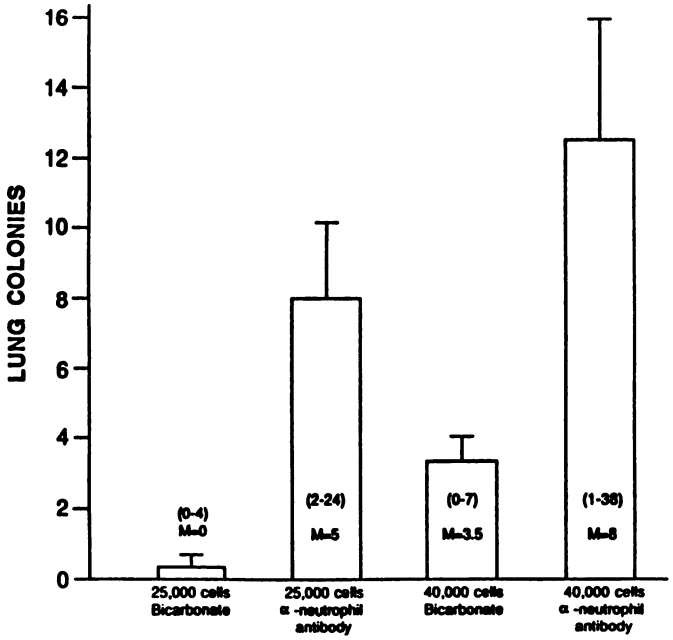

Figure 4. Effect of host pretreatment with antineutrophil antibody on Lewis lung carcinoma pulmonary tumor colony formation. Mice were preinoculated with bicarbonate buffer or antibody against circulating neutrophils 1 h before i.v. injection of 2.5×10^4 or 4×10^4 tumor cells. Host animals were killed 21 days after tumor cell injection, and the incidence of macroscopic pulmonary tumor colonies was determined by visual examination after fixation in Bouin's solution. The incidence of pulmonary tumor colonies was increased in animals pretreated with antineutrophil antiserum.

activation with the synthesis and expression of adhesion molecules (26) and by the release of a variety of humoral products of endothelial metabolism, including interleukin 1. These events are associated with a shift in the metabolism of fatty acids from linoleic acid to arachidonic acid with production of hydroxyeicosatetraenoic acids instead of hydroxyoctadienoic acid (M. R. Buchanan et al., unpublished).

It is possible that these mechanisms are also relevant to metastasis. For example, the motility of human A549 lung carcinoma cells is stimulated in response to endothelial-derived interleukin 1, which suggests a means by which the endothelium could regulate cancer cell motility after arrest (F. W. Orr et al., unpublished). This may be partially responsible for the localization of neoplastic cells at inflamed sites where endothelium is likely to be in an activated state. Other experiments have demonstrated that the adhesiveness of human umbilical vein endothelial cells for the A549 tumor is increased after perturbation of the endothelium with interleukin 1 or peptide chemoattractants. These events are associated with decreased levels of endothelial 13-hydroxyoctadienoic acid, providing further evidence of the similarity and/or association between inflammatory and metastatic mechanisms.

COAGULATIVE RESPONSE ASSOCIATED WITH ARREST

Cancer cell interactions with platelets may facilitate metastasis. Platelet/tumor cell emboli have been proposed to facilitate the entrapment of tumor cells within the microvasculature. In addition, cancer cells shielded within the platelet thrombi may be protected from trauma of types described above. However, contrary to popular belief, in vivo studies (22, 27) suggest that tumor cell-induced platelet aggregation occurs after the initial arrest has occurred. The release of platelet products such as serotonin may induce vasoconstriction, aiding in the trapping of tumor cells or alternatively facilitating endothelial cell retraction (28); platelets also release adhesion glycoproteins, and the platelet membranes themselves may facilitate tumor cell adhesion (29).

Part of the confusion about platelet/cancer cell interactions stems from the fact that the reported times of dissolution of the platelet thrombi and eventual extravasation of the tumor cell vary from one publication to another, even allowing for variation in cancer type. In none of these earlier studies was the extent of platelet involvement in tumor cell arrest quantitated, or the temporal nature of the association between platelets and tumor cells adequately examined. However, recent quantitative studies of platelet involvement in pulmonary arrest and retention of B16 melanoma cells, 3LL cells, and 16C mammary adenocarcinoma cells (22, 30) reveal that at the earliest time intervals examined (10 min), 59% of B16a cells, 21% of 3LL cells, and 51% of 16C cells were in contact with platelet aggregates. Maximum association with pulmonary thrombi were observed by approximately 30 min in all cell lines examined. Platelets remained associated with intravascular arrested tumor cells for approximately 24 h, after which the association with platelets decreased, while tumor cells remained intravascular. At approximately 8 h, endothelial cell retraction occurred and tumor cells initiated attachment to the subendothelial matrix. Tumor cells remained intravascular for 3–5 days, proliferating in the intravascular space (22). As the amount of surface contact between the tumor cell and the subendothelial matrix increased, there was a loss of activated platelets associated with arrested tumor cells. By 24–48 h, tumor cells were observed adherent to the subendothelial matrix of vessel walls, with their luminal surface exposed to blood elements including platelets, which did not show any evidence of activation. This contrasts with cells of the same lines observed during the early arrest phase when massive platelet aggregation occurred. These results suggest that during the process of endothelial cell displacement and cancer cell association with the subendothelial matrix, there is a loss of thrombogenicity on the luminal surface of the tumor cell.

Numerous laboratories have demonstrated that a variety of cancer cell types can induce platelet aggregation. In some cases, this aggregation has been linked to the ability of tumor cells to form lung colonies in vivo; in others, there has been a lack of such correlation (31).

Attempts were made to clarify platelet/cancer cell interactions by electron microscopy at defined phases of the normal aggregation curve (30), and these observations were then correlated with interactions occurring in vivo after tail-vein injection of elutriated tumor cells (22, 30). After the initial limited contact with platelets, cancer cells displayed a rapid decrease in surface microvilli, which remained only at the site of initial platelet attachment to the tumor cell. These microvilli at the attachment site then developed into large cellular processes that extended into the platelet aggregate (30). Thus, not only do platelets respond to cancer cells by aggregating, but cancer cells also respond to platelets with very specific morphological changes, which were not observed with cancer cells and endothelial cells, neutrophils, red blood cells, or lymphocytes (30).

The initiation of aggregation of washed platelets by tumor cells utilizes a thrombin-dependent pathway initiated by a novel procoagulant protein, and requires the presence of calcium (> 1.2 mm) and a small amount of platelet-poor plasma (PPP $< 0.1\%$), which are cofactors necessary for the generation of thrombin (32). This procoagulant protein is neither a tissue factor nor a proteolytic enzyme (32). Recent evidence suggests that the binding of platelets to tumor cell surfaces and the initiation of aggregation are distinct, separable events.

Platelets enhance tumor cell adhesion to extracellular matrix (29). The key factors in platelet-facilitated tumor cell adhesion were an intact plasma membrane and receptor mobility in the platelet plasma membrane (29). It was postulated that platelet-facilitated tumor cell adhesion occurs via the interaction of platelet receptors with both the tumor cell and the subendothelial cell matrix, and further, that the receptors involved in platelet-facilitated tumor cell adhesion are the platelet glycoproteins Ib and the IIb/IIIa complex (33).

Glycoproteins immunologically related to platelet gpIIb/IIIa complex appear to be present in a variety of cancer (33) and noncancer cells, and the translated cDNA sequence for endothelial cells exactly matches the amino acid sequences of platelet gpIIIa (34).

FATE OF ARRESTED CANCER CELLS

Extravasation

In studies of the events leading to metastasis formation, two different patterns of extravasation have emerged: *1*) active migration; and *2*) arrest and proliferation. In support of the first hypothesis, Wood (35) observed the arrest of V2 squamous carcinoma cells and associated thrombus formation within 3–48 h in the rabbit ear chamber model. The tumor cells were often observed to extravasate by following the migration of host leukocytes. Other studies have demonstrated the formation of defects in the vascular wall by tumor cell cytoplasmic projections unrelated to previous passage of host leukocytes (36). The arrest and proliferation hypothesis is characterized by arrest and intravascular proliferation of circulating tumor cells with subsequent erosion of the vessel wall and extravasation by the growing tumor mass rather than extravasation by active tumor cell migration. Microscopy of the lungs after tail-vein injection of B16a melanoma cells (23) revealed that cancer cells were most prevalent in the pulmonary microvasculature 10 min after injection, and their number decreased steadily for 24 h. Platelets were associated with more than half of the arrested tumor cells at the early time intervals, and approximately one-third of these platelets demonstrated degranulation as evidenced by electron microscopic examination. By 48 h, only 2% of the intravascular tumor cells were still associated with platelets. Fibrin association peaked at about 8 h after tumor cell injection. No tumor cells were observed in contact with the basal lamina during the first 4 h after injection. Thereafter, the percentage of tumor cells associated with the basal lamina increased steadily during the first 72 h and remained maximal 120 h after injection of B16a cells. This basal lamina contact was formed after the apparently nontraumatic displacement of the underlying endothelium (23). The percentage of mitotic tumor cells increased dramatically once contact with the basal lamina was established (23). These results suggest that in addition to possibly promoting the mechanical trapping of platelet/tumor cell aggregates within the microvasculature and platelet-facilitated adhesion of tumor cells to the endothelium, platelet/fibrin thrombi may protect arrested tumor cells by a thrombus coat or cocoon and enhance their intravascular growth by the possible release of platelet-derived growth factors. However, cancer cells were observed to proliferate intravascularly long after any protective coat was removed (23).

Neovascularization of metastases

Some of the most important interactions of cancer cells with the microvasculature are those culminating in the vascularization of micrometastases. A hypothesis has been advanced that "once tumor take has occurred, every increase in tumor cell population must be preceded by an increase in new capillaries that converge upon the tumor" (37).

An operational definition of a micrometastasis is a metastatic lesion that obtains its nutrients by diffusion processes. Neovascularization is a major factor promoting the further development of these lesions to metastases with diameters greater than approximately 1 mm. Tumor neovasculature arises from host vessels in the form of capillary sprouts in response to non-cancer-specific and tumor-induced stimuli. Capillary sprouts are formed in sequential steps involving local basement membrane degradation, and the immigration and growth of endothelial cells to form these sprouts that penetrate the tumor to form its neovasculature; alternatively, tumors may acquire their neovasculature by penetration into the dense peritumoral vascular network.

Experiments with rabbits having intraocular tumors have demonstrated that tumor growth is dependent on neovascularization. Implanted tumors growing free in the anterior chambers of the rabbit eye, where they were essentially avascular, attain volumes of approximately 0.5 mm^3 in 14 days. In contrast, tumors of similar type implanted onto the iris, where they rapidly became vascularized, reached volumes of 330 mm^3 over the same time (37).

Although the interactions resulting in angiogenesis can be initiated by products released from cancer cells, they are not cancer-specific but take place in many physiological and pathological situations, particularly during inflammation. Thus, angiogenesis is stimulated by appropriately activated macrophages, particularly in hypoxic environments associated with tissue damage; by (T) lymphocyte products; and by polymorphonuclear leukocytes and mast cells. However, as discussed above, an inflammatory response is commonly associated with different parts of the metastatic process. It is therefore of interest that prostaglandins E_1 and E_2 are angiogenic, although those of the A and F series are not; in addition, prostaglandin synthetase inhibitors inhibit angiogenesis.

An essential step in the angiogenesis sequence is degradation of basement membranes, and in common with other types of invasion, the role of proteolytic enzymes in angiogenesis has been explored. Capillary endothelial cells produce at least two proteolytic enzymes, plasminogen activator (PA) and an inactive form of collagenase. In addition to converting plasminogen to active plasmin, which itself causes tissue proteolysis, plasmin activates latent collagenase, which degrades basement membrane. The degradation products themselves also stimulate angiogenesis. When stimulated in vitro by crude preparations of angiogenesis factor, there was stimulation of the release of PA and collagenase by capillary but not aortic endothelial cells, and not from nonendothelial blood vessel-associated cells. A 500-fold purified, 18-kDa agent was not only a potent stimulator of PA and collagenase production, but also stimulated endothelial cell migration and

growth, demonstrating it to be an angiogenesis factor (38).

Angiogenic factors may act directly on endothelial cells (e.g., acidic and basic fibroblast growth factors and transforming growth factor, TGF-α), or indirectly through cellular intermediates (e.g., TGF-β). In addition, contributions to angiogenesis can be made by agents that are low-molecular-weight endothelial mitogens or chemotactic/chemokinetic factors. It is therefore not surprising that a number of different factors have been described, which probably act through quite different mechanisms. Thus, angiogenin, which is a single-chain polypeptide of 14.4 kDa, appears different from the heparin-binding growth factor; it lacks sequence homology to either acidic or basic fibroblast growth factors, does not bind to heparin, and does not act as a mitogen for vascular endothelial cells (37).

The process of tumor vascularization is interrupted and does not lead to the unrestrained continuous growth of cancer cells. The neovasculature is leaky and the vessels are easily compressed. Thus, tumor expansion, together with the absence of tumor lymphatics that could act as a pressure relief valve, tends to compress the internal neovasculature and lead to local anoxia and necrosis, particularly in rapidly growing tumors. Although this is not the only event leading to inhibition of cancer cell growth or death, the possibility that the growth of metastases could be controlled by antiangiogenesis agents or angiostasis is interesting.

Although neither angiogenesis nor its regulation is fully understood, it seems that these processes constitute a group of cancer cell/microvasculature interactions of major importance in regulating the final development of metastases from cancer cells surviving other interactions with the microvasculature. It is of great interest to view neovascularization of tumors not as an isolated event, but rather as linked to other interactions discussed earlier.

CONCLUSIONS

The blood vascular system provides the major route for cancer dissemination, and cancer emboli from which metastases develop are arrested in the microvasculature. The evidence, briefly reviewed here, shows that in addition to serving these traditional anatomic roles, the microvasculature, by means of a complex series of interactions involving both cancer and noncancer cells, controls the efficiency of critical parts of the metastatic process and thereby acts as one of its major rate regulators. One objective for future research in this area will be to more precisely define the nature of the relationships between cancer cells and vessel walls. In addition, the prospect of pharmacological modulation of endothelial function offers a potential therapeutic strategy for the control of cancer dissemination. FJ

REFERENCES[3]

1. Weiss, L. *Principles of metastasis.* Orlando, FL: Academic; 1985.
2. Liotta, L. A. Tumor invasion and metastases—role of the extracellular matrix. *Cancer Res.* 46: 1-7; 1986.
3. Garbisa, S.; Pozzatti, R.; Muschel, R. J.; Sattiotti, U.; Ballin, M.; Goldfarb, G. H.; Khoury, G.; Liotta, L. A. Secretion of type IV collagenolytic protease and metastatic phenotype: induction by transfection with c-Ha-ras but not c-Ha-ras plus AD2-E1a. *Cancer Res.* 47: 1523-1528; 1987.
4. Weiss, L.; Ward, P. M. Cell detachment and metastasis. *Cancer Metastasis Rev.* 2: 111-127; 1983.
5. Weiss, L.; Dimitrov, D. S. Mechanical aspects of the lungs as cancer cell-killing organs during hematogenous metastasis. *J. Theor. Biol.* 121: 307-322; 1986.
6. Weiss, L.; Dimitrov, D. S.; Angelova, M. The hemodynamic destruction of intravascular cancer cells in relation to myocardial metastasis. *Proc. Natl. Acad. Sci. USA* 82: 5737-5741; 1985.
7. Auerbach, R.; Wei Cheng, L.; Pardon, E.; Gumkowski, F.; Kaminska, G.; Kaminski, M. Specificity of adhesion between tumor cells and capillary endothelium: an in vitro correlate of preferential metastasis in vivo. *Cancer Res.* 47: 1492-1496; 1987.
8. Aumailley, M.; Timpl, R. Attachment of cells to basement membrane collagen type IV. *J. Cell Biol.* 103: 1569-1575; 1986.
9. Hynes, R. O. Integrins: a family of cell surface receptors. *Cell* 48: 549-554; 1987.
10. Terranova, V. P.; Hujanen, E. S.; Martin, G. R. Basement membrane and the invasive activity of metastatic tumor cells. *J. Natl. Cancer Inst.* 77: 311-316; 1986.
11. Dennis, J. W.; Laferte, S.; Waghorne, C.; Breitman, J. L.; Kerbel, R. S. Beta 1-6 branching of ASN-linked oligosaccharides is directly associated with metastasis. *Science* 236: 582-585; 1987.
12. Vollmers, H. P.; Imhof, B. A.; Braun, S.; Waller, C. A.; Schirrmacher, V.; Birchmeier, W. Monoclonal antibodies which prevent experimental lung metastases. *FEBS Lett.* 172: 17-20; 1984.
13. Weiss, L. The hemodynamic destruction of circulating cancer cells. *Biorheology* 24: 105-115; 1987.
14. Sato, H.; Suzuki, M. Deformability and viability of tumor cells by transcapillary passage, with reference to organ affinity of metastasis in cancer. Weiss, L., ed. *Fundamental aspects of metastasis.* Amsterdam: North Holland; 1976: 311-317.
15. Gabor, H.; Weiss, L. Survival of L1210 and Ehrlich ascites cancer cells after mechanical trauma is a random event. *Invasion & Metastasis* 5: 84-95; 1985.
16. Adamson, I. Y. R.; Young, L.; Orr, F. W. Tumor metastasis after hyperoxic injury and repair of the pulmonary endothelium. *Lab. Invest.* 57: 71-77; 1987.
17. Orr, F. W.; Warner, D. J. A. Effects of neutrophil-mediated pulmonary endothelial injury on the localization and metastasis of circulating Walker carcinosarcoma cells. *Invasion & Metastasis* 7: 183-196; 1987.
18. Sloane, B. F.; Rozhin, J.; Ryan, R. E.; Lah, T. T.; Day, N. A.; Crissman, J. D.; Honn, K. V. Cathepsin B-like cysteine proteinases and metastasis. *Dev. Oncol.* 40: 377-397; 1986.
19. Danø, K.; Andreasen, P. A.; Grøndahl-Hansen, J.; Kristensen, P.; Nielsen, L. S.; Skriver, L. Plas-

[3]Editorial restriction on the numbers of references necessitates that citations to reviews are used to cover historical background and precedence. The majority of references are used to cover the recent literature. We apologize to the many apparently neglected authors who are, in fact, cited in the referred publications.

minogen activators, tissue degradation, and cancer. *Adv. Cancer Res.* 44: 139-266; 1985.
20. Lichtenstein, A. Stimulation of respiratory burst of murine peritoneal inflammatory neutrophils by conjugation with tumor cells. *Cancer Res.* 47: 2211-2217; 1987.
21. Glaves, D. Intravascular death of disseminated cancer cells mediated by superoxide anion. *Invasion & Metastasis* 6: 101-111; 1986.
22. Crissman, J. D.; Hatfield, J.; Schaldenbrand, M.; Sloane, B. F.; Honn, K. V. Arrest and extravasation of B16 amelanotic melanoma in murine lungs. *Lab. Invest.* 53: 470-478; 1985.
23. Ishikawa, M.; Koga, Y.; Hosokawa, M.; Kobayashi, H. Augmentation of B16 melanoma lung colony formation of C57BL/6 mice having marked granulocytosis. *Int. J. Cancer* 37: 919-924; 1986.
24. Varani, J.; Orr, F. W. Chemotactic mechanisms and cancer metastasis. *Pathol. Annu.* 17: 307-330; 1982.
25. Leroyer, V.; Werner, L.; Shaughennessy, S.; Goddard, G. J.; Orr, F. W. Chemiluminescence and oxygen radical generation by Walker carcinosarcoma cells following chemotactin stimulation. *Cancer Res.* In press.
26. Jaffe, E. A. Cell biology of endothelial cells. *Human Pathol.* 18: 234-239; 1987.
27. Tanaka, N. G.; Tohgo, A.; Ogawa, H. Platelet-aggregating activities of metastasizing tumor cells. V. In situ roles of platelets in hematogenous metastasis. *Invasion & Metastasis* 6: 209-224; 1986.
28. Skolnik, G.; Ericson, L. E.; Kjellstrom, T.; Rehman, L.; Isperg, B. "Trap-door" effect of the endothelium—a mechanism of tumor cell extravasation. Hellmann, K.; Eccles, S. A., eds. *Treatment of metastasis: problems and prospects.* London: Taylor & Francis; 1985: 283-286.
29. Menter, D. G.; Harkins, C.; Onoda, J.; Craig, R.; Steinert, B. W.; Sloane, B. F.; Taylor, J. D.; Honn, K. V. Platelet enhancement of tumor cell adhesion to subendothelial matrix: role of platelet cytoskeleton and platelet membrane. *J. Natl. Cancer Inst.* In press.
30. Menter, D.; Hatfield, J. S.; Harkins, C.; Sloane, B. F.; Taylor, J. D.; Crissman, J. D.; Honn, K. V. Tumor cell-platelet interactions in vitro and their relationship to in vivo arrest of hematogenously circulating tumor cells. *Clin. & Exp. Metastasis* 5: 65-78; 1987.
31. Estrada, J.; Nicolson, G. L. Tumor-cell-platelet aggregation does not correlate with metastatic potential of rat 13762NF mammary adenocarcinoma tumor cell clones. *Int. J. Cancer* 34: 101-105; 1984.
32. Cavanaugh, P. G.; Sloane, B. F.; Honn, K. V. The role of the coagulation system in tumor cell induced platelet aggregation and metastasis. *Hemostasis* In press.
33. Grossi, I. G.; Honn, K. V.; Sloane, B. F.; Thompson, J.; Ohannesian, D.; Kendall, A.; Newcomb, M. Role of platelet glycoproteins 1b and 11b/111a in tumor cell induced platelet aggregation and tumor cell adhesion to extracellular matrix. *Thromb. Haemostas.* 58: 507; 1987.
34. Fitzgerald, L. A.; Steiner, G.; Rali, S. C.; Lo, S.; Phillips, D. R. Protein sequence of endothelial glycoprotein IIIa derived from a cDNA clone. Identity with platelet glycoprotein IIIa and similarity to "integrin." *J. Biol. Chem.* 262: 3936-3939; 1987.
35. Wood, S. Pathogenesis of metastasis formation observed in vivo in the rabbit ear chamber. *Arch. Pathol.* 66: 550; 1958.
36. Kinjo, M. Lodgement and extravasation of tumor cells in blood-borne metastasis: an electron microscopy study. *Br. J. Cancer* 38: 293-301; 1978.
37. Folkman, J.; Klagsbrun, M. Angiogenic factors. *Science* 235: 442-447; 1987.
38. Moscatelli, D.; Presta, M.; Rifkin, D. B. Purification of a factor from human placenta that stimulates capillary endothelial cell protease production, DNA synthesis, and migration. *Proc. Natl. Acad. Sci. USA* 83: 2091-2095; 1986.