INTRODUCTION

An association between systemic activation of the clotting system and malignant disease has been recognized for more than a century (1, 2). Patients with many types of malignancy, especially in their advanced stages, exhibit a diverse spectrum of coagulopathies that may include thrombophlebitis, hemorrhage, embolism, and disseminated intravascular coagulation. As many as 95% of cancer patients may exhibit some type of clotting abnormality. Fibrin deposition at sites of solid tumor growth was first suspected in the late 1950s (3). Support for this possibility has come from studies of two types. (a) Immunohistochemical approaches have indicated that fibrinogen and/or fibrin accumulates in a wide variety of tumors, both transplantable and autochthonous, and in both humans and experimental animals (4–9). Tumor-associated fibrin takes the form of a gel and is often particularly abundant at the tumor’s growing edge, i.e., at the tumor-host interface. (b) Fibrinogen or fibrin has been localized to solid tumors by means of radiolabeling and radiographic techniques (10, 11).

The mechanisms by which fibrin is deposited in solid tumors have been investigated to some extent. Many tumors have been localized to solid tumors by means of radiolabeling and radiographic techniques (10, 11). However, roles for clotting, fibrin deposition, and fibrinolysis have been proposed in tumor invasion, protection from host immunological defense mechanisms, angiogenesis, and tumor stroma formation (8, 9, 16, 17).

At present, very little is known about the nature and extent of the fibrinogen/fibrin-related proteins that are deposited in tumors. A recent study from our laboratory determined that both the influx of 125I-fibrinogen and the accumulation of radioactive products were many times greater in two transplantable guinea pig hepatocarcinomas than in control subcutaneous tissue (18). Half or more of the labeled products was insoluble in 3 M urea, a property of fibrin which has been cross-linked by clotting factor XIIa (19–21).

In the present study, we sought to generalize the findings of increased fibrinogen influx and fibrin accumulation in guinea pig carcinomas to several types of carcinomas in a different species, the mouse. In addition, we sought to measure fibrinogen influx and accumulation in a premalignant mammary lesion, HAN. A final goal was to characterize more carefully the molecular structure of tumor-associated fibrin by means of polyacrylamide gel electrophoresis and autoradiography in animals that had received an i.v. injection of iodinated fibrinogen.

MATERIALS AND METHODS

Tumors. Lewis lung carcinomas were obtained as frozen solid fragments from the NCI-Frederick Cancer Research Facility, Frederick, MD; TA3/St and MOT ascites tumor cells were the kind gifts of Dr. Barbara Sanford and Dr. Gerald Kolodny, respectively. Lewis lung tumors were passaged in the subcutaneous space of syngeneic C57BL/6 mice. TA3/St and MOT ascites tumors were passaged weekly in syngeneic, 5- to 6-wk-old A/J and C3HeB/FeJ mice, respectively.

We also studied primary mammary adenocarcinomas that arose spontaneously from the D2 HAN line. The D2 HAN is a precancerous mammary lesion that is maintained by serial transplantation in the mammary fat pads of virgin female BALB/c mice after the fat pads have been cleared of host epithelium (22, 23). All of the BALB/c mice were generously provided by Dr. Daniel Medina.

Fibrinogen. Fibrinogen of high quality is difficult to prepare from the mouse because of its small size and limited blood volume. Therefore, GPF and HF were used in these experiments. GPF, either prepared from strain 2 guinea pig plasma (18) or purchased from Sigma, St. Louis, MO, was iodinated by the lactoperoxidase-glucose oxidase method to a specific activity of 0.022 mol of iodine per mol of fibrinogen (24). HF, obtained from Kabi, Greenwich, CT, was similarly iodinated. Autoradiograms of 125I-GPF or 125I-HF that had been electrophoresed on reduced 7.5% SDS-polyacrylamide gels revealed clear, ungraded Aα, Bγ, and β bands (Fig. 1). More than 97% of labeled fibrinogen was precipitated by 10% trichloroacetic acid and clottability, measured as the percentage of radioactivity associated with the washed fibrin clot following coagulation with thrombin, exceeded 80%.

The abbreviations used were: HAN, hyperplastic alveolar nodules; GPF, guinea pig fibrinogen; HF, human fibrinogen; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.
No bands are present in the autoradiogram of control subcutaneous tissue. and 7 chains, all indicative of cross-linked fibrin, are present in the tumor extract. 98,000), poly-α (M, > 200,000) bands, normal B/3(β) chain, and decreased Ao(α) tumor (Lane 3), and normal subcutaneous tissue (Lane 4), all harvested from an A/J mouse 4 h after i.v. I25l-fibrinogen injection. Distinct 7-7 dimer (M, ~ 340,000) fibrinogen preparation prior to injection (Lane 1) and plasma (Lane 2), TA3/St animals were sacrificed with ether and exsanguinated. Tumors and anticoagulant-antifibrinolytic mixture (18). Immediately thereafter, an hemorrhage, or an inflammatory response that often developed as tumor growth progressed.

For influx measurements, animals received 2.5 × 10^6 cpm of 125I-labeled GPF or HF i.v., followed exactly 20 min later by 0.2 ml of an anticoagulant-antifibrinolytic mixture (18). Immediately thereafter, animals were sacrificed with ether and exsanguinated. Tumors and roughly equivalent amounts of control tissues were harvested in tared tubes containing 2 ml of ice-cold 0.01 M phosphate buffer, pH 7.5, supplemented with protease inhibitors (18), prior to determining wet weights.

An important criterion for selection of control tissues was that they possessed a microvasculature of the same structural type as that of the tumors studied, that is, lined by continuous endothelium (Refs. 25 and 26; Footnote 4). Normal mammary tissue, ear skin, and skeletal muscle all have a microvasculature lined by continuous endothelium and therefore were appropriate choices (25, 26).

Accumulation experiments were performed in identical fashion to influx experiments except that 125I-GPF or HF was injected 18 h prior to animal sacrifice.

Extraction of Tumors and Control Tissues. Tumors and control tissues were minced into fine fragments in 2 ml of cold tissue buffer; aqueous and urea extraction were then performed as described (18). The extracts and the urea-insoluble pellets were separately counted for radioactivity. Total GPF accumulating in tumors would be expected to consist of a mixture of fibrinogen, fibrin, and fibrinogen/fibrin degradation products. We have shown previously that fibrinogen and soluble degradation products of fibrinogen or fibrin appear in the aqueous extract; that fibrin polymers that had not been cross-linked by factor Xlla, and some partly degraded cross-linked fibrin, appear in the urea-solubilized extract; and that cross-linked fibrin as well as some early cross-linked fibrin degradation products remain in the urea-insoluble fraction (18).

Calculation of 125I GPF Influx and Accumulation. Total influx was calculated by dividing cpm/g of tissue by cpm/μl of plasma, giving a value whose units are μl of plasma/g of wet tissue; that is, influx represents the number of μl of plasma 125I-GPF that entered each gram of tumor or other tissue over the course of 20 min after i.v. injection of radioactive tracer, a period of time in which efflux of tracer from the tissue was negligible and could be safely ignored (18). Influx is a function of both local blood flow and microvascular permeability. In addition to measuring total influx, we also measured that portion of total 125I-GPF present in tumor or control tissues that was insoluble in 3 M urea.

Accumulation of 125I-GPF in tumors or control tissues was similarly calculated by dividing the specific radioactivity of the tissue (cpm/g wet weight) by the specific radioactivity present in plasma at the time of animal killing (cpm/μl) 18 h later. At this interval, tissue radioactivity reflects a balance between tracer influx and efflux. Because plasma levels of labeled fibrinogen were falling exponentially throughout the 18 h of the experiment, this calculation does not provide a satisfactory estimate of absolute fibrinogen accumulation (18). However, it does provide a useful measure of relative accumulation that allows valid comparisons between different tissues within the same animal or the same inbred strain in which plasma clearance rates of radioactive fibrinogen are identical.

Statistical comparisons of influx and accumulation data were made using Student's t test or the Newman-Keuls test for multiple comparisons (27).

Electrophoresis and Autoradiography of Tumor Extracts. These experiments were performed identically to accumulation experiments except that animals received 2.5 × 10^6 cpm of 125I-GPF i.v. Mice were killed. Tumors and control tissues were minced and solubilized in a mixture composed of 8 μl urea, 2% SDS, 2% dithiothreitol, 0.01 M Tris, pH 6.8, 10 units/ml of heparin, 2 units/ml of hirudin or 5 μg/ml of ζ-phenylalanine-arginine-chloromethyl ketone, 2 mg/ml of EDTA, 0.1 M 2-aminocaproic acid, and 2 mM phenylmethylsulfonyl fluoride at 90°C over the course of 1 h. Samples were then run on 7.5% SDS-PAGE and stained with Coomassie blue. Autoradiograms on XAR-2 film were exposed at -70°C for 1 to 7 days (14). Protein molecular weight markers (Bethesda Research Laboratories, Inc.) were run on each SDS gel; other standards regularly used included purified guinea pig fibrinogen and fibrin clotted with thrombin and cross-linked with factor XIIIa (28).

Migration Properties of Fibrinogen and Fibrin on SDS-PAGE. Native fibrinogen is a M, 340,000 heterodimer composed of three pairs of nonidentical polypeptide chains (α, β, and γ) covalently linked by disulide bonds (29). Reduction of the disulide bonds cleaves fibrinogen into its three types of chain which migrate separately on SDS-PAGE according to their respective molecular weights. Cross-linked or non-cross-linked fibrins have extremely high molecular weights and without reduction do not enter 7.5% polyacrylamide gels (30). Reduction of non-cross-linked fibrin generates a pattern on SDS-PAGE that is similar to that of reduced fibrinogen except that the α and β bands migrate at slightly lower molecular weights after the A and B fibrinopeptides have been cleaved. Reduction of cross-linked fibrin results in a distinctly different and characteristic pattern on SDS-PAGE (30).

To the extent that the fibrin is cross-linked, the γ chains are dimerized to form γ-γ dimers (M, ~ 98,000), and the α chains form high-molecular-weight polymers (poly-α chains) of varying size, some of which are too large to enter the running gel. As a consequence, α and γ chains appear reduced or absent, whereas β chains remain unchanged from fibrinogen.

Immunohistochemistry. Tumors were fixed in periodate-lysine-paraformaldehyde (31) for 5 h at 4°C. Immunoperoxidase staining was performed on 4-μm paraffin-embedded tissue sections after pretreatment with trypsin, using an indirect peroxidase-antiperoxidase technique (32). Primary rabbit antibodies to human fibrinogen (Accurate Chemical and Scientific Corporation, New York, NY) were absorbed (9) to remove contaminating antibodies to fibronectin. Absorbed antibodies reacted with fibrinogen, fibrin, and certain fibrinogen/fibrin breakdown products in tissue sections (9) and only with fibrinogen in Western blots of electrophoresed mouse plasma.3

RESULTS

Influx of 125I-GPF into Transplantable Mouse Carcinomas. Influx of 125I-GPF was 2 to 9 times greater in all three mouse

3 J. Nagy, unpublished data.
tumors than in their respective normal breast or skeletal muscle (P < 0.01) (Table 1). Differences in influx were even greater (3- to 15-fold) when urea-insoluble $^{125}$I-GPF was compared instead of total $^{125}$I-GPF.

Relative Accumulation of $^{125}$I-GPF in Transplantable Mouse Tumors. Accumulation of $^{125}$I-GPF was measured in tumor and control tissues over an 18-h period (Table 2). Accumulation of total $^{125}$I-GPF was consistently and significantly greater (1.6- to 11-fold) in each of the three mouse tumors than in any of the normal control tissues studied (P < 0.01). The differences between tumors and control tissues were greater still (11- to 39-fold) when accumulation of urea-insoluble $^{125}$I-GPF, rather than total $^{125}$I-GPF, was compared. In every case, the fraction of tumor $^{125}$I-GPF that remained insoluble following urea extraction was significantly greater than that for any of the normal control tissues (P < 0.01).

Accumulation of $^{125}$I-GPF was also studied in virgin female BALB/c mice bearing HAN or mammary carcinomas that arose from HAN. As shown in Table 3, total $^{125}$I-GPF accumulation was significantly greater in HAN than in normal mammary tissue (P < 0.01), though the fraction insoluble in 3 M urea was not increased. Both $^{125}$I-GPF accumulation and the fraction of $^{125}$I-GPF that was urea insoluble were greater still in mammary tumors that developed from HAN (P < 0.01 versus both HAN and normal mammary tissue). Once again, the differences became even larger when urea-insoluble $^{125}$I-GPF was compared rather than total $^{125}$I-GPF.

Molecular Characterization of Tumor-associated $^{125}$I-GPF by SDS-PAGE and Autoradiography. TA3/St mammary and Lewis lung carcinomas and plasma were harvested from mice that had received $^{125}$I-GPF or HF i.v., 4 or 18 h previously. These were extracted and electrophoresed on SDS-PAGE under reducing conditions, along with the $^{125}$I-HF starting material. Autoradiograms of the injected radioactive fibrinogen and of plasma taken from animals that had received iodinated fibrinogen 4 h earlier revealed clear Aα, Bβ, and γ chains and no other significant bands (Fig. 1). Thus, neither the starting material nor circulating fibrinogen gave evidence of clotting or degradation. Insufficient radioactivity remained in the circulation at 18 h to evaluate plasma at that interval by autoradiography.

In contrast, tumor extracts revealed prominent Bβ(§) and γγ bands and several bands corresponding to polymerized α chains. Bands corresponding to the Aα(α) and γ chains were reduced in intensity, as would be expected if these had been largely polymerized and cross-linked (30). A single, very prominent additional band (Fig. 1, Lane 3, DP) was also noted along with several lighter bands of lower molecular weight; these correspond to fibrinogen/fibrin degradation products whose nature we are currently investigating. As anticipated from fibrinogen influx and accumulation data, gels of extracts of normal subcutaneous tissue revealed no detectable fibrinogen/fibrin (Fig. 1, Lane 4).

Localization of Fibrin in Mouse Tumors by Immunocytochemistry. All three transplantable tumors were poorly differentiated carcinomas, composed of nodules of loosely connected tumor cells and minimal connective tissue stroma.

Abundant fibrillar deposits reactive with antibodies to fibrinogen/fibrin were observed in all three tumors. Lewis lung and TA3/St carcinoma nodules were enveloped by loose connective tissue containing abundant fibrin (Fig. 2). In MOT tumors, fibrin appeared as a sharply defined, circumferential band that was deposited at the tumor-host interface. This band may have served as a barrier to host lymphocytes which were numerous in the surrounding host tissue but which did not penetrate the fibrin to make contact with tumor cells.

A prominent fibrin meshwork also circumscribed individual Lewis lung and TA3/St mammary carcinoma cells and cell clumps (Fig. 2). These deposits were patchy, being well-developed in some zones and less noticeable in others, and their presence could not be related to tumor cell necrosis nor to local blood vessel frequency.

Connective tissue at a distance from the tumor, normal skin, and normal mammary tissue exhibited no immunohistochemically reactive deposits.

DISCUSSION

Our data indicate that influx of $^{125}$I-GPF into three transplantable mouse carcinomas greatly exceeded that into several normal tissues whose microvasculature was also of the continuous type (25, 26). The differences in influx between tumors and control tissues were even more striking when urea-insoluble $^{125}$I-GPF was compared rather than total $^{125}$I-GPF. At least two factors accounted for the greater sensitivity of the urea-insoluble $^{125}$I-GPF comparison. (α) Urea-insoluble $^{125}$I-GPF measures only that fraction of tracer that had extravasated from blood vessels into the tissues, whereas total influx also includes vari-

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Table 1 Influx of $^{125}$I-GPF into three transplantable mouse carcinomas and normal control tissues

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Tissue</th>
<th>No. studied</th>
<th>125I-GPF influx</th>
<th>Total (µg/g)</th>
<th>Urea insoluble (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>Lewis lung carcinoma</td>
<td>17</td>
<td>93.1 ± 9.4</td>
<td>24.9 ± 5.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal mammary tissue</td>
<td>10</td>
<td>45.8 ± 8.4</td>
<td>1.9 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal muscle</td>
<td>6</td>
<td>30.7 ± 4.9</td>
<td>7.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>A/J</td>
<td>TA3/St breast carcinoma</td>
<td>10</td>
<td>46.7 ± 6.8</td>
<td>12.7 ± 4.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal mammary tissue</td>
<td>4</td>
<td>16.2 ± 0.8</td>
<td>1.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>C3HeB/FeJ</td>
<td>MOT ovarian carcinoma</td>
<td>6</td>
<td>47.9 ± 7.5</td>
<td>4.6 ± 1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal mammary tissue</td>
<td>3</td>
<td>24.9 ± 7.3</td>
<td>0.3 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SE.

Table 2 Relative accumulation of $^{125}$I-GPF in three transplantable mouse carcinomas and normal control tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. studied</th>
<th>Total (µg/g)</th>
<th>Urea insoluble (µg/g)</th>
<th>% urea insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis lung carcinoma</td>
<td>17</td>
<td>589 ± 65.0**</td>
<td>284 ± 41.8</td>
<td>46</td>
</tr>
<tr>
<td>Normal ears</td>
<td>10</td>
<td>367 ± 40.0</td>
<td>26.7 ± 3.4</td>
<td>7</td>
</tr>
<tr>
<td>Normal mammary tissue</td>
<td>11</td>
<td>178 ± 50.6</td>
<td>12.1 ± 3.1</td>
<td>8</td>
</tr>
<tr>
<td>Normal muscle</td>
<td>7</td>
<td>52.1 ± 8.7</td>
<td>7.2 ± 1.3</td>
<td>14</td>
</tr>
<tr>
<td>TA3/St mammary carcinoma</td>
<td>10</td>
<td>470 ± 29.7</td>
<td>224 ± 74.6</td>
<td>46</td>
</tr>
<tr>
<td>Normal ears</td>
<td>10</td>
<td>101 ± 4.8</td>
<td>18.1 ± 2.4</td>
<td>18</td>
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<tr>
<td>Normal mammary tissue</td>
<td>5</td>
<td>88.8 ± 12.3</td>
<td>8.2 ± 1.2</td>
<td>9</td>
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<tr>
<td>MOT ovarian carcinoma</td>
<td>10</td>
<td>302 ± 20.4</td>
<td>135 ± 15.1</td>
<td>44</td>
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<tr>
<td>Normal ears</td>
<td>8</td>
<td>81.6 ± 7.8</td>
<td>10.1 ± 1.6</td>
<td>13</td>
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<tr>
<td>Normal mammary tissue</td>
<td>5</td>
<td>62.4 ± 14.5</td>
<td>5.7 ± 1.1</td>
<td>10</td>
</tr>
</tbody>
</table>

*Mean ± SE.

Table 3 Relative accumulation of $^{125}$I-GPF in D2 HAN tissue and in primary mammary carcinomas arising from the HAN as compared with normal mammary tissue of virgin female BALB/c mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. studied</th>
<th>Total (µg/g)</th>
<th>Urea insoluble (µg/g)</th>
<th>% urea insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary mammary carcinomas</td>
<td>11</td>
<td>160 ± 20.2</td>
<td>56.9 ± 14.7</td>
<td>32</td>
</tr>
<tr>
<td>HAN</td>
<td>15</td>
<td>99.7 ± 6.7</td>
<td>7.0 ± 0.7</td>
<td>7</td>
</tr>
<tr>
<td>Normal mammary tissue</td>
<td>14</td>
<td>48.4 ± 3.4</td>
<td>2.8 ± 0.8</td>
<td>6</td>
</tr>
</tbody>
</table>

*Mean ± SE.
Fig. 2. Immunoperoxidase localization of fibrinogen/fibrin in Lewis lung (a and b), TA3/St (c and d), and MOT (e and f) carcinomas. In all instances, fibrinogen/fibrin reactive products appear as dark-staining fibrils. Lewis lung carcinomas: in a, prominent fibrin deposits are present in the host (H) connective tissue that envelops tumor (T) nodules; in b, in addition, prominent fibrin strands circumscribe individual tumor cells and tumor cell clumps. TA3/St carcinomas: in c and d, the loose host (H) connective tissue enveloping tumor (T) nodules contains lightly staining fibrinogen/fibrin deposits. More extensive fibrillar deposits are found between tumor cells and cell clumps. MOT carcinomas: in e and f, the junction between tumor (T) and enveloping host (H) tissue is delineated by a sharp, linear band of fibrin. Lymphocytes form cuffs around blood vessels (v) in adjacent host tissue, and individual lymphocytes approach, but do not penetrate, the fibrin band to make contact with tumor cells. In contrast to the Lewis lung and TA3/St carcinomas, little or no fibrin is deposited between tumor cells. Sections were lightly counterstained with hematoxylin. a and b, × 340; c × 220; d, × 440; e, × 280; f, × 340.

able amounts of tracer that remain within blood vessel lumina after exsanguination. Residual intravascular tracer may be substantial in tissues such as ear skin that have a relatively large vascular volume that is difficult to drain. Because animals were systemically anticoagulated immediately prior to tumor harvest, residual intravascular tracer was entirely in the form of fibrinogen, as attested by infinite plasma clotting times. (b) A larger fraction of extravasated fibrinogen clotted in tumors than in...
most control tissues (Table 1 and 2).

These data generalize to the mouse earlier studies with guinea pig carcinomas, indicating that influx of plasma proteins into tumors is greater than into normal tissues having comparable continuous microvascular endothelium (18). Using a somewhat different approach, O'Connor and Bale (33) reported increased influx of iodinated IgG into the extravascular compartments of several rat fibrosarcomas, as compared with normal skin and muscle. Dewey (34) and Song and Levitt (35) made similar observations concerning the extravasation of iodinated γ-globulin and albumin into Walker rat carcinomas. However, in none of these instances was the type of microvasculature supplying these tumors defined. Influx is a function of both local blood flow and the extent of local vascular permeability to tracer, but the latter is more important in accounting for the increased influx of plasma proteins into tumors lined by a vascular endothelium of the continuous type (5, 8, 12, 14). High-molecular-weight tracers such as fibrinogen normally penetrate vessels lined by continuous endothelia at only a very low rate (25, 26).

Other reports that tumor vessels have increased vascular permeability are open to interpretation. Thus, Peterson et al. (36) reported an elevated level of vascular permeability to plasma proteins in two chemically induced rat sarcomas, but "influx" was measured over a 7 h interval, i.e., between 1 and 8 h after tracer injection. Increased isotope accumulation obtained over so long a period of time cannot be taken as evidence for increased vascular permeability. The reported findings could reflect normal or even reduced microvascular permeability coupled with increased blood flow or impeded return to the circulation of extravasated tracer. Such an interpretation would not be unreasonable in that extravasated plasma proteins normally return to the circulation by way of lymphatics, structures that are generally absent from tumors. Similar questions arise in the experiments of Ackerman and Hechmer (37) who claimed increased vascular permeability to circulating plasma protein-bound dyes in the case of Walker carcinomas growing in the livers of Sprague-Dawley rats. Increased dye was observed in tumors only after a lag phase of more than 2 h. At earlier intervals, these investigators actually found decreased amounts of dye in tumors, relative to surrounding tissue, thereby indicating decreased vascular permeability in tumors, assuming comparable levels of blood flow. Decreased vessel permeability in this setting may be attributable to the fact that much of the normal hepatic circulation is of the discontinuous type (25, 26) and therefore may have been hyperpermeable to plasma proteins relative to tumors that had induced a vasculature with continuous endothelium. Unfortunately, the tumor microvasculature was not characterized ultrastructurally.

In comparison with normal control tissues, accumulation of 125I-GPF in tumors was even more strikingly enhanced in our three transplantable mouse carcinomas than was influx. Accumulation reflects a net imbalance between tracer influx and efflux. This imbalance is favored by extravascular clotting which serves to retard tracer efflux. Nearly half of tumor-associated tracer was urea insoluble, in contrast to ≤18% in the normal tissues studied. As in influx experiments, therefore, measurements of urea-insoluble 125I-GPF provided a more sensitive measure of relative net fibrin accumulation than did measurements of total 125I-GPF.

Consistent with these observations of 125I-GPF accumulation, deposits reactive with antibodies to fibrinogen were detected in all three of the transplantable mouse tumors studied by immunoperoxidase methods. As in earlier studies of carcinomas from other species (5-9, 38), fibrin deposits were found in the loose connective tissue that envelopes tumor nodules and at the tumor-host interface. More prominent in the Lewis lung and TA3/St tumors than in other carcinomas studied (MOT) (5-9, 38) were fibrillar deposits that outlined and enveloped individual tumor cells and cell clumps.

Accumulation of both total and urea-insoluble 125I-HF was enhanced in HAN, as compared with normal breast tissue, and was still further increased in mammary carcinomas that arose in HAN months after they had been transplanted to cleared mammary fat pads. These findings are of interest for at least two reasons. (a) They provide evidence that fibrin accumulation is a feature of premalignancy. (b) In contrast to the transplantable mouse and guinea pig tumors (5) we have studied, the carcinomas arising from HAN developed as primary tumors in the autochthonous site (mammary tissue), not in the subcutaneous space or other nonnative sites.

We also investigated the biochemical nature of the fibrin deposited in the TA3/St and Lewis lung carcinomas. Tumor extracts that were subjected to reduced SDS-PAGE and autoradiography revealed the characteristic reduced gel pattern of cross-linked fibrin, i.e., prominent γ-γ dimers, polymerized α chains, reduced Aα(α) and γ chains, and normal Bβ(β) chains. These findings suggest that fibrinogen extravasating from blood vessels at tumor sites is clotted and cross-linked in a manner analogous to that occurring when plasma or purified fibrinogen is clotted in vitro with thrombin and clotting factor XIIIa. Fibrin with similar properties has also been found in vivo in pathological thrombi (39), in the skin after local blood vessels have been rendered hyperpermeable (14), and in delayed hypersensitivity (19). Thus, the fibrin deposited in tumors is not a unique molecular species.

In summary, we have demonstrated that the influx and accumulation of 125I-fibrinogen in three transplantable mouse carcinomas greatly exceed those observed in normal tissues having a similar microvasculature. The findings confirm and extend earlier data on fibrinogen influx and accumulation in guinea pig carcinomas. Furthermore, electrophoresis and autoradiography of tumor extracts demonstrated that the bulk of extravasated fibrinogen had been clotted to fibrin and cross-linked by factor XIIIa, a property shared with the fibrin deposited in wounds and inflammatory reactions (8, 16, 40).

We are only now beginning to understand the significance of the fibrin deposited in solid tumors. By analogy, fibrin gels of similar composition that are deposited in healing wounds serve as a provisional matrix and are subsequently ingrown by new blood vessels and fibroblasts, forming granulation tissue (8, 40). It is not unreasonable to suppose that fibrin deposits have a similar role in malignancy. Indeed, fibrin gels have been shown to induce a brisk angiogenic response when implanted in vivo (5, 17). In addition, deposition of fibrin at the interface between tumor and host tissues raises the possibility that this matrix serves a barrier function, as, for example, to the cellular immune response (Fig. 2, e and f). Finally, the finding of increased fibrin deposition in hyperplastic alveolar nodules points to a need for study of the role of fibrin in the transition from premalignancy to malignancy. In a recent study of chemically induced hamster pancreatic carcinomas, fibrin deposits were found in the basement membrane zones of noninvasive carcinomas and of atypical ducts (38). At particularly atypical foci, there was focal loss of basement membrane zone fibrin (and type IV collagen) staining, suggesting a correlation with local invasion. Taken together with the data presented here, these
findings suggest that fibrin deposition may actually begin before the onset of frank malignancy.

ACKNOWLEDGMENTS

We thank Dr. Daniel Medina, Baylor College of Medicine, for providing us the BALB/c mice used in this study.

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Fibrinogen Influx and Accumulation of Cross-Linked Fibrin in Mouse Carcinomas

Lawrence F. Brown, Bonnie Asc, V. Susan Harvey, et al.


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