Granulopoiesis and Thrombopoiesis in Mice Bearing Transplanted Mammary Cancer

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Summary

Growth of a transplantable mammary cancer in CE mice (CE 1460 MA CA) was paralleled by hyperplasia of the granulocytic elements of the bone marrow and peripheral leukocytosis (up to 350,000 leukocytes/cu mm) with a lymphoid-myeloid ratio reversal to 1:4 and a shift to the left. About 5-20% of the granulocytic elements in the blood and bone marrow showed morphologic abnormalities. Hematocrit values decreased 10-15%. Peripheral platelet levels manifested a slight depression in the face of bone marrow megakaryocytopenia and splenic megakaryocytosis. Extramedullary granulopoiesis, with the presence of mitoses and all stages of development of the granulocyte series in diffuse and discrete foci, was observed in the liver, spleen, lungs, lymph nodes, and adrenals of CE 1460 tumor bearers. The development of foci of extramedullary granulopoiesis, despite the absence of metastases in the bone marrow, suggests that the former resulted from direct stimulation of both intra- and extramedullary hematopoietic tissue by a tumor-elicited, granulocyte-specific factor, rather than from a reduction of bone marrow space available for granulopoiesis.

In BALB/C mice, another transplantable mammary cancer (BALB/C 2301 MA CA) induced periodic fluctuation of platelets between base-line and elevated levels and bone marrow megakaryocytosis but no change in splenic megakaryocyte levels. The bone marrow erythroid-myeloid ratio and the peripheral leukocytes' lymphoid-myeloid ratio remained normal. Hematocrit values decreased 10-15%. No foci of extramedullary granulopoiesis developed in the host tissues.

The tumor specificity of the leukemoid and thrombocytocytic responses was suggested by the host response to transplantation of the respective tumors into (BALB/C x CE) F1 hybrid mice and by the regression of the specific CE 1460 tumor-associated and BALB/C 2301 tumor-associated hematologic changes following tumor excision.

Introduction

A recent report from our laboratories (3) described the extraction and partial purification of a granulocytosis-promoting factor from a transplantable mouse mammary tumor (CE 1460 MA CA) that arose as a spontaneous tumor, associated with a severe leukemoidosis and extramedullary granulopoiesis (EMG), in a female mouse of the CE strain (22). CE 1460 MA CA has been carried through more than 100 transplant generations in CE mice and through more than 50 transplant generations in (BALB/C x CE) F1 hybrid mice (hereafter referred to as F1 hybrids); it has shown remarkable stability as regards tumor biology and the tumor-associated hematologic response in both the parent strain (CE) and F1 hybrid mice.

During a partial survey of other transplant mice tumors in our laboratories, we observed a fluctuating increase in circulating platelets but no leukemoid reaction to be associated with another transplantable mammary tumor (BALB/C 2301 MA CA) of spontaneous origin. The specificity of this response was suggested by the finding that none of the other tumors studied in the survey to date evoked a comparable platelet response.

Although tumor-associated hematologic changes—particularly leukemoid reactions and extramedullary hematopoiesis—have been reported frequently, and reviewed extensively by Dunn (5) and Ebbenhorst-Tengbergen and Mühlbock (7), it was felt that the extraction of a granulocytosis-promoting factor from CE 1460 tumor tissue and the consequently implied possibility that another humoral (thrombocytosis-promoting) factor is elaborated by the BALB/C 2301 tumor were of sufficient interest to warrant a study of the biology of these tumors. Hence, the present report attempts (a) to relate the leukemoid and thrombocytocytic responses to tumor characteristics (e.g., growth rate, size, and tumor viability), changes in blood morphology, and host tissue histopathology, and (b) to differentiate between the host strain-dependent and the tumor-dependent aspects of the hematopoietic responses to the 2 tumors in the F1 hybrid system.

Materials and Methods

Hematologic and histopathologic changes were studied in 98 control and 155 tumor-bearing mice of the CE, BALB/C, and F1 hybrid strains. These included: Group I (control mice), (a) 74 intact young adult (6-8 weeks old) and 6 intact 3-month-old CE, BALB/C, and F1 hybrid mice, and (b) 18 intact sham-operated young adult CE, BALB/C, and F1 hybrid mice; Group II (mice bearing CE 1460 MA CA), 42 young adult, 6 3-month-old, and 13 12-month-old CE mice and 45 young adult F1 hybrid hosts; Group III (mice bearing BALB/C 2301 MA CA), 31 young adult BALB/C and 18 young adult F1 hybrid hosts. Mice were kept in groups of 3 per cage, separated as to sex, and given Purina Lab Chow and drinking water ad lib.
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Each host received a s.c. implant (1 cu mm) of fresh donor tumor tissue delivered with a trocar into the right flank on Day 1. Of these mice, 105 animals were killed after 2–3 weeks (Group II) or 9–11 weeks (Group III). Another 48 hosts of Groups II and III were subjected to excision of moderately advanced (16-22 mm in diameter) tumors, while under light ether anesthesia, and were observed for periods up to 30 days following both successful and unsuccessful (i.e., local recurrence or metastases, or both) tumor excision.

Morning samples of caudal vein blood were obtained (a) at weekly intervals in intact controls, (b) prior to transplantation, (c) at weekly intervals thereafter in unoperated tumor-bearing hosts, and (d) prior to operation and at 3-day intervals thereafter in sham-operated controls and tumor-excised hosts. A single 13-μl blood sample, diluted 1:100 with 1.3 ml of ammonium oxalate in an Unopette disposable blood-diluting pipette, sufficed for both total leukocyte and platelet determinations by light microscopy. Heparinized capillary tubes were used for microhematocrit determinations. Wright-Giemsa-stained blood films and bone marrow imprints were prepared for differential leukocyte counts and studies of blood morphology.

Intact control mice (Group Ia) were sacrificed at ages comparable to those of the corresponding nonoperated tumor hosts. Sham-operated control mice (Group Ib) were sacrificed 21 days following surgery.

Tissue samples were fixed in 10% neutral formalin, sectioned at 5 μ, and stained routinely with hematoxylin and eosin as well as with (PAS) plus alician blue. Megakaryocytes were counted in 100 high power fields (X 450) of 5-μ-thick PAS-stained sections of femoral and sternal bone marrow and of spleen—equivalent to 2.35 x 106 cu μ of the fixed tissue sample.

Samples of blood, tumor tissue (with and without central necrosis), liver, and spleen were taken at autopsy, under aseptic conditions, from 12 F1 hybrid mice bearing CE 1460 MA CA and cultured both aerobically and anaerobically on blood agar and in thioglycolate broth. Whenever necessary, further diagnostic culture media were used to identify any microorganisms present.

A preliminary study of 6 other transplantable solid tumors carried in mice of 5 different strains in the Kirschbaum Memorial Laboratory, Baylor University College of Medicine, was undertaken for the purpose of relating possible changes occurring in the hosts’ leukocyte and platelet picture with tumor type, growth rate, and size. These tumors included 1 skin cancer (A' 9966), 3 mammary cancers (BALB/C x CE) F1, 3206, DBA/2/f/ 5199, BALB/C 926), 1 testicular tumor (CE 8), and 1 lymphoma ((BALB/C x CE) F1, 1182). Leukocyte and platelet values in caudal vein blood were determined as described above.

**Results**

**CE Host**

CE 1460 MA CA, an adenocarcinoma Type B according to Dunn's (6) classification, attained a diameter of approximately 25 mm within 14-21 days in 6- to 8-week-old and within 21-28 days in 3- and 12-month-old CE mice of both sexes. Macroscopically, the tumor was a homogeneous white mass that, at a diameter of approximately 15 mm, began to manifest superficial eschar formation and erosion as well as central necrosis and cystic fluid accumulation.

Metastases did not develop in the tissues of young adult CE tumor bearers, but they occasionally occurred in the form of pinpoint to 3-mm foci in the lungs and livers of 3- and 12-month-old CE hosts during the usual 3-4 week survival period. Metastases were also observed in the lungs of several young adult CE mice which survived for exceptionally long periods of time (6-12 weeks) following tumor implantation.

Tumor growth was paralleled by a marked rise in total circulating leukocytes (frequently to 350,000/cu mm and occasionally to 750,000/cu mm) as well as by slight depression of both hematocrit (from 45-54% to 40-46%) and platelet levels (from 1.3-2.2 million/cu mm to 1.0-1.7 million/cu mm) (Chart 1). Circulating leukocytes showed a reversal of the lymphoid-myeloid ratio to 1:4 and a shift to the left (5-15% immature granulocytes).

The bone marrow presented severe, predominantly granulocytic hyperplasia with a shift to the left (Fig. 1b), as well as pronounced megakaryocyte depression (Table 1). Megakaryocyte size remained within normal limits (20-50 μ in diameter).

About 5-20% of the granulocytes in both blood and bone marrow were enlarged up to 1.5-fold and showed other morphologic abnormalities (Fig. 1a) commonly seen in tumor-associated leukemoid reactions, as well as in pernicious anemia, drug toxicity, and radiation disease states. The cytoplasm of the abnormal granulocytes tended towards extreme basophilia in both immature and mature cells and frequently presented irregular coarse granulation. The nuclei manifested swelling, hypersegmentation, clumped and accessory chromatin, and occasionally abnormal mitotic figures.

The tumor size-tumor weight curve of the CE 1460 tumor was linear in transplanted and biphasic in recurrent (postexcision) tumors (Chart 2). In the latter, it increased in a linear fashion up to around the 3.5-gm level, then leveled off as the tumor began to manifest marked eschar formation, erosion, central necrosis, and cystic fluid. Occasionally, tumors underwent central erosion with subsequent peripheral regrowth.

Circulating leukocytes averaged 65,000-80,000/cu mm/gm of tumor weight and 8,000-12,000/cu mm of tumor diameter (Chart 2). Leukocytosis averaging 100,000 leukocytes/cu mm was associated with a 10-mm tumor; yet macroscopic evidence of necrosis was seen only after the tumor had attained a diameter of 15 mm or more. Histologic analysis of CE 1460 tumors during recent transplant generations in F1 hybrid mice showed no consistent relationship between the presence or absence of microscopio foci of necrosis in 5.5- to 6.5-mm tumors and the onset of the myeloid reaction. Nor was the degree of macroscopically evident necrosis in 9.5- to 11.5-mm tumors consistently proportional to the degree of the leukemoid reaction observed.

In cases where necrosis advanced to the stage of tumor erosion, the leukemoid reaction became markedly depressed, and the depression appeared to be reversed only upon renewed growth along the tumor periphery. Cystic-necrotic material (devoid of viable cells) from large CE 1460 tumors, when injected i.p., failed to evoke significant leukocytosis in young adult CE mice (L. Delmonte and R. A. Liebel, unpublished data). The peripheral
leukocyte, or more specifically the peripheral granulocyte level apparently was directly proportional to the volume of viable tumor tissue present.

Mitotically active foci of EMG developed consistently in the liver, spleen and lungs and occasionally in the lymph nodes and adrenals of CE mice bearing CE 1460 MA CA (Figs. 1 c–e; 2 f–k). EMG was more pronounced in 6- to 8-week-old than in 3- and 12-month-old hosts. EMG foci were not usually associated with metastatic proliferation. Granulocytes in EMG foci encompassed both normal and morphologically aberrant forms. Only rarely did erythropoietic and thrombopoietic proliferation occur at sites of EMG.

Hepatic EMG foci occurred mainly within the walls of the larger venous vessels and occasionally among the liver cord cells themselves (Fig. 2f, g). Occasionally megakaryocytes were found lodged in hepatic sinusoids.

The spleen, which doubled or tripled its size within 2-3 weeks of CE 1460 tumor implantation, showed evidence of diffuse EMG that disrupted the splenic architecture and occasionally even displaced the lymphoid elements in germinal centers (Fig. 1c). Megakaryocytes, easily distinguished from other giant cells by their strong affinity for the PAS stain, increased about 3-fold (Table 1); they occurred mainly in discrete nests of both immature and mature cells and occasionally singly within the EMG foci.

The lungs presented diffuse granulocyte permeation of the alveolar walls as well as a few discrete foci abutting bronchioles and small blood vessels and showing a high degree of mitotic granulocytic activity (Fig. 2h, i).

The lymph nodes occasionally presented diffuse granulocyte permeation or discrete round areas consisting entirely of immature and mature granulocytic elements (Fig. 1d, e). These areas of EMG stained a distinctive light purple with hematoxylin and eosin and thus were easily distinguishable from the more deeply staining lymphoid tissue.

The adrenals developed EMG mainly at the corticomedullary junction (Fig. 2j, k). Granulocytic elements appeared to concentrate particularly around clumps of cells whose granules showed a strong affinity for PAS.

**BALB/C Host**

BALB/C 2301 MA CA, an adenoacanthoma according to Dunn’s (6) classification, had the macroscopic aspect of a homogeneous white mass throughout the course of its development, although it occasionally showed eschar formation towards the terminal stage (30–38 mm in diameter).

In young adult hosts of both sexes, the tumor developed to a diameter of approximately 25 mm within 9–11 weeks and at-
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CHART 2. Correlation of leukocytosis, tumor size, and tumor weight in mice bearing transplanted and recurrent CE 1460 MA CA. Upper graph: Ratio of tumor size to tumor weight. Transplant: \( Y = 1.95X + 15.28, r = 0.782, P < 0.001 \). Recurrence: \( Y_1 = 2.63X + 16.76, r_1 = 0.646, P_1 < 0.010 \). Recurrence: \( Y_2 = -2.44X + 38.90, r_2 = 0.812, P_2 < 0.025 \). Interclass: \( r = 0.739, P < 0.001 \). Center graph: Ratio of tumor size to leukocytosis. Transplant: \( Y = 0.03X + 10.93, r = 0.581, P < 0.001 \). Recurrence: \( Y = 0.04X + 8.42, r = 0.624, P < 0.001 \). Interclass: \( r = 0.979, P < 0.001 \). Lower graph: Ratio of tumor weight to leukocytosis. Transplant: \( Y = 0.007X + 1.55, r = 0.569, P < 0.001 \). Recurrence: \( Y = 0.005X + 1.77, r = 0.529, P < 0.200 \). Interclass: \( r = 0.613, P < 0.100 \).

CHART 3. Peripheral blood changes accompanying growth of BALB/C 2301 MA CA in BALB/C mice.

Fig. 1. Leukemoid blood picture and granulopoiesis in tissues of CE 1460 MA CA hosts: a, peripheral blood with aberrant granulocytic forms (X 1000); b, bone marrow (X 150); c, spleen (X 650); d, lymph node (X 300); e, lymph node (X 750).
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and periodically attained peak levels in the former that were up to 50% higher than any ever observed in the latter. At 9–11 weeks, tissue megakaryocyte concentrations manifested an increase of about 50% in the bone marrow and 25% in the spleen (Table 1). The tumor dependence of these changes in the megakaryocyte-platelet axis has been studied extensively and will be reported elsewhere.

The bone marrow of BALB/C 2301 MA CA hosts presented an unchanged erythroid-myeloid ratio. There were no aberrant morphologic forms and no macro- or microscopically discernible metastatic foci.

In F1 hybrid mice, both CE 1460 MA CA and BALB/C 2301 MA CA retained their biologic and specific hematologic characteristics (Chart 4; Table 1).

CE 1460 tumor growth rate in young adult F1 hybrids lagged 1 week behind that observed in young adult CE hosts (Chart 5). This lag, which was significant within 99.9% confidence limits, was reproduced in the development of both the leukemoid response and the platelet depression (Charts 1 and 4), although leukocyte and platelet values as related to tumor size were statistically identical in the parent strain and the F1 hybrid. It is of interest that the CE 1460 tumor growth rate in young adult F1 hybrids was comparable to that observed in 12-month-old CE hosts.

The incidence of lung and liver metastases in CE 1460 tumor bearers appeared to be dependent on time rather than on tumor size or strain. In 3- to 12-week-old CE and in young adult F1 hybrid hosts bearing advanced tumors (20–25 mm in diameter),
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the incidence of metastases was negligible at 2 weeks, slight at 3 weeks, and increasingly marked at 4 or more weeks after tumor transplantation.

BALB/C 2301 tumor growth rate was identical in young adult F₁ hybrid and BALB/C hosts (Charts 3 and 4). No leukemoid reaction occurred in either host strain. The fluctuating platelet elevation was slightly less pronounced in F₁ hybrids than in BALB/C hosts (Charts 3 and 4). Unlike CE 1460 MA CA, BALB/C 2301 MA CA was already associated with a rise in platelet levels by the time a 5-mm tumor diameter had been attained in the F₁ hybrid host.

Hematocrit changes were slight (10-15% maximum decrease) and were comparable for the 2 tumors and all 3 strains of mice (Charts 1, 3, 4).

The bone marrow and EMG and megakaryocyte picture in F₁ hybrids bearing CE 1460 MA CA or BALB/C 2301 MA CA was comparable to that of hosts of the respective strain of tumor origin.

Splenomegaly in F₁ hybrids, as well as in the strain of tumor origin, depended on tumor size. Splenic megakaryocyte levels were elevated more than 3-fold in CE 1460 tumor bearers but only about 1 in BALB/C 2301 tumor bearers (Table 1).

**Tumor Excision**

Moderate transient leukocytosis (25,000–45,000 leukocytes/cu mm)—probably due to a transient increase in leukopoiesis compensating for blood loss at surgery—followed not only CE 1460 and BALB/C 2301 tumor excision in hosts of the respective parent strain (CE and BALB/C) and in F₁ hybrid hosts, but also sham operation in all 3 strains of mice (Table 2).

Renormalization of total and differential leukocyte values and disappearance of aberrant granulocytic forms occurred in CE and F₁ hybrid hosts within 3–9 days of excision of CE 1460 MA CA (Table 2). Excision of a 16–20-mm tumor was usually followed within 5–21 days by tumor recurrence along the line of excision. Recurrence of the leukemoid response was observed as early as 24–48 hr prior to macroscopically evident tumor regrowth. The growth rate of CE 1460 tumors recurring at the site of excision was accelerated by comparison to that of the transplanted tumors (Chart 5).

**TABLE 2**

**Peripheral Blood Picture Following Sham Operation and Tumor Excision in CE, BALB/C and (ALB/C x CE) F₁ Hybrid Mice (Mean ± S.E.)**

<table>
<thead>
<tr>
<th>MOUSE STRAIN</th>
<th>TUMOR</th>
<th>TYPE</th>
<th>EXCISION</th>
<th>DAYS POSTSURGERY</th>
<th>WBC (thousands/cu mm)</th>
<th>PLATELETS (millions/cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>CE</td>
<td>None</td>
<td>Sham</td>
<td></td>
<td>10.6</td>
<td>14.8</td>
<td>14.0</td>
</tr>
<tr>
<td>CE 1460 MA CA</td>
<td>Tumor</td>
<td></td>
<td>185.3</td>
<td>35.9</td>
<td>32.4</td>
<td>21.5</td>
</tr>
<tr>
<td>(BALB/C x CE) F₁</td>
<td>None</td>
<td>Sham</td>
<td></td>
<td>13.3</td>
<td>10.3</td>
<td>13.0</td>
</tr>
<tr>
<td>(BALB/C x CE) F₁</td>
<td>CE 1460 MA CA</td>
<td>Tumor</td>
<td></td>
<td>175.1</td>
<td>44.0</td>
<td>25.7</td>
</tr>
<tr>
<td>BALB/C</td>
<td>None</td>
<td>Sham</td>
<td></td>
<td>9.6</td>
<td>10.3</td>
<td>8.9</td>
</tr>
<tr>
<td>BALB/C 2301 MA CA</td>
<td>Tumor</td>
<td></td>
<td>12.6</td>
<td>24.8</td>
<td>30.5</td>
<td>32.4</td>
</tr>
</tbody>
</table>

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With CE 1460 MA CA recurrence after unsuccessful excision, the tumor size-tumor weight relationship was initially (up to the 3.5-gm level) similar to that observed before excision; then, with the development of a disproportionate weight increase due to massive accumulation of cystic fluid, the ratio began to show a trend towards a linear decrease rather than towards an increase (Chart 2). However, the pre- and postexcision ratios of tumor size to leukocyte number were statistically identical (interclass r = 0.979) (Chart 2). Following recurrence, the relationship of tumor weight to leukocyte number became random (r = 0.329), although it manifested a trend towards the same ratio as that of the pre-excision group (interclass r = 0.613) (Chart 2).

Following CE 1460 tumor excision, pinpoint to 3-mm metastases were found frequently in the lungs and liver and occasionally in the lymph nodes, kidneys, and adrenals, but not in femoral or sternal bone marrow of the mice that presented no recurrence at the tumor implantation site. Microscopically evident foci of lung metastases but no foci or EMG were found upon histologic examination of all CE and F, hybrid hosts with macroscopically and hematologically diagnosed successful CE 1460 tumor excision.

In BALB/C and F, hybrid mice bearing BALB/C 2301 MA CA, tumor excision was usually successful, recurrence being rare within the 21-day postsurgical observation period of our experiments. Neither of 2 mice that manifested incipient (palpable) tumor recurrence presented metastases.

A transitory 2- to 3-fold increase in platelet levels with a peak at 6 days followed both tumor excision and sham operation in all 3 strains of mice, persisted for 9-12 days, and manifested a trend towards normalization by 21 days (Table 2). The immediate reaction, following sham operation or tumor excision, appeared to be dependent on (i.e., compensatory for) blood loss or trauma, or both, at surgery rather than on tumor ablation itself, but it was less pronounced after sham operation than after tumor excision. In all 3 strains of mice, the tumor-dependent platelet changes were totally or partially reversed within 21 days of excision of either CE 1460 MA CA or BALB/C 2301 MA CA.

Microbiology of CE 1460 MA CA

Although all the mice from which these tissue samples had been taken presented a marked leukemoid reaction, only some of the samples of tumor (67%) and spleen (50%), a single sample of blood (8%), and no sample of liver (0%) from mice bearing advanced (20- to 25-mm diameter) CE 1460 tumors manifested evidence of microorganisms in thioglycolate broth or on blood agar. Among the positive cultures, 4 different Gram-negative organisms—Pseudomonas aeruginosa, Aerobacter aerogenes, Proteus vulgaris, and Staphylococcus aureus—were identified; a Bacillus (species not identified) was also isolated. The presence or absence of bacterial contamination had no effect on either the occurrence or the magnitude of the CE 1460 tumor-associated leukemoid reaction.

Hematologic Survey of Other Transplantable Mouse Tumors

The hematologic study of hosts of 5 different strains of mice bearing additional tumors of various types of tissues revealed that neither the tumor tissue type and growth rate nor the host strain and survival time following tumor transplantation appeared to affect the type of hematologic response associated with the tumor: A+ 9966 skin cancer, (BALB/C x CE) F, 3206 mammary cancer, CE 8 testicular tumor, and DBA/2A/ff 5199 mammary cancer hosts survived approximately 4-5, 5-6, 6-7, and 19-20 weeks, respectively; yet all showed normal platelet values. A+ 9966 and (BALB/C x CE) F, 3206 tumors hosts also manifested a leukemoid reaction, whereas CE 8 and DBA/2A/ff 5199 hosts showed a normal leukocyte picture. BALB/C 962 mammary cancer and (BALB/C x CE) F, 1882 lymphoma killed their respective hosts within approximately 4-5 weeks; hosts bearing both these tumors manifested a trend towards platelet depression similar to that seen in CE 1460 tumor hosts, whose survival was 3-4 weeks, but only BALB/C 962 hosts also manifested leukocytosis. None of the tumors studied evoked a recurrent thrombocytosis like that observed in BALB/C 2301 tumor hosts.

Discussion

The tumor specificity of the leukemoid and thrombocytic responses associated with CE 1460 MA CA and BALB/C 2301 MA CA, respectively, was demonstrated by the response of genetically isologous hosts (F, hybrid mice) to the transplantation of these genetically unrelated tumors and by the regression of the specific tumor-associated hematologic responses following tumor excision.

Over the past 50 years, leukocytosis associated with extramedullary hematopoiesis—both leukopoiesis and erythropoiesis—has been reported repeatedly in tumor-bearing mice (1, 2, 5, 7, 13, 14, 19-21, 26-28), rats (21, 23), hamsters (15), rabbits (8), dogs (8), monkeys (8), and man (4, 9, 10-12, 16-18, 24, 25, 29-35). Thrombocytosis associated with leukemoid reactions has been reported both in man and in tumor-bearing mice manifesting extramedullary hematopoiesis (2). A direct correlation between splenic megalakaryocytosis and peripheral leukocytosis has been reported by Parsons et al. (28) in mice bearing transplanted sarcomas. Most of the tumor-associated leukemoid reactions described in the literature have been associated with moderate to extreme concurrent effects on erythropoiesis or thrombopoiesis, or both, and have resulted in extramedullary hemopoiesis involving all cell lines rather than a single one, as is the case for the CE 1460 and BALB/C 2301 tumor systems.

The tumor-host relationship has been shown to be affected by a variety of factors. Whereas the growth rate of CE 1460 MA CA was influenced by factors such as host age and genetics, the development of granulocytosis and EMG foci appeared to be dependent upon the volume of viable tumor tissue present and on the hemopoietic mitotic and maturation potential of intra- and extramedullary host tissue in response to the tumor-elaborated stimulus. The occurrence of EMG foci, despite an actual quantitative increase rather than a reduction of granulocytic tissue in the bone marrow cavities by metastatic or other factors, further supports the concept that the host's granulocytic response is tumor dependent.

Although several investigators including Bateman (2) found that tumor age, necrosis, ulceration, and eschar formation had a greater influence on tumor-associated leukemoid reactions in mice than did tumor tissue viability or volume, our data, like those of Ebbenhorst-Tengbergen and Mühlbock (7), suggested...
that the amount of viable tumor tissue present rather than the presence of necrotic tissue was the prime factor in the development of the tumor-associated leukemoid reaction. In fact, in the CE 1460 tumor system, whenever the ratio of tumor weight to tumor size increased disproportionately owing to the appearance of cystic fluid and necrosis at the core of the tumor, the peripheral leukocytosis tended to level off despite a continuous increase in absolute tumor weight. Furthermore, association of erosion with necrosis, resulting in an even greater loss of viable tumor tissue, was actually accompanied by regression of the leukocytosis, which recurred only upon renewal of growth at the tumor periphery.

The possibility that tumor-associated microorganisms induce the leukemoid reaction has been proposed by Barnes and Sisman (1). Bateman (2) found leukemoid reactions to occur in tumor-bearing mice without demonstrable bacterial infection. In the present study, we found the presence or absence of bacterial contamination of CE 1460 tumor or host tissue to have no effect on the induction or the magnitude of the leukemoid reaction, suggesting that there probably is no bacterial pathogenesis of the tumor-associated leukocytosis and EMG. However, the possibility of viral pathogenesis cannot be excluded, since recent preliminary electron microscope studies in our laboratories (L. Delmonte, T. Smith, and R. A. Liebelt, unpublished data) have revealed the presence of virus-like particles in CE 1460 tumor tissue.

The disparity between the ratios of tumor size to leukocytosis in mice with transplanted and those with recurrent CE 1460 tumors raises an interesting point: Is the disproportionate decrease in this ratio due to reactivation of residual EMG foci that have become dormant following tumor ablation, superimposed on the laying down of new EMG foci during recurrent tumor growth? Or has the host become "sensitized" to hemopoietic stimuli by the original tumor transplant? The phenomenon only serves to emphasize the delicate balance of tumor and host factors in tumor-host relationships.

Similarly to the CE 1460 tumor-associated leukemoid and EMG reaction, the BALB/C 2301 tumor-associated thrombocytosis and bone marrow megakaryocytosis showed direct dependence on the presence of viable tumor tissue. However, it appeared that even a small volume of BALB/C 2301 tumor tissue sufficed to initiate a platelet release—as manifested by the appearance of the 1st platelet peak 2–3 weeks following tumor implantation—but that the bone marrow megakaryocyte build-up (hyperplasia) was a more gradual process paralleling tumor volume increase.

The specificity of BALB/C 2301 MA CA for evoking a fluctuating thrombocytosis is further supported by the lack of consistent relationship found by both ourselves and Bateman (2) between tumor tissue type, size, and growth rate and host strain and survival time, following tumor transplantation, on the one hand, and the development of tumor-associated thrombocytosis (and/or leukocytic) reactions, on the other hand. Further investigation is necessary to determine whether a thrombocytosis-promoting factor can be extracted from BALB/C 2301 tumor, just as a granulocytosis-promoting factor was extracted from CE 1460 mammary tissue (3). The BALB/C 2301 tumor system appears to present us with a biologic model from which we can postulate the probable presence of a tumor-dependent factor influencing circulating platelet levels.

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