Primary Growth of Leukemic Cells as a Factor in Their Metastatic Growth and in Their Lethal Effect on the Mouse*

HORACE GOLDIE, BARBARA R. JEFFRIES, SOLOMON S. GREEN AND MELVIN R. SIMPSON

(Laboratory for Experimental Oncology, Meharry Medical College, Nashville, Tenn.)

The vigorous growth of primary mouse tumors usually overshadows the other essential characteristic of their malignancy—their metastatic spread by blood and lymph routes into organs of the host. Vice versa, in another group of malignancies, the leukemias, the outstanding features are the metastatic transportation of malignant cells in the blood and their infiltrative growth in organs, while the primary growth is often disregarded. The purpose of the work reported below was to investigate the spread of leukemic tumors from three sites, namely, (a) the peritoneal cavity, (b) the subcutaneous tissue of the flank, and (c) the subcutaneous tissue of the scalp. The combination of data on the characteristics of primary growth, on the extent of leukemic cell spread into organs, and on the mortality of the host outlined for each strain of leukemia the effect of primary growth of leukemic cells on their fate and on the fate of their host.

MATERIALS AND METHODS

Tumor and mouse strains.—AKR mice were bred in our animal quarters or received from Carworth Farms, New City, N.Y.; AK4 strain was obtained through the courtesy of Dr. J.H. Burchenal, Sloan-Kettering Institute; mouse strains DBA-2 and C57-6 and tumor strains P1534 and C1498, from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. Data on nonleukemic tumors had been obtained previously (4) and were used for comparison with the leukemias.

Technics.—Methods of tumor cell counts for inoculation of requisite numbers of leukemic cells, of subcutaneous tumor cell implantation from peritoneal exudate (auto-inoculation), of abdominal puncture for withdrawal of exudate specimens, etc., were described elsewhere (4–6).

Estimate of the amount of primary growth.—The total number of cells/c mm of peritoneal exudate and the percentage of tumor cells were determined on the 6th day after inoculation of $10^6$ tumor cells. For tumors induced in the flank or the scalp by graded numbers of cells, the diameters of implants or their anatomical limits were recorded at the animal's death.

Pattern of mortality survey for leukemias induced by various inoculation routes.—Suspensions of leukemic cells were diluted serially with 0.85 per cent NaCl solution to concentrations of $10^7$–$10^4$/ml. From each dilution, immediately after thorough mixing with the diluent, doses of 1 ml. were injected into two mice intraperitoneally, into two mice in the flank, and into two mice in the scalp. The amount of primary growth at the death and the time interval between inoculation and death were recorded for each mouse. The experiment was repeated 3 times for each strain, and the results were pooled for presentation in tables. In control experiments suspensions of normal tissue cells (ground liver, spleen, kidney, and lung) were added to the diluent (about $10^6$ cells/ml), to induce conditions comparable to inoculation of organs from leukemic mice (see below).

Pattern of organ assay graft.—For each strain of leukemias, the animals of two series, each composed of 60 mice, were given inoculations of large numbers of leukemic cells ($10^7$) intraperitoneally (first series) and into the scalp (second series). At intervals after inoculation ($\frac{1}{2}, 1\frac{1}{2}, 3$, and 6 hours; 1, 2, 3, 4, 5, and 7 days), groups of six mice (donors of organs) were sacrificed; from each mouse each organ (spleen, liver, kidney; lung) was ground, suspended in 2 cc. of 0.85 NaCl solution, and injected intraperitoneally into a new mouse (recipient). Microscopic detection of free leukemic cells in the peritoneal fluid of recipients was con-
considered evidence of the presence of leukemic cells in the grafted organs of the donor. The average survival of recipients of organs from each donor was recorded, and the results were pooled and presented in Charts 1–6. These data provided information on the extent of leukemic cell invasion of the organs.

RESULTS

Amount of local primary growth.—Table 1 shows that leukemic cells reached on the 6th day a higher growth level (higher proportion in the cellular composition of the exudate) than lymphoma or sarcoma cells (in the later stage cells of leukemias and of lymphoma constituted more than 90 per cent of the total number of cells in the exudate). Moreover, a large proportion of the free leukemic cells were atypical and sometimes unclassifiable (Figs. 1, 2, and 3).

<table>
<thead>
<tr>
<th>Amount of local primary growth</th>
<th>Tumor cell growth from leukemic and nonleukemic tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>6th day after intraperitoneal inoculation of 10⁶ tumor cells (average of ten mice and range)</td>
<td>Percentage of tumor cells *</td>
</tr>
<tr>
<td>Leukemia AK4</td>
<td>124 (61–170)</td>
</tr>
<tr>
<td>Leukemia P1534</td>
<td>162 (81–212)</td>
</tr>
<tr>
<td>Leukemia C1498</td>
<td>130 (72–191)</td>
</tr>
<tr>
<td>Malignant lymphoma</td>
<td>128 (95–188)</td>
</tr>
<tr>
<td>Sarcoma 37</td>
<td>66 (37–73)</td>
</tr>
</tbody>
</table>

* The nonleukemic cells were leukocytes of various types as indicated elsewhere (4–6).

In the subcutaneous tissue of the flank the inoculated leukemic cells grew by diffuse infiltration without distinct demarcation line. Within 6–8 days AK4 cells induced only flat nodules 0.5–1 cm. in largest diameter, while P1534 cells and, in particular, C1498 cells grew as elevated tumors with the largest diameter over 1 cm.; they have never reached the size of lymphoma or sarcoma. Leukemic cells from the subcutaneous flank spread by lymphatics into the peritoneal cavity and caused accumulation of exudate with abundant free tumor cells. The outstanding feature of AK4 leukemia, as compared to other leukemic strains, was the shorter survival of the hosts as a result of intraperitoneal growth from any size of inoculum and from flank or scalp growth of 10⁶–10⁷ cells.

Mortality survey in leukemic mice.—For each group of mice inoculated with 10⁶–10⁹ AK4 cells (Table 2), the shortest survival periods were recorded in the series with intraperitoneal primary growth and the longest in the series with subcutaneous growth in the flank. The difference was less marked for higher inocula. There was a similar difference with C1498 cells for all groups, and with P1534 cells only for inocula 10⁶, 10⁷ and 10⁸ cells.

The outstanding feature of AK4 leukemia, as compared to other leukemic strains, was the shorter survival of the hosts as a result of intraperitoneal growth from any size of inoculum and from flank or scalp growth of 10⁶–10⁷ cells.

Spread of leukemic cells into organs of the host from various sites of primary growth.—The increase of the inoculum over 10⁶ cells was not followed by significant reduction of survival span. Moreover, in control experiments, addition of suspension of normal organs to the diluent delayed (but never inhibited) the growth of leukemic cells from 10⁶–10⁷ intraperitoneal inocula. For these reasons, Table 2 indicating the survival span after inoculation with graded numbers of leukemic cells can be used only for an approximate estimate of tumor cell numbers in organs of leukemic mice. For instance, if all mice inoculated intraperitoneally with various organs from AK4 leukemic mice were dead within 12 days, the reference to the Table 2 might indicate that each organ contained at least 10⁴ AK4 cells.

Comparison of Charts 1 and 2 shows that organs from mice sacrificed 1–6 hours after intraperitoneal inoculation of AK4 cells caused death of mice recipients much faster, and therefore contained much higher numbers of tumor cells than organs of animals inoculated with the same dose in the scalp.

1 Mr. Wendell Gillett, medical photographer at Vanderbilt Medical School, prepared the photographs.
This difference was less marked in recipients of organs from animals sacrificed after 1–7 days (intrapertoneal series, 7–12 days; scalp series, 9–15 days, Charts 1 and 2). For P1534 leukemia, recipients of organs from mice with intraperitoneal growth 6 hours to 7 days old died within 11–15 days (Chart 3), while organs from the series with scalp tumors of the same age caused the death of recipients within 17–30 days (Chart 4). An analogous range of differences in the survival of recipients, i.e., in the tumor cell content of organs from donors, appeared from a comparison of the intraperitoneal (Chart 5) and scalp series (Chart 6) for C1498 leukemia.

### TABLE 2

**Survival of Mice after Inoculation with Graded Numbers of Leukemic Cells of Various Strains by Various Routes**

<table>
<thead>
<tr>
<th>Route of inoculation</th>
<th>No. cells inoculated</th>
<th>Av. survival (days) and range are indicated for each group.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^7$</td>
<td>$10^6$</td>
</tr>
<tr>
<td>A. Lymphocytic leukemia (AK4) in AKR mice</td>
<td>6 (5–7)</td>
<td>7 (6–8)</td>
</tr>
<tr>
<td>Intrapерitoneal</td>
<td>7 (8–9)</td>
<td>13 (11–15)</td>
</tr>
<tr>
<td>Subcut. (scalp)</td>
<td>8 (9–9)</td>
<td>13 (12–13)</td>
</tr>
<tr>
<td>Subcut. (flank)</td>
<td>7 (6–8)</td>
<td>19 (12–13)</td>
</tr>
<tr>
<td>B. Granulocytic leukemia (C1498) in C37–6) mice</td>
<td>8 (7–8)</td>
<td>11 (10–11)</td>
</tr>
<tr>
<td>Intrapерitoneal</td>
<td>11 (11–12)</td>
<td>14 (10–11)</td>
</tr>
<tr>
<td>Subcut. (flank)</td>
<td>11 (12–13)</td>
<td>16 (12–13)</td>
</tr>
<tr>
<td>C. Lymphocytic leukemia (P1534) in DBA-2 mice</td>
<td>9 (8–10)</td>
<td>11 (11–12)</td>
</tr>
<tr>
<td>Intrapерitoneal</td>
<td>11 (10–13)</td>
<td>13 (11–12)</td>
</tr>
<tr>
<td>Subcut. (scalp)</td>
<td>11 (9–14)</td>
<td>15 (11–10)</td>
</tr>
</tbody>
</table>

---

**Chart 1.** Assay grafts of organs from AKR mice bearing intraperitoneal growth of AK4 cells.

Each graph represents average survival of ten mice after assay inoculation of organs from leukemic mice.

Interval between primary inoculation of leukemia and assay grafts of organs is marked for each graph.

**Chart 2.** Assay grafts of organs from AKR mice bearing subcutaneous growth of AK4 cells in the scalp.

Each graph represents average survival of ten mice after assay inoculation of organs from leukemic mice.

Interval between primary inoculation of leukemia and assay grafts of organs is marked for each graph.
DISCUSSION

It is recognized by clinical and experimental workers (2, 3, 9–11) that “leukocytemia is but an incidental feature of leukemias—a symptom—and that the fundamental changes are to be sought in the tissues rather than in the blood” (9). Accordingly, in our previous work (5, 6) we have investigated the role of free leukemic cells grown in the peritoneal exudate as a factor of leukocytemia and of lethal tissue infiltration with leukemic cells. In the experiments reported above we have studied, for the same purpose, localized primary growth of leukemic cells at various sites and their secondary metastatic growth in organs. It was found that the presence of numerous leukemic cells in organs 3–6 hours after their intraperitoneal inoculation and their extensive invasion in later stages of growth may be accounted for by early implantation of tumor cells from peritoneal exudate into abdominal organs, as it was previously described for other

Chart 3.—Assay grafts of organs from DBA-2 mice bearing intraperitoneal growth of lymphatic leukemia P1534 cells. Each graph represents average survival of ten mice after assay inoculation of organs from leukemic mice. Interval between primary inoculation of leukemia and assay grafts of organs is marked for each graph.

Chart 4.—Assay grafts of organs from DBA-2 mice bearing subcutaneous growth of lymphatic leukemia P1534 in the scalp. Each graph represents average survival of ten mice after assay inoculation of organs from leukemic mice. Interval between primary inoculation of leukemia and assay grafts of organs is marked for each graph.

Chart 5.—Assay grafts of organs from C57-6 mice bearing intraperitoneal growth of granulocytic leukemia C1498 cells. Each graph represents average survival of ten mice after assay inoculation of organs from leukemic mice. Interval between primary inoculation of leukemia and assay grafts of organs is marked for each graph.

Chart 6.—Assay grafts of organs from C57-6 mice bearing subcutaneous growth of granulocytic leukemia C1498 cells. Each graph represents average survival of ten mice after assay inoculation of organs from leukemic mice. Interval between primary inoculation of leukemia and assay grafts of organs is marked for each graph.
tumors (7). The relatively slight difference between these two series for mice with AK4 leukemia early massive blood-borne invasion of organs. For transition from peritoneal exudate was overshadowed by metastatic infiltration of organs by blood route at the expense of primary growth. For all leukemic strains the difference in the amount of organ invasion from various sites was paralleled by a difference in survival (Table e), suggesting that metastatic infiltrative growth of leukemic cells into organs was the essential lethal factor. However, shorter survival of animals with large tumor growths in the scalp as well as their shorter survival (Charts 1 and 2) as well as their shorter survival periods in the intraperitoneal wall and to grow subcutaneously in the scalp and intraperitoneal growth earlier and to a greater extent than metastatic infiltrative growth in organs is a specific characteristic of malignant cells due to their interdependence, while the ability to grow as free cells in body fluids, to be separated from the tissue, and to inject free tumor cells in serous body fluids (Table 1). Thus, primary intraperitoneal growth was higher for leukemic cells than for nonleukemic tumors, inversely to their reduced adhesiveness (1). The purpose of this paper was to study the primary growth in the peritoneal exudate and nonleukemic tumors with regard to their two tendencies to spread extensively into tissue either at the site of inoculation or from the opposite tendencies to implant from peritoneal exudate into abdominal organs by blood route at the expense of primary growth and to be implanted into connective tissue either at the site of inoculation or from the peritoneal exudate. It appears from the results on leukemias AK4, P1534, and C1498; malignant lymphoma, S-37, S-180; carcinomas in C57-6 and in C3H mice) and that the tendency of tumor cells to implant from peritoneal exudate into abdominal organs was overshadowed by metastatic infiltration of organs by blood route at the expense of primary growth. For tissue growth; and of highest importance for malignant cells is an important factor in the amount of tumor cells on the 6th day was higher for leukemic (S-37, lymphoma) tumors. An inverse proportion was found for the tendency of tumor cells to implant from peritoneal exudate into abdominal organs from subcutaneous growth. This was attributed to their high tendency to early spread in the blood (AK4); whereas the shortest survival periods in the intraperitoneal series and the longest in the flank series. The role of primary growth as presumed that tissue growth from benign and malignant cells is based on their interdependence, and nonleukemic tumors with regard to their two periods of their subcutaneous growth. It may be noted that for this strain the role of implantation factor in leukemias. However, shorter survival of animals with large tumor growths in the scalp as well as their shorter survival periods in the intraperitoneal wall and to grow subcutaneously in the scalp and intraperitoneal growth earlier and to a greater extent than metastatic infiltrative growth in organs is a specific characteristic of malignant cells due to their interdependence, while the ability to grow as free cells in body fluids, to be separated from the tissue, and to inject free tumor cells in serous body fluids (Table 1). Thus, primary intraperitoneal growth was higher for leukemic cells than for nonleukemic tumors, inversely to their reduced adhesiveness (1). The purpose of this paper was to study the primary growth in the peritoneal exudate and nonleukemic tumors with regard to their two tendencies to spread extensively into tissue either at the site of inoculation or from the opposite tendencies to implant from peritoneal exudate into abdominal organs by blood route at the expense of primary growth and to be implanted into connective tissue either at the site of inoculation or from the peritoneal exudate. It appears from the results on leukemias AK4, P1534, and C1498; malignant lymphoma, S-37, S-180; carcinomas in C57-6 and in C3H mice) and that the tendency of tumor cells to implant from peritoneal exudate into abdominal organs was overshadowed by metastatic infiltration of organs by blood route at the expense of primary growth. For tissue growth; and of highest importance for malignant cells is an important factor in the amount of tumor cells on the 6th day was higher for leukemic (S-37, lymphoma) tumors. An inverse proportion was found for the tendency of tumor cells to implant from peritoneal exudate into abdominal organs from subcutaneous growth. This was attributed to their high tendency to early spread in the blood (AK4); whereas the shortest survival periods in the intraperitoneal series and the longest in the flank series. The role of primary growth as presumed that tissue growth from benign and malignant cells is based on their interdependence, while the ability to grow as free cells in body fluids, to be separated from the tissue, and to inject free tumor cells in serous body fluids (Table 1). Thus, primary intraperitoneal growth was higher for leukemic cells than for nonleukemic tumors, inversely to their reduced adhesiveness (1). The purpose of this paper was to study the primary growth in the peritoneal exudate and nonleukemic tumors with regard to their two tendencies to spread extensively into tissue either at the site of inoculation or from the opposite tendencies to implant from peritoneal exudate into abdominal organs by blood route at the expense of primary growth and to be implanted into connective tissue either at the site of inoculation or from the peritoneal exudate. It appears from the results on leukemias AK4, P1534, and C1498; malignant lymphoma, S-37, S-180; carcinomas in C57-6 and in C3H mice) and that the tendency of tumor cells to implant from peritoneal exudate into abdominal organs was overshadowed by metastatic infiltration of organs by blood route at the expense of primary growth. For tissue growth; and of highest importance for malignant cells is an important factor in the amount of tumor cells on the 6th day was higher for leukemic (S-37, lymphoma) tumors. An inverse proportion was found for the tendency of tumor cells to implant from peritoneal exudate into abdominal organs from subcutaneous growth. This was attributed to their high tendency to early spread in the blood (AK4); whereas the shortest survival periods in the intraperitoneal series and the longest in the flank series. The role of primary growth as presumed that tissue growth from benign and malignant cells is based on their interdependence, while the ability to grow as free cells in body fluids, to be separated from the tissue, and to inject free tumor cells in serous body fluids (Table 1). Thus, primary intraperitoneal growth was higher for leukemic cells than for nonleukemic tumors, inversely to their reduced adhesiveness (1). The purpose of this paper was to study the primary growth in the peritoneal exudate and nonleukemic tumors with regard to their two tendencies to spread extensively into tissue either at the site of inoculation or from the opposite tendencies to implant from peritoneal exudate into abdominal organs by blood route at the expense of primary growth and to be implanted into connective tissue either at the site of inoculation or from the peritoneal exudate. It appears from the results on leukemias AK4, P1534, and C1498; malignant lymphoma, S-37, S-180; carcinomas in C57-6 and in C3H mice) and that the tendency of tumor cells to implant from peritoneal exudate into abdominal organs was overshadowed by metastatic infiltration of organs by blood route at the expense of primary growth. For tissue growth; and of highest importance for malignant cells is an important factor in the amount of tumor cells on the 6th day was higher for leukemic (S-37, lymphoma) tumors. An inverse proportion was found for the tendency of tumor cells to implant from peritoneal exudate into abdominal organs from subcutaneous growth. This was attributed to their high tendency to early spread in the blood (AK4); whereas the shortest survival periods in the intraperitoneal series and the longest in the flank series. The role of primary growth as presumed that tissue growth from benign and malignant cells is based on their interdependence, while the ability to grow as free cells in body fluids, to be separated from the tissue, and to inject free tumor cells in serous body fluids (Table 1). Thus, primary intraperitoneal growth was higher for leukemic cells than for nonleukemic tumors, inversely to their reduced adhesiveness (1). The purpose of this paper was to study the primary growth in the peritoneal exudate and nonleukemic tumors with regard to their two tendencies to spread extensively into tissue either at the site of inoculation or from the opposite tendencies to implant from peritoneal exudate into abdominal organs by blood route at the expense of primary growth and to be implanted into connective tissue either at the site of inoculation or from the peritoneal exudate. It appears from the results on leukemias AK4, P1534, and C1498; malignant lymphoma, S-37, S-180; carcinomas in C57-6 and in C3H mice) and that the tendency of tumor cells to implant from peritoneal exudate into abdominal organs was overshadowed by metastatic infiltration of organs by blood route at the expense of primary growth. For tissue growth; and of highest importance for malignant cells is an important factor in the amount of tumor cells on the 6th day was higher for leukemic (S-37, lymphoma) tumors. An inverse proportion was found for the tendency of tumor cells to implant from peritoneal exudate into abdominal organs from subcutaneous growth. This was attributed to their high tendency to early spread in the blood (AK4); whereas the shortest survival periods in the intraperitoneal series and the longest in the flank series. The role of primary growth as presumed that tissue growth from benign and malignant cells is based on their interdependence, while the ability to grow as free cells in body fluids, to be separated from the tissue, and to inject free tumor cells in serous body fluids (Table 1). Thus, primary intraperitoneal growth was higher for leukemic cells than for nonleukemic tumors, inversely to their reduced adhesiveness (1).
Free tumor cells in the peritoneal exudate of the mouse on the 5th day after intraperitoneal inoculation of 10^6 cells.

Fig. 1.—AK4 leukemia in AKR mouse. Wright ×600.
Fig. 2.—P1534 leukemia in DBA-2 mouse. Wright ×600.
Fig. 3.—C1498 leukemia in C37-2 mouse. Wright ×600.

Tumors induced in the scalp by subcutaneous inoculation of 5 × 10^7 cells.

Fig. 4.—AK4 in AKR mice.
Fig. 5.—P1534 in DBA-2 mice.
Fig. 6.—C1498 in C37-2 mice.
tumors (7). The relatively slight difference between these two series for mice with AK4 leukemia (Charts 1 and 2) as well as their shorter survival suggests that for this strain the role of implantation from peritoneal exudate was overshadowed by early massive blood-borne invasion of organs. For all leukemic strains the difference in the amount of organ invasion from various sites (charts) was paralleled by a difference in survival (Table 2), suggesting that metastatic infiltrative growth of leukemic cells into organs was the essential lethal factor in leukemias. However shorter survival of animals with large tumor growths in the scalp as compared to those bearing scantier local growths in the flank indicated the role of primary growth as eventually being the direct lethal factor.

The purpose of this paper was to study the transition between various strains of leukemias and nonleukemic tumors with regard to their two opposite tendencies—to spread extensively into organs by blood route at the expense of the primary growth and to be implanted into connective tissue either at the site of inoculation or from the peritoneal exudate. It appears from the results on leukemias reported above and those on other tumors (6) that both tendencies vary only quantitatively for all the mouse tumors investigated (leukemias AK4, P1534, and C1498; malignant lymphoma, S-37, S-180; carcinomas in C57-6 and in C3H mice) and that the tendency of tumor cells to be separated from the primary growth, to be carried as free cells in the blood, and to infiltrate into organs is paralleled by their trend to grow as free cells in serum body fluids (Table 1). Thus, primary intraperitoneal growth was higher for leukemias than for nonleukemic tumors, inversely to the results of their subcutaneous growth. This was attributed to their reduced adhesiveness (1).

SUMMARY
1. Primary growth in the peritoneal exudate (estimated from total cell number and percentage of tumor cells on the 6th day) was higher for leukemias (AK4, P1534, C1498) than for nonleukemic (S-37, lymphoma) tumors. An inverse proportion was found for the tendency of tumor cells to implant from peritoneal exudate into abdominal wall and to grow subcutaneously in the scalp and in the flank.
2. Mortality assay of leukemic strains showed the shortest survival periods in the intraperitoneal series and the longest in the flank series.
3. It was found with the help of organ assay grafts that organs were invaded from intraperitoneal growth earlier and to a greater extent than from subcutaneous growth. This was attributed to cell implantation into abdominal organs from peritoneal exudate.
4. It was concluded that primary localization of leukemic cells is an important factor in the amount of their primary growth, in the extent of metastatic growth and—indirectly and directly—in the death of the host. This factor is least significant for leukemias with a high degree of cell autonomy and high tendency to early spread in the blood (AK4); more important for P1534 and, in particular, C1498 with higher capacities for implantation and for tissue growth; and of highest importance for nonleukemic tumors.

REFERENCES
Primary Growth of Leukemic Cells as a Factor in Their Metastatic Growth and in Their Lethal Effect on the Mouse

Horace Goldie, Barbara R. Jeffries, Solomon S. Green, et al.