Pathologic Studies of Friend Virus Leukemia and the Development of a Transplantable Tumor in BALB/c Mice*

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SUMMARY

The pathology of Friend virus infection in BALB/c mice was studied and was found to be similar to that seen in other highly susceptible strains. In attempts to develop a transplantable tumor variant, 328 BALB/c mice received grafts of either liver or spleen from other BALB/c mice given inoculations of Friend virus 48–124 days previously. Two tumor lines were established from the liver and spleen, respectively, of a single mouse. Both grew rapidly and were readily transplantable. The incubation period fell from 46 to 7 days. Virtually all the mice that developed a tumor showed signs of generalized Friend disease.

Microscopically, the two tumor lines were identical and were classified as reticulum-cell sarcomas. They appeared to have arisen from the same primitive reticulum cells that proliferate in Friend virus infection. The spleens and livers of tumor-bearing animals showed, in addition to the microscopic changes of generalized Friend disease, nodules composed of reticulum cells closely resembling those comprising the tumor. The microscopic impression that these were true tumor metastases was confirmed by showing that some of the cells in the spleens of tumor-bearing animals would themselves give rise to tumors identical histologically and genetically to the original tumor. The tumor could be produced only by injecting living cells. Friend virus was consistently associated with the tumor; no increase in the potential of the virus for tumor induction could be demonstrated.
mals given inoculations intraperitoneally of virus from pools prepared in the manner described previously (2).

In attempts to produce a solid transplantable tumor, mice were killed 43—124 days after inoculation with Friend virus, and the spleen and/or liver was removed aseptically. In some instances liver and spleen were taken from infected animals employed in earlier unsuccessful attempts to produce tumors. The organ to be implanted was cut into small pieces and kept moist in Hanks buffered saline solution containing: penicillin, 500 units/ml; streptomycin, 500 μg/ml; neomycin, 500 μg/ml; and bacitracin, 7.5 units/ml.

Several methods of implantation were employed: (a) mice were anesthetized with sodium nembutal (1—2 mg., intraperitoneally), and 2– to 5-mm. pieces of tissue were implanted surgically into each axilla; (b) a coarse suspension of fragments of tissue, 0.2–1.0 mm. in diameter, was injected subcutaneously into the flank in 0.3 ml. amounts; (c) a similar suspension was injected intraperitoneally in 0.5-ml. amounts; (d) three groups of animals were given inoculations of similar material, intramuscularly, in 0.1-ml. amounts. In three experiments animals given subcutaneous inoculations were given 1 mg. cortisone intramuscularly on the day of inoculation. Animals were observed daily for the development of tumors. Routine passages were made by one of the first three methods described.

Material for histological examination was fixed in formol-saline and stained with hematoxylin and eosin. Selected sections were stained with Van Gieson’s stain, Lillie’s reticulin stain, periodic acid-Schiff, and methyl green pyronine stains. Blood smears were stained with Wright’s stain, and blood counts and peroxidase reactions were performed by standard techniques.

RESULTS

Pathology of Friend virus leukemia in BALB/c mice.—The primary site of the disease was the spleen, where specific changes could be detected as early as 3 days after the intraperitoneal injection of $10^{14} ID_50$ of virus. These specific changes, which were preceded by a slight nonspecific increase in the erythromyeloid elements in the spleen, consisted of small, rounded foci of small actively dividing reticulum cells beneath the capsule or adjacent to the trabeculae (Fig. 1). Varying numbers of erythromyeloid precursors surrounded these foci. At this stage the disease was confined to the red pulp of the spleen, and the lymphoid nodules appeared normal; but later both were replaced by solid masses of actively dividing reticulum cells.

The spleen enlarged rapidly and by 3 weeks weighed nearly 2.0 gm. At 35 days it was not unusual to find spleens weighing as much as 5 gm. and containing 3.5 ml. of blood. At this time infarction of the spleen and death following spontaneous rupture of the spleen were frequent findings.

During the 8th month of the disease, the sinusoids of the liver were usually infiltrated by reticulum cells and by moderately large numbers of erythromyeloid precursors. There was no correlation between the severity of the changes in the liver and the spleen size in individual cases.

Small foci of reticulum cells were found occasionally in the bone marrow. Sometimes small collections of mononuclear cells could be seen between the renal tubules.

The blood picture in BALB/c mice surviving 60 days or longer varied. There was usually anemia and leukocytosis. The latter comprised chiefly typical lymphocytes with, in addition, up to 10 per cent of abnormal mononuclear cells, including cells with doughnut-shaped nuclei (4). Since these contained numerous peroxidase-positive granules, we agree with Metcalf, Furth, and Buffett (7) that they are probably myeloid precursors. Terminally, the number of erythroblasts present varied widely but was usually less than 10 per cent of the total number of circulating nucleated cells.

A small number of BALB/c mice developed ascites during the 8th and 4th weeks of the disease. The ascitic fluid was similar to that described in Swiss mice (4) and would transmit the disease to about half of the mice inoculated.

Development and characteristics of a transplantable tumor.—Thirty-four separate attempts to develop a transplantable tumor are summarized in Table 1. Only two groups of mice developed progressively growing tumors. Both were given inoculations of tissue taken from the same animal; one group received spleen and the other liver. The donor animal had received a splenic implant 93 days previously in one of the earlier, unsuccessful attempts to produce tumors. The splenic tissue used to inoculate that animal, in turn, was taken from a mouse 63 days earlier with Friend virus in its eleventh passage in Swiss mice in our laboratory.

The initial tumors were first noticed between the 42nd and 50th day after implantation, and at autopsy all animals showed evidence of Friend disease.
Two separate lines of tumor were carried in serial passage, one derived from the spleen and the other from the liver of the original mouse (Table 2). The majority of mice receiving grafts developed both tumors at the site of the implant and generalized Friend disease. Of the nine which failed to develop tumors seven showed no evidence of Friend disease.

The average incubation period of the tumors passed subcutaneously decreased from 46 to about 7 days. The rate of growth was such that a typical tumor weighed 0.5 gm. by the 8th or 9th day after it was first detected—i.e., about 16–17 days after grafting—and 1 gm. by the 25th or 26th day after grafting. In extreme cases, 35 days after grafting, the tumor accounted for half of the total body weight of the animal.

Tumor-bearing mice survived, on the average, only about 30 days after implantation compared with about 70 days in the case of mice receiving cell-free virus.

Intraperitoneal injection of tumor cells resulted in the appearance of clusters of small white nodules situated chiefly on the mesentery, and a small quantity of ascitic fluid. As few as 3 X 10^4 viable cells intraperitoneally produced tumors, whereas at least 2.5 X 10^6 cells were required subcutaneously. None of the mice that received fewer than the minimum of cells necessary to produce a tumor by either route developed Friend disease.

We have successfully stored the tumor cells at —70° C., suspended in Eagle's basic medium made up in Hanks balanced salt solution with 10 per cent calf serum and 15 per cent glycerol. These cells produced tumors when implanted into BALB/c mice after storage for as long as 15 weeks.

Pathology of tumor-bearing BALB/c mice.—At autopsy tumors appeared as soft, grey, mobile nodules. Their cut surface was homogeneous, apart from an occasional area of necrosis. Ulceration of the overlying skin was unusual.

The microscopic appearances were strikingly constant (Figs. 2, 5). The tumor was composed of rounded or polygonal cells, 12–14 μ in diameter, with a moderate degree of anisocytosis. Both normal and abnormal mitotic figures were frequent. Moderate numbers of binucleate cells and occasional tumor giant cells were seen, but these did not resemble Sternberg-Reed cells. The supporting stroma was scanty and took the form of fine bands or septae. Reticulin fibers were sparse and were not formed by the tumor cells. Microscopically the tumor cells exhibited only limited powers of local invasion.

In contrast to the spleens weighing 2 gm. or more 21 days after inoculation with cell-free virus,

### Table 1

<table>
<thead>
<tr>
<th>Route</th>
<th>Tissue grafted</th>
<th>No. attempts</th>
<th>No. inoculated</th>
<th>Tumor plus F.D.</th>
<th>F.D. only</th>
<th>Negative</th>
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<tbody>
<tr>
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<td>S.C.</td>
<td>Spleen</td>
<td>21</td>
<td>204</td>
<td>10</td>
<td>179*</td>
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<tr>
<td></td>
<td></td>
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<td>49</td>
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<td>37</td>
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<td>I.M.</td>
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<td>20</td>
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<td>20</td>
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<tr>
<td></td>
<td></td>
<td>Liver</td>
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<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>I.P.</td>
<td>Spleen</td>
<td>4</td>
<td>46</td>
<td>0</td>
<td>46</td>
</tr>
</tbody>
</table>

F.D. = Generalized Friend disease.
* 35 mice of those mice received 1 mg. cortisone I.M. on day of inoculation.

### Table 2

<table>
<thead>
<tr>
<th>Passage</th>
<th>Incubation period, av. in days</th>
<th>No. developing tumors/ no. grafted</th>
<th>Incubation period, av. in days</th>
<th>No. developing tumors/ no. grafted</th>
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</thead>
<tbody>
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<td>43 (42–44)</td>
<td>10/10</td>
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<td>2</td>
<td>16 (12–21)</td>
<td>11/11</td>
<td>20 (18–40)</td>
<td>17/17</td>
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<td>3</td>
<td>14 (8–27)</td>
<td>22/24</td>
<td>15 (7–27)</td>
<td>10/10</td>
</tr>
<tr>
<td>4</td>
<td>10 (7–18)</td>
<td>45/45</td>
<td>11 (7–21)</td>
<td>14/16</td>
</tr>
<tr>
<td>5</td>
<td>10 (7–17)</td>
<td>39/39</td>
<td>12 (9–13)</td>
<td>7/7</td>
</tr>
<tr>
<td>6</td>
<td>9 (7–18)</td>
<td>44/44</td>
<td>7 (7–10)</td>
<td>10/10</td>
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<tr>
<td>7</td>
<td>8 (7–13)</td>
<td>9/9</td>
<td>7 (7)</td>
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<td>9 (8–11)</td>
<td>10/10</td>
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<tr>
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<td>8 (7–12)</td>
<td>18/12</td>
<td></td>
<td></td>
</tr>
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<td>10</td>
<td>11 (8–18)</td>
<td>10/10</td>
<td></td>
<td></td>
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</table>

* Figures in parentheses indicate range.
† All animals developing tumors also showed Friend disease except for three animals in passage 7 and one in passage 8 of the liver-derived tumor. The spleens of the animals in passage 7 were not, however, examined histologically.
Passage Incubation period, av. in days* No. developing tumors

<table>
<thead>
<tr>
<th>Passage</th>
<th>Incubation period, av. in days*</th>
<th>No. developing tumors</th>
</tr>
</thead>
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<td>1</td>
<td>12 (8—20)</td>
<td>11/12</td>
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<td>8 (7—12)</td>
<td>12/13</td>
</tr>
<tr>
<td>6</td>
<td>9 (8—12)</td>
<td>10/10</td>
</tr>
</tbody>
</table>

* Figures in parentheses indicate range.
† All animals developing tumors showed Friend disease except one in passage 4. One of the three not developing a tumor showed Friend disease; the other two were completely negative.

appeared somewhere between the two extremes described above. Thus, in some spleens the microscopic appearances strongly suggested metastatic tumor, but in the majority they were equivocal.

Grossly, the liver in tumor-bearing animals was usually normal, but microscopic examination showed both diffuse sinusoidal involvement similar to that usually seen in Friend disease and development of small, rounded nodules composed of pleomorphic cells similar to those seen in the subcutaneous tumor. Spread from the primary tumor to the regional lymph nodes and thymus was observed occasionally, but spread to other organs was unusual. The arterioles in the lung and sometimes those in the kidney contained a granular hematoxyphilic material. These deposits, which were Feulgen-positive and similar to those described by Buffett and Furth (1), were interpreted as degenerating intravascular tumor emboli.

Although only two groups of mice initially developed progressively growing transplantable tumors, in eleven other mice from six experiments histologic examination showed small reticulum-cell tumors at the site of implantation. None, however, grew beyond 5 mm. in diameter, and they could not be transplanted.

Experimental evidence for tumor metastases.—Although the histologic appearances gave the impression, at least in some instances, that true metastases developed in the spleen and liver, a second interpretation was possible—namely, that the changes in the spleen and liver were due to the action of the virus on the reticulo-endothelial cells in these organs. If the nodules were true cellular metastases, then some of the cells in the spleen should be genetically similar to the tumor cells implanted subcutaneously and should possess the same potential for growth and metastasis as those of the original tumor.

According to the laws governing tissue transplantation (8), a tumor arising in either parent will grow progressively in the F1 hybrid, but a tumor arising in an F1 hybrid will not grow in either parental strain. Since the tumor would be readily transplanted in (BALB/c X A)F1 hybrid

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Tissue transplanted</th>
<th>Results in recipient*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tumor</td>
<td>11/11</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>15/17</td>
</tr>
<tr>
<td>II</td>
<td>Tumor</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>9/9</td>
</tr>
</tbody>
</table>

* Number with tumors/number grafted.

mice (Table 3), groups of those hybrid mice were given subcutaneous grafts of BALB/c-derived tumors, and after 21—28 days the animals were killed and the tumor and spleen removed aseptically. After tissue had been taken for histologic examination BALB/c, strain A, and (BALB/c X A)F1 hybrid mice were given implants subcutaneously of either tumor or spleen. The mice were observed for 60 days for the development of tumors. The results of two separate experiments are given in Table 4. A similar experiment in which (BALB/c X C57BL)F1 hybrids were used is summarized in Table 5. From these experiments it was clear that the spleens of the F1 generation contained cells capable of developing into solid tu-
mors, and these cells were genetically similar to those of the original tumor.

As further evidence that true metastases occurred, a series of experiments were carried out in which the spleens from tumor-bearing animals were grafted subcutaneously. These spleens produced tumors at the site of implantation that were identical histologically to the original tumor. The spleens of these animals were in turn grafted in the same way and again yielded a subcutaneous tumor. Two lines of tumors have been maintained in this way for three and five generations, respectively. A similar experiment has been carried out successfully with liver from tumor-bearing mice. On the other hand, with the exception of the tissues of the one mouse which gave rise to our two tumor lines, repeated attempts to obtain tumors from the livers and spleens of animals given inoculations of cell-free material have failed. These included a series of experiments in which 35 mice were given implants of spleen taken from mice that had been given inoculations 35 days previously of filtered extracts from tumors. None of these mice developed subcutaneous tumors, although all developed generalized Friend disease. Further, none of 1,810 mice given inoculations in titrations of virus from the tumors and spleens of tumor-bearing animals developed tumors.

It was concluded that living cells were essential for the propagation of the tumor and that true cellular metastases were produced. There was no evidence to suggest that virus extracted from tumor-bearing animals had a greater tumor-inducing potential than that obtained from other sources.

**DISCUSSION**

The disease produced by intraperitoneal inoculation of Friend virus in BALB/c mice did not differ fundamentally from that observed previously in Swiss and DBA/2 mice (4, 7). The ascites appeared to be an integral part of the disease as described originally by Friend (4), although subsequently this seems to have been overlooked.

The tumor in BALB/c mice is best regarded as a non-dictiocyte form of reticulum-cell (stem-cell) sarcoma, which we think is derived from the same cells that proliferate in the spleen in response to Friend virus infection. It is similar in its general characteristics to that reported in the DBA/2 and Swiss mice (1, 6). In all reports, only tissue from an occasional mouse surviving for a long time was able to produce tumors. It is of interest that both the mouse from which our tumor lines were developed and another mouse given grafts of the same material developed small nonprogressive reticulum-cell tumors, which observation suggested that possibly a series of changes are required before the cells acquire complete autonomy.

We could not prove beyond doubt that the virus caused the tumor, but the following evidence strongly suggested a close association. The tumors all arose from grafts of the organs of an animal with Friend disease which was confirmed microscopically. The donor was less than 6 months old, an age at which spontaneous neoplasms are unusual. The histologic appearances of the tumor were compatible with an origin from reticulum cells of the same type that appeared in the spleen following infection with Friend leukemia virus. The differences between these cells and those of the tumor can be explained by their dedifferentiation into cells of either the erythroid or myeloid series.

The tumor has always been associated with virus. A series of comparative titrations between the tumors and spleens of the same BALB/c mice are reported in the subsequent paper (3). The tumors have constantly yielded up to $10^{9.1}$ ID$_{50}$/ml of virus, and the spleens up to $10^{9.2}$ ID$_{50}$/ml.

The occurrence of true cellular metastases makes it impossible to be certain whether generalized viral infection has occurred in the host. Since the tumor always contained virus, as presumably did the metastases, assay for virus does not resolve the dilemma.

**ACKNOWLEDGMENTS**

We should like to thank Mr. G. W. Kohr and Mrs. Sandra Smith for their skilled technical assistance.

**REFERENCES**


Fig. 1.—Focus of Friend disease in spleen of BALB/c mouse 4 days after inoculation of 10^4ID_50 of virus. H. & E., ×256.

Fig. 2.—Cellular detail of reticulum-cell sarcoma 30 days after transplantation derived from spleen of BALB/c mouse with Friend disease. H. & E., ×640.

Fig. 3.—Tumor metastasis in spleen from BALB/c mouse with subcutaneous tumor 17 days after transplantation. H. & E., ×400.

Fig. 4.—Friend disease in same spleen as Figs. 3, 6. H. & E., ×256.
Fig. 5.—Reticulum-cell sarcoma 30 days after subcutaneous transplantation. H. & E., ×90.

Fig. 6.—Spleen with metastatic focus. Same spleen as Figs. 3, 4. H. & E., ×90.
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