A New Spontaneous Hamster Carcinoma Associated with a Positive Erythroagglutination Reaction and Anemia*

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SUMMARY

The appearance of a spontaneous carcinoma in an untreated male golden hamster has provided us with a very useful additional experimental model with which to study the anemia of malignancy. The tumor, probably of salivary gland origin, has been serially transplanted in the cheek pouch or flank of normal hamsters through 70 transplant generations. The growth characteristics are stable and predictable, and a growth curve has been established.

Growth of the tumor is associated with the development of a normochromic, normocytic anemia, much like that seen in many human cancer patients. The anemia is not related to blood loss, metastases, cachexia, or infection. The animals develop a positive erythrocyte agglutination reaction, and red cell survival is shortened. The erythrocytes are not intrinsically defective, but an extrinsic destructive mechanism is operative.

The golden hamster (Mesocricetus auratus) has been used as a laboratory animal since the late 1930's, and during this time a variety of spontaneous transplantable tumors have been reported (see references 5, 6, 14 for a review of the literature), including some with associated hematologic abnormalities. An undifferentiated carcinoma arising in the anterolateral neck area is distinctly unusual, however, and the association of this type of tumor in its transplanted generations with anemia, shortened red blood cell survival, and a positive erythrocyte agglutination reaction is apparently unique.

The purpose of this paper, therefore, is to describe in detail the origin, pathology, transplantation, and growth characteristics, and the anemia related to this new spontaneous carcinoma named MAD I. A preliminary report of these data has been presented (8).

MATERIALS AND METHODS

Origin of tumor.—The tumor was discovered in the right anterolateral cervical triangle of an untreated male golden hamster in our stock colony. The animal was sacrificed with an overdose of sodium pentobarbital (Nembutal, Abbott). The tumor was firm, pea-sized, and grossly appeared to involve a salivary gland. No metastases were found at autopsy. The cut surface of the tumor was pink, with small foci of apparent necrosis. A portion of the tumor was fixed in 10 per cent neutral formalin, and the remaining tumor was divided into small portions and transplanted to the cheek pouches of normal hamsters.

Microscopic pathology.—Microscopic study of the original tumor revealed cords and clusters of large polygonal cells, with a moderate amount of lightly eosinophilic, granular cytoplasm, varying moderately in size and shape, interspersed in a scanty, fibrous stroma. Nuclei were predominantly ovoid, with an irregularly clumped chromatin pattern, and with one to three eosinophilic nucleoli. Frequent mitoses were present, many showing asymmetrical metaphase plates and multipolar figures. Variable focal areas of necrosis were evident. The over-all pattern of this anaplastic tumor was that of an undifferentiated carcinoma, and in

* This work was supported in part by a grant (C-4959) from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service; and by U.S. Atomic Energy Commission, Contract AT(30-1)-901, with the New England Deaconess Hospital.

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Received for publication June 24, 1963.
areas its appearance strongly suggested origin from salivary gland. Where there was extensive involvement by tumor, the salivary gland elements were atrophic (Figs. 1, 2).

Transplantation studies.—Golden hamsters (Mesocricetus auratus) of both sexes, 8–10 weeks old, fed Purina Laboratory Chow and water ad libitum, were used. The animals were also fed supplementary carrots and cabbage and were kept in an air-conditioned environment. The method of transplanting homologous tumor to the hamster cheek pouch is a modification of technics previously reported (13, 16). A scalpel is used to cut pieces approximately 1 cu. mm. from the portion of the tumor that appears grossly viable. The tumor fragments are kept moist with a saline-soaked gauze pad. Recipient hamsters are anesthetized with a single intraperitoneal injection of 0.10–0.15 cc. of sodium pentobarbital (Nembutal, Abbott), 60 mg/cc/100 gm body weight. The cheek pouch is then carefully pulled out with forceps and everted over a cork board and pinned into position with the blind end facing the transplanter. The pouch is gently cleaned with cotton swabs soaked in normal saline or benzalkonium chloride (Zephiran). One piece of tumor is implanted into the nonmuscular portion of the pouch with a B-D Yale No. 16 caudal needle that serves as a trocar. Only one pouch is employed for transplantation. Ten hamsters received cheek pouch transplants of the original tumor.

At regular intervals after transplantation each hamster is anesthetized, and the cheek pouch carrying the cancer transplant is everted, pinned out on a cork board, and observed with reflected light at 10 X magnification through a binocular dissecting microscope. During the period of observation the tumors are moistened with normal saline. Particular attention is paid to the tissue reaction about the transplant. An attempt is made to distinguish inflammatory edema from tumor growth. The length and width of the tumor are determined with an ocular micrometer, and the height of the tumor is measured with calipers calibrated in millimeters.

Calculation of tumor volumes is based on the resemblance of a tumor transplant in its logarithmic growth phase to an oblate spheroid. The formula for determining the volume of such a spheroid is \( V = \frac{4}{3} \pi a^2b \); however, since diameters \( c, d, e \) were used, the formula was modified as follows: \( V \approx \frac{a}{2} \times b^{1/2} \times c^{1/2} \text{ or } 0.52 ace \). A similar technic has been used to calculate the volume of heterologous human tumors grown in the hamster cheek pouch (10).

The tumor growth curve for each animal was determined, and an average curve for each transplantation generation established. A minimum of ten animals was used in each new transplantation generation, and in some cases as many as 80 animals were used in a given generation. Particular attention was paid to: (a) the duration of the initial (lag) phase, (b) the duration of the second or logarithmic (log) phase, (c) the slopes of the curves during the log phase, and (d) the tumor volume at the end of the log phase.

In addition to the observations and measurements of the cheek pouch tumors in vivo, selected animals in each transplant generation were sacrificed, an autopsy was performed, the tumor was examined grossly, and a routine histological examination was made of all tissues and tumor. A line of MAD I, derived from the second cheek pouch transplant generation, has also been maintained in the hamster flank by serial transplantation.

Hematologic studies.—Random blood studies performed on the earlier transplant generations indicated that anemia developed in many of the tumor-bearing animals. Studies were then established to ascertain the nature of this anemia and to determine other hematologic abnormalities.

Two blood determinations taken 10 days apart were averaged to establish the “normal” values in any one animal. With the use of standard hematologic technics on cardiac blood, the following tests were done: differential and total white blood cell counts, hematocrits, hemoglobins, total red cell and reticulocyte counts, and platelet estimates (smear). An erythrocyte agglutination reaction was also performed by a modification (19) of the method originally described by Betta et al. (1).

Immediately following the second “normal” determination, a 1-cu. mm. fragment of the MAD I tumor selected from the log phase of growth was transplanted to the cheek pouch, and at 5-day intervals, for the duration of the experiment, cardiac punctures were performed and the blood studies noted above were done. The alterations in blood values were correlated with changes in tumor growth.

In addition to the blood studies just described, red cell survival times were determined by in vitro chromation of erythrocytes and intracardiac return of labeled cells. Chromation was accomplished by withdrawal of 1 ml. of whole blood by cardiac puncture from each of five hamsters and mixing this blood with 0.5 ml. of acid citrate dextrose diluent and then incubating at 37° C. for 30 minutes with 50 \( \mu c. \) of Cr\(^{51} \) (\( Na_2CrO_4 \)). On two occasions during the incubation period the samples were gently agitated. After incubation, the specimens was centrifuged at 2,000 r.p.m. for 10 min-
utes; the plasma was withdrawn, and normal saline was substituted for the plasma. The labeled erythrocytes (0.25 ml.) were then given by intracardiac injection to five recipient hamsters. Blood samples of labeled red cells (0.1 ml.) were obtained by cardiac puncture from each animal on the 1st day and at 3-day intervals thereafter. Hemoglobin radioactivity was determined for each sample.

The erythrocyte survival studies were done on four groups of animals with MAD I tumors that were in the 49th and 50th generations, at a time when the growth characteristics of the tumor were stable and predictable. Group I, composed of eighteen hamsters with MAD I cheek pouch transplants, had autologous red cell survival studies. These animals were all anemic (7.0–9.8 gm. of hemoglobin per 100 ml. of blood) and had positive erythroagglutination reactions. Group II consisted of twenty carcinoma-bearing hamsters which had been given injections of normal (homologous) red cells. In Group III were 41 normal hamsters of comparable age and both sexes. Erythrocyte survival time of autologous red cells was determined in 21 animals, and homologous red cell survivals in twenty animals. In Group IV, fifteen normal hamsters were given injections of labeled red cells from anemic hamsters with growing carcinoma.

RESULTS

The MAD I tumor has now been serially transplanted to the cheek pouch for 70 generations. The tumor growth curve has been stable since the fifteenth transplant generation. The tumor has been 100 per cent transplantable since the sixth generation.

There is now very little macroscopic inflammatory reaction associated with the lag phase. During the first fifteen generations, however, it was not unusual to see edema fluid and hemorrhages around transplants in the lag phase. The tumor now grows very rapidly, with a lag period of no more than 4 days; but through the fifteenth generation the lag period lasted for approximately 1 week. The lag period of growth extends from 5 to 35 days, and during this period the tumor may ulcerate the overlying cheek pouch. The tumor develops in-
features of the tumors grown in the flank are identical to those in the cheek pouch.

Since the 25th cheek pouch tumor generation detailed hematologic data have been collected. The blood values have been consistently reproduced for the past twenty tumor generations. Data from the 60th generation obtained on day 50 following tumor transplantation are given in Table 1. An elevation in white blood cell count occurs at the 20th day following tumor transplantation and persists at levels as high as 25,000/cu mm of blood. Associated with this elevation in white count is an absolute granulocytosis and absolute lymphopenia with a reversal of the normal lymphocyte:polymorphonuclear leukocyte ratio. The platelet values, over all generations studied and as judged by smear, remain within the normal range during the entire period of tumor growth, and there has never been clinical or post-mortem evidence of bleeding. The most striking hematologic abnormality is the development of a normocytic normochromic anemia. The hemoglobin, hematocrit, and red blood cell counts all decline by the 15th day following tumor transplantation and are significantly decreased by the 25th day following carcinoma transplantation. Once anemia ensues it persists and increases in magnitude. The erythroagglutination tests performed on erythrocytes from tumor-bearing hamsters were positive in 68 per cent of the animals after 1 week of tumor growth when the tumor was in its 25th transplant generation. Since the 50th tumor generation, 80 per cent of the hamsters develop positive erythroagglutination tests 1 week to 10 days after tumor transplantation and prior to the onset of anemia. A mild elevation in reticulocytes has been found consistently by the 30th transplantation day.

The average autologous and homologous survival of hamster red blood cells under various experimental and control conditions is summarized in Table 2. The tumor-bearing animals had a shortened autologous erythrocyte survival time when compared with the autologous survival of normal hamster red cells, the survival time being decreased 30—50 per cent. In addition, there was a shortened survival of normal (homologous) erythrocytes in animals with carcinoma when compared with the red cell survival of homologous erythrocytes in normal hamsters. The reduced survival was approximately the same as in the autologous survival studies in anemic carcinoma-bearing hamsters. The red blood cells from anemic tumor-bearing hamsters survived normally when placed in normal, tumor-free animals. There was no evidence of infection, weight loss, debility, or metastasis in

| TABLE 1 |
| HEMATOLOGICAL DATA FOR THE 60TH GENERATION OF MADM I TUMOR-BEARING HAMSTERS |

<table>
<thead>
<tr>
<th>Blood value</th>
<th>Normal*</th>
<th>Tumor-bearing†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC (thousands/cu mm)</td>
<td>5.78 ± 1.29</td>
<td>13.10 ± 3.10</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>29.9 ± 5.50</td>
<td>65.1 ± 4.71</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>66.4 ± 5.90</td>
<td>29.2 ± 6.31</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>2.5 ± 0.80</td>
<td>3.3 ± 1.00</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.1 ± 0.02</td>
<td>1.8 ± 0.05</td>
</tr>
<tr>
<td>Platelets (smear)</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Hemoglobin (gm/100 ml)</td>
<td>16.1 ± 1.30</td>
<td>10.4 ± 2.00</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>48.1 ± 2.10</td>
<td>33.2 ± 3.10</td>
</tr>
<tr>
<td>Total RBC (millions/cu mm)</td>
<td>6.71 ± 0.74</td>
<td>3.44 ± 0.81</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>1.3 ± 0.05</td>
<td>3.61 ± 0.88</td>
</tr>
<tr>
<td>Erythrocyte agglutination reaction</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>(25/25)</td>
<td>(25/25)</td>
<td></td>
</tr>
</tbody>
</table>

* Normal hamsters are comparable to tumor-bearing in regard to age, sex, and number (25).
† Data presented for the 50th day following tumor transplantation.
‡ Average value and one standard deviation.
§ Number and morphology.

| TABLE 2 |
| SURVIVAL OF Cr*-LABELED RED CELLS UNDER VARIOUS CONDITIONS |

<table>
<thead>
<tr>
<th>Group</th>
<th>No. animals</th>
<th>Condition of recipient*</th>
<th>Condition of red cells</th>
<th>T-1/4 of Cr*-labeled RBC in days (mean ± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>18</td>
<td>Tumor-bearing (anemic, EAR-positive)</td>
<td>Autologous</td>
<td>9.6 ± 2.4</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>Tumor-bearing (anemic, EAR-positive)</td>
<td>Normal, homologous</td>
<td>9.8 ± 2.1</td>
</tr>
<tr>
<td>III a</td>
<td>21</td>
<td>Normal</td>
<td>Autologous</td>
<td>14.2 ± 2.5</td>
</tr>
<tr>
<td>b</td>
<td>20</td>
<td>Normal</td>
<td>Normal, homologous</td>
<td>14.4 ± 2.8</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>Normal</td>
<td>Anemic, tumor-bearing homologous</td>
<td>14.0 ± 2.7</td>
</tr>
</tbody>
</table>

* EAR = erythroagglutination reaction.
Fig. 1.—MAD I hamster tumor, showing undifferentiated anaplastic carcinoma separated from salivary gland by a pseudocapsule. Numerous mitoses and pleomorphic tumor cells are seen. X100.

Fig. 2.—MAD I hamster tumor demonstrating cords and clusters of large polygonal cells, varying moderately in size and shape. Ovoid nuclei, irregularly clumped chromatin, frequent nucleoli, and mitotic figures are seen. X430.
any of the animals employed in these red blood cell survival studies.

DISCUSSION

The finding of a spontaneous, undifferentiated, hamster carcinoma of probable salivary gland origin is reported. The growth characteristics of the tumor and its morphology have been described. The tumor’s growth pattern in the hamster cheek pouch is similar to the growth of other induced (15) and spontaneous hamster tumors transplanted to this site (9). However, the tumor has many more mitoses and appears to grow more rapidly than other spontaneous tumors that have been reported (5, 6, 14) or that we have studied (10).

A unique feature of the host response to this tumor is the development of a positive erythrocyte agglutination reaction prior to the onset of anemia. This test has been shown to be positive in human cases of malignancy (2), and, although it appears to indicate a nonspecific red cell alteration, it is of some interest that the test becomes positive prior to the frank development of anemia. The development of a positive direct Coombs test has been noted prior to the onset of human autoimmune hemolytic anemia (4) and by Burnet in the NZB strain of mice prior to the development of autoimmune hemolytic anemia (8). The erythroagglutination reaction differs from the direct antiglobulin or Coombs test, but the possibility exists that the positive erythroagglutination reaction in our hamsters reflects an immunologic basis for the ensuing anemia.

The autologous and homologous red cell survival values reported in this study agree well with those reported by us in previous studies concerning animals with chemically induced sarcomas (18). Since in these current studies normal hamster erythrocytes had a shortened survival time in carcinoma-bearing hamsters, and red cells from anemic, carcinoma-bearing hamsters survived normally in normal hamsters, it would appear that an extrinsic mechanism for erythrocyte destruction exists in the carcinoma-bearing animal. In these animals a hyperactive reticuloendothelial system, characterized by splenomegaly, reticuloendothelial cell hyperplasia and anaplasia, hemosiderosis, and proliferation of plasma cell precursors, would be one possible explanation for the increased destruction. Observations in sarcoma-bearing hamsters have shown this to be the case (7, 10, 17–20), and morphologic observations to be reported on animals bearing the MAD I would also support this contention that a hyperactive reticuloendothelial system is the extrinsic mechanism responsible for red cell destruction.

REFERENCES

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