The Effect of Transplanted Tumors on the Hemolytic Behavior of Red Blood Cells from C3H and C57BL Mice

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In a previous paper (2) it was shown that red blood cells of the tumor-susceptible C3H mice had a different hemolytic behavior from the red blood cells of the tumor-resistant C57BL mice. A hemolytic factor was found associated with the erythrocytes from C3H mice which was not present with erythrocytes from C57BL mice. Furthermore, it was reported that, during development of spontaneous mammary cancer in C3H mice, the red blood cells showed (a) less hemolysis with dilutions of rabbit serum and (b) a higher resistance against the hemolytic effect of suspensions of mammary cancer than those from nontumorous animals. The question arose: Are these findings characteristic only for red blood cells from C3H mice with mammary cancer, or are these findings also valid for other mouse strains and other tumors, including transplants of tumors originally induced with methylcholanthrene?

Experiments were performed to investigate the hemolytic behavior of erythrocytes from C3H and from C57BL mice which carried transplants of various tumors. The red blood cells were tested for hemolysis with dilutions of rabbit serum and with dilutions of suspensions of the tumors used for transplants. Data will be presented which indicate that the growth of some of these tumors affects the hemolytic behavior of C3H and C57BL erythrocytes.

MATERIALS AND METHODS

Mouse strains and tumors employed.—C3H and C57BL mice from our own breeding colony were employed as previously described (1). The animals were 4–5 months old, since at this age the tumor transplants grow better than in older animals. The following tumors, originally produced in the brains of mice by implanting pellets of methylcholanthrene (7), were used for subcutaneous transplantation:

C3H(9).—Meningeal fibrosarcoma in the 98th–112th passage.
C3H(18).—Astrocytoma in the 58th passage.
C3H(19).—Malignant glioma (unclassified) in the 64th–79th passage.
C3H(20).—Glioblastoma multiforme in the 48th passage.
C3H(25).—Fibrosarcoma in the 5th–10th passage.
C3H(26).—Malignant glioma (unclassified) in the 7th and 8th passage.
C3H(31).—Ependymoma in the 5th passage.
C3H(46).—Ependymoma in the 2nd to 5th passage.
C3H(48).—Astrocytoma in the 1st and 2nd passage.
C57(1).—Rhabdomyosarcoma in the 74th–93rd passage.
C57(20).—Malignant glioma (unclassified) in the 49th to 62nd passage.
C57(31).—Polar spongioblastoma in the 11th–26th passage.

The tumors were checked regularly in vivo as to size, appearance, firmness, and possible necrosis, and their growth rate was noted until the death of the animal. They were measured twice weekly in three dimensions and routinely before bleeding. Post mortem examinations were performed regularly to determine incidental pathologic findings in addition to the tumor.

Hemolysis experiments with dilutions of rabbit sera.—The rabbit sera were procured from normal animals, inactivated at 56°C for 30 minutes, and stored at −20°C for various lengths of time until used.

The mouse red blood cells were obtained from the tail of the animal. Each sample was taken from an individual mouse to determine the effect of a specific tumor. A 2% per cent red blood cell suspension was prepared on ice as previously described (1) and used within 1–5 hours after bleeding of the animal.

The tests were performed in the following manner: 0.2 cc. of the red blood cell suspension was
added to 0.2 cc. of twofold serial dilutions of rabbit serum (1:4 to 1:2048). The mixtures were incubated at 37° C. for 60 minutes and then kept at 4° C. Observations were made daily for 4–5 days to determine the initial occurrence, the degree and the titer of hemolysis, and the results were recorded. Complete hemolysis was designated 4 plus; advanced hemolysis with some intact red blood cells, 3 plus; moderate hemolysis with a well defined red blood cell sediment at the bottom of the tube, 2 plus; and a reddish or pink color of the supernatant fluid, 1 plus. The lowest serum dilution in which hemolysis occurred was taken as an arbitrary titer.

Hemolysis experiments with tumor suspensions.—The same types of tumors as were used for the transplants were employed for the preparation of the tumor suspensions. Tumors were used either immediately after the death of the animal or after storage at —20° C. for various lengths of time. The tumor was ground in a mortar with sterile sea sand; a sterile 10 per cent saline suspension was prepared on ice and centrifuged for 5–10 minutes at 3,250 r.p.m. The supernatant, designated below as the tumor suspension, was used undiluted and in dilutions of 1:2 and 1:4. Suspensions of mammary tumors from C3H mice were similarly prepared; these are known to hemolyze mouse red blood cells (2, 4–6) and were used as controls.

The experiments with tumor suspensions were performed and recorded in the same manner as the experiments with rabbit sera; however, hemolysis was recorded only when observed within 24 hours. Within this time there was no hemolysis of the erythrocytes in tests with saline or with dilutions of rabbit serum.

EXPERIMENTAL RESULTS

Hemolysis with rabbit serum.—Two series of tests were run simultaneously, using ten dilutions of two different rabbit sera for each sample of red blood cells. The difference in hemolysis between the two parallel series was within the range of 25 per cent. When there was no hemolysis in any of the dilutions, or a 1 plus in only 1 dilution, the observations were recorded “without hemolysis.” The results are given in Chart 1 for red blood cells from C3H and from C57BL mice with and without tumors.

It can be seen in Chart 1 that the red blood cells from C3H mice carrying a subcutaneous transplant of a rapidly growing malignant glioma—C3H(19)—or a rapidly growing ependymoma—C3H(46)—did not show hemolysis as frequently as those from nontumorous C3H mice. The bars indicate that, in thirteen experiments with the malignant glioma and in fifteen experiments with the ependymoma, no hemolysis was observed in eight instances with the former tumor and in ten with the latter. Hemolysis did occur 5 times with each of the two tumors, but was weak in three of the tests. These findings are similar to those with

![Chart 1](chart1.png)

CHART 1.—Number of tests for hemolysis of erythrocytes from tumor-bearing and nontumor-bearing C3H and C57BL mice. C3H and C57BL denote tests with erythrocytes from normal control animals. The other designations refer to the tumor-bearing mice noted in text.

C3H mice bearing spontaneous mammary tumors previously reported (2). The results with C3H mice bearing transplants of the fast-growing meningeal fibrosarcoma—C3H(9)—also showed less hemolysis than did the nontumorous control mice.

The results with other tumors were not so definite and approximated in general the results with red blood cells from nontumorous C3H mice (see Table 1). Tumor C3H(48), an astrocytoma, was the only tumor used in the first two subcutaneous passages. Tumors C3H(20), a glioblastoma multiforme, C3H(95), a fibrosarcoma, and C3H(91), an ependymoma, were slow-growing in contrast to tumors C3H(19), C3H(46), and C3H(9), pre-
Previously described. Close scrutiny revealed two general facts: (a) Red blood cells from mice with fast-growing tumors showed limited hemolysis, whereas those from mice with slow-growing tumors showed a degree of hemolysis similar to the controls; (b) red blood cells from mice with tumors which had a good growth rate showed no hemolysis during the early and moderately advanced stages of tumor growth but showed marked hemolysis in the far advanced stages.

To investigate this relationship between the stage of tumor growth and the hemolytic behavior of the erythrocytes, a series of sixteen mice was observed continuously during tumor growth.

**TABLE 1**

RESULTS OF HEMOLYSIS EXPERIMENTS WITH RED BLOOD CELLS FROM C3H MICE BEARING SUBCUTANEOUS TRANSPLANTS OF BRAIN TUMORS*

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Passage no.</th>
<th>Rate of growth</th>
<th>No. experiments</th>
<th>With hemolysis</th>
<th>Without hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H(10)</td>
<td>64th-70th</td>
<td>fast</td>
<td>15</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>C3H(16)</td>
<td>2d-5th</td>
<td>fast</td>
<td>15</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>C3H(26)</td>
<td>1st-2d</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>C3H(20)</td>
<td>46th</td>
<td>slow</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>C3H(25)</td>
<td>5th-10th</td>
<td>slow</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>C3H(31)</td>
<td>3d</td>
<td>very slow</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>C3H(19)</td>
<td>50th</td>
<td>fast</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C3H(36)</td>
<td>7th &amp; 8th</td>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* All tumors except C3H(46), a fibrosarcoma, are gliomas.

Twelve of these animals conformed to the observation that no hemolysis occurs in the early stages but does occur in the late stages. A typical example is given in Chart 2 of one C3H mouse with a subcutaneous implant of tumor C3H(46), an ependymoma. It shows that lack of hemolysis or weak hemolysis was present at an early or intermediate stage of tumor growth, 23 and 31 days after subcutaneous implantation, whereas in the far advanced stage, 40 days after implantation, hemolysis had increased. It cannot be stated how soon the tumor affects the reaction of the red blood cells. In one instance of a rapidly growing tumor, very weak hemolysis was observed as early as 4 days after the tumor had been transplanted; a noticeable growth of this tumor was seen on the 7th day. If the transplanted tumors did not grow, the hemolytic behavior of the red blood cells was not affected in any of six tests.

The hemolytic behavior of erythrocytes from nontumorous and tumor-bearing C57BL mice is different from that of C3H erythrocytes as seen in Chart 1. Red blood cells from the nontumorous C3H mice show hemolysis, and hemolysis decreases with tumor growth. With C57BL mice, the red blood cells from the nontumorous animals do not show hemolysis, and hemolysis increases with tumor growth. However, the change in the hemolytic behavior with erythrocytes of C57BL mice is not regular or predictable. Hemolysis occurred with tumor C57(1), a rhabdomyosarcoma, in nine out of eighteen trials; with tumor C57(26), a malignant glioma, in eight out of nineteen instances; with tumor C57(31), a polar spongioblastoma, in seven out of 21 instances. Hemolysis usually occurred on the 4th day, rarely on the 5th. It was by no means a constant phenomenon and was observed more often, though not regularly, with large tumors. No change in the hemolytic behavior was demonstrable with C57BL erythrocytes in the early stage of tumor growth.

Red blood cells from C57BL mice with implanted leukemia did not show hemolysis in any of five experiments. Unsuccessful transplants of brain tumors did not affect the hemolytic behavior of C57BL erythrocytes at any time.

**Hemolysis with tumor suspensions.**—The hemolysis tests with tumor suspensions were done: (a) to determine the hemolytic effect of tumor suspensions on red blood cells from nontumorous mice, (b) to investigate the effect of heterologous tumor suspensions on red blood cells from tumor-bearing mice, (c) to investigate the possibility of a specific effect of homologous tumor suspensions on erythrocytes from tumor-bearing mice.

Suspensions from the following tumors were tested for their hemolytic effect: C3H(9), C3H(19), C3H(20), C3H(26), and C3H(46); C57(1), C57(26), and C57(31). Each suspension was tested simultaneously with five to eight different red blood cell samples of tumorous and nontumorous mice. The hemolytic effect of the tumor suspensions on mouse red blood cells was controlled by the effect on C57BL red blood cells.
which normally do not hemolyze with saline or rabbit serum.

The results showed that with the exception of tumor C57(1), a rhabdomyosarcoma, the suspensions rarely exerted a hemolytic effect on red blood cells from nontumorous C57BL mice. In 25 of the 32 tests, a hemolytic effect was observed in only five instances. With suspensions of tumor C57(1), hemolysis occurred in four out of seven experiments.

There was no specific effect of various homologous tumor suspensions on the red blood cells from tumor-bearing mice, the question (c) raised above. The pattern of hemolysis observed with tumor suspensions was about the same as with rabbit serum. Red blood cells which showed hemolysis with dilutions of rabbit serum also developed it with tumor suspensions. Those which did not hemolyze with rabbit serum failed to hemolyze with tumor suspensions. Occasionally, however, there was a deviation from this general pattern. Red blood cells from C3H mice implanted with tumor CSH(9), a meningeal fibrosarcoma, showed no hemolysis in four out of six instances with homologous tumor suspensions, although they did show hemolysis with rabbit serum.

In about 50 per cent of the control tests with suspensions of CSH mammary tumors there was decreased hemolysis of the red blood cells from CSH and C57BL mice with brain tumor transplants. In some of these tests the difference in hemolysis of the red blood cells from tumor-bearing and nontumorous animals was very impressive, as shown in Figure 1. It is seen that the tumor suspension had a hemolytic effect on red blood cells from nontumorous mice (test tubes 4, 7, and also 6) and that it hemolyzed the erythrocytes from tumor-bearing mice slightly or not at all (test tubes 1, 2, 3, and 5).

DISCUSSION

The difference in the hemolytic behavior of the red blood cells from tumor-bearing and nontumor-bearing CSH mice cannot be explained as yet. Attention should be drawn to the fact, however, that the red blood cells from the tumor-bearing mice behave similarly to mouse red blood cells sensitized with antitumor rabbit serum or anti-mouse red blood cell serum, especially anti-CSH red blood cell serum (9). The sensitized mouse red blood cells show only limited hemolysis with suspensions of CSH mammary tumors compared to nonsensitized red blood cells. The red blood cells from the tumorous mice show limited hemolysis with dilutions of rabbit serum and, in some instances, also with suspensions of mammary tumors. One may speculate, therefore, that the red blood cells from the tumorous mice have taken up an antibody—either a tumor antibody or an auto-antibody—which inhibits hemolysis. Such a concept would also explain the change in the hemolytic behavior during tumor growth. It could be assumed that in the early or moderately advanced stage there is more antibody available and that this antibody is no longer present or is no longer demonstrable in the late stages of tumor growth. Whatever the mechanism may be which causes the change in hemolysis, it should be emphasized that the erythrocytes show a change during tumor growth in CSH mice.

With erythrocytes from C57BL mice the findings are less conclusive. Increase of hemolysis was present only in advanced stages of tumor growth; even this was not a regular observation. No decrease of hemolysis was seen with dilutions of rabbit serum, possibly because the red blood cells from the nontumorous C57BL mice ordinarily show no hemolysis. However, it should be noted that, with suspensions of CSH mammary tumors which hemolyze the erythrocytes from both the nontumorous C57BL and C3H mice, there was limited hemolysis of the red blood cells from the tumor-bearing C57BL mice as well as from the tumor-bearing C3H mice.

SUMMARY

1. Erythrocytes from CSH mice bearing subcutaneous transplants of brain tumors produced with methylicholanthrene showed limited hemolysis. This is in contrast to the marked hemolysis of erythrocytes from nontumor-bearing C3H mice.

2. The hemolytic behavior of the red blood cells of C3H mice bearing brain tumor transplants is similar to that of the red blood cells of C3H mice with spontaneous mammary tumors.

3. The hemolytic behavior was influenced by growth rate and stage of development of the tumor:
   a) Slowly growing tumors had no effect on the hemolytic behavior of the CSH erythrocytes.
   b) Tumors with a good rate of growth decreased the hemolysis of the CSH erythrocytes in the early and moderately advanced stages. In the far advanced stages of tumor growth, hemolysis was not decreased.
   c) The red blood cells from C57BL mice with transplanted brain tumors showed increased hemolysis only in about one-third of the experiments. This would indicate that the effect of the tumor on the hemolytic behavior of red blood cells of C57BL mice is not so definite as with CSH mice.
   d) Suspensions of transplanted brain tumors
Fig. 1.—Effect of suspension of mammary tumor of C3H mice on red blood cells from C3H and C57BL mice with and without tumor transplants.
1.—RBC from C3H mouse with tumor C3H(46)5.
2.—RBC from C3H mouse with tumor C3H(48)2.
3.—RBC from C3H mouse with subcutaneously transplanted mammary tumor (control).
4.—RBC from C3H mouse without tumor.
5.—RBC from C57BL mouse with tumor C57(26)55.
6.—RBC from C57BL mouse, where tumor transplant did not take.
7.—RBC from C57BL mouse without tumor.
rarely exerted a hemolytic effect on mouse red blood cells.

6. The importance of the change in hemolysis of erythrocytes during tumor development is discussed.

REFERENCES
2. ———. Differences in the Hemolytic Behavior of Red Blood Cells of a Tumor-susceptible (C3H) and a Tumor-resistant (C57) Mouse Strain. Ibid., pp. 658–62.
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