Mammary Tumor Immune Enhancement in Mice by Local Hyperemia

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ABSTRACT

The mammary carcinoma MC2 causes a strong immune response in syngeneic, female C3H/He mice, and growing s.c. implants will regress spontaneously in about 20% of untreated hosts. Hyperemia is part of the local immune reaction against MC2 implants. An accelerated local hyperemic reaction at MC2 implants could be created in normal mice by the i.p. injection of blood or plasma from MC2 hosts at an early (Day 17) stage of immunization. In these mice given injections, MC2 growth was enhanced, and the incidence of spontaneous regressions was reduced. Local hyperemia caused by heat also promoted the growth of MC2 implants. In contrast, injections of blood or plasma from MC2 hosts at an advanced (Day 35) stage of immunization reduced the growth of MC2 implants and increased the incidence of their spontaneous regression. It is concluded that increased blood supply to a tumor in the absence of adequate systemic immunity favors tumor growth, and that this represents a new, additional mechanism in the immune enhancement phenomenon.

INTRODUCTION

Some important areas of investigation in tumor immunology are relatively underemphasized. As an example, the role of humoral immunity against neoplasms has received relatively little attention vis-à-vis the study of cellular effector mechanisms. One reason for this uneven research emphasis derives from serum enhancement and blocking antibody theories, which generated immunization reduced the growth of MC2 implants and increased the incidence of their spontaneous regression. It is concluded that increased blood supply to a tumor in the absence of adequate systemic immunity favors tumor growth, and that this represents a new, additional mechanism in the immune enhancement phenomenon.

MATERIALS AND METHODS

Mice. All the animals used in these experiments were 8- to 10-wk-old inbred female C3H/He mice, raised and kept in an infection-controlled environment.

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TUMOR ENHANCEMENT AND HYPEREMIA

RESULTS

Immune Enhancement. Table 1 shows the results of an experiment that compared the growth of MC2 implanted into the left flank of mice in seven groups: Group 1, mice at a moderate level of concomitant immunity by already carrying a 17-day-old MC2 implant; Group 2, like Group 1, but given normal blood; Group 3, like Group 2, but given normal plasma; and Group 7, untreated mice.

The results show that the injection of whole blood or plasma from Day 17 MC2 hosts resulted in enhanced tumor growth. The injection of blood from hosts of the immunologically unrelated mammary carcinoma 21-6, or of blood or plasma from normal mice, had no effect on the recipients. In the Day 17 MC2 hosts (Group 1), the growth of second MC2 implants was inhibited, and rejections and regressions were more frequent than in untreated mice.

Figs. 1 and 2 compare the tumor-stroma interface of 6-day MC2 implants from Groups 1 and 2 (Table 1). Fig. 1 shows a tumor from Group 1 which is separated from an area of active hyperemia by a stromal layer containing numerous lymphocytes and macrophages. The MC2 implant has an area of central necrosis and a peripheral layer of live cells that has moderate mitotic activity and shows little tendency to invade the stroma. Fig. 2 presents a very different situation. The tumor implant from Group 2 is infiltrating the host tissue, growing through the cutaneous muscle layer (panniculus carnosus) toward an area of active hyperemia. Mitotic figures are frequent.

Local Hyperemia. To further study the relationship of passive and active immunization to local hyperemia, the histology of the vascular reaction at s.c. implants of MC2 was surveyed in the following groups of mice: Group 1, mice given injections i.p. with 1 ml of heparinized normal mouse blood 18 h before implantation; Groups 2 to 5, mice already carrying 1- to 7-day-old MC2 implants; and Group 6, mice given injections i.p. with 1 ml of heparinized blood from Day 17 MC2 hosts.

The results presented in Table 2 show the following. (a) The development of active hyperemia at the MC2 implant in mice given normal blood was related to the development of systemic immunity. Systemic immunity has been shown to be demonstrable 1 wk after MC2 implantation (9). (b) In tumor hosts, the development of hyperemia at the MC2 test-implant was shortened by a period close to the length of the immunizing MC2 exposure. (c) The injection of whole blood from MC2 hosts caused a rapid development of hyperemia at the MC2 implant.

To determine the effect of physically induced local hyperemia on tumor growth, the growth of MC2 was compared in heat-treated and in normal s.c. sites on the same animal. Table 3 shows the combined results of two identical tests. The results

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatmenta</th>
<th>Mitotic index</th>
<th>Incidence</th>
<th>Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immunized</td>
<td>3.1 ± 0.2ab</td>
<td>15/32 (47)</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>MC2 host blood</td>
<td>6.4 ± 0.5</td>
<td>25/66 (38)</td>
<td>7.8 ± 1.7</td>
</tr>
<tr>
<td>3</td>
<td>MC2 host plasma</td>
<td>5.4 ± 0.6</td>
<td>19/40 (70)</td>
<td>5.0 ± 1.2</td>
</tr>
<tr>
<td>4</td>
<td>21-6 host blood</td>
<td>4.8 ± 0.3</td>
<td>12/12 (50)</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>Normal blood</td>
<td>4.8 ± 0.2</td>
<td>25/66 (62)</td>
<td>6.2 ± 0.7</td>
</tr>
<tr>
<td>6</td>
<td>Normal plasma</td>
<td>4.8 ± 0.3</td>
<td>9/12 (75)</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>7</td>
<td>Untreated</td>
<td>4.8 ± 0.4</td>
<td>34/49 (69)</td>
<td>6.1 ± 0.6</td>
</tr>
</tbody>
</table>

* Numbers are the results of five separate experiments. Group 3 was included in four of the experiments, and Groups 4 and 6 were included in two of the experiments. Each mouse given an injection received 1 ml of heparinized blood or plasma i.p. 18 h before receiving 2 s.c. MC2 test implants. One implant was removed after 6 days to determine the mitotic index. The second implant remained to determine incidence and mean size.

a Mean ± SE. The two groups are significantly different (P < 0.05).

c Numbers in parentheses, percentage.

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<table>
<thead>
<tr>
<th>Day test implant removed</th>
<th>Normal host blood</th>
<th>Immunizing MC2 implanted host blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>4</td>
<td>±</td>
<td>+1</td>
</tr>
<tr>
<td>5</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>6</td>
<td>+2</td>
<td>+2</td>
</tr>
<tr>
<td>7</td>
<td>+2</td>
<td>+2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MC2 host blood treatments</th>
<th>Incidence</th>
<th>Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heated</td>
<td>17/18 (94)</td>
<td>10.1 ± 1.0</td>
</tr>
<tr>
<td>Normal</td>
<td>13/18 (72)</td>
<td>6.4 ± 0.8</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

a Mean ± SE. The two groups are significantly different (P < 0.05).
show that the repeated application of heat sufficient to cause a first degree burn also prepared the conditions for improved tumor growth.

Passive Immunity. In other studies of the local immune reaction against MC2 implants (11), in vitro lytic activity against MC2 cells developed in the serum of MC2 hosts only after at least 1 mo of immunization, and most predictably in mice with regressing or dormant tumors. In this experiment, blood and plasma from mice that had been cured of 35-day s.c. implants were tested for their ability to transfer passive immunity to MC2 implant recipients.

The results presented in Table 4 show that mice cured of 35-day MC2 implants had become fully resistant to a second MC2 implant. Whole blood, and to a slightly lesser degree, plasma, from cured MC2 hosts transferred a significant degree of resistance. The transfer of blood from mice cured of 21-day implants and the transfer of normal blood and plasma were without effect on the growth of MC2 implants.

DISCUSSION

The phenomenon of immune enhancement of tumor growth has, since it was first described by Casey in 1932 (12) and investigated by Snell in 1954 (13), remained incompletely understood (6) (review by Winn), despite intensive study by a number of investigators. Central inhibition by specific antibody (6), immune suppression by regulatory antibodies (14), and immune suppression by regulatory T-suppressor cells (15–17) are some of the mechanisms that have been proposed.

This paper presents novel findings which suggest that factors in the plasma (and whole blood) of tumor hosts with incompletely developed concomitant immunity can cause active local hypereemia and the enhanced growth of tumors implanted into normal plasma recipients. This condition was only part of the early phase of a normal immune response, and factors in the blood of mice with fully developed concomitant immunity provided enhanced protection to normal recipients. Increased blood supply to a tumor implanted into a nonimmune host would favor tumor growth and is an additional mechanism not previously considered in explanations of the immune enhancement phenomenon. But in an adequately immunized host, on the other hand, hypereemia is an important factor in the defensive reaction. The conditions for demonstrating immune enhancement can therefore only exist in animals without adequate systemic immunity, and enhancement has, in fact, never been observed in preimmunized recipients (6).

The demonstration (Table 3) that tumor growth was enhanced where hypereemia had been induced by heat only shows that this condition in a simple form will also promote tumor growth. The demonstration supports the speculation that hypereemia may be a factor in immune tumor enhancement, but without suggesting any mechanistic relationship between two different methods of demonstration.

Accordingly, it may be suggested that the enhanced tumor growth in Group 2 (Table 1) was related to the active hypereemia shown in Fig. 2. The restrained tumor growth in Group 1 (Table 1) was related to the strong cellular infiltration in Fig. 1, which may have been facilitated by the local hypereemia around the implant.

REFERENCES


Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment*</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immunized</td>
<td>0/27</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>MC2 immune blood</td>
<td>15/26 (58)</td>
<td>6.1 ± 0.6*</td>
</tr>
<tr>
<td>3</td>
<td>MC2 immune plasma</td>
<td>40/65 (62)</td>
<td>6.3 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>21-6 immune blood</td>
<td>20/26 (77)</td>
<td>10.2 ± 0.6</td>
</tr>
<tr>
<td>5</td>
<td>Normal blood</td>
<td>22/26 (85)</td>
<td>11.0 ± 0.7</td>
</tr>
<tr>
<td>6</td>
<td>Normal plasma</td>
<td>62/75 (83)</td>
<td>8.0 ± 0.25</td>
</tr>
<tr>
<td>7</td>
<td>Untreated</td>
<td>56/73 (79)</td>
<td>7.6 ± 0.25</td>
</tr>
</tbody>
</table>

* The numbers are the results of six separate experiments. Groups 1, 2, 4, and 5 were included in three of the experiments. Each mouse given an injection received 1 ml of heparinized blood or plasma i.p. 18 h before receiving the s.c. MC2 test implant.
* Numbers in parentheses, percentage.
* Mean ± SE.

CANCER RESEARCH VOL. 46 JANUARY 1986

388
Fig. 1. Restrained growth of MC2, 6 days after implantation into a mouse carrying a 17-day-old implant. A narrow band of live tumor cells separates the necrotic center (right) and the stroma infiltrated by lymphocytes and macrophages. Bar, 100 μm. H & E, × 100.

Fig. 2. Rapidly growing (from right to left) MC2, 6 days after implantation into mice given injections of whole blood from MC2 hosts. Bar, 100 μm. H & E, × 100.
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