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 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.

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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evaluations of hyperemia around tumors were being determined by the area of blood-filled vessels in 10-μm grid fields of 0.25 mm² (at × 100) each. A 5- to 10-fold increase above normal vascularity (0) was given a value of +1. More than a 10-fold increase was given a value of +2.

**Heat-Induced Hyperemia.** A 30-g piece of copper with a Teflon handle was heated to 62°C in a water bath. The 10-mm-diameter tip of the copper was held for 3 s against the shaved skin of the left flank of the mice. The procedure was repeated 3 times at weekly intervals up to the day of the s.c. implantation of 10⁶ suspended MC2 cells into the treated and in a similar, untreated location on the right side.

**Blood and Plasma.** About 1 ml of blood was collected per donor mouse. The blood was drawn from the right ventricle into a 2.5-ml syringe containing 2 units of heparin. Blood was pooled and injected i.p. into recipient mice without delay. Plasma was separated by centrifugation (70 × g) for 10 min. Pooled plasma was kept in the refrigerator on crushed ice and was used within 24 h. The plasma was brought to 37°C before it was injected i.p.

**Statistical Analysis.** Differences in accumulated tumor incidence between groups of mice were evaluated with the χ² test. Differences in mean tumor size were evaluated with Student’s t test. Differences between groups were considered significant when the P value of comparison was 0.05 or less.

**RESULTS**

**Immune Enhancement.** Table 1 shows the results of an experiment that compared the growth of MC2 implants 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 in the right flank; Group 2, mice given injections i.p. with 1 ml of heparinized blood from Day 17 MC2 hosts 18 h before receiving the left flank implant; Group 3, like Group 2, but given MC2 host plasma; Group 4, like Group 2, but given tumor host blood; Group 5, like Group 2, but given normal blood; Group 6, 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.

**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 area 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

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**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mitotic index</th>
<th>Incidence</th>
<th>Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immune</td>
<td>3.1 ± 0.2b</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>25/35 (71)</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td>3</td>
<td>MC2 host plasma</td>
<td>5.4 ± 0.6</td>
<td>18/20 (90)</td>
<td>7.4 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>21-6 host blood</td>
<td>4.8 ± 0.3</td>
<td>10/12 (83)</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>Normal blood</td>
<td>4.8 ± 0.2</td>
<td>20/25 (80)</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>39/40 (98)</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 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.

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**Table 2**

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

**Table 3**

<table>
<thead>
<tr>
<th>MC2 growth in heat-treated skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Heated</td>
</tr>
<tr>
<td>Normal</td>
</tr>
</tbody>
</table>

*Numbers in parentheses, percentage.*

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*c 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 35-day implants of tumor 21-6 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 hyperemia 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, hyperemia 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 hyperemia 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 hyperemia 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 hyperemia 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 hyperemia around the implant.

REFERENCES


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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