The Influence of the Age of the Host on the Incidence of Blood-borne Metastases

RICHARD ESTENSEN† AND RENATO BASERGA‡

(Department of Pathology, Northwestern University Medical School, Chicago, Illinois)

SUMMARY

The influence of the age of the host on the incidence of metastasis has been studied in mice given injections intravenously of Ehrlich ascites tumor cells. The results indicate that (a) the resistance of mice to the establishment of metastases decreases with age and (b) the influence of age is apparent only at relatively low doses of tumor cells.

Previous studies in mice have shown that the incidence of metastases from intravenously injected tumor cells is influenced by various factors, such as the number of tumor cells injected and the sex of the host (8). Reports in the literature (6, 9, 18) indicate that the age of the host might also be a factor in the "take" of circulating tumor cell emboli.

To determine the influence of age on the incidence of blood-borne metastases, mice of both sexes and of different age groups were given a single intravenous injection of Ehrlich ascites tumor cells. At the termination of the experiment the animals were autopsied, and the incidence and number of metastases per mouse in each group were determined.

MATERIALS AND METHODS

C X A F1 mice of both sexes and different ages were randomized into two groups (Table 1); those in the first group were given injections in the tail vein of $1.5 \times 10^6$ Ehrlich ascites tumor cells suspended in sterile isotonic saline; those in the second group were given a similar injection of $4.4 \times 10^6$ tumor cells. Details of the methods used have been given in a previous paper (3).

It was originally intended to sacrifice all animals on the 30th day after the intravenous injection of tumor cells. However, the oldest females in the second group began to die on the 14th day of the experiment because of extensive replacement of the lungs by tumor masses. Since the purpose of this experiment was a comparison of age groups rather than sexes, all the females were sacrificed on the 17th day, and the males were allowed to live until the 35th day after the injection of tumor cells. Except for the few animals that died, all the others were sacrificed by cervical dislocation. About one-third of the animals given injections of tumor cells had to be discarded because of local tumor growth at the site of injection in the tail or at the root of the tail, and these are not included in the figures given in Table 1. The others were autopsied, and the number of grossly visible metastases was determined by two different observers. Most of the metastases were located in the lungs, but about 1 per cent of the secondary tumor growths were found in the heart, liver, and various locations. Regardless of their location, all metastases were included in the computations.

RESULTS

The influence of the age of the host on the incidence of metastases.—Table 1 shows the incidence of metastases in male and female mice given injections intravenously of Ehrlich ascites tumor cells. It will be noticed that, in the different age groups given injections of $1.5 \times 10^6$ tumor cells, the incidence of metastases and the mean number of metastases per mouse increased linearly, in both sexes, with increasing age. The equations of the least square lines are, for the females:

$$Y = -0.592 + 0.015X$$

and for the males:

$$Y = -0.201 + 0.003X$$
where $Y$ is the mean number of metastases per mouse and $X$ is the age in days. The slopes of the curves indicate that the mean number of metastases in the females will be, on the average, 5 times the number in males (3, 8).

Table 1 also shows that the differences observed in mice given injections of $1.5 \times 10^6$ tumor cells had almost completely disappeared in those mice given injections of $4.4 \times 10^6$ Ehrlich ascites tumor cells. In fact, with the exception of the youngest females, the incidence of metastases and the number of metastases per mouse were nearly similar in both sexes and in all groups given injections of $4.4 \times 10^6$ tumor cells.

The influence of the age of the host on the resistance to the establishment of metastases.—Shock and his co-workers (5, 11, 12) have shown that several physiologic functions in man decrease at a constant rate with increasing age. If the resistance of the animal to the establishment of metastases were dependent upon the physiologic status of the host, a decrease in resistance should follow a pattern similar to that described by Shock for other physiologic functions. For this purpose, the mean number of metastases in mice 740 days of age, which is the average life span in our colony of $C \times A$ $F_1$ mice, was calculated through equations (1) and (2) and designated as the maximum number of metastases per mouse, $M_{\text{max}}$. The $M_{\text{max}}$ for both sexes was taken as 0 resistance, or the lowest point of physiologic function opposing the establishment of metastases. From the data in Table 1, a regression line was calculated indicating the rate of decrease of resistance to the establishment of metastases in mice as a function of age. This regression line is shown in Chart 1. Each experimental point represents the resistance to the establishment of metastasis, at any given age, calculated from the equation:

$$R = \frac{M_{\text{max}} - M_t}{M_{\text{max}}} \times 100 \quad (3)$$

where $R$ is the percentage resistance to the establishment of metastases, $M_{\text{max}}$ is the number of metastases at age 740 days, being 5 times higher in females than in males, and $M_t$ is the mean number of metastases at a given age $t$. It can be seen from Chart 1 that the resistance to metastases

<table>
<thead>
<tr>
<th>No. tumor cells injected</th>
<th>Mean age (days)</th>
<th>Sex</th>
<th>No. animals</th>
<th>Animals with metastases (per cent)</th>
<th>No. metastases per mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.5 \times 10^6$</td>
<td>76</td>
<td>F</td>
<td>27</td>
<td>33.3</td>
<td>1.0</td>
</tr>
<tr>
<td>$1.5 \times 10^6$</td>
<td>168</td>
<td>F</td>
<td>33</td>
<td>39.3</td>
<td>1.0</td>
</tr>
<tr>
<td>$1.5 \times 10^6$</td>
<td>269</td>
<td>F</td>
<td>38</td>
<td>84.2</td>
<td>4.0</td>
</tr>
<tr>
<td>$1.5 \times 10^6$</td>
<td>354</td>
<td>F</td>
<td>23</td>
<td>95.6</td>
<td>5.0</td>
</tr>
<tr>
<td>$1.5 \times 10^6$</td>
<td>78</td>
<td>M</td>
<td>21</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$1.5 \times 10^6$</td>
<td>197</td>
<td>M</td>
<td>21</td>
<td>14.2</td>
<td>0.4</td>
</tr>
<tr>
<td>$1.5 \times 10^6$</td>
<td>279</td>
<td>M</td>
<td>23</td>
<td>60.8</td>
<td>0.7</td>
</tr>
<tr>
<td>$4.4 \times 10^6$</td>
<td>46</td>
<td>F</td>
<td>34</td>
<td>52.9</td>
<td>2.4</td>
</tr>
<tr>
<td>$4.4 \times 10^6$</td>
<td>199</td>
<td>F</td>
<td>25</td>
<td>84.0</td>
<td>5.2</td>
</tr>
<tr>
<td>$4.4 \times 10^6$</td>
<td>257</td>
<td>F</td>
<td>34</td>
<td>88.2</td>
<td>7.9</td>
</tr>
<tr>
<td>$4.4 \times 10^6$</td>
<td>624</td>
<td>F</td>
<td>28</td>
<td>75.0</td>
<td>6.7</td>
</tr>
<tr>
<td>$4.4 \times 10^6$</td>
<td>47</td>
<td>M</td>
<td>26</td>
<td>100.0</td>
<td>6.5</td>
</tr>
<tr>
<td>$4.4 \times 10^6$</td>
<td>210</td>
<td>M</td>
<td>22</td>
<td>95.4</td>
<td>8.8</td>
</tr>
<tr>
<td>$4.4 \times 10^6$</td>
<td>270</td>
<td>M</td>
<td>20</td>
<td>100.0</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* The females were sacrificed 17 days and the males 35 days after injection of tumor cells.
Estensen and Baserga—Age of Host and Blood-borne Metastases

...age decreases at a constant rate with increasing age, the equation of the least square line being:

\[ Y = 107.22 - 0.144X \] (4)

where \( Y \) is the percentage resistance to metastases and \( X \) is the age in days.

For a proper comparison with Shock's data, we must remember that the average life span of these mice is \( \frac{3}{8} \) the average life span of man. When this correction is allowed, it can be seen that the resistance of mice to the establishment of metastases decreased at a rate of 1.5 per cent per \( \frac{3}{8} \) of life span, which closely approximates words, an increase in the number of tumor cells injected was more effective in increasing the number of metastases in younger than in older male mice. This effect, although less pronounced, was also clearly visible in the females. It may explain why the difference in the number of metastases per mouse due to age, quite evident in mice given injections of \( 1.5 \times 10^6 \) tumor cells, disappeared at the higher dose level. This is shown in Chart 2, where the average number of metastases per mouse, in mice given injections of \( 1.5 \times 10^6 \) tumor cells, is plotted against age in days (data from Table 1), and the same average, in mice 4–6 months old, is plotted against the number of tumor cells injected (data from Ref. 3). Although the mean number of metastases increased linearly with increasing age, the response to increasing dose of tumor cells increased even faster than an exponential function. At higher dose levels, then, the effect due to the age of the host will be masked by the more rapidly increasing effect due to the number of tumor cells injected, at least in sample sizes of the magnitude used in the present experiment.

**DISCUSSION**

It is customary to divide the mechanism of formation of blood-borne metastases into three phases (1, 14): the first phase is that during which the tumor cells of the primary growth penetrate...
the vascular endothelium from the outside, the second phase concerns the transportation of the tumor cell emboli by the blood stream, and the third phase is that in which the tumor cells establish organic union with the vessel wall after their arrest, penetrating the endothelium from the inside. It is clear that the method of injecting tumor cells into the tail vein of laboratory animals can mainly be used to study the third phase, the lodging and growth of tumor emboli at the site of arrest. The limitations and advantages of this technic have been pointed out in a previous paper (1), and with these qualifications the following considerations can be made:

A comparison with previous studies.—Loefer and Gilles (9) studied the influence of the age of the host on the incidence of subcutaneous "takes" from a transplantable sarcoma of rats. They found that the incidence of "takes" was highest in rats 1–13 days old and lowest in rats aged from 27 to 39 days, whereas it rose again in older rats. The oldest animals tested were 120 days of age. In our studies, we have omitted newborn mice, because of the special position that newborn animals occupy in the scale of immunological tolerance; otherwise our results are essentially in agreement with those of Loefer and Gilles, despite the different route of administration of the tumor cell suspension. Flaks (6), however, found that lymph node metastases from a transplantable sarcoma of rats were more frequent in younger than in older animals. Similarly, in man, Walther (15) found that the incidence of blood-borne metastases from carcinoma of the lung and lymph node metastases from carcinoma of the stomach decreased in frequency with age. It is conceivable that the discrepancy between the findings of Flaks and of Walther and the present results may be due to an opposing influence of age on the first phase of metastases—that is, the entrance of tumor cells into the blood or lymph stream. In other words, increasing age may increase the "take" of tumor cell emboli lodging in the capillaries and at the same time decrease the entrance of tumor cells into the circulation. As shown in Chart 2, a moderate increase in the number of circulating tumor cells would cancel out and even reverse the effect of the age of the host on the "take" of tumor cell emboli. If this explanation is correct, technics presently in use (10) should be able to demonstrate a larger number of circulating cancer cells in younger than in older tumor-bearing patients.

Our results also suggest that the "take" of intravenously injected tumor cells may serve as a measure of the "aging" induced by a variety of experimental procedures. In this respect it may be interesting to notice that whole-body radiation, a procedure that has often been considered to induce aging, is known to increase the incidence of metastases from both induced and transplantable tumors (4, 7).

Which function is responsible for the resistance of animals to metastases can be, at the present time, only a matter of speculation. The authors are inclined to relate such resistance to the function of the reticuloendothelial system, which has been shown to be actively stimulated by the presence of tumor cells in the body (2, 3), but this can be considered only an educated guess.

ACKNOWLEDGMENTS

The authors wish to acknowledge the technical assistance of Mr. Kenneth Nemeroff.

REFERENCES

The Influence of the Age of the Host on the Incidence of Blood-borne Metastases

Richard Estensen and Renato Baserga