Echinococcus multilocularis in the Cotton Rat: Growth of Intrathoracic Metastases Following Surgical Removal of Subcutaneous Cysts

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Abstract—Rau M. E. and Tanner C. E. 1976. Echinococcus multilocularis in the cotton rat: growth of intrathoracic metastases following surgical removal of subcutaneous cysts. International Journal for Parasitology 6: 151-153. The radical resection of large, established subcutaneous cysts of Echinococcus multilocularis results in a 20-fold increase in the weight of its intrathoracic metastases. While all subcutaneously-infected animals also developed intrathoracic foci, these remained small in the presence of the massive subcutaneous foci. Animals whose subcutaneous cyst masses had been resected were, however, still fully resistant to a subsequent intraperitoneal challenging inoculation with protoscolices.

INDEX KEY WORDS: Metastasis; post-operative; hydatid; Echinococcus multilocularis; cotton rat.

INTRODUCTION

The vegetatively proliferating larval stage of Echinococcus multilocularis bears an intriguing resemblance to a malignant tumor. Indeed, until 1856, when Virchow demonstrated the parasitic nature of these cysts in man, such lesions were commonly diagnosed as carcinomas of the liver. In the cotton rat, Sigmodon hispidus, subcutaneous cysts of E. multilocularis exhibit a surprisingly high incidence of metastasis. While most of this growth and metastasis is confined to the immediate vicinity of the original site of inoculation, distinct metastatic foci are, however, frequently found in the thoracic cavity and lungs of infected animals. Since these metastases remain small, their growth may be suppressed by the concomitant subcutaneous infection especially since we (Rau & Tanner, 1973) have demonstrated that subcutaneous cysts can inhibit the establishment, growth and metastasis of an intraperitoneal inoculum of the same parasite. In vitro studies suggest that this latter inhibition is primarily cell mediated, and that specific antibodies may be involved by furnishing recognition sites for cell action on target protoscolices (Rau & Tanner, in press). The present study was undertaken to determine whether the surgical removal of massive subcutaneous E. multilocularis cysts favours the growth of these distant, intrathoracic metastases.

MATERIALS AND METHODS

Twelve 6-week-old, male cotton rats were each inoculated subcutaneously in the right inguinal region with 30 (2 mm dia) clear acephalic vesicles of E. multilocularis in 1.0 ml of sterile TC Medium 199 (Difco). A group of 6 controls of the same age and sex received Medium 199 only. Ninety days later, the resultant subcutaneous cyst masses were removed surgically from six randomly-picked animals of the infected group; the excised cysts were weighed.

To remove the subcutaneous cyst, a 2 cm incision was made in the skin in the base of the parasite mass. Large blood vessels entering the surgical arena were ligated before the cyst mass was carefully detached from adhering connective tissue by blunt dissection. Care was taken not to rupture any thin-walled vesicles nor to leave any macroscopic foci within that area. The skin incision was subsequently closed with catgut sutures.

The other 6 infected animals, as well as the un inoculated controls were sham-operated. This operation consisted of a 2 cm skin incision in the left inguinal region. A comparable area of subcutaneous connective tissue was separated from the skin by blunt dissection before closing the wound.

Three of the cotton rats whose subcutaneous cysts had been removed, three rats still bearing massive intact cysts, and three uninfected controls were each inoculated intraperitoneally with 50 living protoscolices in 1.0 ml of Medium 199, 14 days after surgery; the remaining nine animals, three within each group, were not challenged but received an intraperitoneal injection of 1 ml of Medium 199. All 18 cotton rats were killed and autopsied 30 days after the challenging inoculation. The cysts found in all infected animals were classified as either intrathoracic, intraperitoneal, or subcutaneous, and then weighed; these data are presented in Table 1.

RESULTS

Autopsy 44 days after the removal of the subcutaneous hydatid cysts revealed that the surgery had been successful: no residual subcutaneous foci were found. All animals that had been inoculated subcutaneously with small vesicles of E. multilocularis 135 days previously (rats 1-12; Table 1)
TABLE I—GROWTH OF INTRATHORACIC E. multilocularis METASTASES AND CHALLENGING PROTOCOLEX INOCULA IN THE PRESENCE OF MASSIVE SUBCUTANEOUS CYSTS AND FOLLOWING THEIR SURGICAL EXCISION

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cyst weight (g) and location—intrathoracic (i. th.)</th>
<th>Subcutaneous cysts intact at autopsy</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subcutaneous cysts removed 45 days prior to autopsy*</td>
<td>Subcutaneous cysts intact at autopsy</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Rat No.  i. th.</td>
<td>i. p.</td>
<td>s. c.</td>
</tr>
<tr>
<td>Challenged intra-peritoneally with 50 protoscolexes</td>
<td>1. 4:5 0 0</td>
<td>7. &lt;0:1 0 49:0</td>
<td>13. 0 1:3 0</td>
</tr>
<tr>
<td></td>
<td>2. 4:0 0:6 0</td>
<td>8. &lt;0:1 0:2 26:0</td>
<td>14. 0 5:1 0</td>
</tr>
<tr>
<td></td>
<td>3. 7:0 0 0</td>
<td>9. 0:6 0 38:0</td>
<td>15. 0 3:6 0</td>
</tr>
<tr>
<td>Not challenged</td>
<td>4. 6:0 0 0</td>
<td>10. &lt;0:1 0 18:0</td>
<td>16. 0 0 0</td>
</tr>
<tr>
<td></td>
<td>5. 5:2 0 0</td>
<td>11. &lt;0:1 0 19:0</td>
<td>17. 0 0 0</td>
</tr>
<tr>
<td></td>
<td>6. 8:0 0 0</td>
<td>12. 0:6 0 20:0</td>
<td>18. 0 0 0</td>
</tr>
</tbody>
</table>

*Weight of the excised subcutaneous cyst mass in rats 1-6: 24, 31, 13, 50, 6, and 19 g respectively.

bore distinct intrathoracic metastases. The extent of this intrathoracic invasion, however, varied greatly. Thus, all cotton rats whose subcutaneous cysts had been removed (rats 1-6) bore massive, intrathoracic cyst masses ranging in weight from 4.0 to 8.0 g (mean = 5.8 g) whereas cotton rats whose subcutaneous cysts had remained intact (rats 7-12) bore very much smaller intrathoracic foci (0.1-0.6 g; mean = 0.3 g). In the most severe cases (rats 3, 4, 6), much of the lung tissue had been replaced by the cyst mass; these animals had shown respiratory distress for two days prior to their death. Half of the control group (rats 13-18), which bore intraperitoneal cysts as a result of the challenge inoculation, exhibited no intrathoracic or subcutaneous infiltration.

The challenge re-inoculation into animals already bearing massive subcutaneous primary or intrathoracic metastatic cysts gave rise to small intraperitoneal infections in only 2 of 6 rats (2 and 8) whereas all previously uninfected controls developed significantly larger intraperitoneal cysts within 30 days of the challenge inoculation. The cotton rats, which had not been given the intraperitoneal challenge did not develop intraperitoneal infections (rats 4-6 and 16-18), nor did intraperitoneally inoculated animals develop intrathoracic or subcutaneous cysts (rats 13-15).

DISCUSSION

The post-operative growth of pulmonary metastases of E. multilocularis finds a close parallel in the experimental tumour systems described by Schatten (1958) and by Gershon, Carter & Kondo (1968). Schatten's study demonstrates that a primary tumour inhibits the growth of its distant metastases after the primary tumour reaches a certain size. Gershon et al. (1968) described concomitant immunity, the phenomenon whereby animals already bearing one tumour are sometimes resistant to a subsequent homologous challenging inoculation. It has been shown by these latter authors that concomitant immunity has important functional implications in the suppression of metastatic growth, since, although viable tumour cells are demonstrable in the blood and regional lymph nodes of infected animals, intense reactive changes in the lymphatic tissues inhibit the establishment of metastatic foci. A weakening of this concomitant immunity by the excision of the primary tumour or by immunosuppression rapidly leads to the appearance of distant foci. Thymectomy and antithymocyte serum treatment also enhance the growth and metastasis of E. multilocularis in mice (Baron & Tanner, 1976).

Alternately, the dramatic increase in the size of the intrathoracic cyst masses following the excision of the subcutaneous focus may only be in part attributable to the growth of the parasite. It is well-known that cyst masses of E. multilocularis are composed of parasite vesicles held tightly in a matrix of host connective tissue and leukocytes; as the parasite grows, host tissues continue to infiltrate between the forming vesicles (Webster & Cameron, 1961). Indeed, leukocytes from infected cotton rats tightly adhere to the metacestode in vitro (Rau & Tanner, in press). It is conceivable that once the main subcutaneous parasite target for these cells was removed surgically, the remaining small metastatic intrathoracic lesions may form the nuclei for a rapid deposition of host tissues and, thus, lead to the enlargement of the existing foci. Thus, Asherson & Allwood (1972) have shown that systemic reaction to antigens generates lymphokines that "activate" phagocytic and/or inflammatory cells; in specifically sensitized animals these cells are attracted to and aggregate about any inflammatory nidus (Boros & Warren, 1973). The unavoidable release of some parasite antigen during surgery in spite of all reasonable precautions may have initiated a somewhat similar process in our study. It seems unlikely that the increase in the size of the pulmonary metastases was due to the transfer of microvesicles
from the subcutaneous to the intrathoracic site during surgery. It is difficult to visualize the transfer of 4–8 g of cyst material.

There is circumstantial evidence in the results of this study for the persistence of protection for at least 2 weeks after the removal of the subcutaneous cyst mass. Thus, while the removal of the subcutaneous focus was accompanied by a “flare-up” of the intrathoracic lesions, the establishment of intraperitoneal inocula of protoscolices was still inhibited: both the “intact” and “resected” group of cotton rats in this study bore intraperitoneal infections that were significantly lighter than those of the corresponding controls. Alternately, it can be argued that the “enhanced” intrathoracic metastases grew rapidly enough to suppress the growth of the intraperitoneal inoculum.

Whatever the aetiology of the massive, intrathoracic lesions following the removal of subcutaneous foci, their effect on the host is unquestionably serious and should be considered in the surgical management of hydatid disease. The relevance of the phenomenon revealed by this study to the control of secondary echinococcosis is currently under active investigation in our laboratory.

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REFERENCES


