Enhancement of Metastases by Antilymphocyte Serum in Allogeneic Murine Tumor System

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SUMMARY

When C57 mice, with foot implants of allogeneic tumor Sarcoma 180, were treated with rabbit antimouse lymphocyte serum, the tumor metastasized extensively to the regional popliteal nodes (98%), inguinal nodes (50%), aortic nodes (25%), and lungs (30%). In untreated control animals and those treated with normal rabbit serum, the incidence of metastases in these sites was less than 3%. The present study illustrates, in a very dramatic fashion, through immunosuppression by antilymphocyte serum, the importance of the host immune response in preventing metastases of such a tumor.

INTRODUCTION

In Swiss mice previously immunized with an implant of the allogeneic tumor Sarcoma 180 (S180), a second implant of the same tumor fails to establish itself. We (3) previously reported that the treatment of such mice with rabbit anti-Swiss mouse lymphocyte serum (ALS) during the period of immunization suppressed the development of immunity and allowed the growth of the second tumor. Recently, we have observed that ALS also exerts a similar effect on the growth of S180 in C57 mice. During the course of these recent studies, we noted a striking increase in the incidence of metastases from the tumor on the foot to the regional popliteal node and other lymph nodes and to lungs in the ALS-treated mice as compared with that in untreated animals or those treated with normal rabbit serum (NRS).

It is well known that the tendency of a specific tumor to metastasize is influenced by many factors, among which the host's immune response is undoubtedly an important one. The present study dramatically illustrates the effect of this factor on metastases through immunosuppression by ALS.

MATERIALS AND METHODS

Mice. Four- to five-week-old C57 male mice were obtained from the Jackson Laboratories, Bar Harbor, Maine.

Rabbits. Albino female rabbits whose weights ranged between 2 and 3 kg, were used for producing anti-mouse ALS.

Sarcoma 180. This tumor was initially obtained from the Jackson Laboratories, Bar Harbor, Maine, and it has been kept growing through serial transfers in mice.

Production of ALS. The rabbits were immunized with C57 mouse thymus cells in the following manner. Thymus tissue was minced in phosphate-buffered saline (pH 7.2) and put through a cytosieve to obtain a cell suspension. Two milliliters of this suspension containing between 150 million and 200 million cells was mixed with 1 ml of complete Freund's adjuvant; the mixture was injected at multiple sites in the appropriate rabbits. In the third, fifth, ninth, and eleventh weeks, the cell injections were repeated. The animals were bled by heart puncture every alternate week between the sixth and the fifteenth week. The sera were pooled, heated at 56°C for 30 minutes, and stored at —20°C.

Treatment with ALS, Implantation of S180, and Study of Metastases. The mice were divided into three groups: ALS treated, NRS treated, and untreated. In the first two groups, ALS or NRS was injected subcutaneously, 0.1 ml daily, starting at four days before the day of implantation of the tumor and continuing throughout the entire experimental period. The tumor was implanted both on the right and left hind feet of the mouse by injecting 0.01 ml of a tumor mince diluted 1:3 in Hanks' solution. At various intervals, certain numbers of mice from each group were killed, and the right and left popliteal nodes, inguinal nodes, aortic nodes, spleen, and lungs were examined grossly and microscopically for the presence of metastatic tumor. In some mice the presence of metastatic tumor at these sites was studied also by injecting portions of these tissues intraperitoneally into normal mice. Both tumor-bearing feet were amputated from each mouse, and the approximate weights of the tumors were determined by subtracting the weight of a normal foot of a control mouse from the weight of the tumor-bearing foot. For microscopic study, the appropriate tissue was treated with Zenker's fixative and the sections stained with hematoxylin and eosin. For pyronin staining, the tissue was fixed in absolute alcohol.

RESULTS

Initially, the presence of metastases was studied both by direct histologic examination and by transplanting the suspected tissue intraperitoneally into a normal mouse. However, since the results of the direct histologic examination were so conclusive, this was the only procedure used in subsequent studies. The results of these experiments are summarized in Table 1. Until Day 5 after the implantation of the tumor, no metastases were seen in any of the experimental groups. Between Day 7 and Day 17 there was a progressive increase in
the incidence of metastases from the tumors in the ALS-treated mice, and there was also a high mortality in this group, most likely because of the presence of metastatic tumor. ALS treatment alone, in another set of experiments, showed no evidence of toxicity or increased mortality. Between Days 14 and 17 almost all mice in the ALS-treated group showed metastases to one or both of the regional popliteal nodes, and a significant number showed metastases to the inguinal and aortic nodes and the lungs. The incidence of metastases to the inguinal and aortic nodes and the lungs in the ALS-treated groups (after Day 14) was 50%, 25%, and 30% respectively, while the combined incidence for the two control groups at the same period was 0%, 0%, and 3% respectively. No metastases were identified in the spleens of any of the three groups. In the untreated control groups and those treated with normal rabbit serum, the incidence of metastases remained small even up to Day 21, when the tumors in this group had reached sizes considerably larger (average weight, 350 mg) than those of the tumors in the ALS-treated group on Days 10 to 14 (average weight, 180 mg).

In the ALS-treated group, the earliest presence of metastatic tumor was noted in the subcapsular sinuses of the popliteal nodes, and between Day 7 and Day 10 this consisted of small colonies of tumor cells. After Day 10 there was progressive replacement of normal lymphoid tissue, and on Day 17 many of the popliteal and inguinal nodes had been completely replaced by tumor. Fig. 1 illustrates the presence of metastatic tumor in the popliteal nodes.

The morphologic changes in the regional popliteal lymph nodes in the ALS-treated, the NRS-treated, and the untreated mice were identical when examined at various times after the implantation of the tumor. There was no evidence of follicular necrosis or cellular depletion in the ALS-treated animals. There was progressive enlargement of the popliteal nodes in all groups, and the sizes and weights of the nodes were comparable in all of the three groups, except after Day 10, when the popliteal nodes from the ALS-treated animals were the largest because of the presence of metastatic tumor. In all the popliteal nodes, microscopic examination on Day 1 showed significant enlargement of the germinal centers, which continued through Day 3, and at Day 5 almost the entire cortex had been replaced by these large immature cells or immunoblasts. On Day 7, cells of the mature and immature plasma cell family began to appear, first in the medullary portion of the node and later, after Day 10, also in the cortical regions. Many of these cells were found to be pyroninophilic. After Day 14, the major cell component in the lymph node was the plasma cell or the plasmacytoid cell. A number of binuclear and other atypical plasma cell forms were also observed at this time. These morphologic cellular changes are illustrated in Fig. 2 A–D. The popliteal nodes from the three groups of mice were indistinguishable morphologically except for the presence of the metastatic tumors in the ALS-treated animals.

### DISCUSSION

The factors that influence metastases of a specific tumor have been investigated in various experimental models. These models fall into two general categories. One model comprised injection of tumor cells directly in the vascular system of animals under various conditions and observation of the effects of these conditions upon the growth of the tumors in the various locations. Another model involved the study of metastases of tumors implanted into animals under various conditions. These extensive studies (1) demonstrated that a number of factors enhanced metastases; these include: (a) size, rate, and duration of tumor growth; (b) genetic relationship of the tumor to the host; (c) various hormonal factors such as adrenocorticotropic hormone, adrenal steroids, growth hormone; (d) trauma and tissue injury; (e) temperature; and (f) treatment with radiation and chemotherapeutic agents. More recent studies of the S180 system by one of us (2) have confirmed the importance of factors such as size and duration of the tumor in determining the incidence of metastases. Enhanced metastases were observed also after removal of the regional popliteal node between Day 7 and Day 10 after implanting the tumor, after ligation of the lymphatics of the leg above the tumor-bearing foot, and after radiation of the regional popliteal node. On the other hand, other procedures, such as squeezing the tumor-bearing foot, partially destroying the tumor by electrocoagulation, applying a tourniquet to the leg immediately before or after implantation of the tumor on the foot, treating with corticosteroids, and introducing inflammatory agents into the tumor, did not increase the incidence of metastases.

In general, the results of most of the previous studies are consistent with the view that the immunologic response of the host to the tumor is an important factor in determining the incidence of metastases. In none of the previous studies, however, was the role of the immune mechanisms or any other factor demonstrated in such a dramatic fashion as that observed in the study reported here. In the ALS-treated animals, 14 days after implantation of the tumor, virtually 100% of the mice showed metastases in the regional popliteal nodes and a significant number showed metastases in the inguinal and aortic lymph nodes and lungs. The control animals, treated with normal rabbit serum and untreated, by contrast, showed metastases in only 2 to 3% of the mice even as late as Day 21 after implantation of the tumor. The size of the tumor was clearly not the determining factor in these results, since in the ALS-treated animals the tumors were noted in the popliteal nodes between Day 7 and Day 10, when the sizes of the tu-

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<th>Time after tumor implant, days</th>
<th>Proportion of popliteal nodes with metastases</th>
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<td>NRS-treated control</td>
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<td>21</td>
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<td>Overall incidence of metastases after Day 14</td>
<td>3/104 (3%)</td>
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Table 1: Proportion of popliteal nodes with metastases

Effect of rabbit anti-Swiss mouse lymphocyte serum (ALS) on metastases of Sarcoma 180 in C57 mice.
mors were considerably smaller than those in the control groups between Day 17 and Day 21. Recently we have made similar observations with respect to the pronounced increase in metastases with ALS treatment in Swiss mice given foot implants of S180. In preliminary experiments with another allogeneic tumor in Swiss mice, Sarcoma 1, we have found that this tumor almost never metastasizes in untreated Swiss mice, but metastasizes extensively by the tenth day not only to the popliteal nodes but to the tail nodes and aortic nodes when the mice are treated with ALS. The same tumor, in isogenic, normal A/aja mice, exhibits a pattern of metastases similar to that observed in allogeneic, ALS-treated Swiss mice. These results suggest that the presence of metastases in such an allogeneic tumor system may serve as yet another qualitative test for the activity of a specific preparation of ALS.

While it may be argued that allogeneic tumors in mice are not comparable to spontaneous tumors in man, the results of studies with such models may have some clinical applications. Recently Wilson et al. (5) reported a case in which bronchogenic carcinoma metastatic to the kidney was inadvertently transferred to a patient when he received a kidney transplant from a cadaveric source. The tumor remained viable and grew while the patient was on immunosuppressive therapy but underwent immunologic rejection when this therapy was discontinued and the major portion of the tumor was removed. These studies have again emphasized the importance of the host’s immune response in determining the behavior of allogeneic transplanted tumor in man, and they suggest that similar mechanisms may be operating in spontaneous neoplastic growths in man, at least in those tumors that may have specific antigenic components.

While the increased incidence of metastases in ALS-treated animals is most likely due to the immunosuppressive effect of this agent, the mechanism of action of this agent has not been clarified by our study. Two possible mechanisms have received serious consideration: one, related to a cytotoxic effect of ALS producing peripheral lymphopenia and severe lymphocyte depletion in lymphoid tissue, and two, related to a sterile activation of the lymphocyte. In our studies, the ALS-treated mice did not demonstrate significant lymphocyte or other cellular depletion in the various lymph nodes and spleens. In contrast, the lymph nodes from the ALS-treated and control animals were indistinguishable, all showing a striking morphologic response with proliferation of immunoblasts, plasmacytoid cells, and plasma cells. Our studies, in this particular model, would thus be more consistent with the sterile activation concept of ALS action.

The mechanical barrier function of the lymph node has received some attention, in the past, with respect to trapping of tumor cells and prevention of metastases. It has been suggested that, depending upon the physical status of these barriers, more or less tumor cells may escape the node. However, Fisher and Fisher (4) recently showed that the regional lymph node is not a completely effective mechanical barrier to the passage of tumor cells in the efferent channels. It is unlikely that ALS has any effect on the mechanical barrier function of the regional lymph node; however, it is possible that ALS enhances metastatic tumor growth in the regional lymph nodes by suppressing tumor immunity.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Metastatic Sarcoma 180 in the regional popliteal node. Note the sheet of tumor cells growing in the subcapsular cortical region. This mouse was treated with rabbit anti-Swiss mouse lymphocyte serum, Day 14. H & E, × 270.

Fig. 2. Cellular changes in the regional popliteal node. A, Normal node. H & E, × 270; B, Node at Day 3 after tumor implant, rabbit anti-Swiss mouse lymphocyte serum (ALS) treated. Note the enlargement of the germinal center. H & E, × 270; C, Node at Day 10 after tumor implant, ALS treated. Note the proliferation of “immunoblasts” in the cortical region and the tumor cells (arrow) in the subcapsular sinus. H & E, × 270; D, Node at Day 14 after tumor implant, ALS treated. Note the predominance of cells of the plasma cell series. Atypical forms (arrow) are also present. H & E, × 270.
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