General Immunocompetence of Rats Bearing Avian Sarcoma Virus-induced Intracranial Tumors

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ABSTRACT

The mitogenic responsiveness of spleen cells obtained from avian sarcoma virus-inoculated Fischer 344 rats was studied. Sixty % of the rats had astrocytomas, 13% had sarcomas, 7% had mixed gliosarcomas, and 20% had no evidence of tumors. Only spleen cells from rats bearing astrocytomas had significantly diminished responses to phytohemagglutinin and concanavalin A (Con A) when compared to control responses. The decreased responsiveness observed with phytohemagglutinin was limited to the optimal concentration range (10 and 20 µg) while a broader concentration of Con A (0.01 to 50 µg) induced significant suppression. Moreover, a more profound immunosuppression was observed with Con A. The results also demonstrated that spleen cells from rats with the largest astrocytomas exhibited the greatest suppression. From the results of this study, it appears the avian sarcoma virus-induced astrocytoma in rats is an immunological parallel of the human disease based on the loss of general immunological competence as assessed by responsiveness of lymphocytes to phytohemagglutinin and Con A.

INTRODUCTION

Patients with primary intracranial tumors are among the best suited for immunological studies because these neoplasms rarely metastasize and because generally the patient is not as debilitated as are those with metastatic disease. In spite of this, however, these patients manifest a variety of immunological deficits, including a marked inability to respond to ubiquitous skin test antigens and a decrease in the ability of their peripheral blood lymphocytes to respond to mitogens (4, 5, 18, 19). Moreover, the percentage of as well as the absolute number of thymus-derived lymphocytes is decreased, whereas complement receptor lymphocytes are increased (6, 7). This decrease in immunological competence may be the result of a soluble suppressor substance(s) present in the plasma of these patients (5, 19). While these immunological deficiencies are readily detectable, it is difficult to study the basic mechanisms involved in the absence of a reliable animal model. Several animal models are available that may serve as suitable immunological parallels of the human disease (3, 9, 20). One particularly well-characterized animal model is the ASV*-induced astrocytoma in the Fischer 344 rat (9). This model has the advantages of a primary tumor with morphology similar to that of human anaplastic astrocytomas (8). However, this tumor model has not been characterized with respect to the immunological features observed in patients with malignant gliomas.

The purpose of this report is to describe the mitogenic responsiveness of spleen cells obtained from rats bearing tumors elicited by intracranial inoculation of ASV. The results demonstrate that spleen cells obtained from rats bearing anaplastic astrocytomas, but not rats bearing sarcomas, have a decreased response to PHA and Con A. Moreover, the spleen cells obtained from rats bearing large astrocytomas are less responsive to these mitogens than are the spleen cells obtained from rats with smaller tumors. These results suggest that the ASV-induced rat astrocytoma model may serve as a useful immunological parallel for the human disease.

MATERIALS AND METHODS

Animals. Male Fischer 344 rats (Charles River Breeding Laboratory, Wilmington, Mass.) 42 days of age were inoculated intracerebrally with 2 µl of ASV as previously described (8).

Cell Cultures. Spleen cell suspensions were prepared in RPMI-1640 (Microbiological Associates, Bethesda, Md.) by extruding the cells by compression against the bottom of a glass Petri dish with the use of a sterile Morton closure. The cells were then passaged through a 20-gauge needle several times, transferred to a 15-ml glass conical tube, and allowed to stand for 3 to 5 min to remove clumps. Finally, the cells were transferred to a 50-ml conical tube with the use of a 20-ml syringe fitted with a 22-gauge needle, washed once in RPMI-1640 by centrifugation at 150 x g for 10 min, and resuspended in 5 ml of RPMI-1640. After the nucleated cells were counted, they were adjusted to 4 x 10⁴/ml in RPMI-1640 supplemented with 10% heat-inactivated normal rat serum, glutamine, nonessential amino acids, vitamins, penicillin, and streptomycin. Two hundred fifty µl of the cell suspension were pipetted into flat-bottomed microculture wells (No. 3040; Falcon Plastics, Oxnard, Calif.), and 10 µl of various concentrations of either Con A (Sigma Chemical Co., St. Louis, Mo.) or PHA 4

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(Difco Laboratories, Detroit, Mich.) were added. Cell cultures were performed in triplicate. The culture plates were incubated at 37°C in a 5% CO2-air atmosphere for 56 hr, at which time 0.25 μCi of [3H]thymidine (New England Nuclear, Boston, Mass.; specific activity, 6.7 Ci/m mole) was added in a volume of 10 μl. The radioactivity incorporated into DNA was determined 16 hr later by harvesting the cells with an automatic cell harvester (Otto Miller Co., Madison, Wis.) and counting them in a liquid scintillation counter. The data were analyzed by the 2-tailed Student’s t test for independent means.

Histopathology. Animals were sacrificed by cardiac puncture under ether inhalation anesthesia at various intervals from 100 to 120 days after inoculation. Their brains were removed and placed in 10% buffered formalin. After fixation, brains were sectioned in a coronal plane, and whole sections of the cerebral hemispheres were embedded in paraffin. The presence of tumors was determined by gross examination and study of hematoxylin- and eosin-stained sections. Masson’s trichrome phosphotungstic acid-hematoxylin, reticulum methods were used for classifications of tumors according to criteria previously established (9). The gross location and microscopic characteristics were similar to those previously described for ASV-induced neoplasms in rats (9); 60% of the tumors were gliomas, 13% were sarcomas, and 7% were mixed gliosarcomas. The gliomas were primarily fibrillar, gemistocytic, or poorly differentiated astrocytomas. The sarcomas were both fibrosarcomas and meningeal sarcomas. Tumor size was graded on a scale of 1 to 3: 1 was used to indicate the presence of a small microscopic intraparenchymal or extraparenchymal tumor; 2 was used to indicate a medium neoplasm occupying a portion of the cerebral hemisphere with or without an extracerebral extension; and 3 was used to indicate a large tumor that occupied major portions of the cerebral hemisphere and/or a large extraparenchymal mass.

RESULTS

Responsiveness of Spleen Cells from Tumor-bearing and Non-Tumor-Bearing Rats to PHA. Spleen cells obtained from control rats, those from rats bearing intracranial astrocytomas and sarcomas, and those from virus-inoculated rats that exhibited no evidence of tumors were stimulated with various concentrations of PHA. Spleen cells from rats bearing astrocytomas had a significantly reduced response to 10 and 20 μg of PHA as compared to the response of normal controls (Chart 1). However, the response of spleen cells from virus-inoculated rats not bearing tumors and of those from rats with sarcomas did not differ from control response at any concentration of PHA.

Those rats with astrocytomas were subdivided on the basis of tumor size and the spleen cell response to PHA as compared to control responses (Chart 2). The results demonstrated that the rats with the largest astrocytomas had significantly lower responses to concentrations of PHA, ranging from 5 to 50 μg, when compared to control responses. However, those rats with small or medium astrocytomas did not have a significantly impaired response to PHA.

Responsiveness of Spleen Cells from Tumor-bearing and Non-Tumor-bearing Rats with Con A. Experiments similar to those performed with PHA were carried out with Con A, and the results are presented in Chart 3. The spleen cell responses of rats bearing astrocytomas were reduced at all concentrations of Con A used with the exception of 0.1 and 50 μg. Comparison of the response of spleen cells obtained from either virus-inoculated tumor-negative rats or rats bearing sarcomas to the responses of control rats revealed no difference.

The Con A responsiveness of spleen cells from rats with small, medium, or large astrocytomas was compared to that of control spleen cells (Chart 4). Regardless of the size of the tumor, decreased Con A responses were observed, and the spleen cells from those rats bearing the larger astrocytomas were most affected. Moreover, this decreased responsiveness was observed at all concentrations of Con A with the exception of 0.1 and 50 μg for the medium-sized astrocytoma group.

DISCUSSION

The results of this study establish that rats bearing ASV-induced intracranial astrocytomas have decreased general immune competence as evidenced by the diminished capacity of their spleen cells to respond to PHA and Con A. Moreover, rats with large astrocytomas were more affected than those with smaller astrocytomas. This impaired mito-
genic responsiveness was a unique feature of animals that harbored primary astrocytomas, inasmuch as it did not occur in rats with intracranial sarcomas or in virus-inoculated animals that did not develop tumors.

The diminished responsiveness was significant only when optimal doses of mitogens were used. Furthermore, it was observed that spleen cells from rats with astrocytomas responded less well to Con A than to PHA. These results may be explicable on the basis of the existence of different subpopulations of splenic thymus-derived lymphocytes (T₁ and T₂) that can be differentiated in part by their responsiveness to mitogens (17). The T₁ subpopulation is stimulated by Con A, while T₂ lymphocytes respond to both Con A and PHA. Thus, it appears that the T₁ lymphocyte subpopulation may not respond normally in rats bearing primary astrocytomas.

More importantly, the results of this study indicate that rats that harbor ASV-induced astrocytomas are immunologically parallel to humans with primary malignant brain tumors. This similarity is based upon impaired general immunological competence as assessed by lymphocyte mitogenic responsiveness (4, 5, 18). The discovery that the in vitro lymphocyte function of ASV-induced astrocytoma-bearing rats is qualitatively similar to that of humans with malignant gliomas now permits exploration of the immunological deficit in a more controlled experimental setting. In addition to the investigation of the mechanism(s) of depressed lymphocyte function, the paradox of "specific" antitumor immunity in humans (12, 13) and in rats (1) in the presence of impaired generalized lymphocyte responsiveness may be explored.
Furthermore, the effects of tumor immunity and alterations in general immunological responsiveness upon host survival can be systematically approached. Presently, an association between impaired or enhanced host immunocompetence, absence or presence of specific antiglioma immunity, and overall survival has not been demonstrated. Prolonged immune suppression in experimental animals with the use of antithymocyte sera has not influenced the incidence, type of tumor, or host survival regardless of the agent used for brain tumor induction (2, 10, 11). Similarly, immunostimulation via intradermal or intracerebral administration of Bacillus Calmette-Guérin to rats with ASV-induced brain tumors did not result in significant increased survival when combined and/or compared with effective chemotherapy (14). Immunotherapeutic approaches in humans with brain tumors also have failed to influence survival significantly (15).

The only correlation of changes in immune status and incidence of primary brain tumors is the association of increased incidence of primary brain lymphosarcomas in chronically immunosuppressed transplant patients (16). The availability of ASV-induced astrocytomas in adult rats that produce lymphocyte abnormalities similar to those observed in human glioma patients may begin to answer some of the perplexing associations between the natural history of glioma-bearing animals, humans, and the immune system.

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