SUMMARY

Antigens from a rat astrocytoma cell line were compared with those from normal rat liver and normal rat brain by the immunodiffusion and immunoelectrophoresis techniques. Antigens that appeared to be specific to the tumor cells were observed. Although these were predominantly in the soluble fraction, a small but distinct amount of the antigens were found to be membrane bound. The existence of tumor-specific soluble and membrane antigens in astrocytoma lends support to the feasibility of immunotherapy for the brain tumors.

INTRODUCTION

Ample evidence has been accumulated in recent years that indicates changes in cellular antigenicity accompanying malignant transformation. Studies on tumor-specific antigens not only will benefit us in the understanding of the malignant process but also should provide us with an invaluable aid in the immunological approach to the therapy of cancer. During the past few years, several cell lines have been established from tumors originating in the nervous system (1—4, 19, 20). Although these cells retain many of the functional and morphological characteristics of the parent cells (10, 12—14, 18, 21, 22), it is likely that, as with tumors from other tissues, antigenic changes could have taken place that might be related to their abnormal growth pattern. In the present study, we compared antigens of a rat astrocytoma cell line with antigens obtained from normal rat tissues.

MATERIALS AND METHODS

An established rat astrocytoma cell line (induced by N-nitrosomethylurea, clone strain RG-6) was grown in monolayer cultures in Falcon plastic tissue culture flasks (surface area, 75 sq cm) in the F-10 medium (11) supplemented with 16% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.), penicillin, 100 units/ml, and streptomycin, 100 µg/ml. The cells used for the experiments were free of infection by pleuropneumonia-like organisms as tested with the Mycoplasma agar medium (Grand Island Biological Co.). The tumorigenicity of the cell line was confirmed in our laboratory by inoculating 1 X 10^7 cells s.c. into young rats (30 g body weight). A palpable tumor ranging from 4 to 8 cm in diameter was invariably produced within 2 weeks.

After growing to confluency in monolayer, the cells were rinsed 3 times in the flask with 0.15 M NaCl containing 0.02 M Tris buffer, pH 7.5, and removed by shaking with glass beads. Cells harvested from 1 flask (approximately 10^9) were pelleted by centrifuging at 1000 X g for 10 min and were resuspended in 4 ml 0.9% NaCl solution. The resuspended cells were homogenized with a glass homogenizer and mixed with an equal volume of complete Freund's adjuvant (Difco Laboratories, Inc., Detroit, Mich.). The mixture was emulsified by forcing it through a 20-gauge needle with a syringe. Each rabbit was given 3 ml of the antigen mixture in the hind footpads and the shoulder regions. A 2nd inoculation was given 3 weeks later, and the antisera was collected after another 10 days. The γ-globulin fraction, which was used for all the immunological studies, was obtained by 3 successive series of 33% ammonium sulfate precipitation and 40% ammonium sulfate wash (25). The final product was dissolved in 0.9% NaCl solution at a concentration of 10 mg of protein per ml as measured by the method of Lowry et al. (16). Antiliver γ-globulin was prepared in the same manner except that a 10% liver homogenate instead of a cell suspension was used for immunization.

The astrocytoma cells to be used as antigens in the immunological reactions were harvested as described above. They were lysed in distilled water for 15 min before homogenization. The homogenate was then centrifuged at 100,000 X g for about 30 min. The supernatant was designated as the “soluble antigen fraction,” and the pellet was designated as the “membrane antigen fraction.” The latter was resuspended in 0.15 M NaCl at 4° and sonically disrupted with a Heat Systems-Ultrasoundis Sonifier (Model W185D) equipped with a microtip. A total of 3 min was accumulated by subjecting the suspension to 30-sec bursts at 50 watts machine output. The sonically disrupted fraction was then centrifuged again at 100,000 X g for 60 min. The final supernatant, which contained the antigens released from the membranes, was designated as the “sonicated membrane fraction.” This and the soluble antigen fraction were used in the immunological reactions. Liver and brain antigens were prepared in an analogous manner from homogenized tissues.

RESULTS

The presence of tumor-specific antigens in the rat astrocytoma cell line was detected by immunodiffusion and
Fig. 1. Comparison of rat astrocytoma and rat liver antigens by immunodiffusion (Ouchterlony technique). Rabbits were immunized against the astrocytoma cell line obtained from tissue culture, or against the normal rat liver. Center wells, antisera; peripheral wells, antigens. A, anti-astrocytoma; L, anti-liver; Well 1, astrocytoma soluble antigen; Well 2, astrocytoma membrane antigen (sonically disrupted); Well 3, liver membrane antigen (sonically disrupted); Well 4, liver soluble antigen. Bottom, precipitin lines.

immunoelectrophoresis. In the former procedure, the astrocytoma cells were compared with the liver cells (Fig. 1). The Ouchterlony plates were made up of 1% Ionagar No. 2 (Colab) in the presence of 0.15 M NaCl and 0.02 M Tris buffer, pH 7.5. Each antibody well contained 1 mg of either anti-astrocytoma or anti-liver γ-globulin fraction, whereas each antigen well contained either 50 µg of astrocytoma soluble or membrane antigen, or 500 µg of the corresponding liver antigen fractions. Fig. 1 shows that, under the conditions used for immunodiffusion, soluble and membrane antigens from the 2 cell types did not cross-react. With the anti-astrocytoma antibody, no precipitin line could be detected against the liver antigens, whereas the astrocytoma soluble antigen gave intense lines even at one-tenth the amount used for the liver. The astrocytoma membrane fraction showed weak but distinct lines. Since sonic disruption releases only a fraction of the total membrane antigens, the result obtained with astrocytoma membranes probably represents a lower limit in antigen detection. Absorption of the anti-astrocytoma antisera with liver antigen or absorption of the anti-liver antisera with astrocytoma antigen did not alter the results of immunodiffusion.

For immunoelectrophoresis, the astrocytoma and normal rat brain antigens were first subjected to electrophoresis in 7.5% acrylamide gels (9). After being run in the cold at 2.5 mA/tube for 3 hr, the gels were sliced longitudinally. Half of each gel was fixed in 10% trichloroacetic acid overnight and stained with Coomassie blue (27). The other half was embedded in 1% Ionagar No. 2 for immunological reaction. The antigens were allowed to diffuse out of the gel for 24 hr before the addition of the antibody fraction to the troughs. The precipitin lines were fixed by soaking the plates in 10% trichloroacetic acid. The anti-astrocytoma γ-globulin fraction was used at 10 mg/ml, whereas 100 µg of astrocytoma protein and 500 µg of brain protein were used in the initial gel electrophoresis. The precipitin pattern following immunoelectrophoresis is depicted in Fig. 2. As was true with the simple diffusion technique, the astrocytoma soluble fraction showed a high degree of reaction (3 lines), while the astrocytoma sonically disrupted membrane fraction revealed only 1 line. However, the line in the membrane fraction appeared to be distinct from any of the lines observed in the soluble fraction. Essentially the same precipitin lines were observed if the γ-globulin fraction was absorbed with normal brain tissue. On the other hand, brain antigens, even those used in an amount larger than that of astrocytoma antigens, did not produce any precipitin line against the anti-astrocytoma antibody under identical experimental conditions.

Although it is known that some common antigens, such as the S-100 protein (a soluble, brain-specific protein found in normal glia and some glial tumors including RGC-6), exist in astrocytoma and normal brain, our results do show that at least some antigens predominantly present in the tumor cells can be demonstrated.

DISCUSSION

Over the last few years, important progress has been made in the field of cancer immunology. It is now generally accepted that tumor immunity resembles transplantation.
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membrane bound, some of which might have come from the plasma membrane. Indeed, differences in cell surface properties between normal and transformed cells have been found, such as in the ability to bind wheat germ agglutinin (8, 17) and concanavalin A (15, 17). With a double isotope labeling technique, differences in surface glycopeptides between normal and malignant cells have been detected (5—7). Although such direct evidence has not been obtained for astrocytoma, our results do indicate indirectly that the same might hold true with brain tumors.

The use of established cell lines provides us with the following advantages: (a) large quantities of a homogeneous cell population can be obtained, making immunological and chemical analysis relatively simple and clear-cut; and (b) the astrocytoma cell line currently under investigation can be grown both in vitro and in vivo. Cells harvested from tissue culture flasks can be transplanted directly into animals to initiate a tumor growth. The procedure of Simmons and Rios (23) can be used to test the feasibility of immunotherapy on this brain tumor model. Furthermore, work is currently under way in our laboratory to purify the tumor-specific surface proteins from these cells, a process which should increase the specificity of the immunological attack. The purified antigens can also be used to obtain antitumor antibodies that, if conjugated with boron or lithium, could be used as an agent for the neutron-capture therapy of the brain tumor (24, 26).

On the other hand, one should be aware of possible limitations when attempting to extrapolate results obtained from established cancer cell lines to the spontaneously occurring tumors in patients because antigens specific to a tumor in 1 individual may differ from those specific to the same tumor found in another individual. Nevertheless, experience gained with the established cell lines should be useful as a guideline for dealing with tumors occurring in patients, particularly with respect to the general feasibility of the immunological approach to the therapy of brain tumors. Whether or not antibodies can get across the blood-brain barrier is another point to be considered in a successful antibody therapy of spontaneous brain tumors. It is conceivable that brain tumors are more accessible to antibodies than normal brain tissue because of increased vascularity and various degrees of cell damage frequently found in neoplastic tissues.

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REFERENCES


Immunological Specificity of Astrocytoma Antigens

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