Common Antigen in Meningioma-Derived Cell Cultures

Abstract. *Serums of patients with intracranial meningiomas reacted in immunofluorescence assays with cell cultures and tumor imprints prepared from human meningiomas. Antibody in these serums appears to be specific for antigens in meningioma tissue and shows some cross-reactivity with neoplastic tissue of glial origin.*

Neoplasms of the mammalian central nervous system can be induced by direct inoculation with a number of RNA and DNA tumor viruses (*1*). These observations suggest that some brain tumors of man may be of viral etiology. Since virus-induced animal tumors contain a common antigen irrespective of tumor histology or anatomic location (*2*), we examined cell cultures prepared from central nervous system tumors for the presence of a common antigen with the use of immunofluorescence techniques. Our results show that cell cultures derived from intracranial meningiomas contain a common antigen demonstrable by fluorescence antibody staining with serums from meningioma patients.

Brain tumor tissue obtained at craniotomy was minced and dispersed with trypsin. Cells were collected by centrifugation at 600*g* for 10 minutes suspended in reinforced Eagle's medium supplemented with 20 percent fetal bovine serum, penicillin (100 unit/ml), and streptomycin (100 μg/ml); seeded into 250-ml plastic tissue culture flasks; and incubated at 36°C. When cells had grown to confluency, secondary cultures were established after dispersion by exposure to a solution containing trypsin and ethylenediaminetetraacetic acid. Cell monolayers to be used for immunofluorescence staining were prepared by seeding approximately 5×10^4 cells onto 35-mm plastic dishes followed by incubation at 36°C in humidified air containing 5 percent carbon dioxide. Prior to staining for immunofluorescence, cell monolayers were fixed in 95 percent methanol and washed with phosphate-buffered saline (PBS), *p*H 7.2.

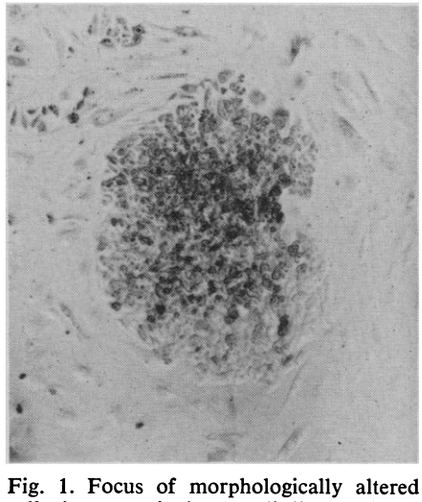

Fig. 1. Focus of morphologically altered cells in a meningioma cell line (subpassage 3). Phase-contrast photomicrograph of unfixed cells (×55).

In the indirect reaction, cells were exposed to unconjugated serum for 60 minutes at 25°C, washed with PBS, and stained for 30 minutes at 25°C with a 1:10 dilution of equine antiserum to human serum globulin conjugated with fluorescein isothiocyanate (*3*). When direct staining was used, cells were incubated under similar conditions with fluorescein-conjugated human serum globulin alone. After staining by either the direct or indirect technique, the cell monolayers were repeatedly washed with PBS, then covered with 10 percent buffered glycerol and examined with a fluorescence microscope (*4*). Preparations stained with fluorescein-conjugated equine antiserum to human serum globulin alone were included as controls in the indirect procedure. Controls for autofluorescence consisted of cells stained with unconjugated human serum or human serum globulin alone.

Serums from adult brain tumor patients were prepared from preoperative blood specimens. Control serums were prepared from the blood of randomly selected blood bank donors and from patients with non-neoplastic neurological disease.

All of the cell lines derived from meningiomas were composed predominantly of fibroblast-like cells. Two of the lines, however, demonstrated occasional distinct foci of cells in which contact inhibition was lost and cellular density was markedly increased (Fig. 1). These areas of apparently transformed cells have been observed in each of eight subpassages in one meningioma-derived cell line. In the other line, these areas were not seen after subpassage 3. These morphologic alterations resemble those observed in monolayer cultures prepared from human sarcomas (*5, 6*).

Table 1 summarizes results obtained when cell cultures derived from intracranial neoplasms were stained by the indirect immunofluorescence technique. Subpassages 1 to 5 from meningioma cell lines and subpassages 1 to 8 from cell lines from other intracranial neoplasms were studied. When each of ten serums obtained from meningioma patients was used at a dilution of 1:8 or greater, fluorescence was demonstrated in two or more meningioma-derived tissue culture cell lines. Four of these ten serums reacted against cell cultures from both homologous and autologous meningiomas. Fluorescence staining in these cultures was strictly limited to the cell cytoplasm and tended to be concentrated in the perinuclear region (Fig. 2). Staining was diffuse and finely granular in quality; at times larger distinct fluorescent granules were observed. When four cell lines derived from intracranial tumors other than meningiomas were treated with serums from meningioma patients, only one cell line demonstrated fluorescence staining.

Two meningioma cell lines were stained with 12 serums at 1:8 dilution from normal blood donors. Cytoplasmic fluorescence was observed in both cell lines after exposure to two of the serums; two additional serums produced fluorescence in one cell line. The remaining three meningioma cell lines were each tested with two serums at 1:8 dilution from normal blood donors; in no instance was fluorescence observed. One meningioma cell line was exposed to serums obtained from five patients with non-neoplastic neurological disease; no fluorescence staining was

Table 1. Indirect immunofluorescence staining of cell cultures derived from human neoplasms. Abbreviations are as follows: Men. Pat., meningioma patients; Cont., blood donor controls; Pat. N-Neo. Neur. Ill., patients with non-neoplastic neurological illness. Results are given as the ratio of the number of serums showing positive reaction to the number of serums tested and are for dilutions of 1:8 or greater.

Cell origin	Serums from:		
	Men. Pat.	Cont.	Pat. N-Neo. Neur. Ill.
Meningioma 1	10/10	4/12	
Meningioma 2	10/10	2/12	0/5
Meningioma 3	9/9	0/2	
Meningioma 4	7/7	0/2	
Meningioma 5	6/6	0/2	
Glioblastoma	7/7	0/2	
Mixed glioma	0/8	0/2	0/5
Metastatic carcinoma	0/8	0/2	
Neurofibroma	0/2	0/2	

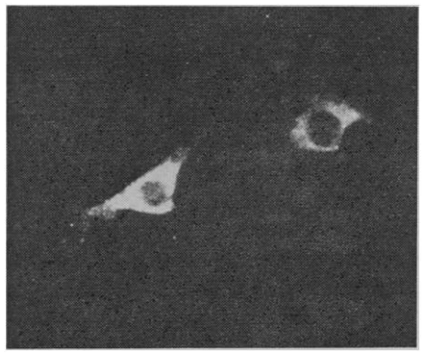

Fig. 2. Cytoplasmic fluorescence in meningioma cell culture stained with homologous serum from meningioma patient by indirect immunofluorescence method (×310).

observed. In addition, cell cultures derived from cerebral tissue obtained by biopsy of a patient with Creutzfeldt-Jakob disease failed to demonstrate immunofluorescence with any of the serums tested: six from meningioma patients, seven from blood donors, and three from patients with Creutzfeldt-Jakob disease.

To test the specificity of the fluorescence antibody reaction, we attempted to inhibit immunofluorescence staining of a meningioma cell culture by treating the culture with unconjugated globulin before the staining procedure. Fluorescein-conjugated globulins from two meningioma patients were used for direct staining; these two preparations stained effectively at dilutions of 1:20 and 1:40 but were used at 1:4 dilution in the blocking experiment. Of the many globulins used, only those derived from two meningioma patients markedly inhibited the staining reaction (Table 2). Staining was moderately inhibited by treatment with globulin from astrocytoma and glioblastoma patients but was completely unaffected by treatment with globulin from apparently normal persons. Both homologous and autologous globulin from meningioma patients inhibited fluorescence staining by the direct method.

To determine whether similar immunofluorescence could be demonstrated in tumor cells before they were cultured, we prepared tumor imprints from two meningiomas by the method of Morton *et al.* (*7*) and examined them by the indirect immunofluorescence technique (Table 3). Treatment of both imprints with autologous serum at titers of 1:32 and 1:128 produced fluorescence. Imprints prepared from one meningioma were stained with serums obtained from eight meningioma patients; treatment with each serum produced fluorescence at a titer of 1:8 or greater. In all instances, the appearance and distribution of the cytoplasmic fluorescence resembled that observed in cell cultures derived from meningiomas. Four of five control serums from blood donors failed to react with the tumor imprints at a 1:8 dilution. Separate portions of serums from three meningioma patients and one normal blood donor were adsorbed (2 hours at 37°C, 18 hours at 4°C) with either finely minced normal brain obtained at autopsy or meningioma tissue obtained at surgery (200 mg of tissue per milliliter of serum). Antibody in serums from three meningioma patients was removed with meningioma tumor tissue as judged by loss of immunofluorescence when the test serum was used at 1:4 dilution. Adsorption with normal brain tissue failed to affect the staining reaction.

Cell cultures derived from human sarcomas contain a common antigen (S-antigen) demonstrable by indirect immunofluorescence staining with human serums (*5, 6, 8*). Although virtually all patients with sarcoma, regardless of type, had high titers of antibody to this common antigen, the antibody was also present to a significant extent in the serums of normal blood donors (26 percent) and of healthy members of sarcoma patients' families (80 percent) (*5*). Another similar study, however, demonstrated the presence of a common antigen in sarcoma cell cultures but failed to clearly show either an increased incidence or increased amount of immunofluorescence antibody in serums from sarcoma patients when compared with serums from normal subjects or patients with other malignancies (*6*).

Our results suggest that the serums of patients with meningiomas contain an antibody which reacts against tumor-derived cell cultures as well as meningioma imprint preparations. Although blocking and adsorption studies of serums from meningioma patients show that some antibody is specific for a meningioma-associated antigen, the antibody may also react with cell cultures derived from other brain tumors, and treatment of meningioma-derived cell cultures with serum globulin from patients with intracranial gliomas may block the immunofluorescence reaction. This suggests that the antibody found in serums of meningioma patients is directed against an antigen of some human brain tumors of varying histologic type. This antigen of brain tumors may be analogous to the sarcoma antigen (*5, 6, 8*). This similarity is not entirely unexpected since meningiomas may represent an intracranial form of fibrosarcoma and can undergo sarcomatous degeneration (*9*). Furthermore, instances of gliomas coexisting with intracranial fibrosarcoma have been reported (*10*). Serum obtained from a patient with osteogenic sarcoma reacted with three meningioma cell cultures in the indirect immunofluoresence test. In addition, four serums derived from meningioma patients reacted with cells derived from the osteogenic sarcoma. These studies may indicate an immunologic relation

Table 2. Inhibition of direct immunofloresence staining of cells derived from a human meningioma by prior treatment with unconjugated serum globulin. Abbreviations are as follows: Unconj. SG, unconjugated serum globulin; and Conj. Men. SG, fluorescein-conjugated meningioma serum globulin. Fluorescein-conjugated globulin from serums of two meningioma patients was used at 1:4 dilution. Intensity of fluorescence was graded 1+ to 4+ by comparing the extent and intensity of cytoplasmic fluorescence with that in replicate monolayers treated with PBS and stained with a 1:4 dilution of the fluorescein-conjugated serum.

Unconj. SG	Conj. Men. SG	Intensity
Meningioma patient 1	No. 1	0
Meningioma patient 2	No. 1	0
Astrocytoma patient 1	No. 1	1+ to 2+
Astrocytoma patient 2	No. 1	1+ to 2+
Glioblastoma patient	No. 1	1+ to 2+
Control blood donor	No. 1	4+
Meningioma patient 2	No. 2	0
Meningioma patient 3	No. 2	0
Control blood donor	No. 2	4+

Table 3. Indirect immunofluorescence staining of meningioma imprint preparations. Numbers in parentheses indicate number of serum specimens. The titer is the reciprocal of highest dilution of serum giving positive immunofluorescence reaction. In the adsorption studies, serum was incubated with the tissue indicated and tested at 1:4 dilution. Control serums were from blood donors.

Serum	Titer	Staining after adsorption with:	
		Meningioma	Normal brain
Imprint preparation 1			
Autologous meningioma	128		
Homologous meningioma	> 10 (3)	Absent (3)	Present (3)
	> 8 (4)		
Control (5)	8 (1)		
	< 8 (4)	Absent (1)	Absent (1)
Imprint preparation 2			
Autologous meningioma	32		
Control (1)	< 8		

between systemic sarcomas and intracranial meningiomas and gliomas.

Approximately 30 percent (4 out of 12) of blood donor serums used as controls gave positive staining reactions against meningioma-derived cell cultures. This incidence is similar to that observed for the antibody directed against the S-antigen (*5*). Therefore, the presence of immunofluorescence antibody in human serums cannot be considered, at present, to be diagnostic of the presence of a meningioma or other brain tumor. Such antibody may result from experience with a relatively common antigen which is preferentially localized in neoplastic cells but may be completely unrelated to the oncogenic process. This hypothesis would also explain why the immunofluorescence reaction was not entirely specific for cell cultures derived from meningiomas. Further definition of this immunologic relationship by other techniques, such as complement-fixation or cytotoxicity, may indicate more specific relationships than are demonstrated here.

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Human Sarcomas Contain RNA Related to the RNA of a Mouse Leukemia Virus

Abstract. *Labeled DNA complementary to the RNA of the Rauscher leukemia virus was hybridized with RNA from the polysome fraction of human sarcomas. Eighteen out of 25 specimens contained RNA possessing homology to the RNA of the mouse leukemia virus but not to that of the unrelated viruses causing mammary tumors in mice or myeloblastosis in chickens. Further, no normal adult or fetal tissues showed significant amounts of RNA specific to mouse leukemia virus. It appears that human sarcomas contain RNA sequences homologous to those found in an agent related to a virus known to cause sarcomas in mice.*

We have recently developed (*1*) means to detect virus-specific RNA in the polysome fraction of fresh and frozen tumors. Use of this technique revealed (*2*) that 67 percent of 29 human malignant breast tumors contained RNA that could hybridize with DNA complementary to the RNA of the mouse mammary tumor virus (MMTV). None of 30 control preparations from normal or benign breast tissues contained detectable quantities of this type of RNA.

Breast carcinoma RNA samples that hybridize with MMTV-DNA showed no ability to hybridize with DNA complementary to an unrelated (*3*) murine leukemia agent, Rauscher leukemia virus (RLV). When we turned our attention to human leukemias, we found (*4*) that 89 percent of 27 leukemic patients contained, within their white blood cells, RNA homologous to that of RLV but not to that of MMTV.

The unrelatedness of virus-specific RNA found in human breast tumors and the virus-specific RNA found in human leukemic cells is precisely what one would expect from the accumulated experience with the corresponding animal oncogenic viruses. In animal systems all naturally occurring sarcoma viruses (from chicken, mouse, cat, and so forth) are defective (*5*) and require related leukemogenic virus as helpers. If the human situation is similar, one would predict that, like the leukemias, human sarcomas would also contain RNA that can hybridize to DNA homologous to the RNA of RLV.

We have now found that 68 percent of human sarcomas do contain RNA that can hybridize with DNA homologous to the RNA of RLV. Again, as with the leukemic cells, the sarcoma RNA does not hybridize with DNA homologous to RNA of either MMTV or of the unrelated avian myeloblastosis virus (AMV).

Figure 1 shows representative density profiles (in Cs_2SO_4 gradients) of the products of annealing reactions between RLV [^{3}H]DNA and polysomal RNA (pRNA) preparations isolated from four different human sarcomas. These results (from 1 to 6 percent hybridization of the input RLV-DNA) are comparable to those obtained if

Table 1. Results of hybridization between RLV [^{3}H]DNA and pRNA isolated from human sarcomas. Between 200 and 500 μg of pRNA from each sample were hybridized to 2000 count/min RLV [^{3}H]DNA, and the reactions were analyzed by Cs_2SO_4 density centrifugation. The amount of DNA banding in the RNA region of the gradient (between densities 1.62 and 1.68 g/ml) was then determined. The results are expressed as the radioactivity (count/10 min) (corrected for background) banding in the RNA region for each sample tested and as multiples of σ, the operational standard deviation (see legend to Fig. 3). The annealing reaction is considered positive only if the number of counts per 10 minutes per RNA region is greater than 3σ. Twenty-five sarcomas tested, 68 percent positive.

Diagnosis	RNA (count/10 min)	(Count/10 min) per σ	Reaction
	Fibrosarcoma		
(1448)	363	6.87	+
(1444)	427	8.08	+
(MV)	403	7.63	+
(1455)	96	1.81	−
	Osteogenic sarcoma		
(1415)	433	8.20	+
(PH)	220	4.16	+
(23–2)	253	4.79	+
(23–5)	908	17.20	+
(1468)	173	3.27	+
(1443)	95	1.79	−
	Osteolytic sarcoma		
(1464)	42	0.795	−
	Liposarcoma		
(1438)	333	6.30	+
(1441)	449	8.50	+
(1458)	252	4.77	+
(1450)	454	8.59	+
(1442)	94	1.78	−
	Leiomyosarcoma		
(1418)	297	5.62	+
(1449)	214	4.05	+
(1447)	157	2.97	−
(1463)	145	2.75	−
(1446)	46	0.87	−
	Neurofibrosarcoma		
(1437)	313	5.92	+
(1456)	182	3.44	+
	Rhabdomyosarcoma		
(21–43)	531	10.05	+
(1459)	99	1.87	−