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Abstract

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IMMUNOFLUORESCENT STUDY OF IMMUNOGLOBULINS AND COMPLEMENT COMPONENTS IN HUMAN BRAIN TUMORS

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Abstract. Using a direct immunofluorescent method, histological locations of immunoglobulins (IgG, IgM, IgA and IgD of heavy chain, and κ and λ of light chain) and complement components (C3 and C4) were studied in 78 brain tumors, which included 24 astrocytomas, 6 metastatic tumors, 5 medulloblastomas, 4 malignant lymphomas, 15 meningiomas, 8 schwannomas, 8 pituitary adenomas, and 8 other miscellaneous brain tumors. IgG-positive cells were observed in the perivascular regions of astrocytomas, but were more marked in those of high grade, metastatic tumors and meningiomas. Malignant lymphomas demonstrated IgG and IgM-positive cells accompanied by either kappa or lambda light chains. C3 and C4 were much less evident in these tumors. Pituitary adenomas showed slight positive stains for both immunoglobulins and complement components on the blood vessel walls. Immune reactions against brain tumors were discussed including the clinical application of autologous lymphocyte infusion in malignant gliomas and combination chemotherapy in intracranial malignant lymphomas.

Key words : immunoglobulin, complement component, brain tumor immunity, immunotherapy, combination chemotherapy.

The presence of tumor-related antigens has been reported in various types of malignant neoplasms, such as colon, cervical, breast and lung carcinomas (1). Among human brain tumors, Siris (2) and Weil and Liebert (3) reported glioblastoma antigen, which was identical with brain tissue antigen. Coakham and Lakshmi (4) found surface antigen(s) common to cultured astrocytoma cells and named it human astrocytoma-associated antigen(s). Wickremesinghe and Yates (5) demonstrated it in benign gliomas and in those of low grade malignancy, but not in the more malignant gliomas. On the other hand, Wahlström *et al.* (6) reported glia-specific antigen(s) in human brain tissue and malignant glioma. Wahlström, *et al.* (7) and Solheid *et al.* (8) subsequently reported the existence of both antigens, namely glioma-associated antigen(s) and glial antigen(s), in gliomas. Additionally, the glioma-associated antigen(s) detected by Wikstrand and Bigner (9) was later shown to share with antigen of fetal brain. In meningiomas, Catalano, Jr., *et al.* (10) and Winters and Rich (11) reported the presence of tumor-associated antigen(s). Weiss *et al.* (12) demonstrated simian virus 40-related antigens in some meningiomas.

The two types of lymphocytes, thymus-derived lymphocytes (T-cells) and bone marrow-derived lymphocytes (B-cells), are thought to recognize antigens specifically. Subsequently, both T-cells and B-cells generate cellular and humoral immune responses through intimate reactions between them. Eventually, T-cells are responsible for cells such as helper, suppressor, killer and amplifier cells; whereas, B-cells produce anti-bodies or immunoglobulins for functions of humoral immunity. Immunoglobulins are composed of four polypeptides : 2 heavy chains (H) and 2 light chains (L). Five different H chains, designated gamma, mu, alpha, delta and epsilon have been found in humans. Each form determines each class of immunoglobulins, namely immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), immunoglobulin D (IgD) and immunoglobulin E (IgE). L chains are classified into 2 types : kappa (κ) and lambda (λ). All classes of immunoglobulin carry either kappa or lambda chains. The complement system consists of at least 15 different serum proteins and plays a part as humoral mediator of antigen-antibody reactions.

It is remarkable that little attention has been paid to immunohistochemical demonstration of immunoglobulins and complement components in human brain tumors. Using a direct immunofluorescent method, this report demonstrates their specific locations in 78 brain tumors of various histological types. The correlation between the findings and brain tumor immunity are discussed.

MATERIALS AND METHODS

Tumor specimens from 78 human brain tumors were obtained at craniotomies performed at Okayama University Hospital. Their histological classification is summarized in Table 1. They consisted of astrocytoma : 24 (low grade : 7 & high grade : 17), metastatic tumor : 6, medulloblastoma : 5, malignant lymphoma : 4, meningioma : 15, schwannoma : 8, pituitary adenoma : 8, and miscellaneous : 8 (hemangioblastoma : 2 &

TABLE 1. HISTOLOGICAL CLASSIFICATION OF 78 BRAIN TUMORS STUDIED BY IMMUNOFLUORESCENCE

Histology	No. of tumors
Astrocytoma	24
Low grade	(7)
High grade	(17)
Metastatic tumor	6
Medulloblastoma	5
Malignant lymphoma	4
Meningioma	15
Schwannoma	8
Pituitary adenoma	8
Miscellaneous	8
Total	78

ependymoma, germinoma, teratoma, ganglioglioma, melanoma and chordoma : 1 in each). In addition, five normal brain tissues obtained on lobectomy were also included. They were studied for immunoglobulins and complement components by a direct immunofluorescent method. Both compositions of immunoglobulin were examined, namely IgG, IgM, IgA and IgD of heavy chain and both kappa and lambda light chains. Among complement components, the third (C3) and fourth (C4) complement components were studied. Fibrinogen (F) was also added as a staining control.

The tumor tissues were sharply cut by razor to a size of about 5 × 5 × 5 mm, then quickly frozen in iso-pentane solution cooled by dryice-acetone mixture. They were further cut to 5μ thickness on a cryostat (Coldtome, Finetechnical Sakura Co., Ltd., Tokyo). The first section was stained by haematoxylin and eosin (H & E) and the histological features were examined by light microscope. If sections demonstrated degeneration, hemorrhage, or necrosis they were not studied by immunofluorescence. Otherwise, each 5μ thick-section was attached to a special glass slide designed for immunofluorescent study (Micro slide glass, Matsunami Glass Ind., Ltd., Osaka) and was air dried for 10 min at room temperature. Following fixation in cold acetone for 5 min the slides were washed three times in cold phosphate-buffered saline (PBS) for 15 min. Subsequently, each section was stained with FITC (fluorescence isothiocyanate)-conjugated rabbit antiserum specific for human IgG, IgM, IgA, IgD, κ, λ, C3, C4 or F (Behring Institute, W. Germany) at 1:10 dilution for 60 min at room temperature. After several rinsings in cold PBS, the slides were mounted in phosphate-buffered glycerine and examined with a Nikon fluorescent microscope. Photographs were taken with Kodak Tri-X pan black and white films and Kodak Ektachrome 400 color films (Eastman Kodak Company, Rochester, New York).

In the control, the sections were stained with FITC-conjugated normal rabbit IgG by the procedure described above.

RESULTS

All five normal brain tissues did not demonstrate any immunoglobulins or complement components in the parenchyma except for the occasional appearance of IgG in the capillaries. Fibrinogen was also seen in the capillaries occasionally, as were other immunoglobulins and complement components minimally.

Twenty-four astrocytomas were studied. The age and sex of the patient, the location of tumor, histological grade and the findings of the immunofluorescent study are summarized in Table 2. Among seven low grade astrocytomas, three tumors demonstrated positive cells for IgG, kappa and lambda sporadically. Fluorescence for IgG was brighter than that for kappa or lambda. The positive cells were irregular in shape and located in the vicinity of blood vessels. IgG, kappa and lambda were also present slightly around blood vessel walls (Fig. 1A). C3 was identified in and on the vessels in four tumors (Fig. 1B). IgM was minimum in blood vessels, while IgA, IgD or C4 was not seen in any portion. In Case 4, several round cells which demonstrated bright fluorescence for IgG, kappa, lambda and C3 were observed in blood vessels. The remaining 17 high grade astrocytomas showed IgG, kappa and lambda constantly, although the in-

tensity of fluorescence was different not only among the tumors but also among several tissue sections of the same tumor. Their locations were basically the same as those seen in low grade astrocytomas. However, IgG-positive cells in perivascular regions were generally higher in incidence and intensity (Fig. 2A). Only three tumors demonstrated C3 on blood vessel walls. In Cases 15 and 17, there were many small cells in the tumor, which were positive for IgG, kappa and lambda (Fig. 2B).

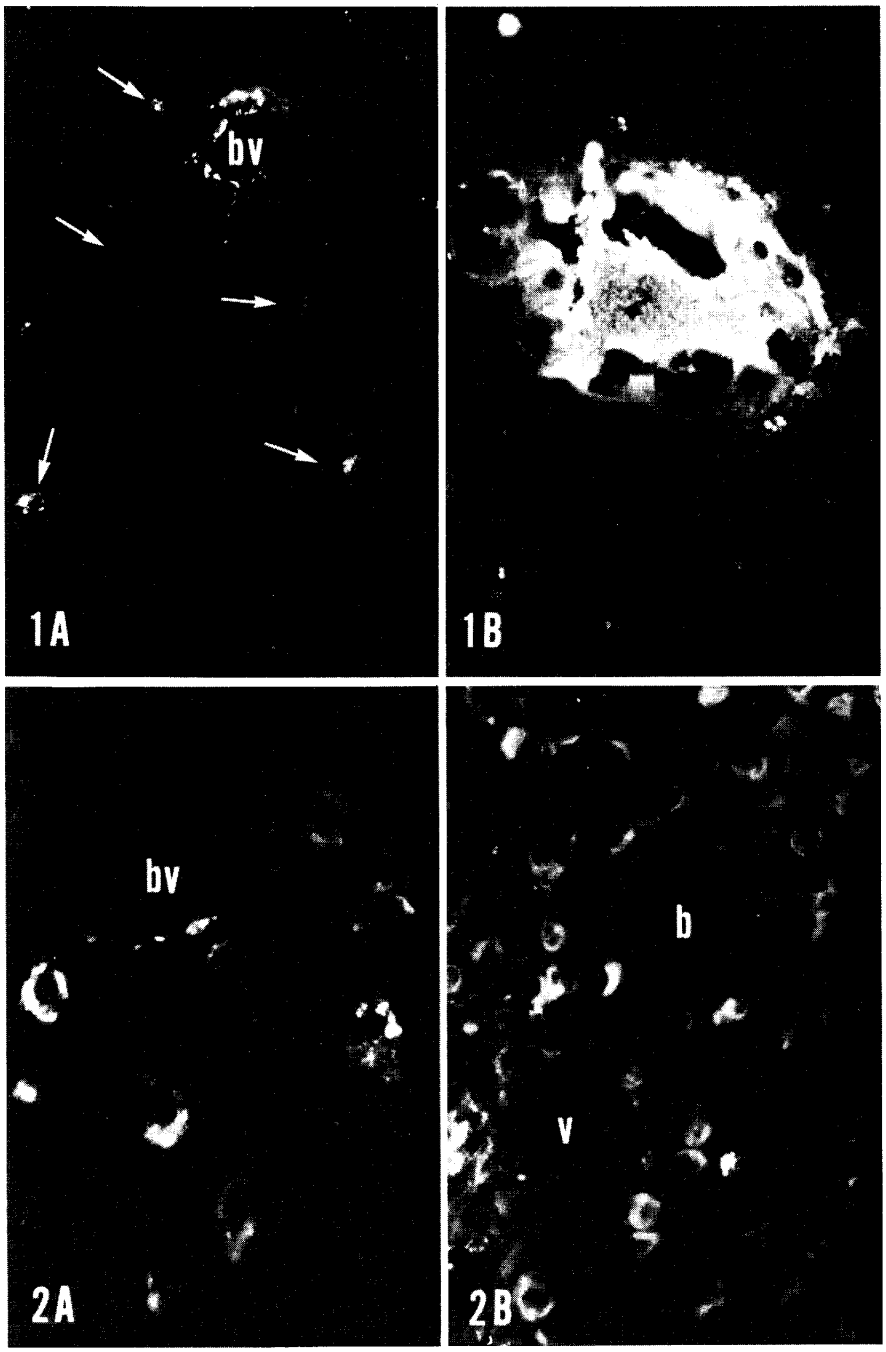
TABLE 2. SUMMARY OF 24 ASTROCYTOMAS

Case no.	Age, (yrs) sex	Tumor location	Grade	Immunofluorescent study*								
				IgG	IgM	IgA	IgD	κ	λ	C3	C4	F
1	22 M	Frontal lobe	Low	−	−	−	−	−	−	−	−	+
2	24 M	Frontal lobe	Low	+	−	−	−	+	+	+	−	++
3	64 M	Temporal lobe	Low	−	−	−	−	−	−	−	−	+
4	13 M	Cerebellum	Low	+	+	−	−	+	+	+	−	++
5	47 M	Temporal lobe	Low	−	−	−	−	−	−	−	−	+
6	47 M	Frontal lobe	Low	+	−	−	−	+	+	+	−	+
7	17 F	Cerebellum	Low	−	−	−	−	−	−	+	−	+
8	36 M	Frontoparietal lobe	High	+	−	−	−	+	+	−	−	+
9	56 M	Occipital lobe	High	+	−	−	−	+	+	−	−	+
10	51 M	Temporal lobe	High	+	−	−	−	+	+	−	−	++
11	52 F	Temporal lobe	High	+	−	−	−	−	+	−	−	+
12	34 F	Temporal lobe	High	+	−	−	−	+	+	−	−	+
13	48 F	Frontal lobe	High	+	−	−	−	+	+	+	−	+
14	13 M	Frontal lobe	High	+	−	−	−	+	+	−	−	+
15	8 F	Brain stem	High	+	−	−	−	+	+	−	−	+
16	53 M	Parietal lobe	High	+	−	−	−	+	+	−	−	+
17	11 F	Cerebellum	High	++	−	−	−	++	−	−	−	++
18	55 F	Frontal lobe	High	++	−	−	+	+	+	+	−	++
19	36 F	Temporal lobe	High	+	−	−	−	+	−	−	−	+
20	23 F	Corpus callosum	High	+	−	−	−	+	+	−	−	++
21	31 M	Parietooccipital lobe	High	++	−	−	−	+	+	−	−	+
22	43 M	Frontal lobe	High	++	−	−	−	+	+	−	−	+
23	35 F	Thalamus	High	+	−	−	−	+	+	−	−	+
24	34 M	Frontal lobe	High	++	−	−	−	+	+	+	−	++

*++ = strongly positive; + = positive; − = negative.

Fig. 1. Immunofluorescent microphotographs of low grade astrocytoma (Case 2). A: Sporadic presence of IgG-positive cells (arrows) in the perivascular region (× 200). bv = blood vessel. B: C3 in and on the blood vessel (× 500).

Fig. 2. High grade astrocytomas (Cases 17 & 21). A: IgG-positive cells in the perivascular region in Case 21 (× 500). B: Diffuse presence of small IgG-positive cells in Case 17 (× 500). bv = blood vessel.


bv
1A
1B
bv
2A
b
v
2B

There were six metastatic tumors which are summarized in Table 3. All tumors were from the metastatic foci of lung cancer and were of various histological types. IgG-positive cells were numerous and were dense in the vicinity of blood vessels. The remaining portions away from the blood vessels were negative for IgG delineating the boundaries between the bright positive and dark negative regions of the tumor. Positive cells were smaller than those in the negative regions (Fig. 3). Both kappa and lambda were also stained with less intensity. IgM was occasionally observed in and around blood vessels. IgA, IgD and C3 were not seen, while C4 was identified in the perivascular regions in two tumors.

All five medulloblastomas did not demonstrate any immunoglobulins or complement components in the parenchyma except for small particles of IgG inside blood vessels.

TABLE 3. SUMMARY OF 6 METASTATIC TUMORS

Case no.	Age (yrs) sex	Primary lesion	Histology	Immunofluorescent study*								
				IgG	IgM	IgA	IgD	κ	λ	C3	C4	F
1	50 M	Lung	Squamous cell ca.	++	+	−	−	+	+	−	+	+
2	65 F	Lung	Small cell ca.	++	−	−	−	+	+	−	−	++
3	58 F	Lung	Adenocarcinoma	++	+	−	−	+	+	−	−	++
4	59 M	Lung	Small cell ca.	++	−	−	−	+	+	−	+	+
5	39 M	Lung	Large cell ca.	++	−	−	−	+	+	−	−	++
6	75 M	Lung	Squamous cell ca.	++	+	−	−	+	+	−	−	++

*++ = strongly positive; + = positive; − = negative.

TABLE 4. SUMMARY OF 4 MALIGNANT LYMPHOMAS

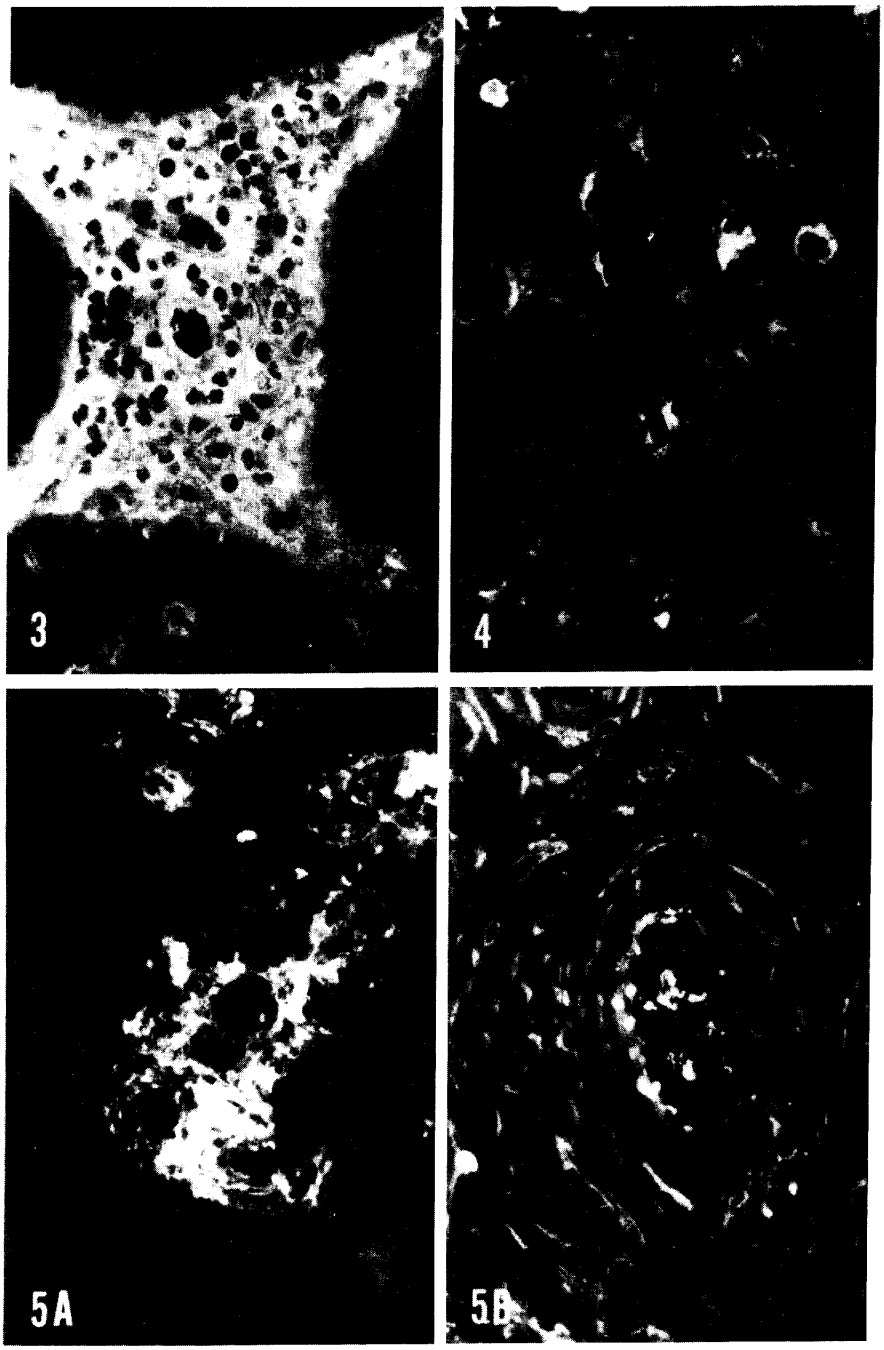
Case no.	Age, (yrs) sex	Tumor location	Immunofluorescent study*								
			IgG	IgM	IgA	IgD	κ	λ	C3	C4	F
1	45 M	Temporal lobe & 3rd vent.	+	++	−	−	−	+	−	−	++
2	51 F	Occipital lobe	++	++	−	−	+	−	−	−	++
3	22 M	Frontal lobe	++	++	+	−	+	−	+	+	++
4	59 M	Bifrontal lobes	++	+	−	−	−	+	+	−	++

*++ = strongly positive; + = positive; − = negative.

Fig. 3. Metastatic tumor (Case 3) demonstrating numerous IgG-positive cells in the perivascular region demarcated from the surrounding negative region. The positive cells are smaller than those in the negative region (× 500).

Fig. 4. Malignant lymphoma (Case 2) demonstrating many kappa-positive cells (× 500).

Fig. 5. Meningiomas (Cases 13 & 14). A: IgG in the perivascular region in Case 14 (× 200). B: IgG-positive cells surrounding the blood vessels in Case 13 (× 500).


3
4
5A
5B

Four cases of intracranial malignant lymphomas are summarized in Table 4. All tumors demonstrated numerous cells positive for IgG and IgM, more marked in the perivascular regions. However, the distribution pattern of IgG-positive cells and IgM-positive cells appeared different. The study of light chains was characteristic. The number of cells positive for light chain was naturally similar to that of IgG and IgM. But the two tumors demonstrated kappa only and the remaining two tumors lambda only (Fig. 4). IgA and IgD were not seen, while C3 and C4 were occasionally seen on blood vessel walls.

The age and sex of the patient, the location of the tumor, histological type and the findings of the immunofluorescent study in 15 meningiomas are summarized in Table 5. IgG was seen around blood vessels and on the surface of tumor cells in the perivascular regions (Figs. 5A & B). It was also present in the whorls and around the psammoma bodies slightly. Both kappa and lambda were similarly present with much less intensity. Other immunoglobulins and complement components were not seen. In Case 15, there were numerous small cells around the blood vessels, which were positive for IgG, kappa and lambda. Some of them were positive even for IgA and rarely for IgM.

Eight schwannomas were studied. IgG was occasionally seen in blood vessels. In addition, two tumors demonstrated cells positive for IgG. These cells were present diffusely and randomly in the tumor. C3 was occasionally present on blood vessel walls.

Among eight pituitary adenomas, six tumors were chromophobe adenoma

TABLE 5. SUMMARY OF 15 MENINGIOMAS

Case no.	Age, (yrs) sex	Tumor location	Histological type	Immunofluorescent study*								
				IgG	IgM	IgA	IgD	κ	λ	C3	C4	F
1	51 F	Falx	Syncytial	+	−	−	−	−	+	−	−	+
2	56 F	Sphenoidal ridge	Angioblastic	+	−	−	−	+	+	−	−	+
3	73 M	Convexity	Syncytial	+	−	−	−	+	−	−	−	+
4	55 M	Sphenoidal ridge	Transitional	+	−	−	−	+	+	−	−	+
5	28 F	Sphenoidal ridge	Syncytial	++	−	−	−	+	+	−	−	+
6	50 F	Parasagittal	Fibroblastic	+	−	−	−	+	+	−	−	+
7	47 F	Sphenoidal ridge	Angioblastic	++	−	−	−	+	+	−	−	+
8	66 F	Tentorial	Transitional	+	−	−	−	+	−	−	−	+
9	44 F	Tuberculum sellae	Transitional	+	−	−	−	+	−	−	−	+
10	44 F	Multiple	Fibroblastic	+	−	−	−	+	+	−	−	+
11	33 F	Tuberculum sellae	Syncytial	+	−	−	−	+	+	−	−	+
12	44 F	Convexity	Angioblastic	++	−	−	−	+	+	−	−	+
13	49 F	Tuberculum sellae	Transitional	+	−	−	−	−	+	−	−	+
14	48 F	Parasagittal	Angioblastic	++	−	−	−	+	+	−	−	+
15	53 F	Convexity	Syncytial	++	−	+	−	+	+	−	−	++

*++ = strongly positive; + = positive; − = negative.

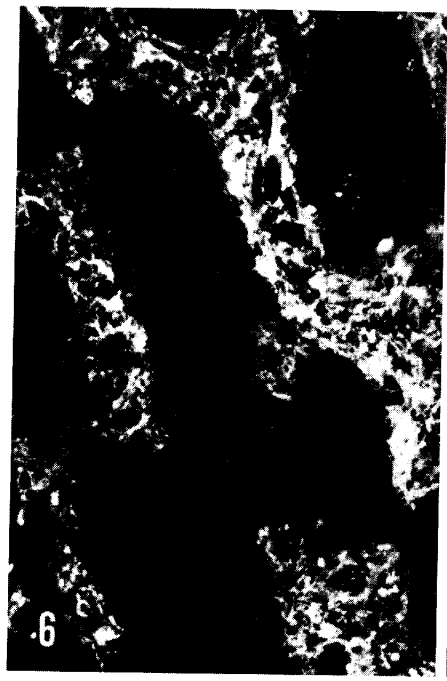


Fig. 6. Hemangioblastoma demonstrating IgG in the perivascular regions (× 200).

Fig. 7. Ependymoma demonstrating bright C3 in the perivascular region (× 500).

Fig. 8. Teratoma demonstrating stain positive for C4 on the cells composing the wall of the lumen (× 200).

and two were eosinophilic adenoma. In both types, the blood vessel walls were stained slightly for IgG and IgM heavy chains and kappa and lambda light chains. IgA, IgD, C3 and C4 were also present on the vessel walls although their staining intensity was minimal.

Among eight other tumors classified as miscellaneous, two hemangioblastomas demonstrated IgG, kappa and lambda around blood vessels (Fig. 6). C3 was also present minimally, while IgM, IgA, IgD and C4 were not seen. In an ependymoma, bright fluorescence for C3 was diffusely present in the perivascular regions (Fig. 7). IgG was also present, but its intensity was much less than that of C3. Other immunoglobulins were essentially negative and C4 was minimal. A germinoma demonstrated many cells positive for IgG, kappa and lambda mainly located close to blood vessels. IgM-positive cells were also seen occasionally. In a teratoma, the cells composing the wall of the lumen were stained for IgG, kappa, lambda and C4 (Fig. 8). The immunofluorescent findings in ganglioglioma, melanoma and chordoma were not remarkable.

DISCUSSION

IgG-positive cells were observed in astrocytomas, but were more marked in high grade, metastatic tumors, meningiomas and some schwannomas in this study. They were present predominantly in the perivascular regions except in schwannomas. Therefore, it was presumed that IgG was originally from the serum representing humoral antibody against tumor associated antigen. Kornblith *et al.* (13) and Phillips *et al.* (14) reported humoral immunological responses against antigens on the surface of astrocytoma cells. Subsequently, Kornblith *et al.* (15) with an improved microcytotoxicity assay, confirmed it in the sera from astrocytoma patients. Additionally they reported positive results in variable numbers of meningioma, acoustic schwannoma, pituitary adenoma and metastatic tumor. As common antigen among astrocytoma, meningioma and schwannoma, they postulated neuroectodermal antigens which were shared by tumors originating from the neuroectodermal germ-layer. However, all five medulloblastomas in this study did not demonstrate any immunoglobulins.

It is also possible that IgG was formed locally by immunoglobulin-producing lymphocytes. Diffuse infiltration of small immunoglobulin-positive cells in Cases 15 & 17 of high grade astrocytoma and in Case 15 of meningioma were probably lymphocytes. In metastatic tumors, IgG-positive cells were uniformly smaller than tumor cells in the negative regions suggesting that the former were lymphocytes. Lymphocyte infiltration in neoplasms has often been reported including intracranial tumors, such as gliomas by Ridley and Cavanagh (16) and meningiomas by Horten *et al.* (17). In regard to the physiological significance of these lymphocytes, Ciembroniewicz and Kolar (18) and Levy (19) reported lymphocyte-mediated cytotoxicity in patients with primary intracranial tumors. Brooks *et al.* (20) and Palma *et al.* (21) reported better clinical prognosis

in malignant gliomas with lymphocyte infiltration.

As long as these humoral and/or cellular immune responses are working properly, high grade astrocytomas and metastatic tumors were supposed to show some regression. But, clinically, they did not. Therefore, it was presumed that the immunoglobulins and complement components were unrelated to these immune responses. When an immunoglobulin molecule is digested by the enzyme papain, two Fab (antigen-binding) fragments and one Fc (crystallizable) fragment are produced. Wood and Morantz (22) reported the existence of Fc receptor-positive cells, mostly macrophages, in human brain tumors. Subsequently, Wood *et al.* (23) demonstrated immunoglobulin bound to these cells *in vitro*. Easty *et al.* (24) and Sutton and Becker (25) reported that cells from brain tumors took up proteins via pinocytosis. Both Fc receptor and pinocytosis support the possibility of non-specific binding of immunoglobulins to tumor cells. However, marked differences in the staining patterns of each immunoglobulin in this study are against this.

Blocking factor was reported to exist in the sera of patients with brain tumor (26, 27). It was considered to be composed of immunoglobulin coating the surface of tumor cells thereby modulating cellular immunity. Therefore, the demonstrated immunoglobulins may represent blocking factor to some degree. The lower incidence of C3 or C4 in this study suggests a low degree of actual antigen-antibody reactions in these tumors.

Prehn (28) developed a theory of immunostimulation since he (29) observed that small numbers of admixed immune spleen cells produced acceleration of tumor growth, while large numbers inhibited it. Similarly, Shearer *et al.* (30) reported that antibodies were stimulatory at low concentrations and cytotoxic at high concentrations. Since then, phenomena of immunostimulation have been reported *in vitro* and *vivo* among chemically induced tumors, virus induced tumors, and transplantable tumors of unknown etiology (31). IgG-positive cells in high grade astrocytomas and metastatic tumors demonstrated in this study were predominantly observed in perivascular regions. The clinical outcome of these tumors was disappointing. It is possible that these tumors were in a condition of immunostimulation; namely, either the demonstrated immunoglobulins in perivascular regions or the negative regions with scanty immunoglobulins were accelerating, instead of inhibiting, tumor growth. Young *et al.* (32) and Neuwelt *et al.* (33) reported treating patients with malignant glioma by infusing autologous lymphocytes into the subarachnoid space or into the tumor bed directly. When the mechanism of immunostimulation is considered, it seems hazardous to apply this procedure unless certain conditions is assured constantly, namely, the number of applied lymphocytes has to be much larger than the number of tumor cells to be treated in any portion where they will occur together.

Primary intracranial malignant lymphomas have been reported in patients with renal transplantation (34, 35), systemic lupus erythematosus (36) and

idiopathic thrombocytopenic purpura (37). Secondary solid intracranial malignant lymphomas were also reported to occur as involvement of the central nervous system in systemic malignant lymphomas (38). The four malignant lymphomas studied were not associated with any of these disorders. Henry *et al.* (39) studied 83 such cases histologically and concluded that the histological patterns observed were analogous to those of systemic malignant lymphomas. Immunological studies of systemic malignant lymphomas often demonstrated multiple heavy chains with a predominant light chain type indicating their monoclonal nature (40, 41). All primary intracranial malignant lymphomas studied by Houthoff *et al.* (42) demonstrated single heavy chains. All four tumors in this study revealed multiple heavy chains accompanied by either kappa or lambda light chains. This finding strongly suggests that the four tumors were of monoclonal origin and were indistinguishable from most systemic malignant lymphomas immunologically. Recently, various types of combination chemotherapy have been reported as improving the survival of patients with systemic malignant lymphomas (43 - 45). We have so far treated 6 cases postsurgical intracranial malignant lymphomas with combination chemotherapy. They are being followed-up at present.

The slight demonstration of immunoglobulins and complement components in pituitary adenomas was probably due to the nature of the adenoma because their main location was on blood vessel walls. In addition, the staining intensity seemed to be in accordance with serum concentrations. The findings in hemangioblastomas, ependymoma, germinoma and teratoma were very interesting but the numbers studied are too small to be discussed at present.

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