A study of the epidemiology of central nervous system neoplasms indicates an association with toxoplasma infection. Experimental and clinical findings support the etiologic nature of this relationship. For this reason this finding deserves further investigation.

RELATIONSHIP OF CENTRAL NERVOUS SYSTEM NEOPLASMS TO TOXOPLASMA GONDII INFECTION

Leonard M. Schuman, M.Sc., M.D., F.A.P.H.A.; Nung Won Choi, M.D., M.P.H.; and Warren H. Gullen, M.D., M.P.H.

Introduction

In our earlier investigations of the epidemiology of toxoplasmosis and particularly the sources of Toxoplasma gondii infection, we were impressed with the highly significant role which contact with chickens and other domesticated fowl played in human infection.[1] In seeking support for an hypothesis involving fowl as a source of Toxoplasma, we were rewarded not only by a number of reports of epizootics of toxoplasmosis in chickens[2,3] and other fowl,[4-7] but by a provocative reference to the observation of "so-called gliomas" in chickens spontaneously and experimentally infected with Toxoplasma.[8] Although human toxoplasma infection is universally distributed, it purportedly rarely stigmatizes with clinically recognizable disease. However, in view of the fact that Toxoplasma gondii apparently has indiscriminate affinities for many mammalian tissues and since a lack of demonstrated association between such infection and a wider spectrum of clinical disease may be more a reflection of our ignorance than of the true state of affairs, it is not difficult to hypothecate a relationship between toxoplasma infection and human primary central nervous system neoplasms.

Design of the Study

To test this hypothesis, the retrospective study design involved the utilization of hospitalized brain tumor cases and a series of hospitalized matched controls, each tested for toxoplasma antibody by the Sabin-Feldman dye-test technic as evidence of infection. This phase of the study was actually part of a larger investigation into the epidemiologic features of brain tumor morbidity and mortality in Minnesota, to be published elsewhere.[9]

Four hospitals in the Minneapolis-St. Paul metropolitan area affiliated with the University of Minnesota were utilized as the sources of cases and controls. These included the University Medical Center, the Veterans Administration Hospital, Hennepin County General Hospital, and Ancker Hospital of Ramsey County which were selected not only because of their cooperativeness and patient accessibility, but because their facilities could be expected to attract a high pro-

portion of the brain tumor cases occurring and residing in Minnesota and provide for a high level of diagnostic accuracy.

The study began in June, 1963. All cases of histologically proven primary central nervous system tumors diagnosed in the four designated hospitals between January 1 and June 20 of that year were located by a search of hospital records for all patients admitted during this period. All those alive at this time and residing within a radius of 150 miles of the Twin Cities were admitted to the study. The geographic area was so delineated for economy in visiting for interviews and obtaining of venous blood specimens for the Sabin-Feldman dye-test. In addition to this retrospective group, another group of brain and spinal cord tumor patients newly admitted from June 21, 1963, until June 30, 1964, coming from all parts of the state and diagnosed by clinical suspicion, but not necessarily proved histologically at the time of their admission, were included in the study group. These were obtained by notification from the respective neurology and neurosurgery departments of the study hospitals and by periodic checking at each service for newly admitted suspects. Since the later group was admitted on clinical suspicion for the most part, a certain number of these would of necessity remain unverified at the termination of the study period.

Selection of a matched control awaited interview of the study case, in order that all information on the matching variables would be available. Control subjects were patients admitted with any condition other than with tumor of any site, neurologic, psychiatric, ophthalmologic (exclusive of simple refractive error) or lymphatic disorders, and were selected from the lists of inpatients registered at the respective time periods. Three control candidates were selected for each case in chronological order of admission after the date of admission of the case. The purpose of this procedure was to provide alternates if the first control failed to meet the diagnostic exclusion criteria after clinical work-up or if he was discharged before interview was possible. Priority of the single control used was based on that chronological order, on the fulfillment of the diagnostic exclusion criteria, and of the designated matching variables.

The matching variables included: hospital of admission, sex, age, race, geographic area of residence and the locale of residence, i.e., urban, rural nonfarm, or rural farm. A minimal degree of leniency was acceptable in the age-matching procedure: for tumor cases up to one year of age the matched control had to be at least as old but not more than two years older; for the subjects aged two to 15 years, at least as old but not more than three years older; for the tumor cases 16 to 25 years of age, at least as old but not more than five years older; for the 26- to 50-year-olds, within five years younger to five years older; and for tumor cases 51 years and older, the matched controls could be no more than ten years younger or older than the case.

For purposes of this study, infection with Toxoplasma gondii was deemed to have occurred if the Sabin-Feldman dye-test was positive in subject serum dilutions of 1:1 or greater. Since the dye-test antibodies are deemed to be quite durable, this serum dilution criterion gives greater assurance that the dye-test negative patients have not been infected with Toxoplasma gondii than would be the case if the "positive" group included only the higher titered positives. Venous blood samples were drawn at the time of the interviews, allowed to clot, the sera separated and deep-frozen until the dye-test was performed. All serological tests were performed by one of us (N.W.C.) without the operator's knowledge of the identifying dis-

Table 1—Types and locations of the central nervous system neoplasms in the Minnesota four-hospital study, 1963-1964

Types of tumors	No.	Location of tumors	No.
Gliomas		Brain (unspecified)	11 (1)
Unclassified and mixed	6	Cerebrum	48 (6)
Astrocytoma	35 (4)	Cerebellum	5 (1)
Glioblastoma	23 (4)	Brain stem	10 (2)
Medulloblastoma	6 (1)	Ventricle	9
Ependymoma	7	Meninges	24 (2)
Acoustic neuroma	8 (1)	Optic chiasm	4
Neurofibromatosis	2	Hypophysis	3
Meningioma	24 (2)	Spinal cord	9
Pituitary tumors	3	Not specified	3
Blood vessel tumor	3	Clinical cases*	31 (2)
Pinealoma	1		
Craniopharyngioma	4		
Tuberous sclerosis	1		
Miscellaneous tumors	3		
Clinical cases*	31 (2)		
Total	157 (14)		157 (14)

* Pathologic diagnosis not yet made.
NOTE: Numerals in parentheses indicate additional cases available to the study for whom controls could not be matched.

tinction between tumor cases and their matched controls.

Test of significance of the difference in positivity among cases and controls was by the exact binomial test for matched pairs. A Type I error of 20 per cent (one-tail) was predesignated because of the exploratory character of this inquiry.

Results

A total of 171 cases of primary central nervous system neoplasms was collected in the 18-month period from the four designated hospitals. Table 1 indicates their distribution by histologic type and anatomic location. It will be noted that 14 cases could not be utilized in the analyses because of matching failures and, of the 157 successful matchings, 31 clinically highly suspect brain tumors remained histologically unverified at the termination of the study. It is of interest that the 126 histologically verified cases reveal an age, sex, and type distribution closely comparable to the distribution of primary CNS neoplasms in the mortality data in Minnesota for the period 1958-1962.[10] Thus the sample of tumor patients may be reasonably representative as well. As of June 30, 1964, only 16 of the 126 cases were dead. All, however, had submitted blood specimens for antibody study.

Tables 2 and 3 reveal the success of our matching procedure in terms of the variables of age and sex. The bimodality of the age distribution is apparent in Table 2.

In Table 4 the toxoplasma dye-test results among verified cases and matched controls may be noted. Matched pair analysis indicates that the excess in proportion of total dye-test positives among the CNS neoplasm cases is statistically significant ($p=0.021$). The distribution of specific titers among the dye-test

positive cases is not significantly different from that among the controls.

When the total verified tumor group was subdivided according to histologic types, some interesting associations with toxoplasma dye-test positivity were noted. These data are presented in Table 5. The group of gliomas as a whole and the astrocytomas as a subgroup reveal significant associations with toxoplasma dye-test positivity. The glioblastomas revealed no disparity and the medulloblastomas were too few for analysis. On the other hand no significant differences were elicited for the meningiomas and the nonverified tumor group. The miscellaneous group consisted of seven distinct histologic types, not one of which had a sufficient number of cases for analysis. The age distribution of the 35 cases of astrocytoma, for which a statistically significant association with dye-test positivity was noted, is presented in Figure 1. Disparities in proportion of dye-test positivity among astrocytoma cases and their matched controls exist for each age group (Figure 2).

Discussion

The data presented herein do, indeed, support the hypothesis that toxoplasma infection and human gliomas are related. Inasmuch as the dye-test, as an indicator of toxoplasma infection, has been shown to be highly sensitive and specific,[11] the significant excess of dye-test positives among the verified gliomas, and particularly the astrocytomas as compared to their matched controls, would tend to relate toxoplasma infection to such tumors.

It should be noted that for several of the histologic subtypes of glioma an inadequate number of cases were available for analysis, and for the glioblastomas and meningiomas no significant differences were elicited, although an excess of dye-test positives was noted for the meningiomas. For evaluation of the lack of demonstrated significance in these latter instances, power functions were calculated and are presented in Table 6. It can be seen that, for both the glioblastomas and the meningiomas, the powers for alternative hypotheses that are less than extreme were not particularly high. This lack of power reflects the limited sample sizes. Thus our data cannot be considered as ruling out a relationship between Toxoplasma gondii infection and either glioblastoma or meningioma.

The fact that multiple hypothesis test-

Table 2—Distribution of 126 histologically verified tumor subjects and their matched controls by age and sex, Minnesota four-hospital study, 1963-1964

Age in years	No. of cases			No. of controls		
	Total	Male	Female	Total	Male	Female
0– 9	17	12	5	16	12	4
10–19	13	7	6	13	6	7
20–29	7	4	3	12	7	5
30–39	21	13	8	17	11	6
40–49	23	14	9	27	16	11
50–59	25	15	10	16	10	6
60–69	13	7	6	18	10	8
70–79	7	5	2	7	5	2
80 and over	0	0	0	0	0	0
Total	126	77	49	126	77	49

Table 3—Comparison of mean ages of study and control subjects by tumor type, Minnesota four-hospital study, 1963-1964

Tumor type	No.	Mean age in years	
		Cases	Controls
Gliomas			
Unclassified and mixed	6	27.2	28.0
Astrocytoma	35	30.8	32.1
Glioblastoma	23	49.2	48.5
Medulloblastoma	6	9.5	9.5
Ependymoma	7	31.6	30.1
Acoustic neuroma	8	45.5	47.3
Meningioma	24	51.4	53.5
Others	17	34.0	34.2
Total histologically verified	126	38.3	39.0
Nonverified	31	48.4	47.6
Total	157	40.3	40.7

ing was employed in analyzing the astrocytomas, glioblastomas, and meningiomas separately, is not overlooked. However, even if there were no true difference between cases and controls for any of the three tumor types, one could expect at least one of three such tests to be significant at the 1.5 per cent level (astrocytoma: $p=0.015$) in only 4.4 per cent of repeated trials.* Such a probability certainly merits further serious consideration.

The biologic significance of this demonstrated association is open to speculation. There are no evidences at the present time that the dye-test may detect antibodies to an antigen common to both Toxoplasma and gliomas or that tumor patients retain toxoplasma antibodies longer than nontumor patients. The possibility that patients with a lower resistance to toxoplasma infection which has a marked affinity for nervous tissue, among others, may be more susceptible to gliomas, cannot be dismissed although there is no evidence to support this view either. That gliomatous tissue serves as a locus of reduced resistance to the *secondary* invasion of Toxoplasma gondii seems even more remote.

However, the possibility that the astrocytoma patients may have acquired either toxoplasma antibodies or organisms through a greater number of blood transfusions for their basic illness than their matched controls had must be explored. Since our test for the significance of the association was by the exact binomial test for matched pairs and this is dependent upon the number of discordant pairs and the proportions of these in which the cases or controls are

Figure 1—Distribution of astrocytoma cases by age, Minnesota four-hospital study, 1963-1964.

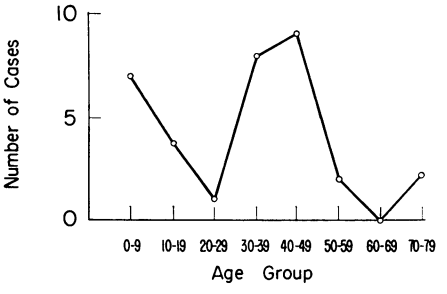
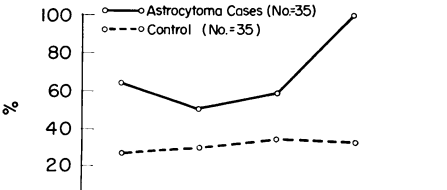


Figure 2—Percentage distribution of toxoplasma dye-test positivity among the astrocytoma cases and their matched controls, Minnesota four-hospital study, 1963-1964



* True Type I error $=1-(1-\alpha)^3=1-(1-0.015)^3=1-(0.985)^3=0.044$.

Table 4—Toxoplasma dye-test* results among 126 histologically verified tumor subjects and their matched controls, Minnesota four-hospital study, 1963-1964

Dye-test result	Cases		Controls	
	No.	%	No.	%
Negative	55	43.7	74	58.7
Positive	71	56.3	52	41.3
1:1 dil.	15	11.9	5	4.0
1:4 dil.	20	15.9	16	12.7
1:16 dil.	22	17.4	21	16.7
1:64+dil.	14	11.1	10	7.9
Total	126	100.0	126	100.0

† n=63, x=23, p=0.021

* Sabin-Feldman method.
† n=total number of discordant pairs,
x=number of discordant pairs in which controls are positive,
p=probability as derived from the exact binomial.

positive, our exploration of a possible passive transfer effect is simplified. There were 18 discordant astrocytoma pairs. Of these, 14 cases in the discordant pairs were dye-test positive. It is only in these latter that a transfusion effect could produce a spurious result. An examination of the clinical and blood bank records for these patients revealed that, although 12 of the 14 cases had received transfusions for surgery, in only five of these were the dye-tests performed on blood samples drawn *after* such transfusion at intervals varying from one to 54 days. However, in three of the five the dye-test titers in the cases were of such a magnitude as to require preposterously high titers in the donor (1:170 to 1:1920) to produce them.

These estimates took into consideration the amounts of transfused blood and the length of the interval between transfusion and sample-bleeding and the half-life of passively transferred antibody. In the remaining two cases the titers were of an order which conceiv-

Table 5 (Part 1)—Toxoplasma dye-test results among subjects with selected histological types of central nervous system neoplasms and their matched controls, Minnesota four-hospital study, 1963-1964

Dye-test result	Total gliomas		Astrocytoma		Glioblastoma		Medulloblastoma	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Negative	33 (42.9)	43 (55.8)	14 (40.0)	24 (68.6)	7 (30.4)	7 (30.4)	5 (83.3)	4 (66.7)
Positive	44 (57.1)	34 (44.2)	21 (60.0)	11 (31.4)	16 (69.6)	16 (69.6)	1 (16.7)	2 (33.3)
1:1 dil.	10 (12.9)	4 (5.2)	6 (17.1)	2 (5.7)	2 (8.7)	2 (8.7)	1 (16.7)	2 (33.3)
1:4 dil.	13 (16.9)	9 (11.7)	6 (17.1)	3 (8.6)	6 (26.1)	6 (26.1)	0 (0.0)	0 (0.0)
1:16 dil.	13 (16.9)	14 (18.2)	5 (14.3)	2 (5.7)	6 (26.1)	8 (34.8)	0 (0.0)	0 (0.0)
1:64+dil.	8 (10.4)	7 (9.1)	4 (11.4)	4 (11.4)	2 (8.7)	0 (0.0)	0 (0.0)	0 (0.0)
Total	77 (100.0)	77 (100.0)	35 (100.0)	35 (100.0)	23 (100.0)	23 (100.0)	6 (100.0)	6 (100.0)
	n=38, x=14; p=0.072		n=18, x=4; p=0.015		n=12, x=6; p=0.613			

Numerals in parentheses are percentages.

Table 5 (Part 2)—Toxoplasma dye-test results among subjects with selected histological types of central nervous system neoplasms and their matched controls, Minnesota four-hospital study, 1963-1964

Dye-test result	Meningioma		Others*		Type not verified	
	Cases	Controls	Cases	Controls	Cases	Controls
Negative	11 (45.8)	13 (54.2)	18 (47.4)	26 (68.4)	16 (51.6)	19 (61.3)
Positive	13 (54.2)	11 (45.8)	20 (52.6)	12 (31.6)	15 (48.4)	12 (38.7)
1:1 dil.	1 (4.2)	0 (0.0)	5 (13.2)	1 (2.6)	1 (3.2)	2 (6.4)
1:4 dil.	3 (12.5)	5 (20.8)	5 (13.2)	2 (5.3)	6 (19.4)	3 (9.7)
1:16 dil.	5 (20.8)	5 (20.8)	6 (15.8)	6 (15.8)	4 (12.9)	4 (12.9)
1:64+dil.	4 (16.7)	1 (4.2)	4 (10.5)	3 (7.9)	4 (12.9)	3 (9.7)
Total	24 (100.0)	24 (100.0)	38 (100.0)	38 (100.0)	31 (100.0)	31 (100.0)
	$n=13$, $x=6$; $p>0.25$				$n=18$, $x=7$; $p=0.24$	

* "Others" includes unclassified and mixed gliomas and ependymomas which are also included in "Total Gliomas." Numerals in parentheses are percentages.

ably might have been the result of passive transfer, but even in these the donors' titers would have had to be of a magnitude which in many general population surveys occurs but seven times in a hundred. Even if these two cases (and their corresponding matched controls) were excluded from the analysis on the basis of a questionable blood transfusion influence, the association between dye-test positivity and astrocytoma remains statistically significant ($p=0.038$).

Table 6—Power functions for matched-pair analyses of glioblastomas and meningiomas, Minnesota four-hospital study, 1963-1964

$\bar{H}$	Power values	
	Glioblastomas $n=12$, $x_o=8$	Meningiomas $n=13$, $x_o=9$
0.55	0.3044	0.2279
0.60	0.4382	0.3530
0.75	0.8424	0.7936
0.80	0.9274	0.9009

$\bar{H}$ = Alternative Hypothesis (probability that a given discordant pair will consist of a positive case and negative control).
n = Number of discordant pairs.
x_o = Number of discordant pairs wth positive cases. necessary to achieve significance at the 0.20 level.

A similar possibility of transfer of organisms cannot be overlooked. In this regard a recent study is pertinent. Kimball, Kean, and Kellner[12] studied 43 patients with thalassemia major ranging in age from 19 months to 21 years who had received a total of 4,805 transfusions of packed red blood cells with 75-80 per cent of the plasma removed; these patients were tested for toxoplasma antibodies. Three patients were consistently positive in relatively high titer, a not unexpected result for their age group, and ten patients at one time or another revealed transient low titer positives, but these had received an average of 5.5 transfusions in the 14 weeks of the study. Thus these conditions were very much more extreme than for the vast majority of the tumor cases in our study.

A causal relationship may thus be entertained. It is of particular interest that in a relatively recent report, Bobowski and Reed, having first described a space-occupying cerebral lesion in an adult human female as an astrocytoma Grade I or II, changed the designation of the lesion to chronic inflammatory

response only after finding Toxoplasma gondii in that lesion.[13] In avian gliomata, initially described with little attempt at subclassification by Belmonte[14] and Jackson,[15] and as astrocytoma by Jungherr and Wolf,[16] the existence of accompanying inflammatory response did not deter these authors from diagnoses of neoplasms. In fact Jackson, in a later report,[17] not only described the gradations of inflammatory response in terms of distance from the neoplastic lesion, but presented evidence of an histopathologic character that "not only does glioma arise from encephalitis, but its continued growth and spread are similarly due to conversion of chronically inflamed brain tissue at its periphery into tumor tissue." The conceptual bridge to the situation prevailing in man is readily crossed: inflammatory response does accompany neoplastic lesions in man and the avian gliomata are structurally related to glioblastoma (spongioblastoma) multiforme of man, being intermediate between astroblastoma and glioblastoma.[17]

In accepting the theory of the infectious origin of and encephalitic precursor stage in avian glioma Jackson sought for exogenous agents and did, indeed, describe A, B, C, and D bodies which he felt were parasitic.[18] The C and D bodies were most often associated with the gliomatous end-phase of the cerebral lesions. Following this, Krynauw and Jackson sought for and found C and D but not A and B bodies in 16 consecutive human brain tumors including medulloblastomas, spongioblastomas, meningiomas, and pituitary tumors.[19] Cautiously, these investigators indicated that they could express no opinion on the identity of these bodies with those in the avian tumors. Although not necessarily relevant to toxoplasma infection specifically, the implications for an infectious origin of gliomatous lesions are clear.

Finally, when our study was nearing completion, our attention was drawn to a report of the concomitant occurrence of a polar spongioblastoma of the left cerebral hemisphere and toxoplasmosis of the central nervous system including pseudocysts, necrosis, and calcification in the right thalamic area of a stillborn infant born to a mother with serum positive to the Sabin-Feldman dye-test in a dilution of 1:4.[20]

Thus some experimental comparative and clinical evidences exist to support a causal interpretation of the association of toxoplasma infection and gliomas. Certainly this finding should be explored further and in more definitive fashion.

Summary

1. An association between toxoplasma infection and central nervous system neoplasms, specifically astrocytomas, seems to exist.

2. The etiologic character of the association is supported by both experimental and clinical findings in both the avian species and man.

3. The provocative character of this initial finding deserves further exploration.

ACKNOWLEDGMENTS — The authors wish to express their appreciation for the significant contributions of the following: Drs. A. B. Baker, S. Chou, L. French, and B. Reiter of the University Hospitals; M. Ettinger of Hennepin County General Hospital; G. Ross of Ancker Hospital; and R. C. Gray, D. Long and J. Story of the Veterans Administration Hospital for providing the cases; the various departments and divisions of these hospitals for providing the control materials; Dr. A. C. Kimball for serving as referee on the laboratory methods; Mr. Harold Fletcher, medical student, for assisting with the bulk of the patient interviews, and the numerous medical students who assisted from time to time in the conduct of the study.

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Dr. Schuman is professor of epidemiology and Dr. Gullen is instructor in epidemiology, University of Minnesota School of Public Health, Minneapolis, Minn. 55455. Dr. Choi was research associate, Department of Biometry and Medical Statistics, Mayo Clinic, Rochester, Minn., and is now associate professor, Social and Preventive Medicine, University of Manitoba School of Medicine, Canada.

This paper was presented in part before a Joint Session of the Conference of Public Health Veterinarians and the Epidemiology Section of the American Public Health Association at the Ninety-Third Annual Meeting in Chicago, Ill., October 21, 1965.

The study was supported in part by Epidemiology Research Training Grant 2G-11.