PREFACE

Among the new treatments currently being investigated for malignant brain tumors, none is as theoretically appealing as immunotherapy, because it offers the potential for high tumor-specific toxicity. Cancer immunotherapy is currently a rapidly developing field, and new discoveries regarding the immune susceptibility of the central nervous system have made the concept of brain tumor immunotherapy an area of active investigation. Enough information has been gained from basic research and clinical trials to allow the conclusion that immunotherapy for brain tumors is feasible, can evoke relevant biologic responses, and can provide important insights into human biology. Brain tumor immunotherapy still faces great hurdles before it becomes an established clinical therapy. However, the accomplishments in this field to date are impressive, and the intuitive logic of this treatment paradigm offers compelling hope that the immunotherapy of brain tumors may someday succeed.

The aim of *Brain Tumor Immunotherapy* is to organize a thorough critical survey of the field, with contributions from leading researchers and clinicians to help convey the many and significant recent accomplishments within this evolving discipline. We hope our book will provide both clinicians and research scientists with a reasonably comprehensive guide to modern brain tumor immunotherapy and thereby enhance future investigation in the area. The scope of this text will detail some of the laboratory experiments and clinical protocols that are currently being investigated, integrate the available information from previous and ongoing research, and help to define the current status of the field.

The feasibility of immunotherapy for central nervous system cancers is just beginning to be studied through clinical trials. Most of our current understanding of brain tumor immunotherapy has been gleaned through the use of transplantable animal brain tumor models, with the primary hope of predicting therapeutic responses in human tumors. Because of the desperate plight of patients suffering from malignant gliomas and the fact that very few treatment modalities have shown clinical efficacy against this deadly disease, it is difficult to prove that any one animal model is necessarily the most exemplary of human primary brain tumors. Nevertheless, we must caution the reader that some of the most widely used animal models of murine and rat

primary glial neoplasms are not well-suited for evaluating immunologic responses to brain tumors since they have inherent histoincompatibilities that can potentially provide misleading results in immune-competent hosts. For example, the commonly used rat C6 glioma cell line has an uncertain genetic background and therefore may not be syngeneic in the animals in which these cells are transplanted. Because of this, favorable immunotherapeutic responses using animal models must always be interpreted with caution, and extreme prudence should be exercised before basing any clinical trial decisions on information obtained solely from such models. New models developed in syngeneic backgrounds with transgenic methodology may be more useful than older models, which are often chemically induced, highly antigenic, and of questionable genetic background. Yet, these models are still far from duplicating the complexities of clinical brain tumors and the human immune system.

With this caveat in mind, *Brain Tumor Immunotherapy* may be used most effectively as a resource text for neurosurgeons, experimental neuroscientists, clinical neuro-oncologists, tumor immunologists, and others who may wish to explore further research in this field. We have attempted to provide sufficient background information about brain tumor immunotherapy strategies, while hoping to capture a contemporary glimpse of the breadth and depth of this field. This book differs from others currently available, as it is probably one of the only texts dedicated specifically to immunotherapeutic approaches for *central nervous system* malignancies.

Whether it is adoptive cellular immunotherapy, radiolabeled antibodies, cytokine gene therapy, or dendritic cell vaccines, almost every leading neuro-oncology program in the world is investigating some form of brain tumor immunotherapy. The number of clinicians and scientists interested in cancer immunotherapy is increasing. Annual meetings of multiple scientific and clinical disciplines have entire sessions dedicated to the immunobiology of brain tumors. Recent developments in our understanding of molecular microbiology and tumor immunology have resulted in increasingly clever and sophisticated immune-based treatment strategies against cancer. It is our sincere hope that dissemination of such information and further research endeavors in this field will someday translate to true therapeutic benefits for our brain tumor patients.

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2 Epidemiology of Primary Brain Tumors

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1. INTRODUCTION

In 1999, it is estimated that 16,800 individuals in the United States were diagnosed with a malignant primary nervous system tumor and that 13,100 of these would die from the disease *(1)*. When benign as well as malignant brain tumors (BTs) are included, the incidence is over twice that for malignant BTs alone; 34,345 individuals were newly diagnosed with a benign or malignant nervous system tumor in 1998 *(2)*. Only about one-half of patients with malignant BTs are still alive one year after diagnosis *(3)*. Controversy continues to surround the issue of whether or not the incidence of BTs, particularly the more lethal subtypes, increased in recent decades *(4–6)*. It appears that trends in childhood *(7,8)* and adult BTs increased because of the introduction of diagnostic improvements, including CT scans in the mid-1970s and magnetic resonance imaging (MRI) scans in the mid-1980s. This issue, and the recent explosion of epidemiological and molecular genetic studies of BTs, has focused attention on this important human cancer, which, up until only a few decades ago, was relatively little studied. Despite this surge of interest, the etiology of the majority of

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nervous system tumors remains unknown. Inherited syndromes that predispose affected individuals to BT development and/or the presence of nervous system tumors in other family members appear to be present in fewer than 5% of BT patients. Some environmental agents, particularly ionizing radiation, are clearly implicated in the etiology of BTs, but also appear to account for few cases. Numerous other physical, chemical, and infectious agents that have long been suspected risk factors have not yet been established as etiologically relevant.

This review focuses on tumors of the brain, cranial nerves, and cranial meninges, which account for 95% of all central nervous system (CNS) tumors. These tumors are unique because of their location within the bony structure of the cranium. Symptoms depend on location of the tumor. Further, histologically benign tumors can result in similar symptomatology and outcome as malignant tumors, because growth of both normal and tumor tissue is confined to the cranial space. For this reason, some cancer registries voluntarily include both benign and malignant intracranial tumors. For simplicity, this group of tumors will be called "brain tumors," or, when benign tumors are excluded, "brain cancer." The term "central nervous system tumors " (or CNS cancer) indicates that tumors of the spinal cord and spinal meninges are included along with BTs, and "nervous system tumors" indicates that tumors of the peripheral nerves are included as well. This review first discusses the descriptive epidemiology of CNS tumors, including the change of incidence rates in different age groups over time; patterns of occurrence by gender, race, geography, and social class; and median survival. Evidence relating to a number of other suggested risk factors is summarized, and prospects for future research explored. For each topic, reference is made only to a few of the numerous relevant papers, but includes a recent paper with a comprehensive bibliography.

2. DESCRIPTIVE EPIDEMIOLOGY

2.1. Variation in Inclusion Criteria

The descriptive epidemiology of CNS tumors has been difficult to study, because of the wide variation in specific tumors included in published rates. Quantitatively, the most important variation is estimated to be approximately 50% and relates to the inclusion or exclusion of benign tumors *(9)*. This critical difference has often been ignored in comparisons across geographic areas. Although reporting of malignant tumors alone eases geographical comparisons, it is unfortunate that incidence rates for benign nervous system tumors are not also reported. For this reason, benign tumors will not be excluded from descriptive data shown here for Los Angeles County. It should be noted that pineal and pituitary tumors, included in some standard definitions of BT and CNS tumors, are not included. In fact, it becomes clear from discussions of analytic studies below, more is known about the etiology of benign histologic types, such as

meningiomas, than about the etiology of neuroepithelial tumors, which are more common than meningiomas and are usually malignant.

Another variation relates to whether or not clinically diagnosed tumors are included. The microscopic confirmation rate of brain and nervous system cancers included in the latest edition of *Cancer Incidence in Five Continents (10)* varies widely across geographic areas, from a high of 99% (e.g., in Los Angeles County Japanese and Koreans) to a low of 0% (e.g., in Setifi, Algeria). Rates vary considerably among registries, as well as among specific population groups within a country. For example, the rates of histologic verification range from 76 to 95% in Switzerland, 27 to 91% in Canada, 45 to 87% in Brazil, 52 to 98% in Japan, and 63 to 99% in the United States *(10)*. Such wide variation suggests that caution in the interpretation of these rates is warranted. In general, for relatively inaccessible cancer sites, a higher rate of microscopic confirmation increases the likelihood that a neoplasm actually existed, and that it was correctly classified. In some registries, however, a high rate of microscopic confirmation of BTs may indicate that clinically or radiologically diagnosed tumors may have been missed. With the advent of radiosurgery, this is an increasing limitation.

2.2. Pathologic Classification

The histologic groups of tumors that occur within the CNS and their corresponding ICD-O codes, are shown in Table 1. A modification of this scheme is proposed for classification of pediatric BTs *(11)*. In both children and adults, neuroepithelial tumors (still more commonly called gliomas) are the most common major histologic type. These are predominantly malignant tumors that arise in the glial cells that comprise the supporting structure of the brain. In Los Angeles, neuroepithelial tumors account for 59% of primary tumors of the brain and cranial meninges, among men and 42% among women. Over 80% of neuroepithelial tumors are astrocytic gliomas (i.e., astrocytomas and glioblastoma multiforme [GBM]). Astrocytic tumors that are grades 1 and 2 are generally classified as astrocytomas, those that are grade 3 are classified as anaplastic astrocytomas (AA), and those with grade 4 are classified as glioblastomas. However, the possibility that this practice is not followed consistently is suggested by the considerable geographic variation in the relative proportions of astrocytic tumors that are classified as glioblastomas. This variation is seen, for example, among the various U.S. registries in the Surveillance, Epidemiology, and End Results (SEER) Program. In comparison with the other SEER registries, Connecticut has a considerably higher proportion of tumors classified as glioblastomas and a correspondingly lower proportion of astrocytomas *(12)*.

The other two most common major histologic types are both predominantly benign. Meningiomas arise in the cranial meninges and account for 20% of all primary BTs in men and for 36% in women. Nerve sheath tumors, called neuro-

Table 1
Anatomic and Pathologic Classification of Tumors of the Central Nervous System

	ICD-O Codes, 1976	*ICD-O Codes, 1991*
Subsite		
Brain	191.0–191.9	C 71.1 – C 71.9
Cranial nerve	192.0	C-72.2 – C72.5
Cerebral meninges	192.1	C-70.0
Spinal cord	192.2	C-72.0
Spinal meninges	192.3	C-70.1
Histologic Type		
Neuroepithelial tumors	9380–9481	
Astrocytoma	9384, 9400–21	
Glioblastoma multiforme	9440–42	
Ependymoma	9391–94	
Primitive neuroectodermal tumor (PNET)	9470–73	
Oligodendroglioma	9450–60	
Other neuroepithelial tumors	9380–83, 9390, 9422–30, 9443, 9472–81	
Meningioma	9530–39	
Nerve sheath tumors	9540–60	
Other	9120–61	
Unspecified	8000–02	
No microscopic confirmation	9990	

mas, neurilemmomas, or schwannomas, arise in the Schwann cells of the nerve sheath. About 8% of BTs in both men and women are nerve sheath tumors. It is curious that about 90% arise in the eighth cranial nerve; these tumors are also known as acoustic neuromas.

Now that improved diagnostic technology is available in many general hospitals in the United States and other industrialized countries, the differential diagnosis of intracranial masses is often made by physicians who are not specialists in neurological diseases. The heterogeneous nature of many CNS tumors makes the assignment of histologic class difficult. In a recent survey in the UK, fewer than one-half of the patients with CT diagnoses were referred to neurosurgeons for histologic confirmation by surgery or biopsy. The positive predictive value of the CT diagnosis was around 90% for neuroepithelial tumors and meningiomas, but only 50% for metastatic tumors *(13)*. The introduction of CT-imaging technology in the United States in the mid-1970s, and MRI in the mid-1980s, appears to have resulted in increases in BT incidence rates, without parallel increases in mortality *(7,8)*. Accuracy of clinical diagnosis of primary BTs will

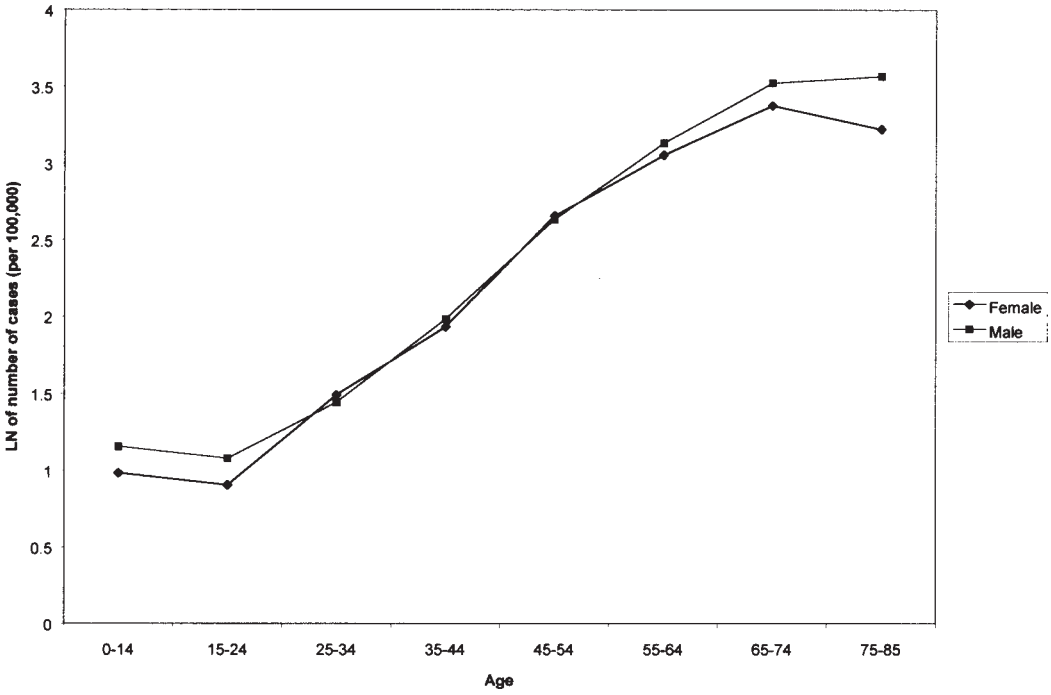

Fig. 1. Average annual age-specific incidence of tumors of the brain, cranial nerves, and cranial meninges (benign and malignant combined) in males and females, Los Angeles County, 1972–1997, whites (excluding Spanish-surnamed). Total cases = 5724 in males and 6180 in females.

continue to vary by geographical region and hospital, even though CT and MRI imaging is now available to a greater proportion of regions in the United States. This may result from variations in how the equipment is used and the degree of training of individuals who interpret the films.

2.3. Distribution by Age and Change in Age Curves and Rates over Time

The average annual age-specific incidence of BTs is shown in Fig. 1. In both males and females, rates decline after a peak in childhood (under age 10), increase after age 25, and level off after age 75. Comparisons of data from different areas of the United States have shown that the shape of the age–incidence curve after age 60 is highly dependent on the autopsy rate *(14)*. Prior to 1955, rates among those over age 55 increased steeply with age in data from Rochester, MN (location of the Mayo Clinic), but decreased after age 55 in data from other areas (e.g., the Second National Cancer Survey, Connecticut and Iowa). Subsequent analyses showed that the proportion of cases first diagnosed at death

Table 2
Average Annual Age-adjusted Incidence Rates (per 100,000) by Major Histologic Type of Primary Brain Tumor by Sex and Ethnic Group, Los Angeles County, 1972–1997

	Neuroepithelial	*Meningiomas*	*Nerve sheath tumors*	*All histologic types*	*(No.)*
Males					
Black	3.9	2.1	0.4	7.0	(679)
Spanish surnamed	4.3	1.3	0.4	6.6	(1200)
Other whites	6.4	1.7	1.0	9.8	(5724)
Chinese	2.6	0.7	0.3	4.3	(85)
Japanese	1.7	0.8	0.9	3.6	(59)
Filipino	2.0	1.6	0.7	4.9	(81)
Korean	2.4	0.4	0.1	3.2	(39)
Other races	1.6	1.2	0.7	3.8	(111)
All races	5.3	1.6	0.8	8.4	(7978)
Females					
Black	2.6	3.0	0.4	6.7	(789)
Spanish surnamed	3.4	2.4	0.4	6.7	(1295)
Other whites	4.3	2.9	1.0	8.9	(6180)
Chinese	1.5	1.6	0.4	3.9	(77)
Japanese	1.1	1.4	0.7	3.5	(68)
Filipino	1.9	1.9	0.6	4.7	(100)
Korean	1.7	1.1	0.3	3.7	(47)
Other races	1.5	1.9	0.8	4.3	(146)
All races	3.6	2.7	0.8	7.8	(8702)

was considerably higher in Rochester than in Connecticut; and, when these cases were excluded from the Rochester data, rates declined after age 65 rather than continuing to rise sharply *(15,16)*. These comparisons suggest that BT incidence continues to increase with age throughout life, but that there is often a significant under-ascertainment of cases in the oldest age groups. Therefore, comparisons of BT rates from different registries may be more meaningful if restricted to age groups under age 65.

2.4. Distribution by Gender, Race, and Geography

In Fig. 1, for all types of BTs combined, rates are higher in males than in females. Table 2 shows the age-adjusted annual incidence rates for the major histologic groups of primary BTs by sex and ethnic group in Los Angeles County, 1972–1997. For all histologic types and races combined, the rate is higher in men than in women. For most ethnic groups, male rates for all histologic types

combined are higher than female rates. The male : female sex ratio (SR) varies considerably, however, by histologic type. In each ethnic group, neuroepithelial rates are higher in males than in females (SR for all races combined = 1.5), and meningioma rates are higher in women (SR = 0.6). The SR in children under age 15 is 1.2 for all tumor types combined. In contrast, primitive neuroectodermal tumors (PNET, formerly called medulloblastomas), which occur almost exclusively in children, have a SR of approx 2 *(17,18)*, but no male excess is seen among U.S. black children *(19)*.

SRs for specific histologic types also vary by anatomic subsite and age group. One of the most interesting examples of this relates to meningiomas. Among non-Spanish surnamed whites in Los Angeles County, spinal meningiomas are 3.5× more common in women than in men (SR = 0.3); cerebral meningiomas are only 1.5× more common in women (SR = 0.7). Similar patterns are seen for meningiomas in Norway *(20)*. Also, the female : male ratio for spinal meningiomas increases with age, but, for cerebral meningiomas, the female excess is greatest during the female reproductive years and declines after age 55. The sex differential for spinal meningiomas suggests the etiologic relevance of some factor related to aging in women. The authors hypothesized that this factor may be vertebral osteoporosis, and a series of three epidemiological studies designed to test this hypothesis do provide it with some, although limited, support *(21)*.

Incidence rates in the Central Brain Tumor Registry of the United States are 12.07 and 10.97/100,000 for males and females, respectively. Overall rates are lower among blacks than among whites (7.72 and 11.6/100,000, respectively) *(22)*. Some of these racial differences vary, however, from one histologic type of BT to another. For example, the rate of neuroepithelial tumors is lower among black males and females than among whites, but the reverse is true for meningiomas (Table 2).

In general, BT rates among whites in Canada, the United States, Europe, the UK, and Australia are relatively similar, although rates are lower in certain Eastern European countries and former Soviet republics (e.g., Russia, Belarus, Krygystan). Rates are lowest in Asian populations in Japan, India, and among Chinese in Singapore. Rates are also lower in Puerto Rico, Costa Rica, and Brazil. Among each racial group, rates are usually higher in migrant populations than in native populations that remain in their country of origin. These differences between migrant and native populations suggest that some change in lifestyle may be occurring in migrant populations that places them at higher risk for BTs, although an increase in diagnostic efficiency may partially explain some of these differences.

2.5. Social Class

Table 3 shows the proportional incidence ratios (PIRs) for primary tumors of the brain and cranial meninges by social class (as determined by census track of

Table 3
Proportional Incidence Ratios (PIRs) for Primary Brain Tumors by Social Class and Total Number of Cases, Los Angeles County, 1972–1997, Non-Latino Whites

Socioeconomic Status	*Proportional Incidence Ratios*		
	Neuroepithelial	*Meningiomas*	*Nerve sheath tumors*
Males			
1 (high)	104.5	93.5	154.1
2	110.6	96.1	113.2
3	106.9	99.2	87.0
4	103.7	100.4	72.9
5 (low)	68.7	114.5	58.8
Females			
1	92.7	96.4	119.0
2	112.7	87.7	121.7
3	108.8	99.1	90.5
4	106.8	114.7	87.0
5	73.7	106.1	70.3

residence) for Los Angeles County non-Spanish surnamed whites. These age-adjusted PIRs represent the ratio of the number of cases observed to that expected in a subgroup. A PIR of 100 indicates that the number observed is the same as the expected number, which was calculated for each 5-yr age group by assuming that the distribution of BTs by social class was the same as that for all other cancer sites combined. There is a clear trend of increasing incidence with increasing social class. For males, this trend is evident for neuroepithelial tumors and nerve sheath tumors. For females, this trend is only clearly evident for nerve sheath tumors. The exception to this is meningiomas, which show the inverse relationship among both males and females. A similar trend of increasing overall brain cancer rates with increasing social class (as determined by occupation) was reported for men in England and Wales a few decades ago *(23)*, and more recently in Washington State *(24)* and New Zealand *(25)*. Because this trend occurs more strikingly among males than among females, it seems unlikely that it may relate to factors such as diagnostic efficiency or exposure to diagnostic radiography of the head (e.g., dental X-rays), both of which might be expected to be greater among those in higher social classes.

2.6. Survival

Recent relative 5-yr survival rates for brain and nervous system cancers are around 25% (24% for whites and 32% for blacks among U.S. cases diagnosed from 1981 to 1986), compared to just under 20% 20 yr earlier (18% for whites

and 19% for blacks diagnosed in 1960–1963) *(26)*. Survival rates for all tumors vary considerably by location, behavior, histologic type, and age *(27)*. For example, astrocytomas that occur in the deep cerebrum have a much lower 5-yr survival rate than those that occur in the frontal lobe (13.3 vs 28.1%, respectively); and survival is lower for malignant vs nonmalignant tumors (21.6 and 72.4%, respectively) *(27)*. The relative 5-yr survival rate in children, ages 0–14 yr, is now 59%, compared to 35% 20 yr ago *(27,28)*. Clinically significant improvements in survival rates are not apparent in patients over the age of 65 yr *(29)*.

In a recent study in Victoria, Australia, 52% of female (compared to 37% of male) BT patients were living 5 yr after diagnosis *(30)*. As might be expected, nerve sheath tumors, tumors in the "other" category (mostly hemangiomas and gangliogliomas not classified as malignant), and meningiomas, all of which are predominantly benign, have the best prognosis; 100, 96, and 92% of patients with tumors of these three types survive 5 years, respectively. Patients with GBM have the poorest prognosis (5% survive 5 years), and the proportion who survive 5 years is also low for patients with unspecified tumors (20%) and those whose tumors were not confirmed microscopically (28%). Survival for the other subtypes of neuroepithelial tumors varies considerably. The proportion of BT patients who are living 5 yr after diagnosis is considerably greater for patients with ependymoma (65%) and oligodendroglioma (61%) than for those with medulloblastoma (43%) or astrocytoma (44%). For most individual histologic types, survival curves for the two sexes are similar. However, females who develop meningiomas are more likely to have benign tumors and survive significantly longer than males with meningiomas (94 vs 87% 5-yr survival in women and men, respectively).

2.7. Summary of Descriptive Epidemiology

Perhaps the most important finding from this review of the descriptive epidemiology of brain tumors is that the patterns of occurrence and survival both vary considerably by histologic type, age, and tumor location. For neuroepithelial tumors: the SR (male : female) is greater than one; incidence declines after an early peak under age 10 and continues to rise again after age 25; rates are higher in whites than nonwhites, and are lowest in Asians; and incidence increases with increasing social class, particularly in males. For meningiomas: the SR is less than one (females > males); the female excess is greatest from ages 25 to 54; and rates in U.S. populations are commonly higher in blacks than in whites.

3. SUGGESTED CAUSES OF HUMAN BT

3.1. Ionizing Radiation

The occurrence of excess BTs after high-dose exposure to ionizing radiation is well established. An updated follow-up of the Israeli cohort who received

scalp irradiation as a treatment for ringworm showed the relative risk (RR) is greatest for nerve sheath tumors of the head and neck (RR = 33.1), intermediate for meningiomas (RR = 9.5), and lowest for neuroepithelial tumors (RR = 2.6) *(31)*. Case–control studies of meningiomas and nerve sheath tumors in adults have found elevated risks associated with exposure to full-mouth dental X-rays decades ago (when doses were relatively high), as well as with prior radiation treatment to the head *(32–35)*. The association with low-dose exposure is more controversial. Prenatal exposure to diagnostic radiography has been related to excess pediatric BTs in several studies, since this association was first reported in 1958 *(36)*, including a study of Swedish twins that found abdominal X-rays of the mother during pregnancy were associated with increased CNS tumor incidence. The findings appeared not to be confounded by mother's age, obstetrical complications, or other factors *(37)*. Exposure to low levels of ionizing radiation during infancy was associated with an elevated risk of intracranial tumors (Standardized Incidence Ratio (SIR) 1.42; Confidence Interval (CI) = 1.13 to 1.75) in a pooled analysis of two Swedish hemangioma cohorts and was highest among infants exposed before 5 mo of age *(38)*.

3.2. Nonionizing Radiation

3.2.1. Electromagnetic Fields

Much controversy in the last 15 years has surrounded the suggestion that exposure to nonionizing electromagnetic radiation, such as power frequency (50–60 Hz) magnetic fields, may contribute to the development of CNS tumors. These fields have not been shown experimentally to be either genotoxic *(39)* or carcinogenic *(40)*, but there is some suggestion that they may act as a tumor promoter *(40)*. Epidemiological evidence is puzzlingly inconsistent, both in studies of residential exposures and pediatric CNS tumors *(41,42)* and in studies of CNS cancer in relation to high levels of job exposure *(43–45)*. Two recent studies *(42,46)* showed no evidence of a link between residential exposure and BTs in children, and a recent review article *(47)* concluded that, overall, there is little evidence for an association.

3.2.2. Radiofrequency Radiation

Studies of the effect of radiofrequency (RF) exposure in humans have included microwave exposures, the use of radar equipment (occupational and handheld), and direct occupational exposures (such as RF heaters, sealers, plastic welders, medical exposures, amateur radio operator exposures, and telecommunication worker exposures). Although some studies suggest a possible effect of RF on all cancers, brain cancer, or specific other types of cancers, the data are equivocal. An association with cellular telephone usage and the development of BTs has been raised in the legal arena. The rapid increase in the use of cellular tele-

phones, combined with their direct exposure to selected regions of the brain, has stimulated ongoing epidemiological studies *(48)*.

3.3. Occupational Exposures

Numerous epidemiological studies have investigated the variation in BT occurrence as it relates to employment, which have been summarized previously *(49)*. Repeated studies in various geographic areas have been completed for only a few groups of workers, including those employed in agricultural *(25,50,51)* or health professions *(52,53)*, and for rubber *(54)*, petrochemical *(55,56)*, and electrical *(44,45)* workers. Various studies of each occupational group have shown conflicting findings. For the most part, no specific chemical or other exposure has been implicated. Even when a particular chemical exposure was investigated, results have been inconclusive, as in studies of job exposure to vinyl chloride, which were prompted by experimental findings *(57,58)*.

Similarly, several studies have investigated possible associations between occupational exposures of parents and the development of BTs in their children, but many associations have been suggested by only single studies. Multiple studies have suggested an increase in pediatric BT risk among children with a parent employed in paint-related, aircraft, electricity-related, agricultural, metal, and construction industries, although these studies have also failed to implicate any particular exposure *(59)*.

The marked inconsistency of these occupational studies may be partially attributed to: the often small sample sizes (leading to imprecise risk estimates); the different geographic areas (with different major industries) studied; the variation across studies in tumor types, ages at diagnosis, years of diagnosis; and the presumed latent period from exposure to tumor diagnosis. The exposure periods of interest also vary, and, thus, for many industries in which procedures changed over time, the exposures vary as well. Variation also relates to the sources of data about and the criteria used for classifying occupations and nervous system tumors. All studies are limited by the fact that occupation is acting as a surrogate for often unidentified specific environmental agents, the true exposures of interest.

3.4. Pesticides

Several epidemiological studies have investigated home and occupational use of pesticides, insecticides, or herbicides as possible etiologic factors for BTs, which have been reviewed *(60,61)*. Excess risk of brain cancer was found in a study of licensed pesticide applicators (Standardized Mortality Ratio SMR = 200) *(62)* and occupational exposure to pesticides (RR = 1.8; 95% CI = 0.6-5.1) *(43)*. Some case–control studies have linked household use and pest exterminations to the development of childhood BTs *(63)*, but few associa-

tions were seen in a recent study of pesticide exposure during gestation and pediatric BTs *(64)*. Associations of CNS tumors with either household or occupational exposures to pesticides are not well established and require further confirmation.

3.5. Nitroso Compounds

Although various chemical, physical, and biological agents can cause nervous system tumors in experimental animals, *N*-nitroso compounds (NOCs), particularly the nitrosoureas, are by far the most effective and the most studied *(65)*. These carcinogens show striking nervous system selectivity in some species, including various primates, and tumors can be produced by relatively low levels of NOC precursors in the animals' food and drinking water. If exposure is transplacental, only one-fiftieth (1/50) the dose of ethyl nitrosourea (ENU) required in adult animals is sufficient to cause 100% tumor induction *(66)*. However, no tumors develop if ascorbate (Vitamin C) is also added to the pregnant dam's diet *(67)*. Because there is no reason to think that man is less susceptible to these compounds, it is likely that NOCs cause cancer in humans as well. Although NOC exposure in some occupational settings (e.g., machine shops, tire and rubber factories) can be substantial, most people have low-level, but virtually continuous, exposure to NOC throughout life. However, because NOCs are the most potent of carcinogens in animals (and probably in humans as well), only small doses are needed to cause cancer.

3.5.1. Population Exposure to NOC

Human exposure to NOC is estimated to derive half from exogenously made and half from endogenously formed compounds *(68)*. Only levels of nitrosamines (not nitrosamides) have been widely measured in human environments and consumer products, even though many of these exposures probably involve both nitrosamines and nitrosamides. Endogenous formation in the stomach or bladder, when both an amino compound and a nitrosating agent are present simultaneously, is likely to be the primary source of human exposure to nitrosamides. Food is a primary source of both highly concentrated nitrite solutions (e.g., from cured meats) and amino compounds (e.g., in fish and other foods, but also in many drugs). Another source of nitrite is reduction (e.g., in the saliva) from nitrate, which comes predominantly from vegetables in the diet. This source is likely to be a far less important contributor to the NOC formed endogenously, because it is highly diluted (and, therefore, less readily reactive), and because vegetables also contain vitamins that inhibit the nitrosation reaction. Drinking water also contains nitrate (in the absence of vitamins), but this is a minor source unless levels are extraordinarily high *(69)*. The level of NOC in the human body is also influenced by other factors, such as the amino compounds present, presence of nitration inhibitors (e.g., vitamins C or E), presence of bacteria or other nitration catalysts, gastric pH, and other physiologic factors. Uncertainties as to

the simultaneous presence of NOC precursors and of inhibitors and/or catalysts of nitration make this hypothesis difficult to study epidemiologically. This difficulty is compounded by further uncertainty about what exposure period during a person's life is most likely to be etiologically relevant.

3.5.2. Epidemiologic Evidence

Epidemiological studies of pediatric *(70–73)* and adult *(74–78)* BT patients have provided limited support for the hypothesis that NOC exposure is related to the development of CNS tumors. Findings that the use of vitamin supplements and/or high intake of fresh fruit or vegetables protect against BT development may also be interpreted as supportive of the *N*-nitroso hypothesis, although this effect may result from another mechanism *(71)*. The experimental model and its potential relevance to humans are sufficiently compelling to encourage further investigation of this hypothesis, despite the fact that it is a difficult one to test epidemiologically. Future studies must include more complete dietary histories, if they hope to differentiate between findings supportive of the NOC/BT hypothesis and those suggestive of other mechanisms of dietary effects.

3.6. Other Dietary Factors

Most dietary investigations among CNS tumor patients have only collected data on dietary sources of NOC exposures, rather than complete dietary histories. Nonetheless, these studies have attempted to evaluate the association between certain dietary micronutrients and BT risk. Adequate evaluation of micronutrient intake will require investigation of complete dietary histories. Use of vitamin supplements (particularly vitamins C, E, and multivitamins), has been found to reduce BT risk in adults in some studies *(77)*, but not others *(78,79)*. In children, risk may be reduced by the child's personal vitamin use *(80,81)*, by the mother's vitamin use during pregnancy *(73,82,83)*, or by her intake of fruit, fruit juice, and vegetables *(71,73,81)*.

Although the findings of reduced risk of BTs in children and adults associated with increased intake of vitamin supplements, fruits, and vegetables may be related to the *N*-nitroso hypothesis by inhibiting endogenous formation of nitrosamines, it is important to consider other potential mechanisms of this effect. In this respect, it is interesting that a study of childhood BTs reported higher RRs associated with the child's consumption of cured meats when the child did not take multivitamins than when they did take multivitamins *(84)*.

Recent studies have investigated the possible associations of BTs with other dietary micronutrients *(71,73)*. In particular, a case–control study of childhood PNET *(73)* found significant protective trends with increasing levels of dietary vitamins A and C, β-carotene, and folate taken by the mother during pregnancy. In a related study of childhood astrocytoma, reduced risks were evident for

dietary vitamins A and C, but these trends were not significant *(71)*. There was no relationship between childhood astrocytoma and dietary β-carotene and/or folate. Although these preliminary results suggest exciting prospects for the possible prevention of childhood BTs, interpretation is difficult, because both studies were primarily focused on the evaluation of dietary NOCs. Thus, evaluation of other micronutrients was limited to the micronutrient composition of NOC-related food items. These results highlight the need to incorporate complete dietary evaluations into future epidemiological studies.

3.7. Prior Head Trauma, Infection, or Other Medical Conditions

3.7.1. Head Trauma

The epidemiological evidence associating head trauma and BTs is strongest for meningiomas. Numerous case reports have presented convincing circumstantial evidence. Case–control studies have found an excess risk of meningiomas in women with histories of head trauma treated medically, in men who boxed as a sport, and in men with histories of serious head injuries *(32–34,83)*. Limited experimental evidence suggests that trauma may act as a co-carcinogen in the induction of neuroepithelial tumors, as well as meningiomas *(85)*. Childhood BTs, which are predominantly neuroepithelial tumors, have sometimes been associated with birth trauma, such as prolonged labor, forceps delivery, and Caesarean section *(46,81,82)*. Because trauma is often regarded by lay persons as related to tumor development, an attempt must be made to limit the reporting of trauma to injuries of a certain minimum severity (such as those requiring medical attention or hospitalization), and thereby limit recall bias.

3.7.2. Acoustic Trauma and Acoustic Neuromas

The observation that over 90% of all nerve sheath tumors arise in the eighth cranial nerve (the acoustic nerve) suggests an exposure unique to this nerve. A case–control study of acoustic neuromas in Los Angeles County residents supports the hypothesis that acoustic trauma may be related to the development of these tumors *(86)*. A dose–response analysis showed an increase in risk related to the number of years of job exposure to extremely loud noise (p for trend = 0.02), with an overall risk of 13.2 (CI = 2.01 to 86.98) for exposure of 20 yr or more, accumulated up to 10 yr before diagnosis. These findings may support the more general hypothesis that mechanical trauma could contribute to tumorigenesis *(84)*.

3.7.3. Viruses and Infectious Agents

Astrocytomas, but not other histologic types of BTs, were previously associated with positive antibody titers to *Toxoplasma gondii*, but a recent study failed to confirm this *(87)*. There are numerous reports in the literature of the isolation of viruses or virus-like particles from human cerebral tumors or tumor cell lines,

but whether these findings may have etiologic implications is uncertain *(88)*. Excess BTs have not been found among those who received polio vaccines contaminated with SV40 or those with mothers who had influenza or various other infections while they were *in utero (89)*. Recently, a reduced risk has been reported between patients with neuroepithelial tumors and chickenpox, shingles, or the associated immunoglogin G antibodies to the *Varicella zoster* virus. This is a novel finding requiring replication *(90)*.

3.7.4. Chronic Diseases

BTs have been associated with various chronic diseases, but none of these associations have been investigated in more than one or two studies. Neuroepithelial tumors, but not meningiomas, occur much less frequently in diabetics *(91)*, who have a lower frequency of all cancers at autopsy *(92)*. Excesses of BTs reported in various cohorts of epileptics probably occur because seizures are a common early BT symptom *(93)*, and studies have found no increase in risk related to *in utero* or childhood exposure to barbiturates after a history of epilepsy was considered *(94)*. Serum cholesterol has been positively related to brain cancer in some, but not all, studies *(95)*; but, because none have evaluated dietary intake, the possibility that an existing BT may cause a spurious rise in serum cholesterol has not been excluded. A deficit of allergic conditions has been found in case–control studies of neuroepithelial tumors alone *(79)*, and of all BTs *(91,96)*.

Clinicians should be aware that an association between meningiomas and breast cancer has been observed *(97)*, so that they will not assume that CNS lesions that are discovered after breast cancer diagnoses are necessarily metastatic. Tissues from meningiomas have been shown to contain hormone receptors, but it is unclear whether or not this finding has etiologic implications *(98)*.

3.8. Predisposing Genetic Syndromes and Familial Occurrence

Some CNS tumors have a relatively clear genetic character, particularly those that occur in association with neurofibromatosis and other phakomatoses, which often display an autosomal dominant pattern of inheritance with varying degrees of penetrance. The occurrence of multiple primary BTs of either similar or different histologic types are associated with the phakomatoses, but also occur in the absence of such syndromes *(99)*.

There are few population-based studies of familial aggregation of CNS tumors. One study found that Connecticut children with CNS tumors more often had relatives with nervous system tumors than did control children. However, this familial occurrence, although statistically significant, was observed for fewer than 2% of the children with CNS tumors *(100)*. Medulloblastomas and glioblastomas were overrepresented among children whose relatives had nervous system tumors *(100)*. Population-based studies that have investigated associa-

tions of BTs with recognized predisposing genetic syndromes, and/or with familial aggregations, have suggested that the proportion of BTs attributable to inheritance is no more than 4% *(101,102)*.

3.9. Other Suggested Risk Factors

A number of other factors have been suggested as being related to BT risk, including barbiturates and other drugs, alcohol, tobacco smoke, and reproductive/hormonal factors *(89)*, but these possible associations have not been studied often or very thoroughly. The few BT studies that have investigated some factors (e.g., alcohol and tobacco) have had contradictory findings. The best one can do in attempting to evaluate their etiologic relevance is to keep them in mind, and hope that future BT studies will also investigate possible associations with these factors.

4. PATHOGENESIS OF NERVOUS SYSTEM TUMORS

Various physical, infectious, and chemical agents appear to relate to the development of cancer because they increase cell proliferation *(103)*. For example, this may explain why acoustic trauma can lead to the development of acoustic neuromas *(86)*. Replication may perpetuate a DNA mutation before it can be corrected in the cell in which it arises. Apparently, various genetic pathways can be involved in the pathogenesis of CNS tumors (as reviewed in Chapter 1 of this volume), and this may be true even for tumors of the same phenotype *(104,105)*. Although many of the inherited syndromes that predispose to CNS tumors were described decades ago, the chromosomal loci of the affected genes have now been identified for most. In the past decade, hundreds of investigators have described molecular events that they have observed in tumor tissue from patients with various types of CNS tumors. A few of the most common of these mutations, which may interact with the environmental epidemiology of brain tumors, are summarized below.

4.1. Molecular Genetic Characteristics

Studies of the molecular biology and cytogenetics of CNS tumors suggest that specific types of tumors have characteristic genetic abnormalities, which have been summarized in review papers *(106–108)*. Such characterization contributes to understanding the pathogenesis of CNS tumors. Glioblastomas, for example, commonly show losses of chromosomes 9p, 10, or 17p, and gains of chromosome 7. Losses of alleles at 17p appear to be the earliest abnormalities that occur in the genesis of these tumors. Most of these tumors express the c-*sis* oncogene, and some express other oncogenes as well. Related characteristics

include the synthesis and secretion of growth factors and/or their receptors that influence mitotic activity *(109)*.

Various CNS tumors (but particularly those of astrocytic origin) have been associated with loss or mutation of the *p53* gene located on the short arm of chromosome 17 *(110)*. *p53* is a tumor suppressor gene, and mutations in this gene appear to play a role in the development of a number of human cancers *(111)*. *p53* mutations have been observed in GBM, as noted *(111)*, in neurofibrosarcoma occurring in association with neurofibromatosis 1 *(111,112)*, and in patients with Li-Fraumeni syndrome *(113)*, which is a rare autosomal dominant genetic syndrome that predisposes those affected to cancers of the brain and other sites *(114)*. This predisposition may relate to germ cell mutations in the tumor suppressor gene, *p53 (113)*. Because benign tumors from patients with neurofibromatosis 1 appear not to have *p53* mutations, it is thought that inactivation of this gene may be associated with the malignant transformation of these tumors *(112)*.

Other tumor types show distinct pathophysiologic features. For example, loss of regions on chr 22 is the characteristic feature of meningiomas. Also, pediatric CNS tumors show different genetic patterns than adult tumors *(28,115)*. For example, astrocytomas (World Health Organization grades II–IV) in children/young adults may demonstrate loss of heterozygosity for chr 17p and/or mutations in *p53 (115–118)*; astrocytomas in older adults often have mutations in chr 10, amplification of the epidermal growth factor receptor, and no mutations in *p53 (116,117)*. The characterization of the various tumor types is still in progress. The etiologic, prognostic, and other implications of specific characteristics remain to be defined. It is anticipated that molecular markers may aid in reducing the known misclassification in the diagnosis of some tumor subtypes.

4.2. Possible Interactions of Genetic and Environmental Factors

For a number of other reasons, epidemiological studies of the hypothesis that nitrosamide exposures relate to BTs are very difficult. For this reason, it is appealing to be able to rely on some biomarker of exposure. Unfortunately, finding a biomarker of *N*-nitroso exposure for use in BT patients (or their mothers), when the relevant exposures occurred years earlier, has not proved easy. Adduct formation by *N*-nitrosoureas in vivo is beginning to be studied, but the extent of damage induced in various tissues does not seem to correlate well with tumorigenicity *(119)*. It may be more promising to identify a genetic polymorphism (one that could easily be assayed in epidemiological studies) for an enzyme or other system that regulates *N*-nitroso metabolism, or that repairs the molecular damage caused by nitroso compounds. One interesting candidate may be alkyltransferase, an enzyme involved in the repair of O^6-alkylguanine, which is

formed and persists in brain DNA after exposure to alkylating agents, such as the nitrosoureas *(120)*. Nitrosoureas produce different types of nervous system tumors in different species. Identifying those histologic types in humans will also make future studies of nitrosamide exposures more efficient.

Many of the problems confronted by epidemiological studies of BTs and nitrosamides also apply to studies of other suspected brain carcinogens, such as the several agents investigated in occupational studies. Although a number of industries have long been noted to have an apparent excess of BTs among workers, it has proved difficult to implicate specific exposures. Simultaneous evaluation, both of exposures to specific chemicals and of individual susceptibility to insult from those chemicals, may be the direction of the future.

5. PROSPECTS

We simply have no idea what causes most nervous system tumors. Certain inherited syndromes may predispose individuals to the development of BT and other nervous system tumors. However, at most, only a few percent of patients with nervous system tumors have one of these rare phakomatoses, or a family member with a nervous system tumor. Studies of such patients and their families have described genetic events that are correlates of nervous system tumor pathogenesis, but the etiologic implications of these findings are unclear.

Ionizing radiation, the only well-established environmental risk factor for nervous system tumors, can cause all three major histologic types of BTs: neuroepithelial tumors, meningiomas, and nerve sheath tumors. However, only a few percent of incident CNS tumors are likely to relate to such exposure, and the association appears weakest for gliomas. Nonetheless, minimizing population exposure to X-rays of the head is, at this point, the best prospect for prevention of all three types of tumors. Beyond this, the environmental etiology of neuroepithelial tumors remains largely unknown. More is known about the etiology of meningiomas and nerve sheath tumors. Ionizing radiation and trauma appear to be important risk factors for both.

Because nitrosamides, especially the nitrosoureas, are the most potent nervous system carcinogens used experimentally, it seems likely that these compounds may also cause nervous system tumors in humans. To date, most epidemiological studies of a possible association of BTs with *N*-nitroso exposures have focused on the other major group of these compounds, nitrosamines. Nitrosamines are easier to study, because reliable assays exist for nitrosamines, unlike nitrosamides, and monitoring of human environments and consumer products for levels of nitrosamines has been done. However, nitrosamines have not caused nervous system tumors in any of the many experimental species tested. Field and laboratory investigations of potential environmental sources of human exposure to nitrosamides and of their precursors (such as alkylamides) are needed.

Given that assays of relevant genetic polymorphism are not yet incorporated into epidemiological studies of nervous system tumors, what further work seems indicated? Diet will be an important focus of the next generation of epidemiological studies of neuroepithelial tumors. Studies to date have included some questions about a limited number of dietary variables, such as the several studies that looked at foods thought likely to be relevant to the *N*-nitroso hypothesis. A number of intriguing associations are emerging from these and other studies, including the suggestion that intake of cured meats, fruit, and vitamin supplements all relate to neuroepithelial tumor risk, with fruit and vitamins being protective. Future studies must include relatively complete dietary surveys, to adequately evaluate associations with various micronutrients, cholesterol, nitrite from cured meats, and other suggested associations.

Are there additional etiologic clues to be gleaned from the descriptive epidemiology of BT? The increase in incidence and mortality rates in recent decades was initially thought by some to suggest the effect of an environmental exposure, but on further consideration appeared to be largely an artifact of improved diagnosis. Compared to other cancer sites, BT rates show relatively little international variation. This suggests that either the relevant environmental exposures are ubiquitous or that endogenous factors are important. The gender differences in distribution by histologic type of BT, namely, the male predominance of neuroepithelial tumors and the female predominance of meningiomas, have long been noted. Although evidence suggesting the importance of hormonal factors is weak, any compelling hypothesis related to this difference would be worth investigating. Most BTs in children are neuroepithelial tumors, and some types such as PNET, occur predominantly in children under age 5 yr. The observation that PNET rates, unlike rates of other pediatric BTs that are similar in the two genders, are up to 2× higher in boys than in girls, also remains unexplained. For neuroepithelial tumors as a major group, as well as for specific glioma subtypes, some of the crucial etiologic questions have not yet been posed.

The etiology of the majority of nervous system tumors remains unexplained. Genetic predisposition, ionizing radiation, and other suggested risk factors each seem to account for only a small proportion of total cases. It may be that there are numerous nervous system carcinogens, such as known animal neurocarcinogens, which have not been fully evaluated in human studies, each with low attributable risk. Continued investigation of suspected brain carcinogens needs to identify and focus on histology-specific associations, and to use improved methods of exposure assessment.

In addition, host factors that influence susceptibility need to be simultaneously considered. In particular, detectable polymorphisms and host immune responses may play important roles in the etiology, progression, and potential treatment of CNS cancers. The relationship of immunological variables and BT develop-

ment has not been well studied in epidemiological investigations. Future studies of such variables are warranted, and need to be considered when interpreting the potential successes and failures of BT immunotherapy trials.

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