The Role of Herpesviruses in Brain Tumor Development

INAUGURAL-DISSERTATION

zur Erlangung des Grades eines

Dr. med. vet.

beim Fachbereich Veterinärmedizin

der Justus-Liebig-Universität Gießen

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Eingereicht von
Sabine Poltermann

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Meiner Omi,
in Liebe

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List of Abbreviations

Ab	Antibody
ACN	Acoustic neurinoma
AEC	3-amino-9-ethylcarbazol
approx.	Approximately
bp	Base pairs
BSE	Bovine spongiforme encephalitis
°C	Degrees Celsius
CBTRUS	Central Brain Tumor Registry of the United States
cf	confer
Chap.	Chapter
CI	Confidence interval
CID	Cytomegalic inclusion disease
CJD	Creutzfeld-Jacob disease
CNS	Central nervous system
CO_2	Carbon dioxide
CSF	Cerebrospinal fluid
CT	Computed tomography
D	DNA ladder
DAE	Deutsche Arbeitsgemeinschaft Epidemiologie
dATP	Deoxy adenosine triphosphate
dCTP	Deoxy cytosine triphosphate
ddH_2O	Double distilled water
dGTP	Deoxy guanosine triphosphate
DKFZ	Deutsches Krebsforschungszentrum
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
dNTP	Deoxy nucleoside triphosphate
dTTP	Deoxy thymidine triphosphate
EA	Early antigen
EB	Electrophoresis buffer
EBMT	European Group for Blood and Marrow Transplantation

EBV	Epstein-Barr virus
EDTA	Ethylenediaminetetraacetic acid
e.g.	Exempli gratia
ELISA	Enzyme Linked Immunosorbent Assay
et al.	et alii (and others)
EtBr	Ethidium bromide
FCS	Fetal calf serum
FH	Fachhochschule
Fig.	Figure
FSME	Frühsommer-Meningoenzephalitis (tick-borne encephalitis)
GAPDH	Glyseraldehyde-3-phosphate dehydrogenase
gB	Glycoprotein B
GBM	Glioblastoma multiforme
GI	Gastrointestinal
h	Hour (s)
HCMV	Human cytomegalovirus
HHV	Human herpesvirus
HIV	Human immunodeficiency virus
HRP	Horseradish peroxidase
HSV	Herpes simplex virus
IARC	International Agency for Research on Cancer
ICD-9	International Statistical Classification of Diseases and Related Health Problems, 9th version
ICD-10	International Statistical Classification of Diseases and Related Health Problems, 10th version
ICD-O-3	International Classification of Diseases for Oncology, 3rd version
IE	Immediate early antigen
i.e.	Id est
Ig	Immunoglobulin (IgA, IgG, IgM)
IHC	Immunohistochemistry
IHV	Industrieverband Heimtierbedarf e. V.

ISCO	International Standard Classification of Occupation
kbp	Kilo base pairs (1 kbp=1000 bp)
LA	Late antigen
M	Molar
$MgCl_2$	Magnesium chloride
min	Minute (s)
ml	Milliliter
MMR	Measles, mumps rubella combined immunization
MOI	Multiplicity of infection
MRI	Magnetic resonance imaging
NC	Negative control
NF	Neurofibromatosis
nm	Nanometer
OR	Odds ratio
PBS	Phosphate buffered saline
PC	Positive control
PCR	Polymerase chain reaction
PNET	Primitive neuroectodermal tumor
POD	Peroxidase
Pp65	Phosphoprotein 65
RKI	Robert Koch Institute
RNA	Ribonucleic acids
rpm	Rotations per minute
RR	Risk ratio
RT	Room temperature
SE	Standard error
sec	Seconds
SES	Socioeconomic status
SP	Seroprevalence
STIKO	Ständige Impfkommission des RKI
SV40	Simian virus 40
Tab.	Table
TBS	Tris buffered saline

TMB	Tetramethylbenzidine
Tris	Tris(hydroxymethyl)aminomethane
U	Unit
UK	United Kingdom
USA	United States of America
UV	Ultraviolet light
v/v (%)	Percent by volume
VZV	Varicella-zoster virus
WHO	World Health Organization
W	Watt
w/v (%)	Percent by weight per volume
y	years

Introduction

Malignant gliomas are the most common primary brain tumors in adults [Becker and Wahrendorf, 1998] and generally rapidly fatal despite current therapies. They are thought to be primarily astrocytic in origin, with the most malignant form being WHO grade IV astrocytoma (also called glioblastoma multiforme, GBM). Although a lot of research has been carried out on their etiology, the only confirmed risk factors are hereditary predisposition and high dose of ionizing radiation. Inconsistently reported risk factors are several occupational exposures (e.g. to chemical substances and metals), head injuries and other medical conditions, environmental and dietary risk factors (e.g. N-nitroso-compounds) [Wrensch et al., 2002; Preston-Martin, 1996].

Among the potential medical risk factors discussed in the literature is the occurrence of common infections, for which an inverse association has been reported in an international multicenter case-control study including glioma patients and population-based controls [Schlehofer et al., 1999]. This inverse correlation might possibly be due to a stimulation of the immune system. Furthermore, viruses and other infectious agents are suggested to be involved in brain tumor pathogenesis or progression since years. Multiple studies focused on the presence of infectious agents in brain tumor tissues as well as on antibodies to several infectious agents. Astrocytomas have been positively associated with antibodies to Toxoplasma gondii by some investigators whereas others could not confirm these findings [Wrensch et al., 2002; Preston-Martin, 1996; Inskip et al., 1995; Ryan et al., 1993]. Furthermore, a Simian virus 40 (SV40) contaminated polio vaccine was administered between 1954 and 1962, which was suggested to increase brain tumor incidence in vaccinated individuals. This polyomavirus is well known to induce brain tumors in hamsters if inoculated intracerebrally; however, epidemiological studies on brain tumor development among numerous subjects vaccinated with this contaminated vaccine remain conflictive [Sabatier et al., 2005a; Brenner et al., 2003; Bondy and Wrensch, 1996; Preston-Martin and Mack, 1996].

Recently, Cobbs et al. [2002] hypothesized that human cytomegalovirus (HCMV, human herpesvirus-5) might be involved in the development or progression of gliomas. In all 27 glioma biopsies examined in their study, multiple HCMV gene products were expressed in

contrast to samples from other brain tumors, several non-tumor brain diseases, and normal brain tissue.

There is strong evidence that human herpesviruses are implicated in the pathogenesis of several human malignancies (e.g. Kaposi's sarcoma, Burkitt's lymphoma, and Hodgkin's disease). Herpesviruses establish latency and reactivation occurs after years and may lead to neoplasms, including brain tumors. Higher titers of anti-herpes simplex virus (HSV) serum antibodies in glioblastoma and pituitary adenoma patients compared to patients with astrocytoma, medulloblastoma, meningioma or metastatic tumors had been reported in a seroepidemiological study [Hadfield et al., 1984]. Furthermore, Wohlrabe et al. [1984] reported that patients with cerebral tumors were more likely to have an acute herpesvirus infection. In contrast, the mean geometric antibody titers against herpesvirus in brain tumor patients did not differ from age- and gender-matched healthy controls in this clinical survey.

In an epidemiological study, infection with some herpesviruses (notably varicella-zoster virus, VZV) was suggested to counteract glioma development; an inverse association between onset of adult glioma and history of chickenpox and shingles was reported by Wrensch et al. [1997a]. In another case-control study by the same study group, an inverse association with immunoglobulin G (IgG) antibodies to VZV in those glioma patients with positive self-reported history of chickenpox was found [Wrensch et al., 1997b]. This inverse association could be specified in another population-based case-control study by the same investigators addressing the occurrence of IgG antibodies to several herpesviruses in brain tumor patients [Wrensch et al., 2001]. In this epidemiological study, glioblastoma cases were significantly less likely to have IgG antibodies to VZV. In addition, glioblastoma cases were less likely to have antibodies to EBV and more likely to have antibodies to HSV and HCMV than controls.

Given the fact that so far few definite etiological factors for brain tumor development have been identified, further research is needed to understand more about brain tumor pathogenesis for the prevention of this disease. As the oncogenicity of several viruses is well known, and ensuing from the above-mentioned studies, a scientific focus on the role of herpesviruses in brain tumor etiology is warranted. The present study was particularly conducted to evaluate the role of herpesviruses as a possible risk factor for the development of gliomas, meningiomas and acoustic neurinomas.

Study Intention

The main intention of this study was to evaluate the role of herpesviruses, especially of HCMV, in the development or progression of primary brain tumors as previously suggested. For this, 76 patients with incident primary brain malignancies could be recruited.

No information on the serological status towards any herpesviruses was available prior to the analyses.

The following questions were addressed in the present study:

- Presence of HCMV molecules in primary brain tumor tissues
- Frequency of HCMV viremia in brain tumor patients' blood
- Prevalences of IgG antibodies to HCMV, HSV, EBV and VZV in brain tumor patients to assess previous infections with these viruses
- Prevalences of IgM antibodies to HCMV, HSV, EBV and VZV to assess acute herpesvirus infections
- Comparison of brain tumor patients' prevalences of IgG antibodies to HCMV, HSV, EBV and VZV with previous publications concerning the serological status in the German population
- Evaluation of previous herpesvirus infections and putative risk factors indicative for a viral pathogenesis of primary brain tumors using a questionnaire inquiring medical and occupational history by telephone or direct interview
- Assessment of demographic data and several other medical risk factors

1 Background and State of the Art

(A) Clinical Background

Because there is no overall German cancer registry, as it is in other countries, and because of the low incidence of primary brain tumors, a major problem in describing these neoplasms is the frequent lack of tumor-type specific descriptive German data. Therefore, this section first describes data available for all primary brain tumors combined, followed by sections about the three histological brain tumor types included in the present study (gliomas, meningiomas, and acoustic neurinomas). Data from countries other than Germany are given if procurable.

1.1 Primary Brain Tumors in Humans

1.1.1 Epidemiology and Classification

Primary brain tumors include a broad variety of histologically different cancers. They comprise tumors of the neuroepithelial tissue, like gliomas (which build the great majority), embryonal tumors (e.g. medulloblastomas), and schwannomas (neurinomas). Furthermore, primary brain tumors include tumors of meningothelial cells (meningiomas), lymphomas and hemopoietic neoplasms, germ cell tumors and tumors of the sellar region [Grisold et al., 2000]. Whereas this definition of primary brain tumors includes all tumors primarily arising in the brain, narrower definitions only include tumors arising from brain tissues.

Brain tumor classification is based on the WHO classification of tumors of the nervous system. Earlier attempts to develop a TNM-based classification were dropped: tumor size (T) is less relevant than tumor histology and location, nodal status (N) does not apply because the brain has no lymphatics, and metastatic spread (M) rarely occurs because most patients with CNS neoplasms do not live long enough to develop metastases [Kleihues et al., 2002]. The first edition of the WHO classification system was published in 1979 by Zülch and it took

almost a decade to complete. Since then, the WHO system has been revised for several times. It classifies all tumors in four grades, from benign (differentiated, slow growing tumor without metastases, WHO grade I) to malignant (undifferentiated, fast growing tumor with metastases, WHO grade IV). However, classification of CNS tumors is a dynamic issue requiring constant review as application of newly established laboratory methods helps to improve diagnostic tools. There is agreement that the WHO grading can only be an estimate of malignancy for most brain neoplasms. However, for diffuse glial tumors, for which a spectrum of progression from low to high grade exists, this system may be a true grading system [Kleihues et al., 2002].

A further attempt is to classify all tumors according to the ICD codes (International Classification of Diseases*). This is a system to encode diseases and causes of death according to their localization. The 9th revision of this classification system (ICD-9) has been replaced by ICD-10 in 2000. Because the ICD-9 codes are still frequently used, Tab. 1 gives ICD-9 as well as ICD-10 codes for neoplasms of the central nervous system.

Table 1: International classification of diseases according to the World Health Organization

Tumor Types	ICD-10		ICD-9	
	Malignant	**Benign**	**Malignant**	**Benign**
Neoplasms of the brain				
excludes: cranial nerves, retrobulbar tissue	C71	D33	191	225.0
Cerebrum, *except* lobes and ventricles	C71.0	D33.0	191.0	225.0
Frontal lobe	C71.1	D33.0	191.1	225.0
Temporal lobe	C71.2	D33.0	191.2	225.0
Parietal lobe	C71.3	D33.0	191.3	225.0
Occipital lobe	C71.4	D33.0	191.4	225.0
Cerebral ventricle; *excludes*: 4th ventricle	C71.5	D33.0	191.5	225.0
Cerebellum	C71.6	D33.1	191.6	225.0
Brain stem; *includes:* 4th ventricle	C71.7	D33.1	191.7	225.0
Overlapping lesion of brain	C71.8	-	191.8	225.0
Brain, unspecified	C71.9	D33.2	191.9	225.0
Neoplasms of the meninges	C70	D32	192	225.2
Cerebral meninges	C70.0	D32.0	192.1	225.2
Spinal meninges	C70.1	D32.1	192.3	225.2
Meninges, unspecified	C70.9	D32.9	192.1	225.2
Neoplasms of the cranial nerves	C72	D33	192	225
Acoustic nerve	C72.4	D33.3	192.1	225.1

* under: http://www.who.int/classifications/icd/en/

Furthermore, all neoplasms are additionally coded according to the "International Classification of Diseases for Oncology" (ICD-O). The ICD-O is a dual classification and coding system for both topography and morphology of a neoplasm. The topography code uses the same three- and four-character categories as ICD-10 for malignant neoplasms (C00.0-C80.9), allowing greater specificity for the site of nonmalignant neoplasms than is possible in ICD-10. The morphology code describes the specific histologic cell type and its behavior. It indicates the specific histologic term. These morphology terms have five-digit codes ranging from M-8000/0 to M-9989/3. The first four digits indicate the specific histologic term. The fifth digit is a behavior code, which indicates whether a tumor is malignant, benign, in situ, or uncertain whether malignant or benign. The specific ICD-O-3 codes (3rd version of this classification scheme) for each brain tumor are given in the respective chapters.

Of all intracranial neoplasms, about 60% are of neuroepithelial origin (gliomas), 28% derive from the meninges and 7.5% are located in the cranial and spinal nerves [IARC and WHO, 2003]. According to the Central Brain Tumor Registry of the US (CBTRUS), more than 44% of all incident primary brain and CNS tumors are gliomas, followed by meningiomas with 27% [2002] (Fig. 1).

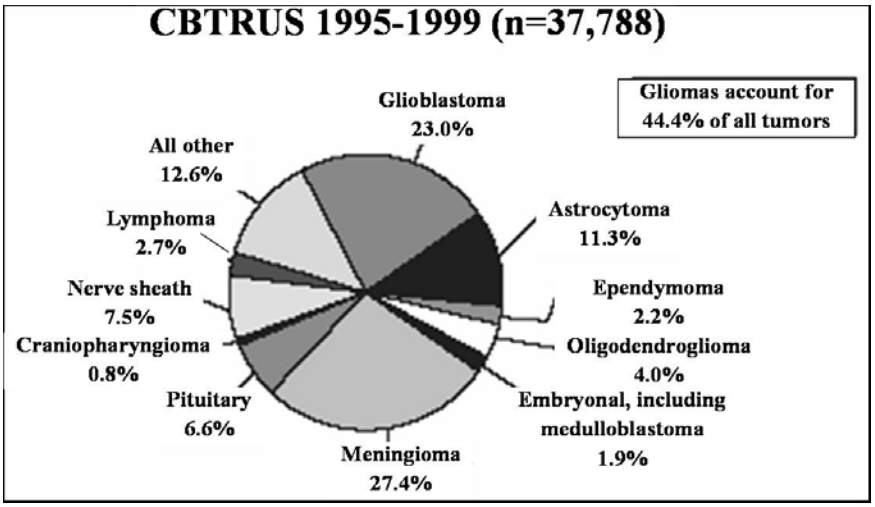

Figure 1: Incidences of brain tumors in the US according to CBTRUS [2002]

In European countries, the age-standardized incidence rate differs from 4-8/100 000 per year [IARC and WHO, 2003], thus, primary brain tumors in humans are a very rare type of cancer. However, age-adjusted incidence rates differ within different publications. In a more recent

review by Ohgaki and Kleihues [2005], incidence rates from 6-11 per 100 000 per year in men and from 4-11 per 100 000 in women were given for western European countries. Of all tumors in Germany, 2.8% (West) and 2.9% (East) in males and 3.3% (West) and 3.2% (East) in females are brain cancers [Becker and Wahrendorf, 1998]. For 2002, the Krebsregister Saarland† reported an age-adjusted incidence for all brain tumors (ICD-9 191) of 5.1 and 4.4 per 100 000 for men and women, respectively. Furthermore, an increase in incidence can be observed over the last decades. There has been some controversy regarding a possible overall increase in incidence during the last decades, especially in developed countries; however, this increase appears to be largely due to the introduction of better diagnostic tools [Ohgaki and Kleihues, 2005].

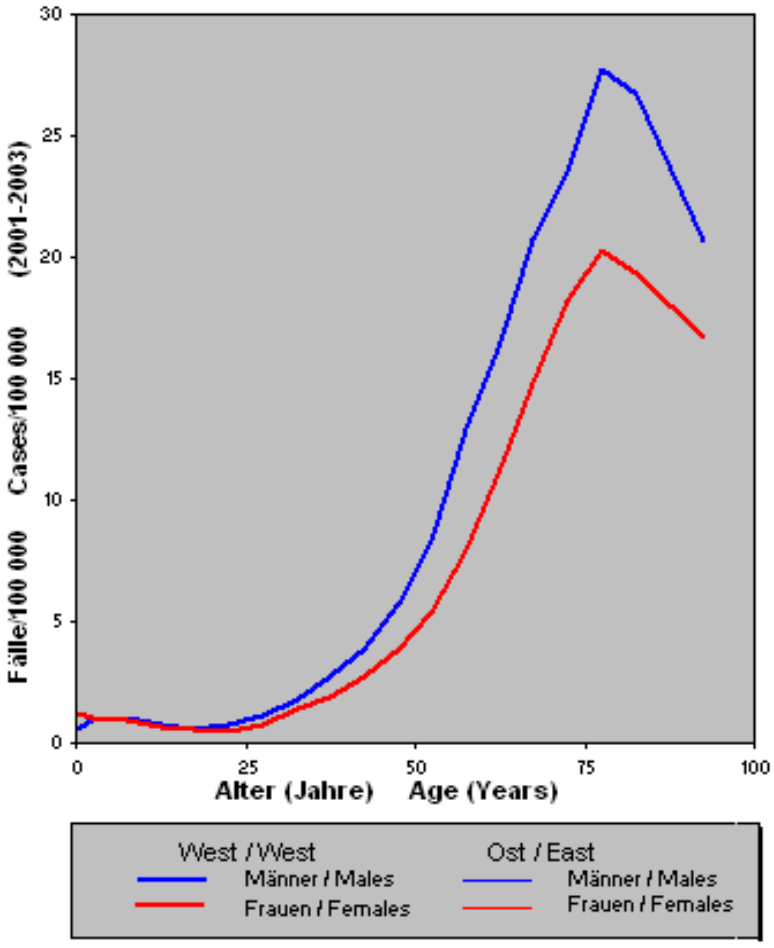

Figure 2: Mortality of brain tumors in the German population, taken from the German Atlas of Cancer Mortality [Becker and Wahrendorf, 1998]

† under: http://www.krebsregister.saarland.de

For all brain tumors combined, rates are higher in men than in women at all ages [Preston-Martin, 1996], because gliomas, which have a higher prevalence in men, are more frequent than meningiomas, resulting in a small overall male predominance for all brain tumors combined [Inskip et al., 1995]. Generally, men were reported to be 1.1-1.7 times more likely to develop any type of primary brain tumor [Bondy and Wrensch, 1996].

Mortality increases with increasing age, with a peak at the age of around 80 years (Fig. 2).

Although brain cancers are very rare, they are among the 20 most frequent causes of cancer deaths in Germany [Becker and Wahrendorf, 1998], in women even under the top ten (Fig. 3).

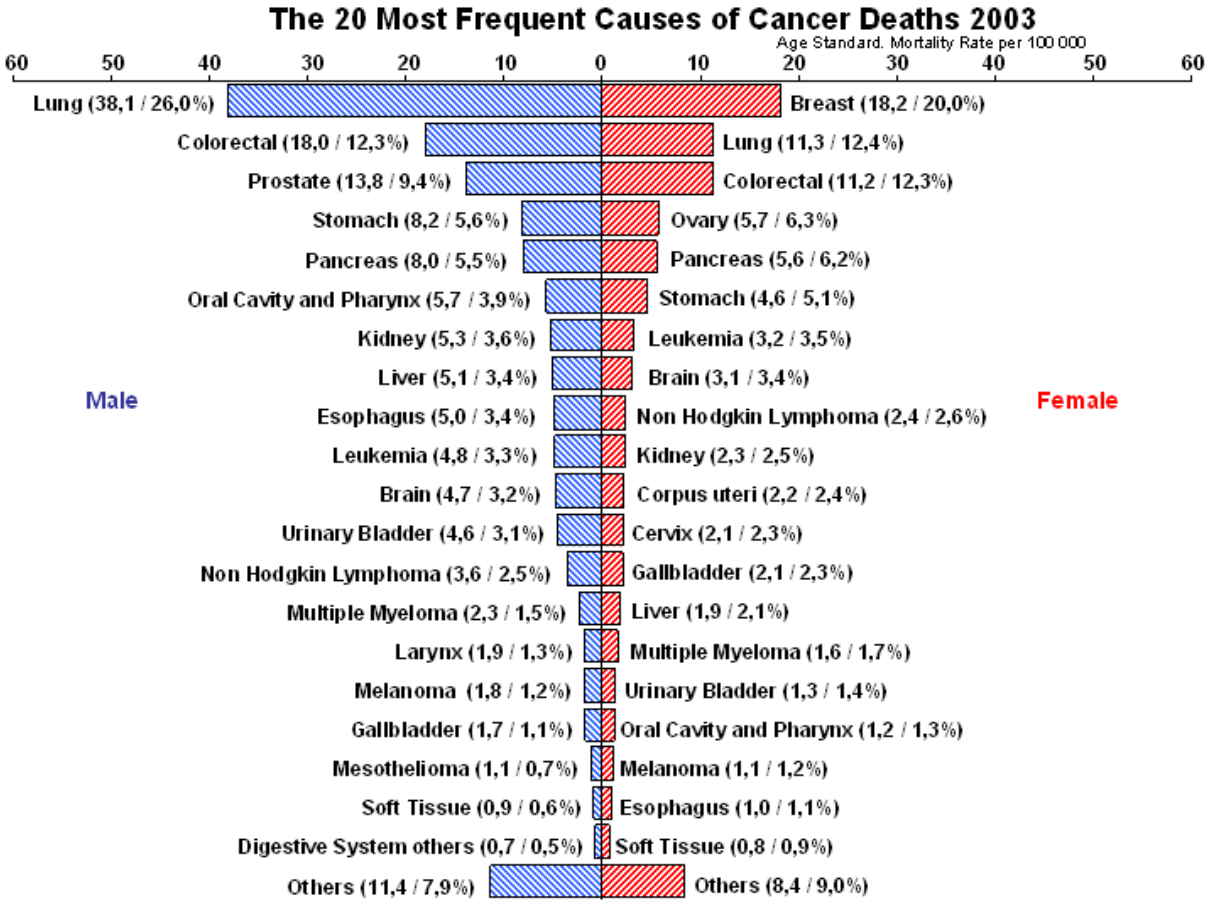

Figure 3: The 20 most frequent causes of cancer deaths in Germany, taken from the Atlas of Cancer Mortality in the Federal Republic of Germany [Becker and Wahrendorf, 1998]

For Western Europe, annual mortality rates are approximately 4-7 per 100 000 in men and 3-5 per 100 000 in women [Ohgaki and Kleihues, 2005]. The age-standardized mortality in the Saarland, Germany, was reported to be 6.5 per 100 000 for men and 4.8 per 100 000 in women in 2002 (Krebsregister Saarland‡).

Due to the heterogeneity of these tumors, prognosis is highly variable. In Germany, the relative 5-year survival rates are 26.8% (West) and 21.5% (East) for males and 33.3% (West) and 21.1% (East) for females [Becker and Wahrendorf, 1998].

Although several of these brain cancers are histologically defined as benign, these benign tumors may result in similar symptoms and clinical outcome as malignant tumors [Preston-Martin and Mack, 1996]. They, too, can be lethal because of their expansive growth inside the cranium thereby increasing intracranial pressure whereby fatal cerebellar herniation through the foramen magnum may occur.

1.1.2 Etiopathogenesis

So far, the only confirmed risk factors besides high dose of ionizing radiation are hereditary cancer syndromes like Li-Fraumeni Syndrome, neurofibromatosis and tuberous sclerosis [Wrensch et al., 2002; Preston-Martin, 1996]. However, there are numerous putative risk factors controversially discussed in the literature.

An association between epilepsy and the occurrence of brain tumors has been reported by several investigations while others could not find an association [Wrensch et al., 1997a; Carpenter et al., 1987; Hochberg et al., 1984; Choi et al., 1970].

Immunomodulation in general is thought to alter the risk of developing brain malignancies [Wrensch et al., 2002; Bondy and Wrensch, 1996; Inskip et al., 1995]. For instance, either the occurrence or treatment of previous cancers might have led to immunosuppression, which is a risk factor for the development of primary brain tumors [Salvati et al., 2003; Schiff et al., 2001; Detry et al., 2000].

Besides that, immunological factors in general are highly suspicious to be involved in brain tumor pathogenesis. In a population-based case-control study, Schlehofer et al. [1999]

‡ under: http://www.krebsregister.saarland.de

reported that subjects who reported a history of common infections (i.e., common colds or flu) had a 30% risk reduction for glioma development. In addition, there is striking, but not yet established evidence that allergic conditions may play a role in brain tumor (especially glioma) development [Brenner et al., 2002; Wiemels et al., 2002; Schlehofer et al., 1999; Cicuttini et al., 1997]. An inverse association between adult onset glioma and history of chickenpox and shingles and an inverse association between glioblastoma cases and the occurrence or levels of IgG antibodies to VZV have previously been reported [Wrensch et al., 2005; 2001; 1997b]. Furthermore, numerous studies tried to assess the possibly higher risk of primary brain tumors in patients vaccinated with a polyomavirus contaminated polio vaccine, but again with inconsistent results [Vilchez and Butel, 2004; Brenner et al., 2003].

Among the various occupational exposures controversially discussed to be involved in brain tumor development too are several immunological factors. As infections can be transmitted from person to person or from animal to person, occupations with frequent contact to people and/or animals might increase the risk of neuro-oncogenic infections. For instance, elevated risks were found for physicians and surgeons [Krishnan et al., 2003; Musicco et al., 1988], among workers in particular industries [Wrensch et al., 2002; Inskip et al., 1995] and farmers and agricultural workers [Khuder et al., 1998; Musicco et al., 1988] in several epidemiological studies. Another case-control study, however, reported a decreased risk for general farm workers [Menegoz et al., 2002].

Furthermore, zoonotic as well as immunologic factors are suspected to influence the development of brain malignancies. In a population-based case-control study, Efird et al. [2003] demonstrated significantly elevated odds ratios for childhood brain tumors in children living on a farm with pigs, horses, dogs or cats. Another case-control study confirmed the findings for contact to pigs and in addition showed an increased risk for contact with poultry [Holly et al., 1998]. In contrast, Menegoz et al. [2002], who investigated in contact to nine species of animals (dairy cattle, beef cattle, pigs, horses, sheep, goats, poultry, dogs and cats), did not find a relationship between either type of tumor (glioma or meningioma) and having contact with farm animals or pets.

However, although numerous studies have been carried out, all these findings are equivocal and need to be scrutinized in further studies.

1.2 Glioma

1.2.1 Epidemiology and Classification

Gliomas, which belong to the group of neuroepithelial brain tumors, are the most common primary brain tumors in humans. According to the International Agency for Research on Cancer [IARC and WHO, 2003], approximately 60% of all intracranial neoplasms are of glial origin. In a great US survey conducted by CBTRUS [2002], 44% of all brain tumors were gliomas (Fig. 3) with glioblastoma (WHO grade IV) being the most common subtype (52%), followed by astrocytomas WHO grade I-III (approx. 26%) and ependymomas (5%; Fig. 4).

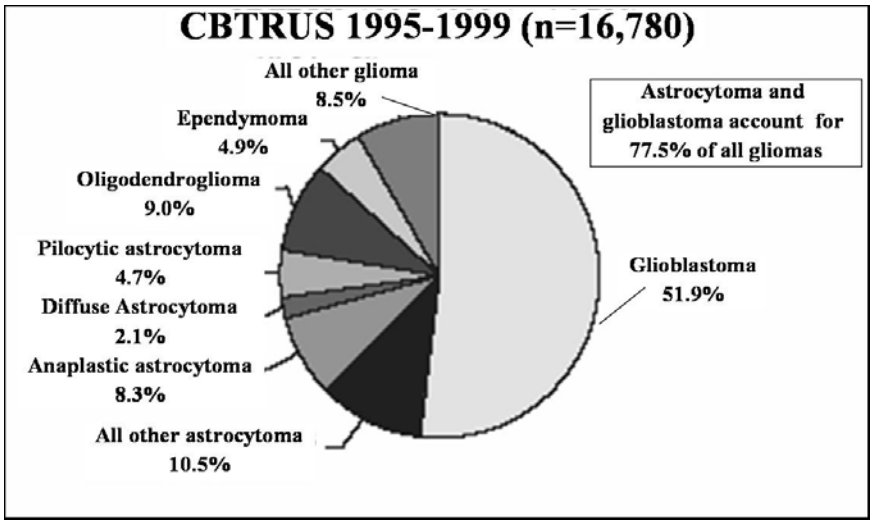

Figure 4: Incidence of gliomas by histology subtypes in the US according to CBTRUS [2002]

Gliomas derive from the neuroglia, a very heterogeneous group of cells originating from the ectoderm [Barres, 2003; Kintner, 2002]. The great majority of these cells are astrocytes (also known as macroglia) and oligodendrocytes. Astrocytes are phagocytosis-competent cells with numerous projections. They are connected with neurons and capillaries, thereby being part of the blood-brain-barrier. Oligodendrocytes are smaller cells with fewer projections than astrocytes. These cells are responsible for the formation of axon-surrounding myelin sheaths. Ependymal cells line the ventricles of the brain and the cavity of the spinal cord. Furthermore,

cells of the plexus chorioideus and the pituitary gland belong to the group of neuroglia. Gliomas can arise from every neuroglia. However, over 80% of gliomas are of astrocytic origin [Preston-Martin, 1996].

The underlying grading system is "The WHO classification of Tumors of the Nervous System" [Kleihues et al., 2002]. Tab. 2 shows an abridged version of the classification of gliomas according to the WHO classification system and the corresponding ICD-O codes.

Table 2: Classification of gliomas according to the WHO classification of tumors of the nervous system and the corresponding ICD-O codes (according to Kleihues et al., 2002)

Astrocytic tumors	**WHO grade***	**ICD-O-3 code**	**Oligodendroglial tumors**	**WHO grade***	**ICD-O-3 code**
Pilocystic astrocytoma	I	M9421/1	Oligodendroglioma	II	M9450/3
Diffuse astrocytoma			Anaplastic oligodendroglioma	III	M9451/3
-Fibrillary astrocytoma	II	M9420/3			
-Protoplasmic astrocytoma	II	M9410/3	**Mixed gliomas**		
-Gemistocytic astrocytoma	II	M9411/3			
Anaplastic astrocytoma	III	M9401/3	Oligoastrocytoma	II	M9382/3
Glioblastoma		M9440/3	Anaplastic oligoastrocytoma	III	M9382/3
-Giant cell g.	IV	M9441/3			
-Gliosarcoma	IV	M9442/3	**Ependymal tumors**		
			Ependymoma	II	M9391/3
			Anaplastic ependymoma	III	M9392/3
			Myxopapillary ependymoma	I	M9394/1
			Subependymoma	I	M9383/1

* WHO, World Health Organization; WHO grade I=benign; WHO grade II=semi-benign; WHO grade III=(semi-) malignant; WHO grade IV=malignant; ICD-O-3, International Classification of Diseases for Oncology, 3rd version

Age at clinical manifestation strongly depends on the histological type of the tumor. The most frequent brain neoplasm in children is pilocystic astrocytoma (74% are younger than 20 years of age). Diffuse astrocytoma is most common in 20 to 44 year-olds, whereas glioblastoma is a tumor of the elderly; 72% are above the age of 45 years at first clinical manifestation [IARC and WHO, 2003]. Anaplastic astrocytomas and glioblastomas often evolve from less malignant astrocytomas. However, some cases appear to arise de novo [Inskip et al., 1995]. For these, hereditary as well as genetic risk factors are discussed [Grisold et al., 2000].

Oligodendrogliomas typically occur in persons above the age of 20 years, whereas ependymomas most commonly develop under 45 years-of-age [IARC and WHO, 2003]. Gradations of anaplasia are also seen in these tumors, although they most often occur as relatively benign forms [Kleihues et al., 2002].

Glioma rates are higher in men than in women [Preston-Martin, 1996]. According to a descriptive epidemiological survey conducted by CBTRUS in the US, gliomas affect about 40% more males than females [Surawicz et al., 1999]; another epidemiological study reported a male:female ratio in the US of 1.5 [Inskip et al., 1995]. However, there is no explanation for this relation up to now.

The overall incidence of gliomas is increasing with increasing age. It has been suggested that, as for other tumors, this increase is a function of length of exposure required for malignant transformation and/or the necessity of multiple genetic alterations preceding malignancy [Bondy and Wrensch, 1996]. However, with two exceptions, these exposures are not characterized yet (see below).

1.2.2 Etiopathogenesis

So far, the only known risk factors for the development of primary brain tumors are high dose therapeutic radiation of the head and some hereditary diseases such as Li-Fraumeni syndrome, neurofibromatosis and tuberous sclerosis. However, these factors only account for less than five percent of all incident primary brain tumors [Inskip et al., 1995].

Epidemiological studies on potential risk factors have produced controversial results. Risk factors that are discussed throughout the last decades include occupational and industrial chemicals (e.g. N-nitroso compounds, pesticides, and formaldehyde), electromagnetic fields, medical conditions (such as allergies, atopic and infectious diseases, and head injuries), medications, and other environmental and lifestyle factors, but the etiological relevance of these factors is not proven up to now [Wrensch et al., 2002; Wrensch et al., 2000; WHO, 2000b; Schlehofer et al., 1999; Bondy and Wrensch, 1996; Preston-Martin and Mack, 1996].

Therefore, further research is needed to recognize etiological pathways to avert this fatal malignancy.

1.2.3 Diagnosis, Therapy and Prognosis

The most common reasons to take medical advice for persons with glial tumors are seizures and headache without improvement after medication. Other signs include paresis, emesis, speech disturbances and personality changes [IARC and WHO, 2003; Grisold et al., 2000; WHO, 2000b]. The presence of symptoms usually leads to a detailed neurological examination, using computed tomography (CT) and magnetic resonance imaging (MRI), during which intracranial masses can be detected. The tentative diagnosis may be confirmed by biopsy [IARC and WHO, 2003; Grisold et al., 2000; WHO, 2000b; Preston-Martin, 1996].

The main target in glioma therapy is a complete surgical removal of the neoplasm. However, due to the commonly infiltrative character and the intracranial location, complete removal is frequently impossible. In this case, partial removal can alleviate symptoms and protract exacerbation. Post-operative radiotherapy may follow. Furthermore, adjuvant chemotherapy has been proven to be effective to remove remaining tumor cells after surgery [Grisold et al., 2000].

Prognosis is strongly related to patients' age and histologic type. Patients with glioblastoma multiforme (WHO grade IV) have the poorest prognosis with less than 3% of the patients still alive 5 years after diagnosis [IARC and WHO, 2003; Preston-Martin, 1996]. In contrast, patients with pilocytic astrocytoma (WHO grade I) have a chance of more than 85% to be still alive after 5 years [IARC and WHO, 2003]. In gliomas of other histology, patients under the age of 44 years have a much better survival than older patients within each histological type [Wrensch et al., 2002]. Overall, higher age at diagnosis is the most powerful negative prognostic factor for gliomas that is operative through all age groups [Ohgaki et al., 2004].

Unfortunately, despite several newly invented diagnostic and therapeutic methods, an Australian study group reported that there seems to be no improvement in the 5-year survival in any age group or histological type [Shugg et al., 1994].

1.3 Meningioma

1.3.1 Epidemiology and Classification

Intracranial meningiomas are the second most frequent type of primary brain malignancies. They account for 27% of all primary brain tumors in the US (CBTRUS, 2002; Fig. 1). Similarly, the International Agency for Research on Cancer reported a proportion of 28% [IARC and WHO, 2003].

These usually benign and slow growing tumors develop from arachnoidal cells in the meninges. Being benign tumors, WHO grade I meningiomas do not infiltrate the brain but may cause symptoms only due to their intracranial location. These meningiomas can often be cured by total surgical resection. However, although this is rarely the case, they can progress to a malignant form. Of all meningiomas, approximately 6-9% are malignant (WHO grade II or III). They can infiltrate the brain, are often recidivating and have a less favorable clinical outcome. Meningiomas are graded according to "The WHO classification of Tumors of the Nervous System" (Kleihues et al., 2002; as shown in Tab. 3).

Table 3: Classification of tumors of meningothelial cells according to the WHO classification of tumors of the nervous system and the corresponding ICD-O codes (according to Kleihues et al., 2002)

Meningiomas with low risk of recurrence and/or aggressive growth		
Meningioma	**WHO grade***	**ICD-O-3 code**
Meningothelial	I	M9531/0
Fibrous (fibroblastic)	I	M9532/0
Transitional (mixed)	I	M9537/0
Psammomatous	I	M9533/0
Angiomatous	I	M9534/0
Microcystic	I	M9530/0
Secretory	I	M9530/0
Lymphoplasmacyte-rich	I	M9530/0
Metaplastic	I	M9530/0

Meningiomas with greater risk of recurrence and/or aggressive growth		
Meningioma	**WHO grade***	**ICD-O-3 code**
Clear cell	II	M9538/1
Chordoid	II	M9538/1
Atypical	II	M9539/1
Papillary	III	M9538/3
Rhabdoid	III	M9538/3
Anaplastic	III	M9530/3

*WHO, World Health Organization; WHO grade I=benign; WHO grade II=semi-benign; WHO grade III=semi-malignant; WHO grade IV=malignant; ICD-O-3, International Classification of Diseases for Oncology, 3rd version

The annual incidence rate of intracranial meningiomas is approximately 6 per 100 000 [WHO, 2000a]. Women are more likely to have benign meningiomas, with a male:female ratio of 0.6 [Inskip et al., 1995]. It has been reported by a descriptive epidemiological study of CBTRUS that meningiomas affect about 80% more females than males [Surawicz et al., 1999]. Interestingly, malignant meningiomas occur about equally in men and women [Inskip et al., 1995].

Primarily, meningioma is a disease of the middle and old age [Inskip et al., 1995] with a peak occurrence between 50-70 years of age [WHO, 2000b]. However, as this is usually a benign and very slow growing tumor, first diagnosis at autopsy occurs with a frequency of 1.4% [WHO, 2000b]. Therefore, epidemiologic and etiologic studies might be confused with determinants of diagnosis [Inskip et al., 1995].

1.3.2 Etiopathogenesis

So far, few factors have been identified to be involved in meningioma pathogenesis. The fact that women are significantly more affected by meningiomas led to the suggestion that hormonal factors are of etiologic relevance [Preston-Martin and Mack, 1996; Inskip et al., 1995]. However, no causative agent could be found up to now.

As in glioma pathogenesis, therapeutical ionizing radiation is the only environmental exposure for which a causal association with meningioma development is established [Yousaf et al., 2003; Strojan et al., 2000; Preston-Martin, 1996]. Childhood radiotherapy has been reported to elevate the relative risk for meningioma pathogenesis 9.5 times by an epidemiological study in Israel [Ron et al., 1988]. The second well-established condition leading to meningioma disease is hereditary neurofibromatosis type 2 [Inskip et al., 1995].

An inverse association of allergic diseases with meningioma has been reported by several large case-control studies [Brenner et al., 2002; Schlehofer et al., 1999]. Epidemiological studies on occupational risk factors, however, showed inconsistent results [Menegoz et al., 2002; Wrensch et al., 2002; Preston-Martin, 1996; Preston-Martin and Mack, 1996; Inskip et al., 1995]. Other factors such as head trauma, smoking, and diet were investigated, but no definite etiological role could be demonstrated for any of these factors [Preston-Martin and Mack, 1996].

1.3.3 Diagnosis, Therapy and Prognosis

Cerebral meningiomas cause clinical symptoms by compression of adjacent tissue. The specific symptoms depend on the location of the intracranial mass. Since meningiomas are slow growing tumors, deficits like headache, seizures or depression occur relatively late [WHO, 2000b].

The preferred therapy is surgical excision. WHO grade I meningiomas are usually curable when resectable. However, the extent of resection depends on the localization, attachment to intracranial structures, and the age of the patient. In case of unresectable or high-grade meningiomas, surgery plus radiation therapy is applied [Grisold et al., 2000].

The proportion of patients who survive 5 years is 69% according to a review of Wrensch et al. [2002] and up to 92% for patients with WHO grade I meningiomas (reviewed by Preston-Martin, 1996).

Malignant meningiomas may relapse locally [IARC and WHO, 2003]. According to the WHO, atypical meningiomas relapse in 29-40% of the cases and anaplastic meningiomas have a recurrence rate of 50-78% [WHO, 2000b]. Furthermore, they are associated with shorter survival times than benign ones. Interestingly, women are more likely to have benign meningiomas and they have a significantly longer survival than men who develop benign meningial tumors [Preston-Martin, 1996].

1.4 Acoustic Neurinoma

1.4.1 Epidemiology and Classification

Acoustic neurinomas belong to the group of schwannomas. These are benign tumors arising from the myelin producing Schwann cells, which enfold the eighth cranial nerve (vestibulocochlear nerve).

Acoustic neurinomas are consistently reported to account for about 8% of all intracranial primary brain tumors by various epidemiological studies [CBTRUS, 2002; WHO, 2000b; Preston-Martin, 1996]. Currently, the worldwide incidence for acoustic neurinoma is reported to be 1-20 per million population per year. An increase in incidence is observed over the last two decades; however, this increase may be due to better diagnostic tools [Howitz et al., 2000; Lanser et al., 1992]. Acoustic neurinoma generally are histologically benign (ICD-10 code D33.3, ICD-O-3 code M9560/0), corresponding to WHO grade I [Kleihues et al., 2002; WHO, 2000b]. Recurrence after surgical resection is very rare.

Neurinomas of the acoustic nerve generally occur in people aged 50 years and above, except for tumors occurring in the course of neurofibromatosis type 2, where acoustic neurinomas often occur in younger persons and bilateral [Lanser et al., 1992]. In contrast to peripheral schwannomas, where no gender predilection can be seen, the female:male ratio in intracranial neurinomas is 2:1 [WHO, 2000b].

1.4.2 Etiopathogenesis

As for gliomas and meningiomas, only two well-established risk factors for acoustic neurinoma development are figured out so far. Therapeutic radiation (e.g. of ringworm of the scalp in childhood) is a strong risk factor contributing to acoustic neurinoma development. This association is the strongest among all brain tumors for which an association was found [Inskip et al., 1995; Ron et al., 1988]. The role of diagnostic radiation, however, remains unclear. Acoustic neurinomas have a relatively clear genetic character. Individuals with the

familial, autosomal dominant cancer syndrome neurofibromatosis (NF) are at high risk for acoustic neurinoma development. Patients with NF type 2 typically develop bilateral acoustic neurinomas [WHO, 2000a]. In patients with NF type 1, however, peripheral rather than central neurinomas are prevalent [Wrensch et al., 2002; Preston-Martin and Mack, 1996; Inskip et al., 1995].

Several other risk factors have been discussed throughout the last decades. These include head and noise trauma, dental x-rays, and mobile phone use [Christensen et al., 2004; Lonn et al., 2004; Hardell et al., 2003; Preston-Martin et al., 1989]. Hay fever and allergies to several substances were reported to increase the risk for acoustic neurinoma development in a hospital-based case-control study [Brenner et al., 2002].

Most of the results, however, were based on small numbers and, in addition, most of the hypotheses are based on one single study and, if more studies had been conducted, the results between the different studies are inconclusive. Therefore, further research is needed to identify additional etiological pathways leading to acoustic neurinoma.

1.4.3 Diagnosis, Therapy and Prognosis

Since schwannomas favor sensory nerve roots, motor symptoms are uncommon. Patients with acoustic neurinoma develop tinnitus, hearing impairments, vertigo and facial palsy [WHO, 2000b]. MRI and CT are the best methods of imaging these tumors [Grisold et al., 2000].

The preferred therapy is a complete surgical resection of the neoplasm, eventually followed by craniospinal irradiation.

Patients with this benign tumor have a chance of 100% to survive 5 years after diagnosis [Preston-Martin, 1996]. Hence, although complications during therapy such as hearing losses or facial paresis are frequent, patients have a normal life expectancy after complete removal.

Acoustic neurinomas rarely undergo malignant transformation. However, patients with the rarely occurring malignant acoustic neurinoma (ICD-10 code C72.4) have a relatively poor prognosis [Grisold et al., 2000].

(B) Virological Background

1.5 The Family of Herpesviruses

Herpesviruses are ubiquitous DNA viruses infecting humans and several vertebrates. Today, approximately 100 different herpesviruses have been detected.

1.5.1 Taxonomy

The family of herpesviruses consists of three subfamilies, which are classified according to their pathogenicity, their target cells and their replication characteristics (alpha [α]-, beta [ß]-, and gamma [γ]-herpesviruses; Tab. 4).

Table 4: The family of herpesviruses in humans

Family	Subfamily	Genus	Species	Designation
Herpesviridae				
	α-herpesviridae	Simplexvirus	Herpes simplex virus 1 Herpes simplex virus 2	HHV-1 HHV-2
		Varicellavirus	Varicella-zoster virus	HHV-3
	ß-herpesviridae	Cytomegalovirus	Human cytomegalovirus	HHV-5
		Roseolovirus		HHV-6 HHV-7
	γ-herpesviridae	Lymphocryptovirus	Epstein-Barr virus	HHV-4
		Rhadinovirus	Kaposi's sarcoma associated herpesvirus	HHV-8

HHV, human herpesvirus

The subfamily of α-herpesviruses is characterized by a variable host range, a short replication cycle and a rapid spread in culture. In vivo, they usually but not exclusively persist in ganglions. In contrast to α-herpesviruses, ß-herpesviruses have a restricted host range and a long replication cycle in cultured cells. Latency occurs in secretory glands, lymphoreticular cells, kidneys, and other tissues. Infected cells become typically enlarged (cytomegalia). The last subfamily, the γ-herpesviruses, typically infects either B- or T-lymphocytes. They, too, are species-specific. Latent virus is frequently demonstrated in lymphoid tissues [Modrow, 2002; Roizman and Pellet, 2001].

Besides the common names α-, ß-, and γ-herpesviruses, the Committee on Taxonomy of Viruses designates herpesviruses in the chronological order of their discovery [Modrow, 2002; Mocarski, Jr. and Courcelle, 2001; Roizman and Pellet, 2001]

1.5.2 Structure

Inclusion in the family of herpesviridae is based on the structure of the virion. A herpesvirion typically has a diameter of approximately 150 to 200 nm and consists of the following:

1. a core containing a linear, double-stranded DNA with a size of up to 230 kbp,
2. an icosadeltahedral capsid, containing 162 capsomeres,
3. an amorphous tegument surrounding the capsid, and
4. an envelope with viral glycoprotein spikes on its surface.

The size of herpesvirions varies between 120 nm and 300 nm. The core of the mature virion contains the viral DNA in form of a torus. The capsid, containing the pentameric capsomeres, is approximately 100-110 nm in diameter. The thickness of the herpesvirus-specific tegument depends on the location of the virion in the infected cell. This tegument is an unstructured protein matrix containing 20 viral proteins. There is evidence that the amount of tegument is more likely to be determined by the virus than by the host. The envelope is the outer membrane of the virus. This lipid membrane contains numerous spikes consisting of glycoproteins, which vary in number and relative amount [Modrow, 2002; Roizman and Pellet, 2001]. The genome range of the linear, double-stranded DNA varies approximately from 125 to 248 kbp. It circularizes immediately upon release from capsid into the nucleus of an infected cell [Modrow, 2002; Roizman and Pellet, 2001].

1.5.3 Replication

Herpesviruses can infect the host cell in two different ways. First, herpesviruses can build virions and cause a lytic infection, i.e., the host cell is killed by the virus. The most interesting property of all herpesviruses, however, is their ability to establish a so-called latent infection without virion production and host cell destruction [Modrow, 2002]. In latency, herpesviruses are able either to establish an antigenically silent form of latent infection or to impair the antigen-processing capacity of lytically infected cells (see below)

1.5.3.1 Lytic Infection

Generally, herpesvirus replication is accompanied by irreversible destruction of the infected host cell. For this so-called “lytic infection”, the adsorption of herpesvirions to the host cell occurs on the cell surface where viral envelope proteins bind to cell surface receptors. Attachment is followed by penetration. Viral nucleocapsids quickly make their way to the nucleus pores to deliver the viral DNA to the nucleus. Upon entry, the genome typically circularizes and integrates in the host cell DNA.

Gene expression can be divided based on time of gene synthesis after infection: immediate early (α), early (ß) and late (γ). Immediate early (IE) proteins are important for ongoing infection. No prior viral protein synthesis is required for their expression. They act as transcriptional trans-activators for the expression of early proteins (early antigens, EA), whose expression is totally independent of viral DNA synthesis. Late proteins (late antigens, LA) are transcribed during DNA synthesis, which is coincident with cellular DNA replication. LAs are responsible for the coding of glycoproteins, which are important for the assembly of nucleocapsid, tegument and covering membrane. DNA packaging follows formation of capsids. Thereafter, progeny virions acquire an envelope from the inner nuclear membrane, are transported in vesicles via the endoplasmatic reticulum and the Golgi apparatus to the cell surface and get released via an exocytotic pathway [Landolfo et al., 2003; Modrow, 2002; Mocarski, Jr. and Courcelle, 2001; Roizman and Pellet, 2001].

1.5.3.2 Latent Infection

Latency is a characteristic feature of all herpesviruses. The viral genome remains with its host for life after primary infection [Modrow, 2002; Roizman and Pellet, 2001].

The sites of latency differ between the herpesvirus subfamilies. α-herpesviruses most commonly persist in ganglions. ß-herpesviruses remain in secretory glands, lymphoreticular cells, kidneys, and other tissues, and latent γ-herpesviruses are usually demonstrated in lymphoid tissues [Modrow, 2002; Roizman and Pellet, 2001]. During latency, no virus replication and no cell destruction occurs. However, the viruses can be reactivated in case of immunosuppression such as infections, pregnancy, malignancies, stress and other medical conditions, and the lytic cycle may be started.

1.5.4 Transmission

Transmission of herpesviruses is cell-associated, and occurs by direct or indirect person-to-person contact. In contrast to most other infectious diseases, the presence of acquired antibodies does not prevent infection.

Because of the relative lability of all herpesviruses after exposure to common environmental conditions such as heat and drying, close or even intimate contact is required for its horizontal spread. Sources of virus include oropharyngeal secretions, urine, cervical and vaginal excretions, semen, breast milk, tears, feces and blood. Without producing clinical disease, virus excretion persists for years after acquired infections, being responsible for an extensive spread of herpesviruses in susceptible populations [Modrow, 2002; Roizman and Pellet, 2001].

1.6 Human Cytomegalovirus

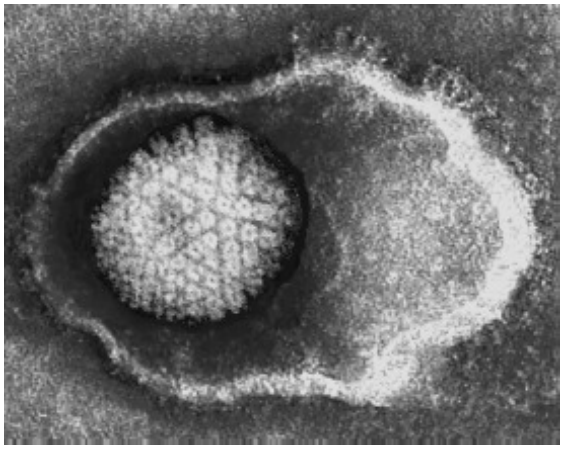

Figure 5: Electron micrograph of a CMV virion[§]

Human cytomegalovirus (HCMV; also designated HHV-5) is a member of the herpesvirus subfamily of ß-herpesviridae.

Characteristically, these viruses produce cell enlargement with intranuclear inclusions, a fact that led to the early designation of the term "cytomegalic inclusion disease" (CID), thereby giving this virus its name [Britt and Alford, 1996].

The HCMV genome is the largest of all herpesviruses, containing approximately 248 kbp, with slight differences in size between the different strains. So far, the DNA of the AD169 laboratory strain is the only completely sequenced HCMV genome [Landolfo et al., 2003].

1.6.1 Epidemiology

HCMV is a ubiquitous virus widespread in the human population. According to the Robert Koch Institute (RKI), Germany [2000a], the prevalence in the German adult population is between 40% and 80%. Another publication assessed a prevalence of 42% in 20-39 year-old persons in the Freiburg area in Southern Germany [Krech, 1973]. The worldwide prevalence has been estimated to vary between 40% in developed countries and higher socioeconomic status (SES) and up to 100% in developing countries and low SES. Immunosuppression, SES and promiscuity are general factors promoting seroconversion [Modrow, 2002; Pass, 2001; de Jong et al., 1998].

[§] from University of Birmingham www.biosciences.bham.ac.uk

HCMV can be transmitted intrauterinely. Up to 2% of infants in developed countries are infected in utero, with approximately 10-15% of them exhibiting long-term neurologic sequelae following this infection. Hence, HCMV is the most common congenital infection in humans. In addition, it is the leading infectious cause of CNS maldevelopment (e.g. hydrocephalus and microcephaly) in children.

Generally, HCMV is transmitted through body fluids, such as semen, blood, cervical and vaginal excretions, breast milk, tears, urine and feces [Landolfo et al., 2003; Pass, 2001]. Before puberty, less than 40% become infected; afterwards, the percentage of infected persons increases only about 1% per year. Two peaks can be observed in HCMV seroprevalence analogous to its transmission pathways. A first increase in seroprevalence occurs during the first three years of life (close physical contact) and a second peak is reached in early adulthood due to sexual contacts [Landolfo et al., 2003].

1.6.2 Pathogenesis

Following oral transmission, HCMV characteristically infects ductal epithelial cells of the salivary glands. The parotidoid gland is most frequently infected. Typically, HCMV-infected cells can be seen with multiple prominent intranuclear inclusions surrounded by a clear halo. These inclusions have given rise to the term “owl’s eyes inclusions”.

A first replication in the salivary glands is followed by hematogenous, cell-associated viremia, where the virus spreads throughout the body to several organs [Sinzger and Jahn, 1996]. Gastrointestinal involvement as well as involvement of the respiratory tract is frequently seen, mainly in immunocompromised individuals. Other sites susceptible for HCMV infection are spleen, kidney, liver, myocard, brain, and bone marrow [Landolfo et al., 2003; Modrow, 2002; Pass, 2001; Ho, 1982]. After primary infection, HCMV may develop latency in macrophage-granulocyte progenitors in the bone marrow and in peripheral monocytes, and can be reactivated under certain conditions [Landolfo et al., 2003]. However, the sites of latency are still largely undefined [Pass, 2001; de Jong et al., 1998].

An important issue for HCMV viremia and disease is the immunological status of the infected person, as described below [Landolfo et al., 2003; de la Hoz et al., 2002].

1.6.3 Clinical Features

HCMV has an incubation period of 4 to 8 weeks. As mentioned above, the immunological status of the infected person is of high importance for the clinical outcome of the disease.

1.6.3.1 Immunocompetent Persons

In immunocompetent persons, HCMV infection usually leads to a clinically inapparent infection. Infrequently, the development of a mononucleosis-like syndrome occurs, which is clinically indistinguishable from an infection with Epstein-Barr virus; about 8% of all mononucleosis cases are caused by HCMV [Pass, 2001]. Cervical adenopathy, myalgia and nonspecific constitutional syndromes are typical, as is persistent fever during 2-5 weeks [Landolfo et al., 2003; Modrow, 2002; Ho, 1982].

1.6.3.2 Congenital Infection

HCMV infection is the most common congenital viral infection in humans. On average, 1% of all newborns are infected with this virus, with geographical differences (0.2% in Europe, and up to 2.2% in the US according to Pass, 2001).

Approximately 0.1% of all children are congenitally damaged by HCMV infection [Modrow, 2002], resulting in mental retardation, hearing impairments, hepatosplenomegaly, jaundice, pneumonia, microcephaly, seizures and thrombocytopenia [Landolfo et al., 2003]. Affected children develop permanent damages in 90% of the cases, mainly neurologic abnormalities. Interestingly, the incidence of hearing loss in otherwise asymptomatically infected newborns is 5-15%. It has been shown that multiple organs of congenitally infected children become infected, with the major target organs being the lung, the pancreas, the kidneys and the liver [Bissinger et al., 2002; de Jong et al., 1998].

The presence of maternal immunity is highly correlated with fetal outcome. 8-10% of primary maternal infections lead to a clinically apparent infection, whereas women with preconceptional immunity rarely deliver symptomatic infants [Modrow, 2002; Pass, 2001].

1.6.3.3 Immunocompromised Individuals

In immunocompromised persons, mainly allograft recipients and persons infected by human immunodeficiency virus (HIV), HCMV infection is a dreaded condition. In fact, HCMV pneumonia is the leading cause of death in HIV-infected patients and bone marrow allograft recipients [Modrow, 2002].

Primary infection of allograft recipients through a seropositive graft can cause a severe mononucleosis syndrome. In the worst case, primary HCMV infection can lead to inflammation or even rejection of the graft. However, even in seropositive recipients, HCMV can be reactivated through treatment-induced immunosuppression, and then lead to manifest HCMV disease [Modrow, 2002; de la Hoz et al., 2002; Pass, 2001]. Fortunately, improved prophylaxis and preemptive antiviral therapy led to a decrease in the incidence of HCMV disease in graft recipients in the past years [de Jong et al., 1998].

In general, immunomodulation is thought to alter the risk for HCMV infection or reactivation [Landolfo et al., 2003; Pass, 2001]. For instance, either the occurrence or the treatment of cancer may cause the necessary immunosuppression [Pass, 2001; Sinclair and Sissons, 1996].

Reactivation of a latent HCMV infection is the major cause for severe disease in HIV-infected individuals rather than primary infection, leading to a manifest mononucleosis syndrome. There is a clear correlation between the severity of HIV immunodeficiency and the development of HCMV disease. Clinically syndromes in affected patients include disease in almost every organ system, e.g. gastrointestinal ulcerations, chorioretinitis, severe pneumonia, and encephalitis [Modrow, 2002; Pass, 2001; de Jong et al., 1998].

1.6.4 Diagnosis

Clinical symptoms are usually unspecific if any are present in immunocompetent persons. Therefore, laboratory analyses are required for adequate diagnostics. Diagnostic methods for the detection of HCMV infection include serological methods (detection of anti-HCMV IgG and IgM antibodies in sera of the patients), measurement of pp65 antigen in leucocytes, virus isolation from several body fluids, histology of tissue sections and detection of viral nucleic acids, generally by polymerase chain reaction (PCR; Pass, 2001; de Jong et al., 1998).

1.6.5 Management of HCMV Infection

In the management of HCMV disease, four different strategies can be distinguished [de Jong et al., 1998]:

1. Prophylactic,
2. preemptive,
3. suppressive, and
4. antiviral treatment.

Prophylaxis is defined as treatment in the absence of detectable virus to prevent primary infection or reactivation of the virus (e.g. prior to an organ transplantation). Preemptive and suppressive treatments aim at limiting treatment to individuals at higher risk of developing a manifest HCMV disease. In this case, virus is detectable, but without causing any clinical symptoms.

Currently available drugs for antiviral treatment are virus polymerase inhibitors like gancyclovir, foscarnet and cidofovir, which inhibit HCMV replication. However, these drugs have severe side effects. Efforts to develop an HCMV vaccine are being made since several decades to reduce morbidity and mortality in individuals affected by HCMV, but so far, no effective HCMV vaccine has been developed [Landolfo et al., 2003; Pass, 2001; de Jong et al., 1998].

1.7 Herpes Simplex Virus

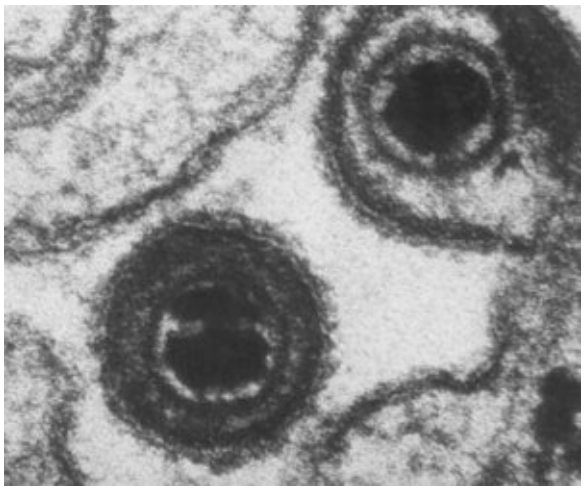

Figure 6: Electron micrograph of herpes simplex virus[**]

Of all human herpesviruses, herpes simplex viruses (HSV) were the first to be discovered. They are members of the herpesviruses subfamily of α–herpesviridae, containing a genome of approximately 152 kbp.

Two species exist, herpes simplex virus 1 (HSV-1, also designated HHV-1), which causes orolabial lesions, and herpes simplex virus 2 (HSV-2, also designated HHV-2), infecting the genital tract.

The virus has been named in reference to the spreading nature of the visualized skin lesions (Greek *herpein*: creep or crawl; Whitley, 1990).

1.7.1 Epidemiology

HSV, like all herpesviruses, is a ubiquitous virus widespread in the human population with the ability to develop latency in the host.

As for other herpesviruses, incidence is strongly associated with the socioeconomic status of the population. Frequency of direct person-to-person contact, indicative of crowding encountered with lower SES, appears to be the major mediator of infection, followed by immunosuppression and promiscuity [Whitley, 1990]. HSV-2 is in fact one of the most prevalent sexually transmitted infectious agents worldwide [Smith and Robinson, 2002]. Generally, individuals from lower SES populations have an HSV-1 seroprevalence of 75-90% by the end of the first decade of life. In contrast, in middle or high SES populations, the proportion is only 30-40% by the middle of the second decade of life [Whitley, 1990].

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In Germany, the seroprevalences of IgG antibodies to HSV-1 was reported to be 64% for men and 71% for women in the age group 15-39 years. Above the age of 40 years, prevalences are 83% and 85%, respectively (Rabenau et al., 2002; Frankfurt am Main area). Hellenbrand et al. [2001] compared seroprevalences of antibodies to HSV-1 in West and East Germany. In the western part of Germany, a seroprevalence of 85% (95%CI 83-87%) compared to 89% (95%CI 87-90%) in the eastern part has been reported.

For HSV-2, a seroprevalence of 13% (95%CI 11-14%) in West Germany and 16% (95%CI 14-18%) in East Germany, respectively, was estimated [Hellenbrand et al., 2001]. A similar seroprevalence of IgG antibodies to HSV-2 of 13% (95%CI 12-14%) was reported for a population of blood donors and hospital patients in the Frankfurt am Main area, Germany [Wutzler et al., 2000].

1.7.2 Pathogenesis

Herpes simplex virus infection requires intimate, personal contact of a seronegative person with an individual excreting the virus.

Infection is initiated when HSV comes into contact with mucosal surfaces or small skin lesions. At the site of infection, a first viral replication takes place including intense inflammatory response and the formation of vesicles containing infectious virus. Through retrograde axonal transport, HSV virions arrive at the dorsal root ganglia, where the virus establishes latency. HSV may become systemic, infecting multiple organs, e.g. in neonatal HSV infection (resulting in herpes neonatorum) and in immunocompromised persons.

An important issue for HSV viremia and disease is the immunological status of the infected person. Immunosuppression and stress are clearly correlated with reactivation from latent status. However, as with primary infection, reactivation may occur in the absence of clinical symptoms [Modrow, 2002; Whitley, 1990].

1.7.3 Clinical Features

The pathologic changes induced by HSV are similar for primary and recurrent infection. Although HSV-1 and HSV-2 are transmitted by different routes and involve different areas of the body, the manifestations of infections caused by HSV are coincident [Whitley, 1990].

1.7.3.1 Oropharyngeal Disease

Infection with HSV-1 may induce HSV-1-specific oropharyngeal disease ("infection above the belt"), the so-called "herpes labialis". However, asymptomatic infection is the rule.

The mean incubation period is 4 days. Primary infection in children lasts from two to three weeks, usually including fever, edema, lymphadenopathy, sore throat, gingivostomatitis, and lesions within the mouth evolving from vesicles. These lesions are followed by ulcerations and erythemas, which progress slowly to healing.

Later in life, primary infection is associated with pharyngitis and a mononucleosis-like syndrome. Recurrent infections start with pain, burning, or itching, followed by vesicles that commonly occur at the vermilion border of the lip. These lesions usually progress to a pustular or ulcerative and crusting stage. Subsequent healing is rapid, generally being complete in 8-10 days [Modrow, 2002; Whitley, 1990].

1.7.3.2 Genital Disease

Genital herpes ("herpes genitalis", "infection below the belt") is caused by HSV-2. Again, asymptomatic infection is the rule. However, primary genital herpetic infection may cause severe clinical disease. Symptoms include fever, dysuria, localized lymphadenopathy, macules and papules, followed by painful vesicles, pustules, and ulcers. Systemic spread is common, especially in women, approaching approximately 70% of all cases, with the most common being aseptic meningitis (10%) and other extragenital lesions. Recurrent disease is associated with a limited number of lesions and an approximate duration of 7-10 days. Neurologic or systemic complications are uncommon [Modrow, 2002; Whitley, 1990].

1.7.3.3 Other Clinical Manifestations

Other clinical manifestations of an infection with HSV include

1. neonatal HSV infection,
2. HSV keratoconjunctivitis,
3. skin infections,
4. infections of the immunocompromised host, and
5. infections of the CNS.

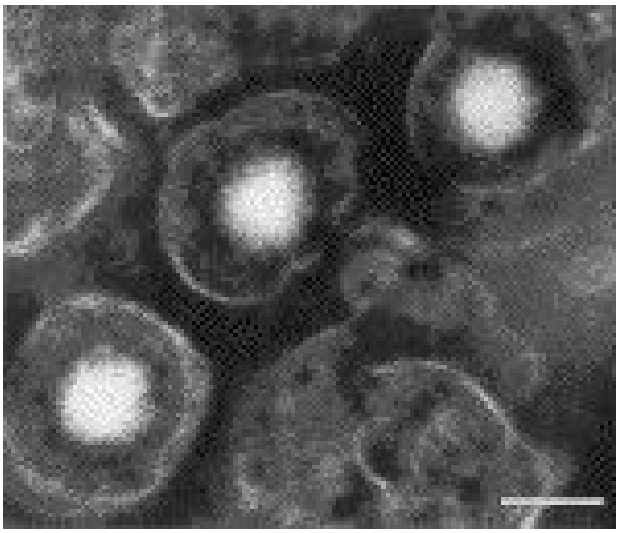
1.7.4 Diagnosis

Diagnosis is based on the clinical disease and on the serological determination of anti-HSV IgG and IgM antibody concentrations. If lesions are present, a scraping of skin vesicles should be made with subsequent virus isolation. In case of doubtful results, viral DNA can be amplified by PCR methods. A distinction between HSV-1 and HSV-2 is possible by determining specific antibodies or via PCR [Modrow, 2002; Whitley, 1990].

1.7.5 Management of HSV Infection

HSV infections can be treated locally or systemically using the virus polymerase inhibitor acyclovir. Exclusive local treatment is recommended only for the treatment of recurrent, circumscribed lesions. Viruses can develop resistance during treatment, but fortunately, resistant viruses are epidemiologically not relevant to date [Modrow, 2002].

1.8 Varicella-Zoster Virus



Varicella-zoster virus, like HSV, belongs to the herpesvirus subfamily of α-herpesviridae.

Among these, VZV is unique in its T-cell tropism, which allows dissemination of the virus to the skin. Its chronological designation is HHV-3 [Arvin, 2001].

Figure 7: Electron micrograph of VZV[††]

1.8.1 Epidemiology

VZV is a ubiquitous virus, producing annual varicella (chickenpox) epidemics during winter and spring in temperate climates. Epidemic years are usually followed by years with a lower incidence. In contrast, reactivation (resulting in shingles) exhibits no seasonal pattern.

Without immunization, the incidence of chickenpox has a peak in early childhood. The IgG seroprevalence to VZV is increasing with increasing age. In temperate climates, only approximately 5% of all individuals remain susceptible for primary VZV infection at the age of 30 years [Arvin, 2001]. Wutzler et al. investigated in the seroprevalence of IgG antibodies to VZV in the German population. They found that from an age of 11 years, more than 90% of the study subjects were positive for VZV IgGs [Wutzler et al., 2001]. Data from the RKI, Germany, confirm these findings; 95% of the study participants were reported to have been VZV antibody-seropositive already at the age of 17 years. At the age of 40 years, seropositivity reaches 100% [RKI, 2000c]. Interestingly, only about 50% of seropositive individuals give a clinical history of varicella, indicating either asymptomatic or mild disease or misdiagnosis [Arvin, 2001].

[††] Image by Dr Frank Fenner, Australian National University, Canberra, http://online.anu.edu.au/

Given the high incidence of VZV infection, most adults are at risk for VZV reactivation, which leads to herpes zoster (shingles). Increased age is strongly correlated with the occurrence of shingles. A population-based, prospective cohort study reported that incidence continued to rise after the age of 60 years, such that the lifetime risk to develop shingles is as high as 50% among those who survive to 85 years [Schmader et al., 1998].

1.8.2 Pathogenesis

Primary infection is presumed to start with inoculation of respiratory mucosa, and a subsequent first viral replication in regional lymph nodes. This is followed by a primary viremia during which VZV is transported to cells of the reticuloendothelial system, and a subsequent second phase of replication. Acute illness occurs at the start of a second viremia, during which VZV is disseminated to cutaneous epithelial cells. Cell-associated viremia continues after the initial skin lesions develop. Cellular tropism of VZV includes T-lymphocytes, skin cells and cells of the dorsal root ganglia. Although infectious virus has rarely been detected from nasopharyngeal secretions during the preeruptive phase, VZV must also be transported back to respiratory mucosa because it can already be transmitted prior to the occurrence of cutaneous lesions. Direct contact with cutaneous lesions provides another mechanism for VZV transmission. After primary infection, epidermal cells become the major target for further replication of the virus, and the typical lesions (liquid-filled, small vesicles) occur. In immunocompromised persons, disseminated infections are seen, including lung, liver, CNS, and other organs.

Primary infection is followed by latency in the dorsal root ganglia, but it is still unknown how the virus reaches this site. Reactivation leads to the so-called shingles (herpes zoster), a vesicular rash usually involving the dermatomal distribution of a single sensory nerve. Pathologic changes include inflammatory necrosis of the dorsal root ganglia, motor and sensory root degeneration, neuritis, and segmental myelitis. In immunocompromised individuals, reactivated VZV disease is similar to progressive varicella [Modrow, 2002; Arvin, 2001; RKI, 2000c].

1.8.3 Clinical Features

1.8.3.1 Varicella

Primary infection of the host leads to varicella, commonly called chickenpox. This is a highly contagious, febrile illness characterized by a generalized, pruritic vesicular rash, which is most prevalent in childhood where it represents the most common preventable infectious disease in Germany [RKI, 2000c].

After an incubation period of 10-21 days, typical varicella exanthemas begin on the scalp, face, or trunk, followed by progression to macules and fluid-filled vesicles, which are usually intensely pruritic. Lesions of mucous membranes are common. The number of days of new lesion formation ranges from 1-7 days followed by final crusting and healing. In children, the most common complications are caused by secondary infections with staphylococcus aureus and streptococcus pyogenes whereas in healthy adults, varicella pneumonia as well as the hemorrhagic form of chickenpox is a serious concern. Generally, immunocompromised persons are at higher risk for progressive varicella than healthy persons [Modrow, 2002; Arvin, 2001].

1.8.3.2 Shingles

Reactivation of VZV leads to herpes zoster, commonly called shingles. Clinically symptomatic varicella disease is not necessary for a reactivation of the virus. Furthermore, shingles can also occur in patients vaccinated against varicella [RKI, 2000c].

Herpes zoster, which is usually observed in elderly or immunocompromised individuals, is a vesicular rash restricted to a dermatomal distribution of one or more adjacent sensory nerves. Cutaneous lesions are often accompanied by pain, acute neuritis, and pruritus, and they may progress to confluent lesions with involvement of the whole dermatome. Final crusting and healing occurs approximately after 2 weeks but may require up to 4-6 weeks.

In the elderly, the most common complication is postherpetic neuralgia, a severe, invalidating, chronic pain. VZV reactivation in immunocompromised individuals leads to a progressive form of shingles whereas complications in younger patients are rare [Modrow, 2002; Arvin, 2001].

1.8.4 Diagnosis

Usually, there is no need for specific diagnostic analyses because the clinical picture is typical for chickenpox or shingles [RKI, 2000c].

If laboratory methods are required, detection of viral proteins, viral isolation in tissue culture and detection of DNA using PCR can be performed. A scraping of skin vesicles with subsequent virus isolation is necessary for a definitive diagnosis. Further processes include the serological determination of anti-VZV IgG and IgM antibody concentrations using enzyme immunoassays [Modrow, 2002; Arvin, 2001].

1.8.5 Management of VZV Infection

The nucleoside analog acyclovir has been proven to be an effective drug for the treatment of primary VZV infection or its reactivation, in healthy as well as in immunocompromised patients. Other nucleoside analogs, which are licensed for treatment of shingles, are famciclovir and valaciclovir [Modrow, 2002; Arvin, 2001]. Preventing VZV transmission is difficult as infected persons are contagious prior to the onset of varicella. Passive IgG antibody preparations are administered to people at risk for serious varicella, but studies are in disagreement about their efficacy [Arvin, 1996].

However, VZV is the first human herpesvirus for which a vaccine has been developed. The burden of disease can be diminished by this immunization using a live attenuated varicella vaccine, which successfully protects against primary infection [Asano, 1996; Arvin and Gershon, 1996]. Furthermore, this vaccine has the potential to lower the risk to develop herpes zoster [Gershon et al., 1996].

1.9 Epstein-Barr Virus

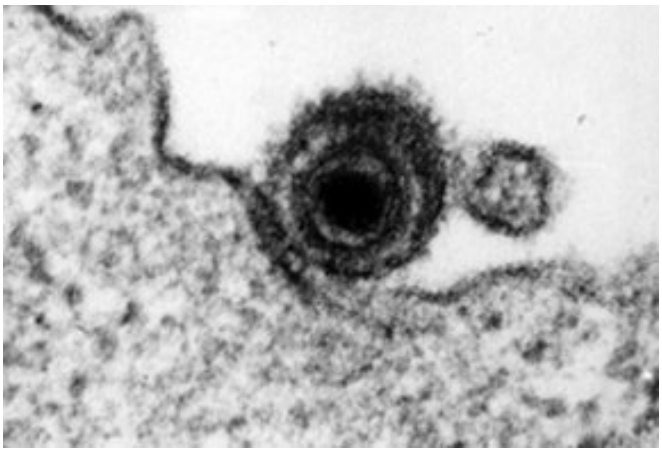


Figure 8: Epstein-Barr virus (dark sphere; Travis, 2002)

Tony Epstein and Yvonne Barr were the first to find Epstein-Barr virus (EBV) in Burkitt's lymphoma in the 1950s, thereby identifying EBV as the first candidate human tumor virus [Rickinson and Kieff, 2001].

Belonging to the herpesvirus subfamily of γ-herpesviridae, genus lymphocryptovirus, its formal designation is HHV-4.

1.9.1 Epidemiology

Like all herpesviruses, EBV is a ubiquitous virus, which is widespread in the human population. Its site of latency are resting memory B cells [Cohen, 2000].

In developing countries, most people become infected within the first three years of life, and EBV antibody seroprevalences reach up to 100% within the first decade of life. In the developed Western world, however, only approximately 50% are seropositive within the first decade of life. They become infected later in life through intimate oral contact, and only about 5% of adults in developed countries remain EBV-uninfected [Berger, 2003; Rickinson and Kieff, 2001]. These seronegative individuals constitute a population from which rare cases of classic infectious mononucleosis are drawn [Rickinson and Kieff, 2001].

Reviewed by Cohen [2000], IgG antibodies to EBV have a worldwide seroprevalence of more than 90% in the adult population.

1.9.2 Pathogenesis

The incubation period for infectious mononucleosis is 4-6 weeks, but little is known about what happens after oral transmission of EBV. One theory is that primary replication takes place at a specialized oropharyngeal site, with a subsequent transfer into the B cell system. An alternative hypothesis is that EBV primarily targets B cells infiltrating the oropharyngeal mucosa, followed by viremia to permissive epithelium. However, EBV infection is not necessarily associated with disease, and even long-term carriers with no history of infectious mononucleosis secrete low levels of infectious virus.

B cells are the site of latency of EBV. Therefore, reactivation can occur at any mucosal site with a B cell infiltrate. After primary infection, EBV-positive B cells remain detectable even in asymptomatic carriers. So far, the complexity of EBV strategies is not fully understood and any current attempt to map EBV pathogenesis must be regarded as preliminary [Rickinson and Kieff, 2001].

1.9.3 Clinical Features

EBV has been linked to several medical conditions for decades, but frequently, its clinical importance for onset of certain diseases or malignancies is only assumed [Rickinson and Kieff, 2001; Okano, 1998; Gaffey and Weiss, 1992].

1.9.3.1 Infectious Mononucleosis

Infectious mononucleosis occurs in rare cases during primary infection with EBV. This disease is more prevalent in adolescents and adults whereas children very rarely develop infectious mononucleosis [Cohen, 2000]. Symptoms can range from mild fever to several weeks of pharyngitis, lymphadenopathy and general malaise [Gaffey and Weiss, 1992]. In about half of the cases, splenomegaly occurs [Modrow, 2002]. However, there is evidence that these symptoms may occur due to immunopathological events (activation of cytokines) rather than be the result of viral replication [Rickinson and Kieff, 2001].

1.9.3.2 Burkitt's Lymphoma

There are three different kinds of Burkitt's lymphoma; (i) an endemic form that was first described by Denis Burkitt in Africa, (ii) a sporadic form causing rare childhood lymphomas, and (iii) a form, which appears in HIV-infected individuals.

The endemic form shows the strongest association with EBV. Endemic Burkitt's lymphoma is a common childhood cancer in Africa that is more common in males than in females. In contrast to other lymphomas, Burkitt's lymphoma occurrs at extranodal sites. This malignancy occurs mainly in the jaw in association with molar tooth development. Other sites include the orbit, abdomen, or CNS. To date, c-myc expression and EBV infection are the best documented etiological factors of endemic Burkitt's lymphoma, for which a multistep pathogenesis is accepted [Rickinson and Kieff, 2001; Gaffey and Weiss, 1992].

1.9.3.3 Lymphomas in Immunodeficient Patients

The clearest evidence for the oncogenicity of EBV in its natural host comes from the fatal B cell lymphoproliferations that are seen in immunocompromised persons with impaired T cell immunity, therefore being unable to control the proliferation of EBV-infected B cells [Cohen, 2000]. EBV-associated lymphomas (especially CNS lymphomas) occur in congenitally immunodeficient patients as well as after transplantations and in HIV-infected individuals, in which the incidence can be as high as 60% [Modrow, 2002; Rickinson and Kieff, 2001].

1.9.3.4 Hodgkin's Disease

EBV is frequently found in Hodgkin's lymphomas' tumor cells. Several studies reported a clearly increased risk for Hodgkin's disease in individuals with a history of infectious mononucleosis. However, a variety of these tumors are EBV-negative and the proportion of EBV to be present in Hodgkin's disease varies between the different histologic types from very few to 80% [Rickinson and Kieff, 2001]. So far, the pathogenetic role of EBV in Hodgkin's disease still remains controversial [Gaffey and Weiss, 1992].

1.9.3.5 Nasopharyngeal Carcinoma

Nasopharyngeal carcinomas are seen all over the world, with an age-adjusted incidence in Europe of less than 0.5 per 100 000 per year. The regular presence of the EBV genome in cells of this type of tumor, and higher antibody titers in tumor patients lead to the suggestion that EBV might be involved in nasopharyngeal carcinoma pathogenesis [Rickinson and Kieff, 2001]. However, there is increasing evidence that this tumor type arises independently of EBV, because the tumor usually occurs in the fourth decade of life or later, and EBV infection is known to occur prior to adulthood in more than 90% of the population [Cohen, 2000].

However, although ongoing studies are still controversial, this possible relation is used as a diagnostic and prognostic tool in disease monitoring. Since patients with nasopharyngeal carcinomas in contrast to healthy persons have elevated anti-EBV IgA, these tumors can be detected at an early stage by screening for these antibodies [Rickinson and Kieff, 2001].

1.9.4 Diagnosis

During primary infection, the immune system of the host produces detectable IgG, IgM and IgA antibodies to early antigens of EBV. The detection of IgM and IgA antibodies is indicative for an acute infection whereas IgG antibodies point at a previous infection. Another diagnostic method is the detection of viral DNA in B cells of the patients [Modrow, 2002].

1.9.5 Management of EBV Infection

Acyclovir used to be the choice treatment of EBV infection, since it was able to inhibit viral replication *in vitro*. However, clinical use did not show any effect on the clinical outcome of EBV infection [Cohen, 2000]. Therapy of EBV infection is restricted to therapy of malignancies caused by this virus. Burkitt's lymphomas show a good respond rate to chemotherapy [Modrow, 2002; Cohen, 2000]. So far, no vaccinations are available for the prevention of primary EBV infection [Modrow, 2002; Rickinson and Kieff, 2001].

2 Material and Methods

The following section describes the recruitment of the study population between December 2002 and April 2004 as well as the samples available for analyses.

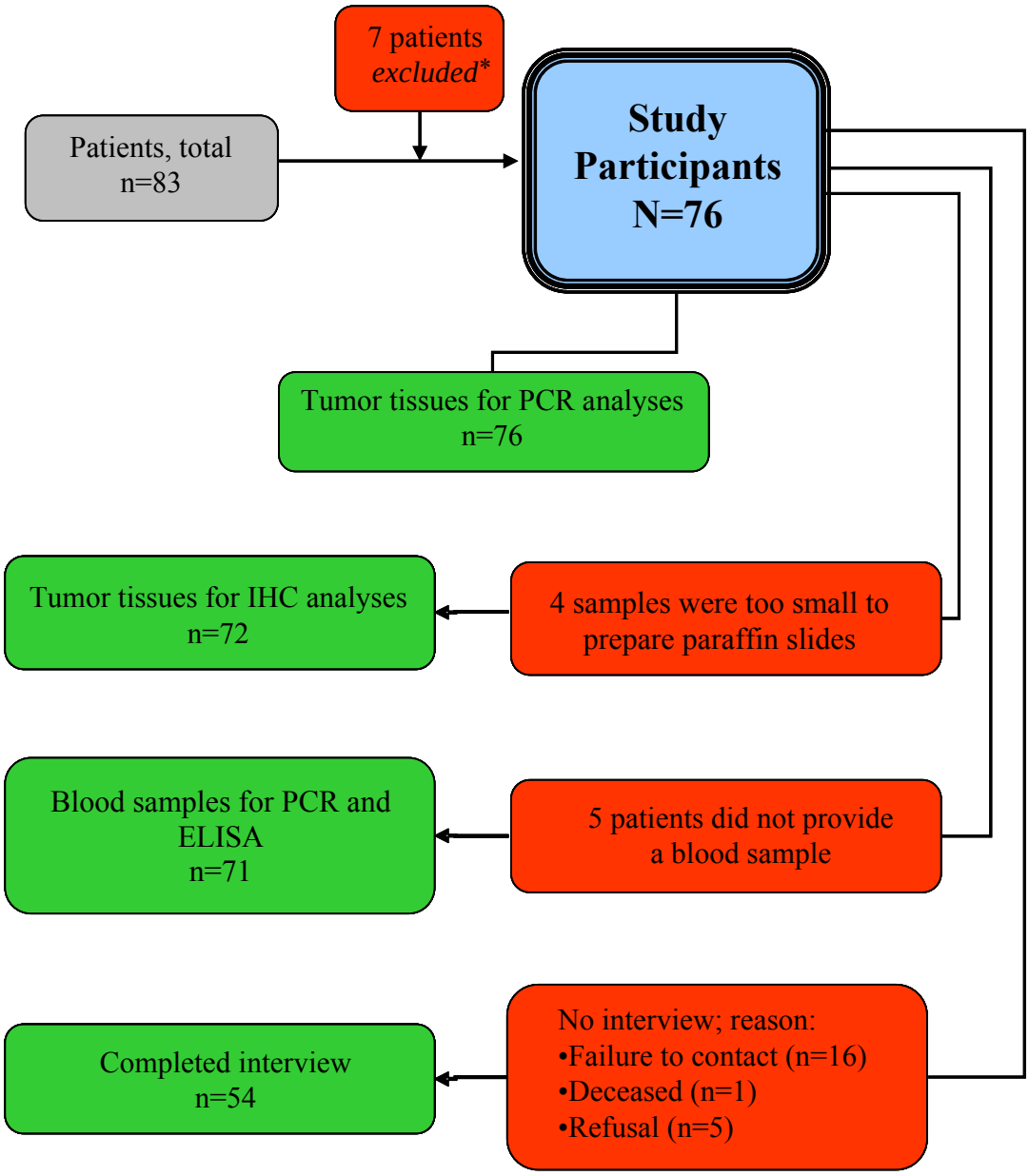


Figure 9: Study design, recruitment of the study population and material available for analyses

* brain tumor diagnosed prior to the present study period (prevalent cases);
PCR, polymerase chain reaction; IHC, immunohistochemistry

2.1 Study Design and Recruitment of the Study Population

The present study included patients, who underwent surgery of primary brain tumors at the Department of Neurosurgery, University of Heidelberg, between December 2002 and April 2004. Inclusion in the study was not dependent on patients' age or residence.

The reference date for all analyses performed was the date of surgery.

Of the tumor samples collected from December 2002 to May 2003, ten were collected anonymously; information was only obtained about histology, gender and year of birth. Inclusion in the study of these samples without informed consent, however, is in concordance with the national ethics commission [Zentrale Ethikkommission Deutschland, 2003].

Surgical specimens were collected from 83 brain tumor patients. Only persons with incident primary brain tumors were included. Therefore, seven patients (4 gliomas, 2 meningiomas, 1 medulloblastoma) had to be excluded from the study, because their tumors were diagnosed prior to the study period. As described above, brain tumors are unequivocally no homogenous group of cancer. Therefore, all analyses were stratified by tumor type (e.g., gliomas, meningiomas, and acoustic neurinomas), and only some analyses were additionally pooled.

The study finally included 76 brain tumor patients. Patients gave informed consent after they received adequate verbal or written clarification.

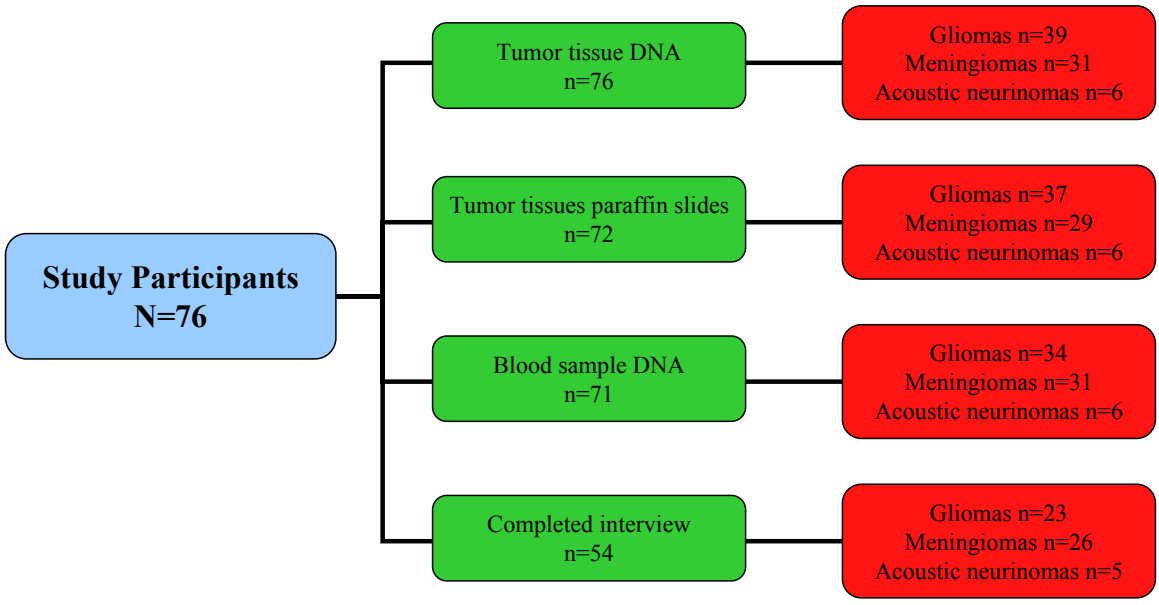


Figure 10: Samples available for study analyses, stratified by tumor type

2.2 Questionnaire

Interviews were performed directly after brain tumor surgery in the hospital if possible. Otherwise, patients were contacted by letter, followed up by up to four more letters and several phone calls if necessary, and telephone interviews were performed.

The standardized questionnaire included questions about previous herpesvirus and common infections, hereditary diseases, previous cancers and other medical conditions. Furthermore, vaccinations as well as contact to animals, a complete occupational history and demographic data were assessed, all of which are controversially discussed as putative risk factors for either brain tumor development or HCMV infection or reactivation.

To exclude that reported seizures, hearing impairments and tinnitus were early symptoms of the present primary brain tumor, these conditions were only taken into account if they occurred two years and more prior to the present tumor.

The question on tinnitus addressed its role as an early symptom of acoustic neurinoma. At first place, only patients affected by acoustic neurinoma were asked about the occurrence of tinnitus. Later on in the study, however, all patients were asked to answer that question.

2.3 Laboratory Material

2.3.1 Brain Tumor Samples

With informed consent of the patients (except of those, who were collected anonymously, cf. above), samples of glioma, meningioma, and acoustic neurinoma were obtained from the Department of Neurosurgery, University of Heidelberg, between December 2002 and March 2004. One part of the sample was immediately stored at -80°C and in addition, if enough tumor tissue was available, a piece was put in formaldehyde for further preparation.

Altogether, 76 primary brain tumor samples were collected (39 gliomas, 31 meningiomas, 6 acoustic neurinomas).

Of the 76 tumor samples, 72 (94.7%) were large enough to prepare a formaldehyde-fixed sample for the preparation of paraffin slides (37 gliomas, 29 meningiomas, 6 acoustic neurinomas).

Furthermore, four of the brain tumor samples were prepared for cell culture, grown in short-term cultures (see below) and frozen in liquid nitrogen.

2.3.2 Blood Samples

In addition to the brain tumor samples, a heparinized or EDTA blood sample (3-7 ml) was provided from 71 (93.4%) of the patients at the time of surgery or two days after surgery at the latest, yet prior to chemotherapy or irradiation (34 gliomas, 31 meningiomas, 6 acoustic neurinomas). Blood clot and serum were separated and stored at -80°C.

2.3.3 Equipment

Centrifuge for Eppendorf tubes 5415	Eppendorf, Hamburg
Electrophoresis chamber, horizontal (Horizon 11•14)	Life Technologies, Gaithersburg, USA
Hood: Template ™ Tamer	Q•Biogene, Heidelberg
Image Master® VDS	Amersham Biosciences, Freiburg
Laboratory scale 1216	MP Sartorius, Göttingen
Measuring cylinder 50-500 ml	VIT LAB, Seeheim-Jugenheim
Microwave 600W	Bosch, Lohr
Nalgene ™ Laptop Cooler	Bender & Hobein AG, Zürich, CH
Picofuge	Stratagene, Amsterdam
Pipettes (Eppendorf)	Eppendorf, Hamburg
Pipettes (Finnpipette)	Labsystems, Helsinki
PTC-100 Programmable Thermal Cycler	MJ Research Inc. California, USA
Table centrifuge Biofuge 13R	Kendro Laboratory Systems, USA
Table centrifuge Sigma 1-15K	Sigma, Osterode am Harz
Vortex Genie 2™	Bender & Hobein AG, Zürich, CH
Power supply	Biotech-Fischer, Pittsburgh, USA

2.3.4 Consumables

Eppendorf tube 0.5; 1.5; 2 ml	Eppendorf, Hamburg
Erlenmeyer flask 250 ml	Schott, Mainz
Falcon tubes 15 ml	Becton/Dickinson, Franklin Lakes, USA
Measuring cylinder	Bürkle, Lörrach
Parafilm “M”	American National Can., Chicago
Pipette tips, unsterile	Greiner Bio-one, Frickenhausen
RNAse-free pipette tips 10/100/1000 µl	Nerbe-Plus, Winsen-Luhe
Tumbler	Schott, Mainz

2.3.5 DNA Extraction

REDExtract N-Amp^{TM} Blood PCR Kit	Sigma-Aldrich Chemie GmbH, Taufkirchen
High Pure PCR Template Preparation Kit	Roche, Mannheim
Isopropanol	Merck, Darmstadt

2.3.6 PCR and Gel Electrophoresis

2.3.6.1 Oligonucleotides

All Oligonucleotides were obtained from Sigma-Ark GmbH, Darmstadt.

Name	**Sequence (5'-3')**
GAPDH1	TTA ACT CTG GTA AAG TGG ATA TTG TTG CCA
GAPDH2	TAT TTG GCA GGT TTT TCT AGA CGG CA
EL	TAA CGG GTA CTG TGG GTG TTG G
ER	ACC AAG TAC CCC TAT CGC GTG T
IL	CTG CCC AGC AGA TAA GTG GTG T
IR	ATC ATC TGC ACC TCG ATG AAG C
EXTL	CGA GGC TAC GCT TCC TAC AC
EXTR	GCG TAC GAG GAA CTC TTT GC
INTL	GAC GAC CCT TTC GAT GAG TG
INTR	GCC CAA CAA CTG GTG GTA AC
IEN1	ACA TCT TTC TCG GGG TTC TCG TTG C
IEN2	GTC CTC TGC CAA GAG AAA GAT GGA C
IEN3	TTG AGG GAT TCT TCG GCC AAC TCT G
IEN4	TCT CCT GTA TGT GAC CCA TGT GCT T
EXT_F	TCC AAC ACC CAC AGT ACC CGT
EXT_R	CGG AAA CGA TGG TGT AGT TCG
INT_F	CGC CGC GGC AGC ACC TGG CT
INT_R	GTA AAC CAC ATC ACC CGT GGA

2.3.6.2 Reagents and Buffers

Agarose	Life Technologies, Eggenstein
Aqua ad injectabila	Braun, Melsungen
DNA 100 bp Ladder	Life Technologies, Gaithersburg, USA
DNA agarose gel 0.3-2% (w/v)	agarose 50x electrophoresis buffer 2 M Tris, pH 7.8 0.25 M sodium acetate 0.05 M EDTA
Electrophoresis buffer	ammonium formate buffer; 0.05 M; pH 4.0: ammonia, concentrated; 2.9 ml formic acid, 98%; 2.0 ml distillated water ad 1000 ml adjust pH-value with concentrated formic acid to pH ≤4.0
Ethidium bromide	Sigma, Deisenhofen 10 mg EtBr/ml H_2O, stored dark at 4 °C
Gene Ruler 100 bp DNA Ladder	MBI Fermentas, St. Leon-Rot
Loading Dye Solution (6x)	MBI Fermentas, St. Leon-Rot 50% sucrose 0.15% bromine phenol blue 0.1% SDS, in H_2O
Oligonucleotides	Sigma-Ark GmbH, Darmstadt
PCR Supermix	Life Technologies, Eggenstein 22 mM Tris-HCl, pH 8.4 5 mM KCl 1.65 mM $MgCl_2$ 220 µM dGTP 220 µM dATP 220 µM dTTP 220 µM dCTP 22 U/ml Taq-Polymerase

2.3.7 Cell Culture, Cell Fixation

PBS	8.0 g NaCl 0.2 g KH_2PO_4 1.2 g Na_2HPO_4 0.2 g KCl ad 100 ml H_2O, pH 7.2-7.5
Dulbecco's Modified Eagle's Medium (DMEM)	Sigma, Deisenhofen
Fetal Calf Serum (FCS)	Sigma, Deisenhofen
Penicillin/Streptomycin (Pen/Strep)	Gibco, Invitrogen Corporation, Karlsruhe
Trypsin EDTA	Gibco, Invitrogen Corporation, Karlsruhe
DMEM+	DMEM 10% FCS 1% Pen/Strep
Methanol	Merck, Darmstadt
Aceton	Merck, Darmstadt
Dimethylsulfoxide (DMSO)	Sigma, Deisenhofen
Freezing medium for cells	60% DMEM 10% DMSO 30% FCS

2.3.8 Immunohistochemistry

Paraffin sections were prepared from formaldehyde-fixed tumor tissue samples at the Division Cellular and Molecular Pathology, DKFZ.

2.3.8.1 Antibodies

anti-Cytomegalovirus, Early Antigen	Novocastra, Newcastle upon Tyne, UK NCL-CMV-EA mouse monoclonal antibody
anti-Cytomegalovirus, pp65 antigen	Novocastra, Newcastle upon Tyne, UK NCL-CMVpp65 mouse monoclonal antibody
anti-Cytomegalovirus, IE-1 antigen	Chemicon, Temecule, USA MAB810 mouse monoclonal antibody

2.3.8.2 Chemicals

AEC+ Substrate-Chromogen	Dako Cytomation, Carpinteria, USA
Antigen Retrieval Accessory Kit	Biogenex, San Ramon, USA
Antigen Retrieval Citra Solution	Biogenex, San Ramon, USA
Aquatex® aqueous mounting medium	Merck KGaA, Darmstadt
Avidin-Biotin Blocking Kit	Biogenex, San Ramon, USA
Common Antibody Diluent	Biogenex, San Ramon, USA
Ethanol, absolute	Riedel-de Haen, Seelze
Negative Control for Mouse Antibody	Biogenex, San Ramon, USA
Peroxide Block	Biogenex, San Ramon, USA
Super Sensitive HRP Label	Biogenex, San Ramon, USA
Super Sensitive Mouse Link	Biogenex, San Ramon, USA
TRIZMA® Pre-set crystals	Sigma, Saint Louis, USA
Xylene	Sigma-Aldrich, Seelze

2.3.9 ELISA Reagents

Enzygnost® System (Dade Behring, Marburg):

IgG Detection	**IgM Detection**
Enzygnost Anti-CMV/IgG test plate	Enzygnost Anti-CMV/IgM test plate
Anti-CMV Reference P/P	
Anti-CMV Reference P/N	Anti-CMV Reference P/N
Enzygnost Anti-VZV/IgG test plate	Enzygnost Anti-VZV/IgM test plate
	Anti-VZV Reference P/P
Anti-VZV Reference P/N	Anti-VZV Reference P/N
Enzygnost Anti-HSV/IgG test plate	Enzygnost Anti-HSV/IgM test plate
	Anti-HSV Reference P/P
Anti-HSV Reference P/N	Anti-HSV Reference P/N
Enzygnost Anti-EBV/IgG test plate	Enzygnost Anti-EBV/IgM test plate
	Anti-EBV Reference P/P
Anti-EBV Reference P/N	Anti-EBV Reference P/N
Anti-Human-IgG/POD Conjugate	Anti-Human-IgM/POD Conjugate
Sample Buffer POD	Sample Buffer POD
Conjugate Buffer Microbiol.	Conjugate Buffer Microbiol.
	RF Absorbent

2.4 Methods

2.4.1 Sample Preparation

2.4.1.1 Brain Tumor Samples

Brain tumor samples were obtained in isotonic sodium chloride (0.9%) immediately after surgery at the Department of Neurosurgery, University of Heidelberg. All brain tumors were histologically diagnosed at the Pathological Institute, University of Heidelberg. The samples were stored at -80°C for further preparation. In case the sample was large enough, one part was stored in formalin (4%) for preparation of paraffin sections. For analyses to validate the methods performed, short-term cultures were prepared from four of the collected tumors from which suitable material was available (two gliomas, two meningiomas; cf. below).

2.4.1.2 Cell Culture

Cell lines provide a useful system for further understanding the biology of certain tissues, e.g. neoplasms. Furthermore, the availability of unlimited numbers of cells and the possibility of performing multiple, repeated experiments over long time intervals provide a valuable resource for studies on pathogenesis or therapy of malignancies.

2.4.1.2.1 Preparation of Short-term Cultures

Surgical specimens of primary brain tumors were obtained immediately after surgery. In this study, two meningiomas WHO grade I, one astrocytoma WHO grade III and one glioma with unknown grading were prepared for short-term culture. For this, one part of the tissue sample was minced to small pieces. Afterwards, hackled tissue was prepared as a fine cell suspension

by digestion with trypsin/EDTA for 5 minutes at room temperature. Cells were washed with DMEM+ (DMEM containing 10% FCS and 1% Penicillin/Streptavidin), and transferred into culture flasks containing DMEM+. All short-term cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2. At the time the cells reached confluence, they were washed with phosphate buffered saline (PBS), treated with trypsin/EDTA and then transferred into new flasks containing DMEM+.

2.4.1.2.2 Cultivation of Human Skin Fibroblasts

Human skin fibroblasts were kindly provided by PD Dr. Tomakidi, Medical Faculty, University of Heidelberg.

Cultivation of these fibroblasts took place at 37°C in a humidified atmosphere of 5% CO_2. DMEM+ was applied. When confluence was achieved, the medium was removed, cells were briefly washed in PBS and the monolayer was removed using trypsin/EDTA at 37°C. Afterwards, cells were transferred into new culture flasks in a ratio of 1:10.

2.4.1.2.3 Freezing and Thawing of Cells

For permanent storing in liquid nitrogen, cells were removed from the culture flask by treatment with trypsin/EDTA, and resuspended in DMEM+. Afterwards, the cell count was determined, cells were centrifuged at 1000 rpm for 10 min and the supernatant was removed. Cells were resuspended in 1 ml of freezing medium on ice and filled into kryo vials. These were enveloped by several tissue plies to assure a slow freezing process. After 24 h in -80°C, kryo vials were taken into liquid nitrogen.

For reculturing, cells were taken out of the liquid nitrogen, resuspended in DMEM+ and centrifuged at 1000 rpm for 10 min. After removal of the supernatant, cells were transferred into a culture flask containing DMEM+ and incubated at 37°C in a humidified atmosphere of 5% CO_2.

2.4.1.2.4 Cell Fixation on Cover Glasses for Immunohistochemistry

Trypsinized cells were transferred into 6-well plates containing two cover glasses each at a concentration of $1x10^5$ cells per well. When confluence was achieved, DMEM+ was removed and fixation of the cells grown on the cover glasses took place by incubation in methanol (10 min at -20°C). Afterwards, methanol was removed and cells were incubated in acetone for 10 min at -20°C. After removal of the acetone, fixated cells were stored at -80°C until further analyses.

2.4.1.2.5 HCMV Infection of Human Skin Fibroblasts

For HCMV infection, medium was removed and virus suspended in DMEM was inoculated to the cell layer at a MOI (multiplicity of infection) of one infectious unit per cell. Fibroblasts were incubated at 37°C (humidified atmosphere, 5% CO_2) for 1.5 h. Afterwards, the virus was removed thoroughly by washing the cells thrice with PBS. Finally, DMEM+ was added, and cells were stored at 37°C in a humidified atmosphere of 5% CO_2.

Fig. 11 shows typically enlarged fibroblasts (cytomegalia) 5 days after HCMV infection, which served as positive controls, and mock-infected fibroblasts, which served as negative controls for immunohistochemical analyses.

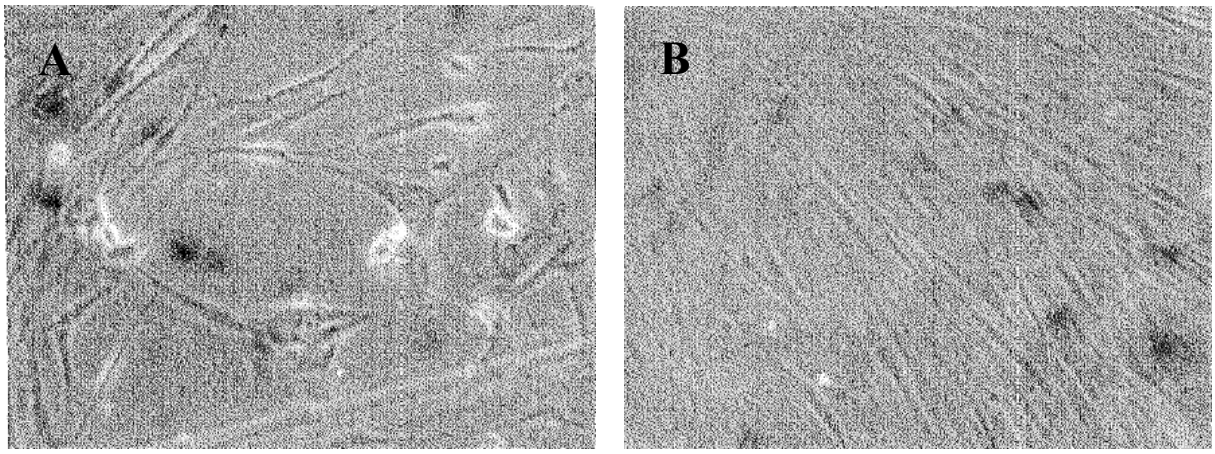


Figure 11: Human skin fibroblasts 5 days after HCMV infection (MOI 1). (A) showing typical cytomegalia and inclusions. In contrast, mock-infected fibroblasts did not show any cytomegalia (B).

2.4.1.3 Blood Samples

3-7 ml of blood samples containing EDTA or heparin were provided by 71 (93.4%) of the brain tumor patients. The samples were centrifuged at 1000 rpm for 10 min. Serum and blood clot were separated, and stored in kryo vials at -80°C for further analyses.

2.4.2 DNA Extraction from Tumor Tissue

DNA isolation took place using the "High Pure PCR Template Preparation Kit". With this method, tissue is lysed by a specific buffer, followed by a short incubation with proteinase K in the presence of chaotropic salts that immediately inactivate all nucleases. Nucleic acids bind to a glass fiber fleece and remain bound, while several washing steps remove contaminating small molecules. Finally, remaining DNA is removed by a low salt elution.

First, minced tissue was taken into a mix of lysis buffer and proteinase K, and incubated at 55°C overnight until all tissue was solubilized. Binding buffer was added and incubation took place at 72°C for 10 min in a prewarmed water bath. After addition of isopropanol, the solution was pipetted into a glass fiber-covered filter tube, centrifuged for 2 min at 8000 rpm and the flowthrough was discarded. Twice adding washing buffer and centrifugation as described above followed this step. Finally, DNA was eluted with twice the recommended amount of elution buffer to increase the final volume of DNA. Now, extracted tissue DNA could directly be used for PCR analyses or stored at 4°C.

2.4.3 DNA Extraction from Blood Samples

DNA isolation was performed using the "REDExtract-N-Amp^{TM} Blood PCR Kit". First, 20 µl lysis buffer were pipetted in Eppendorf tubes, and 10 µl of the whole blood sample were added. After incubation for 5 min at room temperature, 180 µl of neutralization buffer was added and resuspended thoroughly. Finally, extracted DNA could be stored at 4°C or directly used in PCR analyses (as described below).

2.4.4 Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a method for *in vitro*-amplification (multiplication) of specific DNA fragments. The American chemist K. B. Mullis, who received the Nobel price for chemistry for this work in 1993, developed this method. With this very sensitive technique, a defined double-stranded DNA fragment can be amplified (i.e., multiplied) a million times out of a certain double stranded DNA fragment within a few hours.

A template DNA (i.e., DNA that has to be amplified), the thermo-stable taq-polymerase (isolated from the thermo-stable bacterium Thermus aquaticus), specific primers and 2'-desoxynucleosid-5'-triphosphates (dNTPs) for synthesizing new DNA strands are needed for PCR. In addition, free cations (commonly magnesium chloride, $MgCl_2$) are added and a buffer, which keeps the pH-value constantly at 7.0-7.2.

The principle of this method is as follows:
First, the double-stranded template DNA is denaturated. After that, the specific primers added attach to their complementary sequences on the single-stranded DNA ('annealing'). The DNA polymerase starts to synthesize new double strands starting from both primers with the aid of dNTPs ('extension'). This cycle is repeated 30-40 times. At the end, a multitude of amplified template DNA is available. This amplified DNA can now be identified according to its size (measured in base pairs=bp) after electrophoretic separation via agarose gel.

For increasing specificity, PCR products can be blotted and hybridized following PCR. For increasing sensitivity and proving specificity, the so-called nested PCR (nPCR) can be performed, in which the first product is amplified again in a second PCR with primers that lay within the first primer pair.

PCR in all protocols used in this study (except the protocol according to Mangano et al., 1992) was performed using Supermix (Invitrogen), which contains tris-salt acid, potassium chloride, $MgCl_2$, dNTPs and taq polymerase. The basic mix for the first PCR contained 45 µl Supermix, 0.2 µl per primer and 2 µl template DNA. For nested PCR, 1 µl PCR product was used instead of template DNA. In GAPDH PCR, 5 µl of template DNA were applied.

In the protocol according to Mangano et al. [1992], 10 µl of Readymix (Sigma-Aldrich), 7.6 µl double-distilled water, 0.2 µl per primer and 2 µl template DNA (and 1 µl PCR product in the nested PCR, respectively) were used.

Along with the samples being amplified, a positive control (HCMV DNA and HeLa DNA, respectively, for GAPDH PCR) and a negative control (Aqua ad injectabilia) were run to control for contamination and successful DNA amplification.

Primers and temperature protocols of all PCRs performed are described in more detail in the following sections. The same PCR temperature protocols were used for first and second DNA amplification in nested PCR analyses. Amplified DNA fragments were segregated by agarose gel electrophoresis and visualized under UV light.

Primers used for the detection of HCMV DNA were synthesized from HCMV strain AD169. Numbers in brackets state the position of each oligonucleotide within the genome of HCMV. All primers are given in 5'→ 3' direction.

2.4.4.1 Quality Assessment in Brain Tumor DNA

GAPDH is an enzyme of glycolysis, which exists in every human cell. A negative GAPDH PCR result would indicate that the isolated DNA is insufficient for PCR detection or that inhibitors of the PCR are present in the sample. The amplified PCR product with the primers chosen has a length of 670 .

Table 5: PCR protocol and primers for the detection of GAPDH in brain tumor DNA

Primers	**Sequences**			
GAPDH1	TTA ACT CTG GTA AAG TGG ATA TTG TTG CCA			
GAPDH2	TAT TTG GCA GGT TTT TCT AGA CGG CA			
Reaction Mix				
Supermix			45 µl	
Primers, each			0.3 µl	
Template DNA			5.0 µl	
Temperature Protocol				
Initial Denaturation	94°C	3 min		
Denaturation	94°C	45 s	}	40 cycles
Annealing	53°C	45 s	}	
Extension	72°C	1 min	}	
Final extension	72°C	10 min		

2.4.4.2 HCMV DNA Amplification in Brain Tumor DNA

2.4.4.2.1 Protocols for the Detection of HCMV-specific gB (UL55) Gene Sequences

PCR was performed to detect sequences of the HCMV-specific gB (UL55) gene, which encodes for one of the most highly conserved herpesvirus-common proteins [Chee et al., 1989].

The primers EL, ER, IL and IR amplify a segment of gB (UL55) gene. The first PCR product has a length of 419 bp; the second of the nPCR has a length of 167 bp. To confirm PCR results obtained by the first protocol, an alternative PCR was carried out with a subset of the brain tumor DNA samples to detect the HCMV-specific gB (UL55) gene as described by Cobbs et al. [2002]. The primers EXT_F, EXT_R, INT_F and INT_R recognize a segment of gB (UL55) gene. The external primers EXT_F and EXT_R amplified a 268 bp fragment within the coding region of gB (position 655-922). The internal primers INT_F and INT_R amplified sequences of the gB gene from position 704 to 825 (Tab. 6).

Table 6: PCR primers and protocols used for the detection of two different sequences of the HCMV-specific gB (UL55) gene

1st Protocol:				
Primers	**Sequences**			**Genome Position**
EL	TAA CGG GTA CTG TGG GTG TTG G			(83747-83768)
ER	ACC AAG TAC CCC TAT CGC GTG T			(84165-84124)
IL	CTG CCC AGC AGA TAA GTG GTG T			(83933-83912)
IR	ATC ATC TGC ACC TCG ATG AAG C			(84099-84078)
Reaction Mix				
	Supermix	45 µl		
	Primers, each	0.2 µl		
	Template DNA / Product from 1st PCR	2.0 µl / 1.0 µl		
Temperature Protocol				
	Initial Denaturation	94°C	2 min	
	Denaturation	94°C	30 s	
	Annealing	67°C	45 s	35 Cycles
	Extension	72°C	45 s	
	Final extension	72°C	10 min	

Table 6 continued

2nd Protocol (according to Cobbs et al., 2002):

Primers	Sequences	Genome Position
EXT_F	TCC AAC ACC CAC AGT ACC CGT	(634-655)
EXT_R	CGG AAA CGA TGG TGT AGT TCG	(922-943)
INT_F	CGC CGC GGC AGC ACC TGG CT	(684-704)
INT_R	GTA AAC CAC ATC ACC CGT GGA	(825-845)

Reaction Mix

Supermix	45 µl
Primers, each	0.2 µl
Template DNA /	2.0 µl /
Product from 1st PCR	1.0 µl

Temperature Protocol

Initial Denaturation	94°C	5 min	
Denaturation	94°C	30 s	30 Cycles
Annealing	60°C	45 s	
Extension	72°C	45 s	
Final extension	72°C	10 min	

2.4.4.2.2 Protocol for the Detection of HCMV-specific IE-1 Gene Sequences

Additional PCRs were performed to detect sequences of the HCMV-specific IE-1 gene using two different protocols (see Tab. 7).

Table 7: PCR primers and protocols used for the detection of the HCMV-specific IE-1 gene

1st PCR Protocol:

Primers	Sequences	Genome Position
EXTL	CGA GGC TAC GCT TCC TAC AC	(172687- 172626)
EXTR	GCG TAC GAG GAA CTC TTT GC	(172932- 172912)
INTL	GAC GAC CCT TTC GAT GAG TG	(172711 – 172732)
INTR	GCC CAA CAA CTG GTG GTA AC	(172887 – 172868)

Reaction Mix

Supermix	45 µl
Primers, each	0.2 µl
Template DNA /	2.0 µl /
Product from 1st PCR	1.0 µl

Temperature Protocol

Initial Denaturation	94°C	2 min	
Denaturation	94°C	30 s	30 Cycles
Annealing	64°C	45 s	
Extension	72°C	45 s	
Final extension	72°C	10 min	

Table 7 continued
2nd PCR Protocol (according to Mangano et al., 1992):

Primers	Sequences	Genome Position
IEN1	ACA TCT TTC TCG GGG TTC TCG TTG C	(172000 – 172424)
IEN2	GTC CTC TGC CAA GAG AAA GAT GGA C	(172736 – 172760)
IEN3	TTG AGG GAT TCT TCG GCC AAC TCT G	(172461 – 172485)
IEN4	TCT CCT GTA TGT GAC CCA TGT GCT T	(172606 – 172630)

Reaction Mix

Readymix	10 µl
dd H_2O	7.4 µl
Primers, each	0.2 µl
Template DNA /	2.0 µl /
Product from 1st PCR	1.0 µl

Temperature Protocol

Initial Denaturation	94°C	5 min	
Denaturation	94°C	1 min	35 Cycles
Annealing	67°C	2 min	
Extension	72°C	1 min	
Final extension	72°C	7 min	

The primers EXTL, EXTR, INTL and INTR recognize a segment of the Immediate Early-1 (IE-1) gene. The first PCR product has a length of 246 bp; the second of the nested PCR has a length of 177 bp. A subset of DNA samples was amplified using an alternative PCR protocol published by Mangano et al. [1992]. The primers IEN1, IEN2, IEN3 and IEN4 recognize a segment of the IE-1 (UL123) gene. The first PCR product has a length of 350 bp; the second of the nested PCR has a length of 170 bp.

2.4.4.3 HCMV DNA Detection in Blood Sample DNA

To exclude the possibility that positive results in brain tumor tissue were due to a contamination of the tissues with HCMV DNA-positive blood cells, nested PCR of corresponding blood samples of 71 brain tumor patients was carried out, using the protocols published by Cobbs et al. [2002] and Mangano et al. [1992] as described in Tab. 6 and 7.

2.4.5 Agarose Gel Electrophoresis

Agarose gel electrophoresis is a method to identify the size of a DNA strand. Agarose is a linear polysaccharide forming a lattice in polymerized condition. By using gels with different concentrations of agarose, one can resolve different sizes of DNA fragments. Higher concentrations of agarose facilitate the separation of small DNA fragments, while low agarose concentrations allow resolution of larger DNA fragments. Amplified DNA strands migrate through an agarose gel in an electrophoresis chamber towards the anode according to their size (measured in base pairs=bp) and the amplified DNA strand can be identified after comparison with the size of a defined DNA fragment (e.g. “DNA ladder”).

For analyses in the present study, 2.25 g of agarose were weighed out in an Erlenmeyer flask and 150 ml of electrophoresis buffer were added, meeting a gel of 1.5%. Afterwards, the Erlenmeyer flask was microwaved until total solution of agarose. Boiling retardation was refilled with electrophoresis buffer. After a short cooling, 10 µl of ethidium bromide (EtBr), which attaches to DNA double strands during electrophoresis and emits a visible light under UV exposure, were added. The liquid agarose was poured into a horizontal electrophoresis chamber containing a slot-forming comb.

After the gel had solidified, the chamber was filled with electrophoresis buffer and the comb was extracted. 5 µl of loading buffer, containing a fluid with high density (e.g. glycerol) to allow the sample to "fall" into the slots, were added to each PCR product (except for PCR products that were amplified with ‘REDExtract-N-Amp^{TM} Blood PCR Kit’, which already contained loading dye). 20-25 µl of the PCR product containing loading dye were pipetted into the slot. An electric current was applied to the chamber, starting with 80V for 5 minutes, then 60V for about 1.5-2 hours. The negatively charged DNA strands migrated towards the anode. In addition, a so-called “DNA ladder”, which shows bands of defined sizes (generally multiples of 50 or 100 bp), and can therefore be used to determine the size of the separated DNA fragments, was added into one of the gel slots. After electrophoresis, the gel was exposed to UV light at 254 nm for visualization of the bands, and photographed for documentation using a Polaroid camera.

2.4.6 Immunohistochemistry Using the Streptavidin-Biotin Method

Prior to applying immunohistochemical methods, the tissues have to be fixed and embedded in paraffin, cut in sections of 8-10 μm and to be applied to a microscope slide.

The principle of immunohistochemical analyses is as follows:
After tissue preparation, the primary antibody is added. A biotinylated second antibody (so-called 'Link') is attached followed by incubation with a conjugate ('Label') of streptavidin and an enzyme (horseradish peroxidase or alkaline phosphatase). Afterwards, a chromogen binds to the enzyme, leading to a specific staining of the target antigens in the tissue.

Streptavidin isolated from 'Streptomyces avidinii' has many advantages compared to other methods of microchemistry. First, streptavidin has no carbon hydrate side chains that unspecifically bind to lectin-like substances in the tissue. Furthermore, the isoelectric point of streptavidin is near the neutral pH-value. Therefore, streptavidin conjugates show no unspecific binding in contrast to avidin conjugates or avidin complexes.

2.4.6.1 HCMV Protein Detection in Brain Tumor Tissue Sections

The occurrence of false negative results in PCR is common for the detection of HCMV. Several publications reported that great discrepancies can occur in results obtained by PCR and immunohistochemistry [Knosel et al., 2004; Cobbs et al., 2002; Gass et al., 1993]. Therefore, the presence of HCMV-specific proteins in brain tumor samples was further investigated using immunohistochemistry. 72 surgical specimens (94.7%; 32 gliomas, 29 meningiomas, 6 acoustic neurinomas) obtained in paraffin blocks, which were cut (8 μm) and mounted on SuperFrost®Plus slides (Menzel Gläser, Braunschweig, Germany), were available for these analyses.

Test conditions were optimized for each monoclonal antibody (see below) and all immunohistochemical analyses were performed blinded for tumor type. An anti-pp65, anti-EA, and anti-IE antibody were used for analyses. The monoclonal anti-pp65 antibody recognizes an HCMV-specific immediate early (IE) protein, which is an important target for cytotoxic T-lymphocytes. Analysis of this protein is recommended by the Robert-Koch-Institute, Germany, for early detection of reactivation or primary HCMV infection prior to

seroconversion [RKI, 2000a]. The anti-IE-1 antibody detects reactivation of HCMV as well as latent infection. During infection, IE proteins are responsible for the regulation of Early Antigen (EA) expression. Thus, EA are detected later in the viral replication cycle after the expression of IE proteins.

Because no HCMV-positive brain tissues or cells could be obtained, HCMV-positive human lung (Dako) and HCMV-infected fibroblasts, respectively, were stained in parallel as positive controls. In addition, a negative control (uninfected lung tissue and uninfected fibroblasts, respectively) was run.

2.4.6.1.1 pp65 Antigen

In order to assess the possible presence of the HCMV-specific pp65, immunohistochemistry was performed using a monoclonal anti-pp65 antibody.

Paraffin slides were deparaffinized in xylol for 3x10 min. Afterwards, rehydration took place through a decreasing alcohol series (100%, 95%, and 70% ethanol, each for 2x5 min). Remaining ethanol was removed with double distilled water. Endogenous enzyme activity was blocked by incubation with 3% peroxidase for 10 min to prevent background staining that would lead to false positive results, followed by washing in TBS for 3x5 min. Antigen retrieval took place by heating the slides in citrate buffer pH 6.0 (microwave oven, 180 W for 10 min). This treatment increases the specific intensity of the staining and reduces background staining. Avidin-biotin-blocking followed a washing step in TBS (3x5 min). First, slides were incubated with avidin for 15 min, washed with TBS 3x5 min, incubated with biotin for 15 min and again washed with TBS 3x5 min. Then, the monoclonal primary antibody was added (NCL-CMVpp65, Novocastra) in a dilution of 1:200, and incubated at 4°C overnight. After a wash for 3x5 min in TBS, a biotinylated secondary antibody (goat anti-mouse) was applied for 45 min at room temperature. The secondary antibody was removed by washing (TBS, 3x5 min), and incubation with streptavidin-conjugated horseradish peroxidase took place at room temperature for 30 min. After a wash in TBS for 3x5 min, a substrate containing the chromogen AEC (3-Amino-9-Ethylcarbazol) was applied and incubated for 7-10 min. Remaining chromogen was removed with double-distilled water, sections were counterstained in hemalaun, washed with water and mounted using an aqueous medium.

2.4.6.1.2 IE-1 Antigen and Early Antigen

Additional immunohistochemistry was performed using a monoclonal anti-IE-1 antibody and, in another protocol, a monoclonal anti-EA antibody. Paraffin slides were deparaffinized, blocked with 3% peroxidase and treated with citrate buffer as described above.

Afterwards, the primary antibody (NCL-CMV-EA, Novocastra, and MAB810, Chemicon, respectively) was added in a dilution of 1:100, and incubated at room temperature for 2 h. After a wash for 3x5 min in TBS, a biotinylated secondary antibody (goat anti-mouse) was applied for 30 min at room temperature. This secondary antibody was removed by washing (TBS, 3x5 min), and incubation with streptavidin-conjugated horseradish peroxidase took place for 30 min at room temperature. After an additional washing step (TBS for 3x5 min), a substrate containing the chromogen AEC was applied and incubation took place for 7-10 min. Remaining chromogen was removed with double-distilled water and tissue sections were counterstained in hemalaun, washed again with water and mounted using an aqueous medium.

2.4.6.2 HCMV Protein Detection in Cultured Brain Tumor Cells

To control for efficiency of the method and to exclude that the presence of paraffin or other factors in brain tumor tissues interfered with the detection of HCMV in immunohistochemistry, short-term cultures derived from four of the primary brain tumors (two meningiomas, two gliomas) were prepared and fixed as described above.

For immunohistochemistry, cells were first washed for 3x5 min in TBS. Primary antibody (monoclonal anti-pp65 antibody) was diluted 1:200 and cells were incubated overnight at 4°C in a humidified atmosphere. Following a wash in TBS (3x5 min), cells were incubated with a biotinylated secondary antibody (goat anti-mouse) for 30 min at room temperature, and incubation with streptavidin-conjugated horseradish peroxidase took place for 30 min at room temperature. After an additional washing step (TBS for 3x5 min), a substrate containing the chromogen AEC was applied for 5-10 min at room temperature. Afterwards, cells were thoroughly washed with double-distilled water and mounted with an aqueous medium.

2.4.7 Serological Analyses Using the BEP-III®-System

Additional to the evaluation of HCMV molecules in brain tumor tissues and corresponding blood samples, the serological status of the study participants concerning previous or acute infection with HCMV, HSV, EBV and VZV was determined.

Serum samples were obtained from 71 (93.4%) of the 76 study participants. Five glioma patients did not provide a blood sample.

Patients' sera were analyzed for the presence of IgM and IgG antibodies to HCMV, HSV, EBV, and VZV, using an enzyme-linked immunosorbent assay (ELISA) at the Department of Virology, University of Heidelberg (PD Dr. P Schnitzler). Analyses were carried out using a fully automated measuring system. This so-called BEP-III®-system (Dade-Behring) is an ELISA for detection and quantitative determination of human antibodies to viruses in serum.

The performance characteristics of the BEP-III®-system according to the manufacturer are listed in Tab. 8.

Table 8: Sensitivities and specificities of the BEP-III®-system according to the manufacturer

	Detection of IgG antibodies		Detection of IgM antibodies	
	Sensitivity (%)	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**
CMV	99.3	98.2	95.0	100
HSV	100	100	92.0	95.8
EBV	92.0	100	97.3	99.5
VZV	99.3	100	98.5	100

Ig, immunoglobulin; CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus; EBV, Epstein-Barr virus

In analyses performed with the BEP-III®-system, specific antigens for the determination of anti-HCMV antibodies are derived from HCMV-infected human fibroblasts. For determination of anti-HSV antibodies, antigens derived from permanent simian kidney cells infected with HSV are used. VZV-infected cells not further specified by the manufacturer serve as antigens for determination of anti-VZV antibodies, and to state anti-EBV antibody levels, lymphoblastoid cells infected with EBV are used.

2.4.7.1 IgG Detection

Virus-specific IgG antibodies in the test sample bind to the antigen in the wells of the test plate. Afterwards, a conjugate of anti-human IgG and peroxidase binds to this complex. The enzyme component of the conjugate catalyzes a chromogen solution (tetramethylbenzidine, TMB), which produces a blue color. This reaction is determined by addition of a stopping solution, and color changes to yellow, which is measured at 450 nm. The intensity of the yellow color is proportional to the amount of the virus-specific IgG antibodies contained in the sample.

2.4.7.2 IgM Detection

The principle for IgM determination corresponds to the method for the detection of IgG antibodies. The only difference is that a rheumatoid factor absorbent not further specified by the manufacturers is added to the serum sample for 15 minutes prior to the test. This absorbent binds to any rheumatoid factor (which is an IgM antibody present during several non-target diseases), thereby minimizing the occurrence of false positive results. This effect enhances the specificity of the analyses. Afterwards, virus-specific IgM antibodies bind to the antigen in the wells of the test plate and antibody detection continues as described above.

2.4.8 Statistical Analyses

2.4.8.1 Prevalence Estimation

Prevalence is defined as the proportion of occurrence of a disease, condition or characteristic in a certain population at a particular point in time. More precisely, this measure is called the point prevalence.

$$\text{Point prevalence} = \frac{\text{No. of existing cases in the population at one point in time}}{\text{No. of people in the population at the same point of time}}$$

As it is impossible for practical reasons to get the true prevalence in the population, the calculated prevalence can only be an estimate of the true proportion. More precisely, the estimated point prevalence ($\bar{p}$) is given as

$$\bar{p} = \frac{a}{n}$$

with a being the number of cases in the sample, and
n being the number of people in the sample.

The point in time to which it refers must always be specified [dos Santos Silva, 1999].

In the present study, overall prevalences as well as prevalences for the occurrence of IgM and IgG antibodies to HCMV, HSV, EBV, and VZV stratified by 20-year age groups and tumor type, respectively, were estimated. The particular point in time refers to the blood withdrawal, which occurred during surgery or two days after surgery at the latest.

Furthermore, prevalences of several variables stratified by tumor type were obtained by telephone or direct interviews and subsequently compared to population prevalences, where possible.

All prevalences were computed using the FREQ procedure of the statistical software package SAS.

2.4.8.2 Confidence Intervals

As mentioned above, it is difficult to get the true prevalences in all brain tumor patients in Germany. Therefore, a subset of this population is drawn, which has to be representative for all patients with primary brain tumors.

The inferential statistics involved in the construction of confidence intervals (CI) are based on standard error, which reflects the sampling fluctuation of the statistic. In general, the larger the sample size the smaller the standard error and the narrower the confidence interval.

The standard error of a sampling distribution of a proportion *p* is given by

$$\mathrm{SE}(\bar{p}) = \sqrt{\frac{\bar{p}(1-\bar{p})}{n}}$$

with $\bar{p}$ being the estimated point prevalence *a/n*, and

n being the sample size.

Assuming a normal distribution of the prevalence, the 95% confidence interval (95%CI) represents the range of values that has an approximately 95% probability of containing the true and unknown proportion being estimated.

The approximate limits of the 95%CI are given as:

$$95\%\mathrm{CI} = \bar{p} \pm 1.96 \times SE(\bar{p})$$

That means that before drawing a sample, there is an approximately 95% chance that the proportion for the subset of brain tumor patients would lay within 1.96 standard errors of the true population value [dos Santos Silva, 1999].

For small numbers of cases (a<20) or sample sizes (n<40), however, this formula is insufficient and should not be used. Instead, it is better to calculate exact CIs for the binomial proportion using the *F* distribution method given by Leemis and Trivedi [1996] and also described in Collett [2002]. In the present study, exact 95%CIs for calculated prevalences were computed using the FREQ procedure of the statistical software package SAS.

2.4.9 Evaluation of the Questionnaire

A questionnaire was developed to control for factors indicative for previous infections, especially for any possible infection with or reactivation of herpesviruses. Furthermore, the questionnaire addressed the role of several medical conditions as well as the suggestive role of high-level person-person or person-animal contact in brain tumor pathogenesis.

The present study included fast-growing as well as slow-growing tumors with different latency periods. Therefore, with the exception of the questions addressing epilepsy, hearing impairments and tinnitus, which are also considered being early symptoms of brain tumors, no latency period was taken into account for all variables evaluated.

Prevalences of all variables addressed were estimated as described in Chapt. 2.4.8.1.

2.4.9.1 Exclusion of a Selection Bias in the Interviewed Participants

Of the 66 patients in the present study, who could be contacted, 81.2% were willing to complete the questionnaire. Ten samples were collected anonymously and therefore, the patients could not be contacted (cf. above).

In total, 54 of the 76 participants completed the questionnaire, resulting in a participation rate of 71.1%, which was mainly due to the part-time anonymous collection of the samples. The subset of subjects willing to complete the interview, though, could differ from those who were not interviewed, and this could cause much stronger associations between any risk factor and the respective tumor type, but it is also possible that this causes biased results. The main study intentions were related to the histological type of the present brain tumor. Therefore, heterogeneity between those participating in the interview and those not interviewed was tested according to the histological distribution in either group.

Usually, χ^2-testing is applied to measure the extend to which the observed data (or data from the subset) differ from those expected (i.e., from the whole study population) if the two populations were equal. However, for small sample sizes and if the expected value in any of the cells is less than 5, which was the case for the present study, Fisher's exact test will be the appropriate statistical test [dos Santos Silva, 1999].

The heterogeneity of the patients completing the questionnaire and those who could not be interviewed was tested at a significance level of α=0.05 focusing on the distribution of the histological types of the tumors in either population. P-values show the probability that in both subsets the found differences occurred by random where in truth the populations were equal [Kreienbrock and Schach, 2000].

WHO grade III-IV gliomas differ in their clinical and histological behaviors by being more aggressive than any other brain tumors included in the present study. These malign neoplasms may lead to a reduced ability to complete the questionnaire by causing severe disease (or death), and thus are suspected to introduce the most important selection bias. Therefore, these tumors were taken as an extra group, resulting in four groups for this analysis:

- low-grade gliomas (WHO I and II),
- gliomas WHO grade III and IV,
- meningiomas, and
- acoustic neurinomas.

Analyses were performed using the FREQ procedure of the statistical software package SAS.

2.4.9.2 Determination of the Socioeconomic Status

For all participants, the SES was determined according to advises from the "Deutsche Arbeitsgemeinschaft für Epidemiologie" (DAE) for measurement and quantification of sociodemographic characteristics in epidemiologic studies (Tab. 9; DAE, 1997).

In this model, education and training are taken into account, and the SES is categorized in eight classes with category 1 counting for low SES and category 8 counting for high SES. Education is defined as the highest graduation in categories of the national educational system, whereas training is identified according to the national training system (the underlying scheme is shown in Tab. 9).

Table 9: Educational achievement in combination with training level and corresponding categorizing index (taken from the DAE guidelines for evaluation of socioeconomic status, 1997)

	Education: No education	$8^{th}/9^{th}$ grade *	10^{th} grade **	Abitur***	Miscellaneous
Training:					
No training	1	2	3	6	1
Apprenticeship	3	3	4	6	3
Technical school	-	4	5	6	4
FH	-	-	7	7	7
University	-	-	8	8	8
Miscellaneous	-	-	4	7	3

*Hauptschulabschluß; **Realschulabschluß; ***Gymnasialabschluß
FH, Fachhochschule; DAE, Deutsche Arbeitsgemeinschaft für Epidemiologie

The social stratification is characterized by smooth transitions between the single classes. Therefore, the advices for this SES grading scheme do not determine further categorizations into low, intermediate and high SES. However, in the present study, the SES categories of the DAE were categorized into low SES (DAE category 1-3), intermediate SES (DAE category 4-5) and high SES (DAE category 6-8) for a better interpretation and comparison of the data with published results.

For Germany, problems may occur due to the differences in the education and training systems between the Eastern and Western part of Germany. However, this difference was not taken into account in the present study since all participants had grown up in the Western part of Germany.

2.4.9.3 Occupational History

Complete occupational histories from leaving school to the date of interview were obtained from all brain tumor patients completing the questionnaire (n=54). All employments in which a participant was involved for more than one year were taken into account. Exposure assessment included the job title as well as the precise characterization of the occupational activity and its duration in years.

Due to the small sample size, no cumulative working time was calculated. If the subject changed the working place but not the occupational activity, it was considered that other exposures were present at the new place and the occupational category was counted twice in

the analyses. Similarly, a subject could belong to different categories, depending on the activities during its occupational life.

Two separate analyses were performed and evaluated separately:

(1) First, all job titles and occupational activities were coded according to the International Standard Classification of Occupation [ISCO, 2003]. Subsequently, activities of similar profile were classified into 16 a priori defined categories based on exposure criteria (such as chemicals, metals, farming, etc.) as described by Schlehofer et al. [1990].

(2) Additional separate analyses were performed to evaluate the main hypothesis of this study, which refers to a possible causal relationship between neuro-oncogenic infections and the development of primary brain tumors. Therefore, occupational activities were classified into activities with potential high level contact to humans or animals as a measurement of possible transmission of neuro-oncogenic infections as described by Menegoz et al. [2002]. For instance, people working as physicians, teachers, nurses, hairdresser and trained retail sales clerk were considered to have high levels of contact with humans or human tissues. Similar, farmers and cooks were considered to have potential high-level contact to animals or animal tissue. Subjects working for a catering service, in gastronomy (serving and cooking) or as trained retail sales clerk at butchery were considered to have high-level exposure to both, humans and animals. Therefore, these subjects were defined as an extra category.

3 Results

The reference date in the present study for all analyses performed refers to the date of surgery.

(A) Questionnaire Data

3.1 Characteristics of Participants Completing the Questionnaire

A questionnaire was developed to control for risk factors of infection with herpesviruses prior to diagnosis and additional putative risk factors controversially discussed in the literature. Interviews were performed after brain tumor surgery in the hospital if possible. Otherwise, patients were contacted by letter, followed up by up to four more letters and several phone calls if necessary, and telephone interviews were performed.

Of the 76 participants, 54 persons completed the questionnaire either by direct (n=26) or by telephone interview (n=28). The participation rate was 71.1%. Reasons for nonparticipation included refusal (n=5; 6.6%), death (n=1; 1.3%), and failure to contact the study subjects (n=16; 21.1%; percentage due to the part-time anonymous collection of the tumor samples as described above). Among those not interviewed were 16 gliomas (one with unknown grading, one WHO II, five WHO III, nine WHO IV), five meningiomas (four WHO I, one WHO II), and one acoustic neurinoma (WHO I).

To exclude a selection bias in the subset of patients completing the questionnaire, Fisher's exact test was applied to determine whether there was a difference concerning the distribution of the histological findings in those interviewed and those not interviewed. Considering a significance level of α=0.05, no statistically significant heterogeneity in the distribution of tumor types was detected (p=0.09), and the null hypothesis that the two groups were homogenous could not be rejected.

One meningioma patient could not be interviewed because of severe disease; therefore, his son completed the interview (proxy interview). Furthermore, one patient with glioblastoma multiforme did not want do perform an interview because of amblyacousia (hardness of

hearing). Instead, his wife was interviewed, but the study subject remained besides her during the interview and answered the questions if the proxy was not able to (partly proxy interview).

Two meningioma and one glioma patient were interviewed prior to brain tumor surgery. In patients interviewed after brain tumor surgery, the mean time period between the time of surgery and the interview was 53 days in glioma patients, 111 days in meningioma patients, and 87 days in acoustic neurinoma patients.

At surgery, glioma patients had a median age of 54.0 years with a range from 9 to 80 years; of these patients, 57% were male. In meningioma patients, 15% were male; the median age was 51.5 years with a range from 36-83 years. Acoustic neurinoma patients were slightly younger with a median age of 46 years (range 34-66 years). The proportion of men was 80% (Tab. 10).

The distribution of age and gender in subjects completing the questionnaire did not differ significantly from that of the overall study population as described in Chapt. 3.5.

Table 10: Distribution of age and gender in brain tumor patients who completed the questionnaire (N=54)

	n	Age		Gender			
		Median Age (years)	Age Range (years)	Male		Female	
Patients with				**n**	**(%)**	**n**	**(%)**
Glioma	23	54.0	9-80	13	(56.5)	10	(43.5)
Meningioma	26	51.5	36-83	4	(15.4)	22	(84.6)
ACN	5	46.0	34 - 66	4	(80.0)	1	(20.0)

ACN, acoustic neurinoma

For the participants completing the questionnaire, the socioeconomic status (SES) was calculated taking into account educational and training levels (Tab. 11).

Secondary level (Hauptschulabschluß, $8^{th}/9^{th}$ class) was the most common educational level in all patients, independent of the type of tumor. Glioma patients had the smallest proportion of subjects completing Gymnasium and Fachhochschule, respectively. Two of the glioma patients and one meningioma patient reported to have no education.

In training levels, apprenticeship was the most common level for all patients. None of the participants went to Fachhochschule, and only five of all 54 interviewed participants went to university.

Table 11: Education and training level in patients with primary brain tumors completing the questionnaire according to DAE guidelines for evaluation of socioeconomic status [DAE, 1997]

	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
Education Level						
No education	2	8.7	1	3.8	-	-
$8^{th}/9^{th}$ class (Hauptschule)	13	56.5	12	46.2	4	80.0
10^{th} class (Realschule)	5	21.7	6	23.1	-	-
$12^{th}/13^{th}$ class (Abitur/FH-level)	3	13.0	7	26.9	1	20.0
Miscellaneous	-	-	-	-	-	-
Training Level						
No training	4	17.4	8	30.8	-	-
Apprenticeship	16	69.6	13	50.0	4	80.0
Technical school	2	8.7	1	3.8	1	20.0
FH	-	-	-	-	-	-
University	1	4.3	4	15.4	-	-
Miscellaneous	-	-	-	-	-	-

ACN, acoustic neurinoma; FH, Fachhochschule

According to the DAE categorizing index, the most frequent SES category in brain tumor patients was category 3, indicating a low SES (Tab. 12). The highest SES category was present only in one glioma and four meningioma patients.

Table 12: Socioeconomic status in brain tumor patients who completed the interview according to DAE guidelines [DAE, 1997]

low SES

high SES

Category	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
1	3	13.0	1	4.2	-	-
2	2	8.7	6	25.0	-	-
3	10	43.5	7	29.2	4	80.0
4	4	17.4	4	16.7	-	-
5	1	4.3	1	4.2	-	-
6	2	8.7	3	12.5	1	20.0
7	-	-	-	-	-	-
8	1	4.3	4	16.7	-	-

ACN, acoustic neurinoma

The majority of the study participants completing the questionnaire was married or living with a partner (66.7%). Of all 54 persons, four were widowed (7.4%) and one participant was divorced (1.9%). 24.1% of the subjects completing the questionnaire were single.

In meningioma and acoustic neurinoma patients, 77% and 80%, respectively, were married. In glioma patients, the proportion being married was 52% (Tab. 13).

Table 13: Marital status of participants completing the questionnaire (N=54)

	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
Single	9	39.1	3	11.5	1	20.0
Married/partner	12	52.2	20	76.9	4	80.0
Divorced	-	-	1	3.9	-	-
Widowed	2	8.7	2	7.7	-	-

ACN, acoustic neurinoma

3.2 Medical History

The questionnaire addressed information on medical factors possibly indicating an infection or reactivation of herpesviruses as well as on putative medical risk factors for the development of primary brain tumors.

3.2.1 Distribution of Previous Infections

Previous herpesvirus and common infections were assessed using a standardized questionnaire (Tab. 14).

More than 50% of all brain tumor patients reported to have had chicken pox (primary infection with VZV). Five glioma and three meningioma patients could not remember if they ever had this disease. One of those glioma patients who could not remember a history of chickenpox, though, reported the occurrence of shingles 30 years prior to the present tumor. Of the other glioma patients with a positive shingles history, one did not remember at what age this condition occurred; one reported shingles 4 and one 20 years prior to the present tumor. One meningioma patient reported the occurrence of shingles without any history of chickenpox, but could not remember at what age shingles occurred. The second meningioma patient with a history of chickenpox reported its occurrence 14 years prior to the present tumor.

In total, shingles, which is caused by reactivation of VZV, was more common in glioma patients (17%) than in meningioma patients (8%), and absent in all acoustic neurinoma patients. The recurrence rates, i.e., the proportion of shingles in individuals with a history of chickenpox, were 25% for glioma (3 out of 12) and 5.9% for meningioma patients (one out of 17), respectively.

Mononucleosis (EBV infection) was not reported by any of the patients. Only two subjects (one meningioma and one glioma patient) remembered to have had roseola (‘exanthema subitum’, HHV-6 infection). More than 20% of the patients were suffering from an HSV infection (oropharyngeal disease), and more than 20% reported regular common infections like flu, colds and sore throat.

Common infections were distinctly more frequent in meningioma patients (69%) than in glioma patients (25%). In none of the patients, an HCMV titer had been determined previously.

Table 14: Distribution of previous herpesvirus and common infections (e.g. flu, sore throat) in brain tumor patients (N=55)

	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
Chicken pox						
Yes	12	52.2	17	65.4	4	80.0
No	6	26.1	6	23.1	1	20.0
Do not know	5	21.7	3	11.5	-	-
Shingles						
Yes	4	17.4	2	7.7	-	-
No	18	78.3	23	88.5	5	100
Do not know	1	4.3	-	-	-	-
Missing*	-	-	1	3.8	-	-
Mononucleosis						
Yes	-	-	-	-	-	-
No	19	82.6	25	96.2	5	100
Do not know	2	8.7	-	-	-	-
Missing*	2	8.7	1	3.8	-	-
Roseola						
Yes	1	4.3	1	3.8	-	-
No	21	91.3	23	88.5	5	100
Do not know	1	4.3	1	3.8	-	-
Missing*	-	-	1	3.8	-	-
Herpes simplex						
Yes	7	30.4	12	46.2	1	20.0
No	16	69.7	14	53.8	4	80.0
Do not know	-	-	-	-	-	-
Infections						
Yes	6	26.1	18	69.2	1	20.0
No	17	73.9	8	30.8	4	80.0
Do not know	-	-	-	-	-	-
HCMV titer						
Yes	-	-	-	-	-	-
No	18	78.3	22	84.6	5	100
Do not know	5	21.7	4	15.4	-	-

* Missing, patient did not respond to the question;
ACN, acoustic neurinoma; HCMV, human cytomegalovirus

The self-reported history of previous herpesvirus infections was compared to the results of the serological analyses, which are described in more detail in chapter 3.10 (see Tab. 15).

The single meningioma patient and two of three glioma patients, who reported the occurrence of both chickenpox and shingles, were anti-VZV antibody positive. All other glioma patients with a positive history of both did not provide a blood sample.

Table 15: Self-reported history of herpesvirus diseases compared to the serological status concerning these herpesviruses in brain tumor patients providing a blood sample and completing the questionnaire (n=52)

	Glioma patients (n=21*)				**Meningioma patients (n=26)**				**ACN patients (n=5)**			
Self-reported history of**	**IgG**				**IgG**				**IgG**			
	positive		**negative**		**positive**		**negative**		**positive**		**negative**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
HCMV												
Yes	-	-	-	-	-	-	-	-	-	-	-	-
No	7	33.3	9	42.9	13	50.0	9	34.6	3	60.0	2	40.0
Herpes simplex (HSV)												
Yes	7	33.3	-	-	12	46.1	-	-	1	20.0	-	-
No	12	57.1	1	4.8	8	30.8	6	23.1	2	40.0	1	20.0
Mononucleosis (EBV)												
Yes	-	-	-	-	-	-	-	-	-	-	-	-
No	15	71.4	2	9.5	22	84.6	3	15.4	5	100	-	-
Chickenpox and/ or Shingles (VZV)												
Yes	9	42.9	2	9.5	16	61.5	2	7.7	4	80.0	-	-
No	5	23.8	-	-	6	23.1	-	-	1	20.0	-	-

*2 glioma patients did not provide a blood sample;
**patients missing to the overall number of individuals did not know whether they had the respective disease;
ACN, acoustic neurinoma; HCMV, human cytomegalovirus; HSV, herpes simplex virus; EBV, Epstein-Barr virus; VZV, varicella zoster virus; IgG, immunoglobulin G

3.2.2 Distribution of Vaccinations

The proportions of patients being vaccinated against 14 different diseases were evaluated. One glioma patient refused to answer the question about vaccinations (Tab. 16).

Table 16: Distribution of vaccinations against several diseases in brain tumor patients (N=54)

	Glioma patients (n=23*)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	**%**	**n**	**%**	**n**	**%**
Diphtheria						
Ever	10	43.5	12	46.2	3	60.0
Never	10	43.5	11	52.3	2	40.0
Do not know	2	8.7	3	11.5	-	-
Tetanus						
Ever	18	78.3	23	88.5	4	80.0
Never	3	13.1	2	7.7	1	20.0
Do not know	1	4.3	1	3.8	-	-
Pertussis						
Ever	4	17.4	2	7.7	2	40.0
Never	15	65.2	23	88.5	3	60.0
Do not know	3	13.1	1	3.8	-	-
Poliomyelitis						
Ever	12	47.8	17	65.4	4	80.0
Never	8	34.8	9	34.6	1	20.0
Do not know	3	13.1	-	-	-	-
Tuberculosis						
Ever	5	21.7	7	26.9	2	40.0
Never	11	47.8	16	61.5	3	60.0
Do not know	6	26.1	3	11.6	-	-
Rubella						
Ever	3	13.0	7	26.9	-	-
Never	16	69.6	16	61.5	5	100
Do not know	3	13.0	3	11.6	-	-
Mumps						
Ever	3	13.0	3	11.5	-	-
Never	17	73.9	19	73.1	5	100
Do not know	2	8.7	4	15.4	-	-
Measles						
Ever	3	13.0	3	11.5	-	-
Never	17	73.9	20	76.9	5	100
Do not know	2	8.7	3	11.6	-	-

Tab. 16 continued

	Glioma patients (n=23*)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	**%**	**n**	**%**	**n**	**%**
MMR						
Ever	-	-	2	7.7	-	-
Never	20	87.0	23	88.5	5	100
Do not know	2	8.7	1	3.8	-	-
Hepatitis A						
Ever	4	17.4	5	19.2	-	-
Never	17	73.9	20	76.9	5	100
Do not know	1	4.3	1	3.9	-	-
Hepatitis B						
Ever	4	17.4	3	11.5	-	-
Never	17	73.9	22	84.6	5	100
Do not know	1	4.3	1	3.9	-	-
Rabies						
Ever	-	-	2	7.7	-	-
Never	21	91.3	24	92.3	5	100
Do not know	1	4.3	-	-	-	-
FSME						
Ever	2	8.7	3	11.5	-	-
Never	18	78.3	23	88.5	5	100
Do not know	2	8.7	-	-	-	-
Influenza						
Ever	3	13.1	10	38.5	1	20.0
Never	18	78.3	16	61.5	4	80.0
Do not know	1	4.3	-	-	-	-

* one glioma patient refused to answer the question on vaccinations;
ACN, acoustic neurinoma; MMR, measles-mumps-rubella combination; FSME, tick-borne encephalitis

44% to 60% of the patients had been vaccinated against diphtheria and more than 78% of the study subjects were vaccinated against tetanus. For polio, the proportion was 48% for glioma, 65% for meningioma and 80% for acoustic neurinoma patients. Two to four of the patients in each histological subgroup were vaccinated against pertussis. For tuberculosis, mumps, measles, and hepatitis A and B, the proportions were similar. The use of a combination vaccine (measles, mumps, rubella; MMR) was rarely reported.

Of the patients with meningioma, only 8% had been vaccinated against rabies compared to none of the other participants. The proportion of vaccinated subjects against rubella and tick-borne encephalitis (FSME) in meningioma patients was higher as those in glioma patients. The proportion for influenza was 13% in glioma, 39% in meningioma and 20% in acoustic neurinoma patients.

3.2.3 Medical History of the Participants' Children as an Indicator for Parental HCMV Infection

HCMV transmission from mother to child during pregnancy can lead to various diseases in the newborn, becoming apparent during the 1st year of life. Therefore, the questionnaire addressed the occurrence of frequent symptoms of prenatal HCMV infection (e.g. anemia, icterus, pneumonia, gastrointestinal diseases, and malformations/hereditary diseases).

Fifteen glioma, twenty-one meningioma, and two acoustic neurinoma patients reported to have children. The mean number varied from 0.8 to 1.7 in the different tumor types (Tab. 17).

Table 17: Parity in brain tumor patients with offspring (N=54)

	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
Parity	**n**	**%**	**n**	**%**	**n**	**%**
0	8	34.8	5	19.2	3	60.0
1	2	8.7	8	30.8	-	-
2	9	39.1	6	23.1	2	40.0
3	2	8.7	5	19.2	-	-
4	2	8.7	2	7.7	-	-
Mean N°	1.5		1.7		0.8	
Total N°	34		43		4	

ACN, acoustic neurinoma

For analyzing the occurrence of hereditary diseases, malformations and diseases during the offspring's first year of life, and for better handling of the data, patients having offspring with one of the addressed conditions were serially numbered (see Tab. 18).

Patients with acoustic neurinoma did not report any of the diseases in their offspring. Furthermore, none of the study subjects reported anemia in their children.

The most frequent disease was jaundice. One glioma patient had a child affected by jaundice; one offspring of another glioma patient was affected by hereditary amblyopia. In meningioma patients, the occurrence of malformations, hereditary or other addressed diseases was reported by 10 out of 21 meningioma patients with offspring.

Table 18: Numbers of children affected by medical conditions being suspect for HCMV transmission from brain tumor patients to their offspring, and occurrence of IgG antibodies to HCMV

	Total N° of kids	N° of kids affected	Malformation / hereditary disease	Anemia	Icterus	Pneu-monia	Chronic GI disease	IgGs to HCMV in parent
Glioma patients								
1)	2	1	-	-	1	-	-	No
2)	2	1	Amblyopia	-	-	-	-	No
Meningioma patients								
3)	1	1	-	-	1	-	-	Yes
4)	3	1	-	-	1	-	-	No
5)	1	1	Neurodermatitis	-	-	-	-	Yes
6)	2	1	-	-	1	-	-	Yes
7)	1	1	-	-	1	-	-	No
8)	3	2	-	-	2	1	-	No
9)	1	1	Neurodermatitis, Asthma	-	-	-	1	No
10)	2	1	Asthma	-	-	-	-	No
11)	2	1	Amblyopia	-	-	-	-	Yes
12)	3	1	Intestinal obstruction	-	-	1	-	No

GI, gastro-intestinal; IgG, immunoglobulin G; HCMV, human cytomegalovirus

The child of subject (9) was affected by two hereditary conditions and by gastrointestinal disease. Intestinal obstruction in combination with pneumonia was reported in one child of patient (12). Pneumonia and jaundice occurred in one child of patient (8); another one of the three children of patient (8) was affected by jaundice, too.

None of the glioma patients with affected children was seropositive for anti-HCMV IgG. In contrast, four meningioma patients with affected children were seropositive for anti-HCMV IgG.

3.2.4 Distribution of Immunosuppressive Conditions

Immunosuppressive conditions (such as stress, intake of specific drugs, organ transplantation, etc.) may lead to a reactivation of herpesviruses. Self-reported immunosuppression was taken into account only if it occurred at least two years prior to the present brain tumor surgery. Therefore, one glioma and one meningioma patient each, reporting a blood transfusion less than 2 years prior to the present tumor, had to be excluded.

None of the glioma and acoustic neurinoma patients and only one meningioma patient reported immunosuppressive conditions more than 2 years prior to the present tumor, which were due to an intake of immunosuppressive drugs (namely carboplatin chemotherapy during breast cancer therapy 5 years prior to the present tumor). This single patient reporting immunosuppressive conditions was HCMV-seronegative. None of the study subjects reported organ transplantation or infectious diseases leading to an immunosuppression.

HCMV transmission frequently occurs through blood transfusions. Therefore, the occurrence of blood transfusions at least 2 years prior to the present brain tumor surgery was evaluated in all patients. Only six individuals of the total study population reported a blood transfusion for different reasons. One meningioma, one acoustic neurinoma and four glioma patients reported having received a transfusion (Tab. 19). Of the four affected glioma patients, two were seropositive for HCMV IgG. The meningioma and the acoustic neurinoma patients who ever received a blood transfusion were HCMV-seropositive, too.

Table 19: Distribution of blood transfusions and immunosuppressive conditions in brain tumor patients at least 2 years prior to brain tumor surgery (N=54)

	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
Blood Transfusion						
Ever	4	17.4	1	3.8	1	20.0
Never	19	82.6	25	96.2	4	80.0
Immunosuppressive Conditions						
Yes	-	-	1	3.8	-	-
No	23	100	25	32.5	5	100

ACN, acoustic neurinoma

3.2.5 Distribution of Previous Cancers

The occurrence of malignancies prior to brain tumor diagnosis in the present study was assessed to control for previous immunosuppression and for hereditary diseases associated with an increased risk for the development of primary brain tumors (such as neurofibromatosis or tuberous sclerosis).

None of the study subjects reported to be affected by either of these two hereditary diseases.

Seven of the 54 patients reported one previous neoplasm (13.0%) and four (7.4%) reported two previous cancers each (Tab. 20). None of participants with acoustic neurinoma was affected by previous malignancies.

Stratified by brain tumor type, 9% and 19% of the glioma and meningioma patients, respectively, reported one cancer diagnosis before the present brain tumor. Two previous cancers were diagnosed in 9% of the glioma and 8% of the meningioma patients. In glioma patients, the most common cancer was breast cancer. Meningioma patients most often reported previous myoma, followed by breast and thyroid gland cancer.

Altogether, eight out of eleven patients with a positive cancer history were HCMV-seropositive (75.0% of the glioma and 71.4% of the meningioma patients).

Table 20: Distribution of neoplasms prior to the present study in those with a positive cancer history (n=11)

Present neoplasm		1st previous tumor		2nd previous tumor		IgGs to
Tumor Type	Age at Onset	Tumor Type	Age at Onset	Tumor Type	Age at Onset	HCMV
Glioma patients						
GBM IV	62	Myoma	48	Breast cancer	61	No
GBM IV	66	Kidney cancer	57	-	-	Yes
GBM IV	79	Breast cancer	75	Breast cancer	76	Yes
GBM IV	59	Breast cancer	22	-	-	Yes
Meningioma patients						
Meningioma I	36	Fibroadenoma	25	-	-	Yes
Meningioma I	53	Myoma	49	-	-	Yes
Meningioma I	62	Myoma	35	-	-	Yes
Meningioma I	48	Thyroid gland cancer	45	-	-	Yes
Meningioma I	41	Thyroid gland cancer	40	-	-	Yes
Meningioma I	44	Breast cancer	39	Breast cancer	43	No
Meningioma I	47	Myoma	24	Ovarian cancer	41	No

GBM IV, glioblastoma multiforme WHO grade IV; IgG, immunoglobulin G; HCMV, human cytomegalovirus

3.2.6 Distribution of Allergic Conditions

Allergic conditions are controversially discussed to be associated with brain tumors. In total, allergic conditions were reported rarely by all participants (Tab. 21).

The simultaneous occurrence of hay fever, eczema and other allergies was reported by one acoustic neurinoma patient. Asthma did not occur.

One glioma and one meningioma patient reported the occurrence of asthma. The prevalence of hay fever was higher in glioma (13%) than in meningioma patients (8%). Reported eczema distribution was similar in glioma and meningioma patients (17% and 15%, respectively).

35% of all meningioma patients and 22% of all glioma patients reported to have at least one other allergy (such as pollen or dust mite).

Table 21: Distribution of allergic conditions in brain tumor patients (N=54)

	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
Asthma						
Yes	1	4.3	1	4.8	-	-
No	22	95.7	25	96.2	5	100
Hay fever						
Yes	3	13.0	2	7.7	1	20.0
No	20	87.0	24	92.3	4	80.0
Eczema						
Yes	4	17.4	4	15.4	1	20.0
No	19	86.7	22	84.6	4	80.0
Other Allergies						
Yes	5	21.7	9	34.6	1	20.0
No	18	78.3	17	65.4	4	80.0

ACN, acoustic neurinoma

Overall, nine out of 23 glioma patients (39.1%) were suffering from at least one allergic condition. This proportion was 38.5% in meningioma (n=10) and 20% (n=1) in acoustic neurinoma patients (Tab. 22)

Table 22: Number of allergic conditions (asthma, hay fever, eczema, other allergies) reported by brain tumor patients (N=54)

N° of allergic conditions	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
1	5	21.7	4	15.4	-	-
2	4	17.4	6	23.1	-	-
3	-	-	-	-	1	20.0
At least 1 (95%CI)	9	39.1 (20, 61)	10	38.5 (20, 59)	1	20.0 (1, 72)

ACN, acoustic neurinoma; CI, confidence interval

3.2.7 Distribution of Hearing Impairments and Tinnitus

Hearing impairments and tinnitus could be indicators for prenatal HCMV infection, which should be evaluated in the present study. Furthermore, they are also frequently reported as early clinical symptoms of brain tumors. Therefore, their occurrence was only taken into account if it occurred at least two years prior to the present tumor surgery, and three acoustic neurinoma patients had to be excluded from the analyses. At the beginning of the present study, this question addressed only acoustic neurinoma patients, resulting in a high proportion of missing data in glioma and meningioma patients.

None of the patients reported to be affected by tinnitus. Hearing impairments occurred in two of the five acoustic neurinoma patients, in one glioma and in three meningioma patients, who had answered that question, respectively (Tab. 23).

Table 23: Hearing impairments and tinnitus occurring at least 2 years prior to the present study (N=54)

	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
Hearing impairments						
Ever	1	4.3	3	11.5	2	40.0
Never	20	87.0	23	88.5	3	60.0
Missing	2	8.7	-	-	-	-
Tinnitus						
Ever	-	-	-	-	-	-
Never	10	43.5	17	65.4	5	100
Missing	13*	56.5	9*	34.6	-	-

*question was taken up for glioma and meningioma patients later during the study period; ACN, acoustic neurinoma

Concerning the serological status, three of the six patients with a positive history of hearing impairments were HCMV seropositive (one glioma and two meningioma patients).

3.2.8 Distribution of Epilepsy

An association between epilepsy and the occurrence of brain tumors has been suggested in several studies. Therefore, the prevalence of seizures in brain tumor patients was evaluated.

As the possible etiology in brain tumor development was addressed in the present study and as seizures are a frequent first clinical symptom of these tumors, epilepsy occurring at least two years prior to brain tumor surgery was taken into account. Therefore, one glioma and two meningioma patients had to be excluded from this analysis.

None of the other study participants reported the occurrence of seizures two years and more prior to the present tumor.

3.3 Assessment of Occupational History

Tab. 24 shows the distribution of the brain tumor patients in the different occupational categories according to Schlehofer et al. [1990]. Multiple classifications of the subjects to different categories were possible.

None of the participants was working in category "food", and only one person with meningioma was working in the "agriculture" category. In glioma and meningioma patients, most study subjects had worked in jobs belonging to the "office" category (74% and 62%, respectively), and the "service" category (44% and 92%, respectively). All acoustic neurinoma patients reported to have been involved in the "service" category. Working in the "health system" was most common in meningioma patients (65%) whereas working in electrical/electronically industry was most often found in acoustic neurinoma patients (40%). No considerable increase in occurrence in the other categories was seen in either subgroup.

Table 24: Distribution of 54 brain tumor patients in 16 Occupational categories (according to Schlehofer et al., 2005)

Occupational category*	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
Chemical	-	-	3	11.5	1	20.0
Metal	5	21.7	5	19.2	-	-
Office	17	73.9	16	61.5	1	20.0
Health system	3	13.0	17	65.4	1	20.0
Electrical/electronic	5	21.7	7	26.9	2	40.0
Construction	2	8.7	-	-	-	-
Transport	-	-	-	-	1	20.0
Sales/trade	-	-	-	-	-	-
Food	-	-	-	-	-	-
Service	10	43.5	24	92.3	5	100
Agriculture	-	-	1	3.8	1	20.0
Textile	1	4.3	9	34.6	-	-
Wood/paper	2	8.7	2	7.7	-	-
Glass/ceramic	3	13.0	-	-	-	-
Painters	2	8.7	-	-	-	-
Artists	-	-	-	-	-	-

* Multiple assignments possible, percentage related to the respective total numbers of study participants;
ACN, acoustic neurinoma

3.4 Assessment of High-level Contact to Animals and/or Humans

According to the literature, zoonotic as well as immunologic factors are suggested to be involved in the development of brain tumors [Khuder et al., 1998; Musicco et al., 1988]. Therefore, patients' self-reported occupational as well as private contact to animals or pets was evaluated (Tab. 26).

In addition, patients were classified according to a potential high-level contact to humans and/or animals or the respective tissues during work (Tab. 25). This separate analysis was performed according to the classification of Menegoz et al. [2002].

Teachers, sales clerks, traders, and people working in the health system were considered to have had high-level contact to humans or human tissues, whereas farmers and cooks were considered to have had high-level contact to animals or animal tissues. Patients working as sales clerk at a butchery and gastronomists doing both service and cooking were considered to have had high-level contact to both animals and humans.

Differences between self-reported occupational contact to animals (Tab. 26) and potential occupational high-level contact to animals (Tab. 25) are determined by the fact that animal tissues were considered as high-level contact to animals, although the subject would not have reported any vocational contact to animals. For example, a butcher would be considered as having had potential high-level contact to animals (tissue) according to Menegoz et al. [2002] although the subject did not report vocational animal contact.

Table 25: Distribution of 54 brain tumor patients working in occupations with suggested occupational high-level contact to humans or animals (according to the categorization of Menegoz et al., 2002)

Potential high-level contact to*	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
Humans	13	56.5	23	88.5	4	80.0
Animals	1	4.3	6	23.1	-	-
Humans and animals	-	-	6	23.1	-	-
None	39	-	42	-	8	-

* Multiple assignments possible, percentage related to the respective total numbers of study participants;
ACN, acoustic neurinoma

Potential high-level contact to people at work was distributed in the range from 57% to 89% in the different brain tumor types. Only one of the glioma patients had potential high-level contact to animals, whereas the proportion was 23% (n=6) in meningioma patients. Meningioma patients were the only subjects with occupational activities at potential high-level contact to both animals and humans (23%). Four of the acoustic neurinoma patients were considered to have had occupational high-level contact to humans (Tab. 25).

Self-reported private contact frequently occurred in all study subjects; it was reported by 70% and more of all patients. Occupational contact to animals was reported by 4% of glioma and 12% of meningioma patients (Tab. 26).

Table 26: Distribution of self-reported private or occupational contact to animals in brain tumor patients (N=54)

	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
Private						
Ever	16	69.6	19	73.1	4	80.0
Never	7	30.4	7	26.9	1	20.0
Occupational						
Ever	1	4.3	3	11.5	-	-
Never	22	95.7	23	88.5	5	100

ACN, acoustic neurinoma

For analyses stratified by animal type, private and vocational contacts were analyzed together (Tab. 27), because it was defined as unimportant whether the possible transmission of a neuro-oncogenic infection took place during leisure time or at work.

The period of time between first contact to any animal and the present surgery was 22.2 years (range 8-53 years) in glioma patients, 29.0 years (range 4-58 years) in meningioma patients and 27.3 years (range 18-35 years) in acoustic neurinoma patients

Almost 50% of the patients reported having a dog. Fewer subjects reported contact to cats. Contact to rodents (guinea pigs, rats, hamsters), rabbits and birds (budgerigars, poultry, zebra finch) was also frequently reported. Contact to farm animals was commonest in meningioma patients; eight of them reported contact to either pigs or ruminants (goats, sheep, cattle), whereas in other subjects, only two glioma patients reported contact to ruminants.

Table 27: Private and occupational contact to animals stratified by animal species (N=54)

Contact to*	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
Dog	11	47.8	12	45.2	2	40.0
Cat	11	47.8	9	34.6	1	20.0
Horse	2	8.7	3	11.5	-	-
Rodent	4	17.4	2	7.7	2	40.0
Rabbit	3	13.0	6	23.1	2	40.0
Bird	4	17.4	7	26.9	2	40.0
Ruminant	2	8.7	5	19.2	-	-
Pig	-	-	3	11.5	-	-
Fish	1	4.3	-	-	-	-
Turtle	-	-	1	3.8	1	20.0
Cheetah	-	-	1	3.8	-	-

* Multiple assignments possible, percentage related to the respective total numbers of study participants;
ACN, acoustic neurinoma

First contact to animals took place at a median age of 5.0 years (range 0-28 years) in acoustic neurinoma patients, at 31.0 years (range 0-59 years) in glioma patients and 20.0 years (range 0-48 years) in meningioma patients. The mean number of animals to which the study participants had contact varied between 1.7 and 2.0 (Tab. 28)

Table 28: Number of animals to which the study participants completing the questionnaire had contact prior to the present study (N=54)

Number of Animals	Glioma patients (n=23)		Meningioma patients (n=26)		ACN patients (n=5)	
	n	%	n	%	n	%
0	5	21.7	4	15.4	1	20.0
1	6	26.1	7	26.9	1	20.0
2	6	26.1	4	15.4	-	-
3	4	17.4	10	38.5	3	60.0
4	1	4.3	1	3.8	-	-
5	1	4.3	-	-	-	-
Mean N°	1.7		1.9		2.0	

ACN, acoustic neurinoma

(B) Laboratory Results

3.5 Characteristics of Participants Providing Biological Samples

For laboratory analyses, 39 glioma, 31 meningioma and 6 acoustic neurinoma patients were recruited. At the time of surgery, all patients had a median age of 53.5 years (range 9 to 83 years). The median ages were similar for meningioma and glioma patients; acoustic neurinoma patients had a median age of 42 years. 67% of acoustic neurinoma and 56% of glioma patients were male. The majority in meningioma patients was female (Tab. 29).

Table 29: Distribution of age and gender in 76 brain tumor patients included in the study

	N	Age		Gender			
		Median Age (years)	Age Range (years)	Male		Female	
Patients with				**n**	**(%)**	**n**	**(%)**
Glioma	39	55.0	9-80	22	(56.4)	17	(43.6)
Meningioma	31	53.0	32-83	5	(16.1)	26	(83.9)
ACN	6	42.0	34 - 66	4	(66.7)	2	(33.3)

ACN, acoustic neurinoma

3.6 Analyses of the Presence of HCMV DNA Sequences in Blood Samples of the Brain Tumor Patients

To exclude a possible contamination of brain tumor tissues by HCMV DNA positive blood cells, PCR of corresponding blood samples of 71 brain tumor patients was carried out. From five glioma patients, no blood sample was available.

3.6.1 Prevalence of DNA Sequences of the HCMV-specific GlycoproteinB (UL55) Gene

DNA from patients' blood samples was amplified by nested PCR using primers for the detection of sequences of the HCMV-specific gB (UL55) gene as described by Cobbs et al. [2002]. No viral DNA sequences were detected in any of the corresponding blood samples (Fig. 12).

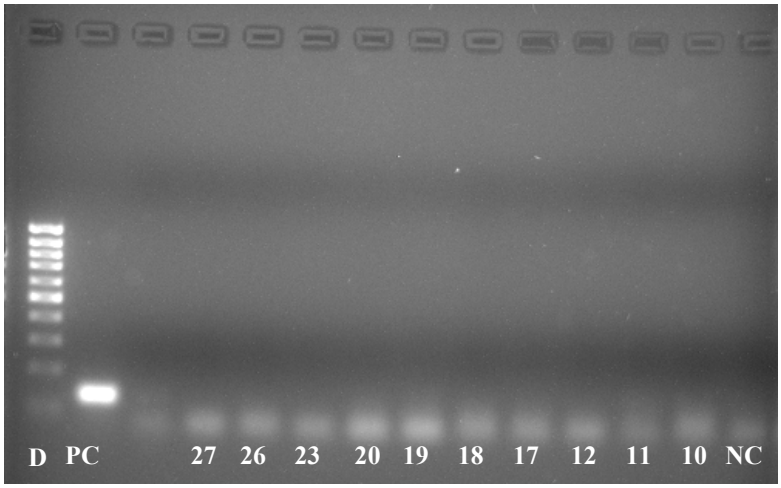


Figure 12: Agarose gel after PCR for the detection of HCMV-specific gB gene as described by Cobbs et al. [2002] in 10 blood samples from patients with primary brain tumor (as an example for 71 blood samples tested); D, DNA ladder (size marker); PC, positive control; NC, negative control

3.6.2 Prevalence of DNA Sequences of the HCMV-specific Immediate Early-1 (UL123) Gene

Another nested PCR protocol was carried out for the detection of DNA sequences of the IE-1 gene as published by Mangano et al. [1992] to validate the results obtained with nested PCR using primers specific for sequences of the gB (UL55) gene. HCMV-specific IE-1 gene sequences were not detected in any of the blood samples (Fig. 13).

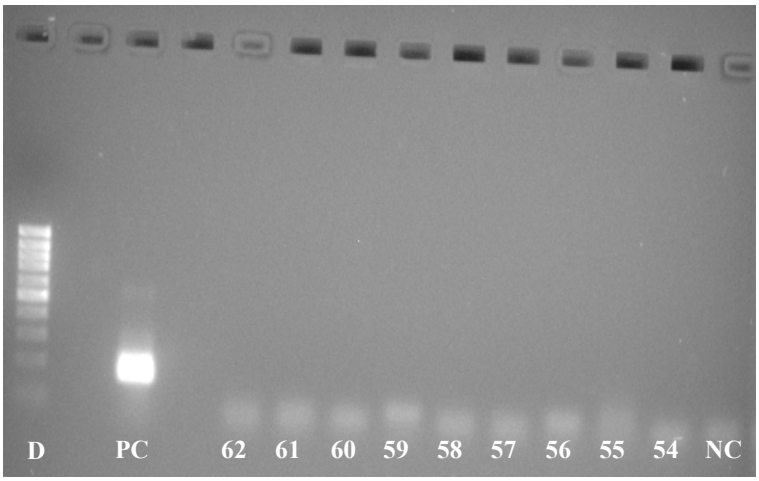


Figure 13: Agarose gel after PCR for the detection of HCMV-specific IE-1 gene as described by Mangano et al. [1992] in 9 blood samples from patients with primary brain tumor (as an example for 71 blood samples tested); D, DNA ladder; PC, positive control; NC, negative control

3.7 Polymerase Chain Reaction in Brain Tumor Tissues

3.7.1 Quality Assessment (GAPDH Detection) in Brain Tumor DNA

All brain tumor samples were examined for the presence of amplifiable cellular DNA by a GAPDH PCR to control for successful DNA extraction. Cellular GAPDH DNA sequences could be amplified in all brain tumor samples, demonstrating that no inhibitors of the PCR were present in the sample. A positive result was defined as a band of 670 bp (Fig. 14).

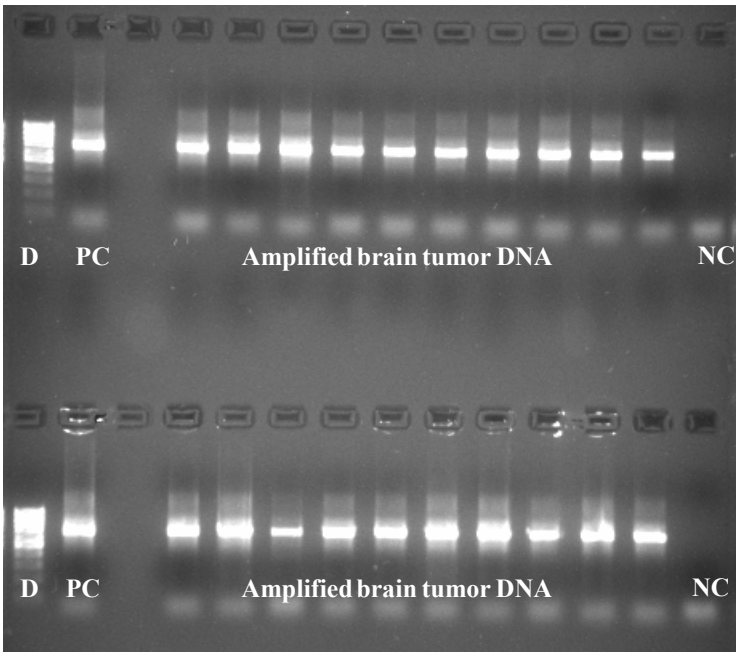


Figure 14: Agarose gel after GAPDH PCR to control for successful DNA extraction of primary brain tumors (representative for 76 brain tumor samples tested); D, DNA ladder; PC, positive control; NC, negative control

3.7.2 Analyses of the Presence of HCMV DNA Sequences in Primary Brain Tumor Tissues

To evaluate the role of HCMV in brain tumor development as previously suggested by Cobbs et al. [2002], the presence of HCMV DNA was assessed in 76 primary brain tumors using different nested PCR protocols.

3.7.2.1 Detection of Sequences of the HCMV-specific Glycoprotein B (UL55) Gene

No HCMV-specific gB (UL55) gene sequences were detected in the tumor samples using two different PCR protocols (Fig. 15). Nested PCR was performed using primers synthesized from HCMV strain AD169 for the detection of gB (UL55) gene and, in a subset of DNA samples, using a protocol as published by Cobbs et al. [2002] to exclude possible measurement errors.

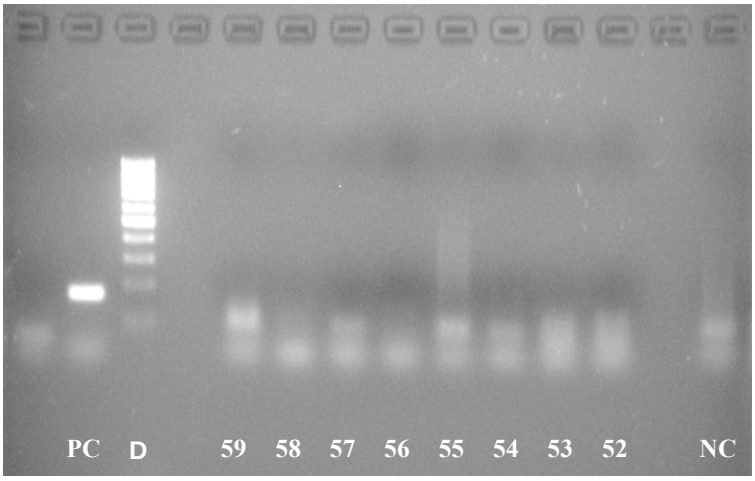


Figure 15: Agarose gel after gB (UL55) PCR for the detection of HCMV-specific gB gene using primers as described in Chapt. 2.4.4 in 8 brain tumor samples (as an example for 76 neoplasms tested); D, DNA ladder; PC, positive control; NC, negative control

3.7.2.2 Detection of Sequences of the HCMV-specific Immediate Early-1 Gene

Sequences of the HCMV-specific IE-1 gene were not found in any of the 76 primary brain tumors investigated using two different PCR protocols (Fig. 16). Primers for the detection of sequences of the IE-1 gene were synthesized from HCMV strain AD169 and, in a subset of DNA samples to control for false negative results, primers as published by Mangano et al. [1992] were used for the detection of such HCMV DNA sequences in brain tumor tissues.

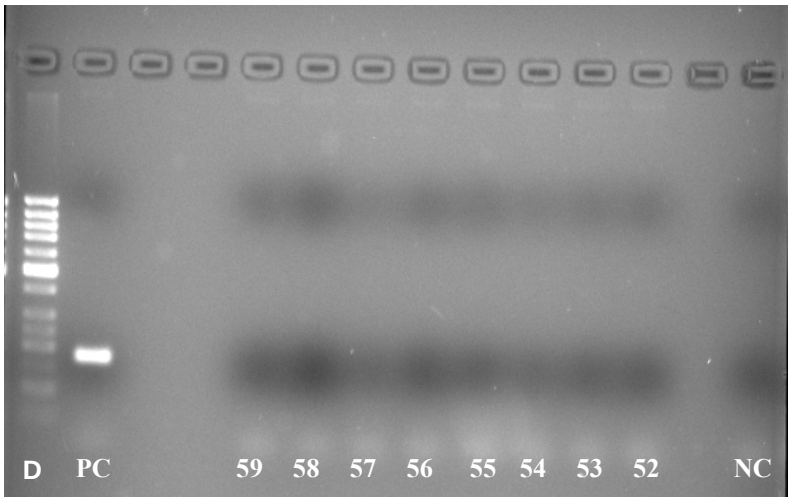


Figure 16: Agarose gel after PCR for the detection of IE-1 gene using primers as described in Chapt. 2.4.4 in 8 primary brain tumors as an example for 76 brain tumors tested; D, DNA ladder; PC, positive control; NC, negative control

3.8 Prevalence of HCMV Proteins in Primary Brain Tumors

Because of published discrepancies between results obtained from PCR and immunohistochemical analyses, the presence of HCMV-specific proteins in brain tumor samples as well as in short-term cultures derived therefrom was investigated additionally.

3.8.1 Detection of the HCMV-specific Phosphoprotein 65 Antigen

No immunoreactivity was detected in sections of 72 primary brain tumors and in four short-term cultures tested by using anti-pp65 monoclonal antibodies. In contrast, the positive controls (HCMV-positive lung tissue and HCMV-infected fibroblasts, respectively) showed clear intracytoplasmatic and intranuclear antigen staining with the protocols used (Fig. 17).

3.8.2 Detection of the HCMV-specific Immediate Early-1 Antigen

Immunohistochemistry was performed using a monoclonal anti-IE-1 antibody to validate the results obtained for pp65. No HCMV-specific IE-1 protein was detected in the brain tumor sections. In contrast, clear intracytoplasmatic and intranuclear antigen staining was seen in HCMV-positive lung tissue and in HCMV-infected fibroblasts, which served as positive controls (Fig. 17).

3.8.3 Detection of the HCMV-specific Early Antigen

To investigate the possibility that EA can be detected in the absence of expression of IE proteins, an immunohistochemical protocol was carried out for the detection of HCMV EA. Again, no immunoreactivity was detected in paraffin slides of brain tumors in contrast to a clear positive staining observed in the positive control (Fig. 17).

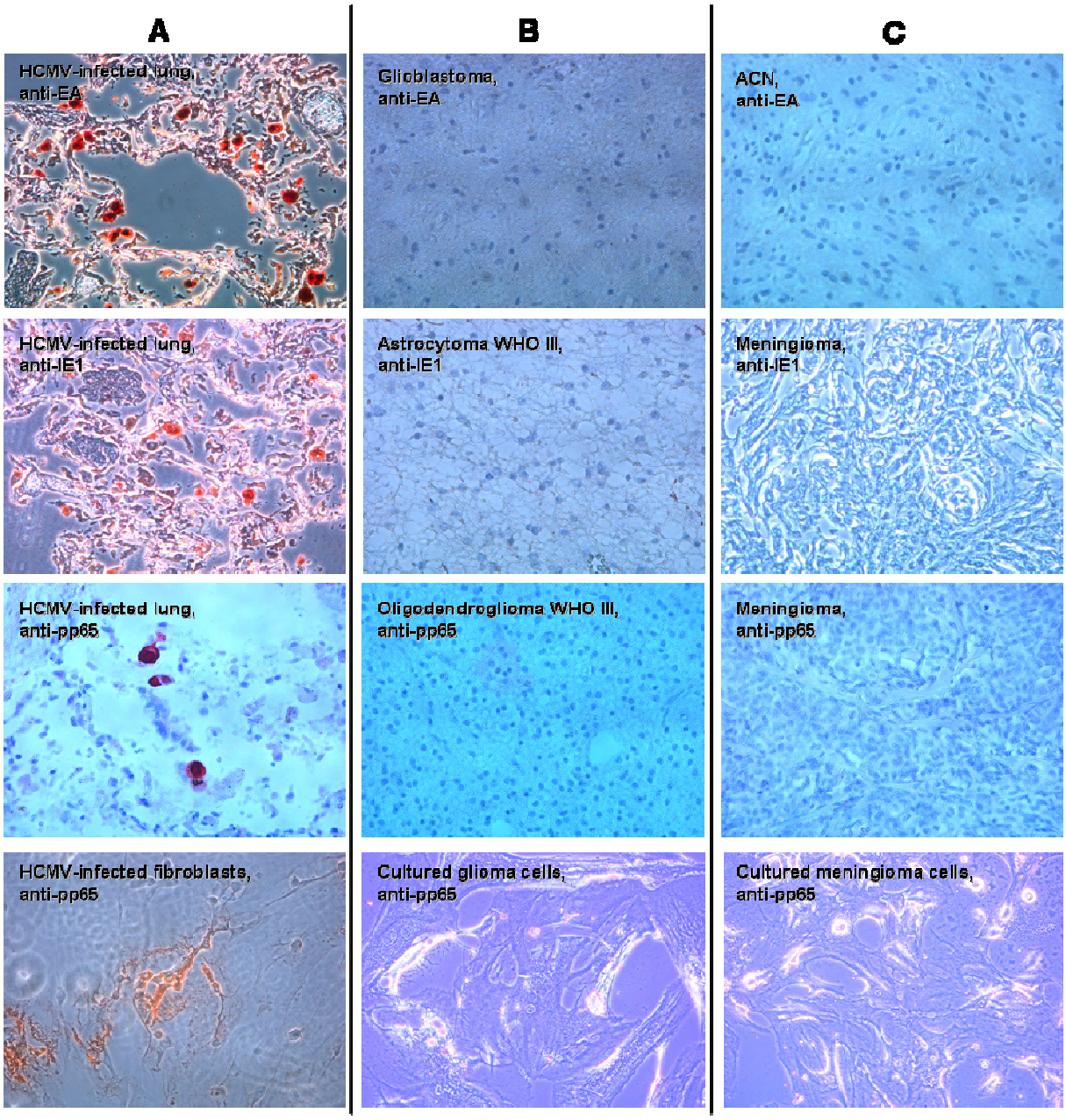


Figure 17: Immunohistochemistry for HCMV detection in brain tumor sections. Tissue types and anti-HCMV antibodies used (pp65, IE1, EA), are indicated in the respective micrograph. Typical enlarged cells can be seen in the positive controls (HCMV-infected lung tissue and HCMV-infected fibroblasts, respectively) with HCMV-immunoreactivity (red staining) in both, cytoplasm and nucleus (column A). The lack of immunoreactivity in brain tumor tissue sections (first, second, and third row) and corresponding short-term cultures (last row) is visible in columns B and C.

3.9 Summary of Analyses for the Detection of HCMV Macromolecules in Brain Tumor Tissues and Blood Samples

Summarizing the results obtained with immunohistochemistry and PCR of brain tumor tissues and obtained with PCR of corresponding blood samples, HCMV macromolecules could be detected neither in the primary brain tumors nor in patients' blood. The results of all analyses performed as well as the histology of all primary brain tumors included in the present study are compiled in Tab. 30.

Table 30: Lack of detection of HCMV molecules in brain tumor tissue and corresponding blood samples using different methods (number of positive samples out of the number of samples tested)

	n	PCR tumor tissue (n=76)		IHC tumor tissue (n=72)*			PCR blood samples (n=71)**	
Tumor type		**Primers specific for**		**Monoclonal antibodies to**			**Primers specific for**	
		gB	**IE1**	**pp65**	**IE**	**EA**	**gB**	**IE1**
Glioblastoma multiforme IV	**23**	0/23	0/23	0/22	0/22	0/22	0/22	0/22
Gliosarcoma IV	**1**	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Anaplastic oligodendroglioma III	**4**	0/4	0/4	0/4	0/4	0/4	0/2	0/2
Astrocytoma II/III	**2**	0/2	0/2	0/1	0/1	0/1	0/1	0/1
Diffuse astrocytoma II	**3**	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Oligodendroglioma II	**1**	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Glioma (not further specified)	**5**	0/5	0/5	0/5	0/5	0/5	0/4	0/4
Transitional meningioma I	**10**	0/10	0/10	0/9	0/9	0/9	0/10	0/10
Meningothelial meningioma I	**5**	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Microcystic meningioma I	**2**	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Fibrous meningioma I	**2**	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Psammomatous meningioma I	**1**	0/1	0/1	0/0	0/0	0/0	0/1	0/1
Secretory meningioma I	**2**	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Meningioma I	**6**	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Atypical meningioma II	**3**	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Acoustic neurinoma I	**5**	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Acoustic neurinoma II	**1**	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Tumors in total	**76**	0/76	0/76	0/72	0/72	0/72	0/71	0/71

*no paraffin slides available of 2 glioma and 2 meningioma;
**no blood samples available for 5 glioma patients;
gB, glycoproteinB; IE, immediate early; pp65, phosphoprotein 65; EA, early antigen; IHC, immunohistochemistry

3.10 Prevalences of IgG and IgM Antibodies in Serum Samples

To estimate the seroprevalences of antibodies to four herpesviruses (HCMV, HSV, EBV, VZV), serum samples of 71 (93%) of the 76 brain tumor patients included in the present study were examined by an automated Enzyme Immunoassay (BEP-III®-system, Dade-Behring).

3.10.1 Prevalences of IgM Antibodies to HCMV, HSV, EBV, and VZV

At the time of surgery, none of the primary brain tumor patients' sera was positive for IgM antibodies to HCMV, HSV, EBV, and VZV, indicating that none of them had an acute herpesvirus infection or reactivation of these viruses at the time of surgery.

3.10.2 Prevalences of IgG Antibodies to HCMV, HSV, EBV, and VZV

The presence of IgG antibodies is an indicator for a previous exposure to the corresponding antigen. To investigate the possible role of previous infections with HCMV, HSV, EBV, and VZV in brain tumor development, the prevalence of anti-herpesvirus IgGs was determined.

Table 31: Overall seroprevalences of IgG antibodies to HCMV, HSV, EBV and VZV in 71 brain tumor patients

	Brain tumor patients (N=71)		
	IgG positive (n)	Prevalence (%)	(95%CI)
HCMV	45	63.4	(51, 75)
HSV	61	85.9	(76, 93)
EBV	63	88.7	(79, 95)
VZV	65	91.6	(83, 97)

HCMV, human cytomegalovirus; HSV, herpes simplex virus; EBV, Epstein-Barr virus; VZV, varicella-zoster virus; CI, confidence interval

Overall prevalences (Tab. 31 and Fig. 18) as well as prevalences stratified by 20-year age groups and tumor type (Tab. 32; Fig. 19; Tab. 34) and the corresponding 95%CI of IgG antibodies to HCMV, HSV, EBV and VZV were evaluated. Additionally, gliomas were stratified according to Wrensch et al. [2001] into WHO grade I-III and WHO grade IV gliomas for additional analyses (Tab. 33).

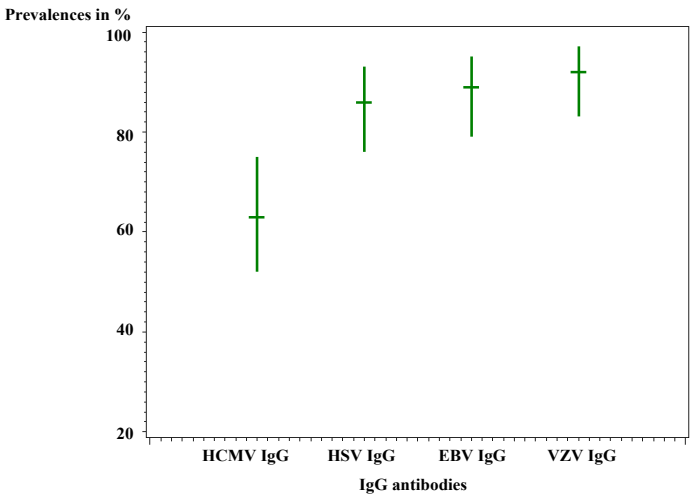


Figure 18: Overall seroprevalences and corresponding 95%CI of IgG antibodies to HCMV, HSV, EBV and VZV in 71 brain tumor patients

3.10.2.1 IgG Antibodies to HCMV

For all brain tumor patients, the seroprevalence of antibodies to HCMV was 63% (95%CI 51%-75%; Tab. 31). Stratified by age, a clear increase in seroprevalence was observed from 50% in the youngest age group (20-39 years) to 73% in the oldest age group (60-84 years; Tab. 32), which could also be observed in analyses stratified by tumor type, though less conspicious (Tab. 34). Stratification of the gliomas by WHO grading did not show any perceivable differences (Tab. 33).

3.10.2.2 IgG Antibodies to HSV

The overall seroprevalence of antibodies to HSV was 86% (95%CI 76%-93%; Tab. 31). An increase in the prevalence from 79% (95%CI 49%-95%) in the youngest to 88% (95%CI 70%-98%) in the oldest age group was observed (Tab. 32), which was still present in meningioma and acoustic neurinoma patients in tumor type stratified analyses (Tab. 34). Stratification by WHO grading did not show any perceivable differences (Tab. 33).

Table 32: Seroprevalences and corresponding 95% confidence intervals (95%CI) of IgG antibodies to HCMV, HSV, EBV and VZV in 71 brain tumor patients

Age Groups		Brain tumor patients	
	n	Prevalence (%)	(95%CI)
HCMV			
20-39y	14	50.0	(23, 77)
40-59y	31	61.3	(42, 78)
60-84y	26	73.1	(52, 88)
HSV			
20-39y	14	78.6	(49, 95)
40-59y	31	87.1	(70, 96)
60-84y	26	87.5	(70, 98)
EBV			
20-39y	14	100	(77, 100)
40-59y	31	90.3	(74, 98)
60-84y	26	80.8	(61, 93)
VZV			
20-39y	14	78.6	(49, 95)
40-59y	31	100.0	(89, 100)
60-84y	26	88.5	(70, 98)

HCMV, human cytomegalovirus; HSV, herpes simplex virus; EBV, Epstein-Barr virus; VZV, varicella-zoster virus; CI, confidence interval; y, years

3.10.2.3 IgG Antibodies to EBV

The overall seroprevalence for anti-EBV IgGs was 89% (95%CI 79%-95%; Tab. 31). In age-stratified analyses, the seroprevalence decreased from 100% (95%CI 77%-100%) in the youngest age group to 81% (95%CI 61%-93%) in patients over 60 years of age (Tab. 32). A similar trend was found only for meningioma patients after stratification by tumor type (Tab. 34). Compared to subjects with gliomas WHO grade I-III, glioblastoma patients had a decreased seroprevalence of IgG antibodies to EBV (Tab. 33).

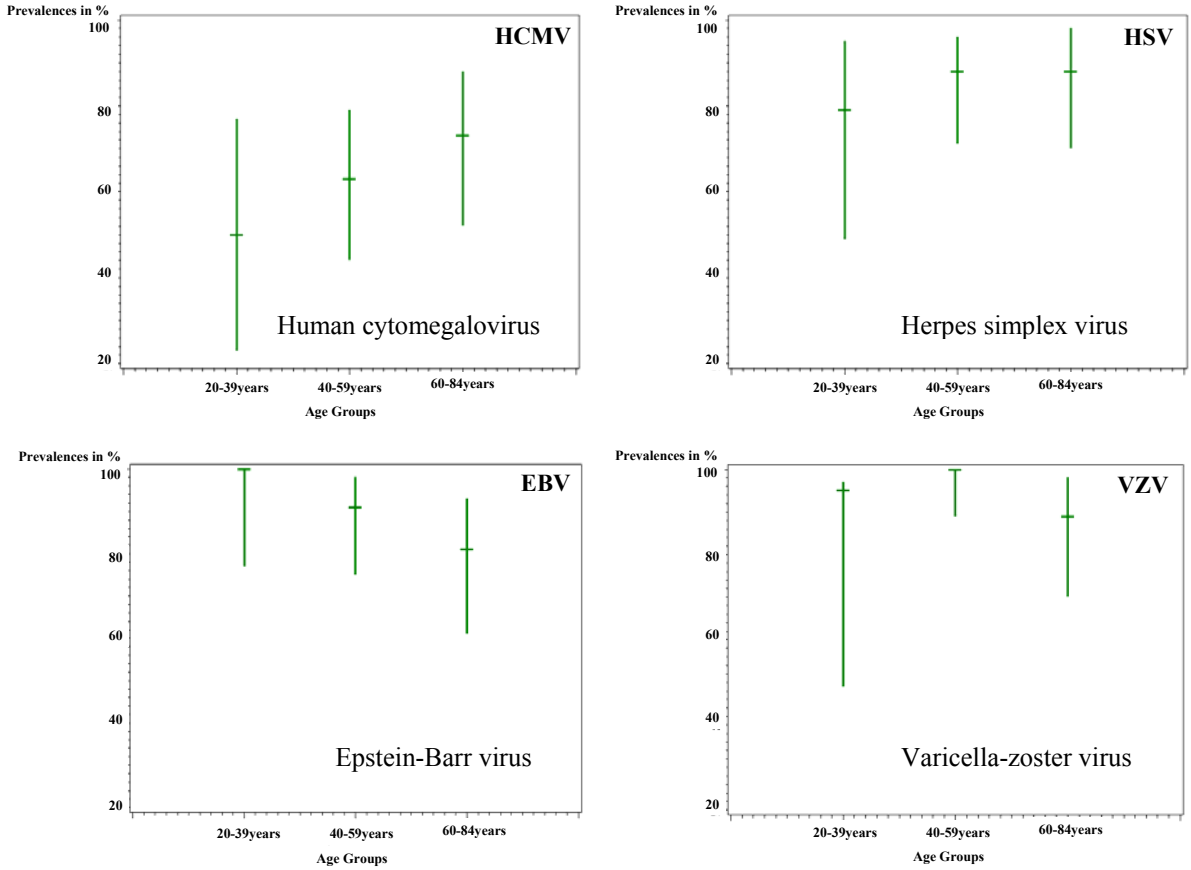


Figure 19: Seroprevalences and corresponding 95%CI of IgG antibodies to HCMV, HSV, EBV, and VZV in brain tumor patients stratified by 20-year age groups (71 serum samples tested)

Table 33: Seroprevalences in 34 glioma patients stratified by WHO grading

Prevalences of IgGs to	Glioma patients in the present study		
	All (n=34)*	GBM IV ** (n=23)	WHO I-III (n=11)
HCMV	58% (41-75%)	57% (35-77%)	64% (31-89%)
HSV	91% (76-98%)	91% (72-99%)	91% (59-100%)
EBV	85% (69-95%)	83% (61-95%)	91% (59-100%)
VZV	91% (76-98%)	91% (72-99%)	91% (59-100%)

*5 glioma patients did not provide a blood sample;
**1 GBM patient with did not provide a blood sample;
WHO, World Health Organization; IgG, immunoglobulin G; GBM, glioblastoma multiforme; HCMV, human cytomegalovirus; HSV, herpes simplex virus; EBV, Epstein-Barr virus; VZV, varicella zoster virus

3.10.2.4 IgG Antibodies to VZV

Overall, the seroprevalence of IgG antibodies to VZV was 92% (95%CI 83%-97%; Tab. 31). Stratified by age, no age-dependent trend was seen (Tab. 32). Patients aged from 40-59 years showed a peak seroprevalence of 100% (95%CI 89%-100%), which was also observed after stratification for tumor type (Tab. 34). In glioma patients, the prevalences did not differ between the different WHO grades (Tab. 33).

Table 34: Seroprevalences of IgG antibodies to HCMV, HSV, EBV, and VZV, stratified by tumor type and 20-year age groups

	IgG antibody-positive patients with								
	Glioma (n=34**)			Meningioma (n=31)			ACN (n=6)		
Virus / age	n	SP (%)	(95%CI)	n	SP (%)	(95%CI)	n	SP (%)	(95%CI)
HCMV									
20-39y	7	42.9	(10, 82)	4	75.0	(19, 99)	3	33.3	(1, 91)
40-59y	12	58.3	(28, 85)	17	58.8	(33, 82)	2	100	(16, 100)
60-84y	15	66.7	(38, 88)	10	80.0	(44, 97)	1	100	(3, 100)
all	**34**	**58.8**	**(41, 75)**	**31**	**67.7**	**(49, 83)**	**6**	**66.7**	**(22, 96)**
HSV									
20-39y	7	85.7	(42, 100)	4	75.0	(19, 99)	3	66.7	(9, 99)
40-59y	12	100	(74, 100)	17	76.5	(50, 93)	2	100	(16, 100)
60-84y	15	86.7	(60, 98)	10	90.0	(56, 100)	1	100	(3, 100)
all	**34**	**91.2**	**(76, 98)**	**31**	**80.7**	**(63, 93)**	**6**	**83.3**	**(36, 100)**
EBV									
20-39y	7	100	(59, 100)	4	100	(40, 100)	3	100	(29, 100)
40-59y	12	83.3	(52, 98)	17	94.1	(71, 100)	2	100	(16, 100)
60-84y	15	86.7	(52, 96)	10	80.0	(44, 97)	1	100	(3, 100)
all	**34**	**85.3**	**(69, 95)**	**31**	**85.7**	**(70, 95)**	**6**	**100**	**(54, 100)**
VZV									
20-39y	7	85.7	(42, 100)	4	50.0	(7, 93)	3	100	(29, 100)
40-59y	12	100	(74, 100)	17	100	(80, 100)	2	100	(16, 100)
60-84y	15	86.7	(60, 98)	10	90.0	(56, 100)	1	100	(3, 100)
all	**34**	**91.2**	**(76, 98)**	**31**	**91.4**	**(77, 98)**	**6**	**100**	**(54, 100)**

**no serum sample available for five glioma patients;
HCMV, human cytomegalovirus; HSV, herpes simplex virus; EBV, Epstein-Barr virus; VZV, varicella-zoster virus; SP, seroprevalence; CI, confidence interval; y, years; IgG, immunoglobulin G

4 Discussion

4.1 Ethical Justification for the Part-time Anonymous Sample Collection

In the present study, laboratory as well as questionnaire data were evaluated. During collection of the brain tumor samples, organizational problems occurred and ten of the samples had to be collected anonymously. This may have been in conflict with ethical issues. However, according to the central ethics commission, it is appropriate to use samples without informed consent of the patients if the following premises are provided [Zentrale Ethikkommission Deutschland, 2003]:

(1) Analyzing the sample will no longer be useful for the respective person (e.g. for diagnostics),
(2) the samples are fully anonymized,
(3) no individual gene analyses will be performed,
(4) the results of the study will be of no individual use for the respective person or its family members,
(5) no controversial issues are under research,
(6) there are no hints for a potential refusal of the concerned patient, and
(7) the informed consent can only be obtained with extraordinary efforts.

In the present study, the respective samples were anonymized in such way that only data on gender and year of birth were obtained. Therefore, the persons could never be traced back.

Furthermore, the study investigated putative etiological pathways in brain tumor development and therefore, as persons were already affected by a brain malignancy, the outcome of the study was of no individual prognostic interest. Similarly, this is true for family members.

Gene analyses were not performed, and there are also no hints that affected persons are not willing to help to ascertain the development of their disease.

Therefore, the use of the ten anonymized samples in the present study is justifiable.

(A) Questionnaire Data

A questionnaire was developed to control for factors indicative of previous infections, especially with herpesviruses, and to control for any possible reactivation of herpesviruses, which previously had been suggested to lead to cancers [Cinatl, Jr. et al., 2004; Michaelis et al., 2004; Roizman and Pellet, 2001; Chan et al., 1999; zur Hausen, 1975; Gahrton et al., 1971].

In addition, the role of several medical conditions as well as the suggestive role of high-level contact to humans or animals in brain tumor development, which are controversially discussed in the literature, were addressed [Ohgaki and Kleihues, 2005; Khuder et al., 1998; Preston-Martin and Mack, 1996].

4.2 Characteristics of Participants Completing the Questionnaire

The present study comprises two parts. First, laboratory analyses were performed to confirm the results of Cobbs et al. [2002] indicating a possible role of HCMV in brain tumor pathogenesis and second, a questionnaire was developed to control for factors indicative of previous infections and for other variables (cf. above).

Unfortunately, 22 of the 76 study participants in the present study did not complete the questionnaire for different reasons, mainly because of the part-time anonymous tumor collection, which could not be avoided during the collection period. Therefore, heterogeneity between patients not interviewed and the subset of patients completing the interview was tested.

Putative risk factors (mainly factors related to infections) were reported to be rather associated to tumor grading than to factors like gender or age. Therefore, and due to the small sample size in this study, WHO grading was determined to be the most important factor for analyses, and the test for heterogeneity between those completing and those not completing the questionnaire focused on the histological distribution of the tumors in these groups.

No heterogeneity at a significance level of $\alpha=0.05$ between those patients completing the questionnaire and those not interviewed was detected using Fisher's exact test. Therefore, it was presumed for the present study that the subset of interviewed persons was probably

representative for the whole study population, although, of course, this result may not have been obtained in a larger study population or if the comparison would have been done by methods of equivalence testing. Therefore, the results in the present study have to be interpreted with care.

Demographic Factors

In the present study, the tendency of both age and gender distribution in glioma and meningioma patients was similar to published descriptive epidemiology. There were more males having glioma and obviously more women were affected by meningioma. Therefore, the study participants affected by glioma and meningioma are a representative subset of brain tumor patients.

Generally, astrocytoma and glioblastoma have a peak in incidence at the age of 65-74 years and oligodendroglioma at the age of 35-44 years. Males are more often affected than females [Wrensch et al., 2002; Davis et al., 1999; Preston-Martin and Mack, 1996]. In meningioma, the incidence rate increases with increasing age [Bondy and Ligon, 1996]. A peak occurrence can be seen between 50-70 years of age [WHO, 2000b], and females are significantly more affected [Wrensch et al., 2002; Davis et al., 1999; Inskip et al., 1995].

Gender and age distribution in acoustic neurinoma patients, though, differed from that reported in the literature as they usually occur in people aged 50 years and above [Lanser et al., 1992] without any association with gender [Muscat et al., 2002]. However, this observation is likely to be due to the small number of acoustic neurinoma patients included in the present study.

Socioeconomic Status

An association between the socioeconomic status and brain tumor development was reported in several studies. Therefore, in the present study, the socioeconomic status was evaluated according to guidelines of the “Deutsche Arbeitsgemeinschaft für Epidemiologie”[DAE, 1997], taking into account educational and training level of each subject. Most subjects were grouped in category 3, indicating a low social class for most study subjects in either tumor type subgroup. This is in contrast to previous studies reporting a positive correlation between social class and all brain tumor types combined [Preston-Martin and Mack, 1996]. In

stratified analyses, higher social class seemed to be positively associated with glioma, meningioma and acoustic neurinoma [Faggiano et al., 1997; Preston-Martin et al., 1993; Preston-Martin, 1989]. Other studies, however, found a negative association between high social class (evaluated by assessing years of schooling) and the occurrence of glioma [Schlehofer et al., 2005; Preston-Martin et al., 1998].

In the present study, few people were on higher social classes in either histological type of brain tumor. For glioma, this corresponds to some of the published results. For meningioma and acoustic neurinoma, however, the present results differ from literature, which may be due to the small number of patients included in the present study.

A case-control study has been conducted by Inskip et al. [2003] to evaluate the association of sociodemographic indicators and the risk of brain tumors. Being single at the time of diagnosis was associated with a significantly reduced risk for meningioma and glioma, but not for acoustic neurinoma. Similarly, most participants in the present study were married or living with a partner. In analyses stratified by tumor type, the proportion of all patients being single was less to those being married or having a partner. An explanation for this reported distribution might be that spouses recognize brain tumor symptoms earlier than affected persons, and force them to seek medical advice. Similar results were reported by Goodwin et al. [1987]. Unmarried persons had a poorer prognosis and they were more likely to be diagnosed at a regional or distant stage as persons living with a partner. Therefore, the results of the present study are in the line with published results.

4.3 Association between Medical History and Primary Brain Tumors

4.3.1 Previous Infections

In the present study, histories of previous infections in brain tumor patients were addressed for two reasons. On the one hand, previous infections with herpesviruses were evaluated to control for a possible involvement of these viruses in brain tumor development. On the other hand, there is strong evidence that common infections like colds and flu may play a decisive

role in brain tumor pathogenesis (see below). Furthermore, the correlation between self-reported history of herpesvirus disease and occurrence of IgGs was evaluated.

As mentioned above, human herpesviruses have been suggested to be involved in the pathogenesis of several tumors. For EBV it is evident that it plays a role in specific lymphoma and sarcoma pathogenesis. In addition, its part in the development of nasopharyngeal carcinoma has become clear during the last years [Rickinson and Kieff, 2001]. HCMV has been linked to several neoplasms, including tumors of cervix, prostate, colon, and brain [Samanta et al., 2003; Cobbs et al., 2002; Harkins et al., 2002; Ho, 1982]. Being a sexually transmitted disease, HSV has been discussed as co-factor for cervical carcinoma pathogenesis, though the results are still inconsistent [Whitley, 1990]. The occurrence of chickenpox (primary VZV infection) and shingles (reactivation of latent VZV) has been reported to be less prevalent in glioma patients than in population-based controls [Wrensch et al., 1997b]. Another case-control study in a similar study population by Wrensch et al. [2001] showed a reduced prevalence of IgG antibodies to VZV in glioma patients with a significant inverse correlation for glioblastoma cases. This was additionally confirmed in another study population, taking into account the levels of anti-VZV IgG [Wrensch et al., 2005].

This section first focuses on the distribution of infectious diseases in the study subjects, followed by a discussion of the self-reported history of disease and the serological result.

Common Infections

In the present study, meningioma patients were more often affected by common infections than all other patients were. Frequent occurrence of infectious diseases was reported by 69% of them, in contrast to 26% in glioma patients and 20% in acoustic neurinoma patients.

Common infections are suggested to be involved in cancer development since several years. In previous studies addressing brain tumors and common infections prior to tumor diagnosis, decreased risks were consistently seen for glioma patients. Decreased tumor risks for people with a history of common infections such as colds and gastroenteric influenza were found by Abel et al. [1991] and subsequently supported by several studies. Schlehofer et al. [1999] reported that population-based controls were more likely to have common infections than glioma and meningioma cases in an international population-based case-control study on brain tumors. Other investigators could not find statistically significant associations, though the trend of the odds ratios for brain malignancies and infectious diseases were also indicative

for an inverse association between common infections and brain tumors [Cicuttini et al., 1997]. This observed correlation, however, is not yet understood. Immunologic factors triggering the immune system are suggested to alter the tumor risk.

A major problem concerning this subject is that "common disease" is a very subjective variable. In the present study, an inverse association with infectious diseases and brain tumor development is likely to be apparent in glioma patients if any is present. The proportion in meningioma patients, though, seems to be elevated, and would therefore rebut previous studies reporting higher prevalences of common infections in healthy individuals (cf. above).

However, there is no comparative literature available about the prevalence of common diseases in the general population. Therefore, and due to the small sample size, the distribution of infectious diseases in the different tumor types of the present study has to be interpreted with care.

Mononucleosis

Mononucleosis is caused by EBV, which has been proven to be an oncogenic virus [Rickinson and Kieff, 2001]. Furthermore, it has been shown *in vitro* that EBV is able to infect astrocytes [Menet et al., 1999]. The seroprevalence of anti-EBV antibodies has been investigated, suggesting previous EBV to be involved in brain tumor pathogenesis [Wrensch et al., 2005; Wrensch et al., 2001]. However, none of the subjects in the present study reported previous mononucleosis disease.

Exanthema Subitum

Only one glioma and one meningioma patient reported previous roseola infantum (exanthema subitum), which occurs after infection with HHV-6 and leads to a generally benign rash illness of infants. This disease is characterized by fever occurring in newborns [Maschke, 1967], which is often not recognized as this specific condition.

Few of the present study's participants knew this disease, mostly those with small children. This might be the reason for the low prevalence in the present study, possibly introducing a high recall bias. For this reason, it is difficult to make a statement about the occurrence of roseola infantum in brain tumor patients in this study.

Herpes Labialis

30% of glioma patients, 46% of meningioma patients and one patient with acoustic neurinoma (20%) reported to be affected by vesicles occurring at the vermilion border of the lip, resulting from a reactivation of HSV.

Reviewed by Whitley [1990], the recurrence rate of herpes labialis is approximately 33% (range from 16% to 38%) with a higher frequency in the upper social classes. Although this virus has been suggested to be involved in tumor pathogenesis, interestingly, an oncolytic (cancer killing) property of HSV has also been seen, and modified HSV may provide a tool for the treatment of malignant gliomas [Shah et al., 2003].

In the present study, there seems to be an elevated number of meningioma patients affected by herpes simplex lesions, especially after considering the low SES in these patients. However, to the author's knowledge, there are no clear-cut prevalence data about recurrent orolabial herpes disease and there is no report suspecting HSV disease to cause primary brain tumors. Thus, and due to the small sample size, results have to be interpreted carefully and further research is needed to evaluate the role of HSV in the etiology of brain malignancies.

Chickenpox and Shingles

In the present study, 52% of glioma, 65% of meningioma, and 80% of acoustic neurinoma patients reported previous chickenpox. The occurrence of shingles was reported by 17% of glioma and 8% of meningioma patients, resulting in recurrence rates (i.e., the proportion of shingles in individuals with a positive history of chickenpox) of 25% and 6%, respectively.

In a population-based case-control study, Wrensch et al. [1997b] reported an inverse correlation between glioma cases and self-reported history of chickenpox and shingles. However, although these findings were confirmed using serological analyses by the same investigators [Wrensch et al., 2005; 2001], no plausible biological explanation has yet been found for this observation. Immunological factors might have an influence on glioma pathogenesis, either by preventing cancer development or by killing existing cancer cells. A decreased risk for histories of common infections and allergies and the development of brain tumors has been reported, supporting this hypothesis (cf. above).

Unfortunately, no prevalence data for chickenpox and shingles in the overall German population are available. An incidence of 42.2 per 10 000 per year was reported for the country city of Ansbach (Bavaria) in 1996 [Paul and Thiel].

Furthermore, results of the present study may not be comparable with that of previous publications since these probably included individuals vaccinated against varicella in recent years and prevented the disease. In contrast, varicella vaccination was unlikely in the present study population because of the median age of the patients and the fact that this vaccination was first recommended by the German Permanent Immunization Committee (Ständige Impfkommission, STIKO) after the present study period. Considering the results of the present study and the hypothesis of frequent infections triggering the immune system and preventing cancer, low prevalences of VZV disease would have been expected, especially for reactivation of the virus (i.e., shingles). Therefore, VZV could be suggested to be involved in glioma etiology because of the low prevalence of chickenpox, and in meningioma pathogenesis, for these reported low proportions of shingles.

On the other hand, it has been reported that cancer is associated with a higher risk for shingles [Arvin, 2001; Whitley, 1990], but it has also been found that herpes zoster in healthy persons is not associated with an increased risk for neoplasms [Ragozzino et al., 1982].

Hence, no definite conclusion can be drawn and further research may be needed to evaluate a potential role of VZV in the pathogenesis of primary brain tumors.

Correlation between Self-reported Disease and Occurrence of IgG Antibodies

Self-reported histories of herpesvirus infections in the present study, however, do not reflect the serological status of the persons concerning anti-herpesvirus antibodies, as these infections are frequently inapparent. Therefore, the seroprevalence of IgG antibodies to HCMV, HSV, EBV, and VZV was evaluated and compared to the self-reported history of the respective diseases. The seroprevalences are discussed in more detail in Chapt. 4.7.

Only few of the patients reporting a negative history of either disease were seronegative for the respective IgG antibodies of the disease-causing agent, for chickenpox/shingles yet none of them. Overall, most individuals reporting never having had the disease were antibody positive. This is explainable by the fact that, with the exception of VZV, the addressed

herpesviruses rarely cause clinical disease after infection of the host (reviewed by Pass, 2001; Arvin, 2001; Rickinson and Kieff, 2001; Whitley, 1990).

Interestingly, each two patients with glioma and meningioma reported a previous occurrence of chickenpox although they were seronegative for IgG antibodies to VZV. There are three possible explanations for this phenomenon:

a) The patients did not exactly remember whether they had chickenpox during childhood and just suggested they had, because they knew it is a common childhood disease (potential recall bias);
b) The patients mistook the disease with another condition occurring during childhood, such as rubella and measles, or with other diseases like smallpox and scabies;
c) Antibody titers decline with increasing age and decrease in immunocompromised individuals [Arvin, 2001]. Taking into account the median age of the patients and cancer as an immunocompromising condition, this could explain the discrepancy between a positive self-reported history of chickenpox and seronegativity.

The presence of IgG antibodies to VZV by reported history of chickenpox has been addressed by Wrensch et al. [Wrensch et al., 1997b] in a subgroup of another population-based case-control study [Wrensch et al., 1997a] for whom serum samples were available. Among those reporting a positive history of chickenpox, glioma cases were more likely to have IgG antibodies to VZV than controls, though not statistically significant. Recently, this finding could be corroborated focusing on the levels of anti-VZV antibodies. Glioma cases had lower levels than population-based controls. In addition, glioblastoma cases were less likely to report a previous chickenpox history [Wrensch et al., 2005].

In the present study, all different tumor types investigated were more likely to have VZV IgG antibodies when they reported a positive history of chickenpox whereas most of the patients were not aware to be affected by any other diseases although they were seropositive for the respective virus (HCMV, HSV, and EBV). This is in the line with the fact that most herpesvirus infections (except VZV) rarely cause manifest disease (cf. above). For VZV, present results are in conformity with several other studies reporting that only about 50% of seropositive individuals give a clinical history of varicella, indicating either asymptomatic or mild disease or misdiagnosis [Ronan and Wallace, 2001; Arvin, 2001].

Only one third of the meningioma patients were IgG-positive despite a negative self-reported history of herpes labialis or genitalis in contrast to more than half of the glioma patients. Clinically apparent diseases have been suggested to have a negative influence towards the development of brain tumors, mainly gliomas [Schlehofer et al., 1999]. However, the seroprevalence of HSV IgGs and the prevalence of apparent orolabial disease in glioma patients in the present study were similar to that reported in the population (cf. above and below).

For other herpesvirus diseases, no differences could be observed in the different tumor types concerning seroprevalence and the respective condition.

Therefore, the conclusion of comparing self-reported history of a disease and the respective serology is that self-reported history of disease can not at all be a surrogate for the occurrence of IgG antibodies to the disease-causing agent.

4.3.2 Vaccinations

Vaccinations support the immune system by improving defense against certain antigens, thereby reducing the possibility of being affected by the respective disease. On the other hand, population prevalences of these diseases are generally low, and vaccinations generally stimulate the immune system by confronting it with an antigen. As discussed in Chapt. 4.3, there is evidence that people frequently affected by common colds are at lower risk for brain tumor development, possibly by stimulation of the immune system. Therefore, it might be possible that frequently vaccinated individuals are at lower risk for tumor development due to a triggered immune system, similar to the suggested inverse association of brain tumor development and allergies or common colds [Brenner et al., 2002; Schlehofer et al., 1999; Schlehofer et al., 1992; Abel et al., 1991].

In Germany, there is no compulsory vaccination, and most people are not aware of how often they were vaccinated against which disease (especially those that are vaccinated in childhood) and if the protection provided by immunization is still sufficient. Therefore, “ever being vaccinated” against a certain disease was addressed in the questionnaire. As participants were adults, some of the vaccinations nowadays applied to children were not inquired. For instance, vaccination against varicella has only been recommended by the Permanent Immunization

Committee (Ständige Impfkommission, STIKO) of the Robert Koch-Institute, Germany, since July 2004, which was after the present study period, and therefore, this vaccination was not assessed in the questionnaire.

Several studies had analyzed vaccination rates in the German population. According to the Robert Koch-Institute, Germany, rates in adults are 33% for diphtheria, and 63% for tetanus [Reiter and Rasch, 2004; RKI, 2002]. Immunity levels against diphtheria, tetanus and poliomyelitis were reported to be 60%, 72% and 79%, respectively, in a study among blood donors in Berlin [Stark et al., 1999].

Tetanus was the commonest vaccination in the study subjects with over 79% being vaccinated, followed by vaccination against diphtheria and poliomyelitis with an overall proportion of 45% and 60%, respectively. It could be suggested that high prevalences of tetanus vaccination are a result of the patients being aware of tetanus vaccination prior to brain tumor surgery, which is common in Germany. Poliomyelitis vaccination seems to be less prevalent in the patients of the present study than in the survey of Stark et al. [1999], which might be due to a potential recall bias as this vaccination is usually administered in early childhood. However, the proportion of brain tumor patients vaccinated against diphtheria was in-between the two studies mentioned above.

Vaccinations against childhood diseases such as rubella, mumps and measles were rarely reported by all brain tumor patients compared to published evaluations in first-year school attendees [Reiter and Rasch, 2004; RKI, 2002; Buxbaum et al., 2001]. This might be explainable by the median age of the study participants and the fact that vaccinating against these diseases first became common when they were already grown up.

In fact, age seems to be the most likely reason for differing vaccination rates. For instance, hepatitis A and B vaccinations are most prevalent in younger individuals who are frequently traveling, as well as in children born during the last decade, where hepatitis B vaccination became usual during nursery. Tuberculosis vaccination rates are supposed to be higher in the elderly than in younger individuals because this vaccination was no longer recommended by STIKO after 1982.

Pertussis vaccination has not been recommended by STIKO between 1974 and 1991; therefore, due to the median age of all patients, vaccination rates should be higher than those reported [RKI, 2000b]. However, pertussis is a disease most prevalent in small children and adolescents; study participants could have been affected by this condition during childhood

and therefore were not vaccinated. On the other hand, patients may presumably not remember being vaccinated during childhood (potential recall bias).

Vaccination against influenza is recommended for persons older than 60 years, individuals with chronic diseases and persons with high-level contact to humans. However, influenza vaccination is still very unpopular in Germany, although medical education is rising. Rates are 20-25% in the German population, and the prevalence is significantly increasing in older persons in which rates can be as high as 37% [Muller et al., 2005; Rehmet et al., 2002]. The overall proportion in the present study was 25% with differences between the different histologic tumor types (range 13% to 39%), being in the line with the data aforementioned.

In the study participants, the mean number of lifetime vaccinations was three to four vaccinations (glioma 3.2, meningioma 3.7, and acoustic neurinoma 3.9). Considering advices from STIKO, which recommends vaccination against multiple diseases (at least 12 during the whole life; RKI, 2004), this seems to be a very low prevalence. This, however, could be carefully interpreted as pointing in the same direction as the suggestion that people with a boosted immune system are at lower risk for tumor development.

In summary, lifetime vaccinations were difficult to obtain from the study participants. Age seemed to be the most important factor for the presumably high recall bias for this question. Therefore, the hypothesis of an influence of immunomodulation in brain tumor development remains elusive due to the small sample size of the present study, and further research may be required to evaluate this hypothesis.

4.3.3 Participants' Children as Indicator for Parental HCMV Infection

The questionnaire addressed malformations and diseases of participants' children during their first year of life to control for a possible prenatal transmission of HCMV from mother to child or a postnatal transmission from father to child. Jaundice, anemia, pneumonia and gastrointestinal diseases are frequently seen after perinatal HCMV infection. In addition, HCMV is the leading infectious cause of congenital malformations [Landolfo et al., 2003; Pass, 2001], and can also lead to hearing loss and mental retardation [Nagy et al., 2004].

None of the children of acoustic neurinoma patients had any malformations or any of the addressed diseases.

Jaundice was the most frequently reported disease occurring in the first year of life in affected offspring of the patients. In general, jaundice is reported to be present in more than 50% of all newborns; however, most cases of neonatal icterus do not need any treatment [Brown et al., 1999]. One can argue that patients reporting jaundice occurring in their children meant the more severe form that needed treatment. On the other hand, “disease” is a highly subjective variable. What is a notably disease for one person might not be recognized as disease by another person. Thus, participants might also have reported the common mild form, thereby introducing a potential recall bias.

In the present study, one single child of a glioma patient was affected by jaundice; however, the parent was seronegative for HCMV. In meningioma patients, six children from five patients were affected by jaundice. However, only two of the parents were HCMV seropositive and the single patient with two jaundice-affected children was seronegative. Therefore, it seems unlikely that HCMV transmission had been the causing factor for jaundice in study participants’ children.

One glioma and one meningioma patient reported the occurrence of hereditary amblyopia in their offspring. This might be indicative for a congenital HCMV infection, which frequently results in neurologic malformations, e.g. of the perceptual organs [Mets, 2001; de Jong et al., 1998]. However, none of the other conditions known to be frequently present after congenital infection (pneumonia, jaundice, anemia, gastrointestinal disease) had occurred in these children. In addition, only the meningioma patient was HCMV seropositive and this sample size is unequivocally too small to draw a definite conclusion.

One child of another meningioma patient was affected by neurodermatitis, asthma and chronic gastrointestinal disease during the first year of life. To the author’s knowledge, there is no report on an association between neurodermatitis and congenital HCMV infection. Asthma has been reported to be present in congenitally HCMV-infected neonates [Nagy et al., 2004] and gastrointestinal disease is a well known condition after prenatal HCMV infection [Pass, 2001]. However, no IgG antibodies to HCMV were found in this patient, thereby ruling out the possibility of a perinatal infection.

One meningioma patient reported the occurrence of intestinal obstruction and pneumonia in the offspring. Both conditions are typical for congenital HCMV infection, indicating maternal, i.e., brain tumor patients’ HCMV seropositivity. Another meningioma patient

reported one child affected by jaundice and pneumonia and another child with jaundice. However, both patients were HCMV seronegative.

In summary, the offspring of glioma and meningioma patients was most commonly affected by jaundice, which, however, is a frequent condition in neonates and therefore may not be a compulsory indicator for congenital HCMV infection.

More interesting is the serological status concerning HCMV IgGs in patients with affected children. None of the glioma patients was seropositive for HCMV IgGs. Therefore, neither jaundice nor amblyopia occurring in glioma patients' children could be traced back to a parental transmission. In total, only 40% (n=4) of the meningioma patients with affected children were HCMV seropositive, of which two had kids suffering from jaundice only.

The serological status of the participants is discussed in Chapt. 4.7. However, the serological status evaluated in the study subjects is likely to differ from that obtained years ago at the time when their offspring was born and may have been even lower at that time. Therefore, no definite conclusion can be drawn. There are some conjecturable conditions present in individuals' children suggesting a parental HCMV infection and subsequent transmission to the offspring. However, it seems unlikely in most of the cases comprising the serological status of the children's parents that the addressed medical conditions were an implication of parental HCMV infection and subsequent transmission to the offspring.

4.3.4 Immunosuppression

HCMV could facilitate tumor pathogenesis and enhance tumor growth, as reported for *in vitro* analyses. It is also well documented that HCMV has the ability to modulate functional properties of neuroblastoma cells [Scholz et al., 1999], and that HCMV IE proteins can block apoptosis and activate replication enzymes [Cinatl, Jr. et al., 1996].

In addition, higher prevalences of brain tumors in graft recipients, in individuals that use immunosuppressive drugs and individuals immunocompromised by viral infections (e.g. HIV infection) are well documented [Kinlen, 2004; Buell et al., 2004; Salvati et al., 2003; Vial and Descotes, 2003; Schiff et al., 2001; Detry et al., 2000]. Therefore, the occurrence of any immunosuppression caused by infections, chemotherapy and drugs, or organ transplantation medication that may have led to HCMV infection or reactivation was evaluated.

Furthermore, the history of previous blood transfusions of the study participants was assessed, as they are a major source of an infection with herpesviruses by transmission from donor to recipient [Kuhn, 2000]. HCMV infection or reactivation is frequently reported in individuals receiving blood transfusion where it can lead to severe disease.

This study included brain tumors with different latency periods. Gliomas are known to have a short latency period, but meningiomas are reported to be diagnosed even years after onset of development [Inskip et al., 1995]. Furthermore, HCMV is a virus with a long replication cycle and it could be presumed that if it were a causing agent in brain tumor pathogenesis, it would take several months to transform normal brain tissues [Roizman and Pellet, 2001]. Therefore, only immunosuppressive conditions indicative for HCMV infection or reactivation occurring at least 2 years prior to the present tumor were taken into account.

Drugs

One glioma and one meningioma patient had to be excluded from this analysis because the intake of an immunosuppressive drug took place less than 2 years prior to brain tumor surgery.

The single meningioma patient supposed to be immunosuppressed had received chemotherapy after each of two breast cancer surgeries. Meningioma had occurred four years after the first chemotherapy. As meningiomas are very slow growing tumors with a latency period of several decades [Gosztonyi et al., 2004; Strojan et al., 2000], it is questionable whether HCMV reactivation influencing subsequent meningioma development could have happened in this short time period. The crucial point is, though, that this patient was HCMV seronegative, clearly indicating that this tumor development was not influenced by HCMV.

Blood Transfusion

Another question addressed the prevalence of blood transfusions in the study participants. Blood transfusions are a major source of an infection with HCMV and other herpesviruses by transmission from donor to recipient [Kuhn, 2000]. Latent HCMV in blood cells of the donor can be reactivated following transfusion when encountering an allogeneic stimulus [Landolfo et al., 2003]. To prevent this transmission, blood donors are commonly screened for HCMV seropositivity.

One acoustic neurinoma patient reported a blood transfusion 30 years ago. Four glioma patients and one meningioma patient had received blood bottles 10 to 40 years prior to the present brain tumor surgery.

Generally, about 10% to 15% of persons receiving HCMV seropositive blood get infected [Albert et al., 1990]. Therefore, HCMV IgG testing is useful at every blood donation to provide a large pool of HCMV negative blood donors [Sibrowski et al., 1990]. At the time of the present tumor surgery, four of the six patients (66.7%) who reported a previous blood transfusion were HCMV seropositive. However, nothing is known about where the subjects received the transfusion and whether the hospital used filtered blood or used blood from seronegative donors. In principle, it is possible that study participants who obtained foreign blood acquired an HCMV infection, reinfection or reactivation during blood transfusion, and that this could have been a risk factor for brain tumor development. However, nothing is known about the serological status of the study subjects prior and shortly after transfusion concerning HCMV. The percentage of patients with a history of blood transfusion positive for anti-HCMV IgGs did not differ from the prevalence given by RKI [2000a], and there are so far no studies addressing time periods of HCMV infection prior to tumor pathogenesis. Therefore, no definite conclusion can be drawn.

4.3.5 Previous Cancers

Hereditary predisposing conditions (e.g. neurofibromatosis, tuberous sclerosis, Li-Fraumeni syndrome) are well-established risk factors for the development of primary brain tumors. However, only 4% of all brain tumor patients are affected by them [Preston-Martin, 1996].

In the present study, patients were asked about the occurrence of neurofibromatosis or tuberous sclerosis, two hereditary diseases associated with primary brain tumors. None of the subjects reported to be affected by either of the hereditary conditions. However, patients might not be aware of being affected by any of the hereditary diseases addressed. Wrensch et al. [2002], for example, suggested that some hereditary syndromes are not readily diagnosed because patients with brain tumors are not routinely referred to a clinical geneticist. Therefore, the proportion of cancer syndromes in brain tumor pathogenesis may be underestimated. To control for possible unrecognized cancer syndromes, cancer histories were

obtained from all study participants. Tab. 35 gives a condensed survey of hereditary cancer syndromes predisposing to CNS tumors and the corresponding cancers at other sites.

No previous cancers were seen in participants with acoustic neurinoma. Breast cancer had occurred in three glioblastoma patients, which could give hints to the presence of Li-Fraumeni syndrome in these subjects. For this syndrome, the frequent occurrence of both glioblastoma multiforme and breast cancer is common [Hisada et al., 1998].

An association between breast cancer and meningioma as seen in one of the patients was reported, and there is a trend towards breast cancer presentation first [Lieu et al., 2003]. An increase in incidence of meningioma of 80% following the diagnosis of breast cancer has been reported in 1975 and subsequently, similar results were seen in several studies [Wahab and Al Azzawi, 2003; Schoenberg et al., 1975], though this trend may simply reflect the slow growth of meningiomas.

The occurrence of thyroid gland cancer, which was the case in two of the meningioma patients, has been reported to have an elevated standardized incidence ratio prior to brain neoplasms by Inskip [2003]. However, their analysis suggested that brain neoplasms were most probably a consequence of cancer treatment of previous malignancies rather than of any genetic factors.

Myomas were more common in meningioma patients, which is not astonishing as more women develop meningiomas.

In summary, it was difficult to determine the possible occurrence of hereditary cancer syndromes in the present study population. Two syndromes were addressed in the questionnaire (neurofibromatosis or tuberous sclerosis) and none of the subjects reported to have either of them. Prevalences of previous cancers are dubious concerning the suggestive occurrence of hereditary syndromes, which patients are not aware, because these cancers of course also occur in individuals not affected by hereditary cancer syndromes. Other cancers (such as breast and thyroid gland cancer) have been reported to be associated with brain tumors. However, the total number of patients in the present study was too small for a definite conclusion concerning this issue.

Table 35: Review of hereditary cancer syndromes (according to Melean et al., 2004)

Syndrome	CNS tumors	Cancer at other sites
Neurofibromatosis type 1	Astrocytoma, meningioma, neurofibroma, neurofibrosarcoma	Hypothalamic tumor, parathyroid adenoma, pheochromocytoma
Neurofibromatosis type 2	Schwannoma, meningioma, ependymoma, astrocytoma, neurofibroma	
Tuberous sclerosis	Ependymoma, giant cell and retinal astrocytoma	Renal carcinoma, renal cysts, gingival fibroma
Li-Fraumeni	Astrocytoma, glioblastoma multiforme	Breast cancer, lymphoma, leukemia, sarcomas
Gorlin	Cerebellar medulloblastoma	Ovarian carcinoma and fibroma, cardiac fibroma, basal cell nevi and carcinoma
Turcot	Medulloblastoma, glioblastoma multiforme, astrocytoma, ependymoma	Colon cancer, basal cell carcinoma, gastric cancer

CNS, central nervous system

Despite giving hints for undiagnosed cancer syndromes, previous malignancies and their treatment are immunosuppressive conditions that might contribute to HCMV infection or reactivation possibly leading to brain tumor development or progression. However, the single meningioma patient who reported the intake of chemotherapeutics was seronegative for anti-HCMV IgG (see Chapt. 4.3.4).

In total, 72.7% of the patients with cancer history were seropositive for anti-HCMV IgGs, with slight differences in analyses stratified by tumor type. This, although the proportion being in the line with RKI prevalences [RKI, 2000a], could be a hint for an immunomodulation leading to HCMV infection or reactivation at the time of the previous neoplasm.

However, the sample size in the study was too small and nothing was known about the serological status of the participants prior and shortly after the previous cancer, to establish a hypothesis. Furthermore, it is conjecturable whether brain tumors are rather a consequence of treatment of previous malignancies than of any genetic factors [Inskip, 2003]. Therefore, further research may be needed to clarify this issue.

4.3.6 Allergic Conditions

An inverse association between the occurrence of allergies and brain neoplasms, mainly gliomas, has been reported in several studies. In fact, this is one of the few consistent factors suggested to be involved in brain tumor development.

In a population-based case-control study, a decreased risk of glioma in those with a history of allergies has been detected [Ryan et al., 1992]. Schlehofer et al. [1992] could confirm this result in a population-based case-control study in the Rhein-Neckar-Odenwald area, suggesting that a general activation of the immune system might be protective for gliomas. Furthermore, in pooled analyses of an international case-control study on brain tumors, in which the data of Ryan et al. [1992] and Schlehofer et al. [1992] were included, a significant risk reduction for subjects reporting a history of allergies was found [Schlehofer et al., 1999].

A study by Cicuttini et al. [1997] could not significantly confirm these results; however, the results in their population-based case-control study also suggested a protective role of allergic conditions in brain tumor pathogenesis. Similarly, more recent case-control studies by Brenner et al. [2002] and Wiemels et al. [2004; 2002], and a cohort study by Schwartzbaum et al. [2003] also reported that adults with glioma were significantly less likely to have a variety of allergic conditions.

A possible explanation for these findings is that frequent activation of the immune system, which occurs in people with allergies, leads to a lifelong hyperresponsiveness to multiple antigens (including tumor cells) and therefore to a more efficient tumor immunosurveillance. However, this hypothesis has not yet been confirmed. In summary, there seems to be an inverse correlation between glioma development and history of allergy, though no causative agent or immunologic pathways could be determined to date.

Therefore, the occurrence of hay fever, asthma, eczema and allergies were addressed in the present study to evaluate this association.

Allergies

The evaluation of the questionnaire in the present study is limited by a relatively small sample size. Therefore, no differences were made in data collection between food and other allergies (like dust, pollen etc.). Hermann-Kunz [1999a] investigated the prevalence of allergic diseases in Germany using data from the German National Health Interview and Examination Survey, 1998. Food allergy and "other allergies" were reported to be prevalent in 6.3% and 16.6%, respectively, in the German population. This would suggest a significant increase in the occurrence of allergies in meningioma patients in the present study (35%), being at variance with the hypothesis of an inverse association between brain tumors and allergies.

Hay Fever and Asthma

With regional differences, hay fever affects 9.5–40.9% of the European population [Heinrich et al., 2002; ECRHS, 1996]. For Western Germany, the German National Health Interview and Examination Survey, 1998 reported a prevalence of 11% [Hermann-Kunz, 1999b]. In the present study, prevalences were 13% for glioma patients and 8% for meningioma patients, being similar to published prevalences. Similar results were found for asthma prevalences; 4% of the glioma and 5% of the meningioma patients reported being affected by asthma, being commensurate to the proportion in the German population (6.1%; Hermann-Kunz, 1999a). Hence, the findings of the present study do not suggest an association of asthma and hay fever and brain tumors as previously reported.

Eczema

Due to the small sample size in the present study, neurodermatitis and urticaria were not distinguished from eczema and contact eczema in contrast to previous studies, resulting in limitations for adequate comparison of the present data with published prevalences. A proportion of 16% for contact eczema and 9% for urticaria were reported in the German population [Hermann-Kunz, 1999a], resembling the prevalences found in the brain tumor patients (17% for glioma and 15% for meningioma patients). From previous publications (cf. above), however, a decreased prevalence would have been expected.

All Allergic Conditions Combined

The occurrence of at least one allergic condition (asthma, hay fever, eczema, or other allergies) was reported by 39% of glioma patients and 39% of meningioma patients. In a survey of Hermann-Kunz et al. [1999a], the prevalence of at least one physician-diagnosed allergic disease in West Germans was assessed to be 42.8%.

It is well known that the frequency of allergies decreases with increasing age. Age-stratified analyses in that survey yielded a prevalence of 50.9% in persons aged 30-39 years, 43.2% in persons from 40-49 years and 40.9% in people at the age from 50-59 years [Hermann-Kunz, 1999a]. A dose-response has been reported for allergy on brain tumor risk in the study of Wiemels et al. [2002]. This was not observed in the present study.

Prevalences in the present study were similar to the study of Hermann-Kunz et al. [1999a], indicating that no differences (especially no decreased occurrence of allergic conditions) occurred, which would have been expected from the above-mentioned studies.

Distribution of Allergic Conditions – Summary

In summary, a suggestive positive correlation was seen in the occurrence of allergies in meningioma patients. This does not support the hypothesis of better tumor immuno-surveillance of people suffering from allergic conditions. However, as an inverse association has frequently been reported by several studies and considering the small sample size in the present study, further research is needed to clarify this possible correlation.

Overall, the results for allergic conditions have to be interpreted carefully due to the lack of adequate comparative data.

4.3.7 Hearing Impairment and Tinnitus

Tinnitus

The prevalences of hearing impairment and tinnitus at least 2 years prior to brain tumor surgery were addressed in the questionnaire. Because this question was added to glioma and meningioma patients' interviews later on in the study, 46% of the glioma and 35% of the meningioma patients did not answer the question concerning tinnitus, resulting in a high number of missing data for this question.

None of the surveyed patients reported the occurrence of tinnitus. In previous reports, prevalences for tinnitus in acoustic neurinoma patients ranged from 8% to 34% [Tos et al., 1998; Deen et al., 1996; Symon et al., 1989], being conflictive to present results. However, due to the small sample size and the fact that this question was included later on in the study, the present result could also be compatible with chance.

Hearing Impairments

The question on hearing impairments at least 2 years prior to brain tumor surgery was intended to evaluate a possible congenital HCMV infection of the patient, after which hearing impairments are frequently reported [Pass, 2001].

Two patients with acoustic neurinomas, one glioma patient and all meningioma patients reporting hearing impairments reported to be affected for more than 10 years (range 13 to 16 years). No previous brain tumors were reported that could have caused this condition. Some patients were rather young at the onset of hearing defects. One participant with acoustic neurinoma was 18 years old (16 years prior to surgery), and one of the glioma patients was 30 years old (16 years prior to surgery), which is relatively young for the occurrence of hearing impairments. On the other hand, acoustic neurinomas as well as meningiomas are very slow-growing tumors and it could be suggested that the occurrence of hearing impairments could be an early symptom for these malignancies [Inskip et al., 1995]. The questionnaire did not address any other conditions that might have caused this disease, such as noise trauma; therefore, it remains unclear how and why these subjects acquired hearing impairments.

Another possible explanation for hearing impairments could be a reactivation of congenitally acquired HCMV infection. In manifest disease of congenital HCMV infection, hearing impairments are one of the most frequent symptoms. In fact, symptomatic congenital HCMV

infection is the most common infectious cause of deafness. Up to 60% of infected infants develop hearing loss [de Jong et al., 1998]. In addition, long-term follow-up studies showed that 7% to 25% of asymptomatically infected infants at birth may develop hearing defects [Landolfo et al., 2003; Pass, 2001]. Three of the six patients affected by hearing impairments were HCMV seropositive. However, even if stratified by tumor type, no increase in the prevalence of IgG antibodies to HCMV could be observed in affected patients, suggesting that HCMV was not the agent causing hearing impairments. However, due to the small sample size, this result could also be due to chance.

4.3.8 Epilepsy

The scientific literature on a possible association between a history of seizures and brain tumors is inconsistent. While some epidemiological studies showed significant associations [Schlehofer et al., 1999; Wrensch et al., 1997a], others failed to detect a correlation [Cicuttini et al., 1997]. Despite being a putative risk factor, seizures are a common first manifestation of brain tumors. Even if convulsions occurred several years prior to brain tumor diagnosis, the chance of a slow-growing tumor causing these symptoms cannot be excluded [Grisold et al., 2000]. In the present study, ever-occurring epilepsy or seizures were addressed. None of the study participants reported epilepsy occurring more than one year prior to brain tumor surgery. From the results of the present study, it seems more likely that epilepsy is rather a symptom than a cause of primary brain tumors.

4.4 Assessment of Occupational History

Occupational risk factors for the development of primary brain tumors had been investigated in several studies, with controversial results (reviewed by Preston-Martin and Mack, 1996). Elevated risks were reported for occupations such as farmers [Inskip et al., 1995] and agricultural exposures [Khuder et al., 1998], whereas other studies found decreased risks for general farm workers [Menegoz et al., 2002]. Increases in brain tumor incidence were also seen for people working in specific industries (e.g. rubber, oil) or white-collar occupations, and health care workers [Inskip et al., 1995].

Most studies used occupation titles as a measure of exposure and did not focus on specific substances. This, however, can act only as a surrogate for potential causal agents in the workplace. Therefore, studies are conflictive about the particular occupational exposures suggested to lead to brain tumors. For example, elevated risks in farmers are suggested to be either due to increased exposure to pesticides [Musicco et al., 1988], or due to an influence of immunological factors (e.g. infections) or animal contact [Menegoz et al., 2002]. In health care workers, an increased risk was suggested to be due to high-level contact to humans ('infectious agents'-hypothesis) as well as to be due to the intake of drugs and drug handling or the use of formaldehyde [De Roos et al., 2003; Krishnan et al., 2003; Menegoz et al., 2002; Inskip et al., 1995].

Schlehofer et al. [2005] investigated 16 occupational categories a priori defined according to the International Standard Classification of Occupations (ISCO). In contrast to other studies reporting an influence of occupations on brain tumor development, this international case-control study did not provide evidence of a strong association between occupational exposures and glioma development.

In the present study, a detailed occupational history was obtained from 54 brain tumor patients. Occupations were classified into 16 categories in accordance to Schlehofer et al. [1990] following ISCO [2003]. The most frequent categories in all participants were 'office' and 'service'.

As the main study intention was to evaluate the risk of neuro-oncogenic infections in brain tumor pathogenesis, 'agriculture' and 'health system' were the most intriguing categories. However, only one meningioma and one acoustic neurinoma patient reported having worked in the 'agriculture' sector. 13% of glioma patients and 20% of acoustic neurinoma patients had worked in the health system. There were obviously more meningioma patients working in that category (65%). It is suggestible whether working in the health system and being exposed to certain substances or infectious agents increases the risk for meningioma development, but further research is needed to clearly establish this hypothesis.

Similarly, obviously more meningioma patients were working in occupations belonging to the 'service' category. Over 90% reported to have worked in jobs such as hairdresser, cleaner, cook, or manager in hotels or public houses, thereby being at risk for acquiring potential neuro-oncogenic infections. However, these findings are difficult to interpret because of the small sample size in the present study.

Few epidemiological studies have evaluated occupations being putative risk factors for meningioma or acoustic neurinoma. Elevated risks were reported for cooks, insurance agents, university lecturers, social workers, computer specialists, glassmakers, chemists, technicians, toolmaker setters and operators, inspectors, carpenters, gas station attendants, motor vehicle drivers, auto body painters, designers, decorators, in military occupations, industrial production, teachers, managers and machine operators [Rajaraman et al., 2004; Menegoz et al., 2002; Navas-Acien et al., 2002; Preston-Martin, 1989; McLaughlin et al., 1987]. However, most studies investigated vocational contact to certain exposures (electromagnetic fields, chemicals, metals, etc.; reviewed in Inskip et al., 1995), which were not addressed in the present study. Furthermore, a major problem for comparing obtained results to previous studies is that studies on brain tumor risk factors frequently combine gliomas and meningiomas in one group. Nowadays, authors try to report results separately for the different histological types because it is likely that gliomas and meningiomas have different pathogeneses.

In summary, no considerable increase was seen in either subgroup except for the elevated number of meningioma patients working in the ‘health’ and ‘service’ category. Nevertheless, the high percentage of people working in the ‘office’ and ‘service’ category, both likely to have potential high-level contact to humans, might be a hint for immunological factors contributing to brain tumors, especially meningiomas. However, due to the small sample size in the present study, this finding has to be further investigated.

The issue of potential high-level contact to animals and/or humans is discussed in the next chapter.

4.5 Contact to Animals and/or Humans and Primary Brain Tumors

For several zoonotic diseases, it is well known that they affect the central nervous system. For example, there is evidence that Borna virus, which most often affects horses, might contribute to psychological changes in humans [Bajramovic et al., 2002; Richt and Rott, 2001]. Furthermore, prions are suggested to lead to neurological damage in humans (e.g. new variant of Creutzfeld-Jacob disease, CJD) after transmission from cattle suffering from Bovine Spongiform Encephalopathy (BSE; Beghi et al., 2004). Rabies and tick-borne encephalitis (Frühsommermeningoenzephalitis, FSME) also affect the CNS after transmission from animal

to human [Lafon, 2005; Sambri et al., 2004]. Furthermore, despite zoonotic diseases, there are several human infectious agents affecting the CNS [Debiasi and Tyler, 2004]. In addition, it is well known since several years that some infectious agents can induce neoplasms (e.g. EBV, or helicobacter pylori), and that inflammation in general contributes to malignancies.

As mentioned above, living on a farm and thus being exposed to several animals as well as exposure to pets has been suggested to be involved in brain tumor pathogenesis for a long time. In people with high-level contact to animals, exposure to potentially hazardous agents may occur, such as animals' feces, body fluids, and contact, inhalation or absorption of zoonotic viruses, bacteria, and other antigens [Inskip et al., 1995].

However, studies on animal contact and brain tumor development are at variance in their findings on the species responsible for tumorigenesis. Preston-Martin et al. [1993] found increased risks for brain tumor development in dairy workers as well as in sheep workers. These findings could not be confirmed in another case-control study, but up to 4-fold elevated risks to develop PNETs (primitive neuroectodermal tumors) were found for children with contact to pigs or poultry [Holly et al., 1998]. Efird et al. [2003] could confirm the findings on contact to pigs. In addition, these investigators found elevated odds ratios for contact with horses, dogs, and cats and the development of childhood brain tumors.

Menegoz et al. [2002] addressed the role of animal contact in brain tumor development in a multi-center case-control study. No association between brain tumors (gliomas and meningiomas) and contact to nine species of animals or working in occupations with high-level contact to animals was observed, except an inverse association of women having contact to horses and meningioma (OR 0.66, 95%CI 0.46-0.94). Analyses on suggested high-level contact to humans or human tissues also did not show any effects. In addition, no increase of the risk with increasing number of animal types was found. However, study centers in this multicenter study were not always homogenous in their results [Menegoz et al., 2002].

In the present study, all occupations reported by the study participants were additionally classified into occupations with high-level contact to animals, humans, both animals and humans, or neither of them (according to Menegoz et al., 2002), to evaluate the possible role of a transmission of infections leading to brain tumor development. People working as physicians, teachers, nurses, hairdresser and trained retail sales clerk were considered to have high levels of contact with humans or human tissues. Farmers and cooks were considered to have potential high-level contact to animals or animal tissue. Subjects working for a catering

service, in gastronomy (serving and cooking) or as trained retail sales clerk at a butchery were considered to have high-level exposure to both, humans and animals.

An obviously higher number of meningioma patients having potential high-level contact to animals or animal tissue and contact to animals and humans were found in the present study. Furthermore, more meningioma patients had potential high-level occupational contact to humans compared to glioma or acoustic neurinoma patients. This result could be interpreted as in the line with the finding that most meningioma patients were working in the vocational categories 'service' and 'health' (see Chapt. 4.4), thus eventually supporting the theory of immunologic factors being related to meningioma etiology.

Furthermore, the present study evaluated self-reported occupational and private contact to animals, extending the analyses according to Menegoz et al. [2002] that were focusing only on occupational contact.

Contact to animals was frequently reported. Over 70% in each group reported private exposure to animals. According to the Industrieverband Heimtierbedarf e. V. (IHV[‡‡]), around 22.7 million domestic animals were living in German households in 2002. Taking 38.7 million households in Germany in 2002 (Statistisches Bundesamt, Germany), this would mean that nearly 60% of all households are exposed to animals. Therefore, and because the questionnaire addressed lifetime contact, the proportion of 70% and more of the study subjects having had contact to animals is quite realistic.

Dogs and cats were the most frequently reported pets, followed by rabbits, rodents and birds. According to the IHV, the population of dogs and cats in Germany is 5 million and 7.2 million, respectively. The number of small animals is given as 5.8 million, and 4.7 million birds are living in German households. The distribution of animal species reported by the participants is in the line with these data, although the proportion of patients having small animals was less than expected. Interestingly, contact to farm animals was most prevalent in meningioma patients. As mentioned above, previous studies were inconsistent about contact to farm animals or living or working on a farm and the association to brain tumors. Infectious microorganisms as well as hazardous agents such as pesticides or insecticides were suggested to be involved in the development of brain malignancies. On the other hand, these immunological factors might be protective as well.

[‡‡] see website: http://www.ihv-online.de

Summarizing the analyses on private and occupational self-reported or suggested high-level contact to animals and/or humans or the respective tissues, there seems to be an association between meningioma and contact to mammalians. Obviously elevated numbers of meningioma patients having high-level contact to animals and humans were found in either analysis. Therefore, it could be suggested that immunological factors (infectious organisms or other antigens) might be a factor correlated with meningioma pathogenesis. However, regarding the small sample size and the inconsistent results in larger studies, this finding could also be due to chance and further research might be needed on this issue.

(B) Laboratory Analyses

4.6 HCMV and Primary Brain Tumors

Since several years, HCMV is controversially discussed to be involved in the pathogenesis of miscellaneous malignancies, including human brain tumors. HCMV is an ubiquitous β-herpesvirus, which is known to be trophic for glial cells, aside from other tissues [Fritschy et al., 1996]. This herpesvirus persistently infects 50-90% of the population [Pass, 2001]. Furthermore, HCMV is well known as the most frequent cause of congenital malformations [Landolfo et al., 2003; Trincado and Rawlinson, 2001]. Infants with congenital HCMV infection are more prone to disorders involving the perceptual organs and the nervous system [Ho, 1990]. In immunocompromised persons, it can cause severe and fatal diseases such as HCMV-encephalitis and graft rejection, and lymphomas and brain tumors occur rather frequently in these individuals.

In vitro, it has been shown that HCMV can be activated in astrocytic cells by inflammatory stimuli [Wolff et al., 1994] and that it can induce malignant transformation [Doniger et al., 1999], possibly by its ability to repress cell growth arrest and p53 mediated apoptosis response [Castillo and Kowalik, 2002; Castillo et al., 2000; Cinatl, Jr. et al., 1999; Lokensgard et al., 1999]. Furthermore, there is evidence that HCMV dysregulates other key cellular pathways such as angiogenesis, cell invasion, and host immune response [Loenen et al., 2001; Scholz et al., 2000; Salvant et al., 1998; Shen et al., 1997]. Additionally, HCMV gene products can transactivate other oncogenic viruses that are suspected to be associated with malignant gliomas (e.g. JC virus), and may synergize with these viruses to promote oncogenesis [Del Valle et al., 2000; Winklhofer et al., 2000; Becker and Wahrendorf, 1998].

Glioma cell lines have been shown to be permissive to *in vitro* HCMV infection [Cheeran et al., 2001; Lokensgard et al., 1999; Fritschy et al., 1996; Kuhn et al., 1995; Poland et al., 1990]. Cinatl et al. [1996] suggested from *in vitro* analyses that HCMV may play the role of an either direct or indirect non-obligate cofactor for neuroblastoma genesis, e.g. by blocking apoptosis, which may be an essential requirement for tumor progression. Furthermore, HCMV may modulate the malignant potential for tumor cells by stimulation of growth factors and/or inhibition of anti-oncogenes by its gene products.

In 2002, Cobbs et al. [2002] examined tissues from primary brain tumors, normal brain tissue and tissues from several other brain diseases to detect HCMV-specific nucleic acids or gene products. In immunohistochemical analyses, all of 27 gliomas examined were positive for various HCMV-specific proteins. In addition, the presence of nucleic acids was demonstrated in a subset of these gliomas. In contrast, tissues from meningiomas and non-tumorous brain diseases (such as Alzheimer's disease, stroke, encephalitis) were not infected by this herpesvirus, leading the authors to the suggestion that HCMV may be involved in glioma pathogenesis. One of the main intentions of the present study was to evaluate this hypothesis.

Prior to PCR analyses of brain tumor tissues, nested PCR for the detection of HCMV DNA was performed in blood samples from the patients to control for the possibility that positive results in brain tumor tissues were due to a contamination of the tissue with HCMV-positive blood cells. It is generally accepted that in seropositive persons, peripheral blood cells such as monocytes and macrophages are a major site of carriage of HCMV DNA [Sinclair and Sissons, 1996]. However, HCMV DNA is not consistently detectable in peripheral blood cells of healthy seropositive individuals. Some studies reported rather high detection rates using PCR, others failed to detect any HCMV DNA in seropositive subjects [Roback et al., 2003; Larsson et al., 1998; Urushibara et al., 1995; Taylor-Wiedeman et al., 1991]. In the present study, only blood clot DNA was extracted for PCR analyses, thereby increasing the density of peripheral blood cells in which HCMV persists during its latent state. However, no HCMV DNA could be detected in the participants' blood samples, though different PCR protocols addressing several gene sequences coding for different HCMV proteins were used. Therefore, possibly obtained positive results in PCR on HCMV DNA in brain tumor samples would have been unlikely to be a result of contamination.

In none of the brain tumor tissues examined in the present study, HCMV DNA was found using nested PCR amplifying sequences of two different regions of the viral genome (IE-1, gB), including protocols as described by Cobbs et al. [2002] and Mangano et al. [1992]. Furthermore, every single tumor DNA sample had been successfully checked for the presence of GAPDH, demonstrating that DNA isolation was sufficient and that no inhibitors of the PCR were present in the sample.

It is well known for HCMV detection that false negative results may occur. In a study by Gass et al. [1993], HCMV DNA sequences could only sporadically be amplified by PCR in immunohistochemically proven HCMV encephalitis. In addition, in the study of Cobbs et al.

[2002], HCMV nucleic acids were found only in 5 out of 7 brain tumor DNA samples, which where positive for several HCMV-specific proteins in immunohistochemistry. The same phenomenon had been observed by other studies [Knosel et al., 2004]. Therefore, immunohistochemistry was also performed in the present study using three different monoclonal antibodies recognizing the HCMV-specific proteins pp65, EA, and IE-1. Paralleling the PCR results, immunohistochemistry failed to demonstrate the presence of HCMV molecules in primary brain tumor tissues. As mentioned above, conflicting results in the same samples using different methods for detecting HCMV molecules are common. In the present study, however, results of PCR and immunohistochemistry were consistent, demonstrating the authenticity of all analyses performed.

It is suggestive whether geographical differences in HCMV prevalence in brain tumor tissues may have led to the conflicting results between the data of Cobbs et al. [2002] and those of the present study. However, a more recent US study by Lau et al. [2005] supports the present results. In their study, 22 gliomas had been investigated for the presence of HCMV, partly using the same protocols as Cobbs et al. [2002]. Additionally, tissues from normal brain and various other malignancies had been examined. Paralleling the results of the present study, all tumors tested demonstrated no evidence of HCMV proteins or nucleic acids. Similarly, a very recent French study could not confirm an association between HCMV and primary brain tumors [Sabatier et al., 2005b]. Therefore, considering the results of these two studies [Lau et al., 2005; Sabatier et al., 2005b] and the results obtained in the present study, the hypothesis of an association between HCMV and the development or progression of primary brain tumors cannot be supported.

4.7 Herpesvirus Infections in Brain Tumor Patients

There are five major classes of antibodies: IgG, IgM, IgA, IgD, and IgE.

IgM is the first antibody present in an immune response after the first contact with an infectious agent. IgM antibodies do not persist for a long time; therefore, IgM antibodies in the serum indicate a primary infection.

The production of IgM antibodies is followed by the raise of IgG antibodies. IgG antibodies are the backup antibodies and respond to repeated exposures and/or infections. In early phases of an infection, it is not unusual to have IgM and IgG antibodies at the same time. IgG antibodies can last up to a very long time greater than one year.

For herpesviruses, there is agreement that estimates of antibody prevalences presented as single summary measures (e.g. mean or median) can be misleading when the age of the population sample is not considered [Smith and Robinson, 2002]. Therefore, despite the small study size, prevalences in the present study were stratified by age (20-years age groups). In addition, overall prevalences were presented despite of the aforementioned publication, because most of the reference values in the scientific literature are given as overall prevalence. Where published data presented differing age groups, additional analyses were performed in the present study for adequate comparison of herpesvirus seroprevalences.

4.7.1 Infection with HCMV, HSV, EBV, or VZV Present at Surgery

The seroprevalences of IgM antibodies to HCMV, HSV, EBV and VZV were evaluated in all study participants providing a blood sample. None of the patients was seropositive for IgM antibodies to any of the viruses investigated, indicating that none of them had a primary infection with these herpesviruses at the time of surgery.

4.7.2 Previous Infection with HCMV, HSV, EBV, or VZV

Seroprevalences of IgG antibodies to HCMV, HSV, EBV, and VZV were assessed to control for previous infections with these herpesviruses in brain tumor patients. The prevalences of IgG antibodies to HCMV, HSV, EBV, and VZV had been investigated in several previous studies on brain tumors.

After the study group of Wrensch et al. found that glioma cases were significantly less likely to report a history of chickenpox and shingles [Wrensch et al., 1997a], they conducted a population-based case-control study to further analyze this issue. Serological analyses were performed with serum samples obtained from a subset of the participants of the first study. These analyses showed that among people reporting a positive history of chickenpox or shingles, glioma cases were less likely to have IgG antibodies to VZV than controls [Wrensch et al., 1997b]. To additionally confirm these results, the same study group investigated the presence of IgG antibodies to HCMV, HSV, EBV and VZV in serum samples of another subgroup of participants from their first study [Wrensch et al., 2001]. With this case-control study, they could show that glioblastoma cases were significantly less likely to have IgG antibodies to VZV than controls. Similarly, though not statistically significant, glioblastoma cases were less likely to have IgG antibodies to EBV. Furthermore, the seroprevalences of IgG antibodies to HSV and HCMV were slightly increased, though not statistically significant. Prevalences for glioblastomas (WHO IV) and gliomas other than glioblastoma (WHO grade I-III) frequently showed converse trends compared to the controls, but none statistically significant (prevalences of Wrensch et al., 2001, are given in Tab. 36).

Recently, the same study group (but this time in a different study population) found no differences in the prevalence of anti-VZV antibodies; however, the levels of anti-VZV antibodies were significantly lower for glioblastoma cases than for population-based controls [Wrensch et al., 2005].

Seroprevalences in the present study were compared to published results (German data if procurable) and additionally compared to the seroprevalences assessed in the study of Wrensch et al. [2001], all of which are shown in Tab. 36.

In addition, the seroprevalences of all glioma patients as well as prevalences stratified by WHO grading are given to be further discussed.

Table 36: Prevalences and 95% confidence intervals (95%CI) of glioma patients (all gliomas, glioblastoma multiforme, and WHO grade I-III) and population-based literature/controls positive for IgG antibodies to HCMV, HSV, EBV and VZV in the present study and in the study of Wrensch et al. [2001]

SP of IgGs to	Glioma patients in the present study			SP in the Literature	Glioma patients in the study of Wrensch et al., 2001			
	All * (n=34)	GBM IV * (n=23)	WHO I-III (n=11)		All (n=134)	GBM IV (n=57)	WHO I-III (n=77)	Controls (n=165)
HCMV	59% (41-75%)	57% (35-77%)	64% (31-89%)	40-80% (Germany)	57%	66%	51%	57%
HSV	91% (76-98%)	91% (72-99%)	91% (59-100%)	64-85% (Germany)	67%	82%	57%	73%
EBV	85% (69-95%)	83% (61-95%)	91% (59-100%)	>90% (World)	88%	86%	90%	92%
VZV	91% (76-98%)	91% (72-99%)	91% (59-100%)	>86% (Germany)	88%	82%	92%	92%

* no blood samples available for 5 glioma patients (one patient with glioblastoma multiforme);
**References: [Rabenau et al., 2002; Wutzler et al., 2001; Hellenbrand et al., 2001; Wutzler et al., 2000; Cohen, 2000; RKI, 2000a; RKI, 2000c; Krech, 1973]
HCMV, human cytomegalovirus; HSV, herpes simplex virus; EBV, Epstein-Barr virus; VZV, varicella-zoster virus; SP, seroprevalence; IgG, immunoglobulin G; GBM, glioblastoma multiforme; WHO, World Health Organization

4.7.2.1 Previous HCMV Infection

In two studies of Wrensch et al. [2005; 2001], the occurrence of anti-HCMV IgGs was reported to be higher in glioblastoma patients than in population controls, but decreased titers compared to population controls were found in glioma other than glioblastoma (Tab. 36). However, none of the results was statistically significant.

The overall seroprevalence for IgG antibodies to HCMV in the present study was 63% (95%CI 51-75%). Stratified by age and by tumor type, seroprevalences increased with increasing age, with a conspicious trend as expected from the literature. The Robert-Koch Institute (RKI), Germany, reported a prevalence of HCMV IgGs between 40% and 80% in the German population [RKI, 2000a]. Infection with HCMV can already be acquired during birth or following breast-feeding. During the first 6 months of life, 8% to 60% of infants become infected by HCMV. Afterwards, infection rates increase steadily in most developed countries. 40% to 80% of children are infected before puberty [Pass, 2001].

In the Freiburg area (Southern Germany), a prevalence rate of 42% has been estimated. In this study, male and female healthy blood donors between 20-39 years of age were included

[Krech, 1973]. Analyses stratified by age in the present study similar to the study of Krech [1973] evaluated a similar proportion. The distribution of HCMV in children and adults in the Munich area was investigated by Peller and Goetz [1978]. In this survey, 48% of subjects aged 17-40 years and 57% of persons aged 40-65 years were positive for HCMV antibodies.

Hence, in contrast to the survey of Wrensch et al. [2001], the overall seroprevalence of HCMV in the present study was in the line with published data. Furthermore, no trend similar to the studies of Wrensch et al. [2005; 2001] could be observed. Therefore, it seems unlikely from the results of the present study that previous HCMV infection contributes to brain tumor development.

4.7.2.2 Previous HSV Infection

The study group of Wrensch et al., who investigated the prevalence of anti-herpesvirus antibodies in glioma patients [2005; 2001], found that gliomas other than glioblastoma cases were less likely to have antibodies to HSV than population-based controls. On the other hand, glioblastoma cases were more likely to have IgG antibodies to HSV.

In the present study, an overall seroprevalence of 86% (95%CI 76-93%) of IgG antibodies to HSV was found. A slight age-dependent increase in prevalence was seen, though not as clearly as expected from the literature [Whitley, 1990]. According to Wutzler et al. [2000], the proportions of HSV IgG-seropositive individuals were 72-83% among blood donors and hospital patients between 20 and 39 years of age. Prevalence increased afterwards up to 92% in persons above the age of 70 years. Overall, in their survey, 73% (95%CI 71-74%) of the subjects were seropositive for HSV IgGs. Furthermore, a cross-sectional survey assessed a proportion of HSV susceptible persons of 30-35% in 20-29 year old persons and a proportion of 9-14% for subjects above the age of 30 years [Pebody et al., 2004]. The seroprevalences of IgG antibodies to HSV in the Frankfurt am Main area, Germany, reported a lower prevalence in hospital attendees aged 15-39 years (62% in male, 70% in female) compared to organ transplant recipients (85% for both sexes). 82-85% of persons above 40 years of age were HSV-seropositive [Rabenau et al., 2002]. In another population-based survey, the prevalence of antibodies to HSV in Western Germany was 85% (95%CI 83-87%; [Hellenbrand et al., 2001]). Age stratification in the present study resulted in a prevalence of 78.6% (95%CI 49%-

95%) for participants under the age of 40 years and a proportion of 87.9% (95%CI 77%-95%) in older subjects, being similar to published data.

In analyses stratified by tumor type, though, an increase in the HSV seroprevalence in glioma patients could be observed, which was still prominent after stratification by age. Analyses separately for gliomas WHO grade I-III and glioblastoma multiforme (WHO grade IV) resulted in equally higher prevalences in either subgroup compared to population-based data. This is conflictive to the results of Wrensch et al. [2001], who found that gliomas other than glioblastoma cases were less likely to have antibodies to HSV than population-based controls. On the other hand, glioblastoma cases were more likely to have IgG antibodies to HSV in this [2001] as well as in the more recent study of Wrensch et al. [2005], supporting the finding of the present study.

Similarly, Hadfield et al. [1984] found that glioblastoma cases had higher serum titers to HSV than controls. Furthermore, HSV is known as causative agent for severe encephalitis [Whitley, 1990], and this virus has been controversially discussed throughout decades to be involved in tumorigenesis [Wu et al., 2005; zur Hausen, 1975]. On the other hand, there is increasing evidence that an involvement of HSV in cancer development is unlikely [Lopez et al., 2003; Chang et al., 2000].

However, considering the studies of Wrensch et al. [2005; 2001] and the results of the present study, further research may be needed to clarify the possible role of HSV in glioma (especially in glioblastoma) development.

4.7.2.3 Previous EBV Infection

EBV is involved in the pathogenesis of several malignancies, e.g. Burkitt's lymphoma, Hodgkin's disease, Kaposi's lymphoma and nasopharyngeal cancer [zur Hausen, 1999; Gaffey and Weiss, 1992]. Furthermore, it is known that EBV can infect astrocytes *in vitro* [Menet et al., 1999], and glioma cases were reported to be less likely to have IgG antibodies to EBV (Wrensch et al., 2001; Tab. 36).

In the present study, a seroprevalence of anti-EBV IgGs of 89% (95%CI 79%-95%) was found in the study subjects. According to Cohen [2000] and Berger [2003], IgG antibodies to EBV have an overall seroprevalence of more than 90% in the adult population.

In age-stratified analyses, antibody titers were slightly decreasing with increasing age (100% in the youngest to 81% in the oldest age group), which was not expected from published studies, in which the seroprevalences in the population rose with increasing age. The observed decrease was consistent after stratification by tumor type and age.

In both studies of Wrensch et al. [2005; 2001], all gliomas combined and glioblastoma cases alone were reported to be less likely to have IgG antibodies to EBV. This was also observed in the present study. Glioblastoma patients were less likely to have anti-EBV antibodies, whereas low-grade gliomas were similar to those reported in the literature. Therefore, further research may be needed to evaluate this suggestive inverse association between previous EBV infection and glioma.

4.7.2.4 Previous VZV Infection

Prior infection with VZV has been suggested to have an influence on the risk of adult glioma since it was reported that glioma patients were significantly less likely to have had chickenpox and shingles prior to tumor diagnosis than population controls [Wrensch et al., 1997a]. In addition, among a subsample selected for serological analyses, those reporting a positive history, glioma cases were less likely to test positive for IgG antibodies to VZV. However, no association was found for those reporting a negative history and for all glioma cases combined [Wrensch et al., 1997b]. In 2001, these findings had been evaluated again in another subset of blood specimens of the 1997 study [Wrensch et al., 1997a]. In this case-control study, addressing seroprevalences of four herpesviruses (HCMV, HSV, EBV, and VZV), glioblastoma cases were significantly less likely to have IgG antibodies to VZV than population-based controls [Wrensch et al., 2001], suggesting an association of anti-VZV IgGs and glioblastoma. Recently, the previous finding concerning self-reported history of chickenpox was corroborated by the same study group in another study population [Wrensch et al., 2005]. In addition, although prevalences of anti-VZV IgGs did not differ between

glioma cases and population-based controls, the levels of antibodies to VZV were lower in glioma (especially glioblastoma) cases.

In the present study, the brain tumor patients showed an overall seroprevalence of IgG antibodies to VZV of 92% (95%CI 83-97%). In tumor type stratified analyses, a decrease of seroprevalences in the present study compared to published data was observed in the youngest meningioma patients. Wutzler et al. [2001] investigated the seroprevalence of IgG antibodies to VZV in the German population. They found that already at the age of 11 years, over 90% of the study subjects were positive for VZV IgGs. Other German data confirmed these findings [RKI, 2000c].

4.7.2.5 Summary of Previous Infections with HCMV, HSV, EBV, and VZV

In summary, the results of the present study showed no considerable differences in the seroprevalence of brain tumor patients compared to the general population. In contrast to the studies mentioned above, an inverse association between VZV seropositivity and brain tumors, especially gliomas, could not be observed. Therefore, the hypothesis of an influence of VZV infection on brain tumor development cannot be confirmed in the present study.

(C) Strengths and Limitations of the Study

4.8 Limitations

Participants in any survey are likely to differ in some of their characteristics from those who do not participate. In prevalence studies, selection bias may occur if the subjects who participate in the study are not representative of the underlying population (i.e., brain tumor cases). However, a selection with regard to the laboratory analyses performed is unlikely in the present study, because the study subjects are not suspected to know their latency status concerning herpesviruses, as these do not cause apparent disease during latency. For the questionnaire evaluation, however, it is essential to try to obtain information about those who could not be interviewed for different reasons. This has been done in the present study, as well as Fisher's exact testing for heterogeneity focusing on the histological distribution of brain tumors in both groups. No significant difference between the two groups was found. Furthermore, in comparison with published data for brain tumor patients, age and gender were equally distributed. Hence, it seems very unlikely that obtained results are distorted by selection bias.

A commonly raised criticism in studies using retrospective questionnaires is the potential recall bias. Whereas laboratory methods are obviously not affected by that kind of bias, subjects with a serious disease are likely to have been thinking hard about possible causes of their condition, and so participants may be inclined to give answers that fit with what they believe is the cause of the disease. However, to the author's knowledge, herpesviruses as potential risk factors for brain tumor development have not yet been published in daily newspapers, so that it seems unlikely that patients thought about herpesviruses as potential brain cancer-causing agents. Therefore, it is unlikely that the occurrence of certain medical conditions was overreported. Furthermore, if the study participants may have been convinced that former medical conditions led to the present brain tumor, this may have led to an increased prevalence. However, the distribution of most variables addressed was similar to that of the general population. Furthermore, the study participants were kept unaware of the hypothesis under the study. Hence, the potential recall bias is likely to be marginal.

The question on tinnitus history was included to control for a possible prenatal HCMV infection in the study subjects. Unfortunately, this question has been included in the study later on during the study period, resulting in an increased amount of missing data for that variable. Therefore, the prevalence of tinnitus in brain tumor patients is most likely to be underestimated. However, various questions as well as the serological analyses were included in the present study to assess the prevalence of HCMV infection in the study participants. Hence, the lack of these data is of less importance for the overall study intention, though of course, the distribution of tinnitus in the study subjects has to be interpreted with great care.

The major limitation of the present study with regard to the evaluation of questionnaire data is the small number of patients that could be interviewed. It was, however, not possible to get more brain tumor patients interviewed due to organizational issues and the part-time anonymous collection of the brain tumor tissue samples. Therefore, the prevalences obtained from interviews can only act as an estimate of the true value in the brain tumor population. Due to the resulting small sample size, the estimated proportions were characterized by large 95%CI. Therefore, it cannot be excluded that the findings of the questionnaire were due to chance.

4.9 Strengths

The present study was a well-designed and thoroughly conducted epidemiological study, combining laboratory as well as questionnaire data analyses. Only incident brain tumors were included and data collection was performed blinded for tumor type in each analysis.

The reliability of the laboratory methods was assured by using PCR and immunohistochemical analyses, and various protocols in both. An advantage of the present study in the PCR analyses was the use of fresh-frozen tissues (stored at -80°C, because HCMV is known to be relatively unstable at temperatures exceeding -70°C; Mocarski, Jr., 1996), which were not formalin-fixed. Formalin fixation has been suggested to decrease PCR specificity in some studies [Wilkens et al., 1994; Rogers et al., 1990], whereas others did not support this thesis [Boeckh et al., 1994]. However, even Boeckh et al. [1994], who found that storage at room temperature for a certain time and formalin fixation did not influence detection results, preferred a direct procession of materials.

Furthermore, the possibility of false positive results due to a contamination of the tissues by HCMV-positive blood cells was excluded by PCR on patients' blood samples. For PCR detection of HCMV in peripheral blood, the European Group for Blood and Marrow Transplantation (EBMT) Infectious Disease Working Party recommends that

1. peripheral blood is collected into EDTA or citrate,
2. a standard number of leucocytes is processed,
3. DNA from a standard amount of DNA is added per PCR,
4. cellular gene control of amplificability of the sample is included,
5. a specificity control (nesting or hybridization) is included, and
6. primers in a conserved part of the HCMV genome are chosen [Grundy et al., 1996].

In the present study, analyses followed most of these recommendations, except that the amount of leucocytes processed and the amount of DNA used was not evaluated. However, to eliminate the possibility of false negative results, all brain tumor DNA samples were additionally checked for the presence of the cellular GAPDH gene, with positive result. Furthermore, blood clot was taken for DNA extraction and subsequent PCR analyses, providing a high density of peripheral blood cells.

This study was performed notably to evaluate the findings of Cobbs et al. [2002], suggesting a role of HCMV in glioma pathogenesis, which included only 27 gliomas. More recent studies by Lau et al. [2005] and Sabatier et al. [2005b], which focused on the same hypothesis, were limited either by a small sample size (22 gliomas in Lau et al., 2005) or by the fact that only few analyses were performed without any quality controls (only one protocol for immunohistochemistry and in situ hybridization in Sabatier et al., 2005b).

Therefore, the major advantages of the present study in contrast to the studies of Cobbs et al. [2002], Lau et al. [2005], and Sabatier et al. [2005b] are

a) the amount of brain tumors included, and
b) the number of analyses performed using different protocols,

and therefore, the hypothesis of an association of HCMV and gliomas can definitely not be supported in the present study.

5 Conclusion

Analyses performed to confirm the hypothesis of Cobbs et al. [2002] clearly demonstrated the absence of HCMV molecules in all primary brain tumors analyzed. This finding is confirmed by two very recent studies, which also did not find HCMV gene sequences and proteins in brain tumor tissues [Lau et al., 2005; Sabatier et al., 2005b]. Therefore, the hypothesis of an association between HCMV and glioma pathogenesis cannot be supported.

Furthermore, the present study could not find an association between previous infections with VZV or HCMV and either different histological type of glioma. Prevalences of IgG antibodies to HCMV and VZV, respectively, were similar to published German data. For glioblastoma patients, a decreased prevalence of anti-EBV antibodies and an increased prevalence of anti-HSV IgGs compared to published data was found, paralleling the trends observed in two previous studies of Wrensch et al. [2005; 2001]. However, because of the small sample size regarding this issue, and the consistency of previous results, the association of previous herpesvirus infections and brain tumors deserves further research.

6 Summary

Herpesviruses are suggested to be involved in cancer pathogenesis since several years. A recent study suggested HCMV to be involved in glioma development or progression, and other studies found an inverse correlation between previous VZV infection and glioblastoma pathogenesis. The present study was conducted to evaluate these assumptions.

Brain tumor tissues from 76 gliomas, meningiomas, and acoustic neurinomas were obtained, together with 71 corresponding blood samples. Nested PCR and immunohistochemistry were performed using several protocols to assess the prevalence of HCMV DNA and proteins in brain tumor tissues, blood samples, and short-term cultures of brain tumor tissues. Additionally, the serological status of 71 brain tumor patients concerning the prevalence of IgM and IgG antibodies to HCMV, HSV, EBV, and VZV was analyzed. Furthermore, a questionnaire was developed to control for putative medical risk factors indicating herpesvirus infection or reactivation, and to control for other putative (mainly medical) risk factors in brain tumor development.

None of the 76 brain tumor tissues was positive for HCMV molecules in any analysis performed. The conflicting results may be due to geographical differences in the prevalence of HCMV; however, very recent studies conducted in the US and in France confirmed the absence of any HCMV molecules in primary brain tumors.

In general, the overall seroprevalences of IgG antibodies to HCMV, HSV, EBV and VZV in brain tumor patients were similar to those of the German population. However, the prevalences of anti-HSV and anti-EBV IgGs in glioma patients showed trends that resemble previous findings.

Overall, the questionnaire did not provide evidence for viral infections being involved in brain tumor development. For some factors such as the occurrence of allergies, hearing impairments, and especially the frequent high-level contact to animals in meningioma patients (private and occupational), the results were indicative but not conclusive for an association with brain tumor pathogenesis, and further research may be needed to clarify these issues.

In summary, considering the results from laboratory analyses and the results of the questionnaire, the hypothesis of an association between herpesviruses and the development or progression of brain tumors cannot be supported.

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Schedular Annexes

Patients' Education

Studie „Umwelt und Gesundheit 2003“

Liebe Patientin, lieber Patient

Die Arbeitsgruppe Umwelt- Epidemiologie des Deutschen Krebsforschungszentrums (DKFZ) in Heidelberg (Leiter: Prof. Dr. Jürgen Wahrendorf) führt mit der Neurochirurgischen Klinik der Universität Heidelberg zurzeit eine Studie zu „Umwelt und Gesundheit 2003“ durch. An dieser Studie beteiligen sich insgesamt 14 Länder unter Leitung der Weltgesundheitsorganisation (**WHO**). Sie ist von der Ethikkommission genehmigt.

Was ist das Ziel der Studie?

Bisher ist wenig über Ursachen von Tumoren des Kopfbereiches bekannt. Mit dieser Studie sollen Faktoren gefunden werden, die zur Entstehung von Hirntumoren beitragen. Dazu untersuchen wir den Einfluss verschiedener Aspekte der Umwelt, von Lebensgewohnheiten, der Arbeitswelt und einiger Vorerkrankungen (z.B. Virusinfektionen). Damit wollen wir einen Beitrag dazu leisten, dass in Zukunft bessere Schutzmaßnahmen zur Verhinderung dieser Erkrankung getroffen werden können.

Warum ich?

Wir nehmen in diese Studie alle Patienten der Neurochirurgischen Universitätsklinik Heidelberg auf, die an einem Hirntumor erkrankt sind. **Die Aussagekraft der Studie hängt von der Teilnahme möglichst aller angesprochenen Patienten ab.**

Wie soll die Mitarbeit aussehen?

Wir möchten mit Ihnen eine kurze telefonische Befragung durchführen. Dafür können Sie auf der beiliegenden Einverständniserklärung einen Wunschtermin angeben, an dem unsere Mitarbeiterin mit Ihnen Kontakt aufnehmen kann, um einen genauen Termin für ein kurzes telefonisches Interview zu vereinbaren.

Im Fall einer operativen Entfernung des Tumorgewebes wird dieses routinemäßig feingeweblich untersucht. Ein Teil dieses Gewebes soll auch auf frühere Infektionen mit Herpesviren getestet werden, ebenso Serum von den Blutproben, die bereits zur Diagnostik entnommen wurden. ***Es werden daher keine zusätzliche Blut- oder Gewebeentnahme vorgenommen; das Ausmaß der Operation ändert sich nicht. Es wird keine AIDS-Diagnostik durchgeführt.***

Ihre Sicherheit!

Wir versichern Ihnen, dass die Einhaltung der Vorschriften über die ärztliche Schweigepflicht und des Datenschutzes im Rahmen dieser Studie gewährleistet ist. Ihre Angaben werden nur anonymisiert, das heißt nur mit Studiennummer und ohne Namensnennung, weiter verwendet und zu Statistiken zusammengefasst.

Die Teilnahme an dieser Studie ist freiwillig. Sie können Ihr Einverständnis jederzeit ohne Angabe von Gründen und ohne Nachteile für Ihre weitere medizinische Versorgung zurückziehen. In diesem Fall werden Ihre persönlichen Daten gelöscht.

Wir bitten Sie herzlich um Ihre Teilnahme!

Für Fragen stehen Ihnen zur Verfügung: die Studienleitung: Dr. med. B. Schlehofer und S. Poltermann, DKFZ Heidelberg (Tel.: 06221/42 2346), bzw. Dr. med. K. Geletneky (Tel.: 06221/56 39672), Neurochirurgische Klinik Uni Heidelberg.

Informed Consent

Studiennummer B |__|__|__|

Studie „Umwelt und Gesundheit 2003“

Einverständniserklärung
Vorname Name: «Vorname» «Nachname»
Anschrift: «Strasse»
«PLZ» «Ort»
Tel. Nr.: _____ / _____ Handy Nr._____

Die schriftliche Patienteninformation habe ich erhalten und gelesen. Darüber hinaus bin ich auf meinen Wunsch hin mündlich aufgeklärt worden. Dabei wurden meine Fragen beantwortet.

Ich möchte mich an der Studie „Umwelt und Gesundheit 2003“ beteiligen. Dies schließt auch Untersuchungen zur Rolle von Herpesvirus- Infektionen bei der Entstehung von Hirntumoren ein.

☐ **ja** Wunsch- Termin _____

Wegen der Terminabsprache für ein Interview bin ich telefonisch zu erreichen am:
Datum _____ Uhrzeit _____
Datum _____ Uhrzeit _____

Datum _____ **Unterschrift**
_____

Ich stimme der Teilnahme freiwillig zu. Ich weiß, dass ich diese Zustimmung ohne Angabe von Gründen jederzeit und ohne Nachteile für meine weitere medizinische Versorgung widerrufen kann.
Ich wurde darüber aufgeklärt, dass die *im Rahmen dieser Studie erhobenen Daten nur in anonymisierter Form dokumentiert und in Form von Statistiken zusammengefasst werden, die keine Rückschlüsse auf Einzelpersonen zulassen.*

☐ **nein**

Falls Sie nicht teilnehmen möchten, bitten wir Sie um eine Begründung:
_____

Questionnaire

DEUTSCHES KREBSFORSCHUNGSZENTRUM

Stiftung des öffentlichen Rechts
in der Helmholtz-Gemeinschaft

Umwelt und Gesundheit 2003
„Die Rolle von Herpesviren bei der Entstehung von Hirntumoren“
Fragebogen

In Zusammenarbeit mit der
Neurochirurgischen Universitätsklinik Heidelberg

A. Allgemeine Informationen I

Die folgenden Fragen müssen vor dem Interview ausgefüllt werden.
Die Angaben müssen mit dem Studienteilnehmer nochmals überprüft werden.

Studiennummer : **B** |__|__|__|
Tag des Interviews: |__|__| |__|__| |__|__|__|__|
Tag Monat Jahr
Uhrzeit: |__|__| : |__|__|
Name des Interviewers: _____
Code des Interviewers: |__|__|__|
OP- Datum |__|__| |__|__| |__|__|__|__|
Tag Monat Jahr

Beginn des Interviews

Ich möchte mit Ihnen Ihre persönlichen Angaben durchgehen, um diese zu ergänzen und mit Ihnen gemeinsam zu überprüfen.

Bitte nennen Sie mir Ihren Familiennamen und Ihren Vornamen:
Familienname: _____
Vorname: _____
Bitte nennen Sie mir Ihr Geburtsdatum: |__|__| |__|__| |__|__|__|__|
Tag Monat Jahr

Nicht fragen, ist vom Interviewer anzukreuzen.
Geschlecht: männlich weiblich

2

Wie lautet die Adresse Ihres jetzigen Wohnortes?
Strasse _____ Haus-Nummer |__|__|__|
Postleitzahl |__|__|__|__|__|
Ort _____ Bundesland: _____

Wie lautet die Adresse Ihres Hauptwohnsitzes?
Ist dieser mit obiger Adresse identisch? Keine Eintragung vornehmen, wenn identisch mit obiger Adresse
Strasse _____ Haus-Nummer |__|__|__|
Postleitzahl |__|__|__|__|__|
Ort _____Bundesland: _____

Wie lauten Ihre Telefonnummern?
Privat: |__|__|__|__|__| / |__|__|__|__|__|__|__|__|__|__|
Vorwahl Rufnummer
Arbeitsplatz: |__|__|__|__|__| / |__|__|__|__|__|__|__|__|__|__|
Vorwahl Rufnummer
Handy: |__|__|__|__|__| / |__|__|__|__|__|__|__|__|__|__|
Vorwahl Rufnummer
Handy: |__|__|__|__|__| / |__|__|__|__|__|__|__|__|__|__|
Vorwahl Rufnummer

Interviewpartner (Nicht fragen, ist vom Interviewer auszufüllen):
Studienteilnehmer/in selbst
oder Proxy (stellvertretend für Teilnehmer)
Ehepartner/In / Lebensgefährte/In
Andere,
bitte geben Sie das Verwandtschaftsverhältnis an: _____

3

Grund, warum nicht der Teilnehmer selbst befragt wird:
verstorben
zu krank, um zu antworten
andere Gründe: _____

Folgende Fragen bitte an den Proxy- Teilnehmer richten:

Bitte nennen Sie mir Ihren vollständigen Namen und Ihre Adresse.
Familienname: _____
Vorname: _____
Adresse nicht ausfüllen, falls identisch mit Adresse des Teilnehmers.
Strasse _____ Haus-Nummer |__|__|__|
Postleitzahl |__|__|__|__|__|
Ort _____ Bundesland: _____

Können Sie mir bitte Telefonnummern nennen, unter denen Sie erreichbar sind:
Privat: |__|__|__|__|__| / |__|__|__|__|__|__|__|__|__|__|
Vorwahl Rufnummer
Arbeitsplatz: |__|__|__|__|__| / |__|__|__|__|__|__|__|__|__|__|
Vorwahl Rufnummer
Handy : |__|__|__|__|__| / |__|__|__|__|__|__|__|__|__|__|
Vorwahl Rufnummer

4

B. Medizinische Vorgeschichte des Studienteilnehmers

Ich möchte Ihnen nun einige Fragen zu Ihrer medizinischen Vorgeschichte stellen! Zu einigen Punkten frage ich Sie auch, wie alt Sie beim Auftreten verschiedener Erkrankungen waren. Hier können Sie mir alternativ auch das Jahrnennen, in welchem die Erkrankung auftrat, wenn Ihnen dies leichter fällt.

1. Ich werde Ihnen nun eine Reihe von Infektionskrankheiten vorlesen.
Wurde eine davon jemals von einem Arzt festgestellt?:

Windpocken?
Nein
Ja **Wie alt waren Sie, als dies zum ersten Mal auftrat?** |__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl
Weiß nicht

Gürtelrose?
Nein
Ja **Wie alt waren Sie, als dies zum ersten Mal auftrat?** |__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl
Weiß nicht

Pfeiffersches Drüsenfieber?
Nein
Ja **Wie alt waren Sie, als dies zum ersten Mal auftrat?** |__|__| Jahre
Sie können mir auch das Jahr nennen
|__|__|__|__| Jahreszahl
Weiß nicht

5

Dreitage-Fieber (Exanthema subitum)?
Nein
Ja **Wie alt waren Sie, als dies zum ersten Mal auftrat?** |__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl
Weiß nicht

Herpes simplex (Bläschen an den Lippen oder an anderen Körperstellen)?
Nein
Ja **Wie alt waren Sie, als dies zum ersten Mal auftrat?** |__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl
Wie häufig treten diese Bläschen auf?
<1x jährlich
1-2x jährlich
>2x jährlich
Weiß nicht

Halsschmerzen, Grippe, Infekte, nicht- eitrige Angina?
Nein

Ja **wenn ja, wie häufig**
<1x jährlich
1-2x jährlich
>2x jährlich
Weiß nicht

6

2. Wurde bei Ihnen jemals ein Zytomegalie-Virus (CMV)-Titer bestimmt?
Nein
Ja **wann?** |__|__| Monat |__|__|__|__| Jahr;
Sie können mir auch das Alter nennen, in dem der Titer bestimmt wurde. |__|__| Jahre

Wie lautete das Ergebnis?
positiv
negativ
weiß nicht
Weiß nicht

3. Leiden Sie an einer der folgenden allergischen Erkrankungen?

Asthma?
Nein
Ja **Wie alt waren Sie, als dies zum ersten Mal auftrat?** |__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl

Heuschnupfen ?
Nein
Ja **Wie alt waren Sie, als dies zum ersten Mal auftrat?** |__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl

7

Ekzeme?
Nein
Ja **Wie alt waren Sie, als dies zum ersten Mal auftrat?** |__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl

Hörten die Ekzeme auf?
Nein
Ja **Wie alt waren Sie, als die Ekzeme aufhörten?**
|__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl

In der Zeit, als Sie Ekzeme hatten, wie lange waren Sie davon betroffen?
den größten Teil des Jahres (mehr als 8 Monate)
etwa die Hälfte des Jahres (4 - 8 Monate)
weniger als die Hälfte des Jahres (weniger als 4 Monate)
gelegentlich/ vereinzelt

Trat das Ekzem nur auf, wenn Sie in Kontakt mit speziellen Stoffen kamen?
Nein
Ja

Wodurch wurde Ihr Ekzem speziell ausgelöst?
Pflanzen
Cremes, Salben oder Kosmetika
Metalle
Chemikalien
andere Stoffe, welche:_____
_____

8

4. Leiden Sie an weiteren Allergien?
Nein
Ja
Wenn ja, an welchen?
1. _____
Seit wann? |__|__| Monat |__|__|__|__|Jahr
Sie können mir auch das Alter nennen, in dem die Allergie zuerst auftrat. |__|__|Jahre
2. _____
Seit wann? |__|__| Monat |__|__|__|__|Jahr
Sie können mir auch das Alter nennen, in dem die Allergie zuerst auftrat. |__|__|Jahre

5. Ich werde Ihnen nun weitere Krankheiten vorlesen. Wurde eine davon jemals von einem Arzt bei Ihnen festgestellt?
Epilepsie?
☐ Nein
☐ Ja **Wie alt waren Sie, als dies zum ersten Mal auftrat?** |__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl
Tuberöse Sklerose?
☐ Nein
☐ Ja **In welchem Alter wurde sie zuerst diagnostiziert?** |__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl

Neurofibromatose?
☐ Nein
☐ Ja **In welchem Alter wurde sie zuerst diagnostiziert?**
|__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl

6. Haben Sie Probleme mit dem Hören?
☐ Nein
☐ Ja
Wie wurden Sie darauf aufmerksam?
durch sich selbst durch Ihre Familie/ Freunde
durch einen Arzt durch andere Personen
Wie alt waren Sie, als Sie darauf aufmerksam wurden?
|__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl
Welches Ohr, bzw. welche Ohren waren davon betroffen?
Linkes Ohr Rechtes Ohr Beide Ohren

7. Leiden Sie unter dauerhaften Ohrgeräuschen?
(z. B. Tinnitus)
Nein
Ja
Wie alt waren Sie, als Sie darauf aufmerksam wurden?
|__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl
Welches Ohr, bzw. welche Ohren waren davon betroffen?
Linkes Ohr Rechtes Ohr Beide Ohren

8. Hat Ihnen ein Arzt jemals gesagt, dass Sie einen Tumor haben? Darunter verstehen wir auch Krebs, Leukämie und Lymphome.
☐ Nein
☐ Ja **In welchem Alter wurde er diagnostiziert?**
|__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl
Was für ein Tumor war dies? _____

Hatten Sie jemals noch einen anderen Tumor?
☐ Nein
☐ Ja **In welchem Alter wurde er zuerst diagnostiziert?**
|__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl
Was für ein Tumor war dies? _____

Hatten Sie jemals noch einen anderen Tumor?
☐ Nein
☐ Ja **In welchem Alter wurde er zuerst diagnostiziert?**
|__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl
Was für ein Tumor war dies? _____

9. Ist bei Ihnen eine Abwehrschwäche/ Immunschwäche bekannt, die zum Beispiel durch eine Infektion, eine Chemotherapie, durch Medikamente oder durch eine Organtransplantation verursacht wurde?
☐ Nein
☐ Ja
Wenn ja, wurde Sie hervorgerufen durch:
eine Infektion? nein ja
|__|__| Monat |__|__|__|__| Jahr
welche? _____
eine Chemotherapie/ Medikamente? nein ja
|__|__| Monat |__|__|__|__| Jahr
welche? (z.B. Kortison) _____
eine Organtransplantation? nein ja
|__|__| Monat |__|__|__|__| Jahr
welches Organ? _____

10. Haben Sie jemals eine Bluttransfusion erhalten?
Nein
Ja
Wenn ja, warum? _____
In welchem Alter haben Sie die Infusion erhalten?
|__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl
Wenn ja, warum? _____
In welchem Alter haben Sie die Infusion erhalten?
|__|__| Jahre
Sie können mir auch das Jahr nennen.
|__|__|__|__| Jahreszahl

Und nun noch eine kurze Frage zu Ihren Impfungen.

11. Können Sie sagen, gegen welche Krankheiten Sie geimpft sind?

Diphterie	Ja	Nein	Weiß nicht
Tetanus	Ja	Nein	Weiß nicht
Pertussis (Keuchhusten)	Ja	Nein	Weiß nicht
Kinderlähmung	Ja	Nein	Weiß nicht
Tuberkulose	Ja	Nein	Weiß nicht
Röteln	Ja	Nein	Weiß nicht
Mumps	Ja	Nein	Weiß nicht
Masern	Ja	Nein	Weiß nicht
MMR – Kombi	Ja	Nein	Weiß nicht
Hepatitis A	Ja	Nein	Weiß nicht
Hepatitis B	Ja	Nein	Weiß nicht
Tollwut	Ja	Nein	Weiß nicht
Frühsommer Meningitis (FSME)	Ja	Nein	Weiß nicht
Influenza	Ja	Nein	Weiß nicht

13

C. Familienanamnese

Ich möchte Sie nun nach einigen Erkrankungen fragen, von denen eventuell Ihre Familienangehörigen betroffen sind oder waren.

Bitte sagen Sie mir zuerst, ob Sie Kinder haben. Nennen Sie mir bitte nur Ihre leiblichen Kinder, keine Stief- oder Adoptivkinder.
Verstorbene Kinder sind ebenfalls mit zu zählen.
Nein
Ja
Wie viele Söhne? |__|__| Anzahl
In welchem Jahr wurden sie geboren? **1.** |__|__|__|__| Jahr
2. |__|__|__|__| Jahr
3. |__|__|__|__| Jahr
4. |__|__|__|__| Jahr
5. |__|__|__|__| Jahr
Wie viele Töchter? |__|__| Anzahl
In welchem Jahr wurden sie geboren? **1.** |__|__|__|__| Jahr
2. |__|__|__|__| Jahr
3. |__|__|__|__| Jahr
4. |__|__|__|__| Jahr
5. |__|__|__|__| Jahr
(Falls keine, bitte 0 eingeben)

14

2. Hatte eines Ihrer Kinder im ersten Lebensjahr eine der folgenden Krankheiten?

Gelbsucht?
☐ Nein
☐ Ja **Bei welchem Kind wurde sie diagnostiziert? Bitte geben Sie mir das Geburtsjahr des betroffenen Kindes an.**
|__|__|__|__| Jahr
|__|__|__|__| Jahr
☐ Weiß nicht

Blutarmut (Anämie)?
☐ Nein
☐ Ja **Bei welchem Kind wurde sie diagnostiziert? Bitte geben Sie mir das Geburtsjahr des betroffenen Kindes an.**
|__|__|__|__| Jahr
|__|__|__|__| Jahr
☐ Weiß nicht

Lungenentzündung?
☐ Nein
☐ Ja **Bei welchem Kind wurde sie diagnostiziert? Bitte geben Sie mir das Geburtsjahr des betroffenen Kindes an.**
|__|__|__|__| Jahr
|__|__|__|__| Jahr
☐ Weiß nicht

15

Chronische Magen- Darmentzündung?
☐ Nein
☐ Ja **Bei welchem Kind wurde sie diagnostiziert? Bitte geben Sie mir das Geburtsjahr des betroffenen Kindes an.**
|__|__|__|__| Jahr
|__|__|__|__| Jahr
☐ Weiß nicht

Hat eines Ihrer Kinder angeborene Krankheiten oder Fehlbildungen?
☐ Nein
☐ Ja

Wenn ja, welche Krankheit? _____
Bitte geben Sie mir das Geburtsjahr des betroffenen Kindes an. |__|__|__|__| Jahr

Wenn ja, welche Krankheit? _____
Bitte geben Sie mir das Geburtsjahr des betroffenen Kindes an. |__|__|__|__| Jahr

Wenn ja, welche Krankheit? _____
Bitte geben Sie mir das Geburtsjahr des betroffenen Kindes an. |__|__|__|__| Jahr

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D. Allgemeine Informationen II
Im letzten Teil des Interviews möchte ich Ihnen einige abschließende allgemeine Fragen stellen:

1. Bitte nennen Sie mir Ihren höchsten Schulabschluss:
Î kein Schulabschluss
Î Hauptschulabschluss
Î Mittlere Reife
Î Abitur / Fachhochschulreife
Î Sonstiges, bitte angeben: |______________________|

2. Bitte nennen Sie mir Ihren Berufsabschluss:
Î keinen
Î abgeschlossene Berufsausbildung (Lehre)
Î Fachschulabschluss
Î Fachhochschulabschluss
Î Hochschulabschluss
Î Sonstiges, z.B. Verwaltungs- oder Beamtenlaufbahn

3. Bitte nennen Sie mir Ihren Familienstand:
Î ledig / Single
Î verheiratet oder mit Partner/In
Î getrennt lebend oder geschieden
Î verwitwet

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4. Bitte nennen Sie mir alle Berufe, in denen Sie für <u>min. ein Jahr</u> gearbeitet haben. Falls Sie für denselben Arbeitgeber in zwei ganz unterschiedlichen Tätigkeitsbereichen gearbeitet haben, dann werten Sie dies bitte als zwei versch. Berufe. Beginnen Sie mit dem zuletzt ausgeübten Beruf.
Falls eine identische Tätigkeit bei unterschiedl. Arbeitgebern durchgeführt wurde, können diese zu einem Beruf zusammengefasst werden. Bezahlte Nebentätigkeiten sind ebenfalls relevant, wenn sie regelmäßig für mindestens ein Jahr ausgeübt wurden. Beispiele wären Berufstätige mit einem Nebenverdienst in der Landwirtschaft oder Hausfrauen/- männer oder Studenten mit regelmäßiger Nebentätigkeit.Bitte fragen nach Zeitraum, Berufsbezeichnung und kurzer Skizzierung typischer Tätigkeiten

Nr.	Datum (Monat/ Jahr)	Berufsbezeichnung und Tätigkeitsbereich
1	Von |__|__| __|__|__|__| Bis |__|__| __|__|__|__|	Berufsbezeichnung:____________ Tätigkeit:__________________ ________________________ ________________________
2	Von |__|__| |__|__|__|__| Bis |__|__| |__|__|__|__|	Berufsbezeichnung:____________ Tätigkeit:__________________ ________________________ ________________________
3	Von |__|__| |__|__|__|__| Bis |__|__| |__|__|__|__|	Berufsbezeichnung:____________ Tätigkeit:__________________ ________________________ ________________________
4	Von |__|__| |__|__|__|__| Bis |__|__| |__|__|__|__|	Berufsbezeichnung:____________ Tätigkeit:__________________ ________________________ ________________________

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5. Haben oder hatten Sie in Ihrem Beruf Kontakt mit Tieren?
Nein
Ja
Wenn ja, mit welchem?___________________________
seit wann? |__|__| Monat |__|__|__|__| Jahr;
bis wann? |__|__| Monat |__|__|__|__| Jahr
bei welcher Tätigkeit?_____________________________

Wenn ja, mit welchem?___________________________
seit wann? |__|__| Monat |__|__|__|__| Jahr;
bis wann? |__|__| Monat |__|__|__|__| Jahr
bei welcher Tätigkeit?_____________________________

6. Haben oder hatten Sie auch privaten Kontakt zu Tieren?
Nein
Ja
Wenn ja, mit welchem?___________________________
seit wann? |__|__| Monat |__|__|__|__| Jahr;
bis wann? |__|__| Monat |__|__|__|__| Jahr
Wenn ja, mit welchem?___________________________
seit wann? |__|__| Monat |__|__|__|__| Jahr;
bis wann? |__|__| Monat |__|__|__|__| Jahr
Wenn ja, mit welchem?___________________________
seit wann? |__|__| Monat |__|__|__|__| Jahr;
bis wann? |__|__| Monat |__|__|__|__| Jahr

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Vielen Dank. Hiermit ist das Interview abgeschlossen.
Ich danke Ihnen für Ihre Geduld und Ihre Mitarbeit.
Sie haben damit einen wertvollen Beitrag zur Unterstützung der Krebsforschung geleistet.
Wenn Sie möchten, können Sie mir nun noch weitere Angaben machen, die für Sie von Bedeutung sind.
Sie können an dieser Stelle auch gerne Kommentare zum Interview geben.

Kommentare:
____
____
____
____
____
____
____
____

Falls Sie später noch weitere Fragen haben, zögern Sie nicht, uns anzurufen.

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F. Interviewerfragen

Diese Fragen sind direkt nach dem Interview zu beantworten (NICHT IM BEISEIN DES STUDIENTEILNEHMERS).

Art des Interviews

- ☐ im Krankenhaus direkt
- ☐ telefonisch

Wie hoch war die Bereitschaft des Studienteilnehmers, die Fragen zu beantworten?

- ☐ überhaupt nicht (nicht interessiert, zurückhaltend)
- ☐ mäßig kooperativ und aufgeschlossen
- ☐ sehr kooperativ, aufgeschlossen und interessiert

Ihrer Meinung nach, wie gut konnte sich der Studienteilnehmer an frühere eigene Erkrankungen erinnern? (nur einmal ankreuzen)

- ☐ sehr gut
- ☐ gut
- ☐ mäßig gut
- ☐ nicht gut
- ☐ überhaupt nicht

Ihrer Meinung nach, wie gut konnte sich der Studienteilnehmer an frühere Erkrankungen seiner Familie erinnern? (nur einmal ankreuzen)

- ☐ sehr gut
- ☐ gut
- ☐ mäßig gut
- ☐ nicht gut
- ☐ überhaupt nicht

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Bitte ergänzen Sie Angaben zum Interview, die Ihnen wichtig erscheinen

Bemerkungen/ Ergänzungen:

_____

_____

In diesem Bereich ist der Status des Interviews festzuhalten.

Geben Sie den Vollständigkeits-Status des Interviews an:

Das Interview kann z.B. unvollständig sein, wenn Sie Sektionen übersprungen haben oder das Interview vorzeitig abgebrochen wurde.

- ☐ Vollständig
- ☐ Unvollständig, weil:
  - ☐ Studienteilnehmer verweigerte die weitere Teilnahme;
    Bitte geben Sie den Grund an:
    _____
    _____
  - ☐ andere Gründe, bitte angeben:
    _____
    _____

Werden Sie das Interview zu einem späteren Zeitpunkt wieder aufnehmen?

- ☐ Ja Wann?_____
  _____
- ☐ Nein Warum?_____
  _____

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Mein Dank gilt Frau Dr. B. Schlehofer und Herrn Prof. Dr. J. R. Schlehofer für die Themenstellung sowie für die wissenschaftliche Betreuung dieser Arbeit. Weiterhin möchte ich mich auch bei Frau Dr. K. Steindorf und Herrn Prof. Dr. J. Wahrendorf für die Betreuung im Rahmen des Graduiertenkollegs der Uni Heidelberg bedanken.

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Zu guter Letzt möchte ich mich zutiefst bei meiner Familie bedanken, die mir während aller Höhen und Tiefen dieser Arbeit immer zur Seite stand, die mich kräftig unterstützt hat und auf die ich mich (wie immer) in allen Situationen hundertprozentig verlassen konnte.

Presentations and Publications

Oral presentations

ESVONC (European Society of Veterinary Oncology) meeting, Hofheim (Taunus),
February 07-08, 2004,
Poltermann S, Schlehofer JR, Geletneky K, Schlehofer B, "Epidemiological-Virological Assessment of a possible Role of Human Cytomegalovirus (HCMV) in the Development of Glioma"

Congress of ECVIM-CA (European College of Veterinary Internal Medicine-Companion Animals), Barcelona (Spain),
September 09-11, 2004,
Poltermann S, Schlehofer B, Geletneky K, Schlehofer JR, "Case-control Study on Brain Tumors and Human Cytomegalovirus (HCMV): Rationale, Study Design and first Results"

Poster Presentations

12. Jahrestagung der Deutschen Arbeitsgemeinschaft für Epidemiologie (DAE),
Freiburg im Breisgau,
September 14-15, 2005
Poltermann S, Schlehofer B, Steindorf K, Schnitzler P, Wahrendorf J, Geletneky K, Schlehofer JR, "The Role of Herpesvirus Infections in Brain Tumor Development"

Publications

"Lack of association of herpesviruses with brain tumors", S. Poltermann, B. Schlehofer, K. Steindorf, P. Schnitzler, K. Geletneky, J.R. Schlehofer, Journal of Neurovirology, 2006 Apr;12(2):90-9.

Declaration of Compliance with Good Scientific Practice

Ich erkläre:

Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nicht veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissentlicher Praxis, wie sie in der "Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis" niedergelegt sind, eingehalten.

Sabine Poltermann