HIV disorders of the brain; pathology and pathogenesis

Luis Del Valle and Sergio Piña-Oviedo

Center for Neurovirology and Cancer Biology, Laboratory of Neuropathology and Molecular Pathology, Temple University, 1900 North 12th Street, Suite 240, Philadelphia, Pennsylvania 19122 USA

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1. ABSTRACT

Infection with HIV-1 has spread exponentially in recent years to reach alarming proportions. It is estimated than more than 33 million adults and 1.3 million children are infected worldwide. Approximately 16,000 new cases are diagnosed every day and almost 3 million people die every year from AIDS, making it the fourth leading cause of death in the world. Since the introduction of highly active anti-retroviral therapy (HAART) in the mid 1990s, the morbidity and mortality associated with HIV-1 infection has significantly decreased and AIDS has become a chronic disorder. However, neuropathological conditions associated with AIDS are still present in approximately 70 to 90% of patients and can be the result of HIV itself or of opportunistic infections. Here we briefly review the pathology and pathophysiology of AIDS-Encephalopathy, of some of the significant opportunistic infections affecting the brain in the context of AIDS, including Progressive Multifocal Leukoencephalopathy (PML) a demyelinating disease caused by the human neurotropic JC virus, Toxoplasmosis, Cryptococcosis and of primary CNS lymphoma, a brain malignancy frequently associated with HIV-1 infection, all of them considered AIDS defining conditions.
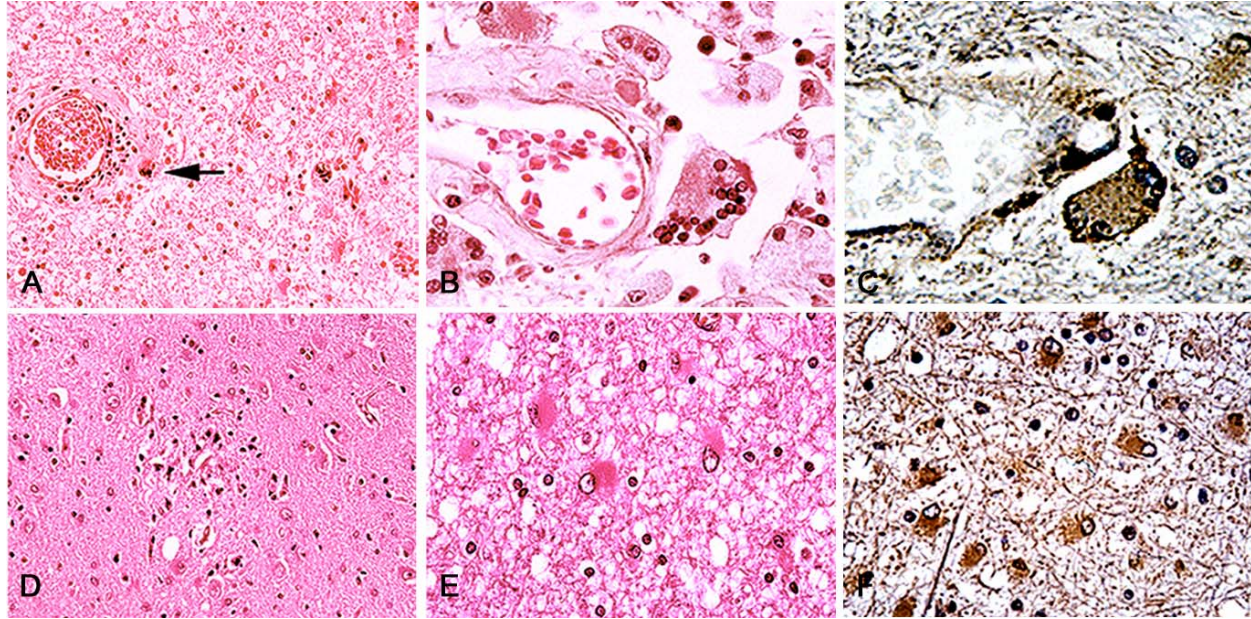
Figure 1. Histopathological Features of HIV Encephalopathy. Low magnification view of the cortical grey matter in a case of HIV-Encephalopathy depicting a blood vessel with intramural and perivascular chronic inflammatory cells, including a giant multinucleated cell (arrow). The vasculitis results in small areas of infarction where foamy macrophages can be seen (Panel A, Hematoxilin and Eosin, original magnification x200). At higher magnification the giant multinucleated cells can be seen in close proximity to blood vessels (Hematoxilin & Eosin, original magnification x1000). Immunohistochemistry for the HIV-1 capsid protein p24 is robustly expressed in the cytoplasm of the giant multinucleated cells, as well as in endothelial cells of the blood vessel (Panel C, original magnification x1000). Another characteristic feature of the HIV encephalopathy are the parenchymal microglial nodules (Panel D, Hematoxilin and Eosin, original magnification x200). Numerous reactive astrocytes are present in areas of damage (Panel E, Hematoxilin and Eosin, original magnification x400). Immunohistochemistry demonstrates expression of the HIV transactivator protein Tat in the cytoplasm of these reactive astrocytes (Panel F, original magnification x400).

2. HIV-ENCEPHALOPATHY

2.1. Definition

Acquired immunodeficiency syndrome (AIDS) is the name given to the disease caused by infection with the human immunodeficiency virus 1 (HIV-1), a lentivirus of the *Retroviridiae* family. Approximately 30% of patients with AIDS will develop a variety of cognitive and motor symptoms, generally late in the course of HIV infection, known collectively as AIDS-dementia complex or HIV-1 Associated Cognitive-Motor Complex (1). HIV encephalopathy is the initial manifestation of AIDS in approximately 3% of the patients, and autopsy reports show that up to 90% of infected individuals have histo-pathological changes compatible with the disease.

2.2. HIV-1 Structure

Although lentiviruses have been known for a long time, HIV-1 was first isolated in 1983 from a patient with lymphadenopathy (2). Like other retroviruses HIV-1 is single-stranded RNA enveloped virus of approximately 100 nm in diameter. The HIV-1 genome is composed of two identical copies of RNA, surrounded by a nucleocapsid. The nucleocapsid is a lipid bilayer rich in envelope glycoproteins. Like other lentiviruses, the HIV-1 genome encodes for structural proteins, such as group specific antigens (Gag), which contains information for the nucleocapsid protein p24 and other internal non-glycosylated proteins, envelope proteins (Env), and enzymatic proteins responsible for reverse transcription and integration (Pol) and viral protease (Pro) (3, 4). However, the genome of HIV-1 is more complex and encodes for 6 other proteins. Vif or viral infectivity factor, essential for macrophage infectivity (5), Tat, a transactivator of RNA synthesis, required for viral replication (6), Vpr, a particle-associated regulatory protein (7), Rev, a regulator of splicing and RNA transport, Nef, a homodimer which has been controversially been associated with viral gene expression and that causes downregulation of CD4 (8), and Vpu, a protein important for virus assembly and release.

2.3. Histopathology

Gross examination of the brain in cases of AIDS Encephalopathy is disappointingly unremarkable. In some cases mild cerebral atrophy, of less that 200 g can be found. The atrophy is characterized by enlarged sulci and slight dilation of the ventricular system (*hydrocephalia ex vacuo*).

Histologically, HIV Encephalopathy is characterized by a subacute encephalitis, with chronic inflammatory infiltrates in close proximity to blood vessels, accompanied by parenchymal microglial nodules and the hallmark of the disease, giant multinucleated cells, which are result of the fusion of macrophages (9, 10). In some cases this vasculitis causes scattered small infarcts in the brain parenchyma, in which reactive astrocytes proliferate (11). Neuronal loss has been described consistently in the cortex of patients with HIV-encephalopathy (12, 13). Figure 1 illustrates the histological characteristics of HIV-Encephalitis.

2.4 Clinical Manifestations

The first exposure of the brain to HIV-1 occurs shortly after systemic infection and results in a usually asymptomatic infection, although in some cases signs of mild encephalitis have been reported (14). The onset of AIDS dementia complex is generally insidious and the signs and symptoms are compatible with a subcortical dementia. In early stages the clinical spectrum include poor concentration, confusion, recent memory loss, fatigue and apathy, which may be misdiagnosed as depression (15). In later stages, more specific cognitive changes develop including personality and behavioral changes, motor impairments, including tremor, weakness, appearance of release reflexes and even ataxia (16, 17). Although the prognosis of patients diagnosed with AIDS-dementia complex has improved after the introduction of HAART therapy, the survival remains poor, ranging from 1 to 3 years.

2.5. Physiopathology

HIV-1 crosses the blood-brain barrier and invades the brain shortly after systemic infection (18). The mechanism of entry involves endothelial pinocytosis through binding of viral gp120 to endothelial cell surface glycoproteins (19). HIV-1 is most likely carried by cells in traffic to the brain, infected monocytes in particular, in what is known as the 'Trojan Horse' hypothesis (20, 21). In support of this model, several studies have demonstrated the presence of HIV-1 in macrophages located in the Virchow-Robin space by *in situ* hibridization and immunohistochemistry (22, 23). Microglial cells may become subsequently infected and along with perivascular macrophages may act as viral reservoirs (24, 25). Viral-envelope proteins expressed in the surface of infected macrophages appear to be responsible for the fusion of HIV-1 infected macrophages to result in the formation of giant multinucleated cells, the hallmark cells of HIV-encephalitis (26). Viral production seem to be limited to these phenotype of cells, since infection of astrocytes have been demonstrated, does not lead to a significant viral replication (27, 28).

The mechanism of neuropathogenesis is not clearly understood, as there is no evidence of HIV-1 infection in neurons. It is possible that neuronal injury occurs as a consequence of the inflammatory process or of direct exposure to HIV proteins released by infected cells, such as gp120, Tat and Vpr, which have been shown to have a toxic effect *in vitro* (29, 30, 31). In addition, gp120 has been shown to cause neuronal damage through an indirect mechanism involving activation of the TNF-α / Caspase cascade of proteins (32). Several studies have also shown the toxic effects of nitric oxide secreted by HIV-1 infected macrophages (33, 34). Another non-exclusive model involves the activation of several cytokines, which results in potentiation of the inflammatory response and in neural damage (35, 36). Interestingly, the degree of inflammatory response and neuronal damage does not always correlate with the severity of the clinical symptomatology (37, 38).

3. PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

3.1. Definition

Progressive Multifocal Leukoencephalopathy (PML) is a sub-acute and fatal demyelinating disease of the CNS, caused by the opportunistic neurotropic virus JCV and seen almost exclusively in patients with immunosuppressive conditions. Before the AIDS pandemic in the early 1980s, PML was a medical curiosity, associated mainly with leukemias and lymphomas or as a complication of chemotherapy for cancer treatment and immunosuppressive therapies in transplant patients. However, recent epidemiological reports indicate that up to 10% of patients with AIDS will develop PML and that 85% of patients with PML are HIV-1 positive. In fact PML is so frequently seen in patients infected by HIV-1, that it is considered an AIDS defining condition (39, 40).

PML is a relatively new disease, originally described by Åstrom and Richardson in 1958 (41), who also first suggested the possibility of an infectious organism as the etiological agent (42), theory that was later confirmed with the discovery of small viral particles in the nuclei of infected cells (43, 44, 45, 46). The next step in the characterization of PML as an infectious disease came with the cultivation and isolation of the virus in a fetal glial cell culture of spongioblasts (47). Finally, experimental animal models aimed to reproduce the demyelinating disease yielded surprising results, as rodents and primates intra-cerebrally inoculated with JCV developed several varieties of brain tumors, leading to the discovery of the oncogenic potential of the virus (48, 49, 50, 51, 52).

3.2. JC Virus Biological Considerations

JCV is a member of the polyomavirus family of DNA viruses, which also includes BK virus, the causative agent of polyomavirus nephropathy (PVN) and the thoroughly studied Simian Virus 40 (SV40). JC Virus is widely spread among the human population, with approximately 85% of adults world wide exhibiting JCV specific hemagglutination antibodies (53, 54). Infection with the virus is usually sub-clinical and occurs in early childhood (55). JCV has not been implicated in disease in healthy individuals and in fact, the virus has been recovered from the urine of pregnant women and renal transplant patients, indicating productive infection, without any overt signs of disease (56, 57). The virus is presumed to remain latent, until it reactivates under immunosuppressive conditions to result in PML. Based on the multifocal nature of the demyelinated lesions it is likely that the virus reaches the brain by hematogenous spread, perhaps carried by white blood cells. Infection of B-lymphocytes with JCV has been demonstrated in patients with PML, so these cells are likely to act as a carrier for the virus (58, 59). JCV exhibits very limited tissue specificity; the virus can replicate most efficiently in primary human fetal glial cells, but is well established that the virus remains latent in the kidney, and more recently several studies support its ability to replicate in B lymphocytes (60). However, due to the species specificity of the DNA polymerase, JCV can only replicate in primates, and humans are thought to represent the natural viral host (61).

3.3. JC Virus Structure

JCV is a small (38-40 nm in diameter) non-enveloped virus the genome of which consists of a closed, circular, double stranded DNA enclosed by an icosahedral

capsid. The prototype strain of JCV, Mad-1, contains 5,130 nucleotides (61) and can be functionally divided into three regions, an early coding region and a late coding region divided by a regulatory (non-coding) region. The control region encodes the viral origin of DNA replication and is the strain that has been isolated from PML, Mad-1, contains a bi-directional promoter composed of two 98 base pair repeats which controls transcription, compared to the archetype strain, CY, which only contains one 98 bp repeat with two insertions of 23 and 68 bp respectively and which is found in latent state in the kidney. Early and late transcription proceeds in opposite directions around the circular DNA. The viral early region encodes the viral regulatory proteins, large and small T-Antigens, and is transcribed before DNA replication, whereas the viral late region encodes for the structural capsid proteins VP-1, VP-2 and VP-3 as well as the small accessory product, Agnoprotein, and is transcribed after DNA replication (61). VP1 is the most abundant protein of the capsid, representing approximately 80% of the virion components, which consists of 360 copies of VP1 arranged in 72 pentamers (62).

3.4. Histopathology

From the pathological point of view, PML is characterized by extensive areas of demyelination in the sub-cortical white matter of the brain, due to the infection and cytolytic destruction of oligodendrocytes, the myelin producing cells of the central nervous system. Although these demyelinated plaques are more frequently seen in the sub-cortical areas of the frontal, parietal and temporal lobes, they can also be found in the white matter any part of the brain, including the basal ganglia, cerebellum and brainstem and in extreme cases the spinal cord (63). In neuroimaging studies, PML usually appears as multiple foci of hyper-intense areas affecting the sub-cortical white matter. In gross examination a demyelination plaque is yellow, of soft consistency, irregular borders and can show small foci of cavitation, which eventually become confluent as the disease progresses. Histologically, the characteristic features of PML are multiple plaques of myelin loss, containing multiple enlarged bizarre reactive astrocytes, which are often atypical, multinucleated and have multiple large processes, and enlarged oligodendrocytes, harboring prominent eosinophilic intranuclear inclusion bodies (64, 65). These inclusion bodies are the result of the viral replication as corroborated by electron microscopy studies, which have consistently revealed the presence of icosahedral viral particles. Other histological characteristics include perivascular cuffing of lymphocytes, microglial nodules and foamy machophages whose function is to phagocyte the myelin lipidic debris accumulated after the lysis of oligodendrocytes. Figure 2 depicts the anatomic and histopathological features of PML.

3.5. Clinical Manifestations

The clinical signs and symptoms depend on the location of the demyelinated lesions and they include headache, motor alterations, visual impairments, sensory loss, cognitive alterations and in the late phase of the disease, dementia. Since the most frequently affected location is the frontal lobe, cognitive and motor

dysfunction are the predominant symptoms (66). Despite significant advances in antiretroviral therapy, thus far, no effective treatment has been developed and PML remains a fatal disease with a poor survival, which ranges from 4 to 6 months after the onset of symptoms (67).

3.6. Physiopathology

The lytic cycle of JCV starts with a direct association between VP1 molecules in the viral capsid and N-linked glycoproteins bearing specific linkages of sialic acid in glial cells, particularly oligodendrocytes (68, 69). Recent evidence has indicated a role for the 5HT2A serotonin receptor in JCV entry (70). After an endocytosis process, viral particles are disassembled and uncoated DNA enters the nuclear compartment, where early transcription proceeds (71). DNA replication occurs with production of approximately 200,000 viral copies. Once DNA replication begins, the infection enters the late phase, where T-Antigen represses the transcription of early genes and stimulates the late genes. Viral capsids are synthetized in the cytoplasm and re-enter the nuclei to assemble with viral DNA, explaining the presence of eosinophilic inclusion bodies in the nuclei of oligodendrocytes.

The significantly higher incidence of PML in patients with AIDS than in patients with any other immunosuppressive conditions, suggests that the presence of HIV-1 in the brain participates, directly or indirectly, in the pathogenesis of this disease (72). One likely candidate to orchestrate the molecular events involved in the activation of JCV is the HIV-1 transactivator protein Tat, a 14 kD protein transcribed early in the HIV-1 infection cycle and important for transcription and replication through interaction with the HIV-1 LTR (73, 74, 75). It has been demonstrated that Tat has the capacity of being secreted by HIV-1 infected cells, such as macrophages, microglial cells and astrocytes and absorbed by neighboring non-infected cells (64, 76) suggesting that reactivation of JCV may not require co-infection of oligodendrocytes by both viruses. Once inside oligodendrocytes, Tat has the ability to bind to specific sequences within the JCV control region, to result in enhancement of JCV promoter transcription and enhancement of viral DNA replication as demonstrated *in vitro* (77, 78). An alternate, but not exclusive mechanism of JCV promoter activation mediated by Tat, may include stimulation of several cytokines, including TGF-β1, which binds to the TGF-β1 Receptor of JCV infected oligodendrocytes, which results in transactivation of the JCV promoter by Smad 3 and Smad 4 (64). In both cases, the transactivation of the JCV promoter by HIV-1 Tat in oligodendrocytes will result in active viral replication, lytic destruction and eventually the development of PML.

4. CRYPTOCOCOSIS

4.1 Definition

Cryptococcal meningitis (CM) is another opportunistic infection of the CNS caused by the encapsulated yeast *Cryptococcus neoformans*. As well as other opportunistic infections, the incidence of CM has increased since the AIDS epidemic (79). CM is the most
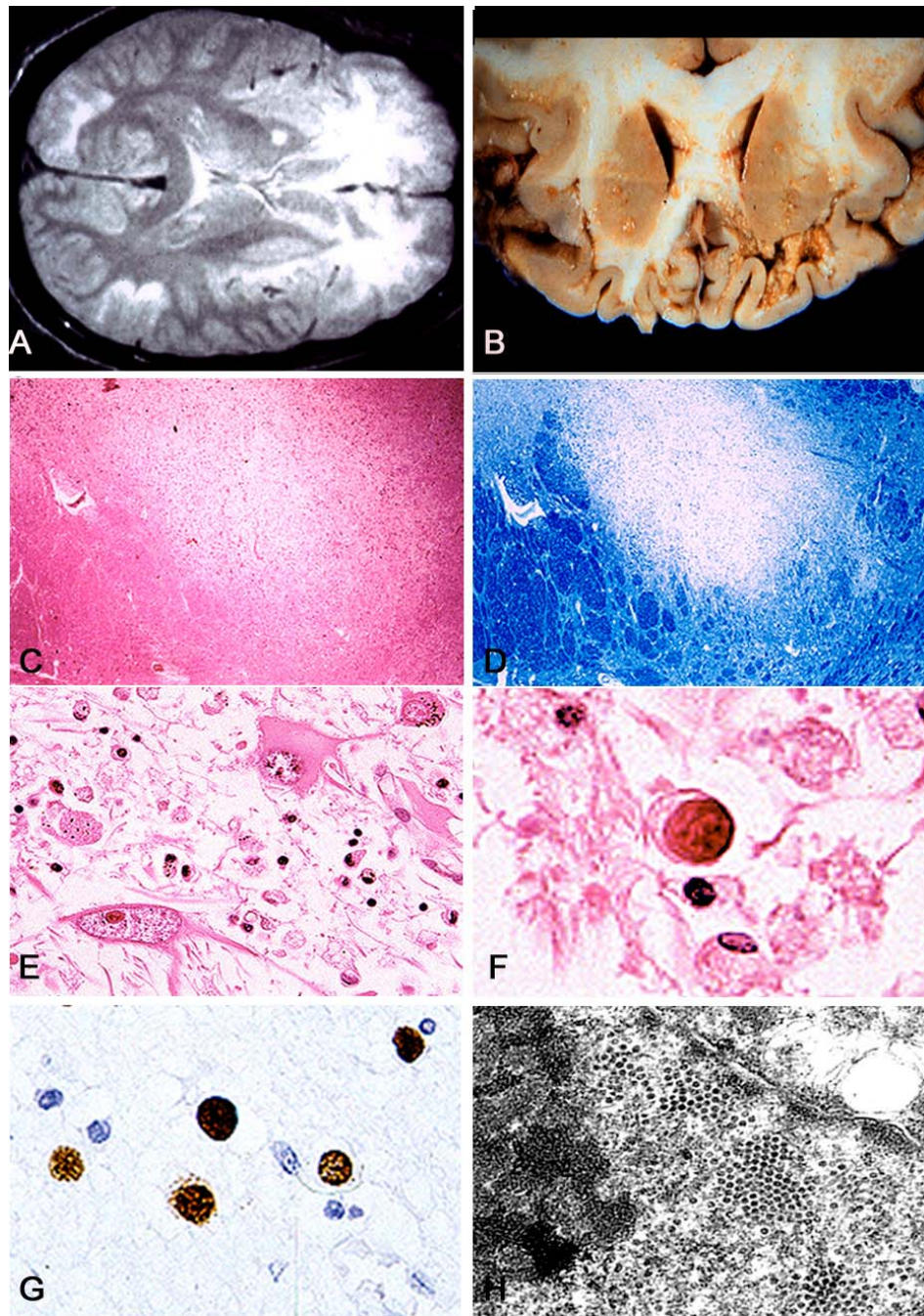
Figure 2. Histopathological Features of Progressive Multifocal Leukoencephalopathy. Axial MRI of the brain showing the characteristic hyper-intense areas located in the sub-cortical white matter of the frontal lobe (Panel A). Coronal section of the same case, at the level of the frontal lobe demonstrating extensive lytic lesions in the sub-cortical white matter particularly prominent in the right olfactory giry (Panel B). Low magnification view of the white matter of a case of PML reveals extensive areas of myelin loss (Panel C, Hematoxylin & Eosin, original magnification x100). The demyelinated areas are highlighted with a special stain for myelin (Panel D, Luxol Fast Blue, original magnification x100). Higher magnification of the demyelinated plaques shows the characteristic cells of PML, giant reactive astrocytes with bizarre atypical nuclei (Panel E, Hematoxylin & Eosin, original magnification x1000), and enlarged oligodendrocytes harboring intranuclear eosinophilic inclusion bodies (Panel F, Hematoxylin & Eosin, original magnification x400). Immunohistochemistry for the JCV capsid protein VP-1 shows robust reactivity in the nuclei of infected oligodendrocytes, demonstrating productive infection (Panel G, original magnification x200). The intranuclear inclusion bodies in oligodendrocytes are composed by numerous icosahedral viral particles as demonstrated by electron microscopy (Panel H).

common fungal infection developed by HIV-1 infected patients and is the third most frequent neurological complication in patients with AIDS. In fact, CM is the initial manifestation of HIV-1 infection in approximately 40% of the patients (80), and 5 to 10% of HIV-1 infected patients will develop CM. Based on these statistics, CM is considered and AIDS defining condition (81). Crytptococcosis can also affect, with a significantly lower incidence, patients with sarcoidosis, lymphoproliferative disorders and individuals undergoing immuossuppressive therapies (80). The incidence of cryptococcal meningitis seems to be particularly common in Southeast Asia (82, 83) and Southern and East Africa (84).

4.2. *Cryptococcus neoformans* Structure

Cryptococcus neoformans, first described simultaneously in the late eighteen hundreds, in isolates from peach juice in Italy and from a tibial lesion of a patient in Germany (85, 86), belongs to the group of fungi known as *Basidiomycete*. Morphologically it is a spherical yeast, of 5 to 10 μm in diameter, with an external polysaccharide capsule, which is the major factor for virulence (79). Two varieties of *Cryptococcus* have been described, *neoformans* and *gattii* (79, 87). From the *neoformans* variety three major capsular serotypes are recognized: A, D, and AD; serotype A is the most commonly isolated in AIDS patients (79). The pathogenicity of *Cryptococcus* requires the presence of a capsule, the temperature of 37°C and the production of phenol oxidase (88, 89).

The main component of the capsule is the Capsular Polysaccharide (CPS), a long, non-branched polymer with monosaccharide branches of xylose and glucuronic acid (glucuronoxylomannan or GXM) (90). The presence of GXM is important, as it has been shown to inhibit both phagocytosis and production of antibodies against *Cryptococcus* (87). Other proteins, not necessarily related to the GXM but present in the capsule, have also been shown to decrease leukocyte migration (91). Virulence has not been correlated with the amount or length of the capsular polysaccharide (92), however, recent studies have shown that organ invasion by *Cryptococcus* was associated not only with changes in the cryptococcal capsule structure and cell size, but also with the characteristics of the organs infected, specially the integrity of the blood-brain barrier (93). In addition, *Cryptococcus* also produces a unique phenol oxidase enzyme, which has the ability to convert a variety of substrates, including catecholamines, into melanin (94, 95), characteristic that may be responsible for the neurotropism of this organism, which has a marked affinity for the brain and the adrenal glands (87, 96). Melanin, can function also as an antioxidant, which may protect C*ryptococcus* from oxidative host defenses (97, 98).

4.3. Histopathology

At gross examination, the brain shows chronic meningitis affecting the basal leptomeninges, which are opaque and thickened. Sections of the brain reveal a gelatinous grayish material within the subarachnoid space and multiple sometime confluent small cysts within the

parenchyma, which are especially prominent in the basal ganglia in the distribution of the lenticulostriate arteries and are the characteristic lesion of cryptococcal infection (99). As the infection spreads the perivascular space (Virchow-Robin) may become distended, filled with this same mucoid, gelatinous material originated from the cryptococcal capsule (100, 101). Larger collections of these same structures are known as gelatinous pseudocysts, more common in adults and rare in children (102).

Histologically, the cystic lesions in the basal ganglia consist of aggregates of organisms confined to the Virchow-Robin spaces, which are enlarged. There is minimal or absent inflammation, however, in rare cases, a granulomatous inflammatory reaction, referred as a "cryptococcoma" can be seen surrounding the microorganisms (103). In tissue sections, the *Cryptococcus* appears as a spherical, encapsulated structure, which is surrounded by large empty spaces result of the abundant mucoid material secreted by the yeasts. The capsule of *Cryptococcus* can be visualized more clearly with special staining methods and silver impregnations, such as Gomori methenamine and Grocott, periodic acid-Schiff (PAS) and mucicarmine (87). In patients with AIDS there seem to be a variability in the size of the microorganisms. Figure 3, Panels A, B and C, illustrates the macroscopic and microscopic lesions of cryptococcosis.

4.4. Clinical Manifestations

Cryptococcal meningitis in AIDS patients usually presents with headache, fever, malaise, and altered mental status over several weeks (79). Meningeal symptoms such as neck rigidity and Kerning' sign however, are not always present. Focal neurological signs and seizures are present in approximately 10% of patients (80). The onset and duration of CM is variable. The duration of symptoms and signs may be shorter in AIDS patients because of poor inflammatory response. Mortality from HIV-associated CM is relatively high, accounting for approximately 10-30% of the cases, mainly as a complication of raised intracranial pressure (104).

4.5. Physiopathology

Crytococcus neoformans is an environmental saprophyte that can be found in the soil and in the manure of certain types of birds. The pathogenesis of cryptococcosis is determined by three main factors: the status of the host immune system, the virulence of the strain, and the size of the inoculum (87). The human infection is an accidental event in the life cycle of the organism. It is acquired through the respiratory tract from inhalation of aerosolized particles present in soil and habitats of birds, particularly pigeons (105, 106), where it can remain latent in the immunocompetent host, or spread into the bloodstream if alveolar macrophage function is impaired, as is the case in HIV-infected patients, finally reaching the brain. The mechanism by which *Cryptococcus* crosses the blood-brain barrier has been recently elucidated and it involves alteration of tight junctions in endothelial cells (107, 108). Once in the perivascular space, the fungi can be located extracellularly or inside phagolysosomes in macrophages, where it has the ability to survive and

replicate (109). Finally, after intracellular replication, the microorganism accumulates in vesicles and produces the mucoid substance, leading to permeabilization of the phagolysosomal membrane and cytotoxicity of macrophages (110).

5. TOXOPLASMOSIS

5.1. Definition

Toxoplasmosis is a disease caused by the coccidian *Toxoplasma Gondii*, an intracellular parasite with world-wide distribution. Infection with *Toxoplasma* results in a wide variety of conditions, ranging from asymptomatic infections in immunocompetent individuals to fatal acute encephalitis in HIV-1 infected patients. In addition *Toxoplasma* causes blindness and mental retardation in congenitally infected children. Serological studies have detected antibodies for *Toxoplasma* in approximately 30 to 40% of adults in Europe and North America and up to 80% in undeveloped countries (111), indication of latent infection. However, the incidence of encephalitis for *Toxoplasma* has dramatically increased in recent years, mainly due to the pandemic of AIDS, after which toxoplasmosis has become the most common opportunistic infection involving the brain (112, 113). In approximately 95% of patients infected with HIV-1, encephalitis is believed to be the result of reactivation of a chronic latent infection, due to the loss of cellular immune response (114, 115).

5.2. *Toxoplasma godii* Structure

Toxoplasma gondii is a ubiquitous intracellular protozoan, member of the family *Apicomplexa*, which also includes *Plasmodium*, the etiological agents of malaria. The discovery of Toxoplasma was made in 1908 at the Pasteur Institute in Tunisia, in the spleen and liver of a small rodent called the gondi (116). The first report of human involvement came a decade later, from a case of congenital toxoplasmosis in Prague (117).

The life cycle of *Toxoplasma* consists of two important phases, tachyzoites and oocysts. Tachyzoites are shaped as a crescent moon, with a conical anterior end and a rounded posterior end, and measure 2 by 6 μm. Ultrastructurally, they are intricate organisms composed by a central nucleus surrounded by numerous organelles and inclusion bodies and delimited by an outer cover or pellicle. The organelles include apical and polar rings, conoid, rhoptries, micronemes, micropore, mitochondria, endoplasmic reticulum, Golgi apparatus, ribosomes, rough and smooth endoplasmic reticulum, dense granules, and a membrane-bound organelle, also termed apicoplast (118, 119, 120). The outer cover consists of three membranes, an external plasmalemma and two close inner membranes (121). Polar rings are thickenings of the inner membrane complex at the anterior end of the tachyzoite, which encircle a cylindrical, truncated cone called the conoid. Between the anterior end and the nucleus, there are 8 to 10 club-shaped organelles called rhoptries (122), which constitute excretory structures and contain proteolytic enzymes (123, 124). Micronemes are rod-like structures, present mostly at the anterior end of the parasite. The function of these structures is not fully understood but is likely associated with penetration of host cells and creation of an intracellular environment permissible for the growth and development of the parasite. On the other hand, oocysts vary in size and bradyzoite contents; young tissue cysts may be as small as 5 μm in diameter and contain only two bradyzoites, while older cyst measure from 70 to 100 μm and may contain hundreds of organisms (125).

5.3. Histopathology

On gross examination, the characteristic lesions of AIDS-related toxoplasmosis are multiple foci of necrosis. The lesions are usually focal, variable in size but generally large, of irregular borders and can affect any part of the brain, with strong predilection for the basal ganglia and brainstem. Necrotic and hemorrhagic areas are frequent (126, 127). The multifocal nature of the necrotic lesions suggests that although encephalitis might be the result of reactivation of a latent infection, the parasite reaches the brain through hematogenous dissemination. Histologically, acute lesions differ from chronic abscesses. Acute lesions are poorly circumscribed and contain necrotic and hemorrhagic areas with scanted inflammatory cells and abundant intracellular tachyzoites and parenchymal oocysts. Chronic lesions developed after treatment, are conformed by well-demarcated cysts with microglial nodules and macrophages in the adjacent brain parenchyma, where parasites are difficult to find (128). Figure 3, Panels D, E and F, depicts the radiological, macroscopic and histological features of toxoplasmosis.

5.4. Clinical Manifestations

The clinical signs and symptoms depend on the location of the lesions. General symptoms include headache, fever, motor deficits, behavioral changes, lethargy and coma, while more focal neurological conditions include hemiparesias, aphasia, convulsive crisis, ataxia, visual field defects and extrapyramidal signs, which once more, reflect the multifocal nature of the lesions (129, 115).

5.5. Physiopathology

Members of the cat family are the definitive hosts of *Toxoplasma*, while humans and other mammals constitute intermediate hosts. Infection can be contracted by ingestion parasites in either of three different stages of the *Toxoplasma* life cycle, oocysts, tachizoites or tissue cysts. The infectious cycle begins with ingestion of tissue cysts in undercooked or raw meat from chronically infected intermediary hosts, such as chicken, pork, goat or sheep, or by ingestion of food or water contaminated with cat feces containing oocysts (130, 131). Domestic cats constitute the major source of contamination, as they can excrete as many as 100 million oocysts per day. Surprisingly, only approximately 1% of the cat population is found to be shedding infectious oocysts (132). These cysts are highly infectious and resilient, as they have the ability of remaining viable in the soil for several years. Once the oocysts reach the stomach, the acid gastric secretions will dissolve the cyst membrane, liberating bradyzoites, which enter intestinal epithelial cells and transform into rapidly dividing tachyzoites. The parasites eventually rupture the
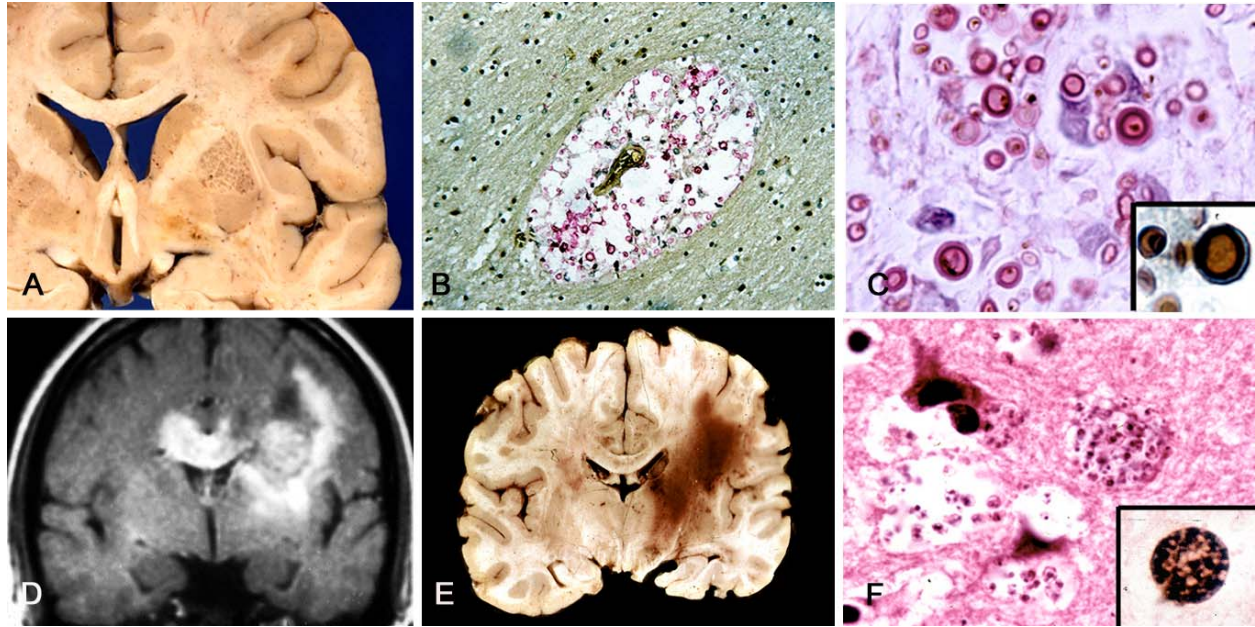
Figure 3. Opportunistic Infections Associated with AIDS. Coronal section of the brain in a case of Cryptococcosis demonstrates the characteristic cystic lesions, some of them confluent in the right Putamen (Panel A). Histologically, *Cryptococcus neoformans* is found confined to the Virchow-Robin space, which is dilated due to the microorganism and its mucoid secretion (Panel B, Mucicarmin, original magnification x100). Special staining methods reveal the structure of Cryptococcus, which is spherical, surrounded by an external capsule (Panel C, PAS, Insert, Groccot, both images original magnification x1000). MRI of the brain reveals an extensive lesion involving the right basal ganglia and corpus callosum in a case of Toxoplasmosis (Panel D). A coronal section of the brain in the same case demonstrates a large, irregularly shaped necro-hemorrhagic lesion, involving the basal ganglia and extending into the subcortical white matter (Panel E). *Toxoplasma gondii* can be found in tissue sections as intracellular tachyzoites in neurons and glial cells (Panel F, Hematoxylin & Eosin), and large oocysts with no inflammatory reaction surrounding them (Insert, both images, original magnification x1000).

host cells and disseminate to other organs, including the lymphatic tissue, myocardium, skeletal muscle, retina and brain, where the cycle starts over. This acute stage is followed by a chronic condition characterized by formation of tissue cysts containing slowly replicating bradyzoites (133).

6. PRIMARY CNS LYMPHOMAS

6.1 Definition

Primary Central Nervous System Lymphoma (PCNSL) is a malignant tumor of lymphocytes arising in the CNS in the absence of tumor outside the CNS. PCNSL is the second most common neoplasm in HIV-1 infected individuals behind Kaposi's sarcoma, and the first intracranial tumor, representing approximately 20% of all lymphomas in patients with AIDS (134, 135). Epidemiological studies have shown that the incidence of primary CNS lymphoma is 3600-fold greater in AIDS patients than in the general population (136, 137), with approximately 2 to 12% of all AIDS patients developing CNS lymphomas (138). Based on these statistics, primary CNS lymphoma is another entity considered as an AIDS defining condition.
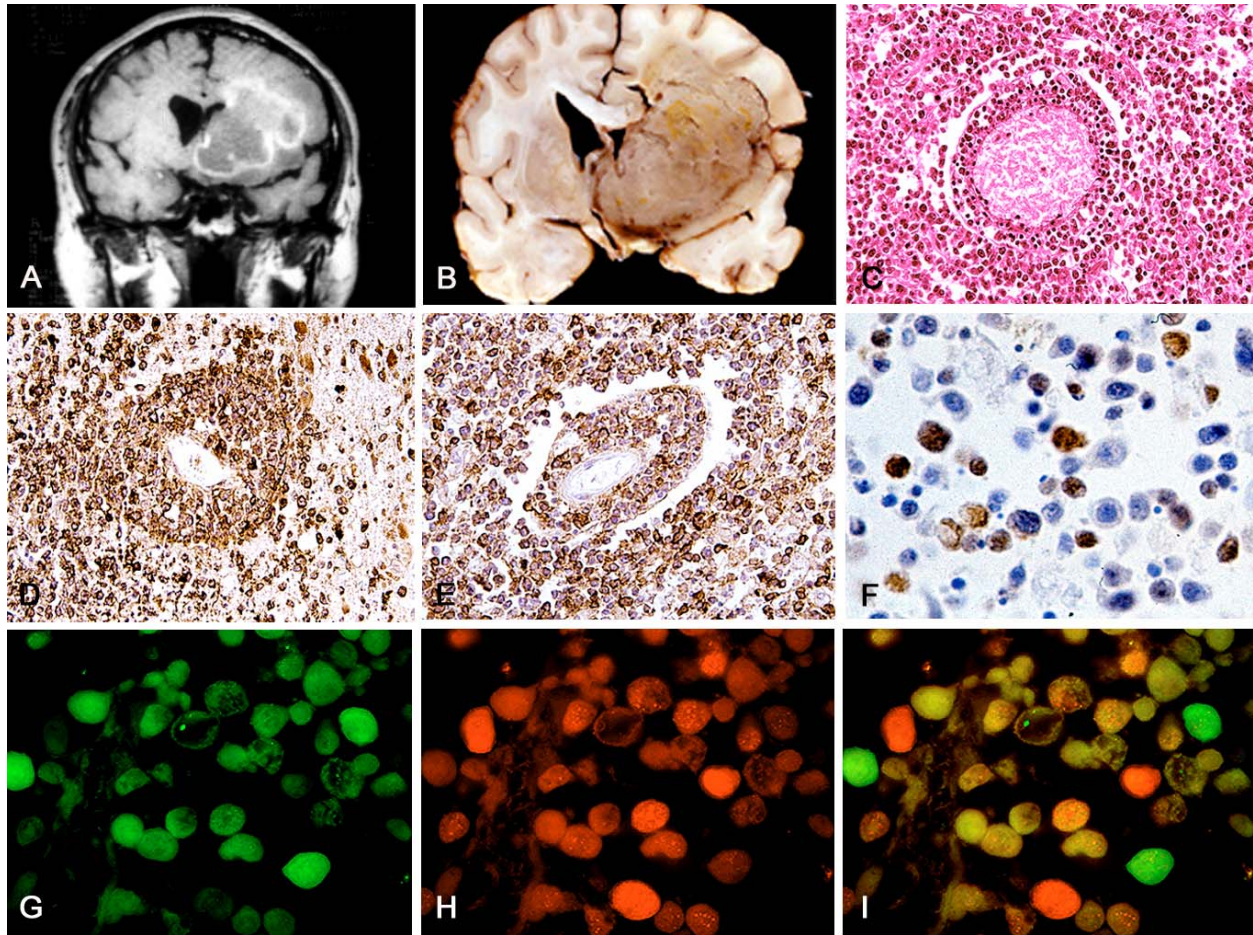
6.2. Histopathology

In contrast with systemic lymphomas infiltrating the brain, which present as leptomeningeal infiltrates, primary CNS lymphomas in the context of AIDS arises in the brain parenchyma. On gross examination CNS lymphomas present either as a single or multiple lesions, and are located in any part of the neuroaxis, with predilection for the cerebral hemispheres particularly the frontal lobe, the basal ganglia and periventricular regions, and the corpus callosum (139). Other locations include the brainstem, the cerebellum and the spinal cord. Approximately 25 to 50% of CNS lymphomas present as multiple masses. In general the tumors are grey-tan, yellowish, of firm and granular consistency, and poorly defined margins. Areas of hemorrhage and necrosis are frequent, especially in patients with AIDS. As mentioned above, the necrotic areas found in AIDS associated primary CNS lymphomas are similar to the ones present in encephalitis for toxoplasmosis, making this a differential diagnosis.

The characteristic histological pattern of primary CNS lymphomas is an angiocentricity of neoplastic cells, which are arranged forming perivascular cuffs in a concentric manner. Deposits of reticulin fibers around blood vessels are responsible for the concentric pattern observed in CNS lymphomas. In early stages, neoplastic cells are confined to the Virchow-Robin space, contained by the reticulin network, but later they diffusely invade the adjacent brain parenchyma. All primary CNS lymphomas grow in a diffuse pattern and no cases of follicular pattern have been described (140, 141). Unlike the complex and

Figure 4. Histopathological Features of Primary CNS Lymphomas. MRI of the brain in a case of primary CNS lymphoma reveals a large necrotic mass involving the right basal ganglia and producing significant brain edema (Panel A). A coronal section of the brain demonstrates a large infiltrating mass that affects the basal ganglia, including the caudate nucleus, the putamen and globus pallidus. The tumor is friable, granular and exhibits areas of necrosis and focal hemorrhage (Panel B). A low power magnification view of the tumor shows abundant neoplastic lymphocytes arranged in a concentric perivascular pattern that diffusely infiltrate the brain parenchyma (Panel C Hematoxilin & Eosin, original magnification x400). Robust cytoplasmic immunolabeling for CD-20 reveals the B-lymphocyte nature of the tumor (Panel D, original magnification x400). Immunohistochemistry for the Epstein-Barr latent membrane protein (LMP) reveals widely expression by neoplastic lymphocytes (Panel E, original magnification x400). The JCV early protein T-Antigen has been found in the nuclei of neoplastic lymphocytes by immunohistochemistry (Panel F, original magnification x1000). Double labeling immunofluorescence for LMP (Panel G, fluorescein) and T-Antigen (Panel H, rhodamin) demonstrates co-localization of both proteins in the majority of neoplastic cells (Panel I). Panels G, H and I original magnification x1000.

heterogeneous systemic lymphomas, primary CNS lymphomas are a homogeneous group of tumors, with approximately 98% expressing B-cell markers, such as CD20 and CD79a, and exhibit immunoglobulin gene rearrangements, which confirms their monoclonal nature (142). Only in rare and sporadic occasions T-cell lymphomas have been described in the context of HIV-1 infection (143). Figure 4 illustrates the spectrum of radiological, anatomic and histological characteristics of primary CNS lymphomas, including the association of Epstein-Barr virus and JCV.

6.3. Clinical Manifestations

Although primary CNS lymphomas can affect all ages, with a peak in the seventh decade of life, immunocompromised patients develop these malignancies at an earlier age, with the peak incidence at age 38. As is the case with other brain lesions associated with AIDS, the clinical signs and symptoms of primary CNS lymphomas are not specific and depend on the location of the tumors. The most common symptoms and signs include headache and seizures, focal neurological deficits, and psychiatric disorders. Unlike their systemic counterparts, B-cell lymphomas originating in the CNS have a poor prognosis with a median survival of only 3 months after diagnosis (144).

6.4. Physiopathology

The pathogenesis and cell of origin in primary CNS lymphomas is still subject of controversy. Two

theories have been proposed (145, 146). In the first scenario, a population of normal lymphocytes is summoned to the brain by an infectious, likely viral process, where is transformed, likely by oncogenic factors produced by viruses. In the second and less likely scenario, lymphocytes undergo malignant transformation outside the brain, where they eventually become trapped and proliferate, while the primary site remains undetected.

The etiology of primary CNS lymphomas also remains unclear. The Epstein-Barr virus (EBV) appears to play a significant role in the development of primary CNS lymphomas, particularly in HIV-1 infected patients, as molecular studies have demonstrated the presence of EBV genomic sequences in approximately 95% of immunosuppressed individuals, compared to only in about 5% of immunocompetent patients (147, 148, 149). Reinforcing this notion, in vitro experiments have demonstrated the ability of EBV to immortalize B cells in culture, and suggest the role of the viral Latent Membrane Protein (LMP) in the oncogenic process (150). The mechanism of malignant transformation involves LMP protection from apoptosis via Bcl-2 and NFκB pathways, providing EBV with a suitable environment for viral replication and latency (151, 152). Recent studies have implicated the previously described JCV. DNA sequences and expression of viral proteins have been detected in cases of primary CNS lymphoma (153). This is not surprising considering the well-established oncogenic potential of the virus, which has been detected in lymphocytes, most likely in a latent state (154, 155). In support of this theory, severe chromosomal damage has been detected in lymphocytes harboring JCV infection, and the presence of JCV infection seems to disrupt proper DNA repair machinery (156). Interestingly, the mechanism of malignant transformation exerted by both EBV and JCV involves the expression of viral proteins, LMP and T-Antigen respectively, which have the ability to bind, sequester and inactivate tumor suppressors p53 and pRb, leading to dysregulation of the cell cycle and uncontrolled proliferation (157, 158, 159, 160). Reactivation of viral promoters under immunosuppressive conditions may be responsible for the cascade of events leading to malignant transformation and to the development of primary CNS lymphomas.

7. ACKNOWLEDGMENTS

8. REFERENCES

1. Albright AV, Soldan SS, & Gonzalez-Scarano F: Pathogenesis of human immunodeficiency virus-induced neurological disease. *J Neurovirol* 9: 222-227 (2003)

2. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vezinet-Brun F, Rouzioux C, Rozenbaum W, & Montagnier L: Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 220: 868-871 (1983)

3. Cullen BR: Regulation of gene expression in the human immunodeficiency virus type 1. *Adv Virus Res* 40: 1-17 (1991)

4. Malim MH, & Cullen BR: HIV-1 structural gene expression requires the binding of multiple Rev monomers to the viral RRE: implications for HIV-1 latency. *Cell* 65: 241-248 (1991)

5. Cohen EA, Terwilliger EF, Sodroski JG, & Haseltine WA. Identification of a protein encoded by the vpu gene of HIV-1. *Nature* 334: 532-534 (1988)

6. Cullen BR: Trans-activation of human immunodeficiency virus occurs via a bimodal mechanism. *Cell* 46: 973-982 (1986)

7. Ogawa K, Shibata R, Kiyomasu T, Higuchi I, Kishida Y, Ishimoto A, & Adachi A: Mutational analysis of the human immunodeficiency virus vpr open reading frame. *J Virol* 63: 4110-4114 (1989)

8. Ahmad N, & Venkatesan S: Nef protein of HIV-1 is a transcriptional repressor of HIV-1 LTR. *Science* 241: 1481-1485 (1988)

9. Brew BJ, Rosenblum M, & Price RW. AIDS dementia complex and primary HIV brain infection. *J Neuroimmunol* 20: 133-140 (1988)

10. Price RW, & Brew BJ: The AIDS dementia complex. *J Infect Dis* 158: 1079-1083 (1988)

11. Kato T, Hirano A, Llena JF, & Dembitzer HM: Neuropathology of acquired immune deficiency syndrome (AIDS) in 53 autopsy cases with particular emphasis on microglial nodules and multinucleated giant cells. *Acta Neuropathol (Berl)* 73: 287-294 (1987)

12. Everall IP, Luthert PJ, & Lantos PL: Neuronal loss in the frontal cortex in HIV infection. *Lancet* 337: 1119-11121 (1991)

13. Johnson RT, Glass JD, McArthur JC, & Chesebro BW: Quantitation of human immunodeficiency virus in brains of demented and nondemented patients with acquired immunodeficiency syndrome. *Ann Neurol* 39: 392-395 (1996)

14. Cooper DA, Gold J, Maclean P, Donovan B, Finlayson R, Barnes TG, Michelmore HM, Brooke P, & Penny R: Acute AIDS retrovirus infection. Definition of a clinical illness associated with seroconversion. *Lancet* 1: 537-540 (1985)

15. McArthur JC, Selnes OA, Glass JD, Hoover DR, & Bacellar H: HIV dementia. Incidence and risk factors. *Res Publ Assoc Res Nerv Ment Dis* 72: 251-272 (1994)

16. Navia BA: Clinical and biologic features of the AIDS dementia complex. *Neuroimaging Clin N Am* (1997); 7: 581-592

17. Price RW: Neurological complications of HIV infection. *Lancet* 348: 445-452 (1996)

18. Gabuzda DH, Ho DD, de la Monte SM, Hirsch MS, Rota TR, & Sobel RA: Immunohistochemical identification of HTLV-III antigen in brains of patients with AIDS. *Ann Neurol* 20: 289-295 (1986)

19. Nottet HS, Persidsky Y, Sasseville VG, Nukuna AN, Bock P, Zhai QH, Sharer LR, McComb RD, Swindells S, Soderland C, & Gendelman HE: Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain. *J Immunol* 156: 1284-1295 (1996)

20. Haase AT: Pathogenesis of lentivirus infections. *Nature* 322: 130-136 (1986)

21. Peluso R, Haase A, Stowring L, Edwards M, & Ventura P: A Trojan Horse mechanism for the spread of visna virus in monocytes. *Virology* 147: 231-236 (1985)

22. Wiley CA, Schrier RD, Nelson JA, Lampert PW, & Oldstone MB: Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. *Proc Natl Acad Sci USA* 83: 7089-7093 (1986)

23. Takahashi K, Wesselingh SL, Griffin DE, McArthur JC, Johnson RT, & Glass JD: Localization of HIV-1 in human brain using polymerase chain reaction / in situ hybridization and immunocytochemistry. *Ann Neurol* 39: 705-711 (1996)

24. Cosenza MA, Zhao ML, Si Q, & Lee SC: Human brain parenchymal microglia express CD14 and CD45 and are productively infected by HIV-1 in HIV-1 encephalitis. *Brain Pathol* 12: 442-455 (2002)

25. Fischer-Smith T, Croul S, Adeniyi A, Rybicka K, Morgello S, Khalili K, & Rappaport J: Macrophage / microglial accumulation and proliferating cell nuclear antigen expression in the central nervous system in human immunodeficiency virus encephalopathy. *Am J Pathol* 164: 2089-2099 (2004)

26. Sharer LR, Cho ES, & Epstein LG: Multinucleated giant cells and HTLV-III in AIDS encephalopathy. *Hum Pathol* 16: 760 (1985)

27. Brack-Werner R: Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis. *AIDS* 13: 1-22 (1999)

28. Sabri F, Tresoldi E, Di Stefano M, Polo S, Monaco MC, Verani A, Fiore JR, Lusso P, Major E, Chiodi F, & Scarlatti G: Nonproductive human immunodeficiency virus type 1 infection of human fetal astrocytes: independence from CD4 and major chemokine receptors. *Virology* 264: 370-384 (1999)

29. Kanmogne GD, Kennedy RC, & Grammas P. HIV-1 gp120 proteins and gp160 peptides are toxic to brain endothelial cells and neurons: possible pathway for HIV entry into the brain and HIV-associated dementia. *J Neuropathol Exp Neurol* 61: 992-1000 (2002)

30. Perez A, Probert AW, Wang KK, & Sharmeen L: Evaluation of HIV-1 Tat induced neurotoxicity in rat cortical cell culture. *J Neurovirol* 7: 1-10 (2001)

31. Piller SC, Jans P, Gage PW, & Jans DA: Extracellular HIV-1 virus protein R causes a large inward current and cell death in cultured hippocampal neurons: implications for AIDS pathology. *Proc Natl Acad Sci USA* 95: 4595-4600 (1998)

32. Garden GA, Budd SL, Tsai E, Hanson L, Kaul M, D'Emilia DM, Friedlander RM, Yuan J, Masliah E, Lipton & SA: Caspase cascades in human immunodeficiency virus-associated neurodegeneration. *J Neurosci* 22: 4015-4024 (2002)

33. Bukrinsky MI, Nottet HS, Schmidtmayerova H, Dubrovsky L, Flanagan CR, Mullins ME, Lipton SA, & Gendelman HE: Regulation of nitric oxide synthase activity in human immunodeficiency virus type 1 (HIV-1)-infected monocytes: implications for HIV-associated neurological disease. *J Exp Med* 181: 735-745 (1995)

34. Dawson VL, Dawson TM, London ED, Bredt DS, & Snyder SH: Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc Natl Acad Sci USA* 88: 6368-6371 (1991)

35. Tyor WR, Glass JD, Griffin JW, Becker PS, McArthur JC, Bezman L, & Griffin DE: Cytokine expression in the brain during the acquired immunodeficiency syndrome. *Ann Neurol* 31: 349-360 (1992)

36. Vitkovic L, da Cunha A, & Tyor WR: Cytokine expression and pathogenesis in AIDS brain. *Res Publ Assoc Res Nerv Ment Dis* 72: 203-22 (1994)

37. Glass JD, Wesselingh SL, Selnes OA, & McArthur JC: Clinical-neuropathologic correlation in HIV-associated dementia. *Neurology* 43: 2230-2237 (1993)

38. Navia BA, Cho ES, Petito CK, & Price RW: The AIDS dementia complex: II. Neuropathology. *Ann Neurol* 19: 525-535 (1986)

39. Berger JR, & Concha M: Progressive Multifocal Leukoencephalopathy: The evolution of a disease once considered rare. *J Neurovirol* 1: 5-18 (1995)

40. Holman RC, Torok TJ, Belay ED, Janssen RS, & Schonberger LB: Progressive multifocal leukoencephalopathy in the United States, 1979-1994: increased mortality associated with HIV infection. *Neuroepidemiology* 17: 303-309 (1998)

41. Åström KE, Mancall EL, & Richardson EP Jr: Progressive multifocal leukoencephalopathy; a hitherto unrecognized complication of chronic lymphocytic leukemia and lymphoma. *Brain* 81: 93-111 (1958)

42. Richardson EP Jr: Progressive multifocal leukoencephalopathy. *New Engl J Med* 265: 815-823 (1961)

43. Schwerdt PR, Schwerdt CE, Silverman L, & Rubinstein LJ: Virions associated with progressive multifocal leukoencephalopathy. *Virology* 29: 511-514 (1966)

44. Silverman L, & Rubinstein LJ: Electron Microscopic Observations on a Case of Progressive Multifocal Leukoencephalopathy. *Acta Neuropathologica* 5: 215-224 (1965)

45. ZuRhein GM, & Chow SM: Particles resembling papovavirions in human cerebral demyelinating disease. *Science* 148: 1,477-1,479 (1965)

46. ZuRhein GM: Polyoma-like virions in a human demyelinating disease. *Acta Neuropathol* (1967); 8: 57-68

47. Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, & Dessel BH: Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet* 1: 1257-1260 (1971)

48. London WT, Houff SA, Madden DL, Fuccillo DA, Gravell M, Wallen WC, Palmer AE, Sever JL, Padgett BL, Walker DL, ZuRhein GM, & Ohashi T: Brain tumors in owl monkeys inoculated with a human polyomavirus (JC virus). *Science* 201: 1246-1249 (1978)

49. Ohsumi S, Motoi M, & Ogawa K: Induction of undifferentiated tumors by JC virus in the cerebrum of rats. *Acta Pathol Jpn* 36: 815-825 (1986)

50. Varakis J, ZuRhein GM, Padgett BL, & Walker DL: Induction of peripheral neuroblastomas in Syrian hamsters after injection as neonates with JC virus, a human polyoma virus. *Cancer Res* 38: 1718-1722 (1978)

51. Walker DL, Padgett BL, Zu-Rhein GM, Albert AE, & Marsh RF: Human papovavirus (JC): Induction of brain tumors in hamsters. *Science* 181: 674-676 (1973)

52. Zu Rhein GM, & Varakis JN: Perinatal induction of medulloblastomas in Syrian golden hamsters by a human

polyomavirus (JC). *Natl Cancer Inst Monogr* 51: 205-208 (1979)

53. Taguchi F, Kajioka J, & Miyamura T: Prevalence rate and age acquisition of antibodies against JC virus and BK virus in human sera. *Microbiol Immunol* 26: 1057-1064 (1982)

54. Walker DL, & Padgett BL: The epidemiology of human polyomaviruses. In: Sever JL, and Madden D (eds.) Polyomaviruses and Human Neurological Disease. Alan R Liss Inc. New York. pp 99-106

55. Padgett BL, & Walker DL: Prevalence of antibodies in human sera against JC virus, an isolate from a case of Progressive Multifocal Leukoencephalopathy. *J Infect Dis* 127: 467-470 (1973)

56. Coleman DV, Gardner SD, & Field AM: Human polyomavirus infection in renal allograft recipients. *Br Med J* 3: 371-375 (1973)

57. Coleman DV, Wolfendale MR, Daniel RA, Dhanjal NK, Gardner SD, Gibson PE, & Field AM: A prospective study on human polyomavirus infection in pregnancy. *J Infect Dis* 142: 1-8 (1980)

58. Houff SA, Major EO, Kats D, Kufta CV, Sever JL, Pittaluga S, Roberts JR, Gitt J, Saini N, & Lux W: Involvement of JC virus infected mononuclear cells from the bone marrow and spleen in the pathogenesis of progressive multifocal leukoencephalopathy. *New Engl J Med* 318: 301-305 (1988)

59. Tornatore C, Berger JR, Houff SA, Curfman B, Meyers K, Winfield D, & Major EO: Detection of JC virus DNA in peripheral lymphocytes from patients with and without progressive multifocal leukoencephalopathy. *Ann Neurol* 31: 456-462 (1992)

60. Monaco MC, Atwood WJ, Gravell M, Tornatore CS, & Major EO: JC Virus infection of hematopoietic progenitor cells, primary B-lymphocytes, and tonsillar stromal cells: implications for viral latency. *J Virol* 70: 7004-7012 (1996)

61. Frisque RJ, & White FA III: The molecular biology of JCV, causative agent of progressive multifocal leukoencephalopathy. In: RP Roos (ed). *Molecular Neurovirology,* (1992); Humana Press, Totowa, NJ, pp 25-158

62. Salunke DM, Caspar DL, & Garcea RL: Self-assembly of purified polyomavirus capsid protein VP1. *Cell* 46: 895-904 (1986)

63. Adams JH, & Short IA: Progressive multifocal leukoencephalopathy. *Scot Med J* 10: 195-202 (1965)

64. Del Valle L, Croul S, Morgello S, Amini S, Rappaport J, & Khalili K: Detection of HIV-1 Tat and JCV Capsid Protein, VP1, in AIDS Brain with Progressive Multifocal Leukoencephalopathy. *J Neurovirol* 6: 221-228 (2000)

65. Major EO, Amemiya K, Tornatore CS, Houff SA, & Berger JR: Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC Virus induced demyelinating disease of the human brain. *Clin Micro Rev* 5: 49-73 (1992)

66. Aksamit AJ: Progressive Multifocal Leukoencephalopathy: A Review of the Pathology and Pathogenesis. *Microsc Res Tech* 32: 302-311 (1995)

67. Albrecht H, Hoffmann C, Degen O, Stoehr A, Plettenberg A, Mertenskotter T, Eggers C, & Stellbrink HJ: Highly active antiretroviral therapy significantly improves the prognosis of patients with HIV-associated progressive multifocal leukoencephalopathy. *AIDS* 12: 1149-1154 (1998)

68. Liu CK, Wei G, & Atwood WJ: Infection of glial cells by the human polyomavirus JC is mediated by an N-linked glycoprotein containing terminal alpha (2-6)-linked sialic acids. *J Virol* 72: 4643-4649 (1998)

69. Eash S, Tavares R, Stopa EG, Robbins SH, Brossay L, & Atwood WJ: Differential distribution of the JC virus receptor-type sialic acid in normal human tissues. *Am J Pathol* 164: 419-428 (2004)

70. Elphick GF, Querbes W, Jordan JA, Gee GV, Eash S, Manley K, Dugan A, Stanifer M, Bhatnagar A, Kroeze WK, Roth BL, & Atwood WJ: The human polyomavirus, JCV, uses serotonin receptors to infect cells. *Science* 306: 1380-1383 (2004)

71. Pho MT, Ashok A, & Atwood WJ: JC virus enters human glial cells by clathrin-dependent receptor-mediated endocytosis. *J Virol* 74: 2288-2292 (2000)

72. Rhodes RH, Ward JM, Walker JL, & Russ AA: Progressive Multifocal Leukoencephalopathy and retroviral encephalitis in acquired immunodeficiency syndrome. *Arc Pathol Lab Med* 112: 1,207-1,213 (1988)

73. Liu Y, Sune C, & Garcia-Blanco MA: Human immunodeficiency virus type 1 Tat-dependent activation of an arrested RNA polymerase II elongation complex. *Virology* 255: 337-346 (1999)

74. Cullen BR: The HIV-1 Tat protein: an RNA sequence-specific processivity factor? *Cell* 63: 655-657 (1990)

75. Frankel AD: Activation of HIV transcription by Tat. *Curr Opin Genet Dev* 2: 293-298 (1992)

76. Frankel AD, & Pabo CO: Cellular uptake of the Tat protein from human immunodeficiency virus. *Cell* 55: 1,189-1,193 (1988)

77. Chowdhury M, Taylor JP, Tada H, Rappaport J, Wong-Staal F, Amini S, & Khalili K: Regulation of the human neurotropic virus promoter by JCV T-antigen and HIV-1 Tat protein. *Oncogene* 5: 1737-1742 (1990)

78. Tada H, Rappaport J, Lashgari M, Amini S, Wong-Saal F, & Khalili K: Transactivation of the JC Virus late promoter by the Tat protein of type 1 human immunodeficiency virus in glial cells. *Proc Natl Acad Sci USA* 87: 3,479-3,483 (1990)

79. Bicanic T, & Harrison TS: Cryptococcal meningitis. *Br Med Bull* 72: 99-118 (2005)

80. Wright D, Schneider A, & Berger J: Central nervous system opportunistic infections. *Neuroimag Clin North Am* 7: 513-525 (1997)

81. Mamidi A, DeSimone JA, & Pomerantz RJ: Central nervous system infections in individuals with HIV-1 infection. *J Neurovirol* 8: 158-167 (2002)

82. Kumarasamy N, Vallabhaneni S, Flanigan TP, Mayer KH, & Solomon S: Clinical profile of HIV in India. *Indian J Med Res* 121: 377-394 (2005)

83. Chariyalertsak S, Sirisanthana T, Saengwonloey O, & Nelson K: Clinical presentation and risk behaviors of patients with acquired immunodeficiency syndrome in Thailand, 1994-1998: Regional variation and temporal trends. *Clin Infect Dis* 32: 955-962 (2001)

84. Holmes CB, Losina E, Walensky RP, Yazdanpanah Y, & Freedberg K: Review of human immunodeficiency virus type 1-related opportunistic infections in Sub-Saharan Africa. *Clin Infect Dis* 36: 652-662 (2003)

85. Sanfelice F: Contributo alla morfologia e biologia dei blastomiceti che si sviluppano nei succhi di alcuni frutti. *Ann Igien* 4: 463-495 (1894)

86. Busse O: Über parasitäre zelleinschlüsse und ihre züchtung. *Zentralbl Bakteriol* 16: 175-180 (1894)

87. Mitchell TG, & Perfect JR: Cryptococcosis in the Era of AIDS-100 Years after the Discovery of *Cryptococcus neoformans*. *Clin Microbiol Rev* 8: 515-548 (1995)

88. Kwon-Chung KJ, Polacheck I, & Popkin TJ: Melanin-lacking mutants of *Cryptococcus neoformans* and their virulence for mice. *J Bacteriol* 150: 1414–1421 (1982)

89. Rhodes JC, Polacheck I, & Kwon-Chung KJ: Phenoloxidase activity and virulence in isogenic strains of *Cryptococcus neoformans*. *Infect Immun* 36: 1175–1184 (1982)

90. Bhattacharjee AK, Bennett JE, & Glaudemans CPJ: Capsular polysaccharides of *Cryptococcus neoformans*. *Rev Infect Dis* 6: 619–624 (1984)

91. Coenjaerts FEJ, Walenkamp AME, Mwinzi PN, Scharringa J, Dekker HAT, Van Strijp JAG, Cherniak R, & Hoepelman AIM: Potent inhibition of Neutrophil Migration by Cryptococcal Mannoprotein-4-Induced Desensitization. *J Immunol* 167: 3988-3995 (2001)

92. Dykstra MA, Friedman L, & Murphy JW: Capsule size of *Cryptococcus neoformans*: control and relationship to virulence. *Infect Immun* 16: 129–135 (1977)

93. Charlier C, Chretien F, Baudrimont M, Mordelet E, Lortholary O, & Dromer F: Capsule structure changes associated with *Cryptococcus neoformans* crossing the blood-brain barrier. *Am J Pathol* 166(2): 421-432 (2005)

94. Ikeda R, Shinoda T, Morita T, & Jacobson ES: Characterization of a phenol oxidase from *Cryptococcus neoformans* var. *neoformans*. *Microbiol Immunol* 37: 759–764 (1993)

95. Polacheck I, & Kwon-Chung KJ: Melanogenesis in *Cryptococcus neoformans*. *J Gen Microbiol* (1988); 134: 1037–1041

96. Jacobson ES, & Emery HS: Catecholamine uptake, melanization, and oxygen toxicity in *Cryptococcus neoformans*. *J Bacteriol* 173: 401–403 (1991)

97. Jacobson ES, & Tinnell SB: Antioxidant function of fungal melanin. *J Bacteriol* 175: 7102–7104 (1993)

98. Polak A: Melanin as a virulence factor in pathogenic fungi. *Mycoses* 33: 215–224 (1990)

99. Wehn SM, Heinz ER, Burger PC, & Boyko OB: Dilated Virchow-Robin spaces in cryptococcal meningitis associated with AIDS: CT and MR findings. *J Comput Assist Tomogr* 13: 756-762 (1989)

100. Everett BA, Kusske JA, Rush JL, & Pribram HW: Cryptococcal infection of the central nervous system. *Surg Neurol* 9: 157-163 (1978)

101. Tien RD, Chu PK, Hesselink JR, Duberg A, & Wiley C: Intracranial cryptococcosis in immunocompromised patients: CT and MR findings in 29 cases. *AJNR Am J Neuroradiol* 12: 283-289 (1991)

102. Caldemeyer KS, Matthews VP, Edwards-Brown MK, & Smith RR: Central Nervous System Cryptococcosis: Parenchymal Calcification and Large Gelatinous Pseudocysts. *AJNR* 18: 107-109 (*1997*)

103. Selby RC, & Lopes NM: Torulomas (cryptococcal granulomata) of the central nervous system. *J Neurosurg* 38: 40-46 (1973)

104. Graybill JR, Sobel J, Saag M, van Der Horst C, Powderly W, Cloud G, Riser L, Hamill R, & Dismukes W: Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. *Clin Infect Dis* 30: 47-54 (2000)

105. Levitz SM: The ecology of *Cryptococcus neoformans* and the epidemiology of cryptococcosis. *Rev Infect Dis* 13: 1163-1169 (1991)

106. Neilson JB, Fromtling RA, & Bulmer GS: *Cryptococcus neoformans:* size range of infectious particles from aerosolized soil. *Infect Immun* 17: 634-638 (1977)

107. Chang YC, Stins MF, McCaffery MJ, Miller GF, Pare DR, Dam T, Paul-Satyasee M, Kim KS & Kwon-Chung KJ: Cyptococcal Yeast Cells Invade the Central Nervous System via Transcellular Penetration of the Blood-Brain Barrier. *Infect Immun* 72: 4985-4995 (2004)

108. Chen SHM, Stins MF, Huang SH, Chen YH, Kwon-Chung KJ, Chang Y, Kim KS, Suzuki K & Jong AY: *Cryptococcus neoformans* induces alterations in the cytoskeleton of human brain microvascular endothelial cells. *J Med Microbiol* 52: 961-970 (2003)

109. Levitz SM, Nong S, Seetoo K, Harrison TS, Speizer R, & Simons E: *Cryptococcus neoformans* resides in an acidic phagolysosome of human macrophages. *Infect Immun* 67: 885-890 (1999)

110. Casadevall A, Steenbergen JN, & Nosanchuk JD: 'Ready made' virulence and 'dual use' virulence factors in pathogenic environmental fungi - the Cryptococcus neoformans paradigm. *Curr Opin Microbiol* 6: 332-337 (2003)

111. Zangerle R, Allerberger F, Pohl P, Fritsch P, & Dierich MP: High risk of developing toxoplasmic encephalitis in AIDS patients seropositive to Toxoplasma gondii. *Med Microbiol Immunol (Berl)* 180: 59-66 (1991)

112. Gray F, Gherardi R, & Scaravilli F: The neuropathology of the acquired immune deficiency syndrome (AIDS). A review. *Brain* 111: 245-266 (1988)

113. Porter SB, & Sande MA: Toxoplasmosis of the central nervous system in the acquired immunodeficiency syndrome. *N Engl J Med* 327: 1643-1648 (1992)

114. Luft BJ, & Remington JS: Toxoplasmic encephalitis in AIDS. *Clin Infect Dis* 15: 211-222 (1992)

115. Renold C, Sugar A, Chave JP, Perrin L, Delavelle J, Pizzolato G, Burkhard P, Gabriel V, & Hirschel B: Toxoplasma encephalitis in patients with the acquired immunodeficiency syndrome. *Medicine (Baltimore)* 71: 224-239 (1992)

116. Nicolle C, & Manceaux L: Sur une infection a corps de Leishman (ou organisme voisins) du gondi. *C R Hebd Seances Acad Sci* 147: 763-766 (1908)

117. Janku J: Pathogenesa a pathologicka anatomie tak nazvaneho vrozeneho kolobomu zlute skvrny v oku normaline velikem a mikrophthalmickem snalezem parazitu v sitnici. Cas Lek Cesk 62: 1021-1027 (1923)

118. de Melo EJT, & de Souza W: A cytochemistry study of the inner membrane complex of the pellicle of tachyzoites of *Toxoplasma gondii*. *Parasitol Res* 83: 252-256 (1997)

119. de Souza W, & Souto-Padrón T: Ultrastructural localization of basic proteins on the conoid, rhoptries and micronemes of *Toxoplasma gondii*. *Z Parasitenkd* 56: 123-129 (1978)

120. Sheffield HG, & Melton ML: The fine structure and reproduction of *Toxoplasma gondii*. *J Parasitol* 54: 209-226 (1968)

121. Sulzer AJ, Strobel PL, Springer EL. Roth IL, & Callaway CS: A comparative electron microscopic study of the morphology of *Toxoplasma gondii* by freeze-etch replication and thin sectioning technique. *J Protozool* 21: 710-714 (1974)

122. Morrissette NS, Murray JM, & Roos DS: Subpellicular microtubules associate with an intramembranous particle lattice in the protozoan parasite *Toxoplasma gondii*. *J Cell Sci* 110: 35-42 (1997)

123. Nichols BA, Chiappino ML, & O'Connor GR: Secretion from the rhoptries of *Toxoplasma gondii* during host-cell invasion. *J Ultrastruct Res* 83: 85-98 (1983)

124. Saffer LD, Mercareau-Puijalon O, Dubrematz JF, & Schwartzman JD. Localization of a *Toxoplasma gondii* rhoptry protein by immunoelectron microscopy during and after host cell penetration. *J Protozool* 39: 526-530 (1992)

125. Dubey JP, Lindsay DS, & Speer CA. Structures of Toxoplasma gondii tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clin Microbiol Rev* 11: 267-299 (1998)

126. Handler M, Ho V, Whelan M, & Budzilovich G: Intracerebral toxoplasmosis in patients with acquired immune deficiency syndrome. *J Neurosurg* 59: 994-1001 (1983)

127. Rose I: Morphology and diagnostics of human toxoplasmosis. *Gen Diagn Pathol* 142: 257-270 (1997)

128. Strittmatter C, Lang W, Wiestler OD, & Kleihues P: The changing pattern of human immunodeficiency virus-associated cerebral toxoplasmosis: a study of 46 postmortem cases. *Acta Neuropathol (Berl)* 83: 475-481 (1992)

129. Gray F, Gherardi R, Wingate E, Wingate J, Fenelon G, Gaston A, Sobel A, & Poirier J: Diffuse encephalitic cerebral toxoplasmosis in AIDS. Report of four cases. *J Neurol* 236: 273-277 (1989)

130. Benenson MW, Takafuji ET, Lemon SM, Greenup RL, & Sulzer AJ: Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. *N Engl J Med* 307: 666-669 (1982)

131. Bowie WR, King AS, Werker DH, Isaac-Renton JL, Bell A, Eng SB, & Marion SA: Outbreak of toxoplasmosis associated with municipal drinking water. *Lancet* 350: 173-177 (1997)

132. Teutsch SM, Juranek DD, Sulzer A, Dubey JP, & Sikes RK. Epidemic toxoplasmosis associated with infected cats. *N Engl J Med* 300: 695-699 (1979)

133. Krick JA, & Remington JS: Toxoplasmosis in the adult - an overview. *N Engl J Med* 298: 550-553 (1978)

134. Beral V: The epidemiology of cancer in AIDS patients. *AIDS* 5 Suppl 2: S99-103 (1991)

135. Levine AM: Acquired immunodeficiency syndrome-related lymphoma. *Blood* 80: 8-20 (1992)

136. Biggar RJ, & Rabkin CS: The epidemiology of AIDS-related neoplasms. *Hematol Oncol Clin North Am* 10: 997-1010 (1996)

137. Rabkin CS: Epidemiology of AIDS-related malignancies. *Curr Opin Oncol* 6: 492-496 (1994)

138. Camilleri-Broët S, Davi F, Feuillard J, Seilhean D, Michiels JF, Brousset P, Epardeau B, Navratil E, Mokhtari K, Bourgeois C, Marelle L, Raphael M, & Hauw JJ: AIDS-related primary brain lymphomas: histopathologic and immunohistochemical study of 51 cases. *Hum Pathol* 28: 367-374 (1997)

139. Schlegel U, Schmidt-Wolf IGH, & Deckert M: Primary CNS lymphoma: clinical presentation, pathological classification, molecular pathogenesis and treatment. *J Neurol Sci* 181: 1-12 (2000)

140. Gill PS, Levine AM, Meyer PR, Boswell WD, Burkes RL, Parker JW, Hofman FM, Dworsky RL, & Lukes RJ: Primary central nervous system lymphoma in homosexual men. Clinical, immunologic, and pathologic features. *Am J Med* 78: 742-748 (1985)

141. So YT, Beckstead JH, & Davis RL: Primary central nervous system lymphoma in acquired immune deficiency syndrome: a clinical and pathological study. *Ann Neurol* 20: 566-572 (1986)

142. Carbone A, Dolcetti R, Gloghini A, Maestro R, Vaccher E, di Luca D, Tirelli U, & Boiocchi M. Immunophenotypic and molecular analyses of acquired immune deficiency syndrome-related and Epstein-Barr virus-associated lymphomas: a comparative study. *Hum Pathol* 27: 133-146 (1996)

143. Bindal AK, Blisard KS, Melin-Aldama H, & Warnick RE. Primary T-cell lymphoma of the brain in acquired immunodeficiency syndrome: case report. *J Neurooncol* 31: 267-271 (1997)

144. Remick SC, Diamond C, Migliozzi JA, Solis O, Wagner H Jr, Haase RF, & Ruckdeschel JC: Primary central nervous system lymphoma in patients with and without the acquired immune deficiency syndrome. A retrospective analysis and review of the literature. *Medicine* 69: 345-60 (1990)

145. Hochberg FH, & Miller DC. Primary central nervous system lymphoma. *J Neurosurg* 68: 835-853 (1988)

146. Shibata S: Sites of origin of primary intracerebral malignant lymphoma. *Neurosurgery* 25: 14-19 (1989)

147. Bignon YJ, Clavelou P, Ramos F, Jouvet A, Tommasi M, Tournilhac M, Dastugue B, & Plagne R: Detection of Epstein-Barr virus sequences in primary brain lymphoma without immunodeficiency. *Neurology* 41: 1152-1153 (1991)

148. List AF, Greer JP, Cousar JP, Johnson DH, Greco FA, Volsky DJ, & Purtilo DT: Primary brain lymphoma in the immunocompetent host: relation to Epstein-Barr virus. *Mod Pathol* 3: 609-612 (1990)

149. MacMahon EM, Glass JD, Hayward SD, Mann RB, Becker PS, Charache P, McArthur JC, & Ambinder RF: Epstein-Barr virus in AIDS-related primary central nervous system lymphoma. *Lancet* 338: 969-973 (1991)

150. Cesarman E: Epstein-Barr virus (EBV) and lymphomagenesis. *Front Biosci* 7: e58-65 (2002)

151. Kenney JL, Guinness ME, Curiel T, & Lacy J: Antisense to the epstein-barr virus (EBV)-encoded latent membrane protein 1 (LMP1) suppresses LMP1 and bcl-2 expression and promotes apoptosis in EBV-immortalized B cells. *Blood* 92: 1721-1727 (1998)

152. Eliopoulos AG, Stack M, Dawson CW, Kaye KM, Hodgkin L, Sihota S, Rowe M, and Young LS: Epstein-Barr virus-encoded LMP1 and CK40 mediate IL-6 production in epithelial cells via an NF-kappaB pathway involving TNF receptor-associated factors. *Oncogene* 14: 2899-2916 (1997)

153. Del Valle L, Enam S, Lara C, Miklossy J, Khalili K, & Gordon J: Primary central nervous system lymphoma expressing the human neurotropic polyomavirus, JC virus, genome. *J Virol* 78: 3462-3469 (2004)

154. Gallia GL, Houff SA, Major EO, & Khalili K: JC virus infection of lymphocytes-revisited. *J. Infect. Dis* 176: 1603-1609 (1997)

155. Tornatore C, Berger JR, Houff SA, Curfman B, Meyers K, Winfield D, & Major EO: Detection of JC virus DNA in peripheral lymphocytes from patients with and without progressive multifocal leukoencephalopathy. *Ann. Neurol* 31: 454-462 (1992)

156. Neel JV, Major EO, Awa AA, Glover T, Burgess A, Traub R, Curfman B, & Satoh C: Hypothesis: "Rogue cell"-type chromosomal damage in lymphocytes is associated with infection with the JC human polyoma virus and has implications for oncogenesis. *Proc Natl Acad Sci USA* 93: 2690-2695 (1996)

157. Allday MJ, Sinclair A, Parker G, Crawford DH, & Farrell PJ: Epstein-Barr virus efficiently immortalizes human B cells without neutralizing the function of p53. *EMBO J* 14: 1382-1391 (1995)

158. Bollag B, Prins C, Snyder EL, & Frisque RJ: Purified JC virus T and T' proteins differentially interact with the retinoblastoma family of tumor suppressor proteins. *Virology* 274: 165-178 (2000)

159. Cannell EJ, Farrell PJ, & Sinclair AJ: Epstein-Barr virus exploits the normal cell pathway to regulate Rb activity during the immortalisation of primary B-cells. *Oncogene* 13: 1413-1421 (1996)

160. Krynska B, Del Valle L, Gordon J, Otte J, Croul S, & Khalili K: Identification of a novel p53 mutation in JCV-induced mouse medulloblastoma. *Virology* 274: 65-74 (2000)

Send correspondence to: Dr Luis Del Valle, Center for Neurovirology and Cancer Biology, Laboratory of Neuropathology and Molecular Pathology, Temple University, 1900 North 12th Street, Suite 240, Philadelphia, Pennsylvania 19122 USA, Tel: 215-204-0631, Fax: 215-204-0679, E-mail: lvalle@temple.edu