Cytomegalovirus Encephalitis: Neuropathological Comparison of the Guinea Pig Model with the Opportunistic Infection in AIDS

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The AIDS epidemic has transformed the importance of cytomegalovirus (CMV) as a pathogen for the adult human central nervous system (CNS). At autopsy, about 25 percent of AIDS cases have cytopathologic evidence of CNS infection by CMV. Since almost nothing is known of the host CNS-viral interactions, we have developed a laboratory model of CMV infection of the brain in the guinea pig. In the present paper, we review the syndromes of CMV infection of the human CNS and compare the neuropathological findings of the opportunistic CMV brain infection in AIDS with the model. Destructive meningoencephalitis, perivascular infiltrates, and subependymal inflammation are found in both, but the glial nodule is the most characteristic feature of each. Thus, we demonstrate that the model faithfully reflects the histopathology of the human disease. Furthermore, since we have found that CNS infection is achieved following systemic infection in the guinea pig, the model recapitulates the sequence of infection in humans.

INTRODUCTION

Prior to the AIDS epidemic, cytomegalovirus (CMV) was not a common pathogen for the adult human central nervous system (CNS). The most important CMV infection of the CNS was the syndrome of cytomegalic inclusion disease of newborn infants. In adults, evidence for CMV infection of the CNS was largely restricted to immunosuppressed patients, particularly allograft recipients. Cases in immunologically intact adults were extremely rare, and those with documentation of virus isolation from the CNS were rarer still.

Cytomegalic Inclusion Disease

Cytomegalic inclusion disease is a devastating multisystem viral infection acquired in utero. Significant hepatic, splenic, and hematologic manifestations occur in addition to CNS involvement [1]. Neurological manifestations include mental retardation, spasticity, and seizures. On gross examination of the brain, microcephaly, ventricular enlargement, cortical atrophy, and polycystic encephalomalacia may be found [2]. The brunt of the CNS disease is borne by the subependymal germinal matrix of the lateral ventricles; however, the majority of infants congenitally infected with CMV are

Abbreviations: AIDS: acquired immune deficiency syndrome CMV: cytomegalovirus CNS: central nervous system CSF: cerebrospinal fluid DHPG: 9-(1,3-dihydroxy-2-propoxymethyl) guanine EEG: electroencephalogram HIV: human immunodeficiency virus i.c.: intracerebral i.p.: intraperitoneal

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asymptomatic at birth. Nonetheless, there is evidence for variable intellectual dysfunction and hearing loss in prospectively studied infants positive for IgM antibody at birth [3]. Hence, there is a range of neurologic dysfunction associated with congenital CMV infection, of which cytomegalic inclusion disease is the most severe and most easily recognized.

**Allograft and Tumor Immunosuppression**

In 1965, Schneck reported neuropathological evidence of CMV infection in the brains of patients who had undergone renal transplantation [4]. In 34 transplant cases, 11 were found to have glial nodules, among whom two were also found to have intranuclear inclusions. No clinical syndrome was identified. Similarly, Dorfman commented on the difficulty of making a diagnosis of cytomegalovirus encephalitis in renal allograft recipients because of the potential multiple causes of diffuse encephalopathy [5]. Furthermore, the cerebrospinal fluid (CSF) was normal in three of the four cases reported by him. In lethal CMV infection following renal transplantation, the brain is only one of several organs to have evidence of infection [6]. Evidence for CMV infection of the brain has also been reported following other causes of immunosuppression. For example, Koeppen et al. have reported the case of a 51-year-old man treated with chemotherapy for a small-cell undifferentiated lymphoma who experienced severe neurological disease and blindness [7]. Autopsy revealed inclusion body retinitis, multiple CNS infarcts associated with occlusive arteritis, and inclusion-bearing cells around foci of necrosis. Hence CMV infection of the CNS may occur in the setting of various causes of immunosuppression.

**Immunocompetent Adults**

Clinically apparent CMV infection of the CNS in immunocompetent adults appears to be a very rare event and the diagnosis difficult to establish. CMV antibody levels fluctuate within normal individuals [8], and CMV replication may be stimulated by a variety of nonspecific immunological challenges. Reliance on serum antibody titers or isolation of the virus outside the CNS, such as from the urine, may be misleading because these may not correlate with the presence of CMV in the brain. Four of the six previously reported cases reviewed by Siegman-Igra et al. relied on serum antibody or virus isolation away from the CNS for diagnosis [9]. Their own case demonstrated a serum antibody rise and the presence of antibody in undiluted CSF. More secure diagnostic findings include local production of anti-CMV antibody in the CSF, and virus isolation from the CSF or the brain. Phillips et al. have reported two cases in immunologically competent adults with CNS viral isolates [10]. CMV was isolated from the CSF of a 52-year-old male with headaches, seven white cells in the CSF, mild right arm weakness, a clouded sensorium, and focal electroencephalogram (EEG) slowing. The second case was that of a 20-year-old woman in whom headache, fever, seizures, CSF pleocytosis, focal EEG abnormalities, and progressive neurologic dysfunction prompted a brain biopsy from which CMV was isolated. Histologically the biopsy revealed microglial and astrocytic proliferation. Both cases demonstrated improvement following treatment with vidarabine.

**AIDS**

As of 1981, at the start of the acquired immunodeficiency syndrome (AIDS) epidemic, CMV was regarded as a pathogen for the fetal CNS and the CNS of
immunocompromised patients, primarily renal allograft recipients. After the start of the epidemic, two facts became clear. First, disseminated CMV infection was the rule in AIDS patients [11] and, second, neurologic complications in AIDS were common [12]. Subsequently, large neuropathological autopsy studies revealed that CMV was the most common opportunistic infection of the CNS in AIDS [13,14]. There have, however, been a variety of clinical-pathological manifestations associated with CMV infection of the CNS, ranging from asymptomatic infection to necrotizing encephalitis [15,16]. Furthermore, little is known of the important host-viral interactions which facilitate CMV brain infection, due in no small part to the absence of a well-characterized animal model. Hence we have developed an experimental model of CMV encephalitis in the guinea pig [17,18]. Because of the principal neuropathologic feature, as in the human infection, we have termed the model glial nodule encephalitis. In this section, we review the current state of knowledge of CMV encephalitis in AIDS patients and in the following section compare the neuropathological characteristics of the opportunistic infection in AIDS patients with the animal model.

Separation from the AIDS Dementia Complex

Soon after the onset of the AIDS epidemic, Snider et al. defined many of the neurological complications seen in AIDS patients [12]. Prominent among these conditions was subacute encephalitis (subacute encephalopathy) manifested by psychomotor retardation and progressing over weeks or months to severe dementia. Based on the presence of disseminated CMV and histopathologic findings such as microglial nodules and inclusions, the authors proposed that CMV might be the cause of this syndrome. Nielsen et al. supported the role of CMV in the subacute encephalitis based on similar autopsy findings in 19 patients [19]; however, in 1985, Shaw et al. demonstrated the presence of HTLV-III (HIV) by in situ hybridization in five of 15 cases [20]. Richard Price and his colleagues performed a careful clinical and pathological analysis to demonstrate that this was a new entity, most probably due to HIV itself, which they termed the AIDS dementia complex [21–23]. These investigators analyzed autopsy material from demented patients according to the presence of multinucleated cells and also the number of microglial nodules [23]. In the subgroup characterized by the absence of multinucleated cells and the presence of abundant microglial nodules, nine of ten cases had cytomegalic inclusions; however, these findings bore no anatomic relationship to white matter pallor and vacuolation, which the authors viewed as key to the pathogenesis of the clinical syndrome. Thus the AIDS dementia complex, as defined by Navia and Price and their colleagues, is based on white matter and subcortical changes and is thought to result from HIV infection of the brain.

Clinical Manifestations of CMV Encephalitis in AIDS

Studies based on histopathologic evidence for CMV, principally cytomegalic inclusion-bearing cells, reveal a spectrum of associated neurological disorders. In a retrospective review of AIDS cases found at autopsy to have evidence for CMV, Post and her colleagues identified progressive encephalopathy as a common presentation [24]. Morgello et al. found that dementia was present in cases with CMV infection characterized by either glial nodules or focal parenchymal necrosis [15]. In addition to dementia, a heterogeneous group of clinical conditions associated with CMV infection of the brain in AIDS patients emerges on review of the literature. The clearest
clinical-pathological correlation of CMV infection in the report of Morgello and her colleagues was necrotizing radiculomyelitis presenting clinically as ascending myelopathy [15]. Illustrative case material presented by Vinters et al. displayed a wide range of clinical manifestations [16]. These included progressive confusion and mental status deterioration, myelopathy, focal cerebral dysfunction, and meningoradiculitis. The authors commented on the heterogeneous nature of CMV-associated pathology, and the coexistence of other pathological processes, including evidence for co-localization of HIV and CMV.

In addition to retrospective clinical pathological analysis, response to antiviral therapy has been used to characterize CMV-associated CNS syndromes. Masdeu et al. have reported the case of a 43-year-old male AIDS patient with multifocal CNS dysfunction which was shown by stereotactic biopsy to be caused by CMV [25]. Experimental antiviral therapy with a guanine derivative (BWB759U) was associated with improvement of focal neurological signs and a decrease in the size of the lesions found on magnetic resonance imaging. Fiala et al. have reported clinical improvement with the use of the anti-CMV agent 9-(1,3-dihydroxy-2-propoxymethyl) guanine (DHPG) in two cases of acute meningoencephalitis [26]. These authors also commented that CMV might augment HIV by increasing the lysis of cells doubly infected with HIV and CMV.

**CMV as a Cofactor for HIV**

There are pathological and virological data supporting the possibility of CMV serving as a cofactor for HIV infection of the brain. Vinters et al. have found evidence for human immunodeficiency virus (HIV) in close proximity to CMV in several cases of CMV infection of the CNS in AIDS [16]. Using several double-labeling techniques, Nelson et al. have shown a significant incidence of double infection, HIV and CMV, in single cells in the CNS of AIDS patients [27].

There are at least three mechanisms whereby CMV could serve as a cofactor for HIV (Table 1). According to the “Trojan horse” theory, HIV-infected circulating monocytes bring HIV into the brain [28]. CMV infection of the brain, whether transient or established, could serve as a cofactor by attracting HIV-infected monocytes. A second mechanism is the provision of a transactivation protein by CMV to enhance HIV replication [27]. Yet another mechanism is immunological. HIV depletes the CD4 helper-inducer subset of T cells, and CMV induces nonspecific immunosuppression mediated by monocytes [29]. Together, the combined immunosuppression may facilitate infection of the brain by one or both agents.

**GUINEA PIG MODEL**

There are many fundamental questions concerning CMV infection of the CNS about which little is known. For example, it is not known how the CNS is infected during the course of systemic infection, the frequency of clinically inapparent infection, the mechanisms of host defense to prevent CNS infection, and the mechanisms which clear CMV once the CNS has been infected. These and other questions of viral-host interaction must be addressed if a logical approach is to be made in preventing and treating CMV encephalitis in AIDS patients. In light of the extensive experience with the guinea pig CMV model in these laboratories [30], we sought to determine whether the model could be extended to study CMV encephalitis.

Following intracerebral (i.c.) inoculation of young guinea pigs, it was found that the
titer of CMV peaked in the brain in the first week after inoculation [17]. Virus was cleared at three to four weeks, about the time of development of serum-neutralizing antibody. Leptomeningitis developed and peaked in the first week of infection, independent of changes in the brain parenchyma. On cytochemical study, it was found that the predominant cell in the leptomeningitis belonged to the monocyte-macrophage series [18]. Parenchymal changes peaked in the second week post-infection. At higher viral doses i.c., meningoencephalitis with necrosis was found [17]. Both Morgello et al. [15] and Vinters et al. [16] have reported necrotizing encephalitis in the human disease. In the example shown in Fig. 1, taken at autopsy from a patient with AIDS, necrotizing encephalitis with florid inclusion-bearing cells is demonstrated. Macrophages, microglia, and astrocytes were found within the intact parenchyma, whereas abundant macrophages were present in necrotic material. A similar pattern has been observed in the guinea pig, except that lesions with very abundant inclusion-bearing cells have not been observed.

The most characteristic finding in the parenchyma in CMV encephalitis, in humans and in the guinea pig model, is the glial nodule (Fig. 2A). It was found in all 30 cases by Morgello and her colleagues [15] and in 28 of the 30 cases studied by Vinters and his colleagues [16]. The nodules consist of collections of cells, often in a swirled pattern, which appear by histologic criteria to be microglia (rod cells) and macrophages. They are found to be associated with intranuclear inclusion-bearing cells in a low percentage of observations in both the model and the human disease. In the model, the majority of cells, both round and rod cells, reacted positively with a histochemical stain for macrophages [18]. This observation is compatible with data showing that microglia are related to monocytes and macrophages [31]. No T cells were identified in the glial nodules [18]. Thus, we found that the host defense response to CMV infection of the CNS in the guinea pig was dominated by cells belonging to the monocyte series.

Another characteristic parenchymal lesion, subependymal inflammation, is seen in both the human and model (Fig. 2B) diseases. It will be recalled that this is a primary region of pathology in cytomegalic inclusion disease of infants (see above). In both the guinea pig and human, ventriculitis and ventriculoencephalitis can also be observed. CNS vasculitis, manifested by inflammatory cells in the vessel wall, is found in the human disease and in the model. While demyelination has been reported in CMV infection of the CNS in AIDS [32], the observation has not been made consistently and remains anecdotal [16]. In all consistent features then, the model reflects the changes in the human brain.

In recent studies, we have extended our observations to infection of the brain following peripheral inoculation [Booss J, Griffith BP, Kim JH: manuscript in preparation]. Two types of evidence for viral infection of the brain were present following intraperitoneal (i.p.) infection. Dependent on the virus inoculum, both histopathologic changes and virus isolation, or histopathologic changes alone, were
FIG. 1. Opportunistic CMV encephalitis in AIDS. The patient was a 32-year-old black woman with a history of intravenous drug abuse. She had been under treatment for brain biopsy-proven toxoplasmosis at the time of death. The figure demonstrates inflammatory infiltrates, focal necrosis, and scattered cytomegalic cells with prominent intranuclear inclusions. Inset (lower right corner) demonstrates an enlarged view of a cytomegalic cell indicated by an arrowhead in the main figure. Hematoxylin and eosin stain. Magnification of the main figure is × 140, of the inset is × 350.
FIG. 2. CMV encephalitis in the guinea pig model.  
A. A microglial nodule composed of loosely arranged microglial cells and scattered round inflammatory cells. Guinea pig sacrificed 14 days after intraperitoneal infection with $10^4$ TCID$_{50}$. Hematoxylin and eosin stain. Magnification × 420.  
B. Focal infiltration of round nuclear inflammatory cells in the periventricular region. An arrowhead indicates the point of a sudden transition between inflammatory cell-infiltrated and normal-appearing periventricular regions. Guinea pig sacrificed 13 days after intraperitoneal infection with $10^6$ TCID$_{50}$. V, ventricle. Hematoxylin and eosin stain. Magnification × 90.
observed. In contrast to i.c. inoculation, leptomenigitis was minimal, but parenchymal changes were similar (Fig. 2). The guinea pig model thus offers the opportunity to study mechanisms of brain infection following systemic infection and the mechanisms of host defense in the brain. These observations could provide the basis for logical prevention and therapy of CMV infections of the CNS in AIDS patients.

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