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Role of Human Immunodeficiency Virus and Cytomegalovirus in AIDS Encephalitis

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Approximately half of patients with advanced acquired immune deficiency syndrome (AIDS) develop a subcortical dementia. The brains of all autopsies on AIDS patients performed at UCSD between 1982 and 1986 (N = 93) were studied. Neuropathologic changes consistent with a viral encephalitis were present in 54 brains (58%). Human immunodeficiency virus (HIV) antigens were detected in 37 of the brains (40%), most frequently in macrophages, multinucleated giant cells, and endothelial cells. Cytomegalovirus (CMV) was detected in 31 of the brains (33%), 22 of which also contained HIV. Cellular localization of CMV antigens suggests that CMV disseminates to the central nervous system hematogenously where the virus can infect endothelial cells, glia, and neurons. While the temporal course of the appearance of these two viruses within the CNS is not clear, the common simultaneous occurrence of both viruses within the brains of AIDS patients suggests that *in vivo* interaction between them may play a role in the pathogenesis of AIDS-associated encephalitis. Given the significant neurologic symptoms described in AIDS patients, the paucity of viral antigens suggests a pathogenic mechanism of indirect CNS damage rather than direct viral infection. (Am J Pathol 1988, 133:73–81)

A VARIETY OF NEUROLOGIC disorders are seen in patients with acquired immune deficiency syndrome (AIDS). Children with AIDS develop a progressive encephalopathy, pyramidal tract abnormalities, and microcephaly.[1–4] Between 10 and 25% of adults with AIDS or AIDS-related complex (ARC) have neurologic symptoms as a presenting complaint. Approximately 50% will develop an encephalopathy during the terminal phases of their disease.[5–8] Initial neurologic symptoms consist of decreased memory and concentration, with sporadic motor difficulties. The neurologic symptoms progress to a subcortical dementia (termed "AIDS dementia complex" by Navia et al[8]), mutism, and paraplegia in advance stages. Computerized tomographic studies show calcific lesion in the brains of children,[3] but studies in adults have shown only a nonspecific cortical atrophy. Cultures of cerebrospinal fluid (CSF)[7,9] have yielded human immunodeficiency virus (HIV) in two thirds of seropositive patients and cytomegalovirus (CMV) in rare cases with neurologic symptoms.

Neuropathologic evaluation of brains of AIDS patients has revealed a variety of disease processes.[10–12] In addition to the frequently-observed opportunistic infections, a subacute encephalitis of presumed viral origin has been noted in up to 50% of AIDS autopsies and more frequently in autopsies of demented AIDS patients. The term "subacute encephalitis" has been used to describe a wide spectrum of central nervous system (CNS) pathology including microglial nodules (with and without multinucleated giant cells), and collections of perivascular chronic inflammatory cells in the cerebral cortex and spinal cord.[1,4,13–17]

Immunocytochemical and nucleic acid probes have become available recently to define the cause of the subacute encephalitis. Immunocytochemical studies

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proved that many cytomegalic cells within microglial nodules contain CMV antigens; however, the low frequency of nodules was discordant with the observed extent of CNS pathology. Following the demonstration of HIV within the CNS of AIDS patients by Southern blot and *in situ* hybridization,[18] the neurologic symptoms have been attributed to direct infection of the nervous system by HIV. However, several groups[19–25] have shown recently that nervous system cells are not frequently infected by HIV. Morphologic studies have shown virion production only within macrophages and multinucleated giant cells.[26–28]

There are many possible causes of the dementia and CNS histopathology seen in AIDS patients. Direct damage to the CNS could be due to infection by HIV, infection by other viral agents (such as CMV, herpes simplex virus [HSV] JC virus), or a combined effect of these viruses.[29] CNS injury could also result from indirect effects of systemic or local viral infection caused by viral or immune factors. To identify the causes of the frequently observed encephalitis, CNS tissue from persons who died with AIDS were examined for the presence of HIV and other viruses commonly associated with AIDS.

Materials and Methods

Brain tissues were from 93 AIDS patients who underwent autopsy at UCSD medical center and San Diego Veterans Administration Hospital between 1982 and 1986. Brains and spinal cords were removed and immersion-fixed in 20% phosphate-buffered formalin. Tissue fixation conditions were standardized, but the interval between death and postmortem examination varied from 4–48 hours. After 7–10 days of fixation, the brains were sectioned coronally, and representative blocks from 13 standard regions of the brain (frontal, temporal, parietal, and occipital cortex, basal ganglia, thalamus, pineal gland, cerebellum, midbrain, medulla, and three levels of spinal cord) in addition to grossly abnormal regions, were embedded in paraffin. Material for electron microscopy was immersion fixed in Trump's fixative. Sections from the brains that demonstrated histopathologic evidence of viral encephalitis (microglial nodules, mononuclear cell infiltrate) were studied further by immunocytochemical staining.[19,30] Rabbit antiserum against glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), HSV, and factor VIII-related antigen were diluted 1:50 according to commercial protocols (Dako, Santa Barbara, CA). Mouse monoclonal antibody to human alveolar macrophages was a gift from Dr. Allen Gowen (University of Washington, Seattle, WA)[31] and was used at a dilution of 1:2000. Mouse monoclonal antibody to gp41 of HIV was a gift from Drs. Kathy Shriver and Lynn Goldstein at Genetic Systems (Seattle, WA) and was used at a dilution of 1:150. Goat polyclonal antiserum to CMV obtained from Polysciences (Warrington, PA) was used at a dilution of 1:50 (this antisera is specific for cytomegalovirus and does not recognize other herpes viruses [unpublished observations]). After incubation in the primary antiserum for 2 hours at room temperature, sections were extensively washed and then incubated for 1 hour at room temperature with appropriate biotinylated secondary antibodies diluted 1:50 (Tago, Burlingame, CA). Sections were again washed extensively before incubation in either an avidin-biotin-horseradish peroxidase complex (Dako) or an avidin-alkaline phosphatase complex (Miles Laboratories, Naperville, IL). Reactions were developed with either aminoethylcarbazole, diaminobenzidine, or fast blue substrates. For double-label immunocytochemistry, sections were first stained for cellular antigens with the phosphatase reaction using a fast-blue substrate. A second round of antibody staining was performed followed by incubations with nonbiotinylated goat anti-mouse immunoglobulin, mouse anti-peroxidase/peroxidase complex, and aminoethylcarbazole or diaminobenzidine substrate. *In situ* hybridization was performed with a commercially available JC virus probe (ENZO Biochem, New York, NY) and stained according to the manufacturer's protocol. This probe consists of a 4kb fragment of biotinylated JC viral DNA.

Results

The brains of 93 autopsied AIDS patients were examined for the presence and intensity of HIV, CMV, JC virus, and HSV infections. The neuropathologic findings in 54 of the brains demonstrated a subacute encephalitis with microglial nodules and chronic inflammatory cells consistent with a viral cause (Table 1). Immunocytochemistry and *in situ* hybridization detected either HIV, CMV, JC virus as well as combinations of these viruses (Table 2). HSV was not detected although this virus has been reported in CNS tissue from AIDS patients.[32–34] The frequency, distribution, and cell types infected by each of these viruses is presented below.

Human Immunodeficiency Virus

Evidence of HIV infection was seen in brains from 37 of the 93 autopsied AIDS patients (40%). When detected by immunocytochemistry, HIV antigens were observed most frequently within the deep white

or gray matter. The lineage of cells containing HIV antigens was assessed by cell morphology and double-label immunocytochemistry for macrophage markers (Table 3 and Figure 1). The most commonly infected cells were macrophages and multinucleated giant cells (Figure 1A,B), and less commonly were endothelial cells (Figure 1C,D). Of the 37 brains with positive immunostaining for HIV gp41, 36 contained positively staining cells identifiable as macrophages and 21 brains showed positive staining in endothelial cells. In approximately one third of the brains containing HIV, the lineage of occasional cells stained positively for HIV gp41 could not be definitively identified. Because these cells were infrequently found, double-label immunocytochemical identification of their lineage was not feasible; however, their morphology was consistent with either astrocytes or oligodendroglia (Figure 1E,F). Positively stained cells with a glial morphology were a frequent finding in only one case.

Few retroviral virion particles were identified in limited ultrastructural studies. In those observations, retrovirus was identified in extracellular spaces in regions of macrophage infiltration or budding from the surface of multinucleated giant cells.

Cytomegalovirus

By immunocytochemical staining, 31 of the 93 brain specimens (33%) contained CMV antigens. Brains containing CMV showed two different patterns of viral dissemination. The first and most common pattern consisted of a diffuse seeding of microglial nodules (some with central cytomegalic cells) throughout the CNS (gray matter more extensively involved than white matter). The second pattern of viral dissemination consisted of microscopic infarctions containing numerous cytomegalic cells. In some of these regions, endothelial cells of small vessels contained cytomegalic inclusions possibly leading to occlusion of the vessel lumen and subsequent infarction (Figure 2A).

Regardless of the pattern of dissemination, once established within the CNS, CMV infection progressed by direct extension of the above described lesions. Microglial nodules and infarcts appeared to expand into a nidus of CMV infection up to several centimeters in diameter, with numerous cytomegalic cells at the perimeter. By morphologic criteria of cytomegaly, CMV caused a productive infection within neurons, glia, and to a lesser extent endothelial cells. In these brains cells infected by HIV were macrophages surrounding cytomegalic cells. In 10% of the patients, the initial nidus of infection was established in the ventricular ependyma (Figure 2B).[30] By cell morphology and double-label immunocytochemistry (using goat antisera directed against CMV and either rabbit antisera directed against GFAP or rabbit antisera directed against NSE), CMV-infected cells were identified as glia and neurons (Figure 3). In other less severe cases, the nidus of infection appeared within a microscopic focus of infarction. By immunocytochemical staining for factor VIII, endothelial cells were also shown to be infected in these regions (Figure 3).

JC Virus

JC virus was detected by *in situ* hybridization in only three of the 93 brains. Each of the three brains

Table 1—Incidence of Viral Encephalitis in AIDS Autopsies*

Year	# Autopsies	Histopathology consistent with viral encephalitis	Virus present		
			JC	CMV	HIV
1982	2	1	–	1	1
1983	4	–	–	–	1
1984	21	9	1	8	5
1985	31	17	1	9	13
1986	35	27	1	13	17
Total	93	54	3	31	37

* The number of cases with neuropathology consistent with a viral encephalitis (mononuclear cell infiltrate, microglial nodules, multinucleated giant cells) are shown for each year. Basal ganglia, cerebral cortex and regions of the CNS showing evidence of encephalitis were assayed for viral nucleic acids or antigens. Presence of JC virus was determined by *in situ* hybridization and confirmed by electron microscopy. Presence of CMV or HIV was determined by immunocytochemistry. No cases contained HSV antigens detectable by immunocytochemistry.

Table 2—Multiple Viral Infections of the CNS*

CMV	+	HIV	+	22
CMV	+	HIV	–	9
CMV	–	HIV	+	13
JC	+	HIV	+	3
JC	+	HIV	–	–

* Brains showing infection by immunocytochemistry (CMV and HIV) or *in situ* hybridization (JC virus) are categorized according to presence or absence of multiple viruses. One brain contained all three viruses.

Table 3—Cellular Localization of HIV in the Central Nervous System of 37 Autopsied AIDS Cases*

	Rare	Occasional	Frequent	Total
Macrophages	7	12	17	36
Endothelial cells	1	12	8	21
Cells of unknown lineage possibly oligodendrocytes	not assessed	13	1	14

* Cell lineage was assessed on the basis of morphology or double-label immunocytochemistry. Sections showing fewer than three cells containing HIV gp41 were classified as "rare." Sections with three to ten cells containing HIV gp41 were classified as "occasional." Sections showing greater than ten cells containing HIV gp41 were classified as "frequent." Fifteen cases contained multinucleated giant cells and in all of these some (but not all) of the multinucleated giant cells stained positively for gp41.

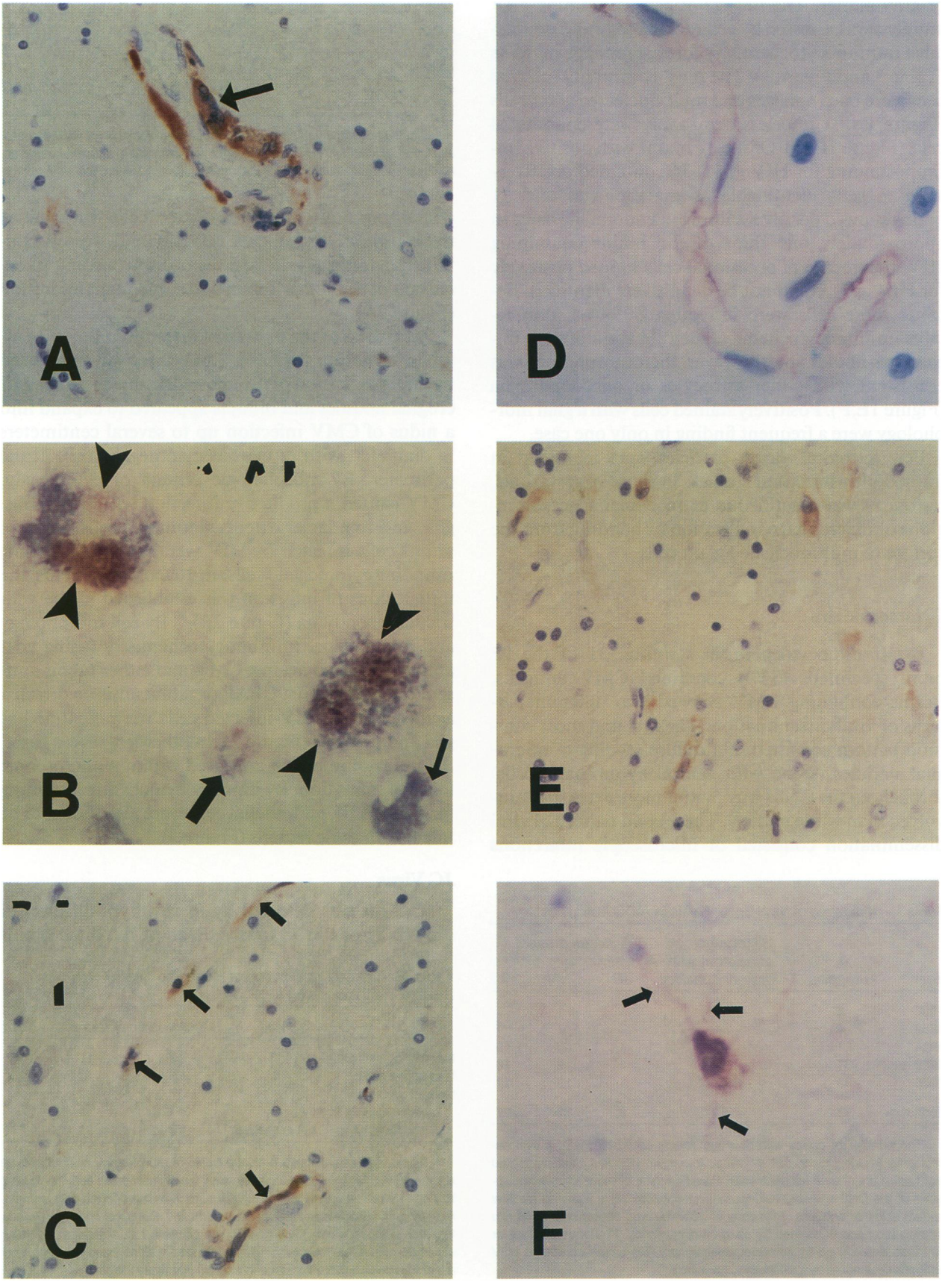
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Figure 1—Paraffin-embedded sections were stained immunocytochemically for HIV gp 41 using a mouse monoclonal antibody. **A**—Perivascular multinucleated giant cells (arrow) and macrophages contain HIV antigen (brown). Counter stained with hematoxylin, ×120 **B**—Double-label immunocytochemistry for HIV gp41 (red) and macrophage antigens (blue). The cytoplasm of several macrophages contain the blue immunoreaction product (arrows). Two large multinucleated giant cells stain blue with the macrophage marker but also contain the red immunoreaction product staining HIV gp41 antigen (arrowheads). Surrounding neuronal and glial cells do not stain for macrophage nor HIV antigens. Not counter stained, ×480 **C**—Low-power light micrograph of vacuolated white matter showing vascular endothelial cells (arrows) staining brown for HIV gp41. Surrounding tissue contains no immunoreaction product. Counterstained with hematoxylin, ×120 **D**—High power light micrograph of white matter showing small vessel endothelial cytoplasm filled with immunoreaction product for HIV gp41 (red). Counterstained with hematoxylin, ×480 **E**—Low-power light micrograph of vacuolated hypercellular basal ganglia with numerous cells containing HIV gp41 (brown). Counterstained with hematoxylin, ×120 **F**—High-power light micrograph of rare cell with spider like processes (arrows) filled with immunoreaction product (red) for HIV gp41. While the lineage of such cells can not be precisely determined, the morphology is consistent with glial cells. Counterstained with hematoxylin, ×480

had the classical histopathologic changes of progressive multifocal leukoencephalopathy, including severe demyelination, macrophage infiltration, oligodendroglia with nuclear inclusions, and anaplastic astrocytic nuclei. The other brains showed no hybridization with the JC virus probe. Double-label immunocytochemistry, *in situ* hybridization, and cell morphology were used to identify the lineage of cells infected with JC virus. Only glial cells proved to contain JC viral nucleic acids. The majority of glial cells that hybridized with the JC virus probe did not stain positively for GFAP, thus identifying the majority of infected cells as oligodendroglia. A few astrocytes were found to contain JC viral nucleic acids (Figure 3F). In some of these cells, JC viral nucleic acids were restricted to the nucleolus. No cells containing either neurofilament antigens or a macrophage/endothelial cell marker[31] hybridized with the JC virus probe.

Discussion

HIV antigens were found in 40% of the brains at autopsy, but they were present in large numbers of cells in only 17 of the 93 brains (Table 3). These antigens were most commonly seen within the deep white matter and basal ganglia. Staining was most frequently observed in macrophages, multinucleated giant cells, and endothelial cells. Injury to the endothelial cell could explain the significant "vasogenic" distribution of gliosis seen in many AIDS brains. Such an injury could lead to intimal proliferation and could explain the presence of microscopic infarctions, as has been suggested by Cho et al.[35] However, at this time there is no direct evidence of an obliterative microvascular change. The origin of numerous HIV-infected macrophages in the CNS remains unexplained. One possible explanation would be that resident microglia within the CNS are infected by HIV that passed through the blood–brain barrier, but the large number of the infected macrophages and their perivascular distribution suggests that these cells arise from systemic monocytes. Macrophages could have migrated into the CNS in response to any of the numerous opportunistic CNS infections characteristic of AIDS (ie, the microglial nodule associated with CMV); how-

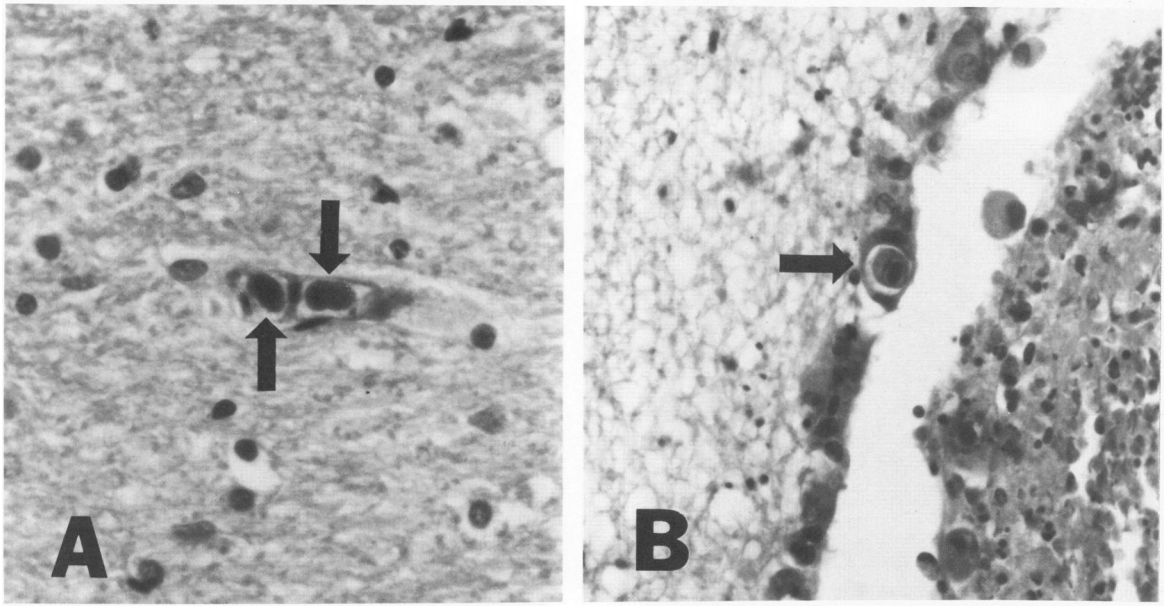

Figure 2—Paraffin-embedded sections stained with hematoxylin and eosin. **A**—The lumen of a small vessel is obliterated by two cytomegalic endothelial cells (arrows). Counterstained with hematoxylin, ×480 **B**—Acutely inflamed ventricular region showing cytomegalic ependymal cells (arrow). Counterstained with hematoxylin, ×120

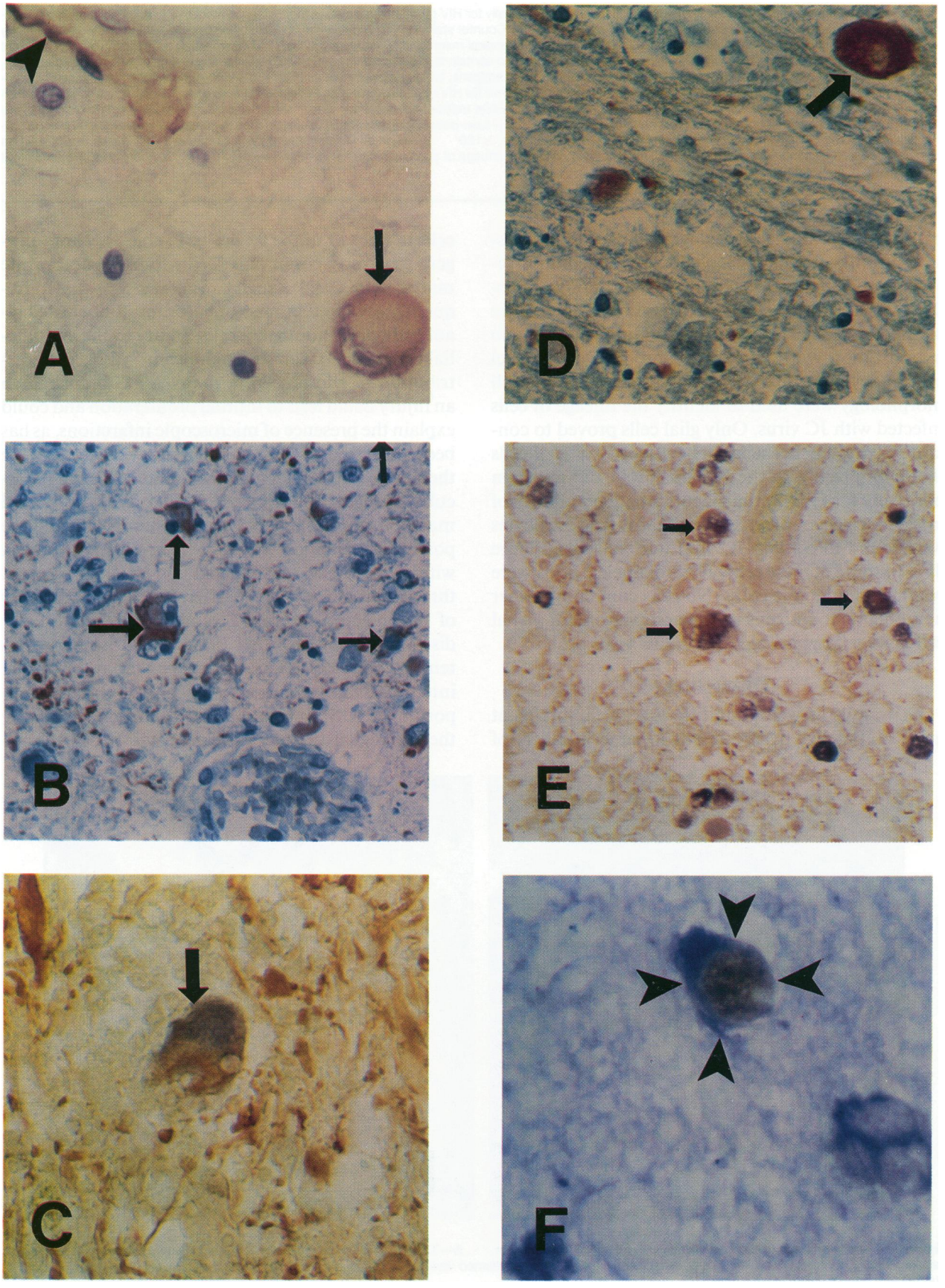
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Figure 3—Paraffin-embedded sections immunostained for cellular and viral antigens. **A**—Endothelial cells (arrowheads) and occasional cytomegalic cells (arrows) stained using immunocytochemistry for factor VIII related antigen. Counterstained with hematoxylin, ×480 **B**—Immunostaining for GFAP (red) shows some cytomegalic cells (arrows) that contain the reaction product denoting their glial origin. Counterstained with hematoxylin, ×120 **C**—Double-label immunocytochemistry for GFAP (brown) and CMV (blue) (arrow). A cytomegalic cell contains both immunoprecipitates confirming that astrocytes are infected by CMV. Not counterstained, ×480 **D**—Immunoperoxidase staining for neuron-specific enolase (red) shows a cytomegalic cell (arrow) containing immunoreaction product. Counterstained with hematoxylin, ×480 **E**—Double-label immunocytochemistry for neuron-specific enolase (red) and CMV (blue) confirms that neurons are infected by CMV (arrows). Not counterstained, ×120 **F**—Immunocytochemical staining for GFAP (blue) and *in situ* hybridization for JC viral nucleic acids (brown), shows occasional double-labeled cells (arrowheads) confirming that astrocytes were infected by JC virus. Not counterstained, ×480

ever, the presence of abundant HIV-infected macrophages within deep gray and white matter in the absence of other pathogens suggests there must be some additional explanation.

Precise identification of some of the HIV-positive cells could not be established. On the basis of location and morphology, these infected cells are most likely glia,[36,37] but in mildly inflamed regions, macrophages can be difficult to distinguish from glia. Nevertheless, neither the pathology nor the clinical symptoms can be explained by the observed frequency of glial cell infection by HIV. Alternatively, the existence of a low level or abortive infection of neuronal or glial cells that is below the detection limit might account for the clinical symptomatology.

While frequently inapparent in CT studies before death,[38] post mortem histopathologic studies have shown that CMV infection of the CNS is common in AIDS (Table 1).[5,6,30,39,40] Dissemination of CMV to the CNS was characterized by two distinct histopathologic patterns: microscopic infarctions and microglial nodules. These two categories subsume the five characteristic lesions described by Morgello et al.[40] Presence of CMV within microinfarcts could be explained by hematogenous infection of CNS endothelial cells[41] followed by swelling of the cell with occlusion of the lumen and infarction of dependent brain tissue, or by secondary CMV colonization around previously infarcted regions. Microglial nodules associated with CMV infection of the CNS are seen frequently in other immunosuppressed patients (eg, organ transplant recipients) as well as AIDS patients. The pathogenic mechanism of this form of diffuse dissemination is unclear but requires a breach of the normal blood-brain-barrier, with penetration of the virus through then endothelial cell and surrounding basement membrane and astrocytic foot processes. Once within the CNS, CMV was capable of productively infecting most CNS cells, and progressing by direct extension from a central nidus.

Even if the microglial nodules without demonstrable CMV antigen are included as a manifestation of CMV encephalitis in AIDS patients, the amount of tissue directly infected by CMV is not sufficient to account for all of the clinical or histologic findings. However, CMV may act indirectly to produce additional pathologic effects. First, infarctions due to CMV infection of endothelial cells could lead to multi-infarct dementia similar to that seen in cerebrovascular disease; however, the low frequency of infarctions is not commensurate with the degree of neurologic dysfunction observed. Second, the presence of CMV could elicit the ingress of latently HIV-infected monocytes as part of the inflammatory response. Differentiation of these monocytes into macrophages in the CNS could then lead to a productive HIV infection.[42]

When explaining the origin of the neurologic symptoms associated with AIDS, one must take into account the absence of significant infection of neuronal and glial cells. The location of HIV antigen and the distribution of the damage within basal ganglia regions would be consistent with other described subcortical dementias in which neuronal loss is observed. Because HIV does not appear to infect glial and neuronal cells, however, the severe gliosis seen in the subcortical region must be an indirect effect of CNS or systemic[43] HIV infection. This damage could result from diminished function of capillary endothelial cells or from factors secreted by macrophages within the CNS environment. Alternatively, viral proteins produced within the CNS may have toxic effects on glia and neurons. Examples of this latter possibility could include disruption of cellular function by retroviral envelope proteins, by circulating immune complexes or by specific immune factors (eg, tumor necrosis factor, neuroleukin[44]). Such effects on endothelial cell function would explain the vasogenic edema and gliosis seen in deep white and gray matter regions. It is also unclear exactly when HIV enters the nervous system. Based on meningitis occurring at the time of seroconversion, some have concluded that HIV infection of the nervous system parenchyma occurs very early.[45,46] However, several viruses (eg, HSV) have been shown to cause a meningitis without parenchymal involvement, so HIV meningitis does not necessarily mean HIV encephalitis.

In the series of 93 autopsies reported here, there was a definite increase in the frequency of CNS infection with both CMV and HIV in 1985 and 1986 (Table

1). This may have resulted from a shift in the patient population being autopsied because, in the first years of the AIDS epidemic, most patients dying of AIDS were autopsied while recently many have chosen to die at home and autopsies are being performed more selectively. Alternatively, there may be a bias toward performing autopsies on patients with prominent nervous system symptoms. While this might explain the high incidence of neuropathologic findings in 1986, the neurologic disorders were not of specific interest in previous years but the increasing percentage of patients with subacute encephalitis is still apparent. Another possible explanation of the increased frequency of CNS infection would be emergence of HIV strains with distinct cellular tropisms leading to greater brain involvement.[47–49] Curiously, there has been a parallel increase in both CMV and HIV infection of the CNS (Table 1). The recent observation[50] of coinfection of individual cells within the CNS by HIV and CMV suggests that *in vivo* interaction between the two viruses may play a role in the pathogenesis of AIDS-associated encephalitis.

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