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Cytomegalovirus (CMV) disease of the brain in AIDS and connatal infection: a comparative study by histology, immunocytochemistry and in situ DNA hybridization*

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Summary. Brain tissues from 45 patients with AIDS and two brains with connatal cytomegalic inclusion body disease were investigated for a cytomegalovirus (CMV) etiology of encephalitic lesions. Nineteen brains showed evidence of CMV infection by histology, immunocytochemistry (ICC) using two different antibodies (mono- and polyclonal), and in situ hybridization (ISH). Fourteen cases with typical cytomegalic cells in conventional histology [eight with focally necrotizing encephalitis/ventriculitis including the two connatal infections and six with nodular encephalitis (NE)] revealed CMV with any method. In 5 of 15 AIDS cases of NE without cytomegalic cells, CMV infection was established by ISH, whereas ICC remained negative in these cases. Typical lesions of human immunodeficiency virus (HIV)-induced multifocal giant cell encephalitis (HIV encephalitis) in 13 brains were never labeled for CMV. In necrotizing encephalitis/ventriculitis, cell types which labeled for CMV, with and without cytomegalic change, comprised neurons, astrocytes, oligodendrocytes, ependyma, choroid plexus, endothelia, and cells in peri- and endoneurium, and in leptomeninges. Both ISH and ICC were able to detect widespread non-cytomegalic CMV-infected cells in normal parenchyma, well beyound the necrotizing lesions, in two AIDS cases. Labeling patterns of nuclei versus cytoplasms varied between the three methods for CMV detection. We conclude that in CNS tissues with cytomegalic cells, ICC and ISH are of comparable sensitivity; however, a diagnosis of CMV disease is possible in such cases by conventional histology. For an in situ diagnosis of CMV infection in NE without cytomegalic cells in AIDS, ISH is the method of choice. A selective vulnerability to CMV infection of any specific cell type of the human CNS is absent. With our detection methods, typical lesions of HIV encephalitis do not show local co-infection by CMV.

Key words: Cytomegalovirus (CMV) – Acquired immunodeficiency syndrome (AIDS) – Encephalitis – DNA hybridization – Immunocytochemistry

Cytomegalovirus (CMV) has been recognized as an important pathogen in all human age groups [1, 7, 8, 12, 17, 24] ranging from the newborn to adults, and from subclinical infection to disseminated disease in the immunocompromised horst [2, 4, 5, 8–11, 16–19, 24, 25, 27]. In acquired immune deficiency syndrome (AIDS) patients, CMV infection of the nervous system

Fig. 1a–f. Connatal cytomegalovirus (CMV) infection. **a–c** Serial sections of cortex (for better orientation, the same vessel is labeled by *asterisks* in **a–c**) labeled for CMV by in situ DNA hybridization (ISH; **a**) and by immunocytochemistry (ICC) with monoclonal (**b**) or polyclonal (**c**) antibodies. *Insets* depict a cytomegalic cell; ISH labels mainly the nucleus (**a**), anti-CMV monoclonal antibody (mAb) immunostains both nucleus and cytoplasm (**b**), and polyclonal anti-CMV immunostains mainly the cytoplasm but not the nucleus at right (**c**). Slight nuclear counterstain with hemalum (*h*). ×63, *insets* ×1000. **d** A bihemispheric section shows calcifying tissue destruction especially in periventricular areas, and polymicrogyric cortex. Hematoxylin-eosin stain, ×1. **e** Cortical-subcortical border zone with plaque-like tissue destruction surrounded by strongly glial fibrillary acidic protein (GFAP)-immunoreactive cytomegalic cells. Peroxidase-antiperoxidase (PAP) technique with anti-GFAP serum, h counterstain, ×63. **f** Immunoreactivity for GFAP of some cytomegalic cells is aggregated in the center of the cell whereas its periphery does not stain. Same stain as in **e**, ×630

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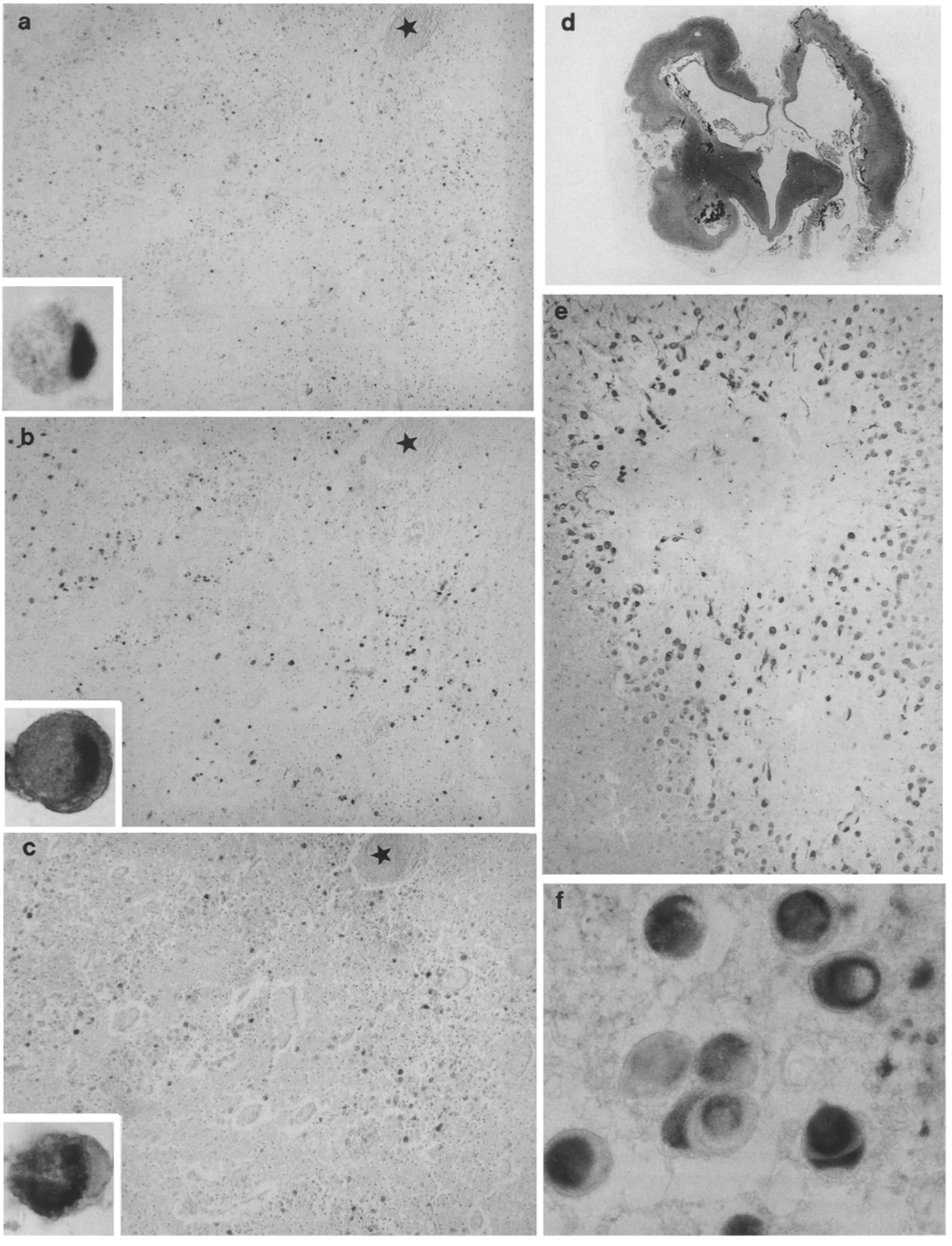
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was described in up to 33% of cases [5, 15, 18–20], including focally necrotizing encephalitis and ventriculitis, vasculitis and nodular encephalitis (NE) with typical cytomegalic cells as diagnostic hallmark [4, 9, 10, 16, 18]. However, many cases with NE in AIDS lack cytomegalic cells [5, 11, 16, 18] and were, therefore, considered to be nonspecific [19], of occasional toxoplasmic origin confirmed by immunocytochemistry (ICC) [5], or were suspected to be due to CMV because of concurrent systemic CMV infection [5].

This retrospective study tries to detect CMV infection by histology, ICC and in situ DNA hybridization (ISH) in connatal CMV disease and in encephalitic lesions of AIDS patients, with or without the presence of cytomegalic cell changes.

Materials and methods

Autopsy tissue from 45 adults with a clinical diagnosis of AIDS, from 2 patients with connatal cytomegalic inclusion body disease, and from 10 control patients was selected according to neuropathological diagnosis. AIDS cases had one or more of the following lesions: focally necrotizing encephalitis and/or ventriculitis with cytomegalic cells (6 cases); NE with cytomegalic cells (6 cases); multinucleated giant cell encephalitis (MGCE) without cytomegalic cells, considered to be caused by human immunodeficiency virus (HIV) [4, 5, 16] (13 cases), NE of uncertain etiology without cytomegalic or multinucleate giant cells (15 cases), necrotizing toxoplasmosis (10 cases), malignant lymphoma (2 cases), diffuse poliodystrophy [5] (7 cases), cryptococcal meningoencephalitis (4 cases), progressive diffuse leukoencephalopathy [4, 5, 16] (6 cases), cerebellar infarction (1 case), meningo-encephalo-radiculitis of uncertain etiology (1 case).

Ten controls (age range 6 months to 76 years) included one case each of Alzheimer's disease, Parkinson's disease, cerebral infarction, cerebral hemorrhage, sudden death in infancy, influenza, myocardial infarction, nephritis, sudden death with brain edema, and herpes zoster. All brain tissue was identically treated; after fixation in neutrally buffered formalin, the material was stored in paraffin blocks. All AIDS patients had died within the last 6 years. Both connatal cases had been stored for 6 years, and the control cases between 2 and 5 years. In most cases, two large blocks were studied. Serial sections were stained by hematoxylin and eosin (H&E), ICC and ISH, and, in selected blocks, by ICC for markers of neurons, glia, hematogenous cells and macrophages, or toxoplasms, as detailed previously [3, 5].

Immunocytochemistry (ICC) for CMV

Two different antibodies were used. Sections were stained by the labeled avidin-biotin technique after enzymatic predigestion with protease, using (a) the immunoglobulin fraction of goat anti-CMV serum (Polysciences, Warrington, Pa, USA) as detailed previously [5], or (b) a mouse anti-CMV monoclonal antibody (mAb) (DAKOpatts, Glostrup, Denmark). Specificity controls included replacement of the antiviral serum by nonimmune serum or anti-herpes simplex virus (HSV) serum, both of which gave negative results. Moreover, sections of HSV encephalitis, and of spinal ganglionitis in a patient with herpes zoster at the respective segment, both containing numerous inclusion bodies, were identically treated with anti-CMV antibodies but remained unlabeled.

In situ hybridization (ISH)

This study was performed with a biotinylated cDNA probe for CMV (ENZO, New York, NY, USA), following a protocol described previously in detail for detection of HSV DNA [21, 22]. Hybridization was performed at room temperature for 60 min. For washing, sections were exposed to 20% deionized formamide in 1 × SSC at 37° C for 10 min.

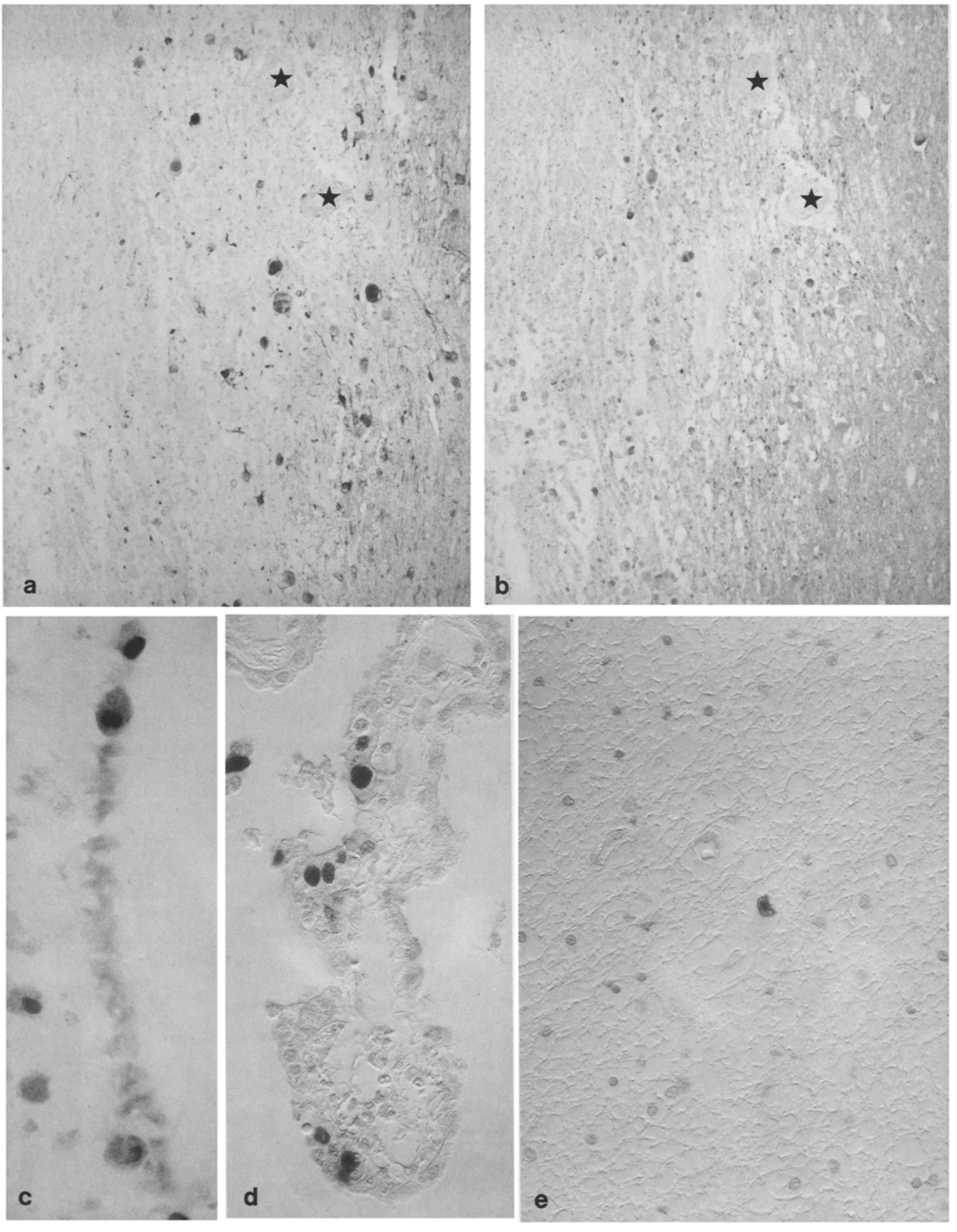
Brain sections with typical changes of connatal CMV infection were well labeled by this procedure but were negative in ISH with a biotinylated HSV cDNA probe as previously described [21, 22]. Sections of a spinal ganglion from a patient with herpes zoster, and brain sections with HSV encephalitis, both with numerous inclusion bodies, were not labeled with our ISH procedure for CMV.

Results

Connatal cytomegalic inclusion body disease

Severe tissue destruction with calcification was most prominent in periventricular areas and in the micropolygyric cortex (Fig. 1 d). ICC and ISH labeled similar numbers in a similar distribution of predominantly cytomegalic cells (Fig. 1 a–c) in patterns identical with those for necrotizing CMV encephalitis in adult AIDS. The cellular site of label differed between the three techniques employed: ISH preferentially labeled the nucleus (Fig. 1 a inset), ICC with anti-CMV mAb stained both nucleus and cytoplasm (Fig. 1 b inset), whereas polyclonal anti-CMV immunostained the cytoplasm but usually spared the nucleus (Fig. 1 c inset). Two other features were remarkable in the connatal CMV infection: first, plaque-like lesions were surrounded by cytomegalic cells with strong immunoreactivity for glial fibrillary acidic protein (GFAP; Fig. 1 e). Second, the intracellular distribution of GFAP was peculiar in cytomegalic cells, occupying a globular area in the cytoplasmic center, but sparing the periphery (Fig. 1 f).

Fig. 2 a–g. Necrotizing CMV encephalitis and ventriculitis in AIDS. **a** Ventriculitis shows discolored softened ventricle walls. **b** Lower oblongata. Preferential superficial distribution of necrotizing changes. Luxol fast blue – nuclear fast red, × 3.5. **c, d** ISH labels mainly the intranuclear inclusion bodies (**c**, *at right*), whereas ICC (anti-CMV mAb), performed on adjacent section, stains both nuclei and cytoplasms of the same cytomegalic cells (**d**). Slight h counterstain, × 630, Nomarski optics. **e** An endothelial nucleus of a periventricular vein in CMV ventriculitis is immunostained with anti-CMV mAb. Slight h counterstain, × 630. **f** Numerous cytomegalic cells in cerebellar cortex; a few ascending dendrites in the molecular layer are also immunoreactive (*at upper right*). Same stain as in **e**, × 250, Nomarski optics. **g** A few cytomegalic cells are labeled in the leptomeninges. Same stain as in **e**, × 100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AIDS brains

Tissue lesions with cytomegalic cells were found in two major patterns: focally necrotizing lesions which tended to prefer periventricular (Fig. 2a) or superficial (Fig. 2b) sites, or encephalitic nodules. CMV could be detected by any method employed in 17 cases.

In all 6 AIDS cases of necrotizing CMV encephalitis/ventriculitis, and in all 6 AIDS cases of NE with typical cytomegalic cells, CMV antigens were detected by ICC. CMV DNA was detected by ISH in the same but 2 cases of NE lacking cytomegalic cells in the ISH preparations. CMV DNA and antigens were invariably found in cytomegalic cells with huge intranuclear inclusions and large-bodied cytoplasms (Fig. 2c, d, f). Also cells with unequivocal or even equivocal inclusions but lacking further features of cytomegalic cells were labeled (Figs. 2e; 3d, e). Labeled cells included neurons and astrocytes which were identified with antisera to GFAP (Fig. 3a), neurofilament proteins (NFP) and neuron-specific enolase (NSE) (Fig. 3b), oligodendrocytes, ependymal cells (Fig. 3c), and rarely (each following site only in one case involved) the choroid plexus (Fig. 3d), endothelia (Fig. 2e), and cells in peri- and endoneurium of cranial nerve roots as well as in leptomeninges (Fig. 2g). The intracellular distribution of label differed between methods (Fig. 2c, d), as described above in the connatal infection. Rarely some dendrites in the cerebellar molecular layer were labeled with anti-CMV mAb (Fig. 2f). In non-cytomegalic cells with small but unequivocal inclusions and otherwise intact morphology, labeling of inclusions and nuclear membranes was prominent with the mAb (Fig. 3e) but inconstant or equivocal with the polyclonal antiserum. Both ISH and ICC were able to detect widespread non-cytomegalic CMV-infected cells in normal parenchyma (Fig. 3e), well beyond the necrotizing lesions, in two AIDS cases. In two other cases, rare isolated cytomegalic cells remote from necrotic lesions were labeled by ICC and ISH.

In NE with inclusions, cytomegalic cells were labeled within nodules (Fig. 4a), as well as some nonmegalic cells with unequivocal inclusions. The latter cells were stained with the mAb but not with the polyclonal antibodies. CMV DNA was also detected within nodules in some nuclei of cells without inclusions.

In NE without cytomegalic cells, 5 of 15 brains hybridized in situ with the CMV cDNA probe, whereas CMV antigens were never found. Labeling was restricted to nuclei of glial or unidentified cells without cytomegalic changes, with equivocal inclusions (Fig. 4b). CMV DNA was mostly restricted to areas with nodular infiltrations and was only exceptionally found in the surrounding normal parenchyma (1 case).

Lesions of HIV-induced MGCE were never labeled by ICC or ISH (13 cases). Any labeling was also absent in other lesions of AIDS brains.

Control brains

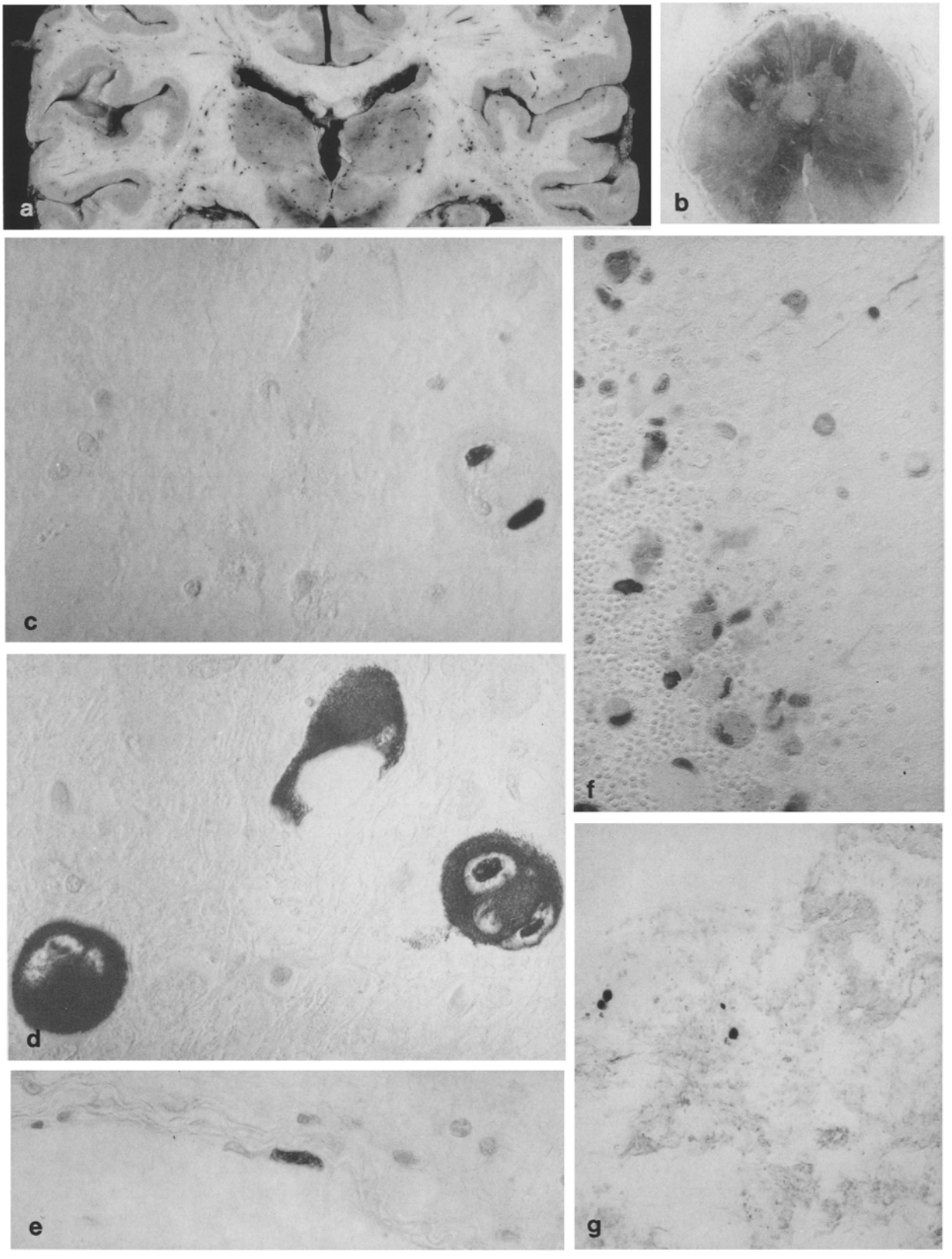
The 10 control brains were unlabeled by any technique for CMV detection.

Discussion

Most recently, CMV infection in AIDS attracted considerable interest by neuropathologists [11, 16, 18, 23, 26]. However, this is the first comparative study of a series of human brains with CMV disease using ICC with two antibodies and ISH. Previous studies of CMV in AIDS brains used only ICC with the polyclonal antiserum [16, 26], supplemented by electron microscopy [18], or used only ISH in single or few cases [17, 23, 27]. Thus the diagnostic potential of different techniques for CMV detection [13] has not been settled for CNS tissue.

In this study, a third of NE cases without cytomegalic or multinucleated giant cells, thus considered of obscure etiology, had significant amounts of CMV DNA detectable by ISH. Its association with inflammatory lesions and the absence of CMV DNA in all control tissues support a CMV etiology of NE in such cases; previously this could be only suspected [5, 14]. The absence of CMV antigens in such cases is in accordance with previous studies [27]. However, a significant portion of etiologically obscure cases remains. We also studied NE without cytomegalic cells by ISH with a HSV cDNA probe as described earlier [21]. In one of the CMV-negative cases, some nuclei of neurons in nodules or in their vicinity were labeled [22]. Thus, in a small number of AIDS cases, NE may be due to HSV rather than to CMV, especially when limited to the brain stem [22].

Fig. 3a–e. Cellular tropism in necrotizing CMV encephalitis/ventriculitis in AIDS. **a, b** Serial sections of severely damaged cortical area (for better orientation, the same two vessels are marked with *asterisks*) demonstrate cytomegalic cells expressing GFAP (**a**) and/or neuron-specific enolase (NSE; **b**). PAP technique with anti-GFAP (**a**) or anti-NSE (**b**) sera, slight h counterstain, ×63. **c** Cytomegalic ependymal and subependymal cells labeled by ISH. Slight h counterstain, ×400. **d** Choroid plexus cells immunoreactive with anti-CMV mAb, slight counterstain, ×400, Nomarski optics. **e** Isolated nucleus immunoreactive with anti-CMV mAb is found within intact parenchyma. Same stain, magnification, and optics as in **d**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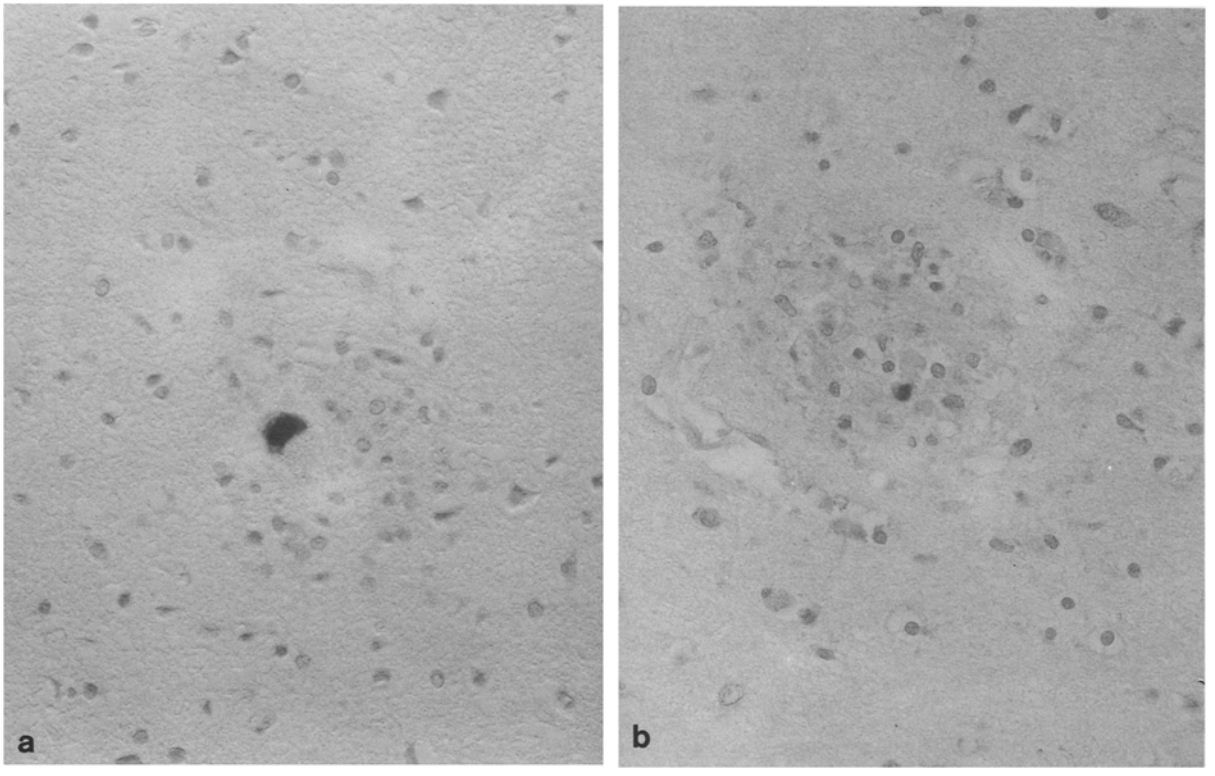

Fig. 4a, b. Nodular encephalitis in AIDS. **a** If present, cytomegalic cells are strongly labeled by ICC (anti-CMV mAb). **b** In some cases without cytomegalic cells, nuclei of otherwise normal cells are labeled only by ISH. **a, b** h counterstain, × 400

CMV was never found in 13 cases of HIV-induced MGCE [3–5]; this confirms a non-CMV etiology of MGCE [4, 5, 18, 19] and argues for separation of MGCE from "subacute encephalitis" in AIDS which may be confused with CMV infection [4]. Considering some speculations on CMV infection as a cofactor for the pathogenesis of HIV encephalopathy [4, 26], our finding of no local co-infection by CMV of typical lesions of HIV encephalitis is remarkable.

In our material, any selective vulnerability to CMV infection of specific cell types of the human CNS was absent. ISH and ICC did not label leukocytes as described previously [17]. Preferential superficial and periventricular localization of necrotizing CMV lesions, as previously noted by others [23, 27], suggests the CSF as important pathway for viral spread in many cases. The mAb labeled nuclei and nuclear membranes with or without cytomegalic change, but also cytoplasms of cytomegalic cells. This reflects the detection of early and late antigens according to the producer's protocol. In contrast, the polyclonal antiserum preferentially labeled the cytoplasms of cytomegalic cells whereas nuclei were only inconstantly labeled, suggesting recognition mainly of late antigens. Thus the mAb seems superior to the polyclonal antiserum for detection of CMV-infected cells without fully developed cytomegalic changes. Moreover, these differences demonstrate the varying properties of commercially available anti-CMV antibodies.

In connatal CMV encephalitis, the peculiar arrangement of cytomegalic cells around plaque-like foci is somewhat reminiscent of the distribution of virus-laden cells in the periphery of plaque-like lesions of another productive virus infection of the human CNS, progressive multifocal leukoencephalopathy. Moreover, heavy staining for GFAP in a peculiar intracellular distribution of cytomegalic cells suggests some type of involvement of cytoskeletal proteins in replication cycles of the virus, as described in some animal virus infections [6].

We conclude that in CNS tissues with cytomegalic cells, ICC and ISH are of comparable sensitivity; however, a diagnosis of CMV disease is possible in such cases by conventional histology. For an in situ diagnosis of CMV infection in NE without cytomegalic cells in AIDS, ISH is the method of choice.

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