to form this intermediate. Both normals, the three heterozygotes, and purified rat liver lysosomal membranes formed acetylated intermediates as expected.

The [^{3}H]-acetylated lysosomal membranes were incubated with 3 m*M* glucosamine for 15 minutes at room temperature. The membranes were spun down and the supernatant was applied to a silica gel thin-layer chromatography plate to separate the [^{3}H]*N*-acetylglucosamine from [^{3}H]-acetate (*7*). Even though the lysosomal membranes from the Sanfilippo C cell lines 92, 96, and 100 could form the acetylated intermediate, they were unable to transfer the acetyl group to glucosamine (Table 3), although cell line 96 appeared to have a very small amount of residual activity. In contrast, acetylated normal, heterozygote, and rat liver lysosomal membranes were all able to form *N*-acetylglucosamine.

Based on the half reaction studies of the mutant cell lines, we can now differentiate two classes of Sanfilippo C mutants. One class, represented by five out of six Sanfilippo C cell lines tested, has the ability to carry out acetyl-CoA/CoA exchange and to form the putative acetyl intermediate, but is unable to transfer the bound acetyl group to glucosamine. (The five lines represent three families, all of whom come from the Netherlands, and thus may have the same mutation.) The second class, composed of, at present, only one mutant cell line, is unable to catalyze acetyl-CoA/CoA exchange or form the acetylated intermediate.

The analysis of the two classes of mutants is the first direct evidence for the proposed transmembrane acetylation mechanism of *N*-acetyltransferase. Since no acetyl-CoA/CoA exchange is observed in the second mutant class, the exchange activity may be entirely due to the *N*-acetyltransferase and not to some other lysosomal membrane protein. The partial enzyme activity observed with the first class of mutants verifies that *N*-acetyltransferase works via a ping-pong mechanism, and that an acetylated enzyme intermediate is formed.

Acetylation of terminal α-linked glucosamine residues inside the lysosome is a required step in the degradation of heparan sulfate. Although acetyl-CoA is the acetyl donor in this reaction, it is unlikely that this cofactor could exist stably in the acidic and hydrolytic environment of the lysosome. *N*-Acetyltransferase provides a means for the cell to utilize cytoplasmically derived acetyl-CoA in this reaction without transporting the intact molecule across the lysosomal membrane. Vectorial transfer of the acetyl group through the lysosomal membrane appears to be a unique solution to a complex enzymatic and compartmental problem.

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Detection of AIDS Virus in Macrophages in Brain Tissue from AIDS Patients with Encephalopathy

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One of the common neurological complications in patients with the acquired immune deficiency syndrome (AIDS) is a subacute encephalopathy with progressive dementia. By using the techniques of cocultivation for virus isolation, in situ hybridization, immunocytochemistry, and transmission electron microscopy, the identity of an important cell type that supports replication of the AIDS retrovirus in brain tissue was determined in two affected individuals. These cells were mononucleated and multinucleated macrophages that actively synthesized viral RNA and produced progeny virions in the brains of the patients. Infected brain macrophages may serve as a reservoir for virus and as a vehicle for viral dissemination in the infected host.

THE ACQUIRED IMMUNE DEFICIENCY syndrome (AIDS) is associated with a variety of clinical disorders involving the peripheral and central nervous systems (CNS) (*1–4*). Premortem neurological findings are detectable in about one-third of AIDS patients, while neuropathological changes are present in over three-fourths of autopsied subjects (*4, 5*). From 17 to 60% of neurologically impaired AIDS patients develop an encephalitis, but only a minority of those cases can be attributed to a recognized opportunistic infection [for example, progressive multifocal leukoencephalopathy (PML) and herpes simplex] (*2, 3, 5*). Of those with encephalitis, the majority develop a subacute encephalopathy with progressive dementia and cerebral atrophy. Recent findings suggest that this form of encephalopathy may be a manifestation of

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Table 1. Clinical and pathological features of patients 1 and 2.

Age, sex, social history	Clinical history	Serology	Gross pathology	Microscopic pathology	Microbiology
			Patient 1		
47-year-old male; heterosexual; businessman	Coronary artery bypass graft with multiple transfusions at age 42; *Pneumocystis carinii* pneumonia at age 45; onset of progressive dementia at age 46; aspiration pneumonia; death	Positive for HIV by ELISA	Normal	Multinucleated giant cells in cerebral cortex and white matter; perivascular mononuclear inflammation; reactive astrocytosis	Negative for bacteria, mycobacteria, fungi, and viruses (CMV, EBV, HSV) by culture
			Patient 2		
45-year-old male; intravenous drug user	Weight loss (30 pounds); lymphadenopathy; dementia for 3 weeks; diarrhea; seizure; aspiration pneumonia; death	Positive for HIV by ELISA; negative by Monospot and for *Toxoplasma*	Slight frontal and parietal atrophy; soft, yellow, granular areas of destruction in temporal, parietal, and frontal white matter	Lipid-laden mononuclear histiocytes and multinucleated giant cells surrounded by atypical astrocytes and oligodendroglial cells with intranuclear inclusions; demyelination and mild axonal loss; PML	Negative for bacteria, fungi, and mycobacteria by culture

infection with human immunodeficiency virus (HIV), the etiologic agent of AIDS. This notion is supported by the identification of HIV in brain tissue and cerebrospinal fluid (CSF) of patients with subacute encephalopathy as monitored by virus isolation, in situ hybridization, or Southern blot analyses (*6–8*).

The AIDS retrovirus is tropic and cytopathic for T cells bearing the CD4 molecule (*9, 10*). HIV replicates in $CD4^+$ T-cell tumor lines and in phytohemagglutinin (PHA)-stimulated peripheral blood T cells (*10–12*). It has therefore been assumed that T helper lymphocytes are the major reservoir of HIV infection in vivo. The possibility that the AIDS retrovirus replicates in non–T cells in antibody-positive individuals is supported by reports that Epstein-Barr virus (EBV)–transformed B cell lines as well as the monocytic line U937 can be infected in vitro with HIV (*10, 13, 14*). Recently, Ho *et al.* (*15*) showed that mononuclear cells, depleted of T lymphocytes, are susceptible to AIDS retroviral infection both in vivo and in vitro. In addition, monocytes in lymph nodes from AIDS patients have been reported to contain retroviral particles by electron microscopy (*16*).

At present, it is unclear whether lymphocytes, monocytes, glial cells, or neurons are infected with HIV in encephalopathic patients infected with the AIDS virus. Epstein *et al.* found viral particles in some multinucleated giant cells that resembled histiocytes or astrocytes in brains of AIDS patients with encephalopathy (*17*). However, definitive identification of the productively infected cell type was lacking. In the present study, we used immunohistochemistry coupled with in situ hybridization techniques and electron microscopy to show that mononucleated and multinucleated macrophages were the major cell type synthesizing HIV in the brains of two patients with AIDS.

As shown in Table 1, both of the patients examined had serological and clinical evidence of HIV infection with associated severe encephalopathy. In patient 1, the AIDS retrovirus was recovered from postmortem homogenized brain tissue cocultured with PHA (1 μg/ml, Sigma)-stimulated normal human peripheral blood mononuclear cells (PHA-PBMC). Syncytia were detected in primary cocultures after 17 days. Supernatants from these cultures were passaged to fresh PHA-PBMC and syncytia formation and reverse transcriptase activity (212,000 counts per milliliter) were detected after 7 days. Comparable nonfixed brain tissue from patient 2 was unavailable.

Tissue sections from the frontal and temporal lobes from patient 1 and the parieto-occipital region from patient 2 were examined by in situ hybridization to determine which cell type (or types) contained AIDS virus RNA. HIV RNA, indicated by silver grains overlying cells, was readily detected in brain sections from both patients. In patient 1, approximately 5 to 15% of multinucleated giant cells in sections from the frontal lobe and 1 to 5% of multinucleated giant cells in the temporal lobe contained HIV RNA (Fig. 1, A, B, and D). The multinucleated giant cells were frequently located near blood vessels or clustered in small groups within the brain parenchyma, although single infected intraparenchymal cells were also detected. Control tissue sections that were hybridized with RNA probes of opposite polarity (sense orientation) exhibited no reaction. Preparations treated with ribonuclease prior to hybridization with probes complementary to the viral messenger RNA (mRNA) also failed to react. In addition to giant cells, rare mononuclear cells localized in perivascular cuffs contained viral RNA.

In patient 2, HIV RNA was detected primarily in the regions of demyelination where an intense mononuclear inflammatory response and multinucleated giant cells were present (Fig. 2A). The majority of cells in these areas were shown to be macrophages, as defined by immunochemical and

Table 2. Characteristics of multinucleated giant cells by histochemical and immunohistochemical stains. Brain tissues, fixed in periodate-lysine-paraformaldehyde-glutaraldehyde (patient 1) or 10% Formalin (patient 2) and embedded in paraffin, were cut (6 μm) sequentially and stained by the avidin-biotin-coupled peroxidase technique (*36, 37*). Reagents included: (i) rabbit antiserum to α_1-antichymotrypsin (*38*) (Dako); (ii) rabbit antiserum to α_1-antitrypsin (*38*) (Dako); (iii) rabbit antiserum to glial fibrillary acid protein (Dako); (iv) mouse monoclonal antibody LN-3, previously shown to detect the HLA-DR (Ia) antigen in paraffin sections (*39*); (v) mouse monoclonal antibody Leu-M1 (Becton Dickinson) recognizing a population of circulating peripheral monocytes and granulocytes (*39*); (vi) rabbit antiserum to lysozyme (Dako) (*38*); (vii) rabbit antiserum to neuron-specific enolase (Polyscience) (*40*); and (viii) monoclonal antibody to myelin-associated glycoprotein, previously shown to react with oligodendroglial cells (*41*). Esterase and acid phosphatase staining was performed as previously described (*42*); ND, not determined.

Polyclonal or monoclonal antibody or other reagent	Patient 1	Patient 2
α_1-Antitrypsin	+	+
α_1-Antichymotrypsin	+	+
LN-3 (HLA-DR)	+	ND
Lysozyme	+	+
Neuron-specific enolase	–	–
Glial fibrillary acidic protein	–	–
Myelin-associated glycoprotein	–	–
Leu-M1	–	ND
Esterase	+	+
Acid phosphatase	ND	+

histochemical criteria (Table 2 and Fig. 2, B and C). In these regions, over 70% of cells contained high levels of viral RNA (Fig. 2D). Rare infected mononucleated cells were detected in gray and white matter outside the lesion (Fig. 2E). The mono- and multinucleated macrophages did not stain with antibodies recognizing primary neuronal or glial cells such as neuron-specific enolase, glial fibrillary acidic protein, and myelin-associated glycoprotein (Table 2).

In situ hybridization for HIV RNA was performed on some tissue sections previously stained immunohistochemically with antibodies to HLA-DR (patient 1) and lysozyme (patient 2) to demonstrate that the cells characterized as macrophages contained HIV RNA. Figure 1C shows localization of silver grains over an HLA-DR–bearing giant cell. From 5 to 15% of HLA-DR positively stained cells contained HIV RNA. Viral RNA in a macrophage containing lysozyme is shown in Fig. 2G.

Transmission electron microscopy (Fig. 3) was performed on brain tissue of patient 2 from areas of greatest demyelination and tissue necrosis. Typical retroviral particles were observed budding from and associated with the plasma membranes of infected cells (*18*). Ultrastructurally, these infected cells were characterized as macrophages by the evidence of phagocytosis and by the presence of lipid vacuoles, lysosomes containing membranous material resembling myelin, and subplasmalemmal linear densities (*19, 20*). Free, immature particles measuring 105 to 115 nm in diameter had a clear 70-nm central zone surrounded by a dense ring and separated from the outer double membrane by a less electron-dense zone. The mature particles had a swollen and distorted outer membrane and contained an eccentric, roughly spherical electron-dense core measuring approximately 40 nm in diameter. When the particles were tangentially and longitudinally sectioned, the cores resembled cones or bars measuring up to 80 nm in length. Retroviral particles were not observed budding from or in association with oligodendroglial cells, astrocytes, plasma cells, lymphocytes, or endothelial cells. Small numbers of viral particles were seen budding from macrophages located in the adjacent white matter.

In addition to the retroviral particles, papovavirus virions measuring approximately 40 nm in diameter and typical of JC virus, the infectious agent implicated in PML, were observed in the nuclei of oligodendroglial cells. Filamentous forms of the virus were regularly observed, while crystalline arrays of particles were only occasionally seen. When these specimens were hybridized with a biotinylated probe to JC viral

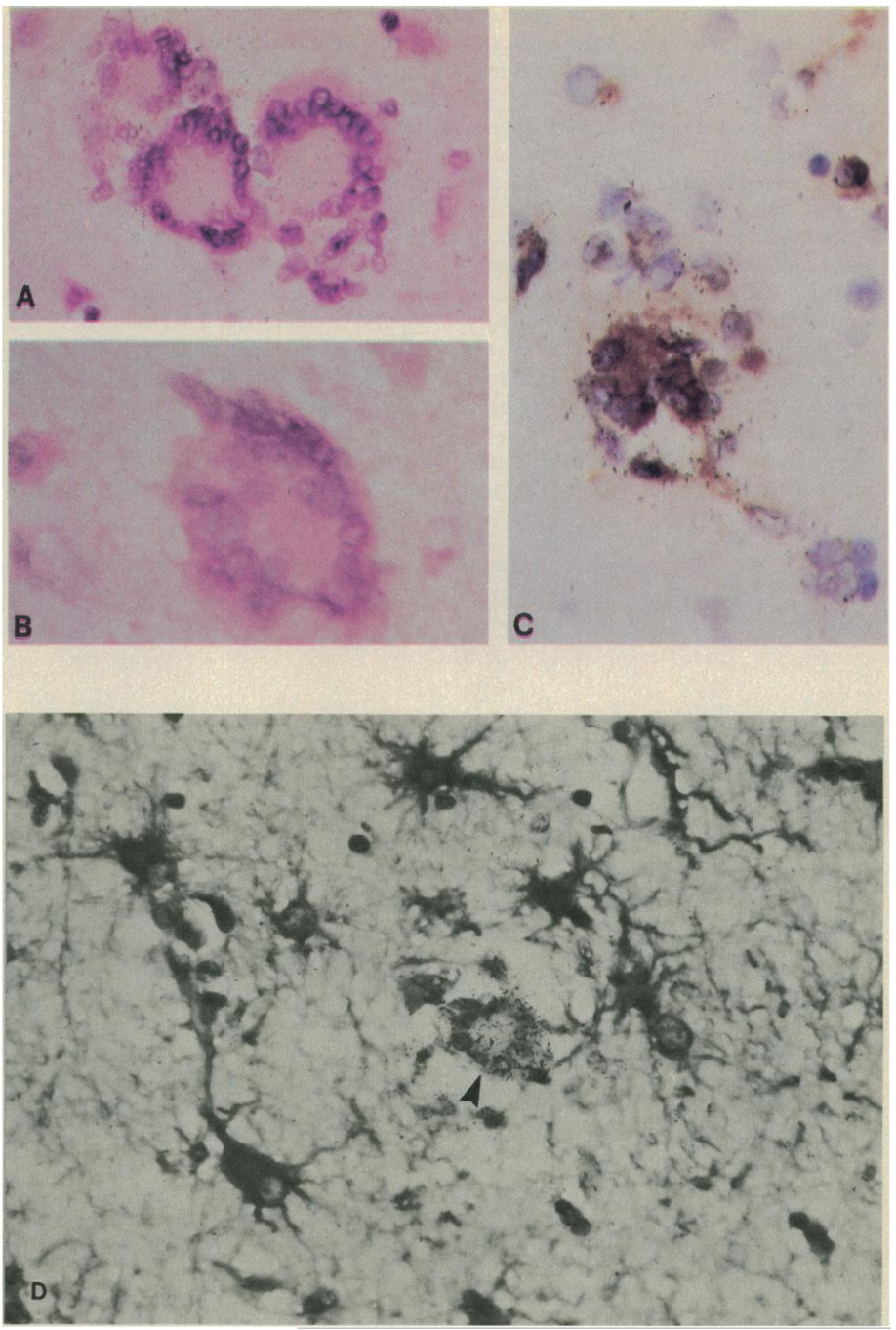

Fig. 1. In situ hybridization with HIV RNA-specific probes on brain tissue from patient 1. (A) Localization of silver grains over multinucleated macrophages indicating AIDS retrovirus RNA. (B) Uninfected giant cell from another field of the same tissue section. (A and B, hematoxylin and eosin, 90×.) (C) Tissue sections stained with the LN-3 specific antibody (to HLA-DR) and hybridized with complementary ^{35}S-labeled HIV RNA probes, showing HLA-DR protein and HIV RNA in the same multinucleated giant cell (hematoxylin, 100×). (D) Astrocytes stained with the rabbit antibody to glial fibrillary acidic protein surrounding an infected multinucleated giant cell (arrow) (hematoxylin and eosin, 100×). For in situ hybridization, tissue sections (6 μm) on polylysine-coated slides were baked at 55°C for 1 hour, passed through xylene and graded ethanols, and processed as described (*30, 31*). Control cells were sedimented onto glass slides, fixed with periodate-lysine-paraformaldehyde-glutaraldehyde (PLPG), and similarly treated (*30, 31*). Subgenomic DNA fragments of the virus that hybridized with a series of probes [pB1 (*32*), pBenn6 (*33*), pB11 (*32*), and a recombinant plasmid (pRG-B) (*31*) that contains a 1.35-kb Hind III fragment mapping between 8.25 and 9.6 kb on the proviral DNA] were subcloned into SP6/T7 vectors (Promega Biotec) and the pooled DNA's were transcribed with ^{35}S-labeled uridine triphosphate (UTP; Amersham). For combined immunocytochemistry and in situ hybridization, preparations were first processed for immunocytochemistry and then for in situ hybridization (*34*). Previous studies demonstrated that specimens processed for immunocytochemistry had reduced in situ hybridization. To assess accurately whether viral genomes were present, we processed serial specimens at the same time for in situ hybridization only.

DNA, viral DNA was detected predominantly in oligodendrocytes, a few astrocytes, but not in mono- and multinucleated macrophages (Fig. 2H).

Thus, in these patients with AIDS, mono- and multinucleated macrophages were the major cell types infected with HIV. The cells had been actively synthesizing AIDS retrovirus RNA and producing progeny virions. Both morphologically mature and immature particles of the virus could be identified. In experiments where combined immunocytochemistry and in situ hybridization were performed, cells synthesizing AIDS retrovirus RNA were predominantly (>93%) of macrophage lineage.

The mono- and multinucleated macrophages described in this report may arise from HIV-infected peripheral blood monocytes that traverse the blood-brain barrier, and terminally differentiate into giant cells in the brain. Recent studies of visna virus–induced CNS disease of sheep suggest the passage of infected monocytes from the circulation into the cerebrospinal fluid (*21–26*). Since biologic, biochemical, and structural similarities exist between visna virus and the AIDS retrovirus, parallel mechanisms of viral pathogenesis may exist. In support of this model, Ho *et al.* have reported (*15*) evidence for monocyte infection in vivo in AIDS patients. Multinucleated giant cells have been described in brains, lymph nodes, and lungs in vivo in patients with AIDS and AIDS-related complex (ARC) (*27, 28*). Thus, a cell of common monocyte/macrophage lineage may become infected, travel to multiple organs, differentiate into mononucleated and multinucleated macrophages, and then serve as a major reservoir for HIV. Recruitment of uninfected monocytes into the brain may also be stimulated in response to infection with other organisms. The large monocytic inflammatory exudate seen in patient 2 occurred in the setting of a coinfection with JC virus. These cells could become targets of HIV infection by other infected macrophages or lymphocytes, or by non–cell-associated free circulating virus.

The localization of HIV in brain macrophages is only an initial step in understanding the pathogenesis of dementia in affected individuals. Infected macrophages may secrete factors that cause extensive tissue injury and edema in white matter and impair neural transmission. Alternatively, the expression of viral antigens on the surface of macrophages may elicit a delayed-type hypersensitivity response and thus precipitate damage in white matter in either an indiscriminate manner or by specific recognition of cross-reacting antigens normally expressed in the brain. Although the macrophage is the predominant cell type infected with HIV in the brains of these individuals, infection of other cells, including glia, neurons, and endothelial cells (*29*), may also play a role in the pathogenesis of AIDS dementia.

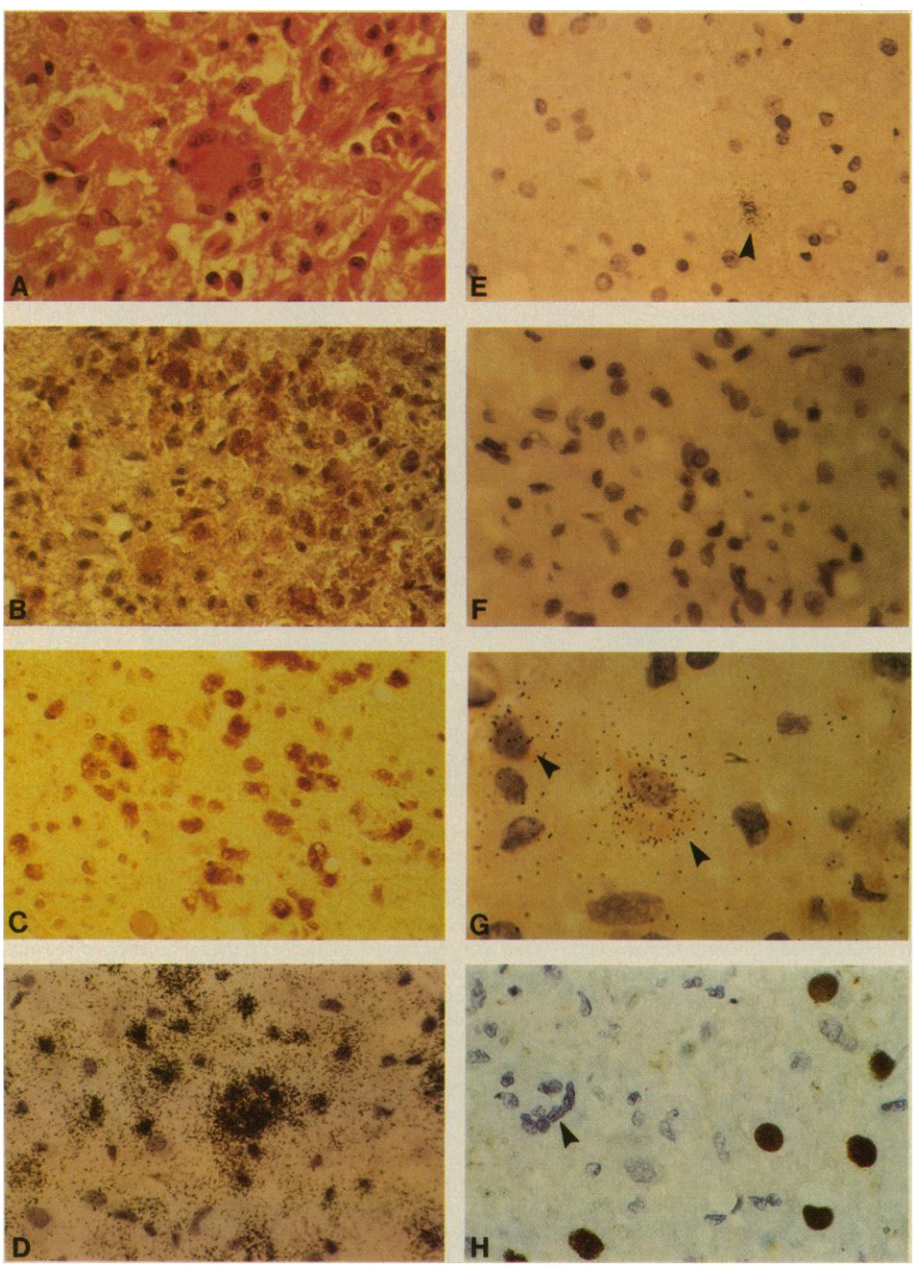

Fig. 2. Photomicrographs of brain sections from patient 2 showing histologic, histochemical, immunohistochemical, and in situ hybridization results. (A) Cerebrum in demyelinated area showing multinucleated giant cell surrounded by mononuclear cells. (B) Adjacent section stained with acid phosphatase shows many mature macrophages within the inflammatory exudate. (C) A demyelinated area stained with nonspecific esterase showing numerous macrophages in the inflammatory exudate. (D) In situ hybridization with ^{35}S-labeled RNA probe complementary to HIV RNA performed on the demyelinated areas. HIV RNA is present in the multinucleated giant cell (center) and in many mononucleated cells and is also located extracellularly. (E) In subcortical white matter rare cells contain viral RNA (arrow). (F) Demyelinated area hybridized with control probe (same orientation as viral mRNA) showing background. (G) Direct identification of HIV RNA in macrophages in section immunochemically labeled for lysozyme protein and then by in situ hybridization for viral RNA. (H) JC viral DNA in oligodendrocytes illustrated by in situ hybridization using a biotinylated DNA probe for JC virus. Many oligodendrocytes contain JC viral DNA (brown cells) at the edge of the demyelinating plaque. Note that an adjacent multinucleated cell (arrow) is unlabeled. JC genomic DNA was nick-translated with biotinylated dUTP (Enzo). The size of the resulting probe was 80 to 300 bp with 21% incorporation. The hybridization procedure, post hybridization washes, and the detection by affinity cytochemistry were performed as described (*31, 35*).

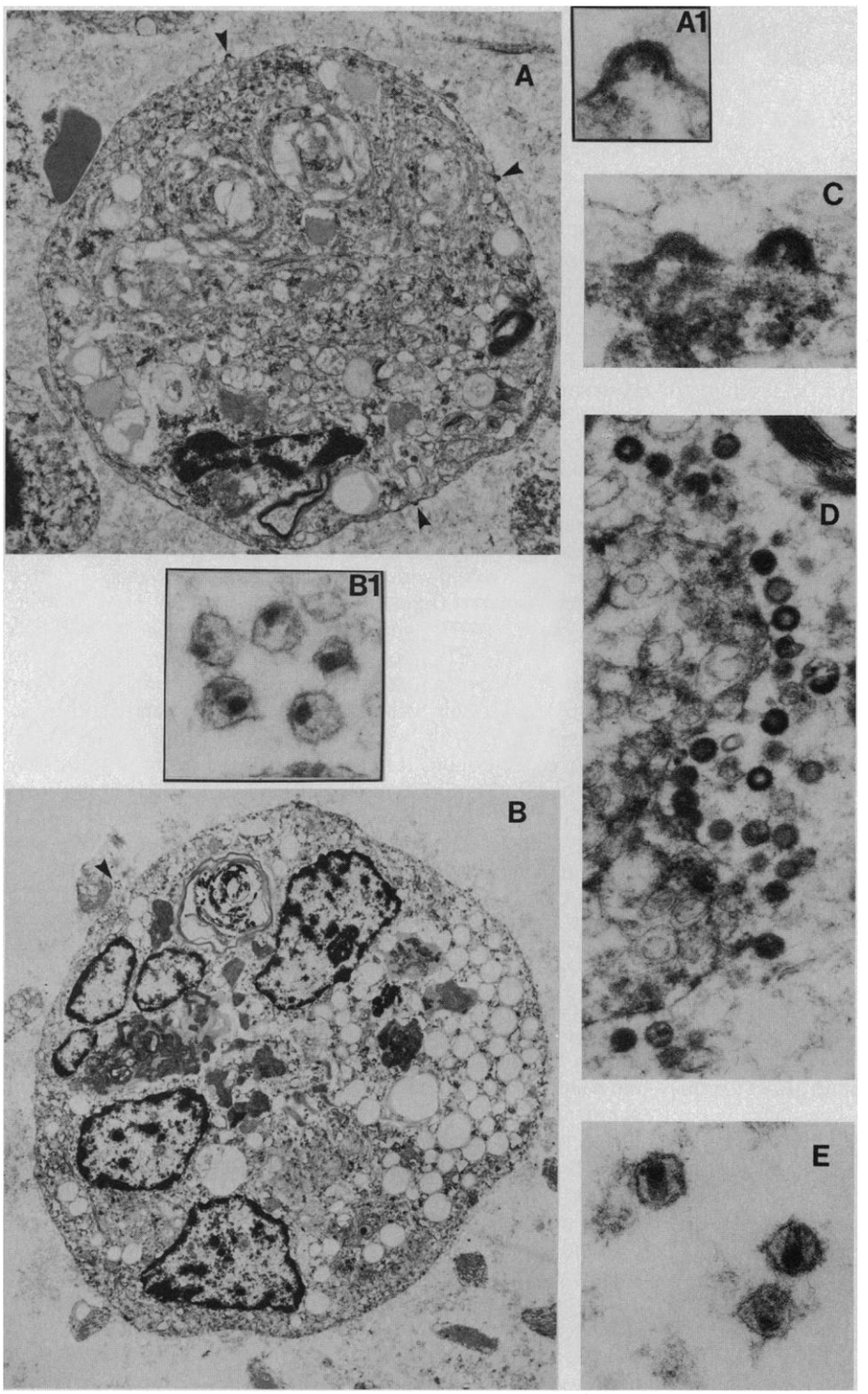

Fig. 3. Transmission electron micrographs of tissue from patient 2. Typical virus-producing (A) mononuclear cells (×9300) and (B) multinucleated cells (×5000) rich in lipids, myelin fibers, and viral particles in varying stages of maturation, are shown. Three particles are budding from the surface of the mononuclear cell (A, arrows); the upper one is enlarged (A1). Two are budding from another cell (C). Late budding and free immature particles with ring-shaped nucleoids are abundant (D). The nucleoids in most mature virions are tangentially or perpendicularly sectioned (B and B1) and only occasional longitudinally sectioned particles showing the conical core shell are seen (E). The outer membrane of the mature virions is usually deformed (B1 and E). (A1, B1, C, and E ×100,000; D ×50,000). At autopsy, fresh tissue samples were fixed in 2.5% glutaraldehyde in phosphate-buffered saline. They were postfixed in 1% OsO_4, dehydrated through graded ethanol, cleared in propylene oxide, and embedded in plastic. Thin sections were stained with uranyl acetate and lead citrate and examined on a Zeiss EM 10H.

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