Acta Neuropathol (Berl) (1986) 69:253–258

Acta Neuropathologica

Multinucleated Giant Cells in Brain: A Hallmark of the Acquired Immune Deficiency Syndrome (AIDS)

H. Budka

Neurological Institute, University of Vienna, Schwarzspanierstr. 17, A-1090 Vienna, Austria

Summary. Multinucleated giant cells (MGCs) were found in the brains of two patients with the acquired immune deficiency syndrome (AIDS), but were absent in five other AIDS brains. In one case there was a distinctive distribution of MGCs in disseminated clusters; damage of brain parenchyma was minor or absent. In another case, MGCs were restricted largely to the perivascular spaces and were accompanied by lesions of toxoplasmosis and cytomegalovirus infection. In paraffin sections, morphological and histochemical-staining characteristics of MGCs were similar to those of macrophages. Occasional immunolabeling of MGCs with monoclonal antibody to leukocyte common antigen suggested a hematogenous origin. MGCs were not stained by immunocytochemistry for neural markers glial fibrillary acidic protein, S 100 protein, neurofilament proteins, neuron specific enolase, and myelin basic protein and, therefore, appear unlikely to originate from the neuroepithelium. In the absence of evidence of other infections in case 1, the peculiar tissue reaction found could be a direct result of infection by the AIDS retrovirus. The formation of MGCs is likely to represent a cytopathic effect of the virus on lymphoid or monohistiocytic cells infiltrating the brain (infection of these cells could occur before or after they entered the brain). These assumptions are supported by the finding of similar MGCs in permissive lymphoid cell cultures after infection with the AIDS retrovirus.

Key words: Acquired immune deficiency syndrome (AIDS) – HTLV-III – Retrovirus – Giant cells – Immunocytochemistry

Introduction

The acquired immune deficiency syndrome (AIDS) has been causally related to infection with the human lymphotropic retrovirus (LAV, Barré-Sinoussi et al. 1983; HTLV-III, Popovic et al. 1984). HTLV-III DNA and RNA have been detected in brains of AIDS patients (Shaw et al. 1985). One cytopathic effect of HTLV-III in permissive human T-cell lines is the production of multinucleated giant cells (Popovic et al. 1984). Most recently, multinucleated giant cells (MGCs) have been briefly described in the brains of three patients with AIDS, and it was suggested that such cells serve as a marker for HTLV-III infection (Sharer et al. 1985). However, the cell or origin of MGCs and their causal versus secondary relationship to brain tissue damage remains to be elucidated. Here, the incidence, distribution and light microscope morphology of MGCs are reported in the brains of patients with AIDS. The characterization of these cells by histochemistry and immunocytochemical markers is described.

Material and Methods

Autopsy brain tissues of seven patients suffering from AIDS, as defined by the Centers for Disease Control (CDC 1983), were studied by light microscopy after formalin fixation and paraffin embedding. Clinical data, the neuropathology of these patients, and the presence of MGCs in brain are summarized in the Table 1. Cases 1, 3–5 and 7 were from the region of Vienna and were studied neuropathologically in detail in this laboratory. Several paraffin blocks of cases 2 and 6 were studied by courtesy of Drs. G. N. Budzilovich (New York, USA) and P. Pilz (Salzburg, Austria). Cases 3–5 (Kristoferitsch et al. 1985) and 6 (Wessely et al. 1984), all lacking MGCs in brain, have been previously reported. In cases 1 and 2, sections from three paraffin blocks containing the largest number of MGCs were studied with the following stains: hematoxylin-eosin, Bodian, luxol fast blue-nuclear fast red, Giemsa, methylgreen-pyronine, PAS, PAS after diastase, alcian blue pH 2,5, Sudan black B, Gram, Grocott, and Ziehl-Neelson. Immunocytochemical staining was performed by the PAP technique (Sternberger 1979), using polyclonal antisera raised against the following antigens (characterization or source in brackets): glial fibrillary acidic protein (GFAP; Herpers et al. 1984), S 100 protein (Kimura et al. 1986), 150 and 200 kDa subunits of neurofilament

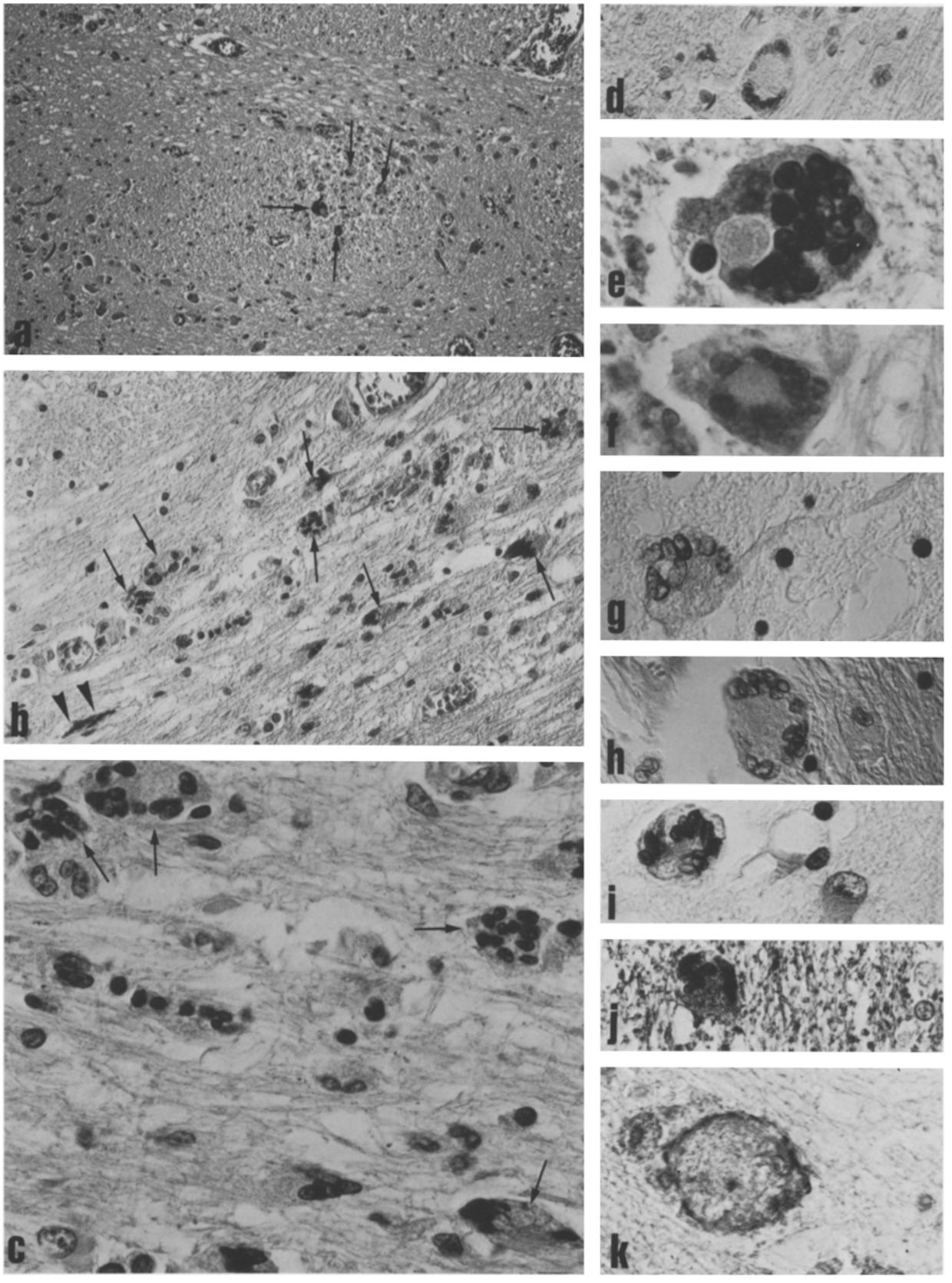

Fig. 1a–k

Table 1. Clinical data, neuropathology, and occurrence of multinucleated giant cells (MGCs) in seven AIDS brains[a]

Case	Sex	Age	Risk group	Neuropathology	MGCs present
1 (29–85)	M	19	Hemophiliac	Multifocal giant cell encephalitis (mainly pons), malignant lymphoblastic lymphoma (mainly basal ganglia)	Yes
2 (138–82)	M	39	Homosexual	Necrotizing toxoplasmosis, foci of cytomegalic cells	Yes
3 (14–83) (Kristoferitsch et al. 1985, Case 1)	M	50	Homosexual	Micronodular encephalitis with cytomegalic cells	No
4 (31–83) (Kristoferitsch et al. 1985, Case 2)	M	56	Homosexual	Nectrotizing toxoplasmosis	No
5 (50–84) (Kristoferitsch et al. 1985, Case 3)	M	41	Homosexual	Necrotizing toxoplasmosis	No
6 (85–84) (Wessely et al. 1985)	M	44	Homosexual	Necrotizing toxoplasmosis	No
7 (121–85)	M	36	Homosexual	Micronodular encephalitis, spongy leukoencephalopathy (cerebellum), posterior tract degeneration, axonal neuroradiculopathy	No

[a] see also: Note added in proof

protein (NFP; Dr. Takeuchi, Fukuoka, Japan), neuron specific enolase (NSE; DAKOpatts, Copenhagen, Denmark), myelin basic protein (MBP; Dr. H. Bernheimer, Vienna, Austria), fibronectin (FN; Kochi et al. 1983), factor VIII-related antigen (FVIIIRAG; DAKO), lysozyme (DAKO), alpha-1-anti-trypsin (AAT; DAKO), alpha-1-antichymotrypsin (AACT; DAKO), and genus-specific papovavirus antigens (Budka and Shah 1983). Monoclonal antibodies to leukocyte common antigen (LCA; Warnke et al. 1983) were used in a labeled avidin-biotin technique. Positive controls included staining of brain parenchymal elements with neural markers, of vessels for FN and FVIIIRAG, of granulocytes for lysozyme, AAT and LCA, and of monohistiocytic cells for AACT. Staining for papovavirus was controlled by concomitant staining of progressive multifocal leukoencephalopathy (PML) brain tissue. Specificity of immunostaining was checked by preparations in which the primary antibody/antiserum was replaced by buffer, normal rabbit serum or non-relevant mouse antibody.

Results

MGCs were readily apparent in brain tissue of cases 1 and 2. Histological review of many sections failed to show typical MGCs in cases 3–7, although occasionally very large mono- or binuclear macrophages or glial cells without typical features of MGCs were present.

Distribution of MGCs

Two patterns were observed. In case 2, MGCs were randomly and widely scattered in the cerebral white matter. Focal accumulations of these cells were not found. They were seen amongst mononuclear macrophages in damaged parenchyma in the vicinity of necrotizing lesions of toxoplasmosis or cytomegalic inclusion-body disease. However, most of these cells were located in the perivascular spaces (Fig. 1h, i) of the white matter, where they occured in areas with severe parenchymal damage, but also in areas with little or no microscopical change.

The second pattern of the distribution of MGCs, found in case 1, was the accumulation in small foci of such cells, especially in the brain-stem white and grey matter. The cells appeared to infiltrate the adjacent parenchyma from perivascular spaces. Loosening of the parenchyma, consistent with edema, was seen in these regions (Fig. 1a). Damage to the involved neural parenchyma was slight. Neurons were intact and no myelin breakdown products or fragmented axons were seen (Fig. 1c). There were, however, large spider-shaped reactive astrocytes within the foci and in unaffected surrounding tissues (Fig. 2a). Up to eight

Fig. 1. a–c Multifocal giant cell encephalitis (case 1). Multinucleated giant cells (MGCs) (*arrows*) are visible in small focus already at low magnification (**a**); there is some edematous attenuation of tissue (**a–c**) within foci, but neural parenchyma appears largely intact (**c**). Nuclei of MGCs appear darker than other nuclei (**b**). Nuclei are arranged at MGC periphery or irregularly in cytoplasm (**c**). Some MGCs are elongated (*arrowheads* in **b**). Hematoxylin & eosin, **a** and **b** with Nomarski optics; **a** ×100, **b** ×250, **c** ×630. **d–i** Cytological appearance of MGCs. **d–f** from case 1, **g–i** from case 2. **d**, **e**, **g–i** hematoxylin & eosin, **f** PAS. **d**, **g** and **h** with Nomarski optics. **d** ×400; **e**, **f** ×1,000; **g–i** ×630. **j** Case 2. Granular sudanophilic material in MGC cytoplasm. Sudan black B, ×250. **k** Case 2. Strong immunolabeling with anti-LCA at the surface of MGC, less strongly of mononuclear macrophage at *left*. ×630

MGCs could be found within one focus at one plane of section (Fig. 1b). In addition to MGCs, variable numbers of other cell types were seen in the foci. These included scattered mononuclear macrophages and a few lymphoid cells. The latter were identified by their strongly positive staining with anti-LCA (Fig. 2b). These cells appeared to infiltrate the adjacent parenchyma from some perivascular cuffs (Fig. 2c).

Cytological Appearance of MGCs

MGCs were similar in both cases. Their outlines were round (Fig. 1d, e, h–k) or, less frequently, polygonal (Fig. 1f). Occasionally the cells were elongated and appeared to conform to the interaxonal spaces (Fig. 1b). Rarely, prominent long cytoplasmic processes were seen (Fig. 1g). Up to 20 nuclei within one plane of section were most often in circular (Fig. 1d, f) or semicircular (Fig. 1h) arrangement at the cell periphery. There were also many MGCs with centrally, or irregularly located nuclei (Fig. 1c, i). Many MGCs contained nuclei with coarse chromatin, which was denser than that of surrounding neurons and glia (Fig. 1b, c). Nuclear inclusion bodies were not seen. The cytoplasm was eosinophilic and showed a diffuse fine granularity; occasional vacuoles or lucent areas were found (Fig. 1e, j).

Histochemistry of MGCs

Staining of MGC cytoplasm was uniform. There was little pyroninophilia, but diffuse staining with alcian blue and PAS was seen (Fig. 1f). Diastase-resistant, strongly PAS-positive granular material was prominent in many cells, which thus were easily detactable at low magnification. There was usually also granular staining with Sudan black (Fig. 1j). However, luxol fast blue and Ziehl-Neelson stains, the latter for lipofuscin, were usually negative. Mononuclear macrophages usually showed similar staining characteristics to those of the adjacent MGCs. No fungi or bacilli were seen in the Gram, Grocott or Ziehl-Neelson preparations.

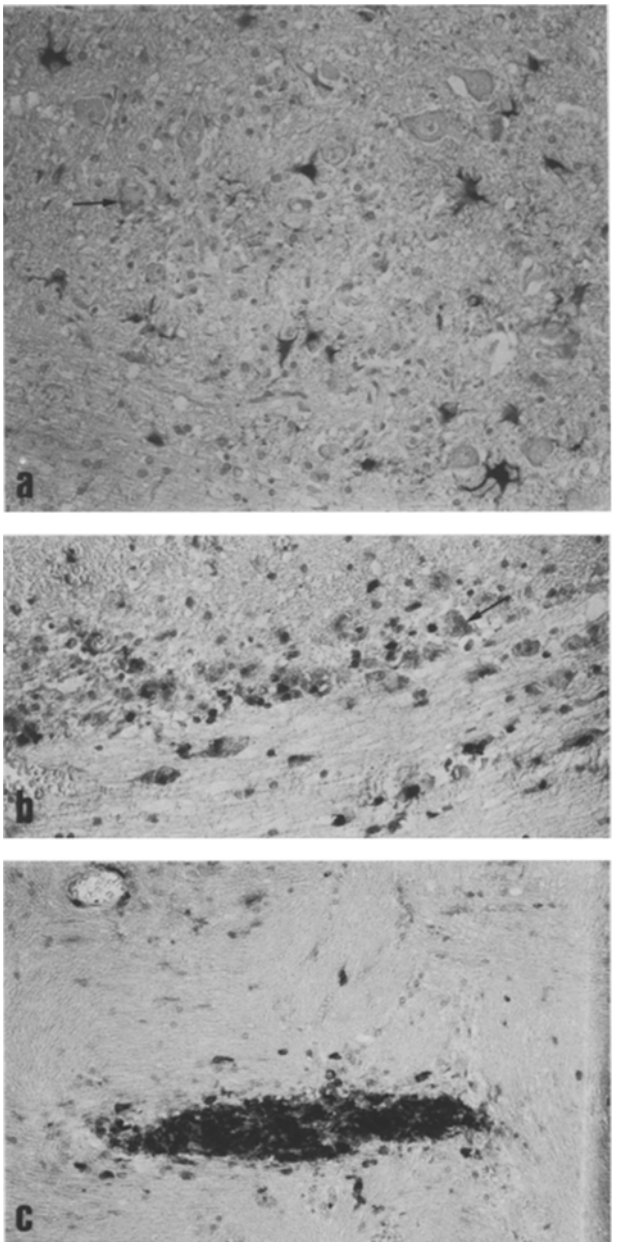

Fig. 2. a–c Multifocal giant cell encephalitis (pons). **a** Some reactive astrocytes in the lesion (*MGC* at *arrow*) and in surrounding tissue. Anti-GFAP, slight nuclear counterstain, ×186. **b** Moderately many hematogenous cells, labeled with anti-LCA, in lesion. MGC (*arrow*) is unlabeled. ×186. **c** Perivascular cuff of lymphoid cells infiltrating in adjacent parenchyma. Anti-LCA, **a–c** with Nomarski optics, ×119

Immunochemistry of MGCs

MGCs were consistently negative for GFAP, S 100 protein, NFPs, NSE, and MBP staining. Neither did they stain for FN or FVIIIRAG. Immunostaining for lysozyme and AAT was also negative. When stained with anti-AACT, some MGCs were not labeled, but others showed moderate diffuse labeling. Prominent granular immunoreactivity, however, was absent. Immunostaining with anti-LCA left most MGCs unstained; occasionally a distinct labeling similar to membrane staining of adjacent mononuclear macrophages was found (Fig. 1k). No MGCs or other cells contained papovavirus antigens.

Discussion

Changes in the central nervous system in AIDS patients have included a wide variety of pathologic lesions (Reichert et al. 1983). These comprise multiple infections, vascular, hypoxic or metabolic conditions, malignant lymphomas (Moskowitz et al. 1984), vacuolar myelopathy (Petito et al. 1985), and progressive diffuse leukoencephalopathy (Kleihues et al. 1985). Since MGCs were found here in two out of seven AIDS brains [1], these characteristic cells seem also to be an important feature of the brains of some patients with AIDS. Most recently, Sharer et al. (1985) described MGCs found in three AIDS brains, and the illustrations in that report appear identical to the appearance of many MGCs in this study. They described MGCs as scattered hapharzardly throughout the gray and white matter, and usually associated with other lesions, such as nodules of macrophages, neuronal loss, or gliosis. They suggested that MGCs resembled macrophages. In two other AIDS brains, Kleihues et al. (1985) found MGCs which were in close association with white matter damage, described as progressive diffuse leukoencephalopathy including the presence of papovavirus antigens. Although I found MGCs also associated with signs of parenchymal damage in some areas in case 2, it was striking in both cases that MGCs were also found without prominent signs of damage to neural tissue. This suggests that the appearance of MGCs is neither the cause, nor the sequel of significant brain parenchyma breakdown. Rather, the histological changes suggest a process of cellular infiltration into the brain which could be, but is not necessarily, attracted by some primary pathology. Immunoreactivity with anti-LCA of MGCs and some adjacent elements is evidence of hematogenous infiltration. Absent immunoreactivity of MGCs for neural markers renders a neuroectodermal origin unlikely.

The presence of clustered MGCs was the principal neuropathologic feature in case 1. Such a state in the apparent absence of tissue damage appears to differ from that described by Sharer et al. (1985) and Kleihues et al. (1985), in which the distribution of MGCs appears to be similar to that in our case 2. There, MGCs loosely scattered mainly in perivascular spaces were less conspicuous than in case 1, were not the hallmark of the tissue syndrome (as they were in case 1), and were overshadowed by other prominent lesions. Similar cases may have gone unnoticed in the past, and a retrospective search for MGCs in histological slides of AIDS brains might yield more data about these cells.

MGCs occuring in AIDS or AIDS-related complex have also been noted in lymph nodes (Domingo and Chin 1983, Joshi et al. 1984), but have not been mentioned in other organs at autopsy (Reichert et al. 1983). In lymph nodes, MGCs were interpreted as lymphoid cells (Domingo and Chin 1983), although until now specific lymphoid cell markers have not been reported for MGCs. In this study, characterization of MGCs by histochemistry and immunocytochemistry applied to paraffin sections revealed a uniform staining behaviour of MGCs in both cases, suggesting that MGCs are derived from one cell type only. By courtesy of Dr. P. Kleihues (Zurich, Switzerland), MGCs occuring in two other AIDS cases, described elsewhere as progressive diffuse leukoencephalopathy (Kleihues et al. 1985), could be studied with the same (immuno)-stains as our cases 1 and 2, and revealed the same results [1]. MGCs are shown here to contain sudanophilic, diastase-resistant PAS-positive and alcianophilic materials similar to those found in macrophages. However, immunocytochemical staining generally associated with macrophages remained inconclusive. The negative result with lysozyme and AAT does not rule out a monohistiocytic/macrophage nature of MGCs in brain, since some of those cells may be negative in paraffin sections (Mason and Taylor 1975; Pinkus and Said 1977; Papadimitriou et al. 1980). Immunoreactivity with anti-AACT was seen diffusely, but not granularly, in MGCs and thus cannot be reliably separated, especially in postmortem tissue, from secondary uptake from the plasma (Meister et al. 1980; Isaacson et al. 1981). Immunolabeling with anti-LCA, as found here on some MGCs, is strongest with lymphoid cells, whereas the reactivity of macrophages is much more variable (Warnke et al. 1983). Other currently available immunocytochemical markers for lymphoid and monohistiocytic cells and their subtypes cannot be reliably applied to paraffin sections. Therefore, it remains to be determined whether MGCs in AIDS brains originate from the lymphoid and/or monohistiocyte/macrophage cell lineages.

Certainly the most intriguing aspect of the occurrence of MGCs in AIDS brains is the possible relation to infection with the AIDS retrovirus. One characteristic effect of HTLV-III in the infected permissive T cell lines is the arrangement in giant cells of multiple nuclei in ring formation (Popovic et al. 1984). These cells were proposed as indicators of HTLV-III infection in clinical specimens (Popovic et al. 1984), including brain (Sharer et al. 1985). They are strikingly similar, if not identical, to the MGCs described here. By tracing HTLV-III DNA and RNA, Shaw et al. (1985) demonstrated frequent infection of brain by HTLV-III as a possible cause of AIDS encephalopathy. However, the histopathological correlates of

[1] see: Note added in proof

brain infection by the AIDS retrovirus remain to be defined. In the absence of other lesions such as opportunistic infections, prominent occurrence of MGCs as in case 1 may correlate directly to HTLV-III infection. Lymphoid or monohistiocytic cells infiltrating the brain from the bloodstream or meninges could manifest the cytopathic effect of the AIDS retrovirus by formation of MGCs. Infection of these cells could occur before or after they entered the brain.

It is not clear why the brain can be more heavily infected by the AIDS retrovirus than other organs, such as lymphatic tissues (Shaw et al. 1985). Since Shaw and co-workers found up to 10% of brain cells infected with HTLV-III, the MGCs in AIDS brains seem to occur in numbers too limited to represent the entire cellular tropism of AIDS retrovirus in brain. Further insight into the mechanisms of brain infection by HTLV-III is urgently needed; it was recently suggested that the AIDS retrovirus, by causing a possible "slow virus" encephalopathy with incubation periods between 2 and 30 years, could be in the process of producing a self-sustaining pandemic (Seale 1985).

Acknowledgements. The author is indebted to Dr. T. Radaszkiewicz (Vienna) for help with histiocytic immunocytochemical markers, to Dr. P. C. Burger (Zurich) for valuable comments and help in editing the English, and to Ms B. Windsperger for excellent technical assistance.

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Received October 7, 1985/Accepted November 11, 1985

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Since submission of this manuscript, histological slides from three more AIDS brains could be studied by courtesy of Prof. B. Volk, Freiburg i. Br., FRG. In one of the latter brains, scattered or focally clustered MGCs were found adjacent to, but also remote from, large necroses of the white matter and showed the morphological characteristics described above in detail.

Thus, in a total of 12 AIDS brains which could be histologically studied by the present author, MGCs were present in 5 cases. This relative frequency further corroborates the importance of MGCs as a morphological hallmark in AIDS brains.