American Journal of Pathology, Vol. 148, No. 4, April 1996


# Immunohistochemical Localization of Capsular Polysaccharide Antigen in the Central Nervous System Cells in Cryptococcal Meningoencephalitis

Sunhee C. Lee,* Arturo Casadevall,† and Dennis W. Dickson*

*From the Department of Pathology (Neuropathology)* and Division of Infectious Diseases of the Department of Medicine,‡ Albert Einstein College of Medicine, Bronx, New York*

***Cryptococcal meningoencephalitis (CME) is caused by the encapsulated fungus* Cryptococcus neoformans *(CN) and is a major cause of mortality and morbidity in patients with AIDS. The polysaccharide capsule of CN is important for virulence, and soluble polysaccharide has the potential to cause immune modulation. To better understand the interactions of central nervous system cells and cryptococcal capsular polysaccharide (CNPS) in the pathogenesis of human CME, postmortem brain tissue from 21 patients with CME (13 AIDS and 8 non-AIDS patients) was analyzed. Histopathology and distribution of tissue CNPS antigen were analyzed using monoclonal antibodies against CNPS in combination with cell type-specific markers (glial fibrillary acidic protein for astrocytes;* Ricinus communis *agglutinin (RCA)-1 for macrophage/microglia and endothelial cells; UCHL-1 for T cells; L26 for B cells). The CN cells showed discrete capsular immunoreactivity as expected; however, diffuse and particulate cellular and tissue staining for CNPS was detected in the brain parenchyma and the meninges in all cases. By quantitative analysis, the CNPS immunoreactive area ranged from 0.1 to 88% of tissue cross sectional area, and tended to be higher in brains of AIDS (median values from two sections ranged from 1 to 57%; mean, 26%) than in non-AIDS (0.1 to 40%; mean, 9.6%) patients. The proportion of CNPS immunoreactive area was positively correlated with the estimated number of CN. None (0/13) of the AIDS patients displayed significant inflammatory responses to CN, whereas most (7/8) non-AIDS patients showed granulomatous inflammatory responses. The phenotype of infiltrating lymphocytes was UCHL-1$^+$/L26$^-$/RCA$^-$, thus consistent with activated T cells, both in AIDS and non-AIDS patients. Double immunolabeling studies revealed that tissue CNPS immunoreactivity was most often localized in macrophages and microglia, less frequently in reactive astrocytes and endothelial cells, but not in lymphocytes. This study demonstrates that CNPS can be detected not only in the serum and cerebrospinal fluid (CSF) of patients, but also in the affected tissue, most often localized in cells of mononuclear phagocyte system. Potential implications of these findings for the pathogenesis of CME are discussed. (Am J Pathol 1996, 148:1267–1274)***

*Cryptococcus neoformans* is a frequent cause of life-threatening meningoencephalitis in immunocompromised patients.[1,2] In recent years there has been a marked increase in the frequency of cryptococcal infections as a result of the AIDS epidemic, since cryptococcosis occurs in 6 to 8% of patients with HIV infection.[3] *C. neoformans* is neurotropic, and most cases of cryptococcal disease are accompanied by central nervous system involvement. Meningeal involvement is often accompanied by parenchymal involvement as a result of spread of infection through Virchow-Robin spaces.[2,4,5]

Supported by NIH grants NIMH MH46676 (SCL and DWD), and AI33774 and AI13342 (AC).

This article was partially presented in abstract form at the XIIth International Congress on Neuropathology in Toronto, Canada, September 1994.

Accepted for publication December 26, 1995.

Address reprint requests to Sunhee C. Lee, M.D., Department of Pathology (Neuropathology), F-717N, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

Table 1. *Summary of Case Information*

| Patient no. | Age/Sex | Underlying illness | Other CNS pathology | Fungal number* | Inflammatory response† | CNPS Immunoreactivity‡ |
|---|---|---|---|---|---|---|
| 1 | 34/M | AIDS | Toxoplasmosis, HIVE§ | Few | Macrophage | <1% |
| 2 | 28/M | AIDS | HIVE | Few | Macrophage | 1% |
| 3 | 26/M | AIDS | *Escherichea coli* meningitis | +++++ | Macrophage | 28 to 86% |
| 4‖ | 28/F | AIDS | HIVE, schizencephaly | Few | Microglial nodule | 15 to 88% |
| 5 | 44/M | AIDS | Basal ganglia infarct, old | ++ | Macrophage | 1 to 4% |
| 6 | 40/F | AIDS | White matter infarcts, acute | ++ | Macrophage ≫ Lymphocyte | 33 to 45% |
| 7 | 33/M | AIDS | Cytomegalovirus ventriculitis | + | Macrophage | 5 to 32% |
| 8 | 30/M | AIDS | HIVE | ++/+++ | Macrophage ≫ Lymphocyte | 41 to 59% |
| 9 | 22/M | AIDS | Toxoplasmosis, treated | ++ | Macrophage ≫ Lymphocyte | 18 to 27% |
| 10 | 28/M | AIDS | None | +++++ | Macrophage/Lymphocyte | 31 to 56% |
| 11 | 36/M | AIDS | None | +++ | Macrophage/Lymphocyte | 26 to 48% |
| 12 | 57/M | AIDS | Lymphoma, HIVE | +/++ | Macrophage/Lymphocyte | 2 to 7% |
| 13 | 47/M | AIDS | HIVE | + | Macrophage/Lymphocyte | 4 to 19% |
| 14 | 42/M | Heroin nephropathy | Cortical infarcts | +/++ | Granuloma | 0 to 9% |
| 15 | 60/F | Rheumatic heart disease | None | + | Lymphocyte > Macrophage | 5 to 13% |
| 16 | 50/F | None | Thalamic infarct | +/+++ | Lymphocyte > Macrophage | 3 to 87% |
| 17 | 93/F | Cancer of kidney | Alzheimer's disease | + | Granuloma | <0.1% |
| 18 | 13/F | Renal transplantation | Uremic encephalopathy | + | Neutrophil > Macrophage > Lymphocyte | 1 to 4% |
| 19 | 51/F | None | None | + | Necrotizing granuloma | 2 to 11% |
| 20 | 73/M | Emphysema | Subdural hematoma, old | Few | Lymphocyte > Macrophage | <1% |
| 21 | 67/F | None | None | +++ | Necrotizing granuloma | 5 to 13% |

*For quantification of fungal numbers, see Materials and Methods.
†For details of degrees and types of inflammatory response, see Results.
‡% $CNPS^+$ area was measured as described in Materials and Methods.
§HIV encephalitis (HIVE) was diagnosed on the basis of published criteria,[32] including the presence of microglial nodules with multinucleated giant cells on hematoxylin and eosin stain.
‖Subject of a separate report.[33]

*C. neoformans* is unusual among fungal pathogens in that it has a polysaccharide capsule which is important for virulence. The capsule inhibits phagocytosis[6] and impairs antigen presentation.[7,8] Polysaccharide antigen is released during infection and can be detected in body fluids such as cerebrospinal fluid and serum.[1,2] The detection of capsular polysaccharide by latex agglutination or enzyme-linked immunosorbent assay (ELISA) is a useful diagnostic test for cryptococcosis. *C. neoformans* polysaccharide (CNPS) is a potential immunomodulator which has been reported to inhibit leukocyte migration,[9] elicit suppressor responses,[10] produce antibody unresponsiveness,[7,8] and enhance HIV infection of T cells *in vitro*.[11]

Despite the importance of CNPS detection in clinical practice and the protean effects of polysaccharide on immune function relatively little is known about the distribution, localization and ultimate fate of this antigen in human infection. Rising CNPS CSF levels have been associated with poor prognosis in human cryptococcal infection.[1] However, the measurement of CNPS antigen levels as an indicator of response to therapy is limited by the fact that CSF often remains antigen-positive despite negative cultures and clinical improvement.[12] This phenomenon suggests the possibility of tissue reservoirs of CNPS. In recent years, several murine monoclonal antibodies (MAbs) have been generated which have been shown to be useful for studying the tissue distribution of CNPS in animal models of cryptococcal infection.[13,14] In this study, immunohistochemical techniques employing MAbs were used to define the tissue and cellular localization of CNPS in the brains of patients with cryptococcal meningoencephalitis.

## Materials and Methods

### Case Selection

Cases were retrieved from the Neuropathology autopsy file at the Albert Einstein College of Medicine by a search of the SNOMED diagnostic code.

Among 27 consecutive cases from 1966 to 1994, paraffin blocks were available for immunocytochemical studies in 21 cases, and these cases were the subject of analysis in this study. For all cases, description of gross and microscopic brain pathology as well as general autopsy findings were available for review. A brief summary of pathology in these patients is presented in Table 1.

### *Analysis of Microscopic Pathology*

Hematoxylin and eosin (H&E)-stained standard sections from cerebral cortex (×2), basal ganglia, thalamus, midbrain, pons, medulla, cerebellum, and spinal cord were evaluated. Sections from grossly abnormal areas, in addition to standard sections, were also analyzed. After reviewing H&E sections, the sections (at least two different regions of the brain) most heavily infiltrated by *C. neoformans* were analyzed by histochemistry for fungi, i.e., periodic acid-Schiff stain (PAS), mucicarmine and modified methenamine silver stain. The number of *C. neoformans* in each case was assigned an arbitrary score of one to five. This was based on a subjective analysis considering both the number of organisms and the size of the fungal lesions (cryptococcomas). If there are regional differences, a range of score is given. In cases where the fungal organisms were too few to be recognized on H&E, the descriptive evaluation "few" was given. Coexisting pathology (toxoplasmosis, cytomegalovirus, lymphoma, HIV-encephalitis) was determined by combined H&E and immunocytochemistry.

### *Immunocytochemistry*

Paraffin sections were stained with a battery of antibodies: glial fibrillary acidic protein (GFAP; rabbit immunoglobulin (Ig)G, Bio Genex Laboratory, San Ramon, CA) for astrocytes, RCA-1 for microglia/macrophages, and HIV-1 gp41 (Genetics System), as described.[15] Sections were also investigated with MAbs to L-26 (DAKO, Carpinteria, CA), UCHL-1 (CD45RO; DAKO) and leukocyte common antigen (LCA; DAKO) as markers of B lymphocytes, T lymphocytes, and leukocytes, respectively, using peroxidase-conjugated isotype-specific secondary antibody methods as described below.

### *Anti-CNPS Immunocytochemistry*

Monoclonal antibody (MAb) 3E5 is a murine IgG3 that binds to *C. neoformans* capsular polysaccharide of all four serotypes.[16,17] Control cases including normals, HIV encephalitis, toxoplasmosis, lymphoma, Alzheimer disease, and infarcts, all without cryptococcal meningoencephalitis (CME), showed no immunoreactivity for CNPS. Briefly, 6 $\mu$m paraffin sections were deparaffinized with xylene and a series of graded alcohol solutions and washed with phosphate-buffered saline (PBS). After 30 minutes incubation in 0.3% hydrogen peroxide solution, the sections were covered with 10% normal goat serum in PBS for 1 hour at room temperature (RT) to block the nonspecific antibody binding. Primary antibody 3E5 was applied at 1:1000 dilution in 10% normal goat serum in PBS and incubated at 4°C, overnight. After washing with PBS twice, sections were incubated with peroxidase-conjugated goat anti-mouse IgG3 (Southern Biotechnology, Birmingham, AL) at 1:250 dilution in PBS for 2 hours at RT. Color was developed with diaminobenzidine (DAB). After final wash in PBS, some sections were briefly counterstained with Meyer's hematoxylin.

### *Double Immunocytochemistry*

For dual immunocytochemistry for CNPS and cell-type specific markers, sections were first immunostained for CNPS using DAB as chromogen as outlined above, and then sequentially reacted with another antibody (GFAP, RCA-1 or others) using isotype-specific secondary antibodies that are conjugated with $\beta$-galactosidase (Southern Biotechnology) at 1:250 dilution in PBS. Color was developed with X-gal as described.[15]

### *Morphometry for CNPS Immunocytochemistry*

The sections of brain immunostained for cryptococcal CNPS revealed grossly identifiable zones of brown staining (Figure 1), which enabled us to measure the percentage of positively stained area in each section. At least two sections were examined from each brain. The glass slides immunostained for CNPS were projected using a slide projector, then positively and negatively stained brain areas were traced on a piece of paper. The traced areas were cut out and weighed on a balance. % $CNPS^+$ areas = 100 × [weight of $CNPS^+$ area/weight of $CNPS^+$ and $CNPS^-$ areas]. The results are expressed as ranges of values from two different sections that showed highest values. The median values were used to calculate the average % $CNPS^+$ area in AIDS and non-AIDS patients.

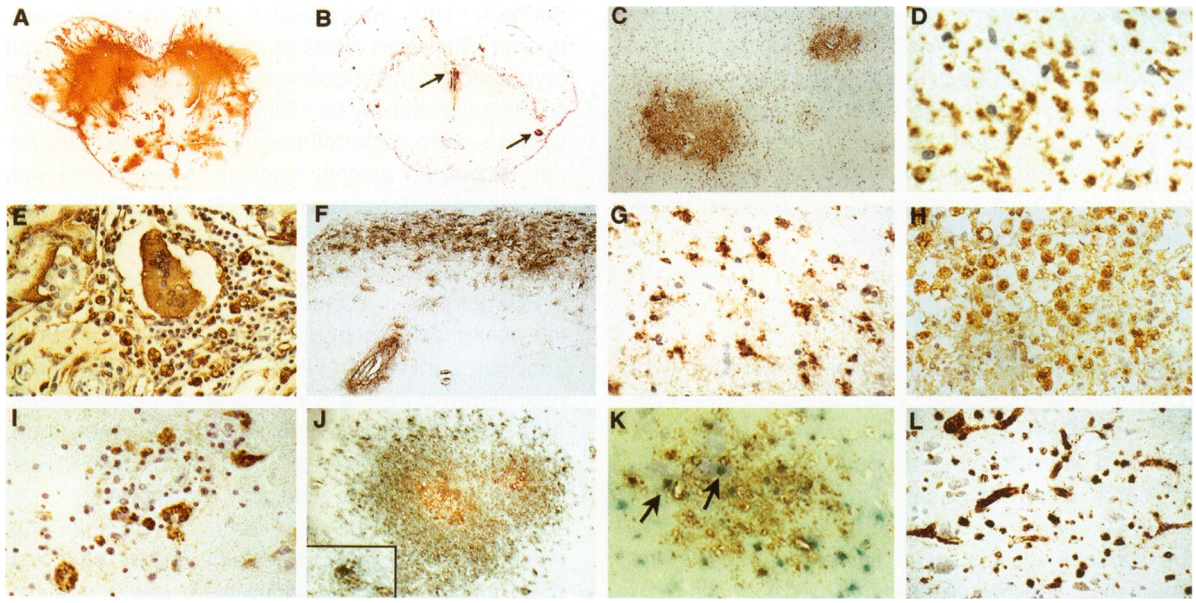


Figure 1. *Tissue CNPS antigen immunocytochemistry in cryptococcal meningoencephalitis. All except* E *and* F *are from AIDS patients.* A: *A whole mount of midbrain section immunoreacted for CNPS showing tissue deposition of cryptococcal antigen along the substantia nigra bilaterally, and multiple perivascular foci in the tegmentum* (×1.5). B: *In contrast to* A, *midbrain from another patient shows minimal tissue immunoreactivity for CNPS, primarily along the large penetrating vessels* (arrows) (×1.5). C: *Low power view of diffuse perivascular tissue CNPS deposits* (×82). D: *Examples of punctate tissue CNPS deposits that appear to be associated with cells but cell types not identifiable* (×300). E: *High power view of granulomatous meningitis with macrophages, multinucleated giant cells, lymphocytes, and plasma cells. CNPS immunoreactivity is associated with a multinucleated giant cell in the center, and many intracellular* C. neoformans, *which show distinct capsular staining* (×210). F: *An example of subpial tissue antigen deposits in a non-AIDS patient with active granulomatous meningitis. Parenchymal CNPS immunoreactivity is localized to the subpial and perivascular glial tissue* (×85). G: *In an AIDS patient, CNPS immunoreactivity was detected in discrete process-bearing cells in subpial regions, despite the limited presence of* C. neoformans *within meninges only* (×230). H: *CNPS deposits within macrophages and activated microglia in a necrotizing abscess. Note large foamy phagocytes with intense granular staining and the faint background staining of the necrotic tissue* (×260). I: *CNPS immunoreactivity is associated with parenchymal multinucleated giant cells in a patient with CNS lymphoma. Perivascular lymphoma cells remain unstained* (×270). J: *Double labeling for RCA-1 (X-gal,* green) *and CNPS (DAB,* brown) *of a parenchymal cryptococcal accumulation shows gross colocalization of the two. The central brown mass represents a collection of CN* (×64). Inset: *Higher magnification* (×400). K: *Double immunolabeling for GFAP (X-gal,* green) *and CNPS (DAB,* brown) *shows some CNPS-containing astrocytes* (arrows) (×240). L: *Microvascular pattern of CNPS immunoreactivity in disseminated cryptococcosis representing antigenemia. Patient 4: Capillary staining for CNPS antigen plus additional punctate parenchymal staining are present in the section of pons* (×260).

## Results

### Specificity of CNPS Immunoreactivity

Microscopic localization of CNPS immunoreactivity varied from discrete capsular staining of the fungi to diffuse and scattered parenchymal cell staining (Figure 1), which sometimes resembled nonspecific tissue binding. Specificity of tissue immunoreactivity was demonstrated by the following: 1) Brains of non-CME patients (normals and other disease controls) were tested for CNPS immunoreactivity, and none showed staining. 2) Anti-CNPS MAbs of several different isotypes including IgG1, IgG3, IgM, and IgA[16,17] were tested on CME tissues and all revealed identical staining patterns (not shown). 3) Immunolabeling was performed with an MAb (IgG3) and an isotype-specific labeled secondary, which gave minimal nonspecific binding. Slides reacted with labeled secondary antibody alone did not reveal staining. Thus, positive immunostaining of parenchymal cells and tissues for CNPS represents true tissue localization of cryptococcal polysaccharide, likely resulting from dissociation of capsular polysaccharide from yeast forms (see below).

### CNPS Immunoreactivity in the CNS

Two extreme examples of CNPS immunostaining in the midbrain in cryptococcal meningoencephalitis are shown in Figure 1, A and B. Grossly visible immunoreactivity resulted from staining of cryptococcal masses as well as diffuse and particulate staining of surrounding parenchymal tissues. As expected, grossly visible $CNPS^+$ parenchymal foci were often perivascular and followed large penetrating vessels (notable in Figure 1, A and B). By morphometry, the percent brain area stained for CNPS varied from negligible (<0.1%) to 87%, and was positively correlated with the number of organisms in tissue for AIDS ($P < 0.05$), but not for non-AIDS patients ($P > 0.05$, Student's *t*-test) (see Table 1). The mean (± SEM) % $CNPS^+$ area in AIDS patients tended to be higher than in non-AIDS patients (26 ± 5.8% *versus* 9.6 ± 5.2%: see Table 1), but the dif-

ferences were not statistically significant ($P > 0.05$, Student's *t*-test).

### *Inflammatory Response to CME*

The inflammatory response in AIDS patients consisted of some macrophages with few lymphocytes. Granulomas were not present in tissue samples from AIDS patients with CME (see Table 1). In contrast, most (6/7) non-AIDS patients in this series showed lymphocyte-rich inflammation with multinucleated giant cells consistent with a granulomatous inflammation. Some showed necrotizing granulomas as well as neutrophils and plasma cells. The only exception was patient 18, who had immunosuppressive therapy after renal transplantation. This patient showed predominant neutrophil and macrophage infiltration with few lymphocytes. All had mucicarmine$^+$ yeast forms (*C. neoformans*) and none had acid-fast bacilli-positive organisms. The immunophenotype of lymphocytes was leukocyte common antigen, UCHL-1$^+$, L26$^-$, and *Ricinus communis* agglutinin-1$^-$, thus activated T cells, both in AIDS and non-AIDS patients. L26$^+$ lymphocytes (B cells) were none too few, with an exception of CNS lymphoma in which tumor lymphocytes were L26$^+$.

### *Microscopic Patterns of CNPS Immunoreactivity*

Several different types of CNPS immunoreactivity were noted. 1) Diffuse tissue immunoreactivity surrounding cryptococcal infiltration was suggestive of both intracellular and extracellular polysaccharide accumulation. This pattern was common in cases where fungal accumulation was present without a significant inflammatory response; thus was the most common pattern in AIDS patients who had active CME at the time of death. Microscopically, multiple foci of scattered or coalescent tissue staining were present, centered around the vessels and/or collections of cryptococci (cryptococcomas) (Figure 1C). The staining had variable combinations of diffuse, reticular, and punctate quality (Figure 1, C and D), and did not localize to readily recognizable cell types by double immunolabeling. Cryptococci within cryptococcomas (so-called "soap bubble" lesions) often showed diffuse weak CNPS immunoreactivity rather than distinct capsular staining (not shown). 2) A discrete meningeal and/or superficial perivascular pattern was seen in CME in non-AIDS patients who had active cellular inflammatory responses to cryptococcosis, or in AIDS patients with minimal cryptococcal activity. Grossly, CNPS immunoreactivity was limited to the meninges or the subventricular regions, and the parenchymal immunoreactivity was limited to the perivascular spaces of large penetrating vessels (Virchow-Robin spaces; eg, Figure 1B). Microscopically, CNPS immunoreactivity in the meninges and Virchow-Robin spaces was primarily associated with macrophages and multinucleated giant cells (Figure 1E). Lymphocytes and plasma cells remained unstained (Figure 1E). Meningeal macrophage staining was due to diffuse or punctate cytoplasmic staining, and staining of polysaccharide capsules of intracellular fungi (Figure 1E). In AIDS patients who had been treated, and had clinically silent CME (eg, patients 1 and 2), only rare fungi were found in the meninges, but CNPS immunoreactivity was always more widely detected. The CNPS was found in the meninges and the subpial and perivascular regions of the brain, often in discrete cells (Figure 1, F and G). The subpial CNPS immunoreactive cells were process-bearing and resembled either astrocytes (Figure 1F) or microglia (Figure 1G); however, double labeling often failed to show cell identity, most probably because of low level expression of RCA-1 or GFAP in these cells (see Discussion). 3) Discrete parenchymal cell staining was seen that was associated with perikarya of reactive cells, most often in RCA-1$^+$ macrophages/microglia, or in GFAP$^+$ gemistocytic astrocytes. When cryptococcal lesions were adjacent to or associated with destructive parenchymal lesions such as necrotizing abscesses, infarcts or lymphoma, CNPS immunoreactivity was clearly present in cells of mononuclear phagocytes including macrophages, activated microglia, and multinucleated giant cells (Figure 1, H and I). Double labeling showed gross colocalization of parenchymal CNPS immunoreactivity more often with RCA-1 (Figure 1J), than with GFAP (Figure 1K). On rare occasions, cells morphologically compatible with neurons and oligodendroglia showed CNPS immunoreactivity (not shown) much less frequently than macrophage/microglia or astrocytes. 4) In cases where there was disseminated systemic cryptococcosis, the brain vessels (especially capillaries) stained positive for CNPS antigen consistent with serum antigenemia (Figure 1L).

## *Discussion*

Detection of CNPS antigen in the serum and the cerebrospinal fluid is useful in making the diagnosis of cryptococcal infection, but little has been pub-

lished on the distribution and tissue localization of the polysaccharide antigen. In 1962 Marshall et al.[18] analyzed 100 cases of cryptococcosis using fluorescent antibody technique and reported that polysaccharide was "liberated into the tissues surrounding a focus of infection," but provided no information on anatomical or cellular localization.[18] In the 1960s, Hirano and co-workers[19–21] published a series of papers in which cryptococcal polysaccharide was implanted in the brains of rats and the fate of polysaccharide was examined by electron microscopy. The authors noted that polysaccharide moved considerable distances in the white matter along the extracellular space, and eventually localized to cells, including macrophages, astrocytes, and oligodendroglia. Bennett and Hasenclever[22] reported that instillation of polysaccharide into rabbit CSF resulted in rapid appearance in serum where it persisted. In another study, mice injected intravenously with CNPS were noted to have CNPS in liver and spleen months after the antigen was cleared from the blood.[23] In experimental cryptococcosis in mice and rats, tissue CNPS deposits are detected in systemic organs.[13,14] In the lung, several patterns of CNPS immunoreactivity emerged including diffuse tissue distribution and discrete cell-associated staining,[14] not dissimilar to those found in human CME. It is notable that in the rat pulmonary cryptococcosis model, diffuse, widespread tissue antigen deposit was associated with early stages of infection in which large numbers of extracellular fungi persisted without notable cellular immune response.[14] Swift relocalization of cryptococcal antigen to macrophages and epithelioid histiocytes followed granuloma formation.[14] These preceding studies provide circumstantial evidence that in cryptococcal infection, polysaccharide antigen would be in brain away from the fungal organisms.

MAb 3E5 reacted with CNPS in human brain. This indicates that CNPS produced *in vivo* is antigenically similar to CNPS produced *in vitro* and in experimental animal infection. In human CME, discrete macrophage localization of CNPS was present in patients who mounted lymphocyte-rich cellular response with granuloma formation. It is remarkable that in all AIDS patients there was no significant tissue inflammatory response. Variable numbers of macrophages were mixed with few or no lymphocytes, often in discordant locations. Variable degree of T cell infiltration is known to occur in the central nervous system of AIDS patients with and without opportunistic infections and with and without HIV encephalitis,[24] further complicating the interpretation. The predominant CNPS immunoreactive pattern in AIDS patients was diffuse, large tissue antigen deposits concentrated around the perivascular or parenchymal cryptococcal mass. In AIDS patients with treated, clinically silent CME, tissue CNPS deposit was limited to discrete cells within the meninges and subpial parenchymal cells. One can infer from these cases that diffuse and widespread tissue antigen is associated with rapidly proliferating fungal organisms, probably as a result of an ineffective inflammatory response. With chemotherapy and clearing of fungi from the brain, tissue polysaccharide antigen appears to be redistributed to meningeal macrophages and CNS glial cells. Intracellular CNPS may persist and serve as a tissue reservoir even after the organisms are cleared from the CNS. The identification of brain tissue antigen reservoirs provides an explanation for the clinical observation that CSF antigen titers may not correlate with clinical improvement, such that antigen titers may remain persistently elevated despite sterile cultures and clinical improvement.[12]

Cellular localization of CNPS in brain parenchyma with double-immunolabeling with cell type-specific markers for microglia (RCA-1) or astrocytes (GFAP) often failed to reveal cell identity. Nevertheless, the pattern of CNPS immunoreactivity often suggested localization within process-bearing cells. Since the expression of lectins and GFAP in glial cells is regulated and linked to cell activation,[25–27] the findings suggest that soluble polysaccharide does not directly activate glia. The fact that CNPS immunoreactivity was found in areas not suspected to have pathology (normal-appearing on routine histochemistry) suggests that CNPS does not elicit inflammation or mediate gross toxic effects on CNS cells. Brain and meningeal phagocytes, on the other hand, showed distinct staining for polysaccharide. It is reasonable to postulate that cellular uptake of CNPS is facilitated by activation of mononuclear phagocytes and upregulation of surface Fc or complement receptors. The occurrence of CNPS-immunoreactive macrophages/activated microglia associated with other CNS processes is compatible with such a hypothesis.

It is noted that in CME, CNPS often localizes to certain regions of the brain, such as basal ganglia and midbrain. A number of features are shared by these anatomic regions, including the presence of large penetrating vessels, the presence of dopaminergic fibers (striatonigral pathway), and frequent localization of HIV antigen.[28] *C. neoformans* uses L-dopa as a substrate for melanin-like pigment production *in vitro*,[29] and melanin-like pigment protects *C. neoformans* from harmful effects of reactive oxy-

gen and nitrogen intermediates.[30,31] Distribution of CNPS in regions that are known to localize HIV gp41 antigen raises the possibility of synergism between HIV and *C. neoformans*. The enhancement of HIV infection in cultured lymphocytes by soluble cryptococcal polysaccharide further strengthens the possibility.[11]

The current study is a correlative systematic investigation of the distribution and cellular localization of cryptococcal polysaccharide in the central nervous system tissues in a series of patients with CME. The results support the view that in CME, capsular polysaccharide dissociates from the fungal organisms resulting in the formation of tissue deposits. The results also imply that brain cells have mechanisms for uptake and clearing of soluble cryptococcal polysaccharide. The finding of tissue cryptococcal antigen is important, given that polysaccharide is a major virulence factor for *C. neoformans*, and a modulator of immune functions *in vivo* and *in vitro*. The modulating effects of cryptococcal polysaccharide on nervous system cells have not yet been investigated. However, given the multitude of biological activities, CNPS is likely to affect the interactions between the CN and the nervous system/immune effector cells, thus contributing to the pathogenesis of CME. The finding of CNPS in brain tissue in CME supports the notion that cryptococcal meningitis is not a process limited to the CSF space, but rather involves the brain parenchymal cells as well.

## Acknowledgments

The authors thank Dr. Meng-Liang Zhao and Dr. Wei Liu for excellent immunocytochemistry and Jorge Bermudas for excellent histochemistry.

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