# Etiology of microglial nodules in brains of patients with acquired immunodeficiency syndrome

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Microglial nodules associated with opportunistic and HIV-related lesions are frequently found in the brains of AIDS patients. However, in many cases, the causative agent is only presumptively suspected. We reviewed 199 brains of AIDS patients with micronodular lesions to clarify their etiology by immunohistochemistry (to *Toxoplasma gondii*, cytomegalovirus, herpes simplex virus I/II, varicella zoster virus and HIV-p24 core protein), PCR (for herpetic viruses and *Mycobacterium tuberculosis*) and electron microscopy. Productive HIV infection was observed in 110 cases (55.1%): 30 cases with *Toxoplasma gondii* encephalitis, 30 with cytomegalovirus encephalitis, eight with multiple cerebral diseases, while in the remaining 42 cases HIV was the only pathogenetic agent. Multinucleated giant cells (hallmark of HIV infection) were found in the MGNs of 85/110 cases with HIV-related lesions; the remaining 25 cases had only p24 positive cells but no multinucleated giant cells. In these latter cases the micronodular lesions had been initially attributed to the main opportunistic agent found in the brain, or defined as subacute encephalitis. Individual microglial nodules positive for an opportunistic pathogen were generally negative for HIV antigens. In 13 cases no opportunistic agent or HIV productive infection was found. In these cases, PCR and electron microscopy examination for HIV and other viral infections were negative. Our data suggest that HIV-immunohistochemistry should be used for the etiological diagnosis of micronodular lesions in AIDS brains, even in the presence of other pathogens. After extensive search, the etiology of the microglial nodules remains unknown in only a small percentage of cases. *Journal of NeuroVirology* (2000) **6**, 46–50.

**Keywords:** microglial nodules; immunohistochemistry; HIV; central nervous system

## Introduction

Micronodular lesions are found in *post-mortem* studies of the central nervous system as non-specific cellular reactions to various agents, bacteria, protozoa and, particularly, viruses (Esiri *et al*, 1982). Microglial nodules (MGNs) are frequently observed in the brains of AIDS patients because of the frequent occurrence of opportunistic cerebral diseases. In these cases, the etiological agent can often be demonstrated within the micronodules at

histological examination. In the cases with no demonstrable agent within the micronodules, their etiology can be hypothesised on the basis of other cerebral lesions or disseminated infections, especially those due to CMV or *T. gondii* (Morgello *et al*, 1987; Vinters *et al*, 1989; Patsouris *et al*, 1993; Lanjewar *et al*, 1998).

In micronodular lesions due to HIV, multinucleated giant cells (MGCs) are a hallmark of HIV infection: if no multinucleated giant cells are found, the presence of immunohistochemically determined HIV antigen is required (Budka, 1986).

Given the lack of literature data concerning the true incidence of particular opportunistic infections as causative agents of MGNs, this study had the aim of clarifying this point as far as possible.

## Results

As can be seen in Table 1, 144 of all cases with MGNs (144/199, 72.4%) had opportunistic brain lesions: *T. gondii* encephalitis in 68 cases, CMV encephalitis in 58, multiple opportunistic lesions in 18 (*T. gondii* and CMV, CMV and primary cerebral lymphoma, *T. gondii* and fungal or bacterial abscesses).

Productive HIV infection was demonstrated in the MGNs of 110 cases (110/199, 55.1%): 30 of the 68 with *T. gondii* necrotizing lesions, 30 of the 58 with CMV encephalitis and eight of the 18 with multiple cerebral diseases. No opportunistic infection was observed in the remaining 42 cases.

MGCs were found in 85/110 cases with productive HIV infection. In the remaining 25/110 cases, HIV was detected only by means of immunochemical positivity to HIV p24 antigens. It is of interest that HIV-IHC stained only a small percentage of the MGNs in 10% of the latter cases.

Free tachizoites/cysts of *T. gondii* or cytomegalic cells within the micronodules (Figures 1 and 2) were observed in 23 cases without MGCs and/or p24 positive cells. In the MGNs of seven cases with the concomitant presence of MGCs and/or p24 positive cells, the MGNs containing the etiological agent were always HIV-p24 negative with the exception of one case, in which a MGC phagocyting toxoplasmic microorganisms was observed (Figure 3). IHC for CMV and *T. gondii* antigens stained only the cytomegalic cells and the toxoplasmic microorganisms of the MGNs respectively. IHC for HSV I/II and VZV antigens was always negative.

In 13 cases (13/99, 6.5%) with MGNs no opportunistic agent or HIV infection was found immunohistochemically. PCR for CMV, HSV I/II and *M. tuberculosis*, and ultrastructural examination for viral, bacterial and protozoal infection, added no further information and the etiology of the micronodular lesions remains unknown.
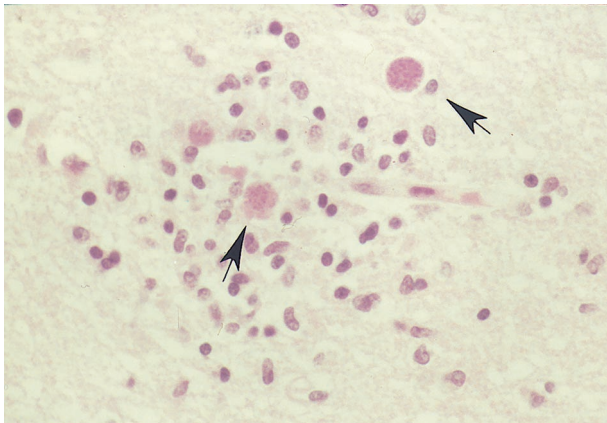


**Figure 1** Microglial nodule containing cysts of *T. gondii* (arrows) in a case of toxoplasmic necrotizing encephalitis. Haematoxylin and eosin, ×400.
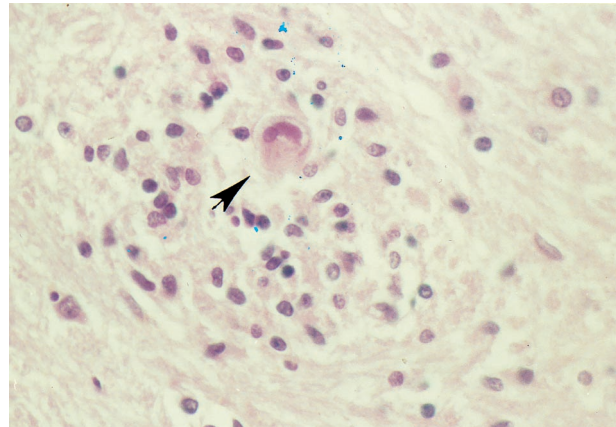


**Figure 2** Microglial nodule in a case of CMV encephalitis: in the center of the nodule a cell with cytopathic changes attributable to the virus (arrow). Haematoxylin and eosin, ×400.

**Table 1** Etiology of cerebral microglial nodules in an autopsy series of 199 AIDS patients

| | | | MGNs without OI (n=55) | T. gon. (n=68) | | CMV (n=58) | | mult. (n=18) | |
| | | | | *col. 1* | *col. 2* | *col. 1* | *col. 2* | *col. 1* | *col. 2* |
|---|---|---|---|---|---|---|---|---|---|
| HIV+ | MGCs+ | p24+ | 40 | 5 | 12 | 1 | 23 | – | 4 |
| HIV+ | MGCs− | p24+ | 2 | 1 | 12 | – | 6 | – | 4 |
| HIV− | MGCs− | p24− | 13 | 18 | 20 | 5 | 23 | – | 10 |

MGNs: microglial nodules; OI: opportunistic infections; *T. gon*: Toxoplasma gondii; CMV: cytomegalovirus; mult: multiple opportunistic lesions; col. 1: column 1 (presence of the etiological agent in the MGNs); col. 2: column 2 (no etiological agent within the nodules); HIV+ MGCs+ p24+: cases with HIV-related brain lesions, presence of multinucleated giant cells (MGCs) in the nodules and immunochemical positivity to HIV p24 antigens; HIV+ MGCs− p24+: cases with HIV-related brain lesions, absence of multinucleated giant cells in the nodules but immunochemical positivity to HIV p24 antigens; HIV− MGCs− p24−: cases without HIV-related brain lesions.
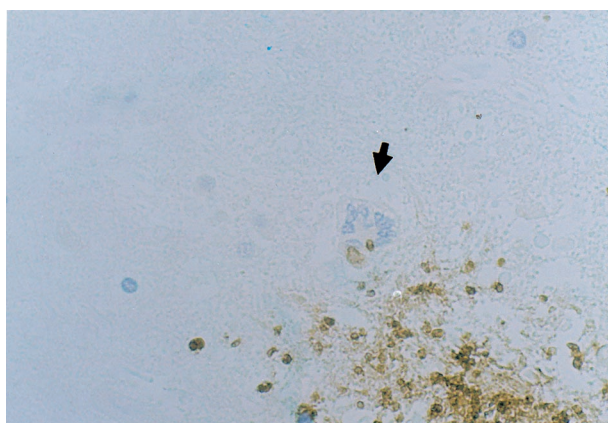
**Figure 3** Immunochemical positivity of toxoplasmic tachizoites phagocyted by an HIV-related multinucleated giant cells at the periphery of a microglial nodule (arrow). A high number of protozoas in the lesion are stained by IHC. Immunoperoxidase, Haematoxylin counterstain, × 400.

## Discussion

MGNs with both HIV and opportunistic infections are a frequent finding in the brains of AIDS patients: autopsy series report MGNs with typical HIV-related MGCs in 10−30% of cases (Masliah *et al*, 1992; Bell *et al*, 1996), although their occurrence in brains affected by CMV and *T. gondii* encephalitis seems to be more variable (Budka *et al*, 1987; Morgello *et al*, 1987; Lang *et al*, 1989).

In our series, MGNs were associated with *T. gondii* necrotising lesions in 34.1% of cases, with CMV encephalitis in 29.1% and with multiple opportunistic brain diseases in 9%. No MGNs were observed in the brains with other opportunistic lesions or neoplasms, although other authors have found the presence of micronodular lesions associated with cryptococcal meningoencephalitis, lymphoma and progressive multifocal leukoencephalopathy (Sharer and Kapila 1985; Kato *et al*, 1987).

HIV-related lesions (p24 positive cells) were observed in 55.2% of the cases (*n*=110), more than three-quarters of which also presented MGCs, which are considered to be histological lesions directly attributable to HIV infection (Budka, 1986). In the remaining 22% of cases (25/110), the diagnosis of HIV encephalitis was made on the basis of immunochemical positivity to HIV antigens as no MGCs were histologically observed in these cases. These data confirm those reported in the literature concerning the importance of using IHC for HIV in the diagnosis of 'AIDS brain diseases'; the number of MGCs does not estimate viral burden as accurately as IHC or *in situ* hybridisation (Wiley and Achim, 1995).

Even without MGCs, MGNs have been previously attributed to HIV (Snider *et al*, 1983; Petito *et al*,

1986; Kato *et al*, 1987; Kure *et al*, 1991). MGNs may also be caused by cerebral opportunistic pathogens (especially CMV or *T. gondii*) (Anders *et al*, 1986; Petito *et al*, 1986; Budka *et al*, 1987; Morgello *et al*, 1987; Patsouris *et al*, 1993) that are also responsible for focal or diffuse necrotising lesions. In cases without any additional neuropathological diseases, the lesions have been defined as aspecific micronodular encephalitis or subacute encephalitis by many authors (Nielsen *et al*, 1984; Lang *et al*, 1989; Kure *et al*, 1991). In cases with extracerebral infections mainly due to viruses, MGNs in the brain strongly suggest a viral origin (Petito *et al*, 1986; Kato *et al*, 1987; Lang *et al*, 1989; Rhodes, 1993).

In our study, IHC for CMV and toxoplasmic antigens did not add any further information to that obtained by histological examination, thus confirming the results of those authors who have previously reported that immunochemical methods (especially those based on CMV antigens) may not be able to detect very low levels of viral cell infection (Kure *et al*, 1991).

A small percentage of the cases with opportunistic lesions and concomitant HIV encephalitis had a micronodular component that was only partially HIV-IHC positive. IHC for the p24 antigen was almost always negative in the MGNs containing cytomegalic cells or tachizoites/cysts of *T. gondii* (except in one case in which a MGC phagocyting toxoplasmic microorganisms was observed). These data may suggest that, HIV infected microglial cells are not involved in immune response to other pathogens during the course of cerebral damage; on the contrary, the inflammatory cells involved in opportunistic lesions do not normally express viral antigens.

No MGCs or HIV antigen positivity were demonstrated in 49.7% of our cases (89/199). Seventy-six of these 89 patients were infected with CMV, *T. gondii* or multiple agents, but cytomegalic cells or *T. gondii* microorganisms were found in the MGNs of only 23 of these 76 cases.

In the last 13 cases without any immunohistochemical evidence of HIV infection in the brain, MGNs were the only neuropathological finding. These cases were defined as subacute encephalitis and hypothetically attributed to the main systemic disease. Four of these 13 brains had systemic CMV infection and four had disseminated tuberculosis. However, the search for CMV or *M. tuberculosis* DNA in these brain tissues and electron microscopy examination to detect other viruses were negative. In conclusion, our data suggest that the correct etiological diagnosis of micronodular lesions requires an immunochemical reaction to HIV antigens. MGCs have been defined as a hallmark of HIV productive lesions (Budka, 1986), but they are not always present in brains with HIV infection. In our study, 25 cases without MGCs were p24 positive. At histological examination, the MGNs in these cases

were initially attributed to the main opportunistic agent found in the brain or diagnosed as aspecific micronodular lesion.

It is interesting that the etiology of the micronodular brain lesions remained unknown in only a few cases (13/199, 6.5%).

## Materials and methods

Brain tissues from 199 AIDS patients with histologically documented MGNs were retrospectively examined. The cases were selected from a large autopsy series (*n*=936 cases) using the presence of MGNs in the brain as the criterion for study inclusion. One hundred-sixty one patients were males (80.9%) and 38 were females (19.1%). The risk factor was intravenous drug addiction in 123 patients (61.8%) and sexual intercourse in 55 (27.6%); it remains unknown in the other 22 (11%). The mean age of the patients was 35 years (range 23–74). In the other 737 cases autopsied at L. Sacco Hospital, between January 1989 and June 1995, neuropathological examination did not reveal any micronodular lesions.

The brain tissues had been fixed in 10% buffered formalin and embedded in paraffin. Haematoxylin-eosin sections from at least ten brain areas were reviewed (the frontal, parietal and temporal lobes, basal nuclei, deep white matter, the cerebellum, the spinal cord, and each macroscopic lesion) and the two areas with the most representative microglial nodules were chosen for immunohistochemistry (IHC) in each case.

IHC was performed using rabbit antiserum to *T. gondii* (1:200, overnight, BioGenex) and/or to herpes simplex virus type I and II (HSV I/II, as previously described) (Vago *et al*, 1996). Mouse antiserum to CMV (as previously described) (Vago *et al*, 1996), HIV core protein-p24 (1:50, overnight, Dako) and varicella zoster virus (VZV, 1:50, overnight, Dako) was used. HIV- and VZV-IHC were performed after microwave pretreatment (780 W, 5 min twice, in 0.01 M citrate buffer, pH 6.0). The reactions were revealed by means of an indirect immunoperoxidase technique, with 3,3′-diaminobenzidine free base as chromogen and haematoxylin counterstaining.

For the reasons explained in the Results section, nested PCR for CMV, HSV I/II and *Mycobacterium tuberculosis* was performed in some selected cases as previously described (Vago *et al*, 1996; 1998). In these cases, a technique for reprocessing paraffin sections for electron microscopy (Yau *et al*, 1985) was applied. After selecting the areas with MGNs by marking them on the back of the slides with a diamond pencil, the slides were soaked in xylol over-night to remove the cover slip. The sections were rehydrated, fixed in osmium tetroxide, dehydrated, covered with resin/propylene oxide 1:1 mixture and then with pure resin. Gelatine capsules filled with resin were placed over the previously marked areas. After polymerisation at 60°C, the capsules with the sections on their surface were removed from the slides by chilling in liquid nitrogen. The cutting and staining of semithin and thin sections were routinely performed.

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