CORRESPONDENCE

I read with great interest the recent *Brain Pathology* article by Trillo-Pazos et al provocatively entitled "Detection of HIV-1 DNA in microglia/macrophages, astrocytes and neurons isolated from brain tissue with HIV-1 encephalitis by laser capture microdissection." Unfortunately, the technology and methods applied in this study support neither the descriptive title nor many of the article's statements (conclusions). The authors used laser capture microdissection (LCM) on 6 paraffin blocks from 4 cases of HIV encephalitis. Pooling material captured from 100 to 200 astrocytes or neurons, they conclude that both cell types are infected at the astonishing frequency of 0.5 to 1%. I do not believe the data in the manuscript support this conclusion nor the manuscript title. While LCM is all the rage, its limitations—particularly given the manner in which it was used in this manuscript—must be acknowledged. Contrary to the author's claim LCM will not ensure "precise capture of individual cells without surrounding matter." Familiarity with the density of the central nervous system and the geometry of 5-μm thick paraffin sections immediately raises issues of specimen contamination by the dense neuropil. Unfortunately, the investigators worsen this problem by "pooling" captured material from hundreds of such specimens and subjecting the isolated material to 40 cycles of PCR. Given the abundance of HIV within the nervous system during encephalitis (1), it would be difficult to sample an encephalitic brain and not capture infected microglial processes that would generate a PCR signal after 40 cycles. HIV infection of the nervous system is complex enough without mudding the waters with inadequate experimental methodology.

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Author's Response

We are glad to respond to Dr Wiley's letter with regards to our study "Detection of HIV-1 DNA in microglia/macrophages, astrocytes and neurons isolated from brain tissue with HIV-1 encephalitis by laser capture microdissection." Before clarifying specific methodological issues, first we will comment on the supposed "mudding the waters" of neuroAIDS research. This phrase implies that "the waters" of HIV neuropathogenesis are "clear" if it were not for studies that diverge from the existing dogma of preeminent role of HIV-1 infection of microglia/macrophages in the brain. In the clinic, the use of highly active combination therapy (HAART) has resulted in a 50% decrease in HIV-1-associated dementia (HAD) (6). Despite these therapeutic benefits, there has been an increase in the number of patients with HAD and high CD4 levels (8). HAART is of limited benefit in 30% of patients with minor cognitive/motor disorder or sensory neuropathy (6). Clearly, some neuropathogenic processes can proceed in the absence of extensive HIV-1 replication and neuroinflammation and need to be investigated.

The letter criticizes the use of LCM and questions whether this technique is able to capture individual cells without surrounding matter. It is precisely this ability to capture individual cells that makes the technique so appealing to an increasing number of researchers. LCM together with immunocytochemistry allows for the unequivocal identification and dissection of specific cell types at the single cell level (Figure 2) (12), and the extraction of nucleic acids and proteins for further molecular analysis (3, 9). LCM in concert with microgenomics is a powerful tool to determine the precise role of each cell type in the pathogenesis of neurological diseases in post-mortem brain tissue (2).

The letter further raises concern over possible specimen contamination from microglial processes from the dense neuropil. This concern is only partially valid because microglial processes are expected to contain viral RNA and not DNA and a contaminant, if present, would not be detected by our DNA based PCR technique. In addition, we purposely used 5-μm sections to obtain an approximate single layer of cells in each section (1). We excluded tissue regions that had demonstrable signs of microgliosis and MNGC in gray matter regions as determined by CD68 staining. Thus, we hand picked specific sections for LCM so as to avoid collecting any contaminating microglia in our samples of astrocytes or neurons. With these caveats in place, we are confident that we have eliminated the possibility of collecting microglial nuclei and have avoided any false positive results upon PCR amplification in this study.

Dr Wiley's letter questions the high frequency of infection of astrocytes and neurons in our study. We chose to use HIV-1 encephalitis (HIVE) cases to maximize our chances of finding viral infection. From the many excellent studies in HIVE it is unclear what constitutes "normal" levels of viral load and inflammation and there is significant variability from case to case and study to study (4, 5, 7). A recent LCM study has demonstrated that microglia and 1 in 50 of hippocampal neurons (2%) contain HIV-1 DNA (11). Accordingly in our study, we find that an approximate 10% of microglia/

macrophages and 0.5 to 1% of astrocytes and neurons carry HIV-1 DNA in our cases with HIVE and neurological impairment. The frequency of HIV-1 DNA positive astrocytes in our study was comparable to that demonstrated by Takahashi et al in an extensive, quantitative survey of brain tissues by in situ PCR (10). There, HIV-1 DNA positive astrocytes constituted 3 to 30% of all HIV-1 DNA positive cells and the overall percentage of HIV-1 positive astrocytes reached 0.7% in some brain regions (10). In one case 2.5% of neurons carried HIV-1 DNA (10).

In conclusion, our study supports the newer and more complex hypothesis that the cellular basis of HIV neuropathogenesis is a dynamic multi-cellular degenerative process that includes infection of microglia, astrocytes and neurons. This infection is associated with the concomitant release of autocrine and paracrine factors that lead to cellular dysfunction and to eventual neuronal demise. Overall, in the era of HAART people are still presenting with cognitive impairments and we need to gain a better understanding of the exact cellular basis of HAD to develop new therapeutic approaches.

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