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LETTER TO THE EDITOR

Letter to Editor

Lena Al-Harthi[1] · Avindra Nath[2]

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Dear Dr. Gendelman;

We read a recently ahead of publication article in JNIP by Ko A et al. entitled "*macrophages but not astrocytes harbor HIV DNA in the brains of HIV-1 infected aviremic individuals on suppressive antiretroviral therapy*" with great interest. This paper addresses a critical question regarding HIV reservoirs, i.e., what are the cells types that harbor HIV in the brain. It is well established that macrophages in the brain are productively infected with the virus. Hence the major contribution of this paper is the determination of whether there is infection of astrocytes. Ko et al. analyzed post-mortem tissue from HIV+ donors for HIV presence using DNA and/or RNA scope. They concluded that astrocytes do not harbor HIV in these samples using this technology.

Below we outline several concerns with this manuscript that negates the conclusion that astrocytes do not harbor HIV in vivo. Most importantly, data shown in Fig. 6 Bof this article actually demonstrates evidence of HIV RNA in astrocytes (e.g. see spot at 3 cm from the right and 1.5 cm from the bottom of the image, another at 1.5 cm from the right and 1 cm from the bottom, another at 1 cm from the left and 2.5 cm from the top when viewing the PDF in print)". Further there are other methodologic concerns. These include: **1)** evaluation of only~40 spots/image/patient, a rather small sample, especially when examining rare events of HIV infection in the CNS under maximal viral suppression and within cellular populations which are thought to be infected at a low frequency; **2)** use of immunohistochemistry for detection of HIV DNA/RNA scope assays but did not use co-localization, as such overlapping signals are difficult to visualize especially when it is hard to discriminate between a brown and a red spot. Further, some "co-localized" CD68/HIV signal does not appear to exactly overlap (**Fig.5, fig. S2)** and due to lack of simultaneous staining for CD68 and GFAP leaves open the possibility that it represents an infected neighboring astrocyte or is background noise. **3)** the manuscript does not show any images with the negative control probe and stated that "*we did not detect any in situ signals in the subjects from negative control group using HIV-1 sense or antisense probes and we also did not detect any in situ signals from the subjects of HIVE group using negative control probe (data not shown)*". This statement is rather absolute and unusual in these assays as there is always a certain level of background noise that is discriminated from true signal by including a no probe control; **4)** the RNA/DNA scope methodology is not described but a "previously reported protocol" is cited. The cited paper (Yuan et al. 2017) does not contain any RNA or DNA scope methodology so we do not know exactly how the assay was performed; and **5)** only a subpopulation of astrocytes immune stain for GFAP and since that is the only marker that was used by the study to identify HIV infected astrocytes, it is another reason why it would be impossible to make the statement that astrocytes are not infected with HIV.

These concerns question the stated conclusion regarding the absence of infection in astrocytes including the title of the manuscript. We recognize that, for some, the issue of HIV infection of astrocytes and its relevance as an HIV reservoir is controversial. Unfortunately, this manuscript adds to unwarranted confusion by making emphatic statements that are simply not supported by their own data and seems to lack rigorous analyses.

✉ Lena Al-Harthi
Lena_Al-Harthi@rush.edu

[1] Department of Microbial Pathogens and Immunity, Rush University Medical Center, Chicago, IL, USA

[2] Section of Infections of the Nervous System, NINDS, NIH, Bethesda, MD, USA