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Astrocytes as an HIV CNS Reservoir: Highlights and reflections of an NIMH-sponsored symposium

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Abstract

This is a summary of a National Institute of Mental Health (NIMH) sponsored symposium that was focused on the role of astrocytes as a reservoir of the human immunodeficiency virus in the brain. The talks were grouped into four themes. The first theme reviewed the evidence for HIV infection of astrocytes and discussed the challenges in the use of traditional methods of immunostaining and in situ hybridization for detection of infected astrocytes. The second theme focused on mechanisms of HIV entry into astrocytes and discussed CD4 independent mechanisms such as receptor mediated endocytosis and transmission of HIV by cell-to-cell contact with infected lymphocytes. The third theme focused on epigenetic regulation of HIV latency in astrocytes and other factors such as cytokines and transcriptional factors regulating HIV replication in astrocytes. The fourth theme focused on therapeutic approaches such as gene editing to block persistently infected astrocytes. A discussion that followed was focused on major unanswered questions in the field and future directions for research.

On April 10, 2018, an International meeting sponsored by the National Institute of Mental Health at the National Institutes of Health, was held in Chicago on “Astrocytes as an HIV CNS reservoir” in conjunction with the 15th International Symposium on NeuroVirology and the 24th Scientific Conference of the Society on Neuroimmune Pharmacology. The purpose of the meeting was to assess published data regarding the role of astrocytes as an HIV reservoir in the CNS. Four primary themes were highlighted. They were: 1) Evidence for HIV infection in astrocytes; 2) How HIV enters astrocytes; 3) Regulation of HIV latency, replication and 4) Therapeutic approaches targeting persistently infected astrocytes. The meeting served as an important venue to identify gaps in knowledge and highlight new areas of investigation.

Astrocytes are the predominant cell type in the brain. They perform vital functions to maintain brain homeostasis, including release of neurotropic factors to support neuronal

health¹. Astrocytes are a frequent target of a wide range of viral infections including retroviruses such as murine leukemia virus², feline immunodeficiency virus^{3,4} human T cell tropic virus type-1 (HTLV-1)⁵ and simian immunodeficiency virus (SIV)^{6,7}. Several laboratories have shown that astrocytes are infected by HIV in vitro^{8–11}. Others demonstrated HIV infection in astrocytes in vivo using in situ hybridization or laser capture dissection microscopy followed by nested PCR^{8,10,12,13}. Some in the field point out that each of these techniques is subject to technical difficulties which may result in false positive cells. Others using traditional methods of viral detection such as immunostaining for HIV core antigens (p24 or gp41) or immunostaining for HIV-Nef protein failed to recognize infected astrocytes in vivo^{14,15}.^{11,16} In this meeting, Narasipura and colleagues demonstrated HIV integration (Alu-PCR of HIV genes) in astrocytes in vitro, which did not exceed 3% of astrocytes¹⁷. Eliseo Eugenin demonstrated HIV infection, including integrated HIV DNA, in post mortem human astrocytes using DNA and RNA scope technology. The rate of infection of post-mortem human astrocytes did not exceed approximately 7% of astrocytes. Given that astrocytes are 40–130 billion cells in the brain, at a frequency of 1% infected astrocytes, it is then estimated that 0.4–1.3 billion astrocytes in the brain are HIV+. But this estimate does not coincide with detected virus in the brain? Several reasons could account for this discrepancy including that HIV infection in astrocytes is mostly non-productive¹⁸. Further, relying on traditional methods for detection of HIV/SIV in post-mortem infected astrocytes (e.g. immunostaining of HIV core antigens) is not useful. Highly sensitive and sophisticated approaches are warranted to probe the rate of HIV infection in astrocytes in vivo, such as DNA/RNA scope technology. Further, a number of groups reported on post-entry host restriction factors that suppress productive HIV replication, even at LTR transcriptional activity, and disruption of such mechanisms is likely to drive burst of HIV release in astrocytes^{18–22}. Nonetheless, even low-rate of HIV infection in astrocytes has profound functional consequences. Eliseo Eugenin showed that a small number of HIV infected astrocytes are sufficient to cause wide disruption of the blood brain barrier by disrupting the integrity of gap junctions and can induce bystander damage to uninfected astrocytes^{23,24}. Al-Harthi's group demonstrated that HIV infection disrupts a key signaling factor (Wnt/ β -catenin) which leads to disruption of glutamate/glutamine cycle, neuronal injury, and heightened inflammatory secretion by astrocytes^{25,26}.

Dr. Gregory Melikian gave an introductory presentation on application of advanced fluorescence microscopy techniques to visualization of HIV-1 entry and fusion with target cells. These include multi-color virus labeling and single particle tracking in living cells. These powerful techniques enable detection of productive HIV-1 entry via conventional, CD4- and co-receptor- dependent route, as well as through a non-conventional entry into cells like astrocytes. HIV entry in astrocytes is not through classical HIV gp120/CD4/chemokine-co-receptor fusion as astrocytes are CD4 negative. Indeed, Quentin Sattentau using an HIV cell fusion assay confirmed that HIV does not fuse with the astrocyte cell membrane²⁷ except under circumstances where CD4 is forcibly expressed on the cell surface²⁸. He further demonstrated that astrocytes may phagocytose infected macrophages. The phagocytosed material was noted by immunofluorescence and electron microscopy. Following phagocytosis of HIV infected macrophages, there was no activation of the HIV reporter virus transfected in the astrocytes indicating that HIV infection of astrocytes does

not occur via the process of phagocytosis^{27,29}. However, this phenomenon of phagocytosis of HIV infected macrophages by astrocytes has not been demonstrated in vivo. Further, highly reactive astrocytes, as reported in HIV post-mortem brain and other neurodegenerative diseases may be that of an A1 phenotype which are impaired in their ability to phagocytose²⁹. Lastly, while it is clear that HIV does not fuse with astrocyte cell membrane, the highly sensitive luciferase assay used by Sattentau may not detect signal from a very small fraction of cells and most critically the assay does not measure HIV integration that is likely to occur through non-classical means. Indeed, a number of speakers at the symposium demonstrated HIV integration in astrocytes using Alu-PCR. Further, HIV LTR-luciferase expression in astrocytes can be silent as a function of cell heterogeneity, as presented by Naraspiura.

Endocytosis of HIV emerged as a potential mechanism of HIV entry into astrocytes. Jonathan Geiger reviewed the literature pertaining to HIV entry into astrocytes via receptor mediated endocytosis^{28,30–32}. Putative receptors include the mannose binding protein³³ and a 65kD membrane protein that may be present in a tetrameric form on the cell membrane³⁴. HIV is thought to enter astrocytes by receptor-mediated endocytosis using mannose receptors or other unknown receptors. Most of the HIV contained in endosomes is likely to be degraded in lysosomes, however events that disrupt endosome maturation to lysosomes can lead to HIV escape from lysosomes and eventual integration into the host DNA. Indeed, agents that increase the pH of the lysosome such as the weak-base chloroquine disrupt endolysosome maturation and lead to higher levels of HIV DNA and p24 detection in astrocytes³⁵. The effects of chloroquine can be negated in Rab 5, 7 and 11 ablated astrocytes confirming the role of endocytosis in HIV entry into astrocytes²⁸. Other lysosomotropic drugs such ammonium chloride, bafilomycin A1 and brefeldinA increase HIV infection by causing de-acidification³³. Deacidification of the endolysosome also enhances Tat mediated HIV transactivation in other cell types as well³⁶. Eliseo Eugenin also presented data confirming the ability of HIV to enter astrocytes via endocytosis. It is important to note that some of the virus escaping endolysosomal destruction may circle out and is released without integrating into host genome and undergoing normal replicative cycle of HIV. For this reason, it is critical that assays that measure HIV in astrocytes should rely on detection of integrated HIV (Alu-PCR) rather than p24 that may be associated with input HIV or HIV that has escaped the lysosome without integration. It also remains to be determined if this mechanism of HIV restriction by the endolysosome is unique to astrocytes or it might be a more ubiquitous defense mechanism against retroviral infections.

Additional mechanisms of HIV entry into astrocytes were summarized by Avindra Nath. He noted that HIV can enter astrocytes by cell to cell contact with HIV-infected lymphocytes. Infection via cell-to-cell contact is independent of CD4 and the transmission of the virus occurs in viral synapses formed by the membranes of the lymphocyte and astrocyte^{37–40}. He also reviewed the literature showing that HIV-infected astrocytes are capable of transmitting HIV to uninfected lymphocytes⁴¹. Transmission of viruses by cell to cell contact is a ubiquitous mechanism preserved in all life-forms particularly in those without a circulatory system. Janice Clements demonstrated CD4 independent entry and replication of SIV in rhesus macaque astrocytes.

Several groups presented data demonstrating HIV latency in astrocytes. Al-Harthi's group, using a cell model where by HIV LTR-luciferase is integrated into astrocytes demonstrated heterogeneity in HIV transcriptional activity, with some astrocytes demonstrating deep latency and others showing HIV LTR- driven luciferase activity. Latently infected astrocytes demonstrated hallmark features of HIV latency including detection of provirus, low level HIV transcripts without protein expression, and epigenetic regulation of HIV latency at the level of histone modification (class I histone deacetylases) and a lysine-specific histone methyltransferase (SU(VAR)3-9)¹⁷. Corinne Barat and Michel Tremblay showed that astrocytes sustain long-term productive infection⁴². These findings demonstrate that astrocyte heterogeneity may be a significant factor in their permissiveness to low level productive replication or latency. The transcriptional status of astrocytes is likely a factor in regulating these two outcomes (low level productive replication vs. LTR transcriptional silence). Indeed, as Naraspiura presented, even within a population of astrocytes in culture some show higher level of HIV LTR transcriptional activity than others. Barrat and Tremblay added that some integrated HIV in astrocytes cannot be induced by conventional HIV viral outgrowth inducers (e.g. HDAC inhibitors/PKC activators). This is consistent with Siliciano's study demonstrating that there are infected memory T cells that harbour replication competent HIV (determined by full genome sequencing) which are not inducible by conventional means⁴³. Eliseo Eugenin also found that latently infected astrocytes had a specific profile of expression of proteins involved in lipid and inositol metabolism. Similar profiles were also seen in vivo in vicinity of the HIV-infected astrocytes and macrophages. He showed that HIV can be reactivated in these cells using a variety of treatments. This is consistent with previous studies that have shown that cytokines such as IL-6 and TNF α and HDAC inhibitors can reactivate HIV in these cells showing the importance of specific transcription factors and epigenetic regulation of HIV in astrocytes^{17,41,44}.

Ruth Brack-Werner developed an Adeno-associated virus-based vector (AAV9P1) with a synthetic surface peptide for transduction of astrocytes. The AAV9P1 vectors contained genes for HIV-1 proviral editing by CRISPR/Cas9. Latently HIV-1 infected astrocytes transduced with these vectors showed significantly diminished reactivation of proviruses. Sequence analysis identified mutations/deletions in key HIV-1 transcriptional control regions. Thus, AAV9P1 could be used for gene delivery to astrocytes and may facilitate inactivation/destruction of persisting HIV-1 proviruses in astrocyte reservoirs⁴⁵.

Significant progress has been made in our understanding of HIV infection of astrocytes. It is clear that the virus has a unique life-cycle in these cells. The study of HIV infection in astrocytes has provided novel insight into the role of cell-to-cell contact in viral transmission, the role of the endolysosome in restricting viral infection post viral entry and the epigenetic silencing of the virus, the ability of the virus to become reactivated and transmission of the virus from astrocytes to lymphocytes. Gene editing tools have also been developed to inactivate the virus in these cells. Yet, there are many unanswered questions the need to be addressed. Lena Al-Harthi and Avindra Nath led the discussion on future directions of this line of research. The discussion highlighted several areas of impactful research. Most studies have relied on using in vitro cultured human fetal astrocytes. There is a greater recognition of heterogeneity of astrocytes that can confound outcome. Therefore, there should be better characterization of astrocytes used in such studies. Additionally, use

of 3D human brain cultures for studying astrocyte infection could be highly valuable. Likewise, use of post-mortem human brain to assess HIV latency in astrocytes is highly valuable. Such studies, however, should move away from relying on traditional HIV core antigen staining, as astrocytes are latently infected and frequency of productive HIV replication is rather low. Advances in tissue probing for target DNA/RNA such as RNA and DNA scope can be incorporated to studies of astrocyte latency and is likely to provide meaningful information. Lastly, animal models that tease out contribution of astrocytes to HIV latency should be utilized. Although animal models cannot recapitulate every aspect of a human condition, they can be highly useful in context of specific questions that were addressed.

Remaining questions include whether there are astrotropic strains of HIV, whether astrocyte phenotype would impact their susceptibility to HIV infection and/or latency status? Whether infected astrocytes produce HIV non-coding RNA? What are the post-entry restriction mechanism(s) for productive HIV in astrocytes? How antiretroviral drugs are taken up in astrocytes and what are their impact on these cells? What is the effect of eradication strategies including gene editing on HIV in astrocytes? to name a few. Targeting research towards these questions will advance the field and be informative beyond glial/HIV interface as it may be more widely applicable to other retroviruses.

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