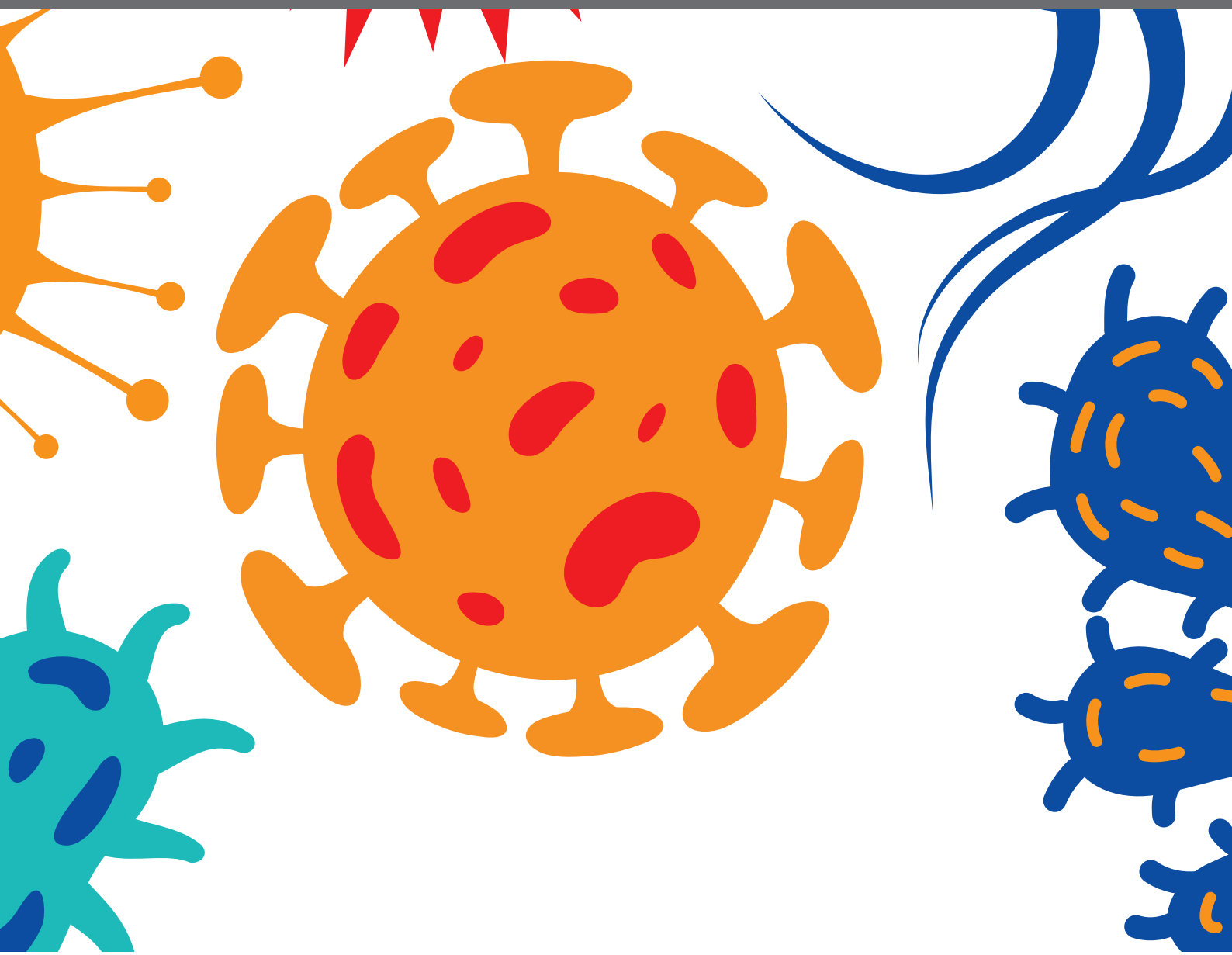
DIRECT AND INDIRECT INTERACTIONS OF HIV WITH HOST CELLS

EDITED BY: Tetsuo Tsukamoto, Santhi Gorantla and Vasco Rodrigues
PUBLISHED IN: Frontiers in Cellular and Infection Microbiology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88971-805-4
DOI 10.3389/978-2-88971-805-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

DIRECT AND INDIRECT INTERACTIONS OF HIV WITH HOST CELLS

Topic Editors:
Tetsuo Tsukamoto, Kindai University, Japan
Santhi Gorantla, University of Nebraska Medical Center, United States
Vasco Rodrigues, Institut Curie, France

Citation: Tsukamoto, T., Gorantla, S., Rodrigues, V., eds. (2021). Direct and Indirect Interactions of HIV With Host Cells. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-805-4

Table of Contents

frontiers in Cellular and Infection Microbiology

EDITORIAL
published: 11 October 2021
doi: 10.3389/fcimb.2021.771370

Editorial: Direct and Indirect Interactions of HIV With Host Cells

Tetsuo Tsukamoto[1]*, *Santhi Gorantla*[2] *and Vasco Rodrigues*[3]

[1] Department of Immunology, Faculty of Medicine, Kindai University, Osaka, Japan, [2] Department of Pharmacology and Experimental Neuroscience, College of Medicine, University of Nebraska Medical Center, Omaha, NE, United States, [3] Institut Curie, PSL Research University, INSERM U932, Paris, France

Keywords: human immunodeficiency virus (HIV), acquired immunodeficiency syndrome (AIDS), virus-host interaction, hematopoietic stem/progenitor cells (HSPCs), NeuroHIV

Editorial on the Research Topic

Direct and Indirect Interactions of HIV With Host Cells

HIV causes AIDS by infection and depletion of $CD4^+$ T cells. HIV can also infect other $CD4^+$ cells such as macrophages, possibly leading to their functional alterations and contribution to viral reservoirs (Hendricks et al., 2021). HIV infection may also result in changes in other hematopoietic cells such as hematopoietic stem/progenitor cells (HSPCs), $CD8^+$ T cells, B cells, natural killer cells, dendritic cells (DCs), and granulocytes (Virgilio and Collins, 2020; Dillon and Wilson, 2021; Madzime et al., 2021). Furthermore, HIV infection can even cause alteration of non-hematopoietic cells. Cells in various tissues and organs, including bone marrow, thymus, gut, genital tract, lung, and central nervous system, have been investigated in depths in HIV-infected hosts (Dillon and Wilson, 2021; Pruitt, 2021). However, the outstanding breadth of the direct/indirect influence of HIV on host cells in different tissues and organs could make it challenging to grasp the whole picture of the HIV disease.

In three articles on this Research Topic, scientists investigated the impact of HIV-1 on host immune cells. Perot et al. extended their previous study on tetraspanin 7 (TSPAN7)-dependent transfer of HIV-1 from immature monocyte-derived DCs (iMDDCs) to $CD4^+$ T cells (Ménager and Littman, 2016). They discovered that TSPAN7 expression is downregulated in the process of DC maturation and that viral replication-independent TSPAN7-dependent trans-infection of HIV-1 in iMDDCs is far more efficient than the replication-dependent, TSPAN7-independent virus transfer mechanism in mature monocyte-derived dendritic cells, further clarifying the previously proposed two-phase trans-infection model. Bharucha et al. analyzed the impact of β-defensins hBD2 and 3 on HIV-1. Defensins are small peptides with potent antimicrobial effects (Brice and Diamond, 2020). The defensins hBD2 and 3 were previously shown to inhibit HIV replication in $CD4^+$ T cells. In this article, the authors demonstrated for the first time that the two defensins also inhibit HIV replication in monocyte-derived macrophages (MDMs). Intriguingly, they described the potential effect of hBD2 treatment on the upregulation of antiviral factors APOBEC3A and APOBEC3G. Further study will clarify whether β-defensins can be used as therapeutics and whether they influence viral reservoirs in MDMs. Badura et al. investigated B-cell defects observed in HIV-1-infected patients. The authors detected altered B-cell dynamics during acute HIV-1 infection caused by impaired bone marrow output of naïve B cells, which correlated with the degree of T-cell activation, and increased B-cell proliferation. The impaired bone marrow output was more profound in patients with severe disease, suggesting the importance of correcting bone marrow

OPEN ACCESS

Edited and reviewed by:
John Hiscott,
Istituto Pasteur Italia Cenci Bolognetti Foundation, Italy

***Correspondence:**
Tetsuo Tsukamoto
ttsukamoro@med.kindai.ac.jp

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *06 September 2021*
Accepted: *23 September 2021*
Published: *11 October 2021*

Citation:
Tsukamoto T, Gorantla S and Rodrigues V (2021) Editorial: Direct and Indirect Interactions of HIV With Host Cells. Front. Cell. Infect. Microbiol. 11:771370. doi: 10.3389/fcimb.2021.771370

abnormalities in HIV-1 infection. Furthermore, the impact of 8-week antiretroviral therapy (ART) on bone marrow B-cell output was limited.

The topic also highlights HSPCs in HIV infection. In his mini-review, Tsukamoto summarizes recent advances in our understanding of the interaction between HIV and HSPCs. He describes the current significant issues on viral reservoirs and the impact of HIV on the number, function, and aging of HSPCs. Bordoni et al. provide evidence on the relationship between chronic HIV infection and aging in circulating $CD34^+$ hematopoietic progenitor cells (HPCs). In the study, peripheral HPCs from HIV^+ patients with low $CD4^+$ T-cell counts (<500/mL blood) showed decreased *in vitro* white colony-forming cell (CFC) capacities. The authors further indicated that increased expression of Period circadian clock 2 (Per2) in HIV^+ patients might have caused lower white CFC counts. Furthermore, they demonstrated the association of HIV infection with the downregulation of Sirtuin 1 (Sirt1), a histone deacetylase and potential anti-aging protein. The Sirt1 inhibitor resveratrol restored the Sirt1 expression levels and caused downregulation of Per2 *in vitro*. These exciting results stress the need to compare HPCs between healthy and HIV^+ individuals using a broader panel of host factors affecting cellular processes such as senescence and inflammatory response. Valverde-Villegas et al. provide data showing the distinct distribution of cells with HSPC-like phenotypes in breast milk and peripheral blood between HIV^+ and HIV^- young women. According to the data, $CD45^+CD34^-CD133^+$ cells showed increased frequencies in milk and blood of HIV^+ women. However, the increase in the frequency of the $CD133^+$ cells was not associated with proinflammatory cytokines in milk except IL-8. The intriguing results will stimulate further research on the origin and the fate of these primitive stem/progenitor-like cells.

The topic further includes three reviews on the central nervous system (CNS). Gorska and Eugenin describe the dysregulation of glutamate in HIV-associated neurocognitive disorders (HAND). The authors detail how overproduction of glutamate, the critical neurotransmitter, can induce cytotoxicity and chronic inflammation. In the CNS, ART cannot block the production of viral proteins gp120, Tat, and Vpr in HIV-infected microglia and astrocytes, which may be associated with inflammatory responses. In addition, persistent activation of glial cells causes increased synthesis and release of glutamate, leading to overstimulation of glutamate receptors and neurotoxicity. Ajasin and Eugenin review previous articles facilitating the current understanding of Tat in the CNS and cardiovascular diseases. The authors detail the role of Tat not only in HIV-infected cells but also in uninfected bystander cells such as microglia and astrocytes. Following the uptake of Tat released from HIV-infected cells, bystander cells produce neurotoxic factors, including proinflammatory cytokines. Lastly, Tice et al. feature the glymphatic system, i.e., a system for waste clearance in the CNS, and the water channel aquaporin 4 expressed on astrocytic endfeet. There has been limited evidence to show changes in AQP4 in HIV infection. However, given the pathological similarities between NeuroHIV and Alzheimer's disease that accompanies altered distribution and expression of AQP4 (Yang et al., 2016) and previous observations with macaque AIDS models, the authors propose that it is time to investigate the potential impact of viral latency/reactivation in the brain on disruption of the glymphatic system.

In summary, our Research Topic brings together nine articles that describe novel findings, up-to-date information, or future directions to advance our understanding and thinking of host cells in connection with HIV infection. Although the funding environment surrounding basic HIV research has become increasingly complex (Levy, 2020), we hope that this Research Topic will help encourage researchers who work on the elucidation of HIV pathogenesis for better prevention and treatment in the future.

AUTHOR CONTRIBUTIONS

TT, SG, and VR edited the Research Topic, wrote, and approved the manuscript.

FUNDING

TT has been supported by JSPS KAKENHI (grant numbers JP19K07581 and JP19H03481) and Mochida Memorial Foundation for Medical and Pharmaceutical Research.

ACKNOWLEDGMENTS

The topic editors sincerely appreciate the contributions to this article collection by all the authors, reviewers, and editors.

REFERENCES

Brice, D. C., and Diamond, G. (2020). Antiviral Activities of Human Host Defense Peptides. *Curr. Med. Chem.* 27, 1420–1443. doi: 10.2174/0929867326666190805151654

Dillon, S. M., and Wilson, C. C. (2021). Gut Innate Immunity and HIV Pathogenesis. *Curr. HIV/AIDS Rep.* 18, 128–138. doi: 10.1007/s11904-021-00544-3

Hendricks, C. M., Cordeiro, T., Gomes, A. P., and Stevenson, M. (2021). The Interplay of HIV-1 and Macrophages in Viral Persistence. *Front. Microbiol.* 12, 646447. doi: 10.3389/fmicb.2021.646447

Levy, J. A. (2020). The Need to Reassess Our Approaches at HIV Cure and Prevention. *Aids* 34, 2141–2143. doi: 10.1097/QAD.0000000000002695

Madzime, M., Rossouw, T. M., Theron, A. J., Anderson, R., and Steel, H. C. (2021). Interactions of HIV and Antiretroviral Therapy With Neutrophils and Platelets. *Front. Immunol.* 12, 634386. doi: 10.3389/fimmu.2021.634386

Ménager, M. m., and Littman, D. r. (2016). Actin Dynamics Regulates Dendritic Cell-Mediated Transfer of HIV-1 to T Cells. *Cell* 164, 695–709. doi: 10.1016/j.cell.2015.12.036

Pruitt, A. A. (2021). Central Nervous System Infections in Immunocompromised Patients. *Curr. Neurol. Neurosci. Rep.* 21, 37. doi: 10.1007/s11910-021-01119-w

Virgilio, M. C., and Collins, K. L. (2020) 127. The Impact of Cellular Proliferation on the HIV-1 Reservoir. *Viruses* 12, 127. doi: 10.3390/v12020127

Yang, C., Huang, X., Huang, X., Mai, H., Li, J., Jiang, T., et al. (2016). Aquaporin-4 and Alzheimer's Disease. *J. Alzheimers Dis.* 52, 391–402. doi: 10.3233/JAD-150949

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cellular and Infection Microbiology

MINI REVIEW
published: 21 February 2020
doi: 10.3389/fcimb.2020.00060

Hematopoietic Stem/Progenitor Cells and the Pathogenesis of HIV/AIDS

*Tetsuo Tsukamoto**

Department of Immunology, Faculty of Medicine, Kindai University, Osaka, Japan

The interaction between human immunodeficiency virus (HIV) and hematopoietic stem/progenitor cells (HSPCs) has been of great interest. However, it remains unclear whether HSPCs can act as viral reservoirs. Many studies have reported the presence of latently infected HSPCs in the bone marrow of HIV-infected patients, whereas many other investigators have reported negative results. Hence, further evidence is required to elucidate this controversy. The other arm of HSPC investigations of HIV infection involves dynamics analysis in the early and late stages of infection to understand the impact on the pathogenesis of acquired immunodeficiency syndrome. Several recent studies have suggested reduced amounts and/or functional impairment of multipotent, myeloid, and lymphoid progenitors in HIV infection that may contribute to hematological manifestations, including anemia, pancytopenia, and T-cell depletion. In addition, ongoing and future studies on the senescence of HSPCs are expected to further the understanding of HIV pathogenesis. This mini review summarizes reports describing the basic aspects of hematopoiesis in response to HIV infection and offers insights into the association of HIV infection/exposure of the host HSPCs and hematopoietic potential.

Keywords: human immunodeficiency virus, acquired immunodeficiency syndrome, hematopoietic stem/progenitor cells, hematopoiesis, senescence

OPEN ACCESS

Edited by:
Samuel K. Campos, The University of Arizona, United States

Reviewed by:
Julià Blanco, IrsiCaixa, Spain
Larisa Y. Poluektova, University of Nebraska Medical Center, United States

***Correspondence:**
Tetsuo Tsukamoto
ttsukamoto@med.kindai.ac.jp

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *19 November 2019*
Accepted: *06 February 2020*
Published: *21 February 2020*

Citation:
Tsukamoto T (2020) Hematopoietic Stem/Progenitor Cells and the Pathogenesis of HIV/AIDS. Front. Cell. Infect. Microbiol. 10:60. doi: 10.3389/fcimb.2020.00060

INTRODUCTION

Human immunodeficiency virus (HIV) infection causes acquired immunodeficiency syndrome (AIDS). The depletion of memory $CD4^+$ T cells preceding the manifestation of AIDS may be mainly due to HIV infection of these cells. However, HIV may also cause reduced production of naïve T cells by infection of $CD4^+$ thymocytes. Although the dynamics of hematopoietic stem/progenitor cells (HSPCs) in response to HIV infection remains unclear, it is well-established that HIV infection is associated with hematological changes, such as anemia and pancytopenia (Parinitha and Kulkarni, 2012; Durandt et al., 2019). Therefore, it is imperative to better elucidate the contribution of altered hematopoietic potential to the disease. The aim of this mini review was to discuss on factors affecting the physiology and pathology of HSPCs by reviewing past publications describing the interactions between HIV and hematopoietic progenitor cells (HPCs) in the bone marrow (BM) and thymus for better understanding the role of hematopoiesis in the pathogenesis.

HSPCs IN THE BM

Adult hematopoietic differentiation occurs in the BM. Hematopoietic stem cells (HSCs) have long-term self-renewing capacity and can differentiate to any type of blood cell (Rieger and Schroeder, 2012). Although HSC niches have not been fully defined (Morrison and Scadden, 2014), a recent study indicated that HSCs reside in a perivascular niche and are supported by various cytokines secreted by endothelial and stromal cells (Ding et al., 2012). BM HSPCs consist of progenitors for all blood cell lineages including those described in **Figure 1** (Rieger and Schroeder, 2012). Proteomic and transcriptomic analyses have reported many potential factors that may work in concert in hematopoiesis, although the significance of individual genes must be further clarified (Liu et al., 2006; Kim et al., 2009; Starnes et al., 2010). Recent evidence indicates that inflammatory signals, such as prostaglandin E2, nitric oxide, granulocyte colony-stimulating factor, interferons, tumor necrosis factor, and Toll-like receptor 4, may be involved in the emergence of HSPCs (He et al., 2015; Luis et al., 2016).

THE ROLES OF HSPCs IN T-LINEAGE DIFFERENTIATION

T-lineage differentiation in the thymus is dependent on a supply of $CD34^+$ progenitors from the BM (Kondo et al., 1997). Early lymphoid progenitors (LPs) are thought to reside in distinct niches from those of HSCs (Ding and Morrison, 2013). $CD34^+CD38^{dim}$, but not $CD34^+CD38^+$, cells can migrate to the thymus and commit to the T cell lineage (Res et al., 1996). Transcriptional regulation of the lymphoid commitment of HSPCs is complex (Laurenti et al., 2013). Notch 1 and its ligands play essential roles in T-lineage commitment

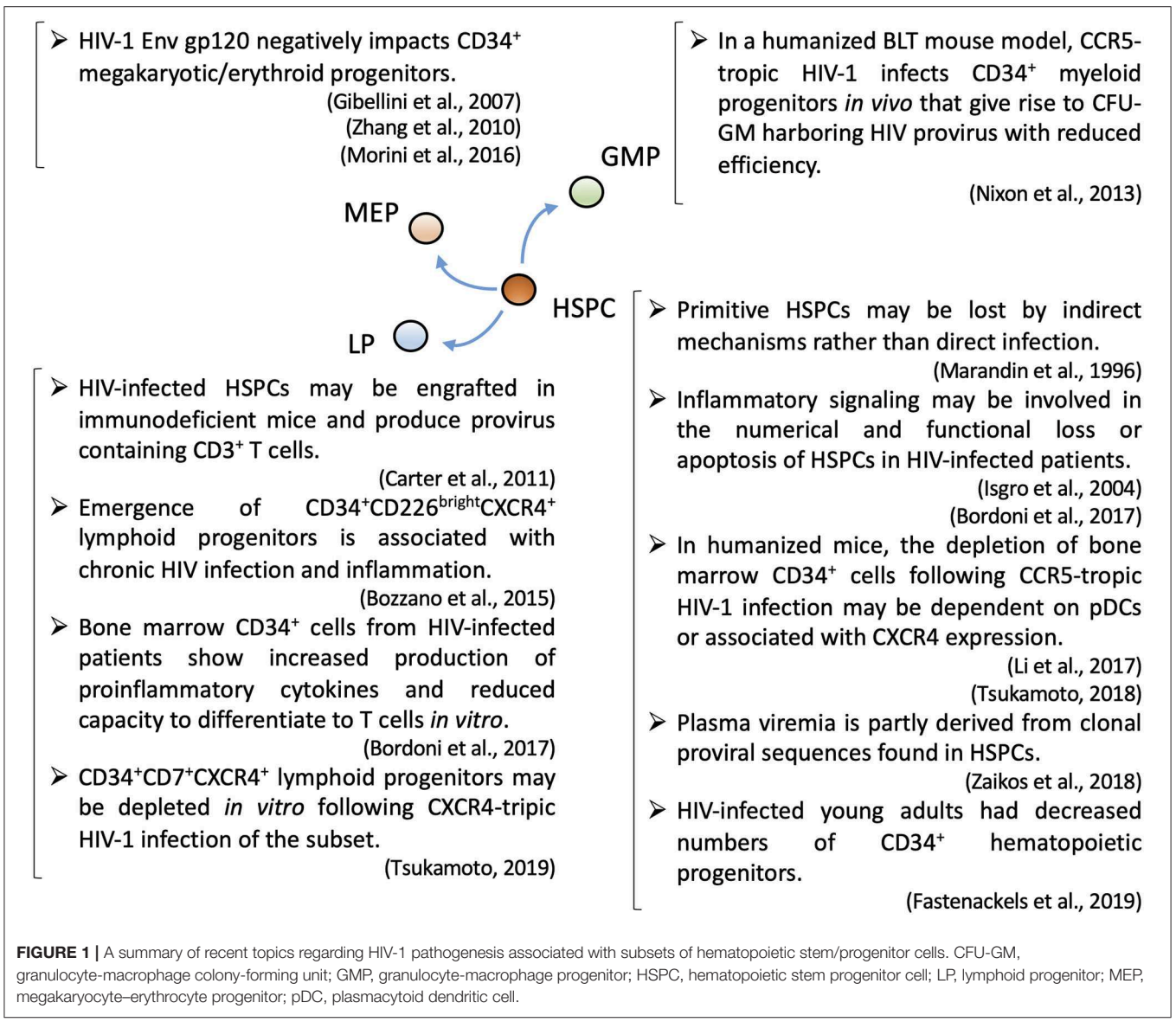


FIGURE 1 | A summary of recent topics regarding HIV-1 pathogenesis associated with subsets of hematopoietic stem/progenitor cells. CFU-GM, granulocyte-macrophage colony-forming unit; GMP, granulocyte-macrophage progenitor; HSPC, hematopoietic stem progenitor cell; LP, lymphoid progenitor; MEP, megakaryocyte–erythrocyte progenitor; pDC, plasmacytoid dendritic cell.

(Radtke et al., 2004). For example, Delta-like 1 (DL1) enhances the repopulation capability of human $CD34^+CD38^-$ cells in the BM and contributes to the generation of thymus-repopulating T-cell precursors (Ohishi et al., 2002). In addition, Delta-like 4 (DL4) induces Notch signaling in the thymus (Hozumi et al., 2008). Accordingly, the stable expression of DL1 or DL4 by OP9 cells allows for the differentiation of human HSPCs to T cells *in vitro* (La Motte-Mohs et al., 2005; Mohtashami et al., 2010). C-X-C chemokine receptor type 4 (CXCR4) also plays a critical role in the localization and differentiation of T-lineage progenitors in the thymus (Plotkin et al., 2003).

HSPC-ASSOCIATED HEMATOLOGICAL CHANGES IN HIV INFECTION

Hematological changes in HIV-infected patients may be at least partly associated with abnormalities in the BM (Dhurve and Dhurve, 2013; Durandt et al., 2019). Because HSPCs generally have limited surface expression of CD4, their abnormalities in HIV infection could be largely explained as an indirect effect of HIV infection, rather than the results of direct infection of HSPCs (Louache et al., 1992; De Luca et al., 1993; Maciejewski et al., 1994; Marandin et al., 1996; Koka et al., 1999). Although antiretroviral therapy (ART) generally improves hematopoiesis in HIV-infected patients (Baillou et al., 2003), the immune function in some patients is insufficient despite successful ART; therefore, such patients are referred to as immunological non-responders (Corbeau and Reynes, 2011; Takuva et al., 2014; Rb-Silva et al., 2019). Indeed, the recovery of $CD4^+$ T cell counts after successful ART may depend on the recovery of $CD34^+$ cell counts (Sauce et al., 2011).

Lymphopoiesis, myelopoiesis, megakaryopoiesis, and erythropoiesis may be altered during the course of HIV infection (**Figure 1**). HIV-1 infection may cause defective myelopoiesis/erythropoiesis as well as the accumulation of myeloid/erythroid precursors (Costantini et al., 2009, 2010). Ineffective platelet production noted in HIV-infected patients (Cole et al., 1998) might be due to a negative impact of HIV on the differentiation of megakaryocyte lineages, leading to thrombocytopenia (Costantini et al., 2006; Sundell and Koka, 2006). The V3 loop region of the HIV-1 gp120 envelope protein was described as a potential inhibitor of megakaryocyte differentiation (Zhang et al., 2010). Furthermore, studies have suggested the influence of HIV-1 gp120/CD4 interaction on $CD34^+$ megakaryocytic/erythroid progenitors (Gibellini et al., 2007; Morini et al., 2016).

THE BIOLOGICAL FUNCTIONS OF HIV CORECEPTORS

HIV-1 uses C–C chemokine receptor type 5 (CCR5) and CXCR4 as coreceptors (Weiss, 1996). CCR5 is expressed on the surface of memory $CD4^+$ T cells and causes the massive depletion of this cell type following HIV-1 infection of the host (Mattapallil et al., 2005). Recent evidence suggests that CCR5 is involved in inflammation (Kitade et al., 2012; Barashi et al., 2013; Duan et al., 2014) because the lack of a functional CCR5 allele is associated with the severity of viral infection, possibly due to altered immune responses (Lim et al., 2008). On the other hand, the pathological roles of CCR5 in various infectious and non-infectious diseases, e.g., autoimmune diseases, have been suggested (Vangelista and Vento, 2017). For example, the depletion of CCR5 was associated with attenuation of the adverse effects of inflammation (Muntinghe et al., 2009), and blockade of CCR5 inhibited leukocyte trafficking and reportedly reduced inflammation in a murine model of colitis (Mencarelli et al., 2016). Thus, these findings address the roles of CCR5 in health and disease.

CXCR4 is specific for stromal cell-derived factor 1 (SDF-1, also known as CXCL12). SDF-1 is produced by BM stromal cells, including CXCL12-abundant reticular cells (Nagasawa, 2015), and allows the homing of HSCs to BM. The interaction between SDF-1 and CXCR4 is essential for hematopoiesis (Karpova and Bonig, 2015). In addition, the SDF-1/CXCR4 axis has multiple essential roles in life (Murphy and Heusinkveld, 2018), such as embryonic (Mcgrath et al., 1999) and vascular (Takabatake et al., 2009; Kim et al., 2017) development, while providing support for the survival and migration of neoplastic cells (Chatterjee et al., 2014). The polymorphisms of SDF-1 might affect the ability to prevent HIV-1 infection (Winkler et al., 1998; Kuipers et al., 1999). However, the effect of SDF-1 polymorphisms on the susceptibility of the host to HIV-1 infection might be moderate (Ding et al., 2018). In contrast to the popularity of the topics of CXCR4 as an HIV-1 coreceptor and SDF-1 as an inhibitor of HIV-1 infection (Arenzana-Seisdedos, 2015), relatively few articles have addressed the intrinsic functions of SDF-1 and CXCR4 in the pathogenesis of HIV-1 infection and AIDS (Ikegawa et al., 2001; Tsukamoto, 2018).

POTENTIAL MECHANISMS UNDERLYING THE LOSS OF OR CHANGES IN HSPCS IN RESPONSE TO HIV INFECTION OF THE HOST

Various potential mechanisms underlying changes in HSPCs during HIV infection have been suggested, such as reduced c-Mpl (thrombopoietin receptor) expression on HSPCs (Koka et al., 2004), elevated plasma SDF-1 levels (Ikegawa et al., 2001), and altered BM niches (Moses et al., 1996). HIV-1 infection results in increased levels of inflammatory cytokines, affecting dynamics and functions (Bordoni et al., 2017) or inducing Fas-mediated apoptosis (Isgro et al., 2004) of HSPCs (**Figure 1**). Importantly, HSPCs require inflammatory signals in their development (Luis et al., 2016), and therefore may contribute to inflammation (Fischer and Agrawal, 2013). A recent study reported the emergence of a $CD34^+CD226(DNAM\text{-}1)^{bright}CXCR4^+$ LP subset in association with chronic HIV infection and inflammation, reflecting altered dynamics of natural killer (NK) cells and α/β T cells (Bozzano et al., 2015; **Figure 1**). Finally, there has recently been an emerging trend to interpret some hematopoietic changes during the course of HIV

infection as the accelerated senescence of HSPCs (Appay and Sauce, 2017; Fali et al., 2018; Fastenackels et al., 2019).

Humanized mouse models provide important resources for the analysis of BM HSPCs following HIV-1 infection. For example, in studies with humanized mice challenged with CXCR4-tropic HIV-1_{NL4-3}, $CD34^+$ cells were depleted and/or exhibited impaired *ex vivo* myeloid and erythroid colony-forming capacities (Jenkins et al., 1998; Koka et al., 1998). Moreover, the reduction in BM HSPC counts in humanized mice was observed even after CCR5-tropic HIV-1 infection (Arainga et al., 2016). Other research groups have reported that the loss of $CD34^+$ cells in CCR5-tropic HIV-1 infection might be dependent on plasmacytoid dendritic cells (pDCs) (Li et al., 2017) or correlated with CXCR4 expression (Tsukamoto, 2018; **Figure 1**). Therefore, it is important to further investigate changes such as altered expression of cytokines in pDCs and other cells residing in BM in HIV infection. The latter could implicate the involvement of SDF-1/CXCR4 axis in the pathogenesis such as accelerated turnover of HSPCs.

THE IMPACT OF HIV ON T-LINEAGE DEVELOPMENT

The involvement of the thymus in HIV pathogenesis has been investigated (Ye et al., 2004). HIV-1 may cause thymocyte depletion mediated by an indirect cytopathic effect and infection of $CD3^-CD4^+CD8^-$ progenitor cells (Su et al., 1995). In an *in vitro* model imitating the thymic environment, thymocyte maturation was inhibited by HIV infection of the $CD44^+CD25^-CD3^-$ cell lineage (Knutsen et al., 1999). Early ART might preserve the lymphopoiesis capability of the host (Bordoni et al., 2015b, 2018; Rb-Silva et al., 2019) and reverse reduced thymic function (Withers-Ward et al., 1997; Levine et al., 2001).

In a BLT (BM, liver, and thymus) mouse model, HIV-1 Nef enhanced HIV-1 replication and caused depletion of $CD4^+CD8^+$ thymocytes (Zou et al., 2012). In another humanized mouse model, HIV-1 infection caused perturbation of cytokine mRNA expression in infected thymocytes. For instance, mRNA levels of interleukin (IL)-6, interferon-γ, and IL-2 were increased, whereas macrophage inflammatory protein (MIP)-1β expression was decreased. On the other hand, HIV infection of human stromal cells increased IL-6 levels, whereas SDF-1 expression levels were unaffected (Koka et al., 2003).

There have also been several reports on the T-lineage differentiation of HPCs, although it may be difficult to interpret all the data collectively. T-lineage progenitors express CXCR4 and are susceptible to CXCR4-tropic HIV infection (Berkowitz et al., 1998). In a study, BM cells infected with HIV before ART initiation had reduced amounts of $CD34^+$ cells, but not $CD34^+CD7^+$ LPs (Muller et al., 2002). Although the data are intriguing, the study lacked information of absolute cell counts, so their notions were not firmly concluded except for reduced $CD34^+$ frequencies. In another study of BM samples from HIV-infected ART-treated immunological non-responders, clonogenic capability and the sizes of primitive HSPCs were altered, which were associated with reduced production of IL-2, increased production of TNF-α, and increased stromal production of IL-7 (Isgro et al., 2008). Another study using a lentiviral vector expressing HIV-1 Nef showed that Nef may impair the differentiation of HSPCs to $CD3\varepsilon^+CD5^+CD1a^+$ T/NK precursors (Dorival et al., 2008).

In a recent study, BM-derived HSPCs from HIV-infected patients exhibited reduced T-cell differentiation potential and increased production of pro-inflammatory cytokines, indicating that they are also produced by non-LPs. However, it remains unclear whether pro-inflammatory cytokine secretion is the cause or consequence of impaired T cell differentiation potential (Bordoni et al., 2017; **Figure 1**). Also, in a macaque model, following challenge with simian immunodeficiency virus, BM-derived $CD34^+$ cells exhibited reduced T-lineage differentiation potential *in vitro* without significant changes in phenotypic analysis of $CD34^+$ subsets (Thiebot et al., 2005). Another recent study suggested that $CD34^+CD7^+CXCR4^+$ cells may be depleted in response to CXCR4-tropic HIV-1 infection in a coculture of HIV-infected umbilical cord-derived $CD34^+$ and OP9-DL1 cells (Tsukamoto, 2019b; **Figure 1**). Despite the evidence of LPs during HIV-1 infection, our understanding of the impact of HIV-1 on LPs remains limited.

DIRECT HIV INFECTION OF HSPCs

HSPCs have limited surface levels of HIV receptors and coreceptors compared with differentiated $CD4^+$ cells. $CD34^+CD133^+$ umbilical cord-derived HSCs may have further limited expression levels of CD4, CXCR4, and CCR5 (Hariharan et al., 1999). In an *in vitro* culture study, BM-derived $CD34^+CD38^-$ primitive HPCs were exposed to HIV-1 or HIV-2, but infection was not observed (Weichold et al., 1998). In another study, HIV-1 exposure had no effect on the *in vitro* expansion/proliferation dynamics of HSPCs (Kaushal et al., 1996). However, accumulating evidence has implicated HIV-susceptible subsets of HSPCs in patients (Louache et al., 1994; Zauli et al., 1994; Chelucci et al., 1995, 1999). In addition, peripheral blood $CD34^+$ cells expressing CXCR4/CCR5 are susceptible to diverse strains of HIV-1 (Ruiz et al., 1998). Another study found that BM $CD34^+CD4^+$ cells are depleted in HIV-infected patients (Banda et al., 1999). Moreover, the HIV-1 Gag protein was expressed by BM HSPCs isolated from HIV-infected patients (Carter et al., 2010). A recent study of patient samples revealed that some HSPC subsets express high levels of CD4 and may harbor both CCR5-tropic and CXCR4-tropic HIV genomes (Sebastian et al., 2017). Furthermore, HSPCs latently infected with cytomegalovirus may have enhanced susceptibility to HIV-1 infection (Cheung et al., 2017). To confirm this evidence, another study using humanized BLT mice demonstrated HIV-1 infection of HPCs *in vivo*. These infected HPCs remained capable of differentiating to myeloid cells *in vitro*, albeit with reduced efficacy (Nixon et al., 2013; **Figure 1**).

Regarding molecular mechanisms preventing HIV infection except limited (co) receptor expression, a recent study suggested

a post-entry mechanism to allow HSPCs to restrict HIV-1 replication prior to conversion of viral RNA into DNA and integration into the host genome (Griffin and Goff, 2015). Variations of tripartite motif-containing protein 5 may also influence the infection efficiency of lentiviruses in human and rhesus HSPCs (Evans et al., 2014). CCR5-ligand β-chemokines, including RANTES and MIP-1β, produced by HSPCs may modify the susceptibility of these cells to CCR5-tropic HIV-1 Env (Majka et al., 1999, 2000).

Some studies on HIV infection of HSPCs have relied on *in vitro* stimulation of cells with 50–100 ng/mL of individual stem cell factors, thrombopoietin, or FMS-like tyrosine kinase 3 ligand to overcome the low permissiveness of these cells to retrovirus/lentivirus infection (Santoni De Sio and Naldini, 2009). Such stimulation may enhance gene expression of the HIV-1 receptor and coreceptors, leading to overestimation of HIV infection/replication levels in HSPCs (Zhang et al., 2009). A method to achieve reproducible *in vitro* infection of HSPCs with CXCR4-tropic HIV-1 with RetroNectin-coated plate, but without strong cytokine stimulation, has been proposed (Tsukamoto and Okada, 2017).

HSPCs AS VIRAL RESERVOIRS

There is no consensus on whether HSPCs are a major HIV reservoir (Von Laer et al., 1990; Stanley et al., 1992; Neal et al., 1995; Kandathil et al., 2016). A relatively recent study of BM HSPCs from eight patients following long-term effective ART found no HIV DNA in the collected cells (Josefsson et al., 2012), suggesting that HIV reservoir surveys of purified $CD34^+$ cells may fail to exclude HIV-contaminated $CD4^+$ T cells (Durand et al., 2012). In contrast, accumulating data support latent HIV infection of HSPCs. Moreover, some BM HSPCs may remain latently infected after successful treatment (Bordoni et al., 2015a). Another study suggested that multiple subsets of HSPCs may be latently infected with HIV-1, including immature ($CD34^+CD38^-CD45RA^-$) progenitors, which are more likely to persist and serve as latent reservoirs following ART (Mcnamara et al., 2012). Humanized mouse models have also been utilized to investigate $CD34^+$ HIV reservoirs. A previous study revealed that the HIV-infected HSPCs may serve as long-term HIV reservoirs in the BM of humanized mice, leading to production of HIV-integrated $CD3^+$ T cells (Carter et al., 2011). Taken together, HSPCs might constitute significant HIV reservoirs, which should be further investigated.

While it remains unclear whether infected HSPCs contribute to residual viremia after ART (Onafuwa-Nuga et al., 2010; Mcnamara and Collins, 2011), a recent article reported that HSPCs in suppressed patients harbor functional HIV proviral genomes that often match residual peripheral viral RNA (Zaikos et al., 2018). If these findings are confirmed, HSPCs might be finally regarded as long-term viral reservoirs, because they are long-lived cells with regulated susceptibility to apoptosis (Durdik et al., 2017). Thus, precise identification of HSPC subsets harboring functional HIV proviral copies could further facilitate these findings and clarify the role of HSPCs in HIV persistence even after successful ART. Furthermore, it is interesting to assess whether early initiation of ART could prevent the establishment of viral reservoirs in HSPCs.

PROTECTION OF HSPCs AGAINST HIV INFECTION

Presently, the best method for treating HIV-infected individuals in terms of protection of HSPCs is to initiate ART as early as possible regardless of the disease stage (World Health Organization, 2015). By interrupting HIV pathogenesis early during infection, it is expected that existing $CD4^+$ T cells and HSPCs as well as the host's hematopoietic capacity will be preserved for long (Bordoni et al., 2015b). However, more treatment options might be helpful for patients who are diagnosed in the chronic phase and/or those who manifest the characteristics of immunological non-responders against the current ART regimens (Rb-Silva et al., 2019).

CXCR4 may be targeted to protect HSPCs against CXCR4-tropic HIV-1 infection, because they express CXCR4 and are considered susceptible to CXCR4-tropic HIV-1 infection. For example, the μ-opioid agonist DAMGO ($C_{26}H_{35}N_5O_6$) was found to downregulate CXCR4 expression and prevent HIV-1 infection of BM HSPCs (Strazza et al., 2014). On the other hand, a clinical study reported that the CXCR4 antagonist plerixafor was not successful for the treatment of HIV-infected patients (Hendrix et al., 2004). Because systemic administration of plerixafor is associated with adverse effects, especially to patients with cardiovascular diseases, further development of CXCR4-tropic HIV-1 entry inhibitors with weaker affinity to CXCR4 than plerixafor is needed (Berg et al., 2018). Other entry inhibitors such as ibalizumab, a humanized monoclonal anti-CD4 antibody that inhibits the binding of HIV gp120, might also be highly effective in preventing HSPCs from infection (Emu et al., 2018). It is unclear whether CCR5-tropic HIV-1 entry inhibitors such as maraviroc are effective in protecting HSPCs because HIV-1 is considered to use CXCR4 to enter those cells (Carter et al., 2011). However, those entry inhibitors can lower viral burden by protecting $CCR5^+$ memory $CD4^+$ T cells and lead to lower risks for indirect damages to HSPCs.

The significance of CXCR4 in HIV-1 infection is not necessarily limited to its function as an HIV-1 coreceptor. For example, it is unclear how the biological roles of CXCR4, including the SDF-1/CXCR4 signaling pathway in the BM and thymus, affect hematopoiesis in response to HIV infection. It has been indicated that elevation of plasma SDF-1 levels may be associated with disease progression (Ikegawa et al., 2001). Another study suggested the use of granulocyte colony-stimulating factor to increase $CD34^+$ and $CD4^+$ cell counts in HIV-infected patients (Nielsen et al., 1998). In addition, a recent humanized mouse study indicated the involvement of CXCR4 in the loss of BM HSPCs in CCR5-tropic HIV-1 infection (Tsukamoto, 2018; **Figure 1**). These results must be further investigated to elucidate whether the loss of HSPCs

following HIV-1 infection can be alleviated by interrupting the SDF-1/CXCR4 signaling pathway.

Recent studies indicate HSPCs as an ideal target for anti-HIV gene therapy aimed to protect hosts' hematopoietic potential (Kitchen et al., 2011; Savkovic et al., 2014). For detailed discussions on recent advances in the field, see a recently published review by this author Tsukamoto (2019a).

CONCLUDING REMARKS

Despite previous efforts and accumulating data to better clarify the interactions between HIV-1 and HSPCs, studies on their involvement in HIV pathogenesis are ongoing. The contribution of latently infected HSPCs to viral persistence should be better described. Regarding HSPC subsets, recent evidence supports the influence of HIV-1 on myeloid progenitor cells. On the other hand, among various steps in T-lineage development, the functional and numerical alteration of $CD34^+$ LPs in HIV-1 infection needs to be further elucidated to improve the current understanding of the degree of impaired $CD4^+$ T-cell generation on peripheral $CD4^+$ T-cell loss and AIDS onset. Humanized mouse models and *in vitro* models including OP9-DL1/OP9-DL4 coculture systems could be used for further analysis of HSPCs in HIV infection in this context. Further investigations in these fields will collectively enhance our understanding on the significance of protecting HSPCs in HIV infection.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

This study was partly supported by a grant from The Imai Memorial Trust for AIDS Research (Tokyo, Japan).

ACKNOWLEDGMENTS

The author thanks Enago (http://www.enago.jp) for the English language review.

REFERENCES

Appay, V., and Sauce, D. (2017). Assessing immune aging in HIV-infected patients. *Virulence* 8, 529–538. doi: 10.1080/21505594.2016.1195536

Arainga, M., Su, H., Poluektova, L. Y., Gorantla, S., and Gendelman, H. E. (2016). HIV-1 cellular and tissue replication patterns in infected humanized mice. *Sci. Rep.* 6:23513. doi: 10.1038/srep23513

Arenzana-Seisdedos, F. (2015). SDF-1/CXCL12: a chemokine in the life cycle of HIV. *Front. Immunol.* 6:256. doi: 10.3389/fimmu.2015.00256

Baillou, C., Simon, A., Leclercq, V., Azar, N., Rosenzwajg, M., Herson, S., et al. (2003). Highly active antiretroviral therapy corrects hematopoiesis in HIV-1 infected patients: interest for peripheral blood stem cell-based gene therapy. *AIDS* 17, 563–574. doi: 10.1097/00002030-200303070-00012

Banda, N. K., Simon, G. R., Sipple, J. D., Terrell, K. L., Archer, P., Shpall, E. J., et al. (1999). Depletion of CD34+ CD4+ cells in bone marrow from HIV-1-infected individuals. *Biol. Blood Marrow Transplant.* 5, 162–172. doi: 10.1053/bbmt.1999.v5.pm10392962

Barashi, N., Weiss, I. D., Wald, O., Wald, H., Beider, K., Abraham, M., et al. (2013). Inflammation-induced hepatocellular carcinoma is dependent on CCR5 in mice. *Hepatology* 58, 1021–1030. doi: 10.1002/hep.26403

Berg, C., Daugvilaite, V., Steen, A., Jorgensen, A. S., Vabeno, J., and Rosenkilde, M. M. (2018). Inhibition of HIV fusion by small molecule agonists through efficacy-engineering of CXCR4. *ACS Chem. Biol.* 13, 881–886. doi: 10.1021/acschembio.8b00061

Berkowitz, R. D., Beckerman, K. P., Schall, T. J., and Mccune, J. M. (1998). CXCR4 and CCR5 expression delineates targets for HIV-1 disruption of T cell differentiation. *J. Immunol.* 161, 3702–3710.

Bordoni, V., Bibas, M., Abbate, I., Viola, D., Rozera, G., Agrati, C., et al. (2015a). Bone marrow CD34+ progenitor cells may harbour HIV-DNA even in successfully treated patients. *Clin. Microbiol. Infect* 21, 290.e295–298. doi: 10.1016/j.cmi.2014.11.003

Bordoni, V., Bibas, M., Viola, D., Sacchi, A., Cimini, E., Tumino, N., et al. (2017). Bone marrow CD34(+) progenitor cells from HIV-infected patients show an impaired T cell differentiation potential related to proinflammatory cytokines. *AIDS Res. Hum. Retroviruses* 33, 590–596. doi: 10.1089/AID.2016.0195

Bordoni, V., Casetti, R., Viola, D., Abbate, I., Rozera, G., Sacchi, A., et al. (2015b). Early ART in primary HIV infection may also preserve lymphopoiesis capability in circulating haematopoietic progenitor cells: a case report. *J. Antimicrob. Chemother.* 70, 1598–1600. doi: 10.1093/jac/dku559

Bordoni, V., Viola, D., Sacchi, A., Pinnetti, C., Casetti, R., Cimini, E., et al. (2018). IL-18 and stem cell factor affect hematopoietic progenitor cells in HIV-infected patients treated during primary HIV infection. *Cytokine* 103, 34–37. doi: 10.1016/j.cyto.2017.12.033

Bozzano, F., Marras, F., Ascierto, M. L., Cantoni, C., Cenderello, G., Dentone, C., et al. (2015). 'Emergency exit' of bone-marrow-resident CD34(+)DNAM-1(bright)CXCR4(+)-committed lymphoid precursors during chronic infection and inflammation. *Nat. Commun.* 6:8109. doi: 10.1038/ncomms9109

Carter, C. C., Mcnamara, L. A., Onafuwa-Nuga, A., Shackleton, M., Riddell, J. T., Bixby, D., et al. (2011). HIV-1 utilizes the CXCR4 chemokine receptor to infect multipotent hematopoietic stem and progenitor cells. *Cell Host Microbe* 9, 223–234. doi: 10.1016/j.chom.2011.02.005

Carter, C. C., Onafuwa-Nuga, A., Mcnamara, L. A., Riddell, J. T., Bixby, D., Savona, M. R., et al. (2010). HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. *Nat. Med.* 16, 446–451. doi: 10.1038/nm.2109

Chatterjee, S., Behnam Azad, B., and Nimmagadda, S. (2014). The intricate role of CXCR4 in cancer. *Adv. Cancer Res.* 124, 31–82. doi: 10.1016/B978-0-12-411638-2.00002-1

Chelucci, C., Casella, I., Federico, M., Testa, U., Macioce, G., Pelosi, E., et al. (1999). Lineage-specific expression of human immunodeficiency virus (HIV) receptor/coreceptors in differentiating hematopoietic precursors: correlation with susceptibility to T- and M-tropic HIV and chemokine-mediated HIV resistance. *Blood* 94, 1590–1600. doi: 10.1182/blood.V94.5.1590

Chelucci, C., Hassan, H. J., Locardi, C., Bulgarini, D., Pelosi, E., Mariani, G., et al. (1995). *In vitro* human immunodeficiency virus-1 infection of purified hematopoietic progenitors in single-cell culture. *Blood* 85, 1181–1187. doi: 10.1182/blood.V85.5.1181.bloodjournal8551181

Cheung, A. K. L., Huang, Y., Kwok, H. Y., Chen, M., and Chen, Z. (2017). Latent human cytomegalovirus enhances HIV-1 infection in CD34(+) progenitor cells. *Blood Adv.* 1, 306–318. doi: 10.1182/bloodadvances.2016000638

Cole, J. L., Marzec, U. M., Gunthel, C. J., Karpatkin, S., Worford, L., Sundell, I. B., et al. (1998). Ineffective platelet production in thrombocytopenic human immunodeficiency virus-infected patients. *Blood* 91, 3239–3246. doi: 10.1182/blood.V91.9.3239

Corbeau, P., and Reynes, J. (2011). Immune reconstitution under antiretroviral therapy: the new challenge in HIV-1 infection. *Blood* 117, 5582–5590. doi: 10.1182/blood-2010-12-322453

Costantini, A., Giuliodoro, S., Butini, L., Silvestri, G., Leoni, P., and Montroni, M. (2009). Abnormalities of erythropoiesis during HIV-1 disease: a longitudinal analysis. *J. Acquir. Immune Defic. Syndr.* 52, 70–74. doi: 10.1097/QAI.0b013e3181af65c5

Costantini, A., Giuliodoro, S., Butini, L., Silvestri, G., Leoni, P., and Montroni, M. (2010). HIV-induced abnormalities in myelopoiesis and their recovery following antiretroviral therapy. *Curr. HIV Res.* 8, 336–339. doi: 10.2174/157016210791208604

Costantini, A., Giuliodoro, S., Mancini, S., Butini, L., Regnery, C. M., Silvestri, G., et al. (2006). Impaired *in-vitro* growth of megakaryocytic colonies derived from CD34 cells of HIV-1-infected patients with active viral replication. *AIDS* 20, 1713–1720. doi: 10.1097/01.aids.0000242817.88086.8c

De Luca, A., Teofili, L., Antinori, A., Iovino, M. S., Mencarini, P., Visconti, E., et al. (1993). Haemopoietic CD34+ progenitor cells are not infected by HIV-1 *in vivo* but show impaired clonogenesis. *Br. J. Haematol.* 85, 20–24. doi: 10.1111/j.1365-2141.1993.tb08640.x

Dhurve, S. A., and Dhurve, A. S. (2013). Bone Marrow abnormalities in HIV disease. *Mediterr. J. Hematol. Infect. Dis.* 5:e2013033. doi: 10.4084/MJHID.2013.033

Ding, J., Zhao, J., Zhou, J., Li, X., Wu, Y., Ge, M., et al. (2018). Association of gene polymorphism of SDF1(CXCR12) with susceptibility to HIV-1 infection and AIDS disease progression: a meta-analysis. *PLoS ONE* 13:e0191930. doi: 10.1371/journal.pone.0191930

Ding, L., and Morrison, S. J. (2013). Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. *Nature* 495, 231–235. doi: 10.1038/nature11885

Ding, L., Saunders, T. L., Enikolopov, G., and Morrison, S. J. (2012). Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature* 481, 457–462. doi: 10.1038/nature10783

Dorival, C., Brizzi, F., Lelievre, J. D., Sol-Foulon, N., Six, E., Henry, A., et al. (2008). HIV-1 Nef protein expression in human CD34+ progenitors impairs the differentiation of an early T/NK cell precursor. *Virology* 377, 207–215. doi: 10.1016/j.virol.2008.04.009

Duan, H., Yang, P., Fang, F., Ding, S., and Xiao, W. (2014). CCR5 small interfering RNA ameliorated joint inflammation in rats with adjuvant-induced arthritis. *Immunol. Lett.* 162, 258–263. doi: 10.1016/j.imlet.2014.09.018

Durand, C. M., Ghiaur, G., Siliciano, J. D., Rabi, S. A., Eisele, E. E., Salgado, M., et al. (2012). HIV-1 DNA is detected in bone marrow populations containing CD4+ T cells but is not found in purified CD34+ hematopoietic progenitor cells in most patients on antiretroviral therapy. *J. Infect. Dis.* 205, 1014–1018. doi: 10.1093/infdis/jir884

Durandt, C., Potgieter, J., Mellet, J., Herd, C., Khoosal, R., Nel, J. G., et al. (2019). HIV and haematopoiesis. *S. Afr. Med. J.* 109, 40–45. doi: 10.7196/SAMJ.2019.v109i8b.13829

Durdik, M., Kosik, P., Kruzliakova, J., Jakl, L., Markova, E., and Belyaev, I. (2017). Hematopoietic stem/progenitor cells are less prone to undergo apoptosis than lymphocytes despite similar DNA damage response. *Oncotarget* 8, 48846–48853. doi: 10.18632/oncotarget.16455

Emu, B., Fessel, J., Schrader, S., Kumar, P., Richmond, G., Win, S., et al. (2018). Phase 3 study of ibalizumab for multidrug-resistant HIV-1. *N. Engl. J. Med.* 379, 645–654. doi: 10.1056/NEJMoa1711460

Evans, M. E., Kumkhaek, C., Hsieh, M. M., Donahue, R. E., Tisdale, J. F., and Uchida, N. (2014). TRIM5α variations influence transduction efficiency with lentiviral vectors in both human and rhesus CD34(+) cells *in vitro* and *in vivo*. *Mol. Ther.* 22, 348–358. doi: 10.1038/mt.2013.256

Fali, T., Fabre-Mersseman, V., Yamamoto, T., Bayard, C., Papagno, L., Fastenackels, S., et al. (2018). Elderly human hematopoietic progenitor cells express cellular senescence markers and are more susceptible to pyroptosis. *JCI Insight* 3:e95319. doi: 10.1172/jci.insight.95319

Fastenackels, S., Sauce, D., Vigouroux, C., Avettand-Fenoel, V., Bastard, J. P., Fellahi, S., et al. (2019). HIV-mediated immune aging in young adults infected perinatally or during childhood. *AIDS* 33, 1705–1710. doi: 10.1097/QAD.0000000000002275

Fischer, K. D., and Agrawal, D. K. (2013). Hematopoietic stem and progenitor cells in inflammation and allergy. *Front. Immunol.* 4:428. doi: 10.3389/fimmu.2013.00428

Gibellini, D., Vitone, F., Buzzi, M., Schiavone, P., De Crignis, E., Cicola, R., et al. (2007). HIV-1 negatively affects the survival/maturation of cord blood CD34(+) hematopoietic progenitor cells differentiated towards megakaryocytic lineage by HIV-1 gp120/CD4 membrane interaction. *J. Cell. Physiol.* 210, 315–324. doi: 10.1002/jcp.20815

Griffin, D. O., and Goff, S. P. (2015). HIV-1 is restricted prior to integration of viral DNA in primary cord-derived human CD34+ cells. *J. Virol.* 89, 8096–8100. doi: 10.1128/JVI.01044-15

Hariharan, D., Li, Y., Campbell, D. E., Douglas, S. D., Starr, S. E., and Ho, W. (1999). Human immunodeficiency virus infection of human placental cord blood CD34+AC133+ stem cells and their progeny. *AIDS Res. Hum. Retroviruses* 15, 1545–1552. doi: 10.1089/088922299309838

He, Q., Zhang, C., Wang, L., Zhang, P., Ma, D., Lv, J., et al. (2015). Inflammatory signaling regulates hematopoietic stem and progenitor cell emergence in vertebrates. *Blood* 125, 1098–1106. doi: 10.1182/blood-2014-09-601542

Hendrix, C. W., Collier, A. C., Lederman, M. M., Schols, D., Pollard, R. B., Brown, S., et al. (2004). Safety, pharmacokinetics, and antiviral activity of AMD3100, a selective CXCR4 receptor inhibitor, in HIV-1 infection. *J. Acquir. Immune Defic. Syndr.* 37, 1253–1262. doi: 10.1097/01.qai.0000137371.80695.ef

Hozumi, K., Mailhos, C., Negishi, N., Hirano, K., Yahata, T., Ando, K., et al. (2008). Delta-like 4 is indispensable in thymic environment specific for T cell development. *J. Exp. Med.* 205, 2507–2513. doi: 10.1084/jem.20080134

Ikegawa, M., Yuan, J., Matsumoto, K., Herrmann, S., Iwamoto, A., Nakamura, T., et al. (2001). Elevated plasma stromal cell-derived factor 1 protein level in the progression of HIV type 1 infection/AIDS. *AIDS Res. Hum. Retroviruses* 17, 587–595. doi: 10.1089/088922201300119680

Isgro, A., Leti, W., De Santis, W., Marziali, M., Esposito, A., Fimiani, C., et al. (2008). Altered clonogenic capability and stromal cell function characterize bone marrow of HIV-infected subjects with low CD4+ T cell counts despite viral suppression during HAART. *Clin. Infect. Dis.* 46, 1902–1910. doi: 10.1086/588480

Isgro, A., Mezzaroma, I., Aiuti, A., Fantauzzi, A., Pinti, M., Cossarizza, A., et al. (2004). Decreased apoptosis of bone marrow progenitor cells in HIV-1-infected patients during highly active antiretroviral therapy. *AIDS* 18, 1335–1337. doi: 10.1097/00002030-200406180-00013

Jenkins, M., Hanley, M. B., Moreno, M. B., Wieder, E., and Mccune, J. M. (1998). Human immunodeficiency virus-1 infection interrupts thymopoiesis and multilineage hematopoiesis *in vivo*. *Blood* 91, 2672–2678. doi: 10.1182/blood.V91.8.2672.2672_2672_2678

Josefsson, L., Eriksson, S., Sinclair, E., Ho, T., Killian, M., Epling, L., et al. (2012). Hematopoietic precursor cells isolated from patients on long-term suppressive HIV therapy did not contain HIV-1 DNA. *J. Infect. Dis.* 206, 28–34. doi: 10.1093/infdis/jis301

Kandathil, A. J., Sugawara, S., and Balagopal, A. (2016). Are T cells the only HIV-1 reservoir? *Retrovirology* 13:86. doi: 10.1186/s12977-016-0323-4

Karpova, D., and Bonig, H. (2015). Concise review: CXCR4/CXCL12 signaling in immature hematopoiesis–lessons from pharmacological and genetic models. *Stem Cells* 33, 2391–2399. doi: 10.1002/stem.2054

Kaushal, S., La Russa, V. F., Gartner, S., Kessler, S., Perfetto, S., Yu, Z., et al. (1996). Exposure of human CD34+ cells to human immunodeficiency virus type 1 does not influence their expansion and proliferation of hematopoietic progenitors *in vitro*. *Blood* 88, 130–137. doi: 10.1182/blood.V88.1.130.130

Kim, B. G., Kim, Y. H., Stanley, E. L., Garrido-Martin, E. M., Lee, Y. J., and Oh, S. P. (2017). CXCL12-CXCR4 signalling plays an essential role in proper patterning of aortic arch and pulmonary arteries. *Cardiovasc. Res.* 113, 1677–1687. doi: 10.1093/cvr/cvx188

Kim, Y. C., Wu, Q., Chen, J., Xuan, Z., Jung, Y. C., Zhang, M. Q., et al. (2009). The transcriptome of human CD34+ hematopoietic stem-progenitor cells. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8278–8283. doi: 10.1073/pnas.0903390106

Kitade, H., Sawamoto, K., Nagashimada, M., Inoue, H., Yamamoto, Y., Sai, Y., et al. (2012). CCR5 plays a critical role in obesity-induced adipose tissue inflammation and insulin resistance by regulating both macrophage recruitment and M1/M2 status. *Diabetes* 61, 1680–1690. doi: 10.2337/db11-1506

Kitchen, S. G., Shimizu, S., and An, D. S. (2011). Stem cell-based anti-HIV gene therapy. *Virology* 411, 260–272. doi: 10.1016/j.virol.2010.12.039

Knutsen, A. P., Roodman, S. T., Freeman, J. J., Mueller, K. R., and Bouhasin, J. D. (1999). Inhibition of thymopoiesis of CD34+ cell maturation by HIV-1 in an *in vitro* CD34+ cell and thymic epithelial organ culture model. *Stem Cells* 17, 327–338. doi: 10.1002/stem.170327

Koka, P. S., Brooks, D. G., Razai, A., Kitchen, C. M., and Zack, J. A. (2003). HIV type 1 infection alters cytokine mRNA expression in thymus. *AIDS Res. Hum. Retroviruses* 19, 1–12. doi: 10.1089/08892220360473916

Koka, P. S., Fraser, J. K., Bryson, Y., Bristol, G. C., Aldrovandi, G. M., Daar, E. S., et al. (1998). Human immunodeficiency virus inhibits multilineage hematopoiesis *in vivo*. *J. Virol.* 72, 5121–5127. doi: 10.1128/JVI.72.6.5121-5127.1998

Koka, P. S., Jamieson, B. D., Brooks, D. G., and Zack, J. A. (1999). Human immunodeficiency virus type 1-induced hematopoietic inhibition is independent of productive infection of progenitor cells *in vivo*. *J. Virol.* 73, 9089–9097. doi: 10.1128/JVI.73.11.9089-9097.1999

Koka, P. S., Kitchen, C. M., and Reddy, S. T. (2004). Targeting c-Mpl for revival of human immunodeficiency virus type 1-induced hematopoietic inhibition when CD34+ progenitor cells are re-engrafted into a fresh stromal microenvironment *in vivo*. *J. Virol.* 78, 11385–11392. doi: 10.1128/JVI.78.20.11385-11392.2004

Kondo, M., Weissman, I. L., and Akashi, K. (1997). Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 91, 661–672. doi: 10.1016/s0092-8674(00)80453-5

Kuipers, H., Workman, C., Dyer, W., Geczy, A., Sullivan, J., and Oelrichs, R. (1999). An HIV-1-infected individual homozygous for the CCR-5 delta32 allele and the SDF-1 3'A allele. *AIDS* 13, 433–434. doi: 10.1097/00002030-199902250-00025

La Motte-Mohs, R. N., Herer, E., and Zuniga-Pflucker, J. C. (2005). Induction of T-cell development from human cord blood hematopoietic stem cells by Delta-like 1 *in vitro*. *Blood* 105, 1431–1439. doi: 10.1182/blood-2004-04-1293

Laurenti, E., Doulatov, S., Zandi, S., Plumb, I., Chen, J., April, C., et al. (2013). The transcriptional architecture of early human hematopoiesis identifies multilevel control of lymphoid commitment. *Nat. Immunol.* 14, 756–763. doi: 10.1038/ni.2615

Levine, A. M., Scadden, D. T., Zaia, J. A., and Krishnan, A. (2001). Hematologic Aspects of HIV/AIDS. *Hematol. Am. Soc. Hematol. Educ. Program* 2001, 463–478. doi: 10.1182/asheducation-2001.1.463

Li, G., Zhao, J., Cheng, L., Jiang, Q., Kan, S., Qin, E., et al. (2017). HIV-1 infection depletes human CD34+CD38- hematopoietic progenitor cells via pDC-dependent mechanisms. *PLoS Pathog.* 13:e1006505. doi: 10.1371/journal.ppat.1006505

Lim, J. K., Louie, C. Y., Glaser, C., Jean, C., Johnson, B., Johnson, H., et al. (2008). Genetic deficiency of chemokine receptor CCR5 is a strong risk factor for symptomatic West Nile virus infection: a meta-analysis of 4 cohorts in the US epidemic. *J. Infect. Dis.* 197, 262–265. doi: 10.1086/524691

Liu, F., Lu, J., Fan, H. H., Wang, Z. Q., Cui, S. J., Zhang, G. A., et al. (2006). Insights into human CD34+ hematopoietic stem/progenitor cells through a systematically proteomic survey coupled with transcriptome. *Proteomics* 6, 2673–2692. doi: 10.1002/pmic.200500032

Louache, F., Debili, N., Marandin, A., Coulombel, L., and Vainchenker, W. (1994). Expression of CD4 by human hematopoietic progenitors. *Blood* 84, 3344–3355. doi: 10.1182/blood.V84.10.3344.3344

Louache, F., Henri, A., Bettaieb, A., Oksenhendler, E., Raguin, G., Tulliez, M., et al. (1992). Role of human immunodeficiency virus replication in defective *in vitro* growth of hematopoietic progenitors. *Blood* 80, 2991–2999. doi: 10.1182/blood.V80.12.2991.2991

Luis, T. C., Tremblay, C. S., Manz, M. G., North, T. E., King, K. Y., and Challen, G. A. (2016). Inflammatory signals in HSPC development and homeostasis: too much of a good thing? *Exp. Hematol.* 44, 908–912. doi: 10.1016/j.exphem.2016.06.254

Maciejewski, J. P., Weichold, F. F., and Young, N. S. (1994). HIV-1 suppression of hematopoiesis *in vitro* mediated by envelope glycoprotein and TNF-alpha. *J. Immunol.* 153, 4303–4310.

Majka, M., Rozmyslowicz, T., Lee, B., Murphy, S. L., Pietrzkowski, Z., Gaulton, G. N., et al. (1999). Bone marrow CD34(+) cells and megakaryoblasts secrete beta-chemokines that block infection of hematopoietic cells by M-tropic R5 HIV. *J. Clin. Invest.* 104, 1739–1749. doi: 10.1172/JCI7779

Majka, M., Rozmyslowicz, T., Ratajczak, J., Dobrowsky, A., Pietrzkowski, Z., Gaulton, G. N., et al. (2000). The limited infectability by R5 HIV of CD34(+) cells from thymus, cord, and peripheral blood and bone marrow is explained by their ability to produce beta-chemokines. *Exp. Hematol.* 28, 1334–1342. doi: 10.1016/s0301-472x(00)00541-5

Marandin, A., Katz, A., Oksenhendler, E., Tulliez, M., Picard, F., Vainchenker, W., et al. (1996). Loss of primitive hematopoietic progenitors in patients with human immunodeficiency virus infection. *Blood* 88, 4568–4578. doi: 10.1182/blood.V88.12.4568.bloodjournal88124568

Mattapallil, J. J., Douek, D. C., Hill, B., Nishimura, Y., Martin, M., and Roederer, M. (2005). Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. *Nature* 434, 1093–1097. doi: 10.1038/nature03501

Mcgrath, K. E., Koniski, A. D., Maltby, K. M., Mcgann, J. K., and Palis, J. (1999). Embryonic expression and function of the chemokine SDF-1 and its receptor, CXCR4. *Dev. Biol.* 213, 442–456. doi: 10.1006/dbio.1999.9405

Mcnamara, L. A., and Collins, K. L. (2011). Hematopoietic stem/precursor cells as HIV reservoirs. *Curr. Opin. HIV AIDS* 6, 43–48. doi: 10.1097/COH.0b013e32834086b3

Mcnamara, L. A., Ganesh, J. A., and Collins, K. L. (2012). Latent HIV-1 infection occurs in multiple subsets of hematopoietic progenitor cells and is reversed by NF- κB activation. *J. Virol.* 86, 9337–9350. doi: 10.1128/JVI.00895-12

Mencarelli, A., Cipriani, S., Francisci, D., Santucci, L., Baldelli, F., Distrutti, E., et al. (2016). Highly specific blockade of CCR5 inhibits leukocyte trafficking and reduces mucosal inflammation in murine colitis. *Sci. Rep.* 6:30802. doi: 10.1038/srep30802

Mohtashami, M., Shah, D. K., Nakase, H., Kianizad, K., Petrie, H. T., and Zuniga-Pflucker, J. C. (2010). Direct comparison of Dll1- and Dll4-mediated Notch activation levels shows differential lymphomyeloid lineage commitment outcomes. *J. Immunol.* 185, 867–876. doi: 10.4049/jimmunol.1000782

Morini, S., Musumeci, G., Bon, I., Miserocchi, A., Alviano, F., Longo, S., et al. (2016). HIV-1 gp120 impairs the differentiation and survival of cord blood CD34+ HPCs induced to the erythroid lineage. *New Microbiol.* 39, 13–23.

Morrison, S. J., and Scadden, D. T. (2014). The bone marrow niche for haematopoietic stem cells. *Nature* 505, 327–334. doi: 10.1038/nature12984

Moses, A. V., Williams, S., Heneveld, M. L., Strussenberg, J., Rarick, M., Loveless, M., et al. (1996). Human immunodeficiency virus infection of bone marrow endothelium reduces induction of stromal hematopoietic growth factors. *Blood* 87, 919–925. doi: 10.1182/blood.V87.3.919.bloodjournal873919

Muller, F., Tjonnfjord, G. E., Nordoy, I., Kvale, D., Mellbye, O. J., Aukrust, P., et al. (2002). Immunophenotypic analyses of CD34(+) cell subsets in bone marrow from HIV-infected patients during highly- active antiretroviral therapy. *Eur. J. Clin. Invest.* 32, 535–540. doi: 10.1046/j.1365-2362.2002.00995.x

Muntinghe, F. L., Verduijn, M., Zuurman, M. W., Grootendorst, D. C., Carrero, J. J., Qureshi, A. R., et al. (2009). CCR5 deletion protects against inflammation-associated mortality in dialysis patients. *J. Am. Soc. Nephrol.* 20, 1641–1649. doi: 10.1681/ASN.2008040432

Murphy, P. M., and Heusinkveld, L. (2018). Multisystem multitasking by CXCL12 and its receptors CXCR4 and ACKR3. *Cytokine* 109, 2–10. doi: 10.1016/j.cyto.2017.12.022

Nagasawa, T. (2015). CXCL12/SDF-1 and CXCR4. *Front. Immunol.* 6:301. doi: 10.3389/fimmu.2015.00301

Neal, T. F., Holland, H. K., Baum, C. M., Villinger, F., Ansari, A. A., Saral, R., et al. (1995). CD34+ progenitor cells from asymptomatic patients are not a major reservoir for human immunodeficiency virus-1. *Blood* 86, 1749–1756. doi: 10.1182/blood.V86.5.1749.bloodjournal8651749

Nielsen, S. D., Afzelius, P., Dam-Larsen, S., Nielsen, C., Nielsen, J. O., Mathiesen, L., et al. (1998). Effect of granulocyte colony-stimulating factor (G-CSF) in human immunodeficiency virus-infected patients: increase in numbers of naive CD4 cells and CD34 cells makes G-CSF a candidate for use in gene therapy or to support antiretroviral therapy. *J. Infect. Dis.* 177, 1733–1736. doi: 10.1086/517434

Nixon, C. C., Vatakis, D. N., Reichelderfer, S. N., Dixit, D., Kim, S. G., Uittenbogaart, C. H., et al. (2013). HIV-1 infection of hematopoietic progenitor cells *in vivo* in humanized mice. *Blood* 122, 2195–2204. doi: 10.1182/blood-2013-04-496950

Ohishi, K., Varnum-Finney, B., and Bernstein, I. D. (2002). Delta-1 enhances marrow and thymus repopulating ability of human CD34(+)CD38(−) cord blood cells. *J. Clin. Invest.* 110, 1165–1174. doi: 10.1172/JCI16167

Onafuwa-Nuga, A., Mcnamara, L. A., and Collins, K. L. (2010). Towards a cure for HIV: the identification and characterization of HIV reservoirs in optimally treated people. *Cell Res.* 20, 1185–1187. doi: 10.1038/cr.2010.140

Parinitha, S., and Kulkarni, M. (2012). Haematological changes in HIV infection with correlation to CD4 cell count. *Australas. Med. J.* 5, 157–162. doi: 10.4066/AMJ.20121008

Plotkin, J., Prockop, S. E., Lepique, A., and Petrie, H. T. (2003). Critical role for CXCR4 signaling in progenitor localization and T cell differentiation in the postnatal thymus. *J. Immunol.* 171, 4521–4527. doi: 10.4049/jimmunol.171.9.4521

Radtke, F., Wilson, A., Mancini, S. J., and Macdonald, H. R. (2004). Notch regulation of lymphocyte development and function. *Nat. Immunol.* 5, 247–253. doi: 10.1038/ni1045

Rb-Silva, R., Nobrega, C., Azevedo, C., Athayde, E., Canto-Gomes, J., Ferreira, I., et al. (2019). Thymic function as a predictor of immune recovery in chronically HIV-infected patients initiating antiretroviral therapy. *Front. Immunol.* 10:25. doi: 10.3389/fimmu.2019.00025

Res, P., Martinez-Caceres, E., Cristina Jaleco, A., Staal, F., Noteboom, E., Weijer, K., et al. (1996). CD34+CD38dim cells in the human thymus can differentiate into T, natural killer, and dendritic cells but are distinct from pluripotent stem cells. *Blood* 87, 5196–5206. doi: 10.1182/blood.V87.12.5196.bloodjournal87125196

Rieger, M. A., and Schroeder, T. (2012). Hematopoiesis. *Cold Spring Harb. Perspect. Biol.* 4:a008250. doi: 10.1101/cshperspect.a008250

Ruiz, M. E., Cicala, C., Arthos, J., Kinter, A., Catanzaro, A. T., Adelsberger, J., et al. (1998). Peripheral blood-derived CD34+ progenitor cells: CXC chemokine receptor 4 and CC chemokine receptor 5 expression and infection by HIV. *J. Immunol.* 161, 4169–4176.

Santoni De Sio, F., and Naldini, L. (2009). Short-term culture of human CD34+ cells for lentiviral gene transfer. *Methods Mol. Biol.* 506, 59–70. doi: 10.1007/978-1-59745-409-4_5

Sauce, D., Larsen, M., Fastenackels, S., Pauchard, M., Ait-Mohand, H., Schneider, L., et al. (2011). HIV disease progression despite suppression of viral replication is associated with exhaustion of lymphopoiesis. *Blood* 117, 5142–5151. doi: 10.1182/blood-2011-01-331306

Savkovic, B., Nichols, J., Birkett, D., Applegate, T., Ledger, S., Symonds, G., et al. (2014). A quantitative comparison of anti-HIV gene therapy delivered to hematopoietic stem cells versus CD4+ T cells. *PLoS Comput. Biol.* 10:e1003681. doi: 10.1371/journal.pcbi.1003681

Sebastian, N. T., Zaikos, T. D., Terry, V., Taschuk, F., Mcnamara, L. A., Onafuwa-Nuga, A., et al. (2017). CD4 is expressed on a heterogeneous subset of hematopoietic progenitors, which persistently harbor CXCR4 and CCR5-tropic HIV proviral genomes *in vivo*. *PLoS Pathog.* 13:e1006509. doi: 10.1371/journal.ppat.1006509

Stanley, S. K., Kessler, S. W., Justement, J. S., Schnittman, S. M., Greenhouse, J. J., Brown, C. C., et al. (1992). CD34+ bone marrow cells are infected with HIV in a subset of seropositive individuals. *J. Immunol.* 149, 689–697.

Starnes, L. M., Sorrentino, A., Ferracin, M., Negrini, M., Pelosi, E., Nervi, C., et al. (2010). A transcriptome-wide approach reveals the key contribution of NFI-A in promoting erythroid differentiation of human CD34(+) progenitors and CML cells. *Leukemia* 24, 1220–1223. doi: 10.1038/leu.2010.78

Strazza, M., Banerjee, A., Alexaki, A., Passic, S. R., Meucci, O., Pirrone, V., et al. (2014). Effect of mu-opioid agonist DAMGO on surface CXCR4 and HIV-1 replication in TF-1 human bone marrow progenitor cells. *BMC Res. Notes* 7:752. doi: 10.1186/1756-0500-7-752

Su, L., Kaneshima, H., Bonyhadi, M., Salimi, S., Kraft, D., Rabin, L., et al. (1995). HIV-1-induced thymocyte depletion is associated with indirect cytopathicity and infection of progenitor cells *in vivo*. *Immunity* 2, 25–36. doi: 10.1016/1074-7613(95)90076-4

Sundell, I. B., and Koka, P. S. (2006). Thrombocytopenia in HIV infection: impairment of platelet formation and loss correlates with increased c-Mpl and ligand thrombopoietin expression. *Curr. HIV Res.* 4, 107–116. doi: 10.2174/157016206775197646

Takabatake, Y., Sugiyama, T., Kohara, H., Matsusaka, T., Kurihara, H., Koni, P. A., et al. (2009). The CXCL12 (SDF-1)/CXCR4 axis is essential for the development of renal vasculature. *J. Am. Soc. Nephrol.* 20, 1714–1723. doi: 10.1681/ASN.2008060640

Takuva, S., Maskew, M., Brennan, A. T., Long, L., Sanne, I., and Fox, M. P. (2014). Poor CD4 recovery and risk of subsequent progression to AIDS or death despite viral suppression in a South African cohort. *J. Int. AIDS Soc.* 17:18651. doi: 10.7448/IAS.17.1.18651

Thiebot, H., Vaslin, B., Derdouch, S., Bertho, J. M., Mouthon, F., Prost, S., et al. (2005). Impact of bone marrow hematopoiesis failure on T-cell generation during pathogenic simian immunodeficiency virus infection in macaques. *Blood* 105, 2403–2409. doi: 10.1182/blood-2004-01-0025

Tsukamoto, T. (2018). Transcriptional gene silencing limits CXCR4-associated depletion of bone marrow CD34+ cells in HIV-1 infection. *AIDS* 32, 1737–1747. doi: 10.1097/QAD.0000000000001882

Tsukamoto, T. (2019a). Gene therapy approaches to functional cure and protection of hematopoietic potential in HIV infection. *Pharmaceutics* 11:114. doi: 10.3390/pharmaceutics11030114

Tsukamoto, T. (2019b). HIV Impacts CD34+ Progenitors involved in T-Cell differentiation during coculture with mouse stromal OP9-DL1 cells. *Front. Immunol.* 10:81. doi: 10.3389/fimmu.2019.00081

Tsukamoto, T., and Okada, S. (2017). The use of RetroNectin in studies requiring *in vitro* HIV-1 infection of human hematopoietic stem/progenitor cells. *J. Virol. Methods* 248, 234–237. doi: 10.1016/j.jviromet.2017.08.003

Vangelista, L., and Vento, S. (2017). The expanding therapeutic perspective of CCR5 blockade. *Front. Immunol.* 8:1981. doi: 10.3389/fimmu.2017.01981

Von Laer, D., Hufert, F. T., Fenner, T. E., Schwander, S., Dietrich, M., Schmitz, H., et al. (1990). CD34+ hematopoietic progenitor cells are not a major reservoir of the human immunodeficiency virus. *Blood* 76, 1281–1286. doi: 10.1182/blood.V76.7.1281.1281

Weichold, F. F., Zella, D., Barabitskaja, O., Maciejewski, J. P., Dunn, D. E., Sloand, E. M., et al. (1998). Neither human immunodeficiency virus-1 (HIV-1) nor HIV-2 infects most-primitive human hematopoietic stem cells as assessed in long-term bone marrow cultures. *Blood* 91, 907–915. doi: 10.1182/blood.V91.3.907

Weiss, R. A. (1996). HIV receptors and the pathogenesis of AIDS. *Science* 272, 1885–1886. doi: 10.1126/science.272.5270.1885

Winkler, C., Modi, W., Smith, M. W., Nelson, G. W., Wu, X., Carrington, M., et al. (1998). Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. ALIVE study, hemophilia growth and development study (HGDS), multicenter AIDS cohort study (MACS), multicenter hemophilia cohort study (MHCS), San Francisco City cohort (SFCC). *Science* 279, 389–393. doi: 10.1126/science.279.5349.389

Withers-Ward, E. S., Amado, R. G., Koka, P. S., Jamieson, B. D., Kaplan, A. H., Chen, I. S., et al. (1997). Transient renewal of thymopoiesis in HIV-infected human thymic implants following antiviral therapy. *Nat. Med.* 3, 1102–1109. doi: 10.1038/nm1097-1102

World Health Organization (2015). *Guideline on When to Start Antiretroviral Therapy and on Pre-Exposure Prophylaxis for HIV*. Geneva: WHO.

Ye, P., Kirschner, D. E., and Kourtis, A. P. (2004). The thymus during HIV disease: role in pathogenesis and in immune recovery. *Curr. HIV Res.* 2, 177–183. doi: 10.2174/1570162043484898

Zaikos, T. D., Terry, V. H., Sebastian Kettinger, N. T., Lubow, J., Painter, M. M., Virgilio, M. C., et al. (2018). Hematopoietic stem and progenitor cells are a distinct HIV reservoir that contributes to persistent viremia in suppressed patients. *Cell Rep.* 25, 3759–3773.e3759. doi: 10.1016/j.celrep.2018.11.104

Zauli, G., Furlini, G., Vitale, M., Re, M. C., Gibellini, D., Zamai, L., et al. (1994). A subset of human CD34+ hematopoietic progenitors express low levels of CD4, the high-affinity receptor for human immunodeficiency virus-type 1. *Blood* 84, 1896–1905. doi: 10.1182/blood.V84.6.1896.1896

Zhang, J. C., Sun, L., Nie, Q. H., Huang, C. X., Jia, Z. S., Wang, J. P., et al. (2009). Down-regulation of CXCR4 expression by SDF-KDEL in CD34(+)

hematopoietic stem cells: an anti-human immunodeficiency virus strategy. *J. Virol. Methods* 161, 30–37. doi: 10.1016/j.jviromet.2009.05.009

Zhang, M., Evans, S., Yuan, J., Ratner, L., and Koka, P. S. (2010). HIV-1 determinants of thrombocytopenia at the stage of CD34+ progenitor cell differentiation *in vivo* lie in the viral envelope gp120 V3 loop region. *Virology* 401, 131–136. doi: 10.1016/j.virol.2010.03.005

Zou, W., Denton, P. W., Watkins, R. L., Krisko, J. F., Nochi, T., Foster, J. L., et al. (2012). Nef functions in BLT mice to enhance HIV-1 replication and deplete CD4+CD8+ thymocytes. *Retrovirology* 9:44. doi: 10.1186/1742-4690-9-44

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

REVIEW
published: 25 February 2020
doi: 10.3389/fcimb.2020.00061

HIV-1 Tat: Role in Bystander Toxicity

David Ajasin† *and Eliseo A. Eugenin**†

Department of Neuroscience, Cell Biology, and Anatomy, University of Texas Medical Branch, Galveston, TX, United States

HIV Tat protein is a critical protein that plays multiple roles in HIV pathogenesis. While its role as the transactivator of HIV transcription is well-established, other non-viral replication-associated functions have been described in several HIV-comorbidities even in the current antiretroviral therapy (ART) era. HIV Tat protein is produced and released into the extracellular space from cells with active HIV replication or from latently HIV-infected cells into neighboring uninfected cells even in the absence of active HIV replication and viral production due to effective ART. Neighboring uninfected and HIV-infected cells can take up the released Tat resulting in the upregulation of inflammatory genes and activation of pathways that leads to cytotoxicity observed in several comorbidities such as HIV associated neurocognitive disorder (HAND), HIV associated cardiovascular impairment, and accelerated aging. Thus, understanding how Tat modulates host and viral response is important in designing novel therapeutic approaches to target the chronic inflammatory effects of soluble viral proteins in HIV infection.

Keywords: connexin, HIV-1, Tat, HIV-1 latency, cardiomyopathy, neuro-HIV

OPEN ACCESS

Edited by:
Tetsuo Tsukamoto,
Kindai University, Japan

Reviewed by:
Dianne T. Langford,
Temple University, United States
Stanley A. Thayer,
University of Minnesota Twin Cities, United States

****Correspondence:***
Eliseo A. Eugenin
eleugeni@utmb.edu

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *05 December 2019*
Accepted: *06 February 2020*
Published: *25 February 2020*

Citation:
Ajasin D and Eugenin EA (2020) HIV-1 Tat: Role in Bystander Toxicity. Front. Cell. Infect. Microbiol. 10:61. doi: 10.3389/fcimb.2020.00061

INTRODUCTION

In 2017, it was estimated that there are about 38 million people living with Human Immunodeficiency Virus-1 (HIV) worldwide, with about 1.8 million new infections in the same year (UNAIDS). Despite these large numbers, HIV-infection, and associated deaths are decreasing mainly due to the advent of combination antiretrovirals (ARVs). The longer lifespan of HIV-infected individuals on antiretroviral therapy (ART) opened new challenges resulting in chronic diseases that are represented by accelerated aging diseases such as dementia, Alzheimer's/Parkinson like diseases, stroke, and cardiovascular diseases.

HIV is the causative agent of acquired immunodeficiency syndrome (AIDS). Currently, AIDS is only observed in countries with minimal infrastructure for detection, follow-ups, and ART access. AIDS corresponds to a significant decline in $CD4^+$ T cells levels below the critical threshold of 200 cells/mm^3, resulting from immune exhaustion and inability of the immune system to replenish $CD4^+$ T cells faster than the rate of HIV-mediated depletion of the same cells, thereby resulting in opportunistic infections and cancer-related diseases (Lackner et al., 2012). While AIDS is not common in most developed countries due to access to quality health care the same cannot be said for some under-developed countries where access to quality healthcare and therapy is limited.

The development and introduction of combinatory antiretroviral therapy (cART), which is a combination of three to four antiviral drugs targeting multiple stages of the viral replication cycle is critical in inhibiting HIV replication, maintaining $CD4^+$ T cells at near-normal levels, and preventing onset of AIDS which has resulted in longer live-spans of the HIV-infected population (Moutouh et al., 1996). Despite these successes, cART does not cure HIV because the virus, early in the acute phase of the infection, colonizes different tissues, infecting resident cells that have a

longer half-life than circulating immune cells and remain for extended periods in a latent state by becoming silent and preventing immune detection. These silent nature of the circulating and tissue associated viral reservoirs become evident because upon ART interruption, the virus rebounds or re-emerges from tissues. Thus, the new focus of the HIV field is on identifying reagents and strategies to cure HIV-infection by eradicating the viral reservoirs (Deeks et al., 2012; Barre-Sinoussi, 2013; Barre-Sinoussi et al., 2013; Abulwerdi and Le Grice, 2017; Cafaro et al., 2018). In addition, the success of cART and maybe the presence of viral reservoirs has exposed several HIV-associated chronic pathological conditions that were not very apparent before the development of the therapy. Currently, people with HIV live longer with nearly undetectable viral loads (Barouch and Deeks, 2014), however, conditions such neurocognitive and cardiovascular impairments persists in a considerable proportion of the population (Fiala et al., 2004; Coll et al., 2006; Becker et al., 2009; Clifford and Ances, 2013; Anand et al., 2018; Alcaide et al., 2019; Estrada et al., 2019; Eyawo et al., 2019). It is estimated that about 50–60% of people with HIV develop HAND and the incidence of cardiovascular disease is probably higher in the same population. Hence, there is a need for more research to understand these HIV-associated impairments and how to eradicate them. We propose that some of these toxic and chronic effects are mediated by soluble viral proteins, including Tat, that continue to be produced and circulated despite adherence to cART.

HIV-1 REPLICATION CYCLE

HIV is a lentivirus that infects $CD4^+$ cells that expresses both or either of the coreceptors (CCR5 or CXCR4). Infection starts with the virus binding to CD4 receptors on the surface of susceptible cells via the viral gp120 protein (Dalgleish et al., 1984; Maddon et al., 1986). The interaction between the virus gp120 and host protein CD4 induces conformational changes that expose the V3 loop in gp120. The V3 loop binds either CCR5 or CXCR4 and causes the exposure of gp41, particularly, the fusion peptide. This exposed fusion peptide inserts into the plasma membrane of the target cell leading to the tethering of the viral and target membranes. gp41 folding drives the two membranes closer to each other, then the membranes mix and form fusion pore through which the virus core is delivered into the cytoplasm. The delivered viral core undergoes reverse transcription into pro-viral DNA, and translocation to the nucleus occurs (the sequence of this is still debated). The translocated pro-viral DNA is incorporated into the chromosomes, and it is transcribed by the host RNA polymerase II into viral mRNA, which can be un-spliced, singly spliced, or multiply splice. The multiply spliced mRNA is translated into of the accessory and regulatory proteins, the singly spliced is translated into the envelope (env), or gp160 (gp120 and gp41), and the un-spliced mRNA is either translated into Gag or serves as the genomic RNA (Schwartz et al., 1990). All viral components are assembled at the plasma membrane into immature virions. These virions bud from the plasma membrane undergo maturation that allows for the cycle to be repeated.

HIV-1 GENOME STRUCTURE AND GENES

HIV genome is ∼10 kb long, and it is flanked by long terminal repeats (LTRs) on both the 5′ and 3′ ends. The 5′ LTR serves as the promoter region for transcription of viral genes, and it encodes sequences for multiple transcription factors like NFκB, Sp1, NFAT, and others. Also, it encodes the Tat-binding trans-activation response (TAR) elements, which is critical for the transcription of the integrated HIV proviral DNA. Other regions of the genome encode for: Gag polyprotein which is the structural protein of HIV made up of Matrix (MA), Capsid (CA), Nucleocapsid (NC), and p6; Pol polyprotein that codes for the enzymes: Reverse Transcriptase (RT), Integrase (IN), and Protease (Pr); Envelope (Env) for gp120 and gp41; and other accessory and regulatory proteins. Transcription of these HIV mRNAs to make the different viral proteins starts with Tat activity. Hence, Tat is a critical protein for HIV.

HIV TAT PROTEIN

Tat protein is a small protein, 86–102 amino acids (aa), and it is predicted to have about 14–16 kDa molecular weight (Dayton et al., 1986; Bohan et al., 1992; Debaisieux et al., 2012; Clark et al., 2017). Tat is encoded by two exons spliced together, as shown in **Figure 1**. The first exon (1–72 aa) is conserved within most of the HIV subtypes and well-characterized but the second exon (73–102 aa) is not as well-conserved as the first exon, and it contains an RGD binding site (Chiozzini and Toschi, 2016).

Multiple studies (Garcia et al., 1988; Ruben et al., 1989; Albini et al., 1998a; Benelli et al., 1998; Monini et al., 2012) have shown that Tat protein contains multiple domains. The N-terminal domain (1–48 aa) is critical for activating transcription of HIV genomic DNA due to the cysteine-rich motif required for dimerization, protein structure stabilization, metal binding, and the hydrophobic core motif that is known to be critical for its transactivation activity through its binding to transactivation

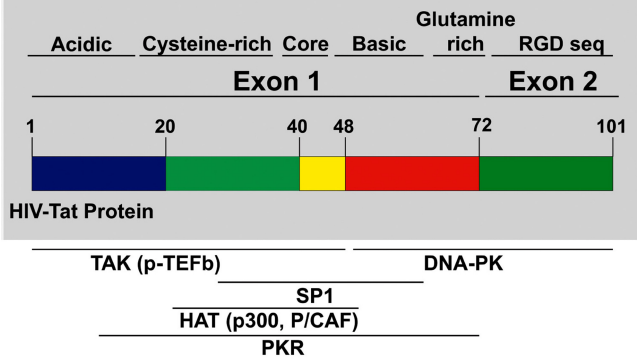


FIGURE 1 | HIV Tat domain structures: tat protein is encoded from exons. Exon 1 encodes for multiple domains like the cysteine-rich, core, the basic domain, and the glutamine-rich domain. Exon 2 encodes for the Arginine rich domain. Most of the p-TEFb complex interactions, as well as other interactions necessary for transcription of HIV provirus, are mediated by domains in Exon 1.

response RNA element (TAR) of the newly transcribed HIV genomic RNA (Chiozzini and Toschi, 2016). The arginine-rich or basic domain is the second domain of Tat (49–58 aa) that is important for localizing Tat to the nucleus, binding of Tat to TAR element, and the internalization of Tat protein into bystander cells by its interaction with surface proteins such as heparan sulfate proteoglycans (Tyagi et al., 2001; Ruiz et al., 2019). The next domain is the glutamine-rich domain, which has been linked to Tat interaction with the TAR element and important for the Tat-apoptosis function (King et al., 2006; Loret, 2015). The second exon of Tat is mostly important for the replication of HIV in both T cells and macrophages. The first motif in the second exon is the arginine-glycine-aspartic acid (RGD) sequence, which is important for Tat interaction with integrins.

Tat is known to be the viral protein that controls HIV-transcription (Dayton et al., 1986). First, transcription starts with short and abortive transcription by RNA Polymerase II (RNA POL II) (Kao et al., 1987). The short transcripts are translocated to the cytoplasm where they are translated into Tat and Rev proteins. Newly translated Tat enters the nucleus to activate RNA POL II driven transcription elongation by binding to P-TEFb (complex made up of CDK-9, Cyclin T1) (Karn and Stoltzfus, 2012; Asamitsu and Okamoto, 2017; Asamitsu et al., 2018). The Tat-P-TEFb complex binds the TAR element on the RNA, which leads to increased processivity of RNA-POL II. Apart from this interaction with P-TEFb, Tat also recruits histone acetyltransferases (HATs) like CNP/p300 complex to the viral promoter to activate the acetylation of nucleosomes to promote transcription of HIV RNA (Nekhai and Jeang, 2006; Vardabasso et al., 2008).

When there is no activation of the above complex, the infected cell becomes latent, as seen in resting $CD4^+$ T lymphocytes and monocytes-macrophages, which are called viral reservoirs (Karn, 2011; Donahue et al., 2012; Kumar and Herbein, 2014; Kamori and Ueno, 2017; Khoury et al., 2018). The establishment of latency or generation of viral reservoirs is mainly due to several cellular processes that restrict access of the transcription machinery to the HIV-1 promoter region (Karn, 2011). Latency also can be established by proviruses that carry transactivation-defective tat sequence (Tat-C22G) that has been shown to prevents effective expression of viral proteins (Wang et al., 1996), which eventually represses Tat expression and/or function. Recent findings have demonstrated that latently HIV-infected cells can be reactivated by adding Tat and one of the proposed cure strategies against preventing reactivation of latently infected cells is to repress the provirus and prevent access of Tat to the promoter region of the provirus by using Tat inhibitors (Easley et al., 2010; Karn, 2011; Mbonye and Karn, 2011, 2017; Siliciano and Greene, 2011; Deeks et al., 2012; Donahue et al., 2012; Desplats et al., 2013; Tabarrini et al., 2016; Kamori and Ueno, 2017; Asamitsu et al., 2018; Khoury et al., 2018). However, our focus for this review will be on how Tat can be released from infected cells and taken up by both uninfected and HIV-infected cells mimicking the transcriptional and cytotoxic effects of the protein. It is important to note that none of the current cART prevents the transcription and/or synthesis of Tat protein.

Tat is secreted from HIV-infected cells and perturbs both HIV-infected and uninfected cells in the surrounding microenvironment (Chang et al., 1997; Debaisieux et al., 2012; Bagashev and Sawaya, 2013; Berks et al., 2014; Clark et al., 2017). Most of the scientific communications described two different actions of this early viral protein, a nuclear and a membrane/cytoplasm associated function. Secretion of Tat into the extracellular space leads to the generation of B cells and T cells antibody responses, as seen in about 20% of HIV-infected patients that are seropositive for Tat antibodies, and studies have shown that seroconversion is associated with low to no progression to AIDS, thereby, indicating the important role Tat plays in HIV-1 replication, infection, and pathogenesis (Re et al., 1995; van Baalen et al., 1997; Allen et al., 2000; Rezza et al., 2005). It also shows that getting rid of extracellular Tat, as is the case with these generated antibodies, might help to slow down the progression of HIV disease. Most of the antibodies against Tat maps to the epitopes in the basic and cysteine-rich regions in the N-terminus (Re et al., 1995; Rezza et al., 2005; Chiozzini et al., 2014).

Tat uptake has been shown to lead to the activation of several transcription factors through phosphorylation or other means (Montano et al., 1997; Karn and Stoltzfus, 2012). Tat-induced activated transcription factors like Sp1, NF-κB, and others have been shown to modulate the expression of both HIV and host genes. Several cellular genes, mostly pro-inflammatory cytokines (like TNF-α, CCL2, IL-2, IL-6, and IL-8), adhesion molecules and sometimes, pro- and anti-apoptotic factors (Albini et al., 1998b; Eugenin et al., 2005; El-Hage et al., 2006a; Lawrence et al., 2006; Youn et al., 2015) is upregulated by these transcription factors via Tat activities. Soluble Tat, in the absence of the virus, has been shown to cause: induction of apoptosis, the release of neurotransmitters, oxidative stress, and inflammation. These mechanisms will be discussed below. Tat modulation of several genes involved in the processes, as mentioned earlier, all contribute to chronic inflammation in people with HIV, and it has been linked to several comorbidities observed in the HIV-infected population, including HIV-associated neurocognitive (HAND) and cardiovascular impairment (Clifford and Ances, 2013; Anand et al., 2018).

Secreted Tat protein has been detected in cerebrospinal fluid (CSF), sera and tissues of HIV-infected people, even in individuals with no detectable viral load (Ensoli et al., 1990; Westendorp et al., 1995; Xiao et al., 2000; Choi et al., 2012). Since most of the HIV-infected individuals are on ART with minimal to undetectable viral replication, still, circulating levels of Tat, in some cases can reach nanomolar concentrations (Ensoli et al., 1990; Westendorp et al., 1995), probably due to the ongoing abortive viral transcription that favor transcription of early genes like Tat (Bachani et al., 2013).

TAT RELEASE FROM HIV-INFECTED OR TRANSFECTED CELLS

Tat accumulates at the plasma membrane of both HIV-infected or Tat-transfected cells, and due to the cytotoxicity of Tat

protein, infected, or transfected cells release most of the cellular Tat as demonstrated in infected or transfected $CD4^+$ T cells (Chen et al., 2002; Rayne et al., 2010). Tat is secreted by an alternate mechanism that is dependent on its ability to bind to phosphatidylinositol 4,5-bisphosphate (PI (4,5) P2) through a tryptophan residue (W11) and negatively charged patches on the inner leaflet of the plasma membrane (Chang et al., 1997). In addition to secretion, Tat is biologically active both extracellularly and intracellularly when taken up, as seen in multiple studies that performed trans-well transactivation of reporter genes under the control of HIV-1 LTR (Fittipaldi et al., 2003; Vendeville et al., 2004; Fittipaldi and Giacca, 2005; Ruiz et al., 2019).

TAT UPTAKE BY BYSTANDER CELLS

The secretion of Tat into the extracellular environment can lead to viral and cellular responses. As mentioned earlier, several groups showed that Tat could act as a viral chemokine attracting monocytes and macrophages into areas of active infection (Albini et al., 1998b; Rao et al., 2013). Furthermore, most cells analyzed including monocytes, macrophages, microglia, $CD4^+$ T lymphocytes, astrocytes, neurons, and cardiomyocytes have been demonstrated to be able to take up Tat protein (Liu et al., 2000; Tyagi et al., 2001; Eugenin et al., 2007; Aksenova et al., 2009; Yao et al., 2010). Their ability to take up Tat has been linked to different surface receptors or proteoglycans such as heparan sulfate proteoglycans (HSPGs), chemokine receptors, integrins, and lipoprotein receptor-related protein-1 (LRP-1) to name a few that can interact with the basic region and the RGD motif on Tat protein. These interactions allow Tat to be endocytosed into bystander cells through two major pathways. First, there is the clathrin-mediated endocytosis pathway, which is dependent on the AP-2/clathrin/dynamin-2 pathway (Vendeville et al., 2004). Vesicles that originate from this canonical pathway depend on acidification for the maturation of the endosomes which can be perturbed by either keeping cells at 4 degrees Celsius or adding ammonium chloride to prevent acidification. Several studies have shown that Tat uptake is significantly inhibited when cells are subjected to either condition, further demonstrating that Tat uptake is dependent on this pathway (Richard et al., 2005; Ruiz et al., 2019). The second pathway is the caveolar pathway, which is an alternative endocytosis pathway for Tat uptake as it was demonstrated for the uptake of Tat-GFP in HeLa as well as CHO cells (Ferrari et al., 2003; Fittipaldi et al., 2003). Immediately Tat is taking up by cells. The protein is mostly seen in the Rab-5 positive late endosome with a pH of <6.0. The release of Tat from the lumen of these vesicles remains unknown but it has been suggested that the same Tryptophan residue (W11) plays a critical role in its release from the lumen of the endosome to the cytosol (Vendeville et al., 2004). The uptake and sometimes, binding of Tat to some surface receptors leads to transcellular signaling, and this has been demonstrated in multiple cell types (Vogel et al., 1993; Rao et al., 2008, 2013, 2014). The ability of Tat to mediate this signaling has been shown to promote pro-inflammation beyond the low level of viral replication that is seen in present-day HIV-infected patients. Thus, further research to understand the role of viral soluble proteins such as Tat is urgent to prevent bystander damage.

TAT EFFECTS ON IMMUNE CELLS, NEURONS, AND ENDOTHELIAL CELLS

As indicated above, Tat has been shown to have chemotactic activities in monocyte-derived dendritic cells (MDDCs), monocytes, and microglia (Lafrenie et al., 1996a,b; Mitola et al., 1997; Benelli et al., 1998; McManus et al., 2000a; Eugenin et al., 2005), but the specific membrane receptors or mechanism that participate in this migration are unknown. It is expected that chemokine receptors participates in this observed Tat-induced chemotaxis. It has been proposed that, because Tat is a highly basic protein, the basic motif may allow it to interact non-specifically with anionic molecules. The limited conformational and structural studies of full length or regions of Tat protein (Bayer et al., 1995) further limit our understanding to discover novel and specific ways to perturb Tat-associated effects with potential cellular targets.

In monocytes, microglia, and MDDCs, Tat induced chemotaxis is blocked by neutralizing antibodies to CCL2 (McManus et al., 2000b; Eugenin et al., 2005), suggesting that Tat-induced chemotaxis is through increased expression and secretion of CCL2. The increased CCL2 mediates chemotaxis of these cells, mostly to the site of infection. CCL2 is, to date, the most potent monocyte chemoattractant and is chemotactic for activated T cells (Yla-Herttuala et al., 1991; Koch et al., 1992; Villiger et al., 1992a,b; Brown et al., 1996), microglia, and macrophages (Cross and Woodroofe, 1999). Experiments indicate that CCL2 is an essential chemokine for monocyte transmigration into the brain parenchyma (Fuentes et al., 1995; Gonzalez et al., 2002) and elevated levels of CCL2 have been detected in the CSF and sera of HIV-1 infected individuals with HAND (Conant et al., 1998; Kelder et al., 1998). However, some of these findings on the rapid release of chemokines like CCL2 may involve rapid release from pre-storage. For instance, the treatment of endothelial cells with histamine resulted in the release of CCL2, eotaxin, GROα, and IL-8 that were stored in small vesicles and Weibel-Palade bodies within 15 min post-treatment (Oynebraten et al., 2004). In another study, the treatment of $CD8^+$ cells with anti-CD3 plus anti-CD28 antibodies or PMA plus ionomycin resulted in rapid RANTES (CCL5) release (Catalfamo et al., 2004). These results suggest that, apart from the Tat-induced upregulation at the transcriptional level of some cytokines, especially, beta-chemokines, there could be an alternative mechanism of Tat-induced fast release of chemokines that induces transmigration. In addition to this spontaneous release of stored chemokines, as earlier stated, Tat has been shown to induce the expression of some beta-chemokines of which CCL2 is one.

Also, Huang et al. showed that the addition of Tat to leukocytes significantly increased the expression of CCR5 and CXCR4 in a dose-dependent manner, thereby suggesting that Tat secretion could be making these cells susceptible

to HIV-infection (Huang et al., 1998). Tat also reprograms immature dendritic cells to express chemo-attractants for activated T cells and macrophages, suggesting a role for Tat in the differentiation of dendritic cells and in the recruitment of activated cells into areas of infection, where HIV replicates and is released (Izmailova et al., 2003). In addition to these Tat effects, some HIV-infected individuals abuse drugs, which results in enhanced activity of Tat on cell migration, inflammation, and neuronal toxicity (Gurwell et al., 2001; Nath et al., 2002; El-Hage et al., 2005). However, the mechanism that mediates these enhanced effects is unknown. Therefore, further experimental approaches are required to investigate the mechanism behind how drug abuse enhances Tat activities or effects on the multiple HIV associated co-morbidities.

In uninfected cells, the effect of Tat has been explored and shown to be multifaceted. Tat has been shown to affect the large protein quality control machinery, which is one of the protein degradation complexes referred to as the proteasome. Two studies have shown that Tat protein alters the activity of the proteasomal complex to induce increased or decreased ubiquitination and targeting some cellular proteins for degradation while reducing degradation of other cellular and viral proteins. These studies also suggested that there is a direct interaction between Tat and the proteasomal complex which can lead to inhibition of proteasomal activity (Huang et al., 2002; Apcher et al., 2003). These experiments ultimately show that Tat can directly alter the degradation of cellular proteins and enhance the accumulation of viral and perhaps, other cellular proteins that favors the formation of new virions in HIV-infected cells.

Tat has also been shown to have effects on endothelial cells (ECs), leading to multiple defects through activation of VEGF/KDR receptors, chemokine receptors, HSPGs, and integrins. Recently, it was demonstrated that Tat binds to $\alpha_v\beta_3$ integrins inducing FAK phosphorylation that triggers the activation of different intracellular messengers (Urbinati et al., 2005). This FAK activation has been associated with migration, enhanced permeability (Avraham et al., 2004), and may explain the alterations in tight junction proteins such as Connexins, Claudin-5, and ZO-1 observed after Tat treatment (Andras et al., 2005; Pu et al., 2005). Tat treatment of endothelial cells can alter the formation of actin filaments, tight junctions, and adhesion molecules, such as ZO-1, JAM-A, PECAM-1, and CD99 (Williams et al., 2013). Results obtained by confocal microscopy demonstrated that, Tat treatment for 12 h induced an aberrant, non-linear, actin filaments, and PECAM-1 is redistributed into the surface of the endothelial cells compared to the untreated cells (**Figure 2**). These changes, in addition to ECs migration, also can be associated with the pro-invasive activities of Tat. Experiments in transgenic mice or metastatic cells expressing Tat showed an enhanced release of Matrix Metalloproteinases (MMPs) that are associated with leukocytes, monocytes, or metastatic cells infiltration, suggesting that Tat in concert with other factors, enhance cell migration (Albini et al., 1994; Zocchi et al., 1997; Prakash et al., 2000). Another almost unexplored form of migration is the neuronal or stem cell migration. This form of migration is important during the regeneration of tissue and synaptic plasticity. They are both critical in NeuroHIV and

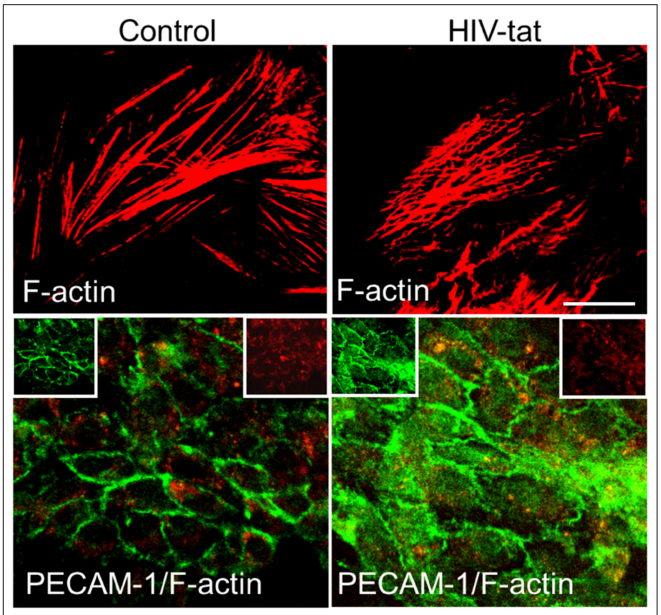


FIGURE 2 | Tat alters actin cytoskeleton and PECAM expression. Cells were treated as empty media and HIV-1 Tat containing media. In control cells, actin cytoskeleton (Cy3-red) remains intact, and PECAM expression (green) is as expected mainly localized to the plasma membrane. In Tat treated samples, actin cytoskeleton is disrupted, and PECAM expression and distribution changes to be in the cytoplasm and at the plasma membrane.

are perturbed by Tat protein. First, Tat has been described to: alter nerve growth factor (NGF) signaling pathway, decrease expression of p35, a neuron-specific activator of CDK5, a cyclin that phosphorylates several neuronal proteins involved in cell survival, migration and differentiation (Peruzzi et al., 2002). Specifically, p35, CDK5, and other cyclins have been described to be essential in normal neuronal migration because mice deficient in cdk5 expression have been shown to have perturbed neuronal migration pattern (Ohshima et al., 1996; Gilmore et al., 1998; Gilmore and Herrup, 2001). Also, *in vivo* experiments have shown alterations in FGF-1 and BDNF expression in neurons that survived in the brain of patients that suffered HIV-encephalitis (HIVE) (Soontornniyomkij et al., 1998; Everall et al., 2001). Embarking on studies to understand the potential contribution of decreased neuronal differentiation or migration to the areas compromised with HIV will be an excellent addition to our knowledge on how HIV causes neuro-cognitive decline.

MECHANISM OF NEUROTOXICITY

HIV enters the CNS soon after infection via transmigration of HIV-infected leukocytes and monocytes into the brain (Joseph et al., 2015). Even with cART (Kranick and Nath, 2012), HIV-1 remains in the brain. Patients on cART with no detectable viral load and high $CD4^+$ T cell counts still suffer from HAND. It is currently estimated that about 50% of HIV-1 infected individuals have HAND irrespective of their cART status (Rojas-Celis et al., 2019), suggesting a mechanism CNS specific (Kusdra et al., 2002). These HIV-infected cells release Tat that can induce neurotoxicity

by two mechanisms; act directly on neurons or act through other cell types like macrophages/microglia and astrocytes, triggering inflammation. The findings that Tat-subtype B, which is the predominant subtype in the USA, but not subtype C (predominant in India), results in significantly higher levels of HIV dementia, 15–30% in the USA compared to 1–2% in India both in pre-therapy era, suggest that neurotoxic potential of Tat protein from different subtypes may vary (Antinori et al., 2007; Heaton et al., 2010, 2011). This finding has been explored by multiple groups, and there have been several studies that have validated this suggestion. Neurotoxic potentials of the Tat protein have been shown by some studies to be associated with some specific molecular signatures that can influence different activities of Tat protein. For instance, Tat plays a key role in the recruitment of monocytes/macrophage cells into the brain and has direct association with HAND (Abraham et al., 2003; Li et al., 2008; Constantino et al., 2011; Bagashev and Sawaya, 2013; Burdo et al., 2013; Chompre et al., 2013; Fields et al., 2015a,b; Niu et al., 2015). It has been shown that Tat with the critical CC motif; mimics chemokines, binds to chemokine receptors, and can recruit monocytes and macrophages to the CNS while Tat without the intact CC motif does not bind to chemokine receptors and it is unable to recruit these cells (Ranga et al., 2004; Mishra et al., 2008). Another molecular signature that has been identified and linked to Tat neurotoxicity is the R57 residue in the basic domain of Tat (Eguchi et al., 2001; Hashida et al., 2004; Ruiz et al., 2019). The basic domain, which is in the same as the cell-penetrating peptide (CPP), mediates cellular uptake of Tat, and the loss of one basic amino acid has been shown by several groups to perturb cellular uptake. A recent study has shown, there is a natural polymorphism seen in Tat mostly in HIV-1 subtype C which significantly reduces cellular uptake of Tat with S57. It was equally shown that less cellular uptake of Tat-S57, translated to less induction of pro-inflammation and less neurotoxicity (Ruiz et al., 2019). On the other side, some studies suggest that Tat protein from subtype C is a better transactivator when compared to subtype B (Kurosu et al., 2002; Johri et al., 2015). These findings show that polymorphisms in Tat can have significant effects on the neurotoxic potential as well as transactivating activity of the protein.

We and others have described the neurotoxic effects of Tat, in part by activation of diverse neuronal players, such as receptors and/or channels like: the N-methyl-D-aspartate (NMDA), non-NMDA receptors, sodium channels, and intracellular pathways (caspase-3) (Eugenin et al., 2007; Li et al., 2008; Aksenova et al., 2009; Hu, 2016). However, conflicting results have been reported, in part due to differences in the culture system, species analyzed, section of the brain examined, and methods to determine the neurotoxicity (Chiozzini and Toschi, 2016). Published data from our laboratory, obtained in human primary neurons indicates that Tat-induced apoptosis is a process that is dependent on glutamate receptor, NMDA receptor, the low-density lipoprotein receptor-related protein-1 (LRP), and nNOS (Eugenin et al., 2003). The intracellular protein that organizes these players is PSD-95. As shown in **Figure 3**, PSD-95 can interact with different membrane and intracellular proteins, such as LRP, NMDA receptors, nNOS, adhesion molecules, guanylate kinase, and diverse tyrosine kinases, including Fyn, and Pyk2. A critical example of the importance of these interactions in pathological conditions and the potential crossroad with Alzheimer's and Parkinson's diseases is the strong correlation of ApoE4 alleles with dementia and survival in the three different diseases (HAND, Alzheimer, and Parkinson). Currently, in the ART era, HAND has some pathological features that are like Alzheimer's and Parkinson's diseases. For example, ApoE4 is a ligand for the lipoprotein receptor-related protein (LRP), and ApoE4 polymorphisms are associated with poor prognoses in numerous neurodegenerative diseases, including Alzheimer's disease, stroke, hemorrhage, trauma, as well as HIV dementia/neuropathology. However, in HIV-neuropathogenesis, the viral protein Tat has been shown to bind to LRP (Liu et al., 2000), the same receptor that binds ApoE, and to induce similar neurotoxicity as ApoE (Eugenin et al., 2003, 2007). Tat and ApoE may have similar or overlapping signaling pathways, and thus Tat needs to be considered as important in the pathogenesis of dementia in HAND. This Tat-mediated neurotoxicity through LRP-1 signaling pathways involve activation of LRP-1, the receptor for both ApoE and Tat on neurons, NMDAR, a synaptic scaffold protein, that leads to the production of glutamate, as well as nitric oxide (NO) (Eugenin et al., 2007) (see **Figures 3**, **4**). Furthermore, Tat through these mechanisms can amplify inflammation and neurotoxicity at long range due to the diffusion of NO into vast areas of the brain. We propose that these amplification methods can partly explain some of the vast synaptic compromise observed in the majority of the 50% of the HIV-infected individuals with HAND despite effective ART.

The neurotoxicity of Tat also involves apoptosis. Tat-induced apoptosis is a bi-phasic process. First, a process dependent on LRP, PSD-95, and NMDA receptors to mediated Tat-internalization and then the second process to trigger and amplify

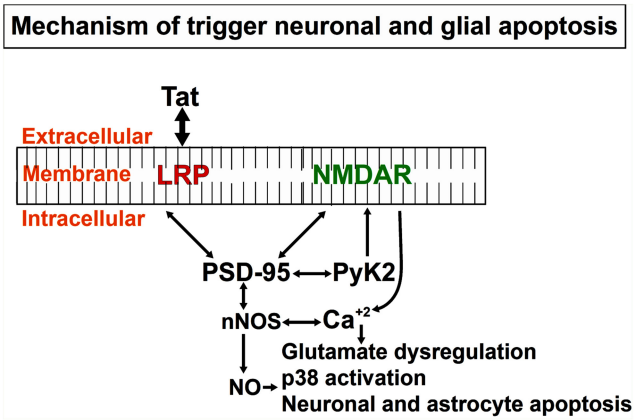


FIGURE 3 | Mechanistic depiction of Tat induced neuronal and astrocytic apoptosis: Tat protein taken up by bystander cells induces the formation of nNOS that leads to the formation of Nitric Oxide (NO), significant release of calcium from intracellular calcium stores that triggers other downstream mediators of apoptosis in both neurons and astrocytes. Apoptosis of both cell types (neurons and astrocytes), as well as others like axonal/dendritic shrinkage, causes a neurocognitive decline in HIV-infected individuals.

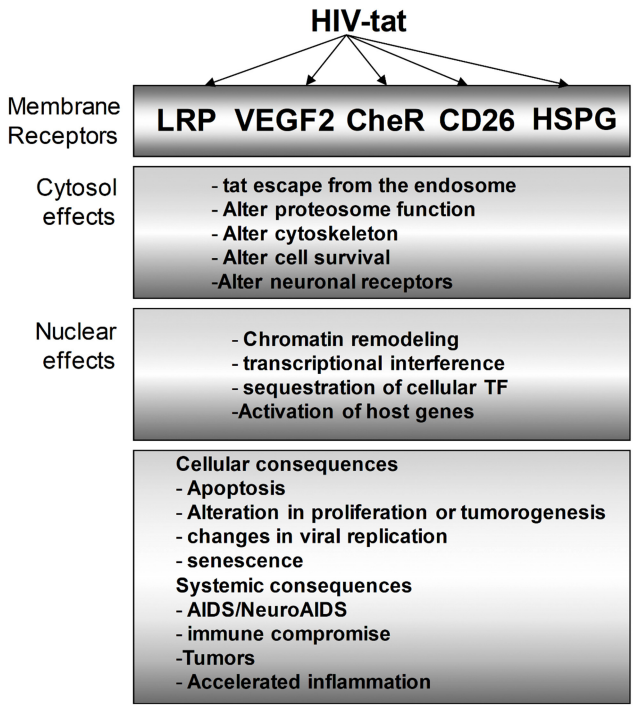


FIGURE 4 | Multiple intracellular mediators of the Tat effect on bystander cells. Tat protein modulates HIV-infected cells as we as uninfected bystander cells. In the cytosol, the effect of Tat ranges from altering the proteasomal degradation pathway to favor viral and host proteins that support viral replication, cell survival, and other identified phenotypes. Similarly, Tat mediates multiple changes in the nucleus to effect transcriptional changes that can vary cellular effects such as apoptosis, inflammation, and many more.

Tat-toxicity from neurons to astrocytes by a mechanism that involves at least glutamate dysregulation, NO generation and diffusion, as well as calcium overload (**Figure 3**). Typically, primary neuronal culture has 35 % of NMDA positive neurons and when these cultures are treated with Tat, it results in 80% of apoptosis at 24 h in the neurons and 20 % in the astrocytes suggesting that Tat first target NMDA receptor-positive neurons and then spread apoptotic signals to astrocytes. The use of NMDA receptor blockers reduced apoptosis in both cell types (Eugenin et al., 2003).

Some reports have suggested that Tat can interact directly with NMDA receptors on the Zn^{+2} binding site (Prendergast et al., 2002; Self et al., 2004; Chandra et al., 2005). Another study has shown that Tat can interact with NMDA receptors through the intact CC-motif in its basic domain, which leads to increased calcium release and retraction of synapses during excessive firing (Li et al., 2008). However, there are no conclusive experiments that have shown Tat binding directly to NMDA receptors, especially to the Zn^{+2} sensitive sites. Also, it is known that Tat can induce the activation of inositol 1,4,5-triphosphate (IP_3), which mobilizes intracellular release of calcium from the endoplasmic reticulum (ER) stores (Borgatti et al., 1998; Haughey et al., 1999, 2001; Mayne et al., 2000; Feligioni et al., 2003; Fotheringham et al., 2004). This excessive calcium release contributes to neurotoxicity. Another mechanism of Tat-induced neurotoxicity is the interaction of Tat and LRP-1, thereby causing the internalization of LRP-1 and decreased uptake of LRP-1 ligands, in this case, amyloid-β peptide and Apolipoprotein E. Data from our laboratory and others have shown that specific LRP blockers like receptor-associated protein (RAP) that is an endoplasmic chaperone for LRP-1 and tightly binds the NMDA receptor when applied extracellularly, block the binding, and prevents the uptake of all known LRP ligands (Prasad et al., 2015), including Tat (Herz and Strickland, 2001; Strickland et al., 2017), suggesting, almost all the initial toxic effects of Tat are mediated by LRP, but we cannot discard a cross-activation or sensitization of NMDA receptors as an additional mechanism (see **Figure 4**).

A report established a connection between the dementias induced by HIV-infection and Alzheimer's with regards to neprilysin (NEP). NEP is the major enzyme that degraded Aβ in the brain. NEP is a membrane surface zinc metalloendopeptidase, also known as CD10, that cleave bradykinin, substance P, and insulin. Tat has been shown to inhibit NEP (Turner et al., 2001). This inhibition results in increased Aβ levels and its accumulation. Besides, it was demonstrated that individuals with long term HIV-infection had increased Aβ. Also, LRP has been associated with controlling extracellular activities for MMPs (Emonard et al., 2005). Both LRP and NEP are considered risk factors in Alzheimer's disease and may equally have connections with the mechanism of HAND.

We think that Tat enhances or induces the formation of apoptosis promoting complex in the surface of neurons made up of Tat, LRP, PSD-95, NMDAR, nNOS, and Pyk2. While there is no Tat in AD, we speculate that a similar complex might be formed in AD and associated with either the beginning or progression to AD. ApoE4 is another factor that induces significant low levels of apoptosis in neurons and astrocytes compared to control cells but is not comparable to the apoptosis induced by Tat. The association of ApoE4 polymorphisms with worse prognoses in numerous neurodegenerative diseases, such as AD, stroke, hemorrhage and HIV dementia, and neuropathy (Strittmatter et al., 1993; Roses et al., 1995; Slooter et al., 1997; Corder et al., 1998), suggest a key role of LRP and their ligands in association with dementia. Intracerebroventricular injection reduces LTP by an NMDA dependent mechanism, suggesting an alteration in neurons. Tat has also been shown to have effects on lipid peroxidation, which are significantly reduced while the generation of reactive oxygen species (ROS) is increased in neurons and synaptosomes from APOE4 KO mouse, suggesting there could be a mechanism that modulates the levels of these two factors. A study demonstrated that Tat and APP colocalize in the brains of SIV-infected macaques suggesting again that may these two diseases share toxic pathways to generate dementia.

However, why is Tat different from other LRP ligands that trigger massive apoptosis? One of the key differences that can be observed from the calcium imaging recording experiment shows, Tat induces general dysregulation of intracellular calcium (Haughey et al., 1999) compared to ApoE4 treatment, but not α_2M or lactoferrin, which results in modest neurotoxicity (Qiu et al., 2003). Also, in contrast to other LRP ligands, Tat works differently, because it induces the recruitment of the membrane

protein-like NMDA receptor, PSD-95, nNOS, and Pyk2 to form a membrane complex that amplifies apoptosis, not observed with other LRP ligands (**Figure 3**). Another difference is that, Tat after it internalization can escape from endosomes and localize to the nucleus where it can alter transcription of cellular genes suggesting that Tat, after internalization can alter the synthesis of key proteins such as transcription of synaptic (Eugenin et al., 2003) or survival proteins that finally trigger massive apoptosis. However, we think one of the key processes of Tat-induced neurotoxicity is the interaction of Tat with NMDA, glutamatergic neurons because early blocking of these channels or blocking the formation of the complex (especially nNOS activation) results in a reduction of Tat toxicity. All these possibilities are under current investigation in our laboratory. We speculate that under normal conditions, this complex exists, and the addition of or exposure to Tat only enhances the complex by increasing the recruitment of more factors involved in the formation of this complex at the membrane.

Published studies from our laboratory show that Tat treatment leads to an elevation in intracellular calcium of human fetal neurons and we observed that this Tat-induced calcium elevation is biphasic in both neurons and astrocytes (King et al., 2006). Also, this calcium elevation is characterized as a single, sustained, and oscillatory spike. We think the cascade of activities that leads to the calcium elevation is; first calcium from IP_3-dependent release and a subsequent sustained glutamate receptor-mediated mechanism (Haughey et al., 1999, 2001). But it is still unclear in human cells, if glutamate activity can alter IP_3-activity like the report in chicken neurons which demonstrated that glutamate could modulate IP_3-mediated calcium release. In the same direction, other published works from our laboratory have shown that early NMDA glutamate receptor activation is not enough to induce neuronal apoptosis because the addition of MK801 after 3 h of Tat treatment still blocks apoptosis, suggesting that early calcium spike is not related to apoptosis. So far, no study has established or demonstrated the link between IP_3, NMDA receptor, astrocytes, and apoptosis, as well as gap junctions. However, gap junctions are permeable to IP_3 and calcium. Also, gap junctions control glutamate metabolism and are a critical communication system between neurons-neurons and neurons-astrocytes (Eugenin et al., 2007). We speculate that the co-application of Tat and gap junction blockers will reduce Tat-induced apoptosis. This will suggest that gap junctions are actively involved in transmitting secondary messenger(s) that may mediate bystander killing to other cells (that are not exposed to Tat but receive these secondary messengers. While we do not know this secondary messenger, glutamate could be a good candidate because it is known to mediate neurotoxicity. Identifying this secondary messenger will be a significant finding that may potentially open the avenue for tackling HAND.

Tat has also been shown to affect the expression of some miRNAs in neurons. For instance, Tat upregulates the expression of miR-34a, a miRNA that targets CREB (Zhang et al., 2012; Zhan et al., 2016). The upregulation of miR-34a leads to the promotion of neuronal dysfunction (Hu et al., 2017; Periyasamy et al., 2019). It also has been proposed that Tat can mediate indirect neuronal toxicity by inducing microglia/macrophagic and/or astrocytes to release toxic factors such as cytokines, chemokines and others (Eugenin et al., 2005; El-Hage et al., 2006a,b; Bagashev and Sawaya, 2013; Joseph et al., 2015; Hu et al., 2017; Periyasamy et al., 2019). The indirect neurotoxic effect of Tat is based on its ability to be taken up by uninfected bystander cells. One's Tat protein is endocytosed, it is released from the endosome as the endosome undergoes maturation. After the release of Tat from the lumen of endosomes to the cytosol, Tat interacts with multiple intracellular factors, which lead to modulation and changes in cellular response. Many of the interacting cellular factors have been extensively discussed in several studies and reviews. Tat is also known to cause transcriptional changes that lead to either downregulation or up-regulation of target genes.

For example, Tat interacts with the NF-κB inhibitor IκB-α, that leads to the release of NF-κB and its translocation into the nucleus. Translocation of NF-κB leads to the transcription of several genes (Fittipaldi and Giacca, 2005; Easley et al., 2010; Zhang et al., 2012). Some of these target genes have significant effects on HIV-1 pathogenesis. Specifically, Tat has been shown to upregulate transcription of genes like CXCR4, CCR5 in PBMCs, which are critical for spreading HIV-infection (Huang et al., 1998; Zheng et al., 2005). Also, some pro-inflammatory cytokine genes like TNF-α, CCL2, and anti-inflammatory genes like IL-4 and IL-10 in MPs. Tat has also been linked with inducing anti-proliferation factors from macrophages, which can suppress the activation of naïve $CD4^+$ T cells and downregulate anti-apoptotic proteins. This ability of Tat to induce both pro- and anti-inflammatory factors is one of the reasons for some controversies associated with the role of Tat in HIV- pathogenesis (Gandhi et al., 2009).

However, there are no controversies in the role of Tat in HAND. The direct and indirect neurotoxic effect of Tat has been well-established, as seen in the significant reduction in cell viability. In the CNS, the indirect effect of Tat has been characterized by several studies that revealed secreted Tat from HIV-infected cells are taken up by uninfected bystander cells like microglia and astrocytes. For instance, in microglia and astrocytes, uptake of Tat leads to the upregulation and/or release of several neurotoxic factors. The released neurotoxic factors damage the neuron in addition to the direct damages. The secretion of neurotoxic factors mostly in the form of cytokines like TNF-α, IL-6, IL-8, IL-1β, and CXCL1, among others (Ruiz et al., 2019). That is not to say, cytokines are the only neurotoxic factors that mediate Tat indirect neurotoxic effects.

Only recently, we identify that a key mechanism of bystander apoptosis and a neuronal and endothelial compromise was mediated by gap junction channels. Usually, gap junction channels are shutdown under inflammatory conditions (Eugenin et al., 2007; Berman et al., 2016). We found that latently HIV-infected astrocytes maintain connexin43 expression despite the inflammatory phenotype (Berman et al., 2016). Connexin43 (Cx43) formed functional Cx43 containing gap junction channels that enable small molecules generated in latently HIV-infected astrocytes to diffuse into neighboring uninfected cells. We identify that some of these toxic molecules corresponded to calcium, IP_3, and cytochrome C related signals; however, the mechanism of dysregulation and diffusion of these molecules is

unknown. To identify the viral component(s) involved in the Cx43 maintenance, we tested all HIV proteins in uninfected cultures of human astrocytes. Only Tat increased expression of Cx43 and increased cell-to-cell communication enabling the toxic signals generated in the few latently infected astrocytes to diffuse longer distances as compared to uninfected cultures. Also, we observed that Tat binds to the Cx43 promoter and keep its mRNA expression high. This finding is unique and explains the large areas compromised by HIV reservoirs even in the current ART era. In conclusion, the combination of direct and indirect effects of Tat leads to neurobehavioral deficits, which are one of the hallmarks of HAND (Prevedel et al., 2017).

MECHANISM OF TAT-MEDIATED CARDIOVASCULAR DISEASE

Since HIV-infection is now a chronic disease, several comorbidities like atherosclerosis and cardiovascular disease all related to aging have become common within the population of people with HIV (Gebo et al., 2010; Clifford and Ances, 2013; Fields et al., 2013; Buggey et al., 2019; Ciccarelli et al., 2019). It is known that HIV-infected individuals have 1.5–2 times higher risk of cardiovascular disease (CVD) due to several factors like chronic inflammation, dyslipidemia, lipid abnormalities like low high-density lipoproteins (HDL), increased triglycerides, and prolonged use of ART (Wang et al., 2015; Zungsontiporn et al., 2016; Prevedel et al., 2017; Ciccarelli et al., 2019; Estrada et al., 2019; Heravi et al., 2019; Palma Reis, 2019). HIV-associated CVD is characterized by extensive loss of cardiomyocytes, increase in fibrous tissues and significant infiltration of immune cells to the heart (Prevedel et al., 2017). Most of the studies that have examined the effect of HIV-infection on CVD have shown that there is an increased rate of coronary events and another cardiomyopathy in HIV-infected individuals (Thompson-Paul et al., 2019). The only debate that is still lingering is, does ART contributes to CVD or not and if it does, to what extent does it contribute. A study of a multicenter AIDS cohort study (MACS) showed that there was a significant reduction in LDL and HDL from HIV-infected individuals. Commencement of ART in these individuals led to a significant increase in total cholesterol and LDL to pre-infection levels while HDL remained low (Heravi et al., 2019). Interestingly, the strategies for management of antiretroviral therapy (SMART) study that examined the effect of continuous HIV-replication on CVD events showed that continuous HIV replication in individuals that discontinued therapy increased the risk of CVD compared to individuals that adhered to their therapy, clearly showing the effect of HIV replication on CVD (Siwamogsatham et al., 2019).

In a recent study, HIV-infected individuals with detectable viral loads and low CD4 T cell counts were found to have an increased risk of heart failure, increased risk of cardiovascular death when compared to HIV-infected patients with high $CD4^+$ T cell counts and undetectable viral loads (Siwamogsatham et al., 2019). This confirms that HIV, on its own, is a risk factor for CVD either with or without ART.

Cardiomyocytes are the most abundant cells in the heart with some fibroblasts. These cells are not infected by HIV, but they can be exposed to both the full virus or specific viral proteins that are shed by HIV-infected cells. Similarly, as is the case with NeuroHIV, uninfected bystander cells that are exposed to HIV or shed viral proteins like Tat and gp120 can secrete pro-inflammatory factors which can be toxic to cardiomyocytes and the heart (Coll et al., 2006; Wang et al., 2015; Zungsontiporn et al., 2016; Anand et al., 2018; Jiang et al., 2018). Also, the vascular tissue, which is important for the cardiovascular system, is made up of endothelial cells that are susceptible to cytotoxic factors induced by HIV-infections or viral proteins. Also, infiltration of the heart by macrophages and lymphocytes in response to the presence of HIV or HIV protein can contribute further to the assault of cardiomyocytes (Zungsontiporn et al., 2016). Therefore, the combination of HIV-replication, shed viral proteins, infiltration by infected and uninfected macrophages-lymphocyte, pro-inflammatory, and cytotoxic factors shed by bystander cells as well as the lipid dysregulation combines to significantly cause systemic dysregulation that leads to cardiomyopathies.

Several studies have shown that gp120 and Tat, two shed viral proteins that are seen in patients, can cause activation of some pathways that lead to apoptosis of cardiomyocytes and endothelial cells. Inhibition of these pathways, for instance, the MAPK/ERK kinase pathway (MEK inhibitors), prevents apoptosis of cardiomyocytes, and endothelial cells (Lee et al., 2011; Cipolletta et al., 2015). The role of Tat, specifically in HIV-associated CVD, is becoming more appreciated as multiple studies have shown that Tat causes transcriptional changes in different cell types that make up the heart, and some of these transcriptional changes are similar to what has been observed in some non-HIV-associated CVDs. Cardiomyocytes, as the predominant cell type of the heart is the cell type that contracts and relaxes in response to action potentials that must be synchronized effectively from the first cell to the last. The speed of contraction and relaxation requires more effective cell-to-cell communication than diffusion. Therefore, cardiomyocytes have robust cell-to-cell connections in the form of gap junctions that allows for the efficient spread of signals from the initial cardiomyocyte to the last. These gap junctions are at the intercalated discs in between cardiomyocytes, and they are made up of several proteins that allow the formation of these junctions (Zhao et al., 2019). While some of these proteins are anchoring proteins at the plasma membranes, others make intercellular contacts with neighboring cells. One protein family known to be involved in intercellular contacts is the connexins and Cx43, particularly localized mainly at the intercalated discs (Prevedel et al., 2017; Macquart et al., 2018; Schultz et al., 2019). We have shown in a recent publication that Tat induces the upregulation of Cx43 mRNA and proteins in cardiomyocytes and increases in levels of lipofuscin, a known aging heart biomarker, and both observations are seen in non-HIV CVDs as well (Prevedel et al., 2017).

Interestingly, in human hearts, it was also observed that there was lateralization of the increased Cx43 protein expressed in response to Tat exposure, suggesting that Cx43 that under

normal condition is localized at the intercalated discs is either targeted to another part of cardiomyocytes or the machinery that targets Cx43 to the intercalated discs has been perturbed. The consequence of mis-localizing Cx43 away from the intercalated discs will be, impairment of cell to cell communication, which is critical for synchronization of contraction and relaxation of the heart. Furthermore, Tat-induced apoptosis of cardiomyocytes led to the formation of plaques of fibrous tissues, accumulation of mitochondria, which suggest the cardiomyocyte apoptosis is through a mitochondria-controlled pathway (see **Figure 4**).

We and others identified a significant increase in the levels of secreted ATP by a hemichannel dependent mechanism (Seror et al., 2011). High circulating levels of ATP correlates with cognitive impairment and endothelial damage (Seror et al., 2011). Furthermore, the exposure of cardiomyocytes to increasing concentration of ATP *in vitro* caused a significant increase in action potentials, shortening of the time between each contraction and calcium flux in the cells. These findings show that HIV-infection and viral protein exposure lead to multiple damages to the heart and CVD (Prevedel et al., 2017; Tahrir et al., 2018).

The endothelium, made up of endothelial cells, is another part of the cardiovascular system that is known to not support active replication of HIV but, it is exposed to both viral proteins and several inflammatory factors released by both infected cells and bystander cells. The effect of such exposure ranges from increased adhesiveness, permeability, apoptosis, oxidative stress, and more cytokine secretion which can lead to recruitment of lymphocytes, monocytes, and macrophages and the initiation of atherosclerosis (Wang et al., 2015; Anand et al., 2018; Chen and Dugas, 2019). Our group determines that HIV can infect cells of the vascular wall such as smooth muscle cells that contribute to the accelerated atherosclerosis observed in the HIV-infected population (Eugenin et al., 2008). In these samples obtained from HIV-infected individuals, a concentric proliferation of smooth muscle cells was observed, a unique characteristic of the plaque that is not observed in uninfected individuals. Thus, we propose that this mechanism of accelerated atherosclerosis also contributes to heart dysfunction in the HIV-infected population.

CONCLUSION AND FUTURE PERSPECTIVES

This review points to the multiple roles of Tat in HIV-replication and HIV-associated comorbidities with specific emphasis on replication, NeuroHIV, and HIV-associated CVD. Various studies discussed in this review have shown that secreted Tat contributes to all if not most of these comorbidities through complex intracellular interactions that lead to chronic immune activation and inflammation that assaults both the CNS and the cardiovascular system. The HIV-infected population is aging faster than their uninfected age-matched pairs due to the assault of all systems by HIV and viral proteins like Tat in multiple ways as shown in **Figure 4**. More work is needed in understanding how HIV and viral proteins, especially Tat, affects all systems particularly, CNS and cardiovascular system. With increasing longevity of HIV-infected individuals, there is a need to find therapies that will allow them to live normal or near-normal lives with little to no HIV-associated pathologies. The need to identify useful biomarkers for quick diagnosis and drugs for better therapy cannot be overstated to treat and reverse this population that is aging faster than age-matched pairs.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was funded by the National Institute of Mental Health grant, MH096625. The National Institute of Neurological Disorders and Stroke, NS105584, and UTMB funding (to EE).

REFERENCES

Abraham, S., Sawaya, B. E., Safak, M., Batuman, O., Khalili, K., and Amini, S. (2003). Regulation of MCP-1 gene transcription by Smads and HIV-1 Tat in human glial cells. *Virology* 309, 196–202. doi: 10.1016/S0042-6822(03)00112-0

Abulwerdi, F. A., and Le Grice, S. F. J. (2017). Recent advances in targeting the HIV-1 Tat/TAR complex. *Curr. Pharm. Des.* 23, 4112–4121. doi: 10.2174/1381612823666170616081736

Aksenova, M. V., Aksenov, M. Y., Adams, S. M., Mactutus, C. F., and Booze, R. M. (2009). Neuronal survival and resistance to HIV-1 Tat toxicity in the primary culture of rat fetal neurons. *Exp. Neurol.* 215, 253–263. doi: 10.1016/j.expneurol.2008.10.006

Albini, A., Benelli, R., Giunciuglio, D., Cai, T., Mariani, G., Ferrini, S., et al. (1998a). Identification of a novel domain of HIV tat involved in monocyte chemotaxis. *J. Biol. Chem.* 273, 15895–15900. doi: 10.1074/jbc.273.26.15895

Albini, A., Ferrini, S., Benelli, R., Sforzini, S., Giunciuglio, D., Aluigi, M. G., et al. (1998b). HIV-1 Tat protein mimicry of chemokines. *Proc. Natl. Acad. Sci. U.S.A.* 95, 13153–13158. doi: 10.1073/pnas.95.22.13153

Albini, A., Fontanini, G., Masiello, L., Tacchetti, C., Bigini, D., Luzzi, P., et al. (1994). Angiogenic potential *in vivo* by Kaposi's sarcoma cell-free supernatants and HIV-1 tat product: inhibition of KS-like lesions by tissue inhibitor of metalloproteinase-2. *AIDS* 8, 1237–1244. doi: 10.1097/00002030-199409000-00004

Alcaide, M. L., Rodriguez, V. J., Abbamonte, J. M., Pallikuth, S., Langlie, J., Soni, M., et al. (2019). HIV and carotid atherosclerosis: a mediational model. *AIDS Care* 23:1–5. doi: 10.1080/09540121.2019.1668527

Allen, T. M., O'Connor, D. H., Jing, P., Dzuris, J. L., Mothe, B. R., Vogel, T. U., et al. (2000). Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. *Nature.* 407, 386–390. doi: 10.1038/35030124

Anand, A. R., Rachel, G., and Parthasarathy, D. (2018). HIV proteins and endothelial dysfunction: implications in cardiovascular disease. *Front. Cardiovasc. Med.* 5:185. doi: 10.3389/fcvm.2018.00185

Andras, I. E., Pu, H., Tian, J., Deli, M. A., Nath, A., Hennig, B., et al. (2005). Signaling mechanisms of HIV-1 Tat-induced alterations of claudin-5 expression in brain endothelial cells. *J. Cereb. Blood Flow Metab.* 25, 1159–1170. doi: 10.1038/sj.jcbfm.9600115

Antinori, A., Arendt, G., Becker, J. T., Brew, B. J., Byrd, D. A., Cherner, M., et al. (2007). Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* 69, 1789–1799. doi: 10.1212/01.WNL.0000287431.88658.8b

Apcher, G. S., Heink, S., Zantopf, D., Kloetzel, P. M., Schmid, H. P., Mayer, R. J., et al. (2003). Human immunodeficiency virus-1 Tat protein interacts with distinct proteasomal α and β subunits. *FEBS Lett.* 553, 200–204. doi: 10.1016/S0014-5793(03)01025-1

Asamitsu, K., Fujinaga, K., and Okamoto, T. (2018). HIV tat/P-TEFb interaction: a potential target for novel anti-HIV therapies. *Molecules* 23:933. doi: 10.3390/molecules23040933

Asamitsu, K., and Okamoto, T. (2017). The Tat/P-TEFb protein-protein interaction determining transcriptional activation of HIV. *Curr. Pharm. Des.* 23, 4091–4097. doi: 10.2174/1381612823666170710164148

Avraham, H. K., Jiang, S., Lee, T. H., Prakash, O., and Avraham, S. (2004). HIV-1 tat-mediated effects on focal adhesion assembly and permeability in brain microvascular endothelial cells. *J. Immunol.* 173, 6228–6233. doi: 10.4049/jimmunol.173.10.6228

Bachani, M., Sacktor, N., McArthur, J. C., Nath, A., and Rumbaugh, J. (2013). Detection of anti-tat antibodies in CSF of individuals with HIV-associated neurocognitive disorders. *J. Neurovirol.* 19, 82–88. doi: 10.1007/s13365-012-0144-8

Bagashev, A., and Sawaya, B. E. (2013). Roles and functions of HIV-1 Tat protein in the CNS an overview. *Virol. J.* 10:358. doi: 10.1186/1743-422X-10-358

Barouch, D. H., and Deeks, S. G. (2014). Immunologic strategies for HIV-1 remission and eradication. *Science* 345, 169–74. doi: 10.1126/science.1255512

Barre-Sinoussi, F. (2013). Functional HIV cure is no pipe dream, says codiscoverer of the virus. *BMJ* 346:f2947. doi: 10.1136/bmj.f2947

Barre-Sinoussi, F., Ross, A. L., and Delfraissy, J. F. (2013). Past, present and future: 30 years of HIV research. *Nat. Rev. Microbiol.* 11, 877–883. doi: 10.1038/nrmicro3132

Bayer, P., Kraft, M., Ejchart, A., Westendorp, M., Frank, R., and Rosch, P. (1995). Structural studies of HIV-1 Tat protein. *J. Mol. Biol.* 247, 529–535. doi: 10.1016/S0022-2836(05)80133-0

Becker, J. T., Kingsley, L., Mullen, J., Cohen, B., Martin, E., Miller, E. N., et al. (2009). Vascular risk factors, HIV serostatus, and cognitive dysfunction in gay and bisexual men. *Neurology* 73, 1292–1299. doi: 10.1212/WNL.0b013e3181bd10e7

Benelli, R., Mortarini, R., Anichini, A., Giunciuglio, D., Noonan, D. M., Montalti, S., et al. (1998). Monocyte-derived dendritic cells and monocytes migrate to HIV-Tat RGD and basic peptides. *AIDS* 12, 261–268. doi: 10.1097/00002030-199803000-00003

Berks, B. C., Lea, S. M., and Stansfeld, P. J. (2014). Structural biology of Tat protein transport. *Curr. Opin. Struct. Biol.* 27, 32–37. doi: 10.1016/j.sbi.2014.03.003

Berman, J. W., Carvallo, L., Buckner, C. M., Luers, A., Prevedel, L., Bennett, M. V., et al. (2016). HIV-tat alters Connexin43 expression and trafficking in human astrocytes: role in NeuroAIDS. *J. Neuroinflamm.* 13:54. doi: 10.1186/s12974-016-0510-1

Bohan, C. A., Kashanchi, F., Ensoli, B., Buonaguro, L., Boris-Lawrie, K. A., and Brady, J. N. (1992). Analysis of Tat transactivation of human immunodeficiency virus transcription in vitro. *Gene Expr.* 2, 391–407.

Borgatti, P., Zauli, G., Cantley, L. C., and Capitani, S. (1998). Extracellular HIV-1 Tat protein induces a rapid and selective activation of protein kinase C (PKC)-α, and ϵ and -ζ isoforms in PC12 cells. *Biochem. Biophys. Res. Commun.* 242, 332–337. doi: 10.1006/bbrc.1997.7877

Brown, A. R., Covington, M., Newton, R. C., Ramage, R., and Welch, P. (1996). The total chemical synthesis of monocyte chemotactic protein-1 (MCP-1). *J. Pept. Sci.* 2, 40–46. doi: 10.1002/psc.46

Buggey, J., Yun, L., Hung, C. L., Kityo, C., Mirembe, G., Erem, G., et al. (2019). HIV and pericardial fat are associated with abnormal cardiac structure and function among Ugandans. *Heart* 106, 147–153. doi: 10.1136/heartjnl-2019-315346

Burdo, T. H., Weiffenbach, A., Woods, S. P., Letendre, S., Ellis, R. J., and Williams, K. C. (2013). Elevated sCD163 in plasma but not cerebrospinal fluid is a marker of neurocognitive impairment in HIV infection. *AIDS* 27, 1387–1395. doi: 10.1097/QAD.0b013e32836010bd

Cafaro, A., Sgadari, C., Picconi, O., Tripiciano, A., Moretti, S., Francavilla, V., (2018). cART intensification by the HIV-1 Tat B clade vaccine: progress to phase III efficacy studies. *Expert Rev Vaccines* 17, 115–126. doi: 10.1080/14760584.2018.1418666

Catalfamo, M., Karpova, T., McNally, J., Costes, S. V., Lockett, S. J., Bos, E., et al. (2004). Human CD8+ T cells store RANTES in a unique secretory compartment and release it rapidly after TcR stimulation. *Immunity* 20, 219–230. doi: 10.1016/S1074-7613(04)00027-5

Chandra, T., Maier, W., Konig, H. G., Hirzel, K., Kogel, D., Schuler, T., et al. (2005). Molecular interactions of the type 1 human immunodeficiency virus transregulatory protein Tat with N-methyl-d-aspartate receptor subunits. *Neuroscience* 134, 145–153. doi: 10.1016/j.neuroscience.2005.02.049

Chang, H. C., Samaniego, F., Nair, B. C., Buonaguro, L., and Ensoli, B. (1997). HIV-1 Tat protein exits from cells via a leaderless secretory pathway and binds to extracellular matrix-associated heparan sulfate proteoglycans through its basic region. *AIDS* 11, 1421–1431. doi: 10.1097/00002030-199712000-00006

Chen, D., Wang, M., Zhou, S., and Zhou, Q. (2002). HIV-1 Tat targets microtubules to induce apoptosis, a process promoted by the pro-apoptotic Bcl-2 relative Bim. *EMBO J.* 21, 6801–6810. doi: 10.1093/emboj/cdf683

Chen, Y. F., and Dugas, T. R. (2019). Endothelial mitochondrial senescence accelerates cardiovascular disease in antiretroviral-receiving HIV patients. *Toxicol. Lett.* 317, 13–23. doi: 10.1016/j.toxlet.2019.09.018

Chiozzini, C., Collacchi, B., Nappi, F., Bauer, T., Arenaccio, C., Tripiciano, A., et al. (2014). Surface-bound Tat inhibits antigen-specific CD8+ T-cell activation in an integrin-dependent manner. *AIDS* 28, 2189–2200. doi: 10.1097/QAD.0000000000000389

Chiozzini, C., and Toschi, E. (2016). HIV-1 TAT and immune dysregulation in aids pathogenesis: a therapeutic target. *Curr. Drug Targets* 17, 33–45. doi: 10.2174/1389450116666150825110658

Choi, J. Y., Hightower, G. K., Wong, J. K., Heaton, R., Woods, S., Grant, I., et al. (2012). Genetic features of cerebrospinal fluid-derived subtype B HIV-1 tat. *J. Neurovirol.* 18, 81–90. doi: 10.1007/s13365-011-0059-9

Chompre, G., Cruz, E., Maldonado, L., Rivera-Amill, V., Porter, J. T., and Noel, R. J. Jr. (2013). Astrocytic expression of HIV-1 Nef impairs spatial and recognition memory. *Neurobiol. Dis.* 49, 128–136. doi: 10.1016/j.nbd.2012.08.007

Ciccarelli, N., Baldonero, E., Milanini, B., Fabbiani, M., Cauda, R., Di Giambenedetto, S., et al. (2019). Cognitive impairment and cardiovascular disease related to alexithymia in a well-controlled HIV-infected population. *Infez. Med.* 27, 274–282.

Cipolletta, E., Rusciano, M. R., Maione, A. S., Santulli, G., Sorriento, D., Del Giudice, C., et al. (2015). Targeting the CaMKII/ERK interaction in the heart prevents cardiac hypertrophy. *PLoS ONE* 10:e0130477. doi: 10.1371/journal.pone.0130477

Clark, E., Nava, B., and Caputi, M. (2017). Tat is a multifunctional viral protein that modulates cellular gene expression and functions. *Oncotarget* 8, 27569–27581. doi: 10.18632/oncotarget.15174

Clifford, D. B. and Ances, B. M. (2013). HIV-associated neurocognitive disorder. *Lancet Infect. Dis.* 13, 976–986. doi: 10.1016/S1473-3099(13)70269-X

Coll, B., Parra, S., Alonso-Villaverde, C., de Groot, E., Aragones, G., Montero, M., et al. (2006). HIV-infected patients with lipodystrophy have higher rates of carotid atherosclerosis: the role of monocyte chemoattractant protein-1. *Cytokine* 34, 51–55. doi: 10.1016/j.cyto.2006.03.013

Conant, K., Garzino-Demo, A., Nath, A., McArthur, J. C., Halliday, W., Power, C., et al. (1998). Induction of monocyte chemoattractant protein-1 in HIV-1 Tat-stimulated astrocytes and elevation in AIDS dementia. *Proc. Natl. Acad. Sci. U.S.A.* 95, 3117–3121. doi: 10.1073/pnas.95.6.3117

Constantino, A. A., Huang, Y., Zhang, H., Wood, C., and Zheng, J. C. (2011). HIV-1 clade B and C isolates exhibit differential replication: relevance to macrophage-mediated neurotoxicity. *Neurotox. Res.* 20, 277–288. doi: 10.1007/s12640-011-9241-3

Corder, E. H., Robertson, K., Lannfelt, L., Bogdanovic, N., Eggertsen, G., Wilkins, J., et al. (1998). HIV-infected subjects with the E4 allele for APOE have excess dementia and peripheral neuropathy. *Nat. Med.* 4, 1182–1184. doi: 10.1038/2677

Cross, A. K., and Woodroofe, M. N. (1999). Chemokines induce migration and changes in actin polymerization in adult rat brain microglia and a human fetal microglial cell line *in vitro*. *J. Neurosci. Res.* 55, 17–23. doi: 10.1002/(SICI)1097-4547(19990101)55:1<17::AID-JNR3>3.0.CO;2-J

Dalgleish, A. G., Beverley, P. C., Clapham, P. R., Crawford, D. H., Greaves, M. F., and Weiss, R. A. (1984). The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* 312, 763–767. doi: 10.1038/312763a0

Dayton, A. I., Sodroski, J. G., Rosen, C. A., Goh, W. C., and Haseltine, W. A. (1986). The trans-activator gene of the human T cell lymphotropic virus type III is required for replication. *Cell* 44, 941–947. doi: 10.1016/0092-8674(86)90017-6

Debaisieux, S., Rayne, F., Yezid, H., and Beaumelle, B. (2012). The ins and outs of HIV-1 Tat. *Traffic* 13, 355–363. doi: 10.1111/j.1600-0854.2011.01286.x

Deeks, S. G., Autran, B., Berkhout, B., Benkirane, M., Cairns, S., Chomont, N., et al. (2012). Towards an HIV cure: a global scientific strategy. *Nat. Rev. Immunol.* 12, 607–614. doi: 10.1038/nri3262

Desplats, P., Dumaop, W., Smith, D., Adame, A., Everall, I., Letendre, S., et al. (2013). Molecular and pathologic insights from latent HIV-1 infection in the human brain. *Neurology* 80, 1415–1423. doi: 10.1212/WNL.0b013e31828c2e9e

Donahue, D. A., Kuhl, B. D., Sloan, R. D., and Wainberg, M. A. (2012). The viral protein Tat can inhibit the establishment of HIV-1 latency. *J. Virol.* 86, 3253–3263. doi: 10.1128/JVI.06648-11

Easley, R., Van Duyne, R., Coley, W., Guendel, I., Dadgar, S., Kehn-Hall, K., et al. (2010). Chromatin dynamics associated with HIV-1 Tat-activated transcription. *Biochim. Biophys. Acta*. 1799, 275–285. doi: 10.1016/j.bbagrm.2009.08.008

Eguchi, A., Akuta, T., Okuyama, H., Senda, T., Yokoi, H., Inokuchi, H., et al. (2001). Protein transduction domain of HIV-1 Tat protein promotes efficient delivery of DNA into mammalian cells. *J. Biol. Chem.* 276, 26204–26210. doi: 10.1074/jbc.M010625200

El-Hage, N., Gurwell, J. A., Singh, I. N., Knapp, P. E., Nath, A., and Hauser, K. F. (2005). Synergistic increases in intracellular Ca2+, and the release of MCP-1, RANTES, and IL-6 by astrocytes treated with opiates and HIV-1 Tat. *Glia* 50, 91–106. doi: 10.1002/glia.20148

El-Hage, N., Wu, G., Ambati, J., Bruce-Keller, A. J., Knapp, P. E., and Hauser, K. F. (2006b). CCR2 mediates increases in glial activation caused by exposure to HIV-1 Tat and opiates. *J. Neuroimmunol.* 178, 9–16. doi: 10.1016/j.jneuroim.2006.05.027

El-Hage, N., Wu, G., Wang, J., Ambati, J., Knapp, P. E., Reed, J. L., et al. (2006a). HIV-1 Tat and opiate-induced changes in astrocytes promote chemotaxis of microglia through the expression of MCP-1 and alternative chemokines. *Glia* 53, 132–146. doi: 10.1002/glia.20262

Emonard, H., Bellon, G., de Diesbach, P., Mettlen, M., Hornebeck, W., and Courtoy, P. J. (2005). Regulation of matrix metalloproteinase (MMP) activity by the low-density lipoprotein receptor-related protein (LRP). A new function for an "old friend." *Biochimie* 87, 369–376. doi: 10.1016/j.biochi.2004.11.013

Ensoli, B., Barillari, G., Salahuddin, S. Z., Gallo, R. C., and Wong-Staal, F. (1990). Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. *Nature* 345, 84–86. doi: 10.1038/345084a0

Estrada, V., Domingo, P., Suarez-Lozano, I., Gutierrez, F., Knobel, H., Palacios, R., et al. (2019). Risk of cardiovascular disease in patients with HIV infection undergoing antiretroviral therapy. *Rev. Clin. Esp.* doi: 10.1016/j.rceng.2019.05.012. [Epub ahead of print].

Eugenin, E. A., D'Aversa, T. G., Lopez, L., Calderon, T. M., and Berman, J. W. (2003). MCP-1 (CCL2) protects human neurons and astrocytes from NMDA or HIV-tat-induced apoptosis. *J. Neurochem.* 85, 1299–1311. doi: 10.1046/j.1471-4159.2003.01775.x

Eugenin, E. A., Dyer, G., Calderon, T. M., and Berman, J. W. (2005). HIV-1 tat protein induces a migratory phenotype in human fetal microglia by a CCL2 (MCP-1)-dependent mechanism: possible role in NeuroAIDS. *Glia* 49, 501–510. doi: 10.1002/glia.20137

Eugenin, E. A., King, J. E., Nath, A., Calderon, T. M., Zukin, R. S., Bennett, M. V., et al. (2007). HIV-tat induces formation of an LRP-PSD-95- NMDAR-nNOS complex that promotes apoptosis in neurons and astrocytes. *Proc. Natl. Acad. Sci. U.S.A.* 104, 3438–3443. doi: 10.1073/pnas.0611699104

Eugenin, E. A., Morgello, S., Klotman, M. E., Mosoian, A., Lento, P. A., Berman, J. W., et al. (2008). Human immunodeficiency virus (HIV) infects human arterial smooth muscle cells in vivo and in vitro: implications for the pathogenesis of HIV-mediated vascular disease. *Am. J. Pathol.* 172, 1100–1111. doi: 10.2353/ajpath.2008.070457

Everall, I. P., Trillo-Pazos, G., Bell, C., Mallory, M., Sanders, V., and Masliah, E. (2001). Amelioration of neurotoxic effects of HIV envelope protein gp120 by fibroblast growth factor: a strategy for neuroprotection. *J. Neuropathol. Exp. Neurol.* 60, 293–301. doi: 10.1093/jnen/60.3.293

Eyawo, O., Brockman, G., Goldsmith, C. H., Hull, M. W., Lear, S. A., Bennett, M., et al. (2019). Risk of myocardial infarction among people living with HIV: an updated systematic review and meta-analysis. *BMJ Open* 9:e025874. doi: 10.1136/bmjopen-2018-025874

Feligioni, M., Raiteri, L., Pattarini, R., Grilli, M., Bruzzone, S., Cavazzani, P., et al. (2003). The human immunodeficiency virus-1 protein Tat and its discrete fragments evoke selective release of acetylcholine from human and rat cerebrocortical terminals through species-specific mechanisms. *J. Neurosci.* 23, 6810–6818. doi: 10.1523/JNEUROSCI.23-17-06810.2003

Ferrari, A., Pellegrini, V., Arcangeli, C., Fittipaldi, A., Giacca, M., and Beltram, F. (2003). Caveolae-mediated internalization of extracellular HIV-1 tat fusion proteins visualized in real time. *Mol. Ther.* 8, 284–294. doi: 10.1016/S1525-0016(03)00122-9

Fiala, M., Popik, W., Qiao, J. H., Lossinsky, A. S., Alce, T., Tran, K., et al. (2004). HIV-1 induces cardiomyopathyby cardiomyocyte invasion and gp120, Tat, and cytokine apoptotic signaling. *Cardiovasc. Toxicol.* 4, 97–107. doi: 10.1385/CT:4:2:097

Fields, J., Dumaop, W., Eleuteri, S., Campos, S., Serger, E., Trejo, M., et al. (2015a). HIV-1 Tat alters neuronal autophagy by modulating autophagosome fusion to the lysosome: implications for HIV-associated neurocognitive disorders. *J. Neurosci.* 35, 1921–1938. doi: 10.1523/JNEUROSCI.3207-14.2015

Fields, J., Dumaop, W., Rockenstein, E., Mante, M., Spencer, B., Grant, I., et al. (2013). Age-dependent molecular alterations in the autophagy pathway in HIVE patients and in a gp120 tg mouse model: reversal with beclin-1 gene transfer. *J. Neurovirol.* 19, 89–101. doi: 10.1007/s13365-012-0145-7

Fields, J. A., Dumaop, W., Crews, L., Adame, A., Spencer, B., Metcalf, J., et al. (2015b). Mechanisms of HIV-1 Tat neurotoxicity via CDK5 translocation and hyper-activation: role in HIV-associated neurocognitive disorders. *Curr. HIV Res.* 13, 43–54. doi: 10.2174/1570162X13666150311164201

Fittipaldi, A., Ferrari, A., Zoppe, M., Arcangeli, C., Pellegrini, V., Beltram, F., et al. (2003). Cell membrane lipid rafts mediate caveolar endocytosis of HIV-1 Tat fusion proteins. *J. Biol. Chem.* 278, 34141–34149. doi: 10.1074/jbc.M303045200

Fittipaldi, A., and Giacca, M. (2005). Transcellular protein transduction using the Tat protein of HIV-1. *Adv. Drug. Deliv. Rev.* 57, 597–608. doi: 10.1016/j.addr.2004.10.011

Fotheringham, J., Mayne, M., Holden, C., Nath, A., and Geiger, J. D. (2004). Adenosine receptors control HIV-1 Tat-induced inflammatory responses through protein phosphatase. *Virology* 327, 186–195. doi: 10.1016/j.virol.2004.07.007

Fuentes, M. E., Durham, S. K., Swerdel, M. R., Lewin, A. C., Barton, D. S., Megill, J. R., et al. (1995). Controlled recruitment of monocytes and macrophages to specific organs through transgenic expression of monocyte chemoattractant protein-1. *J. Immunol.* 155, 5769–5776.

Gandhi, N., Saiyed, Z., Thangavel, S., Rodriguez, J., Rao, K. V., and Nair, M. P. (2009). Differential effects of HIV type 1 clade B and clade C Tat protein on expression of proinflammatory and antiinflammatory cytokines by primary monocytes. *AIDS Res. Hum. Retroviruses* 25, 691–699. doi: 10.1089/aid.2008.0299

Garcia, J. A., Harrich, D., Pearson, L., Mitsuyasu, R., and Gaynor, R. B. (1988). Functional domains required for tat-induced transcriptional activation of the HIV-1 long terminal repeat. *Embo J.* 7, 3143–3147. doi: 10.1002/j.1460-2075.1988.tb03181.x

Gebo, K. A., Fleishman, J. A., Conviser, R., Hellinger, J., Hellinger, F. J., Josephs, J. S., et al. (2010). Contemporary costs of HIV healthcare in the HAART era. *Aids* 24, 2705–2715. doi: 10.1097/QAD.0b013e32833f3c14

Gilmore, E. C., and Herrup, K. (2001). Neocortical cell migration: GABAergic neurons and cells in layers I and VI move in a cyclin-dependent kinase 5-independent manner. *J. Neurosci.* 21, 9690–9700. doi: 10.1523/JNEUROSCI.21-24-09690.2001

Gilmore, E. C., Ohshima, T., Goffinet, A. M., Kulkarni, A. B., and Herrup, K. (1998). Cyclin-dependent kinase 5-deficient mice demonstrate novel developmental arrest in cerebral cortex. *J. Neurosci.* 18, 6370–6377. doi: 10.1523/JNEUROSCI.18-16-06370.1998

Gonzalez, E., Rovin, B. H., Sen, L., Cooke, G., Dhanda, R., Mummidi, S., et al. (2002). HIV-1 infection and AIDS dementia are influenced by a mutant MCP-1 allele linked to increased monocyte infiltration of tissues and MCP-1 levels. *Proc. Natl. Acad. Sci. U.S.A.* 99, 13795–13800. doi: 10.1073/pnas.202357499

Gurwell, J. A., Nath, A., Sun, Q., Zhang, J., Martin, K. M., Chen, Y., et al. (2001). Synergistic neurotoxicity of opioids and human immunodeficiency virus-1 Tat protein in striatal neurons in vitro. *Neuroscience* 102, 555–563. doi: 10.1016/S0306-4522(00)00461-9

Hashida, H., Miyamoto, M., Cho, Y., Hida, Y., Kato, K., Kurokawa, T., et al. (2004). Fusion of HIV-1 Tat protein transduction domain to poly-lysine as a new DNA delivery tool. *Br. J. Cancer* 90, 1252–1258. doi: 10.1038/sj.bjc.6601680

Haughey, N. J., Holden, C. P., Nath, A., and Geiger, J. D. (1999). Involvement of inositol 1,4,5-trisphosphate-regulated stores of intracellular calcium in calcium dysregulation and neuron cell death caused by HIV-1 protein tat. *J. Neurochem.* 73, 1363–1374. doi: 10.1046/j.1471-4159.1999.0731363.x

Haughey, N. J., Nath, A., Mattson, M. P., Slevin, J. T., and Geiger, J. D. (2001). HIV-1 Tat through phosphorylation of NMDA receptors potentiates glutamate excitotoxicity. *J. Neurochem.* 78, 457–467. doi: 10.1046/j.1471-4159.2001.00396.x

Heaton, R. K., Clifford, D. B., Franklin, D. R. Jr., Woods, S. P., Ake, C., Vaida, F., et al. (2010). HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology* 75, 2087–2096. doi: 10.1212/WNL.0b013e318200d727

Heaton, R. K., Franklin, D. R., Ellis, R. J., McCutchan, J. A., Letendre, S. L., Leblanc, S., et al. (2011). HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. *J. Neurovirol.* 17, 3–16. doi: 10.1007/s13365-010-0006-1

Heravi, A. S., Etzkorn, L., Urbanek, J., Crainiceanu, C., Punjabi, N. M., Ashikaga, H., et al. (2019). HIV infection is associated with variability in ventricular repolarization: the multicenter AIDS cohort study (MACS). *Circulation* 141, 176–187. doi: 10.1161/CIRCULATIONAHA.119.043042

Herz, J., and Strickland, D. K. (2001). LRP: a multifunctional scavenger and signaling receptor. *J. Clin. Invest.* 108, 779–784. doi: 10.1172/JCI200113992

Hu, G., Liao, K., Yang, L., Pendyala, G., Kook, Y., Fox, H. S., et al. (2017). Tat-mediated induction of miRs-34a &−138 promotes astrocytic activation via downregulation of SIRT1: implications for aging in HAND. *J. Neuroimmune Pharmacol.* 12, 420–432. doi: 10.1007/s11481-017-9730-0

Hu, X. T. (2016). HIV-1 tat-mediated calcium dysregulation and neuronal dysfunction in vulnerable brain regions. *Curr. Drug Targets* 17, 4–14. doi: 10.2174/1389450116666150531162212

Huang, L., Bosch, I., Hofmann, W., Sodroski, J., and Pardee, A. B. (1998). Tat protein induces human immunodeficiency virus type 1 (HIV-1) coreceptors and promotes infection with both macrophage-tropic and T-lymphotropic HIV-1 strains. *J. Virol.* 72, 8952–8960. doi: 10.1128/JVI.72.11.8952-8960.1998

Huang, X., Seifert, U., Salzmann, U., Henklein, P., Preissner, R., Henke, W., et al. (2002). The RTP site shared by the HIV-1 Tat protein and the 11S regulator subunit α is crucial for their effects on proteasome function including antigen processing. *J. Mol. Biol.* 323, 771–782. doi: 10.1016/S0022-2836(02)00998-1

Izmailova, E., Bertley, F. M., Huang, Q., Makori, N., Miller, C. J., Young, R. A., et al. (2003). HIV-1 Tat reprograms immature dendritic cells to express chemoattractants for activated T cells and macrophages. *Nat. Med.* 9, 191–197. doi: 10.1038/nm822

Jiang, Y., Chai, L., Fasae, M. B., and Bai, Y. (2018). The role of HIV Tat protein in HIV-related cardiovascular diseases. *J. Transl. Med.* 16:121. doi: 10.1186/s12967-018-1500-0

Johri, M. K., Sharma, N., and Singh, S. K. (2015). HIV tat protein: is tat-C much trickier than Tat-B? *J. Med. Virol.* 87, 1334–1343. doi: 10.1002/jmv.24182

Joseph, S. B., Arrildt, K. T., Sturdevant, C. B., and Swanstrom, R. (2015). HIV-1 target cells in the CNS. *J. Neurovirol.* 21, 276–289. doi: 10.1007/s13365-014-0287-x

Kamori, D., and Ueno, T. (2017). HIV-1 tat and viral latency: what we can learn from naturally occurring sequence variations. *Front. Microbiol.* 8:80. doi: 10.3389/fmicb.2017.00080

Kao, S. Y., Calman, A. F., Luciw, P. A., and Peterlin, B. M. (1987). Anti-termination of transcription within the long terminal repeat of HIV-1 by tat gene product. *Nature* 330, 489–493. doi: 10.1038/330489a0

Karn, J. (2011). The molecular biology of HIV latency: breaking and restoring the Tat-dependent transcriptional circuit. *Curr. Opin. HIV AIDS* 6, 4–11. doi: 10.1097/COH.0b013e328340ffbb

Karn, J., and Stoltzfus, C. M. (2012). Transcriptional and posttranscriptional regulation of HIV-1 gene expression. *Cold Spring Harb Perspect. Med.* 2:a006916. doi: 10.1101/cshperspect.a006916

Kelder, W., McArthur, J. C., Nance-Sproson, T., McClernon, D., and Griffin, D. E. (1998). β-chemokines MCP-1 and RANTES are selectively increased in cerebrospinal fluid of patients with human immunodeficiency virus-associated dementia. *Ann. Neurol.* 44, 831–835. doi: 10.1002/ana.410440521

Khoury, G., Darcis, G., Lee, M. Y., Bouchat, S., Van Driessche, B. D., and Van Lint, C. (2018). The molecular biology of HIV latency. *Adv. Exp. Med. Biol.* 1075, 187–212. doi: 10.1007/978-981-13-0484-2_8

King, J. E., Eugenin, E. A., Buckner, C. M., and Berman, J. W. (2006). HIV tat and neurotoxicity. *Microbes Infect.* 8, 1347–1357. doi: 10.1016/j.micinf.2005.11.014

Koch, A. E., Kunkel, S. L., Harlow, L. A., Johnson, B., Evanoff, H. L., Haines, G. K., et al. (1992). Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. *J. Clin. Invest.* 90, 772–779. doi: 10.1172/JCI115950

Kranick, S. M., and Nath, A. (2012). Neurologic complications of HIV-1 infection and its treatment in the era of antiretroviral therapy. *Continuum* 18, 1319–1337. doi: 10.1212/01.CON.0000423849.24900.ec

Kumar, A., and Herbein, G. (2014). The macrophage: a therapeutic target in HIV-1 infection. *Mol. Cell Ther.* 2:10. doi: 10.1186/2052-8426-2-10

Kurosu, T., Mukai, T., Komoto, S., Ibrahim, M. S., Li, Y. G., Kobayashi, T., et al. (2002). Human immunodeficiency virus type 1 subtype C exhibits higher transactivation activity of Tat than subtypes B and E. *Microbiol. Immunol.* 46, 787–799. doi: 10.1111/j.1348-0421.2002.tb02766.x

Kusdra, L., McGuire, D., and Pulliam, L. (2002). Changes in monocyte/macrophage neurotoxicity in the era of HAART: implications for HIV-associated dementia. *Aids* 16, 31–38. doi: 10.1097/00002030-200201040-00005

Lackner, A. A., Lederman, M. M., and Rodriguez, B. (2012). HIV pathogenesis: the host. *Cold Spring Harb. Perspect. Med.* 2:a007005. doi: 10.1101/cshperspect.a007005

Lafrenie, R. M., Wahl, L. M., Epstein, J. S., Hewlett, I. K., Yamada, K. M., and Dhawan, S. (1996a). HIV-1-Tat protein promotes chemotaxis and invasive behavior by monocytes. *J. Immunol.* 157, 974–977.

Lafrenie, R. M., Wahl, L. M., Epstein, J. S., Hewlett, I. K., Yamada, K. M., and Dhawan, S. (1996b). HIV-1-Tat modulates the function of monocytes and alters their interactions with microvessel endothelial cells. A mechanism of HIV pathogenesis. *J. Immunol.* 156, 1638–1645.

Lawrence, D. M., Seth, P., Durham, L., Diaz, F., Boursiquot, R., Ransohoff, R. M., et al. (2006). Astrocyte differentiation selectively upregulates CCL2/monocyte chemoattractant protein-1 in cultured human brain-derived progenitor cells. *Glia* 53, 81–91. doi: 10.1002/glia.20261

Lee, E. O., Kim, S. E., Park, H. K., Kang, J. L., and Chong, Y. H. (2011). Extracellular HIV-1 Tat upregulates TNF-α dependent MCP-1/CCL2 production via activation of ERK1/2 pathway in rat hippocampal slice cultures: inhibition by resveratrol, a polyphenolic phytostilbene. *Exp. Neurol.* 229, 399–408. doi: 10.1016/j.expneurol.2011.03.006

Li, W., Huang, Y., Reid, R., Steiner, J., Malpica-Llanos, T., Darden, T. A., et al. (2008). NMDA receptor activation by HIV-Tat protein is clade dependent. *J. Neurosci.* 28, 12190–12198. doi: 10.1523/JNEUROSCI.3019-08.2008

Liu, Y., Jones, M., Hingtgen, C. M., Bu, G., Laribee, N., Tanzi, R. E., et al. (2000). Uptake of HIV-1 tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nat. Med.* 6, 1380–1387. doi: 10.1038/82199

Loret, E. (2015). HIV extracellular Tat: myth or reality? *Curr. HIV Res.* 13, 90–97. doi: 10.2174/1570162X12666141202125643

Macquart, C., Juttner, R., Le Dour, C., Chatzifrangkeskou, M., Schmitt, A., Gotthardt, M., et al. (2018). Microtubule cytoskeleton regulates connexin 43 localization and cardiac conduction in cardiomyopathy caused by mutation in A-type lamins gene. *Hum. Mol. Genet.* 28, 4043–4052. doi: 10.1093/hmg/ddy227

Maddon, P. J., Dalgleish, A. G., McDougal, J. S., Clapham, P. R., Weiss, R. A., and Axel, R. (1986). The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* 47, 333–348. doi: 10.1016/0092-8674(86)90590-8

Mayne, M., Holden, C. P., Nath, A., and Geiger, J. D. (2000). Release of calcium from inositol 1,4,5-trisphosphate receptor-regulated stores by HIV-1 Tat regulates TNF-α production in human macrophages. *J. Immunol.* 164, 6538–6542. doi: 10.4049/jimmunol.164.12.6538

Mbonye, U., and Karn, J. (2011). Control of HIV latency by epigenetic and non-epigenetic mechanisms. *Curr. HIV Res.* 9, 554–567. doi: 10.2174/157016211798998736

Mbonye, U., and Karn, J. (2017). The molecular basis for human immunodeficiency virus latency. *Annu. Rev. Virol.* 4, 261–285. doi: 10.1146/annurev-virology-101416-041646

McManus, C. M., Liu, J. S., Hahn, M. T., Hua, L. L., Brosnan, C. F., Berman, J. W., et al. (2000a). Differential induction of chemokines in human microglia by type I and II interferons. *Glia*. 29, 273–80. doi: 10.1002/(SICI)1098-1136(20000201)29:3<273::AID-GLIA8>3.0.CO;2-9

McManus, C. M., Weidenheim, K., Woodman, S. E., Nunez, J., Hesselgesser, J., Nath, A., et al. (2000b). Chemokine and chemokine-receptor expression in human glial elements: induction by the HIV protein, Tat, and chemokine autoregulation. *Am. J. Pathol.* 156, 1441–1453. doi: 10.1016/S0002-9440(10)65013-4

Mishra, M., Vetrivel, S., Siddappa, N. B., Ranga, U., and Seth, P. (2008). Clade-specific differences in neurotoxicity of human immunodeficiency virus-1 B and C Tat of human neurons: significance of dicysteine C30C31 motif. *Ann. Neurol.* 63, 366–376. doi: 10.1002/ana.21292

Mitola, S., Sozzani, S., Luini, W., Primo, L., Borsatti, A., Weich, H., et al. (1997). Tat-human immunodeficiency virus-1 induces human monocyte chemotaxis by activation of vascular endothelial growth factor receptor-1. *Blood* 90, 1365–1372. doi: 10.1182/blood.V90.4.1365.1365_1365_1372

Monini, P., Cafaro, A., Srivastava, I. K., Moretti, S., Sharma, V. A., Andreini, C., et al. (2012). HIV-1 tat promotes integrin-mediated HIV transmission to dendritic cells by binding Env spikes and competes neutralization by anti-HIV antibodies. *PLoS ONE* 7:e48781. doi: 10.1371/journal.pone.0048781

Montano, M. A., Novitsky, V. A., Blackard, J. T., Cho, N. L., Katzenstein, D. A., and Essex, M. (1997). Divergent transcriptional regulation among expanding human immunodeficiency virus type 1 subtypes. *J. Virol.* 71, 8657–8665. doi: 10.1128/JVI.71.11.8657-8665.1997

Moutouh, L., Corbeil, J., and Richman, D. D. (1996). Recombination leads to the rapid emergence of HIV-1 dually resistant mutants under selective drug pressure. *Proc. Natl. Acad. Sci. U.S.A.* 93, 6106–6111. doi: 10.1073/pnas.93.12.6106

Nath, A., Hauser, K. F., Wojna, V., Booze, R. M., Maragos, W., Prendergast, M., et al. (2002). Molecular basis for interactions of HIV and drugs of abuse. *J. Acquir. Immune Defic. Syndr.* 31(Suppl 2):S62–S69. doi: 10.1097/00126334-200210012-00006

Nekhai, S., and Jeang, K. T. (2006). Transcriptional and post-transcriptional regulation of HIV-1 gene expression: role of cellular factors for Tat and Rev. *Fut. Microbiol.* 1, 417–426. doi: 10.2217/17460913.1.4.417

Niu, F., Yao, H., Liao, K., and Buch, S. (2015). HIV Tat 101-mediated loss of pericytes at the blood-brain barrier involves PDGF-BB. *Ther. Targets Neurol. Dis.* 2:e471. doi: 10.14800/ttnd.471

Ohshima, K., Suzumiya, J., Tasiro, K., Mukai, Y., Tanaka, T., Kato, A., et al. (1996). Epstein-Barr virus infection and associated products (LMP, EBNA2, vIL-10) in nodal non-Hodgkin's lymphoma of human immunodeficiency virus-negative Japanese. *Am. J. Hematol.* 52, 21–8. doi: 10.1002/(SICI)1096-8652(199605)52:1<21::AID-AJH4>3.0.CO;2-8

Oynebraten, I., Bakke, O., Brandtzaeg, P., Johansen, F. E., and Haraldsen, G. (2004). Rapid chemokine secretion from endothelial cells originates from 2 distinct compartments. *Blood* 104, 314–320. doi: 10.1182/blood-2003-08-2891

Palma Reis, R. (2019). Cardiovascular risk in HIV-infected patients. *Rev. Port. Cardiol.* 38, 471–472. doi: 10.1016/j.repc.2019.08.007

Periyasamy, P., Thangaraj, A., Bendi, V. S., and Buch, S. (2019). HIV-1 Tat-mediated microglial inflammation involves a novel miRNA-34a-NLRC5-NFκB signaling axis. *Brain Behav. Immun.* 80, 227–237. doi: 10.1016/j.bbi.2019.03.011

Peruzzi, F., Gordon, J., Darbinian, N., and Amini, S. (2002). Tat-induced deregulation of neuronal differentiation and survival by nerve growth factor pathway. *J. Neurovirol.* 8(Suppl. 2), 91–96. doi: 10.1080/13550280290167885

Prakash, O., Tang, Z. Y., He, Y. E., Ali, M. S., Coleman, R., Gill, J., et al. (2000). Human Kaposi's sarcoma cell-mediated tumorigenesis in human immunodeficiency type 1 tat-expressing transgenic mice. *J. Natl. Cancer Inst.* 92, 721–728. doi: 10.1093/jnci/92.9.721

Prasad, J. M., Migliorini, M., Galisteo, R., and Strickland, D. K. (2015). Generation of a potent low density lipoprotein receptor-related protein 1 (LRP1) antagonist by engineering a stable form of the receptor-associated protein (RAP) D3 domain. *J. Biol. Chem.* 290, 17262–17268. doi: 10.1074/jbc.M115.660084

Prendergast, M. A., Rogers, D. T., Mulholland, P. J., Littleton, J. M., Wilkins, L. H. Jr., Self, R. L., et al. (2002). Neurotoxic effects of the human immunodeficiency virus type-1 transcription factor Tat require function of a polyamine sensitive-site on the N-methyl-D-aspartate receptor. *Brain Res.* 954, 300–307. doi: 10.1016/S0006-8993(02)03360-7

Prevedel, L., Morocho, C. M., Bennett, V. L., and Eugenin, E. A. (2017). HIV-associated cardiovascular disease: role of connexin 43. *Am. J. Pathol.* 187, 1960–1970. doi: 10.1016/j.ajpath.2017.05.011

Pu, H., Tian, J., Andras, I. E., Hayashi, K., Flora, G., Hennig, B., et al. (2005). HIV-1 Tat protein-induced alterations of ZO-1 expression are mediated by redox-regulated ERK 1/2 activation. *J. Cereb. Blood Flow Metab.* 25, 1325–1335. doi: 10.1038/sj.jcbfm.9600125

Qiu, Z., Crutcher, K. A., Hyman, B. T., and Rebeck, G. W. (2003). ApoE isoforms affect neuronal N-methyl-D-aspartate calcium responses and toxicity via receptor-mediated processes. *Neuroscience* 122, 291–303. doi: 10.1016/j.neuroscience.2003.08.017

Ranga, U., Shankarappa, R., Siddappa, N. B., Ramakrishna, L., Nagendran, R., Mahalingam, M., et al. (2004). Tat protein of human immunodeficiency virus type 1 subtype C strains is a defective chemokine. *J. Virol.* 78, 2586–2590. doi: 10.1128/JVI.78.5.2586-2590.2004

Rao, V. R., Neogi, U., Talboom, J. S., Padilla, L., Rahman, M., Fritz-French, C., et al. (2013). Clade C HIV-1 isolates circulating in Southern Africa exhibit a greater frequency of dicysteine motif-containing Tat variants than those in Southeast Asia and cause increased neurovirulence. *Retrovirology* 10:61. doi: 10.1186/1742-4690-10-61

Rao, V. R., Ruiz, A. P., and Prasad, V. R. (2014). Viral and cellular factors underlying neuropathogenesis in HIV associated neurocognitive disorders (HAND). *AIDS Res. Ther.* 11 13. doi: 10.1186/1742-6405-11-13

Rao, V. R., Sas, A. R., Eugenin, E. A., Siddappa, N. B., Bimonte-Nelson, H., Berman, J. W., et al. (2008). HIV-1 clade-specific differences in the induction of neuropathogenesis. *J. Neurosci.* 28, 10010–10016. doi: 10.1523/JNEUROSCI.2955-08.2008

Rayne, F., Debaisieux, S., Yezid, H., Lin, Y. L., Mettling, C., Konate, K., et al. (2010). Phosphatidylinositol-(4,5)-bisphosphate enables efficient secretion of HIV-1 Tat by infected T-cells. *Embo J.* 29, 1348–1362. doi: 10.1038/emboj.2010.32

Re, M. C., Furlini, G., Vignoli, M., Ramazzotti, E., Roderigo, G., De Rosa, V., et al. (1995). Effect of antibody to HIV-1 Tat protein on viral replication in vitro and progression of HIV-1 disease *in vivo*. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 10, 408–416. doi: 10.1097/00042560-199512000-00003

Rezza, G., Fiorelli, V., Dorrucci, M., Ciccozzi, M., Tripiciano, A., Scoglio, A., et al. (2005). The presence of anti-Tat antibodies is predictive of long-term nonprogression to AIDS or severe immunodeficiency: findings in a cohort of HIV-1 seroconverters. *J. Infect. Dis.* 191, 1321–1324. doi: 10.1086/428909

Richard, J. P., Melikov, K., Brooks, H., Prevot, P., Lebleu, B., and Chernomordik, L. V. (2005). Cellular uptake of unconjugated TAT peptide involves clathrin-dependent endocytosis and heparan sulfate receptors. *J. Biol. Chem.* 280, 15300–15306. doi: 10.1074/jbc.M401604200

Rojas-Celis, V., Valiente-Echeverria, F., Soto-Rifo, R., and Toro-Ascuy, D. (2019). New challenges of HIV-1 infection: how HIV-1 attacks and resides in the central nervous system. *Cells* 8:1245. doi: 10.3390/cells8101245

Roses, A. D., Saunders, A. M., Alberts, M. A., Strittmatter, W. J., Schmechel, D., Gorder, E., et al. (1995). Apolipoprotein E E4 allele and risk of dementia. *JAMA*. 273, 374–5; author reply 375-6. doi: 10.1001/jama.1995.03520290026015

Ruben, S., Perkins, A., Purcell, R., Joung, K., Sia, R., Burghoff, R., et al. (1989). Structural and functional characterization of human immunodeficiency virus tat protein. *J. Virol.* 63, 1–8. doi: 10.1128/JVI.63.1.1-8.1989

Ruiz, A. P., Ajasin, D. O., Ramasamy, S., DesMarais, V., Eugenin, E. A., and Prasad, V. R. (2019). A naturally occurring polymorphism in the HIV-1 tat basic domain inhibits uptake by bystander cells and leads to reduced neuroinflammation. *Sci. Rep.* 9:3308. doi: 10.1038/s41598-019-39531-5

Schultz, F., Swiatlowska, P., Alvarez-Laviada, A., Sanchez-Alonso, J. L., Song, Q. A., and Gorelik, J. (2019). Cardiomyocyte-myofibroblast contact dynamism is modulated by connexin-43. *Faseb J.* 33, 10453–10468. doi: 10.1096/fj.201802740RR

Schwartz, S., Felber, B. K., Benko, D. M., Fenyo, E. M., and Pavlakis, G. N. (1990). Cloning and functional analysis of multiply spliced mRNA species of human immunodeficiency virus type 1. *J. Virol.* 64, 2519–2529. doi: 10.1128/JVI.64.6.2519-2529.1990

Self, R. L., Mulholland, P. J., Nath, A., Harris, B. R., and Prendergast, M. A. (2004). The human immunodeficiency virus type-1 transcription factor Tat produces elevations in intracellular Ca2+ that require function of an N-methyl-D-aspartate receptor polyamine-sensitive site. *Brain Res.* 995, 39–45. doi: 10.1016/j.brainres.2003.09.052

Seror, C., Melki, M. T., Subra, F., Raza, S. Q., Bras, M., Saidi, H., et al. (2011). Extracellular ATP acts on P2Y2 purinergic receptors to facilitate HIV-1 infection. *J. Exp. Med*. 208, 1823–1834. doi: 10.1084/jem.20101805

Siliciano, R. F., and Greene, W. C. (2011). HIV latency. *Cold Spring Harb. Perspect. Med.* 1:a007096. doi: 10.1101/cshperspect.a007096

Siwamogsatham, S., Chutinet, A., Vongsayan, P., Samajarn, J., Putcharoen, O., Aponpong, T., et al. (2019). Low CD4 cell counts are associated with carotid plaque and intima-media thickness in virologically suppressed HIV-infected asians older than 50 years. *AIDS Res. Hum. Retroviruses* 35, 1160–1169. doi: 10.1089/aid.2019.0126

Slooter, A. J., Tang, M. X., van Duijn, C. M., Stern, Y., Ott, A., Bell, K., et al. (1997). Apolipoprotein E epsilon4 and the risk of dementia with stroke. A population-based investigation. *JAMA*. 277, 818–821. doi: 10.1001/jama.277.10.818

Soontornniyomkij, V., Wang, G., Pittman, C. A., Wiley, C. A., and Achim, C. L. (1998). Expression of brain-derived neurotrophic factor protein in activated microglia of human immunodeficiency virus type 1 encephalitis. *Neuropathol. Appl. Neurobiol*. 24, 453–460. doi: 10.1046/j.1365-2990.1998.00134.x

Strickland, M., Ehrlich, L. S., Watanabe, S., Khan, M., Strub, M. P., Luan, C. H., et al. (2017). Tsg101 chaperone function revealed by HIV-1 assembly inhibitors. *Nat. Commun.* 8:1391. doi: 10.1038/s41467-017-01426-2

Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G. S., et al. (1993). Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. 90, 1977–1981. doi: 10.1073/pnas.90.5.1977

Tabarrini, O., Desantis, J., and Massari, S. (2016). Recent advances in the identification of Tat-mediated transactivation inhibitors: progressing toward a functional cure of HIV. *Fut. Med. Chem*. 8, 421–442. doi: 10.4155/fmc.16.3

Tahrir, F. G., Shanmughapriya, S., Ahooyi, T. M., Knezevic, T., Gupta, M. K., Kontos, C. D., et al. (2018). Dysregulation of mitochondrial bioenergetics and quality control by HIV-1 Tat in cardiomyocytes. *J. Cell Physiol*. 233, 748–758. doi: 10.1002/jcp.26002

Thompson-Paul, A. M., Palella, F. J. Jr., Rayeed, N., Ritchey, M. D., Lichtenstein, K. A., Patel, D., et al. (2019). Excess heart age in adult outpatients in routine HIV care. *Aids* 33, 1935–1942. doi: 10.1097/QAD.0000000000002304

Turner, A. J., Isaac, R. E., and Coates, D. (2001). The neprilysin (NEP) family of zinc metalloendopeptidases: genomics and function. *Bioessays* 23, 261–9. doi: 10.1002/1521-1878(200103)23:3<261::AID-BIES1036>3.0.CO;2-K

Tyagi, M., Rusnati, M., Presta, M., and Giacca, M. (2001). Internalization of HIV-1 tat requires cell surface heparan sulfate proteoglycans. *J. Biol. Chem*. 276, 3254–3261. doi: 10.1074/jbc.M006701200

Urbinati, C., Bugatti, A., Giacca, M., Schlaepfer, D., Presta, M., and Rusnati, M. (2005). $\alpha_v\beta_3$-integrin-dependent activas tion of focal adhesion kinase mediates NF-κB activation and motogenic activity by HIV-1 Tat in endothelial cells. *J. Cell Sci*. 118, 3949–3958. doi: 10.1242/jcs.02518

van Baalen, C. A., Pontesilli, O., Huisman, R. C., Geretti, A. M., Klein, M. R., de Wolf, F., et al. (1997). Human immunodeficiency virus type 1 Rev- and Tat-specific cytotoxic T lymphocyte frequencies inversely correlate with rapid progression to AIDS. *J. Gen Virol.* 78 (Pt 8):1913–1918. doi: 10.1099/0022-1317-78-8-1913

Vardabasso, C., Manganaro, L., Lusic, M., Marcello, A., and Giacca, M. (2008). The histone chaperone protein nucleosome assembly protein-1 (hNAP-1) binds HIV-1 Tat and promotes viral transcription. *Retrovirology* 5:8. doi: 10.1186/1742-4690-5-8

Vendeville, A., Rayne, F., Bonhoure, A., Bettache, N., Montcourrier, P., and Beaumelle, B. (2004). HIV-1 Tat enters T cells using coated pits before translocating from acidified endosomes and eliciting biological responses. *Mol. Biol. Cell*. 15, 2347–2360. doi: 10.1091/mbc.e03-12-0921

Villiger, P. M., Terkeltaub, R., and Lotz, M. (1992a). Production of monocyte chemoattractant protein-1 by inflamed synovial tissue and cultured synoviocytes. *J. Immunol*. 149, 722–727.

Villiger, P. M., Terkeltaub, R., and Lotz, M. (1992b). Monocyte chemoattractant protein-1 (MCP-1) expression in human articular cartilage. Induction by peptide regulatory factors and differential effects of dexamethasone and retinoic acid. *J. Clin. Invest*. 90, 488–496. doi: 10.1172/JCI115885

Vogel, B. E., Lee, S. J., Hildebrand, A., Craig, W., Pierschbacher, M. D., Wong-Staal, F., et al. (1993). A novel integrin specificity exemplified by binding of the alpha v beta 5 integrin to the basic domain of the HIV Tat protein and vitronectin. *J. Cell Biol*. 121, 461–468. doi: 10.1083/jcb.121.2.461

Wang, T., Yi, R., Green, L. A., Chelvanambi, S., Seimetz, M., and Clauss, M. (2015). Increased cardiovascular disease risk in the HIV-positive population on ART: potential role of HIV-Nef and Tat. *Cardiovasc. Pathol*. 24, 279–282. doi: 10.1016/j.carpath.2015.07.001

Wang, Z., Morris, G. F., Rice, A. P., Xiong, W., and Morris, C. B. (1996). Wild-type and transactivation-defective mutants of human immunodeficiency virus type 1 Tat protein bind human TATA-binding protein *in vitro*. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol*. 12, 128–138. doi: 10.1097/00042560-199606010-00005

Westendorp, M. O., Frank, R., Ochsenbauer, C., Stricker, K., Dhein, J., Walczak, H., et al. (1995). Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. *Nature* 375, 497–500. doi: 10.1038/375497a0

Williams, D. W., Calderon, T. M., Lopez, L., Carvallo-Torres, L., Gaskill, P. J., Eugenin, E. A., et al. (2013). Mechanisms of HIV entry into the CNS: increased sensitivity of HIV infected CD14+CD16+ monocytes to CCL2 and key roles of CCR2, JAM-A, and ALCAM in diapedesis. *PLoS ONE* 8:e69270. doi: 10.1371/journal.pone.0069270

Xiao, H., Neuveut, C., Tiffany, H. L., Benkirane, M., Rich, E. A., Murphy, P. M., et al. (2000). Selective CXCR4 antagonism by Tat: implications for *in vivo* expansion of coreceptor use by HIV-1. *Proc. Natl. Acad. Sci. U.S.A*. 97, 11466–11471. doi: 10.1073/pnas.97.21.11466

Yao, H., Yang, Y., Kim, K. J., Bethel-Brown, C., Gong, N., Funa, K., et al. (2010). Molecular mechanisms involving sigma receptor-mediated induction of MCP-1: implication for increased monocyte transmigration. *Blood* 115, 4951–4962. doi: 10.1182/blood-2010-01-266221

Yla-Herttuala, S., Lipton, B. A., Rosenfeld, M. E., Sarkioja, T., Yoshimura, T., Leonard, E. J., et al. (1991). Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. *Proc. Natl. Acad. Sci. U.S.A*. 88, 5252–5256. doi: 10.1073/pnas.88.12.5252

Youn, G. S., Ju, S. M., Choi, S. Y., and Park, J. (2015). HDAC6 mediates HIV-1 tat-induced proinflammatory responses by regulating MAPK-NF-kappaB/AP-1 pathways in astrocytes. *Glia* 63, 1953–1965. doi: 10.1002/glia.22865

Zhan, J., Qin, S., Lu, L., Hu, X., Zhou, J., Sun, Y., et al. (2016). miR-34a is a common link in both HIV- and antiretroviral therapy-induced vascular aging. *Aging* 8, 3298–3310. doi: 10.18632/aging.101118

Zhang, H. S., Chen, X. Y., Wu, T. C., Sang, W. W., and Ruan, Z. (2012). MiR-34a is involved in Tat-induced HIV-1 long terminal repeat (LTR) transactivation through the SIRT1/NFκB pathway. *FEBS Lett*. 586, 4203–4207. doi: 10.1016/j.febslet.2012.10.023

Zhao, G., Qiu, Y., Zhang, H. M., and Yang, D. (2019). Intercalated discs: cellular adhesion and signaling in heart health and diseases. *Heart Fail Rev*. 24, 115–132. doi: 10.1007/s10741-018-9743-7

Zheng, L., Yang, Y. D., Lu, G. C., and Salvato, M. S. (2005). Extracellular HIV Tat and Tat cysteine rich peptide increase CCR5 expression in monocytes. *J. Zhejiang Univ. Sci. B* 6, 668–672. doi: 10.1631/jzus.2005.B0668

Zocchi, M. R., Poggi, A., and Rubartelli, A. (1997). The RGD-containing domain of exogenous HIV-1 Tat inhibits the engulfment of apoptotic bodies by dendritic cells. *Aids* 11, 1227–1235. doi: 10.1097/00002030-199710000-00005

Zungsontiporn, N., Tello, R. R., Zhang, G., Mitchell, B. I., Budoff, M., Kallianpur, K. J., et al. (2016). Non-classical monocytes and monocyte chemoattractant protein-1 (MCP-1) correlate with coronary artery calcium progression in chronically HIV-1 infected adults on stable antiretroviral therapy. *PLoS ONE* 11:e0149143. doi: 10.1371/journal.pone.0149143

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: 28 February 2020
doi: 10.3389/fcimb.2020.00070

Dendritic Cell Maturation Regulates TSPAN7 Function in HIV-1 Transfer to CD4$^+$ T Lymphocytes

Brieuc P. Perot[1,2], *Victor García-Paredes*[1,2], *Marine Luka*[1,2] *and Mickaël M. Ménager*[1,2*]

[1] *Inflammatory Responses and Transcriptomic Networks in Diseases, Institut Imagine, Paris, France,* [2] *Inserm U1163, Paris, France*

Dendritic cells (DCs) serve a key function in host defense, linking innate detection of microbes to activation of pathogen-specific adaptive immune responses. DCs express cell surface receptors for HIV-1 entry, but are relatively resistant to productive viral replication. They do, however, facilitate infection of co-cultured T-helper cells through a process referred to as trans-infection. We previously showed that tetraspanin 7 (TSPAN7), a transmembrane protein, is involved, through positive regulation of actin nucleation, in the transfer of HIV-1 from the dendrites of immature monocyte-derived DCs (iMDDCs) to activated CD4$^+$ T lymphocytes. Various molecular mechanisms have been described regarding HIV-1 trans-infection and seem to depend on DC maturation status. We sought to investigate the crosstalk between DC maturation status, TSPAN7 expression and trans-infection. We followed trans-infection through co-culture of iMDDCs with CD4$^+$ T lymphocytes, in the presence of CXCR4-tropic replicative-competent HIV-1 expressing GFP. T cell infection, DC maturation status and dendrite morphogenesis were assessed through time both by flow cytometry and confocal microscopy. Our previously described TSPAN7/actin nucleation-dependent mechanism of HIV-1 transfer appeared to be mostly observed during the first 20 h of co-culture experiments and to be independent of HIV replication. In the course of co-culture experiments, we observed a progressive maturation of MDDCs, correlated with a decrease in TSPAN7 expression, a drastic loss of dendrites and a change in the shape of DCs. A TSPAN7 and actin nucleation-independent mechanism of trans-infection, relying on HIV-1 replication, was then at play. We discovered that TSPAN7 expression is downregulated in response to different innate immune stimuli driving DC maturation, explaining the requirement for a TSPAN7/actin nucleation-independent mechanism of HIV transfer from mature MDDCs (mMDDCs) to T lymphocytes. As previously described, this mechanism relies on the capture of HIV-1 by the I-type lectin CD169/Siglec-1 on mMDDCs and the formation of a "big invaginated pocket" at the surface of DCs, both events being tightly regulated by DC maturation. Interestingly, in iMDDCs, although CD169/Siglec-1 can capture HIV-1, this capture does not lead to HIV-1 transfer to T lymphocytes.

Keywords: HIV-1, trans-infection, TSPAN7, actin nucleation, dendritic cell maturation, kinetic of transfer

OPEN ACCESS

Edited by:
Vasco Rodrigues, Institut Curie, France

Reviewed by:
Andrew Nicholas Harman, University of Sydney, Australia
Nicolas Ruffin, Institut Curie, France

***Correspondence:**
Mickaël M. Ménager
mickael.menager@institutimagine.org

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *26 November 2019*
Accepted: *12 February 2020*
Published: *28 February 2020*

Citation:
Perot BP, García-Paredes V, Luka M and Ménager MM (2020) Dendritic Cell Maturation Regulates TSPAN7 Function in HIV-1 Transfer to CD4$^+$ T Lymphocytes. Front. Cell. Infect. Microbiol. 10:70. doi: 10.3389/fcimb.2020.00070

INTRODUCTION

Dendritic cells (DCs) are key players in the mounting of both innate and adaptive immunity. They are present, in an immature state, in organs and can sequentially: engulf elements from their environment; sense engulfed pathogens, which may trigger inflammatory cytokine secretion and their maturation; migrate to lymph nodes and present antigens to T lymphocytes (Merad et al., 2013). Maturation leads to a decrease in antigen uptake, to an increase in major histocompatibility complex and co-stimulatory protein expression and to the secretion of pro-inflammatory cytokines (Alloatti et al., 2016). Peptide-loaded MHCs (pMHCs), co-stimulatory and inflammatory proteins all participate in the stimulation of T cells harboring a cognate receptor to the pMHC allowing the priming of an efficient adaptive immune response (Jain and Pasare, 2017).

DCs of the genital and anorectal tract mucosa are thought to bind and capture HIV-1 and to participate in translocating HIV-1 to patients' *Milieu Interieur*, thereby participating in HIV-1 infection (Ahmed et al., 2015). Interestingly, DCs have also been shown in *in vitro* and *ex vivo* settings, to increase the infection of $CD4^+$ T lymphocytes through cis-infection and trans-infection mechanisms (Wu and KewalRamani, 2006; Bertram et al., 2019a). Cis-infection is the process whereby productively-infected DCs produce virus progeny, which augments the amount of virions available to infect $CD4^+$ T lymphocytes (Bracq et al., 2018). Trans-infection (sometimes referred to as trans-enhancement) does not involve DC infection but rather the binding of HIV-1 to molecules on DC surface followed or not by the internalization of virions in non-degradative compartments within cells that may or may not communicate with the extracellular milieu (Kijewski and Gummuluru, 2015; Bracq et al., 2018). This binding and/or internalization of HIV-1, with limited infection of DCs, increases $CD4^+$ T cell infection *in vitro* and may involve immunological synapse formation (contact zone between DCs and $CD4^+$ T cells) thereby polarizing HIV-1 delivery to $CD4^+$ T cells (Bracq et al., 2018).

Human DCs express HIV-1 restriction factors among which is SAM domain and HD domain-containing protein 1 (SAMHD1), the major protein responsible for the relative resistance of DCs to HIV-1 infection (Antonucci et al., 2017). SAMHD1 is a phosphohydrolase that is capable of degrading the cellular pool of deoxynucleoside triphosphates, thereby interfering with reverse transcription (Antonucci et al., 2017). Although DCs are relatively resistant to infection by HIV-1, strong DC subtype susceptibility exists (Martin-Gayo and Yu, 2019).

Cyclic GMP-AMP synthase (cGAS) is a sensor for HIV-1 DNA, product of reverse transcription, that is expressed by DCs. DCs however do not respond to HIV-1 infection owing to the block of reverse transcription resulting from SAMHD1 presence (Landau, 2014). Interestingly, when SAMHD1 is degraded experimentally, DCs are able to sense HIV-1 and to produce type I interferons in response to HIV-1 and lose their trans-infection capacity (Manel et al., 2010). Another mechanism by which HIV-1 limits IFN induction in infected DCs and macrophages, is through Vpr and Vif-mediated limitation of TBK1 (TANK-binding kinase 1) phosphorylation, in turn limiting IFN induction (Harman et al., 2015). By limiting its detection by the innate sensors of DCs and using these cells for transfer of virions to T cells (as discussed above), the virus may evade, at least in part, the first line of defense of the immune system in mucosal tissues and use DCs as early cell reservoirs to traffic from the peripheral tissues to lymph nodes to establish and amplify infection of $CD4^+$ T cells using the trans-infection process. This hijacking of DCs by HIV-1 is sometimes referred to as the trojan horse hypothesis (Izquierdo-Useros et al., 2010).

Although strong evidence concerning the *in vivo* occurrence of HIV-1 trans-infection are lacking owing to the absence of optimal animal models and obvious difficulties to perform experiments (e.g., poor reconstitution of human myeloid compartment in humanized mouse models), *ex vivo* experiments revealed that endogenous myeloid DCs can trans-infect $CD4^+$ T cells with HIV-1 (Shen et al., 2014; Reyes-Rodriguez et al., 2016; Trifonova et al., 2018). Langerhans cells, vaginal epithelium DCs and other mucosal $CD11c^+$ DCs have also been shown to transfer HIV-1 to $CD4^+$ T lymphocytes in *ex vivo* experiments (Nasr et al., 2014; Pena-Cruz et al., 2018; Bertram et al., 2019b). Interestingly, subcutaneous injection of murine leukemia virus (MLV) or HIV-1 in mice results in their uptake by macrophages expressing CD169/Siglec-1, through interaction with this protein (Sewald et al., 2015). Importantly, MLV does not infect $CD169^+$ macrophages in mice but, when loaded with MLV and after injection, these cells participate in infecting B lymphocytes and the two cell types form immunological synapses (Sewald et al., 2015). These observations support the trojan horse hypothesis and reinforce the need for a better comprehension of the mechanisms of HIV-1 transfer from DCs to T cells.

As immature DCs (iDCs) and mature DCs (mDCs) differ in their phenotype, function and localization, as exposed above, HIV-1 may encounter them in both states (e.g., immature circulating DCs, immature and mature mucosal DCs, and mature DCs in the draining lymph nodes of HIV-1–infected individuals). Researchers have proposed different mechanisms of trans-infection from iDCs vs. mDCs. Indeed, iDCs can mediate trans-infection through the interaction of several proteins belonging to the C-type lectin receptor (CLRs) family [such as DC-SIGN, mannose receptor (MR) (also known as CD206), langerin (CD207), and syndecan-3] with HIV-1 GP120 protein (Kijewski and Gummuluru, 2015). These interactions, although not leading to potent HIV-1 fusion/entry, are involved in trans-infection by presenting virions to $CD4^+$ T cells; however, it has been proposed that the engagement of this CLRs also efficiently directs bound virions to endolysosomal compartment for degradation (Kijewski and Gummuluru, 2015). DCIR, which is highly expressed by immature monocyte-derived DCs (iMDDCs) in particular, was shown to bind HIV-1 particle leading to an increase of both cis- and trans-infection potential of DCs (Lambert et al., 2008).

In mDCs, CLRs are downregulated while the I-type lectin CD169/Siglec-1 is highly upregulated and was shown to be involved in HIV-1 uptake and subsequent cis- and trans-infection of $CD4^+$ T cell through interaction with a-2,3-linked sialic

acid residues on the glycosphingolipid GM3 (host cell-derived glycosphingolipid that is incorporated in HIV-1 virions) (Puryear et al., 2013; Akiyama et al., 2017). The exosome secretion pathway has also been proposed as a mechanism for trans-infection by mature DCs (Kijewski and Gummuluru, 2015).

Aiming at better understanding the mechanisms at play during trans-infection, we previously demonstrated that tetraspanin 7 (TSPAN7), a transmembrane protein, is involved, through positive regulation of actin nucleation, in the transfer of HIV-1 from the dendrites of iMDDCs to activated $CD4^+$ T lymphocytes without HIV-1 internalization (Ménager and Littman, 2016). TSPANs form a large family of transmembrane proteins that play a scaffolding role, are organized in TSPAN-enriched microdomains (TEMs) and are involved in several biological processes such as cell migration, adhesion and virus entry in cells (Saiz et al., 2018). Some TSPANs, such as TSPAN6 and CD9, have been shown to interfere with the detection of viruses by cells through direct interaction with proteins involved in sensing of PAMPs (Saiz et al., 2018). Other published functions are HIV-1 entry regulation (e.g., CD63 regulates CXCR4 HIV co-receptor expression on cell surface), reverse transcription (e.g., CD81 regulates SAMHD1 turnover), assembly and trans-infection (Suárez et al., 2018). TSPAN7, in addition to its recently discovered role in control of HIV transfer through regulation of actin nucleation (Ménager and Littman, 2016), has been found to be mutated in some forms of X-linked intellectual disabilities, which may be explained by the role of this protein in neuronal morphogenesis (Bassani et al., 2012; Usardi et al., 2017). Indeed, TSPAN7 regulates dendritic spines and filopodia formation in neurons and promotes axonal branching (Bassani et al., 2012; Usardi et al., 2017). This protein is also believed to play a role in cancer as it promotes migration and proliferation of lung cancer cells and limits myeloma tumor development (Cheong et al., 2015; Wang et al., 2018). Of note, TSPAN7, which is highly expressed in pancreatic beta cells, is a target for autoantibodies in patients with type 1 diabetes although contribution of these antibodies in the development of pathology is still unclear (McLaughlin et al., 2016).

Using a CCR5-tropic HIV-1 strain (able to infect both DCs and $CD4^+$ T lymphocytes, unlike CXCR4-tropic strains, which do not infect DCs), it has been shown that transfer of HIV-1 from MDDCs occurs in a replication-independent mechanisms at first (in the first 20 h of co-culture with $CD4^+$ T lymphocytes) and is taken over by a replication-dependent phase (cis-infection phase) partially relying on HIV replication in MDDCs (Turville et al., 2004). This two-phase model, was later confirmed in *ex vivo* derived Langerhans cells (Harman et al., 2009; Nasr et al., 2014).

Here we show that the TSPAN7/actin nucleation dependent transfer of HIV-1 from the dendrites of iMDDCs, is mostly taking place within the first 20 h of co-culture between $CD4^+$ T cells, MDDCs and a CXCR4-tropic replicative-competent HIV-1, expressing GFP in the open reading frame of Nef (X4–HIV-1–GFP), independently of HIV-1 replication. Of note, a CXCR4-tropic virus strain was used to focus on trans-infection occurrence rather than cis-infection as DCs are particularly resistant to infection by these strains (Wu and KewalRamani, 2006). In the course of co-culture experiments, a progressive maturation of MDDCs was observed, leading to a decrease in TSPAN7 expression and to a subsequent loss of dendrites and changes in DC shape. At this stage, a TSPAN7 and actin nucleation-independent mechanism, relying on HIV-1 replication, is at play. We also investigated the contribution of TSPAN7/actin nucleation in HIV-1 transfer from LPS-matured MDDCs and found that, as previously described, this mechanism relies on the capture of HIV-1 by the I-type lectin CD169/Siglec-1 and the formation of big invaginated pockets at the surface of DCs, both events tightly regulated by DC maturation. Interestingly, in iMDDCs, although HIV-1 can be captured by CD169/Siglec-1, this capture does not lead to HIV-1 transfer to T lymphocytes.

RESULTS

Tetraspanin 7 and Actin Nucleation Are Involved in Early Replication-Independent Steps of HIV-1 Transfer From Immature MDDCs to Activated $CD4^+$ T Lymphocytes

To monitor HIV-1 transfer from iMDDCs to $CD4^+$ T lymphocytes and the contribution of TSPAN7 relative to the kinetic of transfer and HIV-1 replication, we used an experimental model of co-culture between MDDCs differentiated from circulating monocytes of healthy donors, autologous $CD4^+$ T lymphocytes activated by PHA and Il-2 (at a 1:1 ratio) with a replicative-competent CXCR4-tropic HIV-1 encoding the GFP in place of Nef (X4–HIV-1–GFP) (Ménager and Littman, 2016) in the presence or absence of an HIV protease inhibitor (Nelfinavir, NFV), to block HIV replication (**Figure 1A**, **Figure S1A**). We observed that most of the T lymphocytes (around 75% of the total number of GFP-expressing T cells, measured after 40 h) were becoming infected during the first 20 h of co-culture, independently of NFV treatment (**Figures 1B,C**, **Figure S1B**). A second phase of HIV-1 transfer, which was dependent on HIV-1 replication (blocked by NFV) was identified during the last 20 h of the co-culture (**Figures 1B,C**, **Figure S1B**). As we previously demonstrated (Ménager and Littman, 2016), TSPAN7 impacts HIV-1 transfer from iMDDCs to $CD4^+$ T cells and most of its effect was observed during the first 20 h, independently of HIV-1 replication (**Figure 1D**, **Figures S1C–E**). The Actin Related Protein 2/3 (ARP2/3) inhibitor CK-666 (which can efficiently reduce HIV-1 transfer by blocking actin nucleation; Ménager and Littman, 2016) has the same kinetic of action on X4–HIV-1–GFP transfer as TSPAN7, with most of its effect observed during the first 20 h of co-culture (Ménager and Littman, 2016; **Figure 1E**, **Figure S1F**).

Taken together, these results suggest that the previously reported role of TSPAN7 on HIV-1 transfer, through positive regulation of actin nucleation, is mostly effective during the first 20 h of co-culture, in a replication-independent phase, during which most of the T cells are getting infected. TSPAN7 has limited, if any effect on a second phase (from 20 to 40 h of co-culture) of T cells infection, which relies on HIV-1 replication.

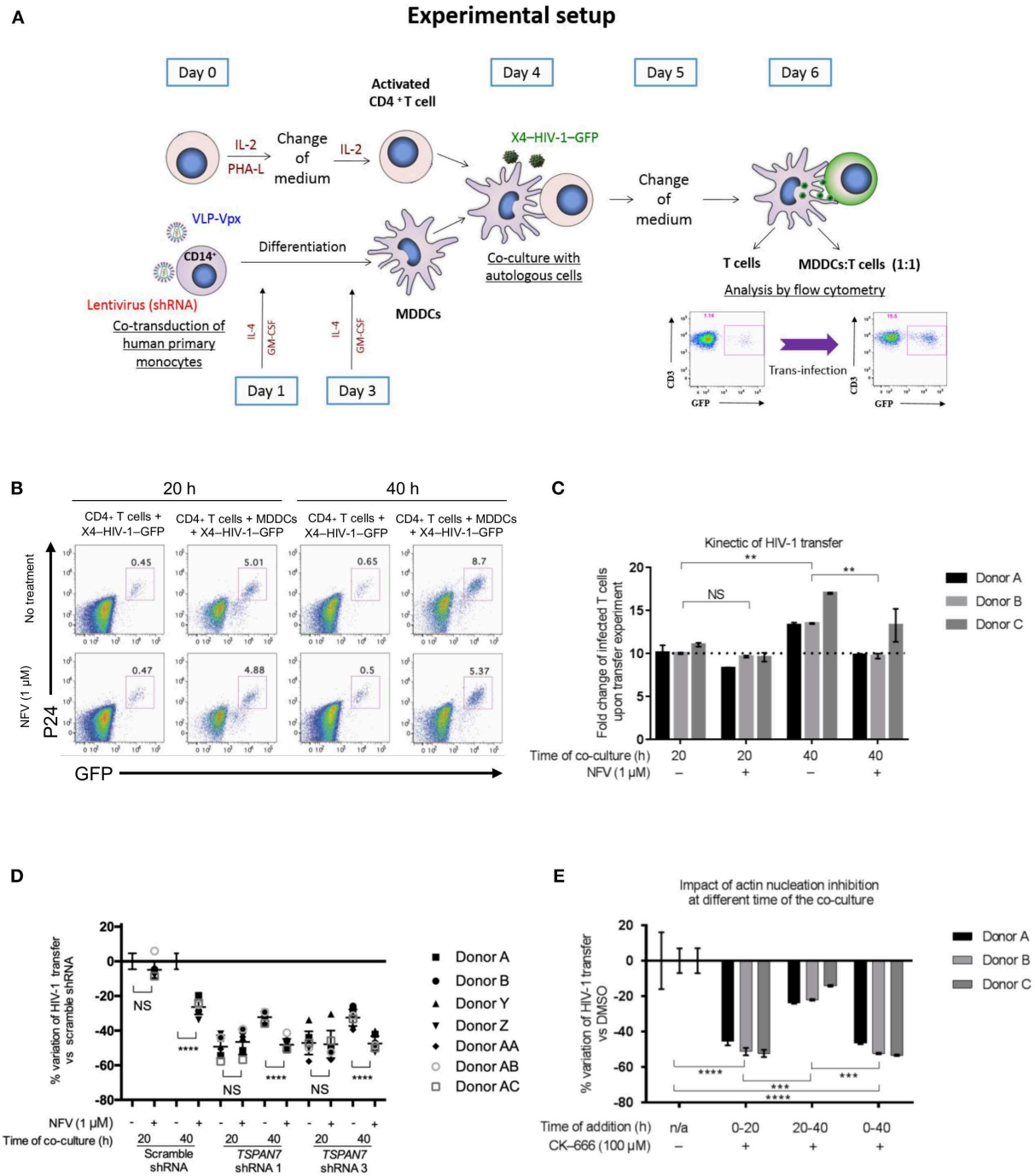


Figure 1

FIGURE 1 | Kinetics of TSPAN7/actin nucleation-dependent mechanism of HIV-1 transfer from immature MDDCs to CD4$^+$ T lymphocytes. **(A)** Scheme depicting the experimental layout used throughout this manuscript to assess MDDC-mediated HIV-1 trans-infection of CD4$^+$ T cells. MDDC, monocyte-derived dendritic cell; VLP, virus-like particles; IL-2, interleukin-2; PHA-L, phytohaemagglutinin/leucoagglutinin; IL-4, interleukin-4; GM-CSF, granulocytes-macrophage colony-stimulating factor. **(B)** Flow cytometry plots showing GFP and P24 expression levels in cells pre-gated on SSC FSC, living CD3$^+$ singlets, 20 or 40 h after the start of co-culture; percentage of CD4$^+$ T cells infected by X4–HIV-1–GFP (GFP$^+$ and P24$^+$) is shown above the gates. Top panel: T cells were cultured with X4–HIV-1–GFP for 20 or 40 h in the absence or presence of MDDCs at a 1:1 ratio and with (bottom panel) or without (top panel) 1 µM Nelfinavir (NFV; an HIV protease inhibitor). **(C)** Bar graph showing the fold increase in T cell infection by X4–HIV-1–GFP following co-culture with MDDCs as compared to CD4$^+$ T cells alone [i.e., % infected T cells (GFP$^+$ P24$^+$) cultured with MDDCs/% infected T cells without MDDCs] as measured by flow cytometry (see **B**). For example, the first bar graph on the left means that, for donor A, 10 times more CD4$^+$ T cells were infected when MDDCs were present than when T cells were cultured alone with X4–HIV-1–GFP. Mean ± Standard

(Continued)

FIGURE 1 | Deviation (SD) of technical triplicates are presented for three healthy blood donors **(D)** Percentage of variation of X4–HIV-1–GFP transfer with MDDCs transduced with two different shRNAs against *TSPAN7* (shRNA 1 and 3) previously validated (Ménager and Littman, 2016) vs. a non-specific shRNA (scramble shRNA), observed by flow cytometry at 20 or 40 h of co-culture. Dot plots on the right of each time point represent variation of transfer when, in addition to knockdown, 1 μM of NFV was added to the co-culture. Mean ± Standard Deviation (SD) of seven healthy blood donors in the context of 4 independent experiments. **(E)** Bar graph showing the percent variation of X4–HIV-1–GFP transfer (based on fold increase in $CD4^+$ T cell infection) between condition where DMSO (drug carrier) was used [0% variation ± Standard Deviation (SD)] vs. 100 μM CK-666 to inhibit the ARP2/3 complex and actin nucleation. CK-666 was added during the first or the last 20 h of co-culture or during the entire experiment. The experiment was performed in three unrelated healthy blood donors, with the mean ± SD of technical triplicates in each condition and for each donor. This experiment is representative of three independent experiments. **(C–E)** NS, not significant. $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

A Change in Shape and Number of Dendrites Is Observed Among MDDCs During Co-culture Experiments

As the role of TSPAN7 in HIV-1 transfer was reported to be through actin-rich dendrites formation (Ménager and Littman, 2016), we next sought to monitor MDDCs morphology and dendrite formation at 4, 20, and 40 h following initiation of HIV-1 trans-infection experiments. Through actin staining, we observed a drastic change in MDDC morphology between the first and second 20 h of co-culture (**Figure 2A**). An increase of MDDC size in general and of their cytosol in particular, correlated with a drastic loss of dendrites, could be seen during the last 20 h of co-culture (**Figure 2A**). Of note, the morphology of MDDCs at the end of the transfer experiments resembles the one observed in MDDCs following *TSPAN7* knockdown by shRNA (**Figure 2B**). Stimulation of MDDCs with lipopolysaccharide (LPS) a TLR4 agonist, also resulted in a profound cell morphology and shape modification (**Figure 2C**). In order to quantify these changes in morphology, we used a circularity index based on changes of perimeter and area (**Figure S2A**) and quantified the length and number of dendrites observed by confocal microscopy. As a result, between 20 and 40 h, an increase of MDDCs circularity index could be quantified and was reaching levels observed following decrease of *TSPAN7* expression through shRNA or after LPS treatment (**Figure S2B**). This change in shape observed during the last 20 h was accompanied by a drastic reduction of size and number of dendrites per cell (**Figures S2C–E**).

Tetraspanin 7 Expression Is Downregulated During Dendritic Cell Maturation

After studying the kinetic of HIV-1 transfer and changes in cell morphology, we observed, by flow cytometry, an increase in CD86 expression at the surface of MDDCs (an indicator of DC maturation), which was particularly pronounced during the last 20 h of the co-culture experiments (**Figure 3A**, **Figures S3A,B**). We next sought to investigate the impact of MDDC maturation status on *TSPAN7* expression, following various innate immune stimuli, such as LPS, polyinosinic:polycytidylic acid [poly(I:C)], a synthetic double-stranded RNA-mimicking (TLR3 agonist). We also induced MDDCs maturation through productive infection of iMDDCs with vesicular stomatitis virus-pseudotyped HIV-1 expressing GFP (VSV-G–HIV-1–GFP), delivered together with virus-like particle carrying the HIV-2 protein Vpx, which allows SAMHD1 degradation thereby facilitating HIV RNA reverse transcription, innate sensing by cGAS, type I interferon production and subsequent MDDC maturation (Manel et al., 2010; **Figure 3B**). As previously reported (Ménager and Littman, 2016), we detected an increase in the expression of *TSPAN7* during the differentiation of monocytes into iMDDCs, which was drastically shutdown upon MDDC maturation through various innate stimuli (**Figures 3C,D**). This shutdown of *TSPAN7* expression was also observed after co-culture between iMDDCs and the X4–HIV-1–GFP used during transfer experiments (**Figures 3C,D**). A specific downregulation of *TSPAN7* expression, as compared to its paralog *TSPAN6* was confirmed by bulk RNA sequencing following DC maturation, in response to the same innate stimuli [LPS, poly(I:C) and VSV-G–HIV-1–GFP; **Figure S3C**].

These results suggest that the changes in cell morphology and in the number of dendrites observed during the last 20 h of HIV-1 transfer experiments, are due to a decrease in actin nucleation activity, driven by the decrease of TSPAN7 expression, observed subsequently to MDDCs maturation (**Figure S3D**).

Tetraspanin 7 and Actin Nucleation Have Limited Impact on Trans-infection of T Lymphocytes by Mature MDDCs

In the next set of experiments, we interrogated actin nucleation and TSPAN7 function in HIV-1 transfer from LPS-matured MDDCs (mMDDCs) to activated $CD4^+$ T lymphocytes. Our results show only a limited contribution of actin nucleation and TSPAN7 when HIV-1 was transferred from mature MDDCs (**Figures 4A,B**, **Figures S4A,B**). As previously described (McDonald, 2010), mMDDCs, in our co-culture experiments, were capable of infecting more T lymphocytes through HIV-1 transfer, as compared to iMDDCs, although timing of MDDCs maturation seemed to be key (**Figure 4C**). Stimulating MDDCs, with LPS 48 h before HIV-1 transfer experiments was required to obtain optimal HIV-1 transfer, and correlated with the appearance of large HIV-1/CD169 (Siglec-1) puncta at the surface of MDDCs, resembling the "big invaginated pocket," previously reported to be responsible for HIV-1 transfer (**Figure 4D**, middle panel; McDonald, 2010). Adding LPS at the same time of co-culture experiments, resulted in the loss of dendrites, with a limited formation of Siglec-1/CD169-puncta and an overall decrease

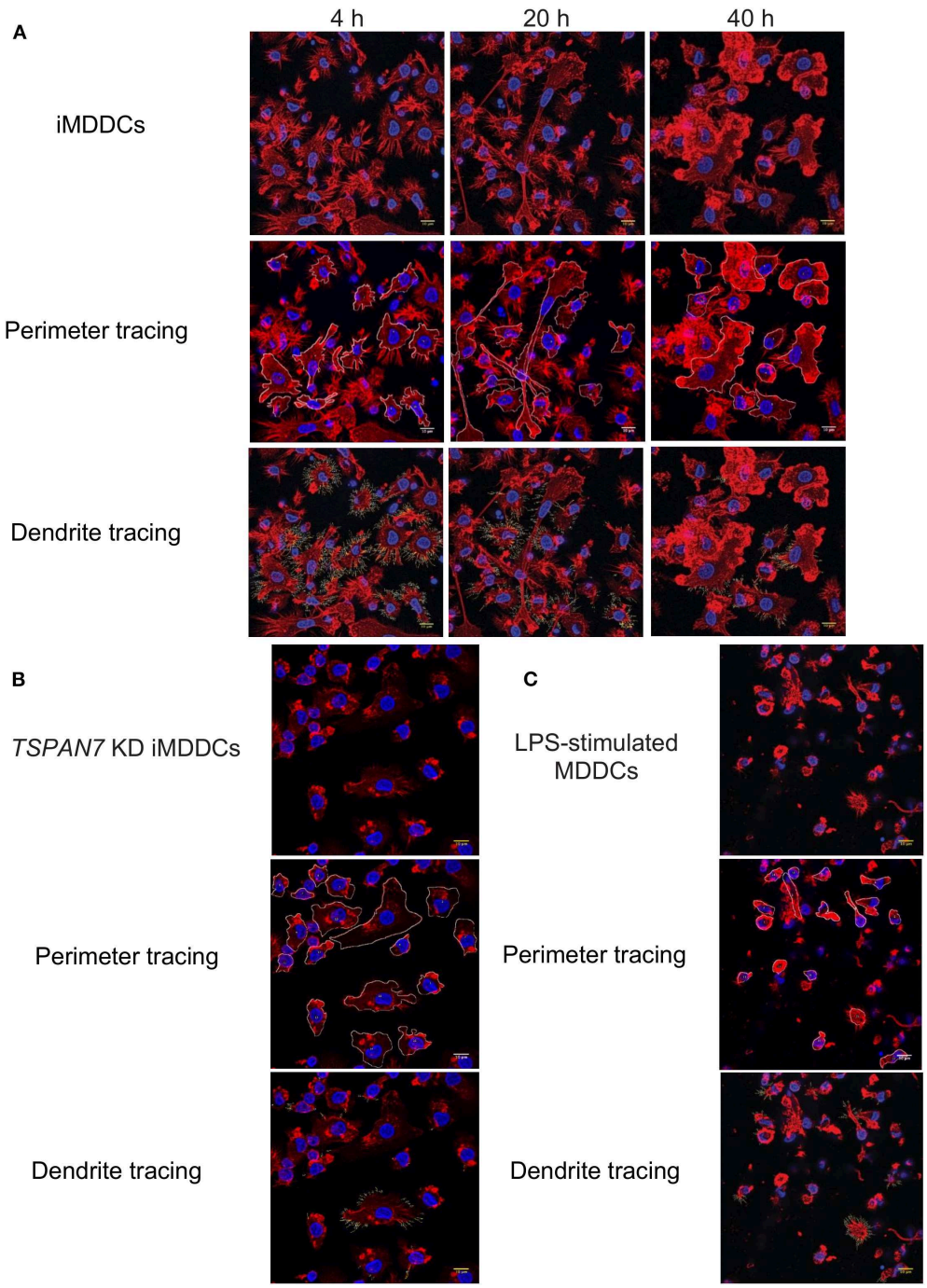


FIGURE 2 | Change of shape and loss of dendrites for MDDCs during HIV-1 transfer experiments. **(A)** Four hundred nanometers of Z-stacks showing by confocal microscopy, iMDDCs and $CD4^+$T cells co-cultured with X4–HIV-1–iGag-GFP for 4, 20, and 40 h. Actin was detected in red following phalloidin staining and nuclei stained in blue by DAPI. Using ImageJ software, perimeter of each MDDC was manually traced in white (middle panel) alongside dendrites (bottom panel). **(B)** Same as in **(A)** with iMDDCs transduced with *TSPAN7* shRNA. **(C)** Same as in **(A)** with MDDCs stimulated with 1 μg/ml LPS for 24 h before analysis by confocal microscopy. **(A–C)** Data are from a donor representative of five unrelated donors studied in three independent experiments.

in X4–HIV-1–GFP transfer when compared to iMDDCs (**Figure 4D**, right panel; **Figures S4D,E**). Although LPS-treated MDDCs were able to capture at least 10 times more viruses than their immature counterparts (**Figure S4C**), the TSPAN7-independent mechanism of HIV transfer appeared to be 5–10 times less efficient than the one observed in iMDDCs with, at most, an increase of only 50% (1.5-fold) in infected T cells (**Figure S4D**).

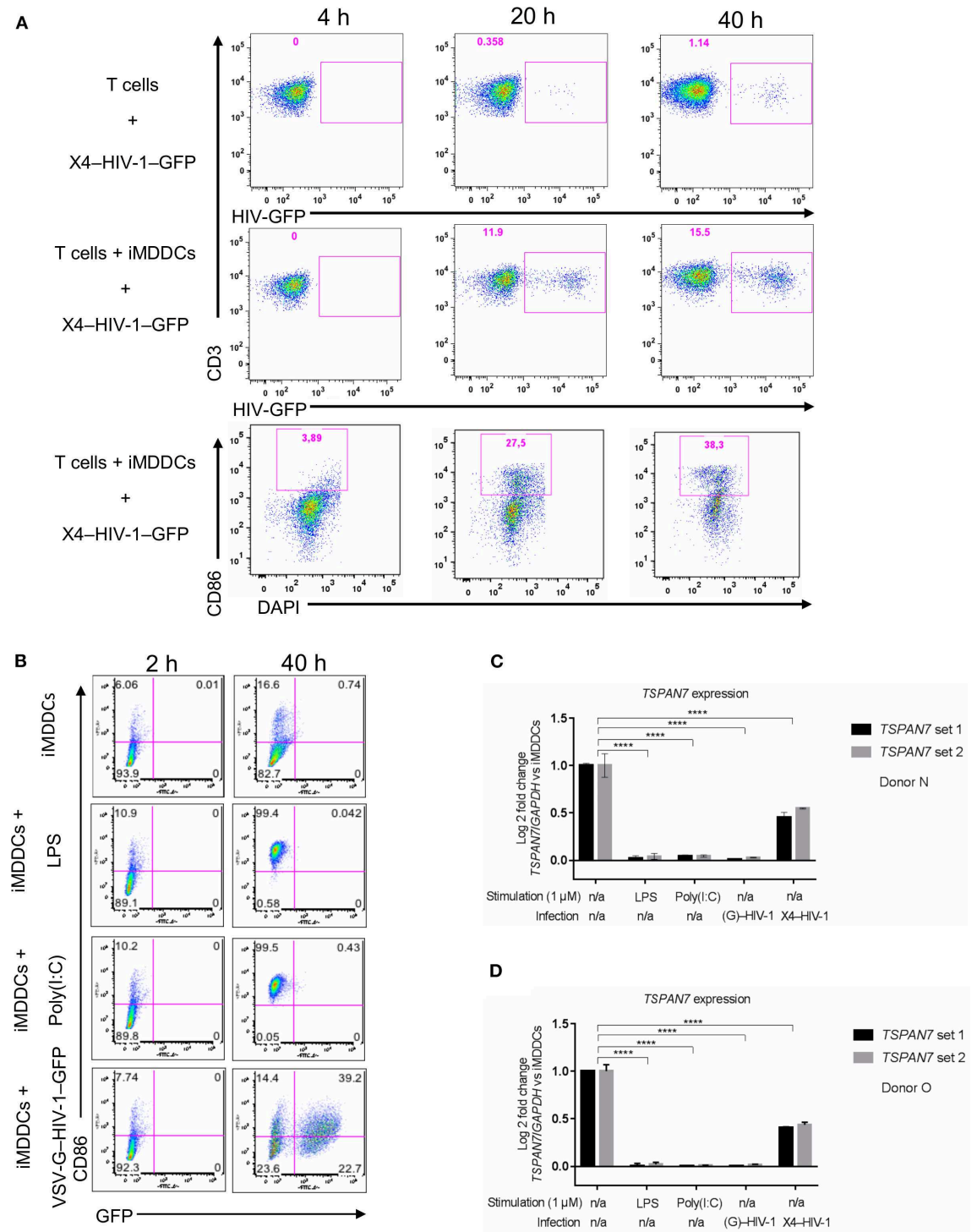


FIGURE 3 | Impact of MDDC maturation on *TSPAN7* expression and function. **(A)** Flow cytometry plots showing CD4$^+$ T cells infected with X4–HIV-1–GFP, in the absence (top panel) or presence (middle panel) of iMDDCs. In the bottom panel, the maturation state of MDDCs was evaluated by gating on CD86 expression, upon co-culture with CD4$^+$ T cells and X4–HIV-1–GFP measured at 4, 20, and 40 h. Cells were pre-gated as follows: SSC FSC, singlets, living cells, CD3$^+$ T cells (top and middle panel), or DC-SIGN$^+$ MDDCs (bottom panel) **(B)** Maturation state of MDDCs at 2 and 40 h following treatment with Poly(I:C) (1 µg/ml), LPS (1 µg/ml) or infection with a VSV-G-pseudotyped single-round HIV-1–GFP (VSV–G-HIV-1-GFP), represented as (G)–HIV-1 on the figure, in the presence of the viral protein Vpx to allow infection and innate sensing. CD86 monitoring by flow cytometry was used to assess maturation status and GFP expression for HIV replication. MDDCs were pre-gated following the same gating strategy as mentioned in **(A)**. **(C,D)** Quantitative PCR (qPCR) measurement of the Log2 fold change of *TSPAN7* mRNA normalized by the housekeeping gene *GAPDH*, 40 h after MDDCs stimulation by LPS (1 µg/ml)/Poly(I:C) (1 µg/ml), infection with VSV-G–HIV-1–GFP + Vpx or in the

(Continued)

FIGURE 3 | presence of X4–HIV-1–GFP (represented as X4–HIV-1) or left unstimulated. Fold change expression was normalized to the level of *TSPAN7* detected in iMDDCs. Experiments were performed 3 times in 6 independent blood donors. Donor N in **(C)** and donor O in **(D)** are representative of an experiment performed in six unrelated blood donors in the context of six independent experiments, using two different sets of qPCR primers to detect specific expression of *TSPAN7*. NS, not significant. $^{****}p < 0.0001$.

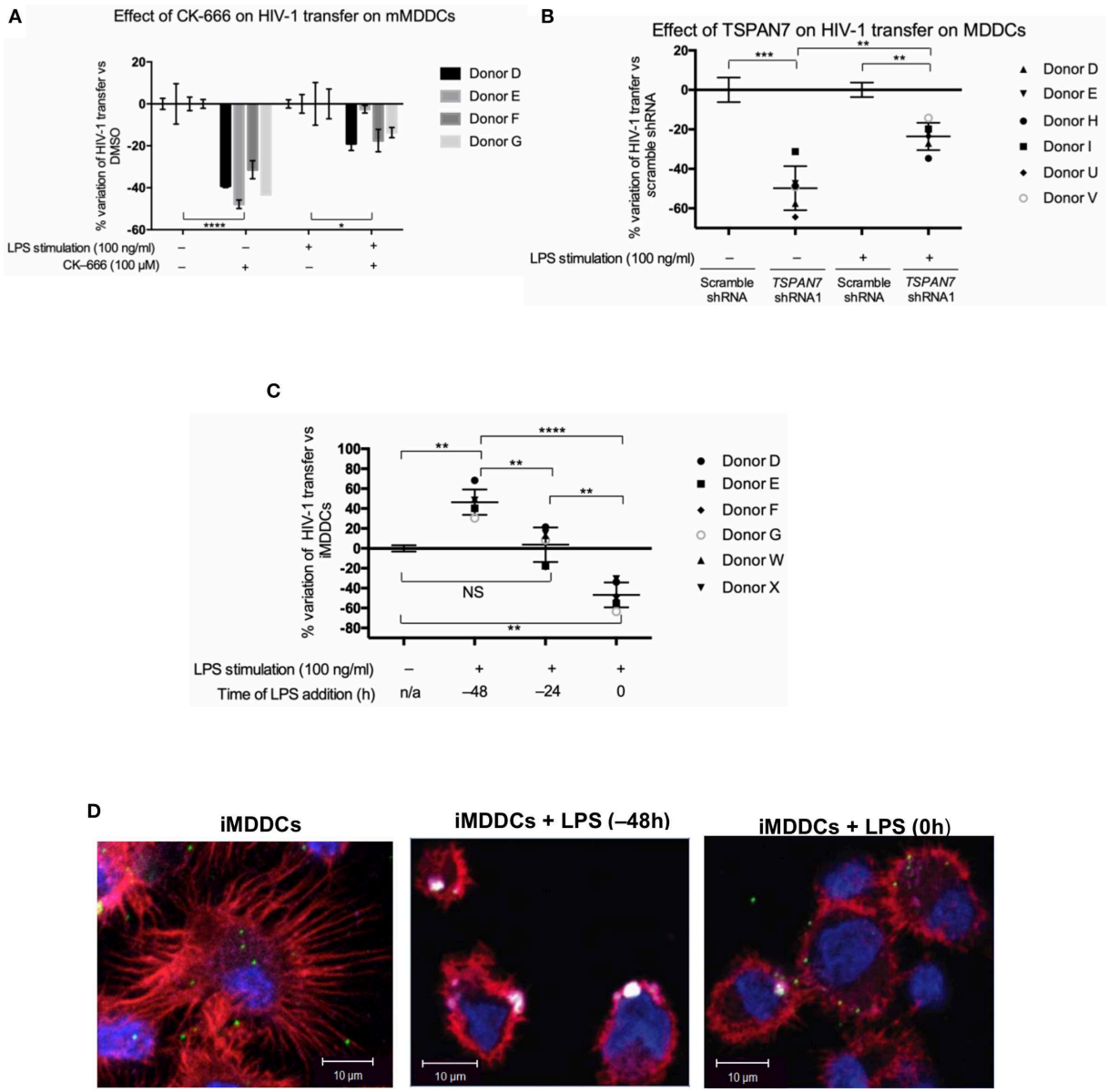


FIGURE 4 | Role of TSPAN7 and actin nucleation in HIV transfer from mature MDDCs to T cells. **(A)** Percentage change of HIV-1 transfer following inhibition of actin nucleation by CK-666 (100 µM), compared to DMSO-treated cells; in immature MDDCs (iMDDCs) and MDDCs matured by 100 ng/ml LPS, 48 h before transfer experiment (mMDDCs). Mean ± SD of triplicates for four different healthy blood donors are represented. **(B)** Percentage change of X4–HIV-1–GFP transfer following *TSPAN7* knockdown compared to scramble shRNA-expressing cells, in iMDDCs and MDDCs matured by 100 ng/ml LPS, 48 h before transfer experiments. Mean ± SD of six different healthy blood donors. **(C)** Percentage change of X4–HIV-1–GFP transfer according to kinetic of maturation of MDDCs [LPS added 48 or 24 h before co-culture or at the time of co-culture (time 0)], compared to iMDDCs. Mean ± SD of six different healthy blood donors. **(A–C)** NS, not significant. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$. **(D)** Confocal microscopy images of MDDCs at different stages of maturation: iMDDCs (left panel); mMDDCs stimulated by LPS (100 ng/ml) 48 h before (middle panel) or at the same time of start of co-culture (right panel). Actin filaments and nuclei were stained with phalloidin (red) and DAPI (blue), respectively and Siglec-1/CD169 was detected using an anti-CD169 antibody conjugated to APC (magenta). Incoming X4–HIV-1–Gag-iGFP particles are seen in green based on (GFP presence). Four hundred nanometers of Z-stacks were taken 20 h after the start of the co-culture with $CD4^{+}$ T cells and X4–HIV-1–Gag-iGFP. The pictures presented here are from a representative donor from four unrelated blood donors.

In Co-culture Experiments, CD169/Siglec-1 Mediates Transfer of HIV-1 From Mature MDDCs but a Different Receptor Is Required for Optimal Transfer From Immature Cells

CD169/Siglec-1 was strongly induced at the surface of MDDCs following LPS stimulation prior to co-culture experiments, whereas a small decrease of DC-SIGN was observed (**Figure 5A**, **Figure S5A**). Using anti-Siglec-1/CD169 neutralizing antibodies, we were able to block a significant portion of HIV-1 transfer from fully mature MDDCs (i.e., MDDCs stimulated by LPS 48 h prior to co-culture experiments; **Figure 5B**, **Figures S5B,C**). In comparison, although blocking Siglec-1/CD169 at the cell surface with antibodies, led to an almost 4-fold decrease in HIV-1 capture by iMDDCs, it had no impact on HIV-1 transfer (**Figure 5B**, **Figure S5D**). Our results suggest that Siglec-1/CD169, although expressed in iMDDCs (at a lower level), cannot efficiently contribute to the transfer of HIV-1 from iMDDCs to $CD4^+$ T cells. In comparison, blocking CD169/Siglec-1 in mature MDDCs can have a synergistic effect on the limited impact of knocking down TSPAN7 expression, suggesting, once again, that a Siglec-1/CD169–dependent but TSPAN7-independent mechanism is at play during HIV-1 transfer from mMDDCs to $CD4^+$ T lymphocytes (**Figure 5C**). In line with these results, no colocalization was monitored between CD169 and X4–HIV-1–Gag-iGFP captured at the tip of actin-rich dendrites in immature MDDCs, as compared to the colocalization seen in the big invaginated pocket described in mMDDCs (**Figure 5D, Figures S5E–G**). Our results support the requirement for another HIV-1 receptor, different from Siglec-1, for the capture of HIV-1, by iMDDCs, at the tips of actin-rich dendrites, followed by subsequent efficient transfer to $CD4^+$ T lymphocytes.

DISCUSSION

By studying the kinetic of HIV-1 transfer from iMDDCs to $CD4^+$ T lymphocytes, we revealed that the previously described role of TSPAN7 and actin nucleation in dendrites formation, required for an efficient transfer, was mostly happening during the first 20 h of co-culture experiments (see proposed model in **Figure 6**). A second phase of HIV-1 transfer, observed during the next 20 h of co-culture experiments and accounting for less than a quarter of total T cells infection, is happening in an HIV replication-dependent manner and seems to rely less on TSPAN7 and actin nucleation. We observed that this second phase of HIV-1 transfer was accompanied by a drastic change in DC size and a reduction of actin-rich dendrites due to a decrease in TSPAN7 expression subsequent to DC maturation (**Figure 6**). We confirmed that the transfer of HIV-1 from LPS-matured MDDCs, in co-culture experiments, as previously described in other experimental settings (Landau, 2014), is mostly dependent on the capture of HIV-1 by CD169/Siglec-1 and the formation of a "big invaginated pocket," rather than through TSPAN7 and actin nucleation. We therefore revealed and confirmed the existence of at least two different mechanisms of HIV-1 transfer in co-culture experiments, depending on MDDCs maturation status: (a) a TSPAN7 and actin nucleation dependent and CD169/Siglec-1–independent mechanism in immature cells vs. (b) a CD169/Siglec-1–dependent, and less TSPAN7 and actin nucleation-dependent process in LPS-matured MDDCs (**Figure 6**). Of note, although the same level of CD169/Siglec-1 induction at the cell surface following MDDCs maturation with LPS during 24 or 48 h prior to transfer experiments, a strong CD169/Siglec-1-dependent transfer is only measured when maturation is initiated 48 h before co-culture experiments. Based on our observations and quantifications performed by confocal microscopy, we believe that this may reflect an incomplete formation of the big invaginated pocket proposed previously (McDonald, 2010), after only 24 h LPS treatment, rather than a weaker induction of CD169/Siglec-1 expression.

We confirmed that, in our co-culture experiments, as previously demonstrated (McDonald, 2010), mMDDCs can transfer more HIV-1 to activated T lymphocytes than iMDDCs. However, although mMDDCs capture at least 10 times more viral particles, the infection of T cells is only increasing by 1.5-fold, suggesting that the mechanism of transfer used by iMDDCs, through actin-rich dendrites is far more efficient than the CD169/Siglec-1–dependent big invaginated pocket mechanism.

In our experimental settings, to study the transfer of HIV-1 from DCs to T cells, instead of first loading DCs with HIV-1, washing away unbound viruses and co-culturing with activated $CD4^+$ T cells, we have chosen, as we previously described, to co-culture at the same time, replicative-competent HIV-1, MDDCs and T cells (Ménager and Littman, 2016). We think that this model can better account for situations potentially encountered at mucosal surfaces where few HIV-1 particles may be captured by iMDDCs actin-rich dendrites and efficiently transferred from the tips of the dendrites to $CD4^+$ T cells present in the surrounding areas. Virological synapses would then be established creating an environment where DCs, T cells and HIV-1 are dynamically interacting altogether.

As we did previously, we used DCs derived from human primary monocytes obtained from healthy donors, as an *in vitro* model to study HIV-1 transfer (Ménager and Littman, 2016). Compared to conventional circulating DCs isolated from blood (cDC1, cDC2), mucosal DCs such as Langherans cells and the more recently describe circulating pre-DCs (which may play an important role in HIV-1 physiopathology; Ruffin et al., 2019), MDDCs can be obtained in large quantities and are easier to transduce with lentiviruses. Although it would be really interesting to compare the roles played by the different subsets of isolated blood DCs, they are quite susceptible to experimental manipulation, and studying them in their immature state represents quite a challenge. Recent data indicate that pre-DCs may be one of the key circulating myeloid cell subsets capable of productive infection and trans-infection as they constitutively express CD169/Siglec-1 (Ruffin et al., 2019); as such, they may constitute a promising cell type to investigate when studying trans-infection. Anyhow, in non-inflamed conditions, it is thought that some DC subsets from the dermis and the intestine originate from monocytes, and

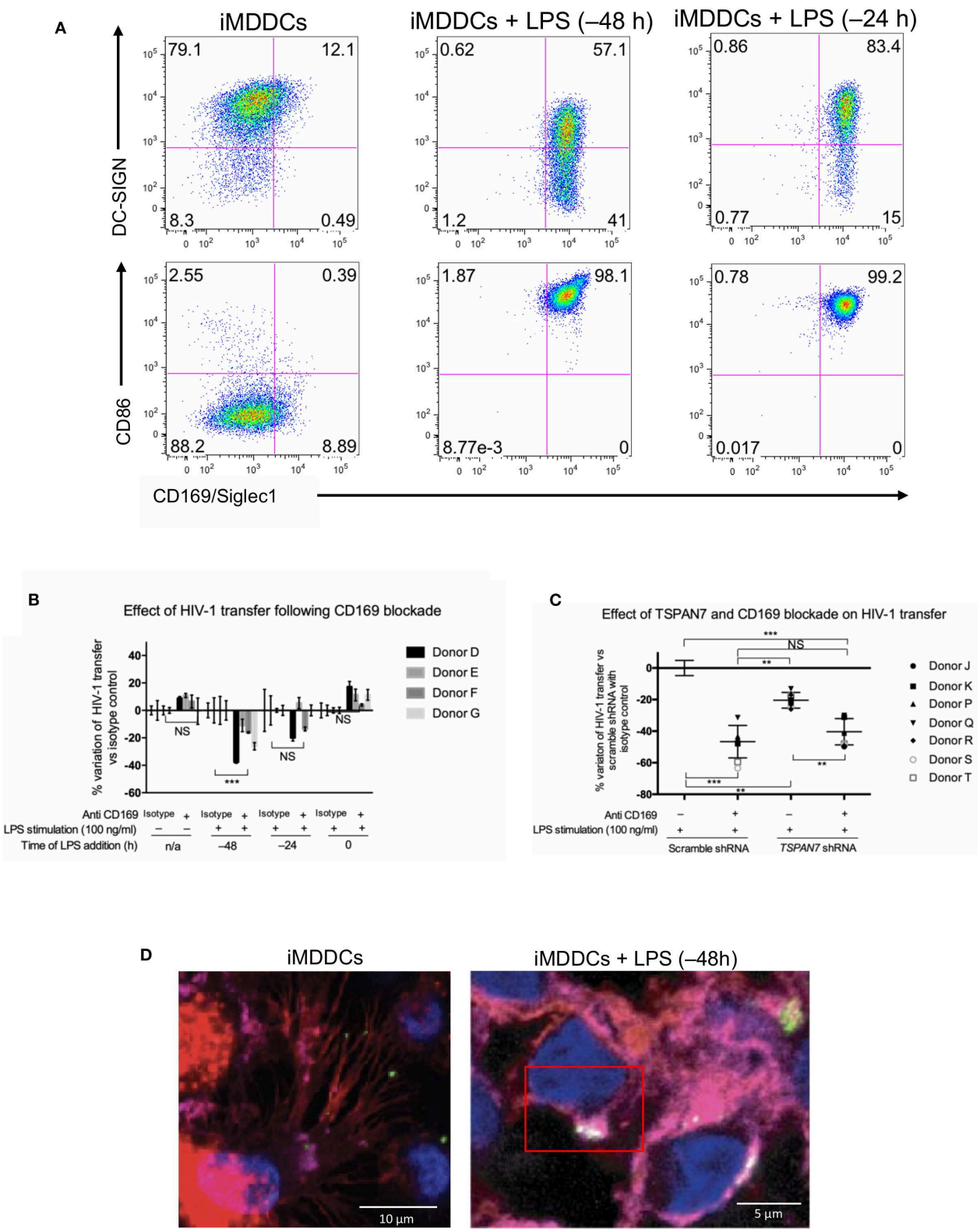


FIGURE 5 | CD169, as an HIV-1 receptor, mostly impacts transfer from mature MDDCs rather than immature MDDCs. **(A)** Flow cytometry plots showing CD86, DC-SIGN, and CD169 expression levels on MDDCs (pre-gated on SSC FSC, living cells, $CD3^{-}$ cells and singlets). Panels show the expression of these proteins in iMDDCs (left panel) and MDDCs with LPS pretreatment at 100 ng/ml for 48 or 24 h before co-culture (middle and right panels, respectively). **(B)** Percentage of

(Continued)

FIGURE 5 | variation of HIV-1 transfer when using iMDDCs or LPS-treated MDDCs (100 ng/ml LPS for different lengths of time) incubated with a blocking antibody against CD169 as compared to an isotype control for each condition. Results are displayed for 4 different blood donors with the mean ± SD of technical triplicates. **(C)** Percent of variation in HIV-1 transfer to assess the impact of blocking CD169 and *TSPAN7* knockdown as compared to scramble shRNA on MDDCs matured with LPS for 48 h treated by an isotype control. Mean ± SD of seven different blood donors in 4 experiments. **(B,C)** NS, not significant. $^{**}p < 0.01$; $^{***}p < 0.001$. **(D)** Confocal microscopy images of iMDDCs (left panel) and mature MDDCs (mMDDCs) right panel, to assess the degree of colocalization between CD169 (magenta) and incoming X4–HIV-1–Gag-iGFP (green). Actin filaments and nuclei were stained with phalloidin (red) and DAPI (blue). Four hundred nanometers of Z-stacks were taken 40 h after the start of the co-culture with $CD4^+$ T cells and X4–HIV-1–Gag-iGFP. The pictures presented here are from a representative donor from four unrelated blood donors.

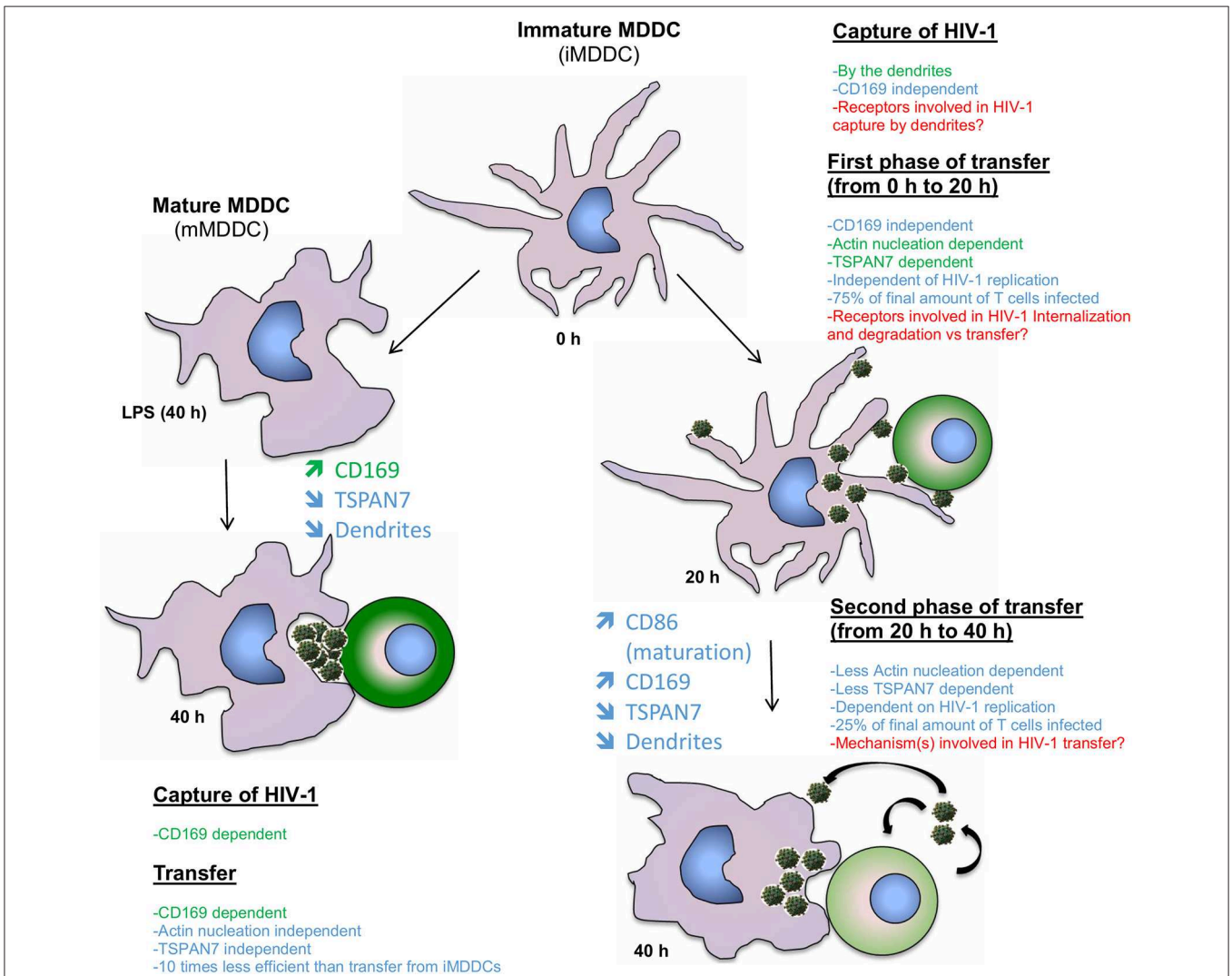


FIGURE 6 | Model comparing the contribution of TSPAN7 and actin nucleation during the different identified phases of HIV transfer when performing co-culture experiments with iMDDCs vs. LPS-matured MDDCs. On the left: mechanism showing the transfer of HIV-1 from LPS-matured MDDCs to $CD4^+$ T lymphocytes; as described in the literature, the transfer relies mostly on the capture of HIV-1 by Siglec-1/CD169 and the formation of a big invaginated pocket. TSPAN7 and actin nucleation roles seem rather limited. On the right, a first phase of transfer, dependent on TSPAN7, actin nucleation and dendrite formation is observed during the first 20 h of co-culture with iMDDCs. Seventy-five percent of the transfer is taking place during this first phase, independently of HIV-1 replication and capture by CD169/Siglec-1. A second phase of transfer, less dependent on TSPAN7 and actin nucleation, but dependent on HIV-1 replication is observed during the last 20 h of co-culture. Of note, a decrease of *TSPAN7* expression correlated with a maturation of MDDCs was detected after 20 h of co-culture and leads to a decrease in actin nucleation, inducing a shortening of dendrite and a change in shape of the cells. Previously reported scientific data are shown in green and the new results presented in this manuscript are in blue. Points that will require further investigation in this model are stated in red.

that, in inflamed tissues, inflammatory DCs are generated from monocytes (Guilliams et al., 2014). MDDCs therefore represent putative DC subtypes that may encounter HIV-1 in the case of infection and in the chronic phase of infection, which leads to persistent inflammation, even under antiretroviral therapy (ART) (Zicari et al., 2019). Of note, *ex vivo* generated MDDCs were

shown to be transcriptionally similar to $CD14^+$ dermal myeloid cells, which were first thought to be a subtype of DCs, but have been recently demonstrated to be short-lived monocyte-derived macrophages (Harman et al., 2013; McGovern et al., 2014). MDDCs generated *ex vivo* from primary monocytes also represent a well-established model to study both HIV-1 transfer and innate sensing by DCs (Manel et al., 2010).

For this study, we have used, as previously reported (Ménager and Littman, 2016), a CXCR4-tropic HIV-1 strain. Due to the lack of expression of CXCR4 at the surface of DCs, these viruses have even more limited capacity to enter and infect DCs, allowing us to focus on trans- (by monitoring only the transfer of incoming viral particles) rather than cis-infection (due to the transfer of newly production HIV-1 in DCs). We have observed that TSPAN7 and actin nucleation were also involved in DC-mediated trans-infection of $CD4^+$ T lymphocytes using transmitted/founder strains of HIV-1 (data not shown), which are CCR5-tropic and infect DCs more efficiently (Kariuki et al., 2017; Bertram et al., 2019a). Interestingly, as mentioned in the introduction, a two-phase trans-infection model has already been proposed for CCR5-tropic HIV-1 strains with the late phase being virus replication-dependent in *in vitro*-generated MDDCs and in mucosal DCs studied *ex vivo* (Turville et al., 2004; Harman et al., 2009; Nasr et al., 2014).

It is interesting to think about the existence of different mechanisms of HIV-1 transfer depending on the maturation status of DCs. DCs are known to switch from being professional pathogens catchers, in their immature state, to potent antigen presenters upon maturation and are then able to establish long-lasting contacts to potently stimulate T cells thereby bridging innate and adaptive immunity (Merad et al., 2013). HIV-1 may have evolved strategies to adapt itself to the changes happening during DC maturation, to exploit DC functions and to be efficiently captured and transferred to T lymphocytes with minimal detection by the immune system. In immature DCs, the actin-rich dendrites would probe the environment and capture HIV-1 through a receptor different from CD169/Siglec-1, which remains to be determined. The virus could then be transferred through dendrites to surrounding T cells, which may result, at some point, as we have observed in our experiments, in DC maturation. DC maturation in turn will lead to some morphological and functional changes, which correlates with an increase in expression of CD169/Siglec-1, a shortening of dendrites and the formation of a big invaginated pocket, which may accumulate HIV-1. Mature DCs, as reported, would then migrate into lymph nodes with a high density of T cells, form long lasting virological synapses with T cells, allowing a CD169/Siglec-1-dependent, TSPAN7/actin nucleation-independent process of HIV-1 transfer to T cells (Kijewski and Gummuluru, 2015; Reyes-Rodriguez et al., 2016). As it has been shown that human DCs are relatively resistant to innate sensing, due to a blockade of HIV-1 viral RNA reverse transcription by SAMHD1, hence of HIV-1 infection, all processes of HIV-1 transfer could happen with minimal stimulation of the innate system, making DCs as the perfect trojan horses for HIV-1 (Antonucci et al., 2017).

As observed in our experiments, during the last 20 h of co-culture, maturation of DCs is happening alongside an increase in circularity and a shortening of the dendrites. These changes are linked to a decrease in actin nucleation activities subsequent to a decrease in TSPAN7 expression. This control of TSPAN7 expression does not seem to be specific to our co-culture experiments, as it is induced by a bacterial TLR4 agonist (LPS), synthetic dsRNA [poly(I:C)] and also observed upon HIV-1 sensing experiments. It would be interesting to unravel the molecular mechanisms by which TSPAN7 expression is shutdown during/as a result of the maturation process, as, to our knowledge, it has not been reported yet in any members of the mammalian tetraspanin family. In particular, we have shown in our study, that TSPAN6, a paralog of TSPAN7, does not seem to be regulated by the same immune stimuli. Such a regulation upon DC maturation also suggests that it may be interesting to investigate TSPAN7 roles in other functions of DCs, in particular the ones modulated upon DC maturation, such as mobility, antigen presentation, production of inflammatory cytokines and expression at the surface of co-stimulatory molecules. We believe that, in our co-culture experiments, the maturation of MDDCs may occur through paracrine or autocrine effects of pro-inflammatory cytokines that may be secreted by T lymphocytes or DCs themselves, initiation of co-stimulatory signals or through microvesicles secreted alongside HIV-1 virions as previously demonstrated (Mercier et al., 2013).

As mentioned in the introduction, most of the experiments demonstrating that trans-infection is a potent way for HIV-1 to propagate have been done in *in vitro* or *ex vivo* settings. However, some experiments performed in *in vivo* conditions suggest that trans-infection may be key for the virus to disseminate *in vivo* especially at early stages of infection and to escape ART agent actions (Bracq et al., 2018). We therefore think that our work contributes to a better understanding of the different mechanisms at play during HIV transfer and highlights the importance of DC maturation status. Such knowledge is required to allow the development of new approaches to tackle HIV-1.

MATERIALS AND METHODS

Cells

HEK293FT cells (Invitrogen) were cultured in DMEM (Gibco), 10% fetal bovine serum (FBS, heat inactivated) (Sigma), 0.1 mM MEM non-essential Amino Acids, 6 mM L-glutamine, 1 mM MEM sodium pyruvate, and penicillin/streptomycin/gentamycin (Gibco).

Peripheral blood mononuclear cells were isolated from buffy coats obtained from healthy human blood donors [Etablissemnt Français du Sang (EFS), agreement N°18/EFS/030] using mononuclear cell isolation with Ficoll-Paque separation media (GE Healthcare). Monocytes were obtained after purification of PBMCs with anti-human CD14 magnetic beads (Miltenyi) and cultured in RPMI (Gibco), 10% FBS (heat inactivated, Sigma), 10 mM Hepes, 55 µM β-mercaptoethanol, 6 mM L-glutamine and penicillin/streptomycin/gentamycin (Gibco) in the presence of 10 ng/mL of human recombinant GM-CSF and 50 ng/mL of human recombinant IL-4 (Miltenyi), in order to differentiate them in monocytes derived dendritic cells (MDDCs). $CD4^+$ T lymphocytes were isolated from the $CD14^-$ fraction after a

purification using anti-human CD4 magnetic beads (Miltenyi). Cells were cultured in the same medium as MDDCs but activated using 2 μg/mL phytohaemagglutinin (PHA-L; eBioscience 00-4977-03) for 48 h combined with human IL-2 treatment (100 U/ml; Miltenyi biotec, 130-097-742). Purity was checked by flow cytometry for both $CD14^+$ and $CD4^+$ cells and was more than 99%. Fresh media and cytokines were added after 24 and 72 h. By day 4, monocytes were differentiated into MDDCs and were mixed with activated autologous $CD4^+$ T lymphocytes and HIV-1 for transfer experiments. MDDC differentiation was assessed by flow cytometry based on DC-SIGN upregulation and CD14 downregulation.

Plasmids and Viral Particle Production

X4–HIV-1–GFP is a fully replication-competent virus derived from the NL4-3 clone with enhanced Green Fluorescent Protein (GFP) (Clontech, Palo Alto, California, United States) cloned in place of the Nef gene, as previously described (Unutmaz et al., 1999; Motsinger et al., 2002); X4-HIV-Gagi-GFP is derived from the same clone modified to insert the GFP protein between the MA and CA domains of Gag as previously described (Hübner et al., 2007); pLK0.1 GFP was generated by replacing the puromycin resistance gene by GFP in the pLK0.1 puro plasmid (Moffat et al., 2006).

The protein Vpx, as described in Mangeot et al. (2002) and Manel et al. (2010) was delivered using pSIV3+ to generate VSV-G-pseudotyped SIVmac239 virus-like particles.

pCMV-ΔR8.91 and pCMV-VSV-G were used for packaging and pseudotyping of viral particles (Manel et al., 2010).

HEK293FT cells were transfected in 10 cm plates with 1.2 ml DMEM, 18 μg DNA and 48 μl TransIT-293 (Mirus Bio) per plate in 10 mL final volume to produce viral particles; for VSV-G–pseudotyped SIVmac VLPs/Vpx, 2.4 μg pTRIP CMV-VSVg and 15.6 μg pSIV3+; for shRNA viral particles, 9.6 μg pLk0.1, 6 μg pCMV-ΔR8.91, and 2.4 μg pTRIP–CMV-VSV-G; HEK293FT cells were transfected in 15 cm plates with Promega kit for calcium phosphate transfection; for X4-HIV-1-GFP, 60 μg of NL4-3 CXCR4-HIV-1-GFP; for X4–HIV-1–GAG-iGFP, 60 μg of NL4-3 CXCR4-HIV-1-iGAG-GFP. Media was changed after 1 day of transfection and viral supernatants were collected after 48 h of transfection and filtered at 0.45 μm.

Transduction

Blood monocytes were plated at 1×10^6 cells/mL in 5 mL of medium with cytokines (hGM-CSF and hIL-4) and 8 μg/ml polybrene (Merck Millipore). They were transduced with 2.5 mL of SIVmac VLP/Vpx (Mangeot et al., 2002) and 4 mL of viral supernatant containing shRNA coding lentiviruses. Fresh media and cytokines were added to cells (40% of total volume) 1 day and 3 days after $CD14^+$ cell isolation. On day 4, cells differentiated into MDDCs, were collected, resuspended in fresh media with cytokines and used for transfer experiments.

Trans-infection/HIV Transfer Experiments

After 4 days of stimulation with cytokines 50,000 MDDCs (1 million/mL) were mixed with 50,000 (1 million/mL) autologous $CD4^+$ T cells and 150 μl X4-tropic HIV-1 encoding GFP (50 ng $p24^{GAG}$, quantified by ELISA) per well in 96-well round bottom plates. Media was replaced after 24 h. Depending on experimental conditions, $CD4^+$ T lymphocyte infection was measured by flow cytometry by monitoring GFP and P24 (HIV viral capsid protein) expression, following 20 and/or 40 h of co-culture. In each HIV transfer experiments, MDDC and $CD4^+$ T cell viability and maturation/activation states were analyzed by flow cytometry.

Flow Cytometry Analysis

For flow cytometry analysis, the staining was performed with anti-CD3 AF700 (for T cells) (eBioscience; 56-0038-82), anti–DC-SIGN PE (for MDDCs) (Clone, 120507; R&D systems, catalog number: FAB161P), anti-CD169 APC (for CD169/Siglec-1; Biolegend; 346007), and DAPI for live/dead gating and in some cases anti-P24 PE (Beckman Coulter, 6604667). Unless otherwise mentioned, antibodies were used at a concentration of 1/200. To assess changes in cell number, CountBright Absolute Counting Beads (Life Technologies) were used in each sample. For some experiments (transfer and/or capture), BD cytofix/cytoperm buffer and fixation protocol was used to detect intracellular P24 staining in both MDDCs and T cells. Flow cytometry was performed on a BD LSRII using a 96-well plate HTS reader. Data were then analyzed using FlowJo software (FlowJo LLC). For each donor, MDDC differentiation was monitored based on DC-SIGN induction, CD14 downregulation and minimal maturation ($CD86^+$ cells <10%) [anti-CD86 PE (eBioscience, 12-0869-42)].

Immunofluorescence and Confocal Microscopy

After different co-culture times with X4-HIV-1-Gag-iGFP and/or T cells, cells were put on coverslips previously coated with poly-L-lysine solution (0.1% w/v in H2O, Sigma), for 30 min at 37°C. Surface staining was performed in PBS containing 1% BSA, for 30 min at room temperature. Cells were incubated with warm paraformaldehyde A in PHEM Buffer [2X PHEM Buffer (500 ml): 18.14 g PIPES; 6.5 g HEPES; 3.8 g EGTA; 0.99 g MgSO4; pH to 7.0 with 10M KOH], for 10 min at room temperature. Following fixation, cells were permeabilized in Triton 0.1% diluted in PHEM buffer for 5 min at RT. Blocking was done in casein overnight at 4°C. For intracellular staining, antibodies were diluted in PBS containing 1% BSA and incubated for 1 h at room temperature. For nucleus detection, cells were then stained with DAPI (1/5,000 dilution from stock at 5 mg/ml in H2O), 5 min at room temperature. After extensive washing in PBS containing 0.1% BSA, cells were mounted in a home-made DABCO-PVA medium [2.5% Dabco, 10% polyvinylalcohol (PVA), 5% glycerol, 25 mM tris buffer pH 8,7]. Microscopy was performed on a Zeiss LSM 710 confocal with 405, 488, 543, and 633 nm lasers, a 63X N.A. 1.40 lens, and the pinhole set to 1 Airy unit as defined by Zeiss. Sequential scanning was used to assure no spillover of channels. Z series were taken at intervals of 400 nm. The following antibodies were used for confocal microscopy, at a concentration of 1/200. Primary antibodies: Mouse anti Human CD169 Alexa Fluor® 647 (AbD Serotech; MCA2517A647T); Alexa Fluor® 568 Phalloidin (Molecular Probes®; A12380).

Image Processing, Colocalization, and Quantification

ImageJ software was used for all image adjustments and quantification. All the pictures were smoothened and in some case CLAHE plugin (Contrast Limited Adaptive Histogram Equalization) was applied. At least 100 cells, with a minimum of 10 Z-stacks per cell, were individually analyzed for each sample. To measure and quantify dendrites length, cell shape and perimeter, there were manually drawn using the freehand line tool in ImageJ, with a width of 10. Then areas and perimeters were calculated using analyze function (measure, set measurements: Area and perimeter) for each cell on the image. Pearson correlation coefficient was calculated using ImageJ coloc2 plugging tool, in order to quantify colocalization between CD169/Siglec1 and HIV-1 in MDDCs.

Pharmacological Drugs

All drugs were resuspended in DMSO and used at the concentrations mentioned below. CK-666 (Sigma-Aldrich, SML0006, 100 μM); Nelfinavir (Sigma-Aldrich, CDS021783, 1 μM).

Innate and inflammatory stimuli were used at the indicated concentrations: poly(I:C) (InvivoGen, 10 μg/ml); LPS (List Biological Laboratories, INC, 100 ng/ml).

Quantitative RT–PCR

RNA was extracted from 3×10^5 MDDCs using TRIZOL® RNA isolation protocol for gene expression quantification. Real time quantitative PCR (qPCR) was performed after reverse transcription (RT) using a Roche LightCycler 480 with Roche 480 SYBR Green I master reagent according to manufacturer specifications. The relative abundance of each target mRNAs was calculated based on the standard curve and normalized using *GAPDH* as a control.

For qPCR, the primers used were the following:
TSPAN7 set 1 F 5′-CCTTCGTCTTCTGGATCACTGGGG-3′;
TSPAN7 set 1 R 5′-CATGGTCCACTGCCCGGCTC-3′;
TSPAN7 set 2 F 5′-CATCGCTGGAGTGGCGTTTGGA-3′;
TSPAN7 set 2 R 5′-TGCACGTTGTGGGGTAAGGGG-3′;
GFP F 5′- ACGTAAACGGCCACAAGTTC-3′;
GFP R 5′-AAGTCGTGCTGCTTCATGTG-3′.

HIV-1 Capture Experiments

Four days after transduction with shRNAs, MDDCs were mixed with X4–HIV-1–GFP using the same conditions as with trans-infection experiments but without T cells. After 20 h co-culture, MDDCs were washed in ice cold PBS to remove unbound HIV-1 and RNA was extracted. After RT, the amount of HIV-1 RNA was detected by qPCR with primers for *GFP* (encoded inside the HIV genome) and normalized to *GAPDH*.

RNA Sequencing

Transcriptional profiling of MDDCs was performed using whole genome RNA sequencing. MDDCs from four healthy blood donors were infected or stimulated as indicated in the figure legends, stained with anti-human CD86 (BD Biosciences) and a live/dead viability dye (ThermoFischer) before sorting into serum on a FACSAria II (BD Biosciences). Cells were lysed in TRIzol reagent (Thermo Fisher), RNA was isolated according to the manufacturer's instructions, and the samples were submitted to HudsonAlpha Institute for Biotechnology (https://hudsonalpha.org/) for library preparation and RNA-sequencing. Fifty bp-length single-end sequences were aligned to the human genome (hg38) using STAR version 2.4.2a. Samtools 0.1.19 was used to filter alignments to a MAPQ score threshold of 30. Counts per gene were called using feature Counts version 1.4.6 and gencode v24 genome annotation. Samtools 0.1.19 was used to filter alignments to a MAPQ score threshold of 30.

Statistics

Unless otherwise specified, statistical analyses were done using the Holm-Sidak multiple comparison test following one-way Anova (NS, non-significant; $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$). For most experiments, two different human blood donors were used and the average of triplicates and standard deviation was calculated for each blood donor.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Etablissemnt Français du Sang (EFS), agreement N°18/EFS/030. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MM performed most of the experiments, wrote the manuscript, generates the figures, and obtained the funding necessary for this project. BP wrote the manuscript and performed some experiments. VG-P helped with the design of the figures and some of the experiments. ML helped writing the material and methods and provide her technical expertise with some experiments. All authors approved the manuscript for publication.

FUNDING

This work was supported by fellowships from EMBO, the Cancer Research Institute, INSERM (MM); ATIP-Avenir INSERM (BP), Fondation Imagine (ML and VG-P), and by grants from ATIP-Avenir and Fondation Imagine (MM).

ACKNOWLEDGMENTS

We thank Dan R. Littman for his support at the initiation of this project, which is a follow up study on the experiments performed

in his laboratory; Nicolas Manel and Jarrod Johnson for their helpful discussions and comments.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2020.00070/full#supplementary-material

Figure S1 | Experimental strategy and kinetic of HIV-1 trans-infection following *TSPAN7* shRNA knockdown. Related to **Figure 1**. **(A)** Experimental design to evaluate the effect of viral replication on HIV-1 transfer from iMDDCs to $CD4^+$ T lymphocytes. Red arrows indicate Nelfinavir (NFV, an HIV protease inhibitor) treatment at 1 μM during co-culture experiments. **(B)** Percentage of variation of X4–HIV-1–GFP transfer as compared to the 20 h time point, in the presence or absence of NFV (1 μM). Mean ± SD of technical triplicates from three different healthy blood donors. **(C–E)** Percentage of $CD4^+$ T cells infected by X4–HIV-1–GFP upon co-culture with iMDDCs, transduced by scramble or *TSPAN7* shRNA (1 and 3), in the presence (black bars) or absence (white bars) of NFV (1 μM). T cell infection was measured by flow cytometry and data represent mean ± SD of technical triplicates from three healthy blood donors (donor A in **C**, donor B in **D**, and donor AC in **E**). **(E)** Data from **(D)** represented as variation of X4–HIV-1–GFP transfer as compared to MDDCs transduced by scramble shRNA, at 20 h. **(B–E)** NS, not significant. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. **(C–E)** Donors are representatives of 8 unrelated donors tested in the context of 4 independent experiments. **(F)** Experimental design to evaluate the effect of actin nucleation inhibition on X4–HIV-1–GFP transfer from iMDDCs to $CD4^+$ T lymphocytes, at 20 and 40 h, using CK-666 as an ARP2/3 inhibitor (100 μM).

Figure S2 | Quantification of changes in MDDC shape and loss of dendrites. Related to **Figure 2**. **(A)** Arithmetic formula and illustration to assess cell shape by calculating circularity index ($0 \leq$ circularity ≤ 1), taking into account the area and perimeter of each cell. A value of 1 represents a cell perfectly circular, whereas 0 indicates a cell with a shape of a line. After manually tracing the perimeter of each cell, areas and perimeters were calculated using Analyze function (Analyze, measure, set measurements: area and perimeter) in ImageJ. **(B)** Box plot of circularity index calculated per cell for each group and at different time (4, 20 h and 40 h) and treatment (LPS-stimulated, *TSPAN7* shRNA-transduced or control cells). Every dot represents a cell and the number of cells analyzed in each condition is indicated on top. **(C,D)** Box plots showing the number and average length of dendrites, respectively, per cell for each group mentioned above. **(E)** Box plot displaying the length of all examined dendrites through this data analysis. Every dot now represents the measurement of one dendrite. Number of total dendrites analyzed in each condition is displayed on top.

Figure S3 | *TSPAN6*and *TSPAN7* expressions relative to MDDCs maturation. Related to **Figure 3**. **(A)** Flow cytometry plot showing the gating strategies to analyze the level of maturation of MDDCs and percent infection of T cells. **(B)** Histogram depicting the mean fluorescent intensity (MFI) of CD86 expression in MDDCs as gated in **Figure 3A** (bottom panel). **(C)** Expression of *TSPAN6* and *TSPAN7* measured by bulk RNA sequencing, under different conditions: stimulation with LPS (1 μg/ml), poly(I:C) (1 μg/ml), or infection with VSV-G–HIV-1–GFP in the presence of Vpx, measured after 2, 4, 20, and 40 h post stimulation/infection. For the 20 h time point, MDDCs infected by HIV-1 were sorted on GFP^+ and GFP^- populations. After 40 h, GFP^+ MDDCs were sorted based on their level of maturation ($CD86^{high}$ vs. $CD86^{low}$). Data are displayed in FPKM (Fragments per Kilobase of gene per Million Reads aligned). **(D)** Schematic summarizing the inverse correlation between MDDC maturation and *TSPAN7* expression, upon co-culture experiments and the two different phases of HIV-1 transfer identified in our experimental settings.

Figure S4 | Efficiency of HIV-1 transfer based on MDDC maturation status. Related to **Figure 4**. **(A)** Scheme depicting the experimental layout used to evaluate the effect of adding 100 ng/ml of LPS at different time before the start of co-culture with $CD4^+$ T lymphocytes and X4–HIV-1–GFP (48, 24 h before or at the same time). Forty hours after the start of co-culture experiments, infected T cells were monitored by P24 and GFP expression using flow cytometry. **(B)** Histogram depicting the fluorescent intensity linked to the expression of CD86 on LPS-stimulated MDDCs vs. iMDDCs. The red line shows CD86 expression on iMDDCs and the blue one CD86 expression following LPS treatment: 48 h before co-culture (left panel), 24 h before co-culture (middle panel) and at the start of co-culture (right panel). **(C)** Quantification of HIV-1 capture by MDDCs 20 h after culture with X4-HIV-1-GFP, using two different donors. The amount of *GFP* RNA (expressed in the open reading frame of the viral protein Nef) was measured by reverse transcription and, subsequently, quantitative real time PCR with specified primers for *GFP* or *GAPDH* (housekeeping gene). Each condition is compared to the amount of *GFP* detected in immature MDDCs after 20 h. **(D)** Comparison of efficiency of HIV-1 transfer to $CD4^+$ T lymphocytes when using iMDDCs vs. mMDDCs. Efficiency of transfer was calculated as follow on 2 different blood donors: ratio of captured HIV-1 by LPS-treated MDDCs compared to iMDDCs divided by the variation of transferred HIV-1 to T lymphocytes (variation of transferred HIV-1 from LPS-treated MDDCs to $CD4^+$ cell compared to iMDDCs). For donor D, from **Figure S4C**, we measured close to 15 times more viruses captured by mature MDDCs but only 1.5 more T cells infected (**Figure 4C**). Transfer from mMDDCs appears to be 10 times less efficient than from iMDDCs and is therefore represented by negative values. Each bar represents mean ± SD of triplicates. **(E)** iMDDCs were treated with LPS (100 ng/mL) for 48 h before co-culture, at the time of co-culture (0 h) or left untreated. The percentages of cells with a CD169/Siglec1 and HIV-1 aggregates were evaluated in 4 unrelated donors.

Figure S5 | CD169 expression in iMDDCs vs. mMDDCs. Related to **Figure 5**. **(A)** Cell density histogram depicting the fluorescent intensity (MFI) of CD169 expression in MDDCs detected in **Figure 5A** (bottom panels). The gray area represents cells stained with the isotype IgG1, the blue, red, and orange lines show CD169 expression on iMDDCs and LPS-treated MDDCs, respectively. **(B)** Diagram displaying experimental conditions and protocol to block CD169 function in HIV transfer experiments as in **Figures 5B,C**. **(C)** Scheme showing the experimental layout used to assess CD169 role on HIV-1 transfer at different states of MDDC maturation, as described above. **(D)** Quantification of HIV-1 capture in iMDDCs transduced with scramble shRNA and treated with an anti-CD169 antibody as compared to isotype control. The amount of X4–HIV-1-GFP captured was measured after 20 h, by quantitative real time PCR with specified primers for *GFP* and *GAPDH* (housekeeping gene). Each bar represents mean ± SD of triplicates. NS, not significant. $**p < 0.01$. **(E,F)** Detailed image and magnification of confocal microscopy images, respectively, shown in **Figure 5D** to assess the degree of colocalization between HIV-1 (GFP) and CD169 (magenta), 4 h after the start of co-culture experiments between iMDDCs **(E)** or LPS-treated MDDCs **(F)** with X4–HIV-1–Gag-iGFP and activated $CD4^+$ T lymphocytes. In **(E)**, the circles represent HIV particles detected on the dendrites or at the plasma membrane. Cellular components were stained as mentioned above. ZEISS LSM 710 microscopy. 63X/oil objective. 0.4 μM section and 0.13 μM/pixel. **(G)** Pearson coefficient was calculated for four independent donors on immature and LPS-stimulated MDDCs in order to highlight co-localization of CD169/Siglec1 and X4–HIV-1–GAGiGFP. The dotted line represents $Y = 0.5$. Paired *t*-test was applied ($P < 0.05$).

REFERENCES

Ahmed, Z., Kawamura, T., Shimada, S., and Piguet, V. (2015). The role of human dendritic cells in HIV-1 infection. *J. Invest. Dermatol.* 135, 1225–1233. doi: 10.1038/jid.2014.490

Akiyama, H., Ramirez, N.-G. P., Gibson, G., Kline, C., Watkins, S., Ambrose, Z., et al. (2017). Interferon-inducible CD169/Siglec1 attenuates anti-HIV-1 effects of alpha interferon. *J. Virol.* 91:e00972-17. doi: 10.1128/JVI.00972-17

Alloatti, A., Kotsias, F., Magalhaes, J. G., and Amigorena, S. (2016). Dendritic cell maturation and cross-presentation: timing matters! *Immunol. Rev.* 272, 97–108. doi: 10.1111/imr.12432

Antonucci, J. M., St. Gelais, C., and Wu, L. (2017). The dynamic interplay between HIV-1, SAMHD1, and the innate antiviral response. *Front. Immunol.* 8:1541. doi: 10.3389/fimmu.2017.01541

Bassani, S., Cingolani, L. A., Valnegri, P., Folci, A., Zapata, J., Gianfelice, A., et al. (2012). The X-linked intellectual disability protein TSPAN7

regulates excitatory synapse development and AMPAR trafficking. *Neuron* 73, 1143–1158. doi: 10.1016/j.neuron.2012.01.021

Bertram, K. M., Botting, R. A., Baharlou, H., Rhodes, J. W., Rana, H., Graham, J. D., et al. (2019b). Identification of HIV transmitting CD11c+ human epidermal dendritic cells. *Nat. Commun.* 10:2759. doi: 10.1038/s41467-019-10697-w

Bertram, K. M., Tong, O., Royle, C., Turville, S. G., Nasr, N., Cunningham, A. L., et al. (2019a). Manipulation of mononuclear phagocytes by HIV: implications for early transmission events. *Front. Immunol.* 10:2263. doi: 10.3389/fimmu.2019.02263

Bracq, L., Xie, M., Benichou, S., and Bouchet, J. (2018). Mechanisms for cell-to-cell transmission of HIV-1. *Front. Immunol.* 9:260. doi: 10.3389/fimmu.2018.00260

Cheong, C. M., Chow, A. W. S., Fitter, S., Hewett, D. R., Martin, S. K., Williams, S. A., et al. (2015). Tetraspanin 7 (TSPAN7) expression is upregulated in multiple myeloma patients and inhibits myeloma tumour development *in vivo*. *Exp. Cell Res.* 332, 24–38. doi: 10.1016/j.yexcr.2015.01.006

Guilliams, M., Ginhoux, F., Jakubzick, C., Naik, S. H., Onai, N., Schraml, B. U., et al. (2014). Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat. Rev. Immunol.* 14, 571–578. doi: 10.1038/nri3712

Harman, A., Kraus, M., Bye, C. R., Byth, K., Turville, S. G., Tang, O., et al. (2009). HIV-1-infected dendritic cells show 2 phases of gene expression changes, with lysosomal enzyme activity decreased during the second phase. *Blood* 114, 85–94. doi: 10.1182/blood-2008-12-194845

Harman, A. N., Bye, C. R., Nasr, N., Sandgren, K. J., Kim, M., Mercier, S. K., et al. (2013). Identification of lineage relationships and novel markers of blood and skin human dendritic cells. *J. Immunol.* 190, 66–79. doi: 10.4049/jimmunol.1200779

Harman, A. N., Nasr, N., Feetham, A., Galoyan, A., Alshehri, A. A., Rambukwelle, D., et al. (2015). HIV blocks interferon induction in human dendritic cells and macrophages by dysregulation of TBK1. *J. Virol.* 89, 6575–6584. doi: 10.1128/JVI.00889-15

Hübner, W., Chen, P., Del Portillo, A., Liu, Y., Gordon, R. E., and Chen, B. K. (2007). Sequence of human immunodeficiency virus type 1 (HIV-1) Gag localization and oligomerization monitored with live confocal imaging of a replication-competent, fluorescently tagged HIV-1. *J. Virol.* 81, 12596–12607. doi: 10.1128/JVI.01088-07

Izquierdo-Useros, N., Naranjo-Gómez, M., Erkizia, I., Puertas, M. C., Borràs, F. E., Blanco, J., et al. (2010). HIV and mature dendritic cells: Trojan exosomes riding the Trojan horse? *PLoS Pathog.* 6:e1000740. doi: 10.1371/journal.ppat.1000740

Jain, A., and Pasare, C. (2017). Innate control of adaptive immunity: beyond the three-signal paradigm. *J. Immunol.* 198, 3791–3800. doi: 10.4049/jimmunol.1602000

Kariuki, S. M., Selhorst, P., Ariën, K. K., and Dorfman, J. R. (2017). The HIV-1 transmission bottleneck. *Retrovirology* 14:22. doi: 10.1186/s12977-017-0343-8

Kijewski, S. D., and Gummuluru, S. (2015). A mechanistic overview of dendritic cell-mediated HIV-1transinfection: the story so far. *Future Virol.* 10, 257–269. doi: 10.2217/fvl.15.2

Lambert, A. A., Gilbert, C., Richard, M., Beaulieu, A. D., and Tremblay, M. J. (2008). The C-type lectin surface receptor DCIR acts as a new attachment factor for HIV-1 in dendritic cells and contributes to trans- and cis-infection pathways. *Blood* 112, 1299–1307. doi: 10.1182/blood-2008-01-136473

Landau, N. R. (2014). The innate immune response to HIV-1: to sense or not to sense. *DNA Cell Biol.* 33, 271–274. doi: 10.1089/dna.2014.2378

Manel, N., Hogstad, B., Wang, Y., Levy, D. E., Unutmaz, D., and Littman, D. R. (2010). A cryptic sensor for HIV-1 activates antiviral innate immunity in dendritic cells. *Nature* 467, 214–217. doi: 10.1038/nature09337

Mangeot, P.-E., Duperrier, K., Nègre, D., Boson, B., Rigal, D., Cosset, F.-L., et al. (2002). High levels of transduction of human dendritic cells with optimized SIV vectors. *Mol. Ther.* 5, 283–290. doi: 10.1006/mthe.2002.0541

Martin-Gayo, E., and Yu, X. G. (2019). Role of dendritic cells in natural immune control of HIV-1 infection. *Front. Immunol.* 10:1306. doi: 10.3389/fimmu.2019.01306

McDonald, D. (2010). Dendritic cells and HIV-1 trans-infection. *Viruses* 2, 1704–1717. doi: 10.3390/v2081704

McGovern, N., Schlitzer, A., Gunawan, M., Jardine, L., Shin, A., Poyner, E., et al. (2014). Human dermal $CD14^+$ cells are a transient population of monocyte-derived macrophages. *Immunity* 41, 465–477. doi: 10.1016/j.immuni.2014.08.006

McLaughlin, K. A., Richardson, C. C., Ravishankar, A., Brigatti, C., Liberati, D., Lampasona, V., et al. (2016). Identification of tetraspanin-7 as a target of autoantibodies in type 1 diabetes. *Diabetes* 65, 1690–1698. doi: 10.2337/db15-1058

Ménager, M. M., and Littman, D. R. (2016). Actin dynamics regulates dendritic cell-mediated transfer of HIV-1 to T cells. *Cell* 164, 695–709. doi: 10.1016/j.cell.2015.12.036

Merad, M., Sathe, P., Helft, J., Miller, J., and Mortha, A. (2013). The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu. Rev. Immunol.* 31, 563–604. doi: 10.1146/annurev-immunol-020711-074950

Mercier, S. K., Donaghy, H., Botting, R. A., Turville, S. G., Harman, A. N., Nasr, N., et al. (2013). The microvesicle component of HIV-1 inocula modulates dendritic cell infection and maturation and enhances adhesion to and activation of T lymphocytes. *PLoS Pathog.* 9:e1003700. doi: 10.1371/journal.ppat.1003700

Moffat, J., Grueneberg, D. A., Yang, X., Kim, S. Y., Kloepfer, A. M., Hinkle, G., et al. (2006). A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. *Cell* 124, 1283–1298. doi: 10.1016/j.cell.2006.01.040

Motsinger, A., Haas, D. W., Stanic, A. K., Van Kaer, L., Joyce, S., and Unutmaz, D. (2002). CD1d-restricted human natural killer T cells are highly susceptible to human immunodeficiency virus 1 infection. *J. Exp. Med.* 195, 869–879. doi: 10.1084/jem.20011712

Nasr, N., Lai, J., Botting, R. A., Mercier, S. K., Harman, A. N., Kim, M., et al. (2014). Inhibition of two temporal phases of HIV-1 transfer from primary Langerhans cells to T cells: the role of langerin. *J. Immunol.* 193, 2554–2564. doi: 10.4049/jimmunol.1400630

Pena-Cruz, V., Agosto, L. M., Akiyama, H., Olson, A., Moreau, Y., Larrieux, J.-R., et al. (2018). HIV-1 replicates and persists in vaginal epithelial dendritic cells. *J. Clin. Invest.* 128, 3439–3444. doi: 10.1172/JCI98943

Puryear, W. B., Akiyama, H., Geer, S. D., Ramirez, N. P., Yu, X., Reinhard, B. M., et al. (2013). Interferon-inducible mechanism of dendritic cell-mediated HIV-1 dissemination is dependent on Siglec-1/CD169. *PLoS Pathog.* 9:e1003291. doi: 10.1371/journal.ppat.1003291

Reyes-Rodriguez, A. L., Reuter, M. A., and McDonald, D. (2016). Dendritic cells enhance HIV infection of memory CD4(+) T cells in human lymphoid tissues. *AIDS Res. Hum. Retroviruses* 32, 203–210. doi: 10.1089/aid.2015.0235

Ruffin, N., Gea-Mallorquí, E., Brouiller, F., Jouve, M., Silvin, A., See, P., et al. (2019). Constitutive Siglec-1 expression confers susceptibility to HIV-1 infection of human dendritic cell precursors. *Proc. Natl. Acad. Sci. U.S.A.* 116, 21685–21693. doi: 10.1073/pnas.1911007116

Saiz, M. L., Rocha-Perugini, V., and Sánchez-Madrid, F. (2018). Tetraspanins as organizers of antigen-presenting cell function. *Front. Immunol.* 9:1074. doi: 10.3389/fimmu.2018.01074

Sewald, X., Ladinsky, M. S., Uchil, P. D., Beloor, J., Pi, R., Herrmann, C., et al. (2015). Retroviruses use CD169-mediated trans-infection of permissive lymphocytes to establish infection. *Science* 350, 563–567. doi: 10.1126/science.aab2749

Shen, R., Richter, H. E., and Smith, P. D. (2014). Interactions between HIV-1 and mucosal cells in the female reproductive tract. *Am. J. Reprod. Immunol.* 71, 608–617. doi: 10.1111/aji.12244

Suárez, H., Rocha-Perugini, V., Álvarez, S., and Yáñez-Mó, M. (2018). Tetraspanins, another piece in the HIV-1 replication puzzle. *Front. Immunol.* 9:1811. doi: 10.3389/fimmu.2018.01811

Trifonova, R. T., Bollman, B., Barteneva, N. S., and Lieberman, J. (2018). Myeloid cells in intact human cervical explants capture HIV and can transmit it to CD4 T cells. *Front. Immunol.* 9:2719. doi: 10.3389/fimmu.2018.02719

Turville, S. G., Santos, J. J., Frank, I., Cameron, P. U., Wilkinson, J., Miranda-Saksena, M., et al. (2004). Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. *Blood* 103, 2170–2179. doi: 10.1182/blood-2003-09-3129

Unutmaz, D., KewalRamani, V. N., Marmon, S., and Littman, D. R. (1999). Cytokine signals are sufficient for HIV-1 infection of resting human T lymphocytes. *J. Exp. Med.* 189, 1735–1746. doi: 10.1084/jem.189.11.1735

Usardi, A., Iyer, K., Sigoillot, S. M., Dusonchet, A., and Selimi, F. (2017). The immunoglobulin-like superfamily member IGSF3 is a developmentally regulated protein that controls neuronal morphogenesis. *Dev. Neurobiol.* 77, 75–92. doi: 10.1002/dneu.22412

Wang, X., Lin, M., Zhao, J., Zhu, S., Xu, M., and Zhou, X. (2018). TSPAN7 promotes the migration and proliferation of lung cancer cells via epithelial-to-mesenchymal transition. *Onco Targets Ther.* 11, 8815–8822. doi: 10.2147/OTT.S167902

Wu, L., and KewalRamani, V. N. (2006). Dendritic-cell interactions with HIV: infection and viral dissemination. *Nat. Rev. Immunol.* 6, 859–868. doi: 10.1038/nri1960

Zicari, S., Sessa, L., Cotugno, N., Ruggiero, A., Morrocchi, E., Concato, C., et al. (2019). Immune activation, inflammation, and non-AIDS co-morbidities in HIV-infected patients under long-term ART. *Viruses* 11:E200. doi: 10.3390/v11030200

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

REVIEW
published: 24 June 2020
doi: 10.3389/fcimb.2020.00261

The Glutamate System as a Crucial Regulator of CNS Toxicity and Survival of HIV Reservoirs

*Anna Maria Gorska and Eliseo A. Eugenin**

Department of Neuroscience, Cell Biology, and Anatomy, The University of Texas Medical Branch, Galveston, TX, United States

Glutamate (Glu) is the most abundant excitatory neurotransmitter in the central nervous system (CNS). HIV-1 and viral proteins compromise glutamate synaptic transmission, resulting in poor cell-to-cell signaling and bystander toxicity. In this study, we identified that myeloid HIV-1-brain reservoirs survive in Glu and glutamine (Gln) as a major source of energy. Thus, we found a link between synaptic compromise, metabolomics of viral reservoirs, and viral persistence. In the current manuscript we will discuss all these interactions and the potential to achieve eradication and cure using this unique metabolic profile.

Keywords: reservoirs, cure, NeuroHIV, dementia, glutamate, glutamine, HIV

OPEN ACCESS

Edited by:
Tetsuo Tsukamoto, Kindai University, Japan

Reviewed by:
Maria Teresa Sanchez-Aparicio, Icahn School of Medicine at Mount Sinai, United States
Nathalie Chazal, Université de Montpellier, France

****Correspondence:***
Eliseo A. Eugenin
eleugeni@utmb.edu

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *30 December 2019*
Accepted: *04 May 2020*
Published: *24 June 2020*

Citation:
Gorska AM and Eugenin EA (2020) The Glutamate System as a Crucial Regulator of CNS Toxicity and Survival of HIV Reservoirs. Front. Cell. Infect. Microbiol. 10:261. doi: 10.3389/fcimb.2020.00261

INTRODUCTION

Neurons are not able to perform *de novo* synthesis of the neurotransmitters glutamate (Glu) and gamma-aminobutyric acid (GABA) from glucose due to the lack of enzymes involved in their metabolism. Thus, a multicellular metabolic pathway known as the Glu/GABA-glutamine (Gln) cycle maintains the balance among these metabolites to support neuronal synaptic transmission (Bak et al., 2006).

Glu is the most abundant excitatory neurotransmitter in the central nervous system (CNS) (Zhou and Danbolt, 2014), and GABA is considered an inhibitory neurotransmitter in adulthood (McCormick, 1989; Harris-Warrick, 2005). A balance between these two neurotransmitters plays a critical role in several brain functions including learning and memory, development, pain, synaptogenesis, motor stimuli, and synaptic signaling (Petroff, 2002; Allen et al., 2004; Bak et al., 2006; Bonansco and Fuenzalida, 2016; Ford et al., 2017). Alterations in this balance have been associated with brain damage and several neurodegenerative diseases including Alzheimer's, Parkinson's, and NeuroHIV. In the particular case of NeuroHIV, the equilibrium of Glu/GABA/Gln is altered and contributes to neuronal and glial dysfunction as well as to cognitive impairment observed in at least half of the HIV-1-infected population, even in the current antiretroviral treatment (ART) era (Sailasuta et al., 2009; Cohen et al., 2010; Ernst et al., 2010; Young et al., 2014; Mohamed et al., 2018; Cysique et al., 2019). Briefly, the pathogenesis of NeuroHIV involves the early (7–10 days post-injection) transmigration of leukocytes carrying the virus across the blood-brain barrier (BBB) using chemokine gradients only sensed by HIV-1-infected monocytes due to their enhanced expression of key chemokine receptors such as CCR2 (Eugenin et al., 2006; Williams et al., 2013). Upon crossing the BBB, the few transmigrated HIV-1-infected leukocytes infect CNS resident cells such as microglia, macrophages, and a small population of astrocytes. If systemic HIV-1 replication is not controlled by ART, localized HIV-1-CNS replication and infection results in HIV-1 encephalitis and dementia (Gatanaga et al., 1999; Bingham et al., 2011; Gelman et al., 2013; Gelman, 2015; de Almeida et al., 2017; Mangus et al., 2018). However, in the current

ART era, CNS damage is mild due to controlled peripheral and CNS replication as well as limited HIV-1 infection; despite this, 50% of the HIV-1-infected individuals still show significant signs of cognitive impairment, but the mechanism of CNS dysfunction is unknown (Eggers et al., 2017; Yoshimura, 2017; Bandera et al., 2019; Fernandes and Pulliam, 2019; Kim-Chang et al., 2019; Paul, 2019; Portilla et al., 2019; Swinton et al., 2019; Angelovich et al., 2020). Several groups have proposed that CNS damage in the current ART era corresponds to a combination of HIV-1 reservoirs within the brain, low level expression and secretion of viral proteins, as well as associated inflammation (Wong and Yukl, 2016; Veenstra et al., 2017, 2019). However, the nature and size of the viral reservoir within the CNS under effective ART is unknown (Churchill et al., 2006, 2009; Eugenin et al., 2011a; Russell et al., 2017; Al-Harti et al., 2018; Ko et al., 2019; Wallet et al., 2019). Our laboratory demonstrated that myeloid and glial cells within the CNS are viral reservoirs. These few viral reservoirs, despite the low to undetectable viral replication, are able to amplify toxicity and inflammation to neighboring uninfected cells by a gap junction, hemichannel, and tunneling nanotube mediated mechanism (Eugenin et al., 2009a,b; Berman et al., 2016; Malik and Eugenin, 2016, 2019; Ariazi et al., 2017; Okafo et al., 2017; Valdebenito et al., 2018).

Another potential mechanism of toxicity is mediated by the low-level production of viral proteins, not blocked by ART, and subsequent secretion into neighboring cells such as neurons and glia (Nath, 2002; Kovalevich and Langford, 2012; Sami Saribas et al., 2017). However, the extent and concentrations of viral proteins in the CNS and other tissues are unknown, but nanograms/ml of viral proteins (Nef, Tat) were detected in the serum or plasma of HIV-1-positive individuals (Westendorp et al., 1995; Goldstein, 1996; Xiao et al., 2000). Almost all HIV-1 proteins are neuro- or glial-toxic. For example, neurotoxicity has been described for several viral proteins including gp120 and the transactivator of transcription (Tat) (**Table 1**), as well as for host factors such as TNF-α, IL-1β, and IL-6 released from latently HIV-1-infected or -activated cells (Koller et al., 2001; Zhou et al., 2017). In addition, several ART drugs have been demonstrated to generate toxicity on their own (Brier et al., 2015; Underwood et al., 2015; Latronico et al., 2018). Thus, the combination of HIV-1-infection, low-level secretion of viral proteins, and ART toxicity probably contribute to CNS dysfunction.

Another accepted mechanism of toxicity is Glu-mediated toxicity. Glu is a neurotransmitter dysregulated in HIV-1 infection that can contribute to HIV-associated neurocognitive disorder (HAND) (Huang et al., 2011; Potter et al., 2013; Vazquez-Santiago et al., 2014). In addition to synaptic dysregulation, a supplemental source of Glu in HIV-1 conditions is the persistently activated resident microglia and invading macrophages, which have been shown to increase Glu synthesis and to release into the extracellular space (Potter et al., 2013; Wu et al., 2015). Furthermore, in several cell types, HIV-1 infection leads to mitochondrial membrane destabilization and the release of phosphate-activated glutaminase (PAG) from the mitochondria matrix into the cytosol through the transition pore (Erdmann et al., 2007, 2009; Huang et al., 2011; Tian et al., 2012). Glutaminase (GLS) is an enzyme responsible for *de novo* synthesis of Glu from Gln resulting in the increased levels of intracellular and extracellular Glu due to mitochondrial dysfunction.

In individuals with NeuroHIV the extracellular level of Glu is elevated in cerebrospinal fluid (CSF) and plasma in correlation with severity of brain atrophy and dementia (Ferrarese et al., 2001; Cassol et al., 2014; Anderson et al., 2015). However, MRI/MRS imaging data are controversial. Some reports indicated that HIV-1-infected individuals with dementia have decreased levels of Glu in frontal gray and white matter as well as the basal ganglia in correlation with cognitive deficits (Sailasuta et al., 2009; Ernst et al., 2010; Mohamed et al., 2018). In contrast, other publications using MRS indicate that Glu is increased in the frontal white and gray matter and basal ganglia in HIV-1-infected individuals before and after initiation of ART as compared to uninfected individuals. However, treatment of HIV-1-infected individuals for extended periods of time with ART results in the reduction of extracellular levels of Glu, suggesting a positive effect of ART treatment (Young et al., 2014). Thus, most of these changes in Glu assessed by brain imaging are probably due to individual-to-individual variability, HIV-1 time course, HIV-1 replication, time of infection, ART, and several other potential CNS complications. Despite the mechanism of Glu dysregulation, we recently identified that glial and myeloid viral reservoirs within the brain generate energy from unusual sources of energy such as Glu and Gln, both of which are highly abundant in the brain, providing them with an almost endless source of energy. We recently demonstrated that blocking some of these metabolic pathways results in the effective killing of viral reservoirs, even in the absence of HIV-1 reactivation.

In this review, we will describe the overall Glu/GABA synaptic system, its dysregulation, and the role of HIV-1 in CNS dysfunction with a major focus on viral reservoirs.

AN INTRODUCTION TO THE GLUTAMATE/GAMMA-AMINOBUTYRIC ACID NEUROTRANSMITTER SYSTEM

Glu binding activates ionotropic and metabotropic receptors expressed on neuronal, glial, and immune cells (Zhou and Danbolt, 2014). Membrane-specific Glu receptors (GluRs) are expressed in neurons and glial cells, and they mediate most, but not all, excitatory and inhibitory effects (D'Antoni et al., 2008; Petralia, 2012; Skowronska et al., 2019). The activation of ionotropic Glu receptors mediates fast excitatory synaptic transmission, whereas the metabotropic receptors play a modulatory role (Lau and Tymianski, 2010).

Ionotropic Glu receptors (iGluRs) are proteins composed of different subunits that form ion channels. They are divided into three groups based on their structural similarities and named according to the type of agonist that activates them: amino-3- hydroxy-5-methylisoxazole-4-propionate (AMPAR), N-methyl- D-aspartate (NMDAR), and 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (kainate, KA) receptors (KAR) (Traynelis et al., 2010). The NMDAR family is composed of seven receptor families. They are divided in three main

TABLE 1 | Effect of HIV-1 proteins on neurotoxicity induced by glutamatergic system dysregulation.

HIV-1 protein	Effect on Glu-related neurotoxicity	References
Gp120	Induce synaptodendritic degeneration by activation of co-receptors CXCR4 and CXCR5.	Kaul et al., 2007
Gp120	Induces a massive calcium release and changes in the morphology of neuronal mitochondria.	Avdoshina et al., 2016; Rozzi et al., 2017
Gp120	Increase the synaptic damage *via* NMDAR based on enhanced of NR2A- and NR2B-mediate EPSCs.	Yang et al., 2013; Zhou et al., 2017
Gp120	In HIV/gp120-tg mice pharmacological blockade of NMDAR was protective against gp120 neurotoxic properties involved in causing neuronal damage and synaptic loss.	Nakanishi et al., 2016
Gp120	Modulate the intracellular trafficking and surface clustering of NMDAR.	Scott et al., 2003; Xu et al., 2011; Ru and Tang, 2016
Gp120	Potentiate neuronal cell death and prolonged increased in level of intracellular calcium induced by Tat.	Nath et al., 2000
Gp120	In primary human astrocytes HIV-1 and gp120 impaired the clearance of Glu by reducing the expression of EAAT2.	Wang et al., 2003; Melendez et al., 2016
Gp120	Reduced astroglial cell viability. In addition, gp120 reduced both Gln concentration in astroglial cell supernatants and GS expression.	Visalli et al., 2007
Gp120	Increase of AEG-1 expression in gp120-treated astrocytes, followed by impaired Glu homeostasis due to down-regulation of EAAT2 in astrocytes.	Kang et al., 2005; Lee et al., 2011
Tat	Stimulate NMDARs by direct cysteine-cysteine interaction of Tat with the extracellular domain of the receptor.	Prendergast et al., 2002; Li et al., 2008
Tat	Promotes the phosphorylation of the NMDAR and triggers the calcium efflux and receptors stimulation.	Haughey et al., 2001
Tat	Induce cell death in human neuroblastoma cells (SH-SY5Y) by *a* mechanism involving H_2O_2 release and NMDAR activity.	Capone et al., 2013
Tat	Amino acids 31–61 of Tat are necessary to cause neurotoxicity. Tat Δ31–61 mutant protein were not able to bind to the NMDAR and induce neurotoxicity.	Li et al., 2008
Tat	The changes in Tat protein structure prevent the interaction with the NMDAR, also the immune complex of the mutant Tat or nitrosylated Tat and anti-Tat antibody block neurotoxicity caused by NMDAR agonist.	Rumbaugh et al., 2013
Tat	Tat binding to LRP on the neuron's membrane initiate the formation of a macro molecular complex among tat-LRP-PSD-95 (as an intracellular adaptor protein)-NMDAR. LRP antagonist, blocked the Tat-dependent NMDAR potentiations.	Eugenin et al., 2007; Krogh et al., 2014b
Tat	Application of Tat into human fetal neurons result in calcium release *via* stimulation of IP_3 receptor and activation of neuronal nitric oxide synthetase (nNOS).	Haughey et al., 1999
Tat	Induced the macrophage/microglia activation and microglia-mediate neurotoxicity, caused by Tat-dependent activation of NADPH oxidase.	Minghetti et al., 2004; Turchan-Cholewo et al., 2009
Tat	Induced the dose-dependent Glu release from microglial which was associated with increased expression of the X_{c-} glutamate-cystine antiporter, and this effect was blocked by NADPH oxidase and glutamate-cystine inhibitors.	Barger et al., 2007; Gupta et al., 2010
Tat	Induced the Nrf2 activation and X_{c-} system up-regulation, which could be a potential source of excitotoxicity induced my Glu release.	Fogal et al., 2007; Bridges et al., 2012a,b; Mastrantonio et al., 2019
Vpr	Induced apoptosis in human neuronal cells *via* activation of caspase-8.	Patel et al., 2000
Vpr	Decreased antioxidants pool in astrocytes *via* increased production of reactive oxygen species due to an increase in the level of oxidized glutathione (GSSG).	Ferrucci et al., 2012
Vpr	Up-regulates the GLS isoform C expression, resulting in an increased level of Glu in a media of HIV-1-infected macrophages.	Erdmann et al., 2009; Datta et al., 2016
Vpr	Altering protein involved in glycolytic and citrate pathways in human derivative macrophages.	Barrero et al., 2013
Vpr	Glu production and release is mediated by glucose-dependent metabolism following by activation of glycolytic and TCA cycle in Vpr overexpressing macrophages.	Datta et al., 2016

subunits, GluN1, GluN2, and GluN3, which present different subtypes: GluN2–N2A, N2B, N2C, N2D; GluN3–N3A, N3B (Kumar, 2015). For a functional channel, an NR1 and an NR2 subunit are required. Glu binds to the NR2 subunit while the co-agonist, glycine, binds to the NR1 subunit. The binding of the co-agonist enables the removal of the magnesium blockade (Guo et al., 2017; Gibb et al., 2018). Similar to NMDAR, the family of AMPA receptors is composed of four subunits, GluR1/2/3/4, and the GluR2 subunit plays a critical role in Ca^{2+} permeability (Lomeli et al., 1994). The KAR family is composed of two types of subunits: GluR5/6/7 and KA1 and KA2, which form homo- or heterotetramers. Depending on the receptor's subunit composition, the channel will have different electrophysiological properties (Contractor et al., 2011).

Metabotropic Glu receptors (mGluRs) have been subdivided in regard to sequence similarity and signaling into three

groups (group I: mGluR 1 and 5; group II: mGluR 2 and 3; and group III: mGluR 4, 6, 7, and 8). The mGluRs are G protein-coupled receptors that are linked to various intracellular second messenger cascades. Group I mGluRs are coupled to G_q, and signaling through these receptors is carried out by the phospholipase C and stimulation of intracellular calcium (Ca^{2+}) release. Receptors belonging to this group (mGluR1 and mGluR5) are located predominantly postsynaptically. However, the presynaptic receptors control Glu release. Thus, depending on the receptor location, they have different activities. The second (mGluR2 and mGluR3) and the third (mGluR4, 6, 7, and 8) group are coupled to $G_{i/o}$ proteins, and they inhibit the activity of adenylate cyclase, resulting in a reduction of the intracellular level of cyclic AMP and the inhibition of protein kinase A (Pilc et al., 2008; Wieronska and Pilc, 2009; Niswender and Conn, 2010). In contrast to group I, receptors from the second and third groups are located predominantly presynaptically, and they inhibit the function of glutamatergic neurons, but the receptors from the third group also activate GABA release (Conn and Pin, 1997).

mGluRs receptors modulate neuronal excitability via regulation of voltage-sensitive calcium channels, G protein-coupled inwardly rectifying potassium (GIRK) channels, and GABA, AMPA, and NMDA receptors (Conn and Pin, 1997). Moreover, in several brain regions, iGluRs were identified on synaptic terminals (i.e., presynaptic receptors) as functional autoreceptors to modulate Glu and GABA release (Duguid and Smart, 2004; Fiszman et al., 2005). Interestingly, all these receptors are also expressed in immune cells, non-excitable, providing a unique neuroimmune cross talk that still is under examination (Eck et al., 1989; Pacheco et al., 2007; Moroni et al., 2012; Yang et al., 2013) and can further contribute to NeuroHIV development.

Characteristic of Glutamate Transporters

The release of intracellular Glu into the extracellular space can be achieved by four different mechanisms: (1) calcium-dependent exocytosis of neurotransmitter from vesicular storage; (2) reverse action of Glu transporters located pre- and postsynaptically on neurons and astrocytes plasma membrane; (3) reverse cystine/glutamate transporter activity on the plasma membrane; and (4) hemichannels (Allen et al., 2004; Santello et al., 2012; Li et al., 2014).

The first system corresponds to the synaptic regulation of the release and uptake of Glu. Glu in the CNS is synthetized and stored (millimolar concentration) in synaptic vesicles of glutamatergic neurons. The millimolar concentration of Glu in the synaptic cleft is kept in nanomolar range after synaptic stimulation by reverse excitatory amino acid transporters (EAATs). The second system corresponds to Glu transporters belonging to solute carrier family (SLC): Na^+- dependent (X_{AG}) excitatory amino acid transporter (EAAT-1), also known as Na^+-dependent glutamate/aspartate transporter (GLAST) and EAAT-2 or glutamate transporter 1 (GLT-1). These transporters work based on the maintainence of the Na^+ gradient between intracellular space and extracellular fluid by $Na^{+/}K^+$-ATPase. In the CNS, the Glu transporters are located on astrocytes (Rothstein et al., 1996), neurons (Kanai and Hediger, 2004), and the BBB (Kanai and Hediger, 2004), and their main function is to remove the excess of Glu from the synaptic cleft after signal transduction (Nedergaard et al., 2002; Grewer et al., 2008). The third system, X_{c-}, is an exchanger of intracellular Glu for extracellular cystine. Cystine is reduced to glutathione (GSH) in the cytoplasm (Lu, 2013). It was published that disruption in *the* X_{c-} the system may lead to an increase of extracellular Glu concentration, which is a source of oxidative stress and cell death (Bridges et al., 2012b; Lewerenz et al., 2013). The fourth system correspond to hemichannels composed of connexin or pannexin proteins. They are expressed on the surface of glial cells including astrocytes and microglia/macrophages, which allow for the rapid intercellular exchange of ions and metabolites. Opening of hemichannels occurs only under stress conditions to serve as a backup system for Glu buffering, calcium wave propagation, and synaptic plasticity (**Figure 1**). In astrocytes, unopposed hemichannels on astrocytes membrane are responsible for the release of gliotransmitters, including ATP, Glu, nicotinamide adenine dinucleotide (NAD), and D-serine to the extracellular space. Under physiological conditions, hemichannels are in a closed state. However, upon inflammation and damage, hemichannels become open and have been associated with several neurodegenerative disorders (Xing et al., 2019) including NeuroHIV (Eugenin et al., 2011a; Orellana et al., 2014; Berman et al., 2016; Malik and Eugenin, 2016, 2019). Only recently, we identified that circulating levels of ATP can be used to provide an early detection of cognitive impairment in HIV-infected individuals (Velasquez et al., 2020).

Pathways of Glutamate/Gamma-Aminobutyric Acid Synthesis—Neuro-Glia Interaction

Neurons cannot synthesize Glu *de novo* due to the lack of expression of glutamine synthetase (GS), which converts Glu to Gln, and pyruvate carboxylase (PC), which converts Gln into Glu (Pardo et al., 2011). In contrast, astrocytes express both enzymes (Bak et al., 2006). After the release of neurotransmitters to the synaptic cleft, the excess of Glu or GABA is removed by neighboring astrocytes using specific transporters and both neurotransmitters are metabolized into Gln via GS or transformed into Glu and then to α-KG to become part of the tricarboxylic acid cycle (TCA cycle, Krebs cycle) (Bak et al., 2006). In glutamatergic neurons, Glu is synthetized on three pathways: (1) from α-KG, a product from the TCA cycle, which is converted by the action of glutamate dehydrogenase (GDH) and aspartate aminotransferase (AAT) into Glu. GDH is a reversible enzyme that can transform a-KG, ammonia, and NADH or NADPH into Glu (Hara et al., 2003). (2) Gln from astrocytes is delivered into neurons by amino acids transporters (SLC6A14 and SLC1A5) and metabolized directly via enzyme glutaminase (GLS)/phosphate-activated GLS (PAG) into Glu. GLS is a main Gln-utilizing enzyme in the brain, converting the Gln to Glu, and it is located on the inner membrane of mitochondria (Holcomb et al., 2000). The

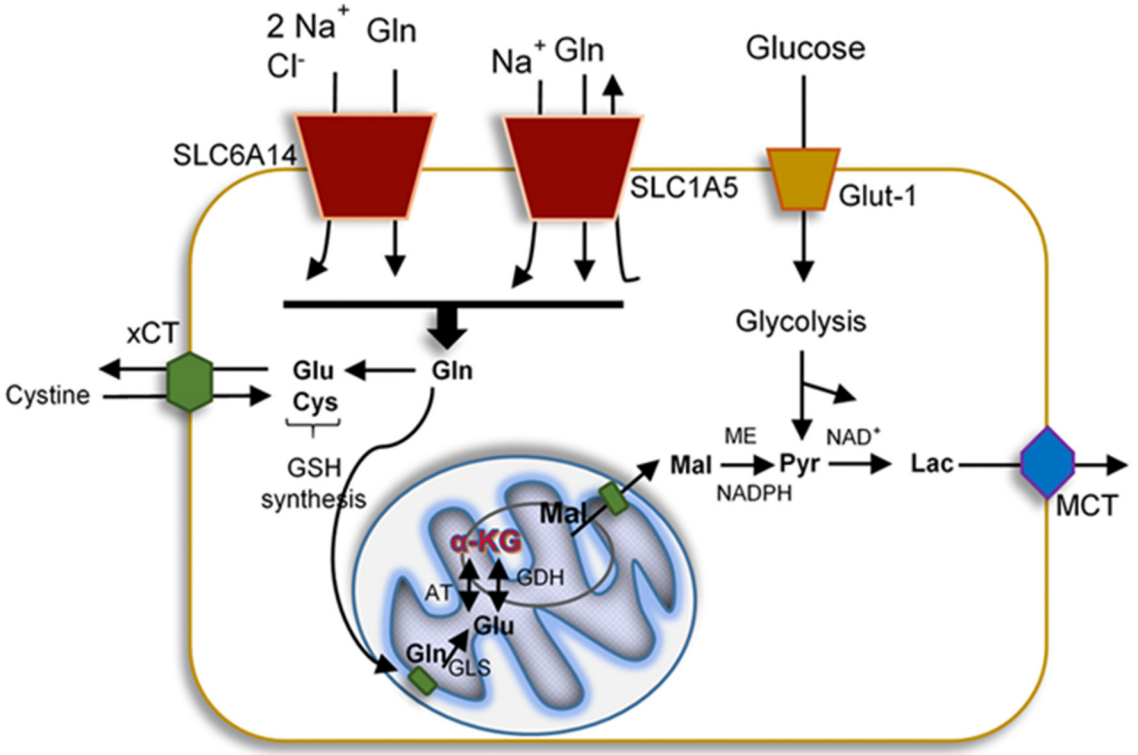


FIGURE 1 | Representation of the uptake and mitochondrial delivery system of glucose, glutamate, and glutamine. Glucose enters the cells by glucose transporter-1 (Glut-1), cystine, Gln, and Glu also are uptake by specific transporters such as cystine/glutamate antiporter (xCT), Na^+ and Cl^--dependent neurotransmitter transporter (SLC6A14), and neutral amino acid transporter (SLC1A5), respectively. All proteins affected by HIV-1 infection. Upon uptake, these molecules can become part of the glycolysis, GSH synthesis, or the TCA. In the brain, metabolism of these mediators generates lactate that exits the cells via the monocarboxylete transporter (MCT).

two types of GLS are well-characterized as the "kidney type" (GLS1) and "liver type" (GLS2). In the human brain, GLS1 has two isoforms: kidney-type glutaminase (KGA) and glutaminase C, which are expressed in the various cell types including neurons, microglia, macrophages, and astrocytes (Cardona et al., 2015). GLS is known to promote cancer cell proliferation and differentiation (Erickson and Cerione, 2010). Moreover, GLS is an initial enzyme in the glutaminolysis pathway, providing the energy source for protein and lipid synthesis. During glutaminolysis, Gln is converted to Glu and subsequently into α-KG, which then enters the TCA cycle (**Figure 1**). Also, Glu can be converted into GSH that can protect the cells from oxidative stress and promote their survival. The third pathway (3) of Glu synthesis is from Glu reuptake from extracellular space and transformation into Gln to go back to neurons (Peng et al., 1993; Schousboe et al., 2013) (**Figure 2**). GABA, similarly to Glu, is metabolized on two pathways: (1) reuptake from extracellular space and incorporation into the TCA cycle and then conversion to α-KG via GABA-transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH), and (2) from Gln, which is converted to Glu, and next via decarboxylation (glutamate decarboxylase enzyme, GAD) to GABA (Schousboe et al., 2013) (**Figure 2**). The synaptic pool of Glu can be divided into two compartments: (1) intracellular/cytosolic, in which Glu is synthetized from Gln inside glutamatergic neurons via enzyme GLS; and (2) mitochondrial, in which Glu synthesis from Gln is followed by Glu transformation to α-KG, which enters into the TCA cycle. Inside the mitochondria, Gln, even if it is considered as a source of energy, is called the "Trojan horse" because it is not the preferred source of energy. During the metabolism of Glu from Gln, Gln-derived ammonia (NH_{4+}) is also generated and affects mitochondrial function by increasing the production of ROS and inducing mitochondrial permeability transition (MPT). This process introduces the collapse of the inner mitochondrial membrane and leads to mitochondrial dysfunction and free radicals production (Zoratti et al., 2005; Albrecht and Norenberg, 2006) (**Figure 3**). Later we will discuss why HIV-1-reservoirs prefer this metabolic pathway to survive for extended periods of time within the CNS. Also, glucose, the main brain energy substrate, is involved in the Glu/GABA synthesis cycle. In astrocytes, glucose is metabolized to lactate and pyruvate, which is a substrate for the TCA cycle, which produces α-KG and Gln, the fundamental substrate for Glu/GABA synthesis (Hertz and Chen, 2017). These tight connections between neurotransmission, neurotransmitter metabolism, and energetics are used in pathological conditions

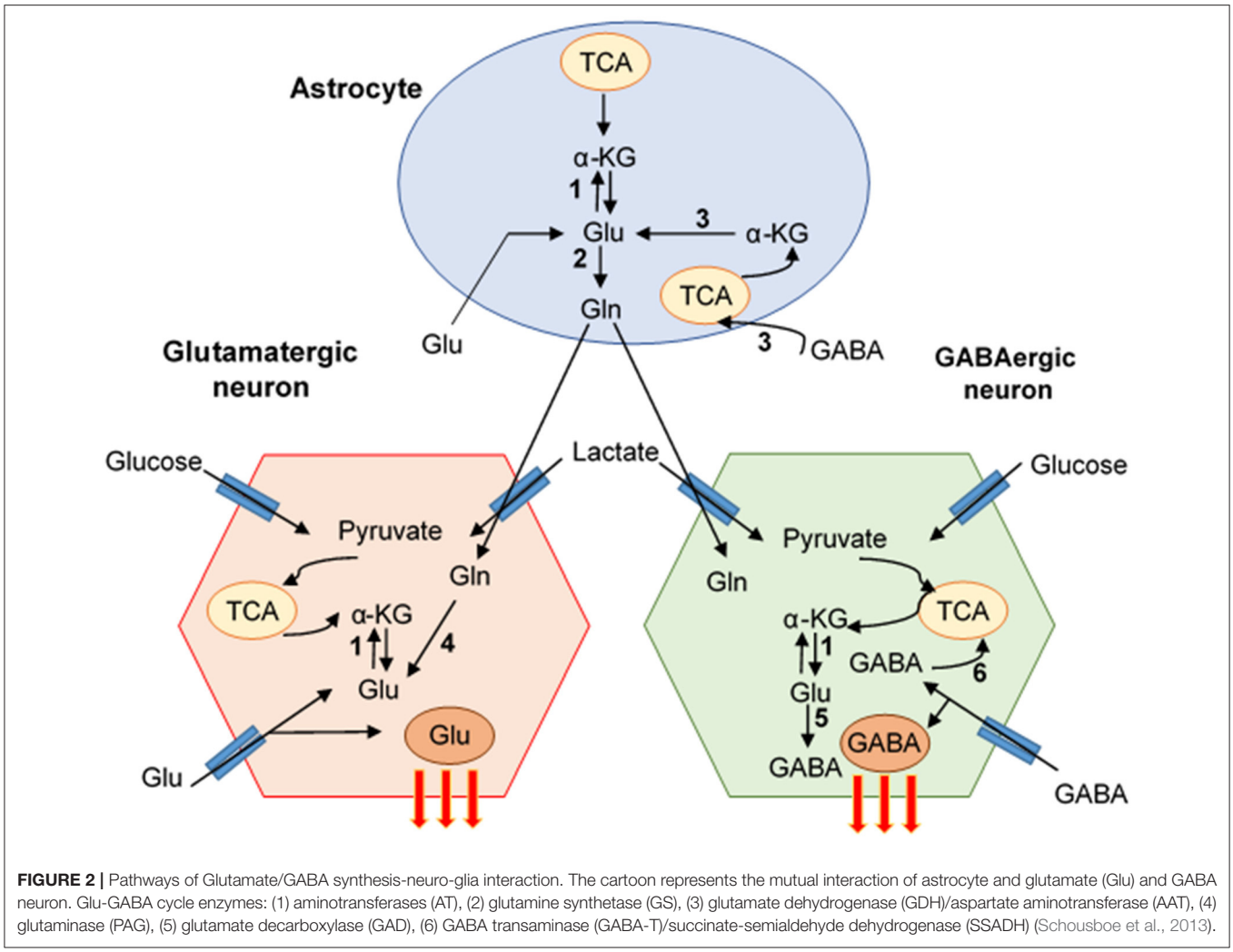


FIGURE 2 | Pathways of Glutamate/GABA synthesis-neuro-glia interaction. The cartoon represents the mutual interaction of astrocyte and glutamate (Glu) and GABA neuron. Glu-GABA cycle enzymes: (1) aminotransferases (AT), (2) glutamine synthetase (GS), (3) glutamate dehydrogenase (GDH)/aspartate aminotransferase (AAT), (4) glutaminase (PAG), (5) glutamate decarboxylase (GAD), (6) GABA transaminase (GABA-T)/succinate-semialdehyde dehydrogenase (SSADH) (Schousboe et al., 2013).

to promote the survival of glioblastoma stem cells as well as HIV-1-CNS reservoirs.

ESTABLISHMENT OF THE VIRAL RESERVOIRS: LINK TO NEURONAL AND GLIAL METABOLISM

HIV-1 infection cannot be eradicated due to the early generation of viral reservoirs in several tissue compartments (Wong and Yukl, 2016). Despite common knowledge that the elimination of viral reservoirs is essential to cure HIV-1, the nature, characteristics, and mechanisms of latent and long-lasting HIV-1-reservoirs are unknown. Currently, the best-characterized viral reservoirs are different circulating subpopulations of $CD4^+$ T lymphocytes; however, several groups propose that circulating viral reservoirs correspond to a poor representation of the real reservoir pool that is present in several tissues (Chomont et al., 2009; Soriano-Sarabia et al., 2014; Kandathil et al., 2016; Abreu et al., 2019).

In most cases, within the first few weeks of HIV-1 infection, the viral reservoirs are established in multiple tissues and anatomic compartments (Wong and Yukl, 2016). Compartmentalization of HIV-1 occurs as a result of differential selective pressures between tissues or restricted virus flow within organs (Borderia et al., 2007). Moreover, the different concentrations of antiretroviral drugs within compartments can result in divergent viral evolution (Zarate et al., 2007). It was shown that, in individuals diagnosed with HIV-1 infection, the pick level of HIV-1 replication was established in blood, oropharyngeal, and genital tract tissue, but not in the CSF (Pilcher et al., 2001). Furthermore, upon ART interruption, viral rebound was different between the blood and CSF, suggesting a differential compartmentalization (Garcia et al., 1999; Gianella et al., 2016; Mukerji et al., 2018). Early on in HIV-1-infection, the CNS becomes a unique compartment due to the presence of the BBB and the blood-CSF barrier, both of which restrict virus trafficking between the CNS and blood compartment, resulting in the rise of at least two separate HIV-1 compartments with different viral and cell evolutions (Smit et al., 2001; Wang et al.,

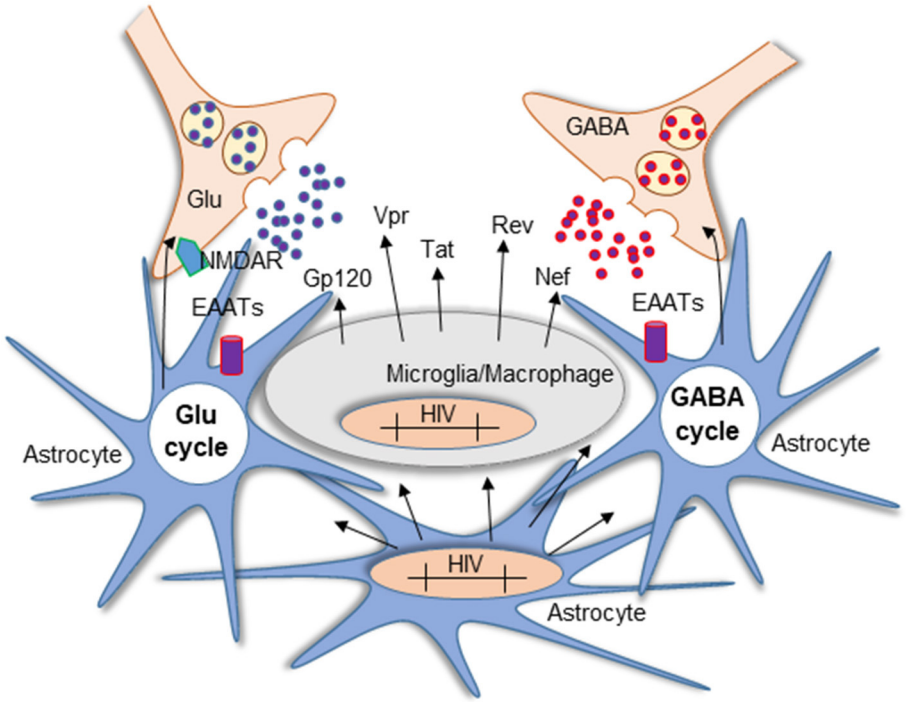


FIGURE 3 | Mechanism of Glu neurotoxicity in NeuroHIV pathogenesis. Glu is a critical neurotransmitter dysregulated in HIV-1 infection and HAND. Imbalance in the Glu/GABA cycle impaired the excitatory/inhibitory state of the neurons resulting in persistent stimulation of NMDARs. The diagram represents the activated state of astrocytes and microglia/macrophages. In the CNS, microglia, macrophages, and a small population of astrocytes become infected and carry HIV-integrated DNA. Although astrocytes cannot produce the virus, they contribute to neurotoxicity mediated by the production of viral proteins that are not blocked by ART and the subsequent secretion into neighboring cells such as neurons and glia. Another source of toxicity is the persistent activation of resident glial cells that increase Glu synthesis and release into the extracellular space.

2001; Ohagen et al., 2003; Abbate et al., 2005; Ritola et al., 2005; Yukl et al., 2013; Ganor et al., 2019). These differences in reactivation time and evolution can also be explained by the cell type infected, tissue microenvironment, access to ART, type of ART, pK distribution, proliferating cell properties, antigen dependency, or independent activation (Alexaki et al., 2008; Bingham et al., 2011; Pinkevych et al., 2015, 2016; Gianella et al., 2016).

Viral reservoirs are defined as cells with an integrated HIV-1 genome that have the capacity to silence viral replication, survive for extended periods of time, and become competent upon reactivation (Blankson et al., 2002). The best-described viral reservoirs are resting $CD4^+$ T lymphocytes, including central memory T lymphocytes, transitional T lymphocytes, and effector memory T lymphocytes, which maintain the infection by homeostatic proliferation in central memory and transitional memory T lymphocytes (Lee and Lichterfeld, 2016). It was shown that activated T lymphocytes have a higher copy number of HIV-1-integrated DNA as compared to lymphocytes in the resting state of individuals with or without ART. Further characterization of the types of T lymphocytes indicate that HIV-1-DNA is present mostly in resting central memory and transitional memory T lymphocytes. These results indicate that both T lymphocyte populations are the circulating viral reservoirs (Chomont et al., 2009; Josefsson et al., 2013). Using the quantitative viral outgrowth assay (QVOA), it has been demonstrated that replication-competent viruses reside in transitional memory T lymphocytes even in samples obtained from HIV-1-infected individuals on long-lasting ART (Siliciano et al., 2003; Soriano-Sarabia et al., 2014). During early infection, $CD4^+$ T lymphocytes are the major target cells (Haase, 1999). Next, within the first few weeks of primary HIV-1 infection, the virus population doubles every 6 to 10 h, resulting in infection of another 20 cells for each cell infected early on (Nowak et al., 1997; Little et al., 1999). During the symptomatic primary infection, the level of circulating virus in the blood (often $>10^6$ to 10^8/ml viral particles) and infected cell are high, and this initiates the compartmentalization early on in several tissues including the gut-associated lymphoid tissue (GALT). In 1995 studies by Wei et al. (1995) and Ho et al. (1995) showed viral evolution was a dynamic process because replacement of the wild-type virus can be achieved in 14 days, suggesting that viral replication and cell turnover can contribute to viral variability and the generation of reservoirs. In these studies, treatment with the HIV-1 reverse transcriptase and protease inhibitors (nevirapine and ritonavir) prevented infection of new cells but did not block virus replication in the cells already carrying integrated HIV-1 DNA. Both anti-viral drugs induced

a decrease in plasma virus levels in the first 2 weeks of therapy. Further studies using mathematical models to calculate HIV-1 replication, half-life of replicating and reservoirs cells, as well as the potential contribution of tissue associated viral reservoirs indicates that ART is extremely effective in reducing systemic viral replication after 1–2 weeks after initiation by preventing new infections. However, a second phase of replication was detected that corresponded to the loss of long-lasting infected cells. However, a pool of tissue resident cells still maintained a slow decay in systemic replication. The authors estimate that 2.3 to 3.1 years are required to eliminate these HIV-1 cells from the tissue compartment, however, this cleaning does not mean eradication (Perelson et al., 1997). It was calculated that the resting T helper lymphocytes population in HIV-1 patients on ART may contain ∼1 replication competent viral genome per 10^6 $CD4^+$ lymphocytes. Considering that the half-life on the latently infected memory T lymphocytes is around 4 years, 88 years of treatment would be required to reach a level of cure using current ART (Finzi et al., 1999; Shan and Siliciano, 2013; Hill, 2018).

HIV-1 enters the CNS early after primary infection (7–10 days), which contributes to the establishment of viral reservoirs in the CNS (Fischer-Smith et al., 2001; Valcour et al., 2012). $CD14^+CD16^+$ monocytes enter the CNS using physiological gradients of CCL2 from the brain and an upregulated expression of CCR2 on HIV-1-infected cells despite ART (Eugenin et al., 2006). More specifically, the mature $CD14^+CD16^+$ monocytes are strongly associated with CNS inflammation and HAND development (Williams et al., 2012, 2013). It was shown that in HIV^+ $CD14^+CD16^+$ monocytes preferentially transmigrate across the BBB, and also junctional proteins JAM-A and ALCAM support their transmigration. Blocking of JAM-A and ALCAM by specific antibodies and the dual inhibitor of chemokine receptors CCR2/CCR5 prevent preferential transmigration of HIV^+ $CD14^+CD16^+$ monocytes. Moreover, the surface of CCR2 is increased on HIV-1-infected $CD14^+CD16^+$ monocytes from individuals who manifested HANDs, independently of ART status, viral load, and CD4+ T lymphocytes count (Williams et al., 2014). The increase of CCR2 on $CD14^+CD16^+$ monocytes correlate with a higher level of HIV-1 DNA and neuronal damage, suggesting that $CD14^+CD16^+$ monocytes could have a key role in HAND pathogenesis and neuronal damage observed in infected individuals. After entering into the brain, HIV-1-infected monocytes differentiate into macrophages resulting in the release of hosts and viral components inducing infection of host cells such as microglia and astrocytes (Churchill et al., 2006).

Pericytes have a crucial role in the formation and maintenance of BBB integrity. Reduction in pericyte coverage is a typical feature of BBB impairment, observed in Alzheimer's disease (AD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and also HAND (Sengillo et al., 2013; Winkler et al., 2013; Persidsky et al., 2016). The main cause of loss of pericyte integrity observed in these diseases is associated with loss of adhesion into the basal membrane and dedifferentiation of smooth muscle cells resulting in their detachment from endothelial cells (Persidsky et al., 2016). HIV-1 Tat also induce changes in pericytes, suggesting that the loss of pericyte coverage and their detachment from the endothelium contribute to loss of BBB integrity in HAND (Niu et al., 2014). Within the CNS, microglia, and macrophages are the main cells supporting HIV-1 replication, however, astrocytes also are infected but HIV-1 replication is minimal to undetectable. Even though only ∼5% of the astrocytes become infected, their HIV-1-infection, but not replication, contributes to loss of BBB integrity (Churchill et al., 2006; Eugenin et al., 2011a). In pericytes, after HIV-1-infection, the viral replication decreased. Viral infection was demonstrated by Alu-PCR and infection was subjected to reactivation (Persidsky et al., 2016).

ART is a key factor in viral reservoir formation within the CNS. It has been demonstrated that initiation of ART less than 6 months after primary infection is associated with lower levels of T-cells activation and smaller circulating reservoir size during long-term therapy, supporting the hypothesis that reservoirs are formed early during infection (Jain et al., 2013; Abrahams et al., 2019). Moreover, by using mathematical models, it has been demonstrated that ART interruption with subsequent drug "washout" induces virus rebound (Hill et al., 2014, 2016; Pinkevych et al., 2015, 2016). Despite the efforts in re-using the success of ART to cure the disease, it is clear at this point that HIV-1-infected cells, including HIV-1 reservoirs, also have an alternative mechanism of survival, not only depending on silent or active replication. Some of these viral mechanisms are anti-apoptotic, metabolic, proliferating, and tissue sequestrating, and there is a variability between people and infected cells. All these mechanisms contribute to perpetuating the virus.

HIV-1 AND GLUTAMATE NEUROTOXICITY—THE ROLE OF GLUTAMATE RECEPTOR ACTIVATION AND GLUTAMATE METABOLISM

HIV-1 and Glutamate Receptors

There are several viral and host proteins that have been described as mediating the over-activation of the Glu/GABA neurotransmission system (Eugenin et al., 2007; Ru and Tang, 2017). Some of these viral proteins are HIV-1 Tat and gp120. Gp120 is a viral envelope protein with neurotoxic activity. HIV-1 isolates from the CNS are mainly macrophage (M)-tropic (Gonzalez-Perez et al., 2012), but also the T-cell-tropic form was detected. The gp120 M-tropic and T-tropic glycoproteins can induce synaptodendritic degeneration by activation of chemokine co-receptors CXCR4 and CCR5, respectively (Kaul et al., 2007), localized on neurons and non-neuronal cells (Westmoreland et al., 2002). Gp120 induces a massive calcium release and changes in the morphology of neuronal mitochondria (Avdoshina et al., 2016; Rozzi et al., 2017). Also, gp120 is a glycoprotein with the ability to activate the NMDA receptors and increase the synaptic damage by overactivation. Direct mechanisms of neuronal injury induced by gp120/HIV via NMDAR involved NR2A and NR2B subunits based on enhanced NR2A- and NR2B-mediated EPSCs (Zhou et al., 2017). In the HIV/gp120-tg administration of the drug to mice, nitromemantine (a blocker of NMDAR), was protective against gp120 neurotoxic properties causing neuronal damage and

synaptic loss. In the same studies, researchers demonstrated the crucial role of the NR3A subunit of NMDAR because the genetic overexpression of the NR3A subunit enhanced the synaptic injury in both gp120-tg and WT mice (Nakanishi et al., 2016). NMDAR phosphorylation in the cytoplasmic tail of this receptor has emerged as an important mechanism regulating its function and trafficking (Chen and Roche, 2007). Phosphorylation of Ser896 and Ser897 are crucial for channel activity but also for the promotion of trafficking from the Golgi to the plasma membrane (Scott et al., 2003). The T-cell-tropic gp120 promotes the trafficking and surface clustering of NMDAR, and this process was associated with the phosphorylation of the NMDA subunit NR1 at C terminal Ser896 and Ser897 (Xu et al., 2011). On the other hand, the M-tropic gp120 transiently decreased the level of pSer896 and pSer897 of the NR1 subunit, having the opposite effect on the T-tropic gp120 (Ru and Tang, 2016). Treatment with NMDAR and AMPAR antagonists prevents the effect of M-tropic gp120 on NR1 down-regulation, suggesting that phosphorylation of NR1 is dependent on synaptic activity and NMDAR activation (Ru and Tang, 2016). Moreover, both gp120 tropic forms present different neuropathological profiles—M-tropic gp120 has a weaker toxic property than T-tropic. Also, M-tropic (CCR5-preferring) is present in the early phase of HIV-1 infection, while T-tropic (CXCR4-preferring) in the late stage in patients with developed HAND (Bachis et al., 2010).

Tat is a potent excitotoxin and it stimulates NMDARs by direct cysteine–cysteine interaction with the extracellular domain of the receptor (Prendergast et al., 2002; Li et al., 2008). Moreover, Tat promotes the phosphorylation of the NMDA receptor and triggers the calcium efflux and receptor's stimulation (Haughey et al., 2001). Also, Tat can induce cell death in human neuroblastoma cells (SH-SY5Y) by stimulation of H_2O_2 release dependent on NMDAR activation (Capone et al., 2013). Amino acids 31–61 of Tat are necessary to cause neurotoxicity (Nath et al., 1996), and their cysteine residues are crucial for direct interaction with the NMDAR (Li et al., 2008). The role of Tat Δ31–61 was shown in studies with Tat Δ31–61 mutant protein where the modified protein was not able to bind to the NMDAR. Moreover, in the same studies, no interaction between Tat and NMDAR was observed (Li et al., 2008). The changes in the Tat protein structure prevented the interaction with the NMDAR, also the immune complex of the mutant Tat, nitrosylated Tat or anti-Tat antibody with NMDAR block neurotoxicity caused by NMDAR agonist (Rumbaugh et al., 2013). Tat as a transactivator of the HIV-1 long-terminal repeat (LTR) is necessary for viral replication (Moses et al., 1994; Taube et al., 1999). Tat causes neural injury by entering into the neurons by a low-density lipoprotein receptor-related protein-1 (LRP) (Liu et al., 2000; Eugenin et al., 2007). Tat binding to LRP on the neuron's membrane initiates the formation of a macro molecular complex among tat-LRP-PSD-95 (as an intracellular adaptor protein)-NMDAR, later nNOS is recruited to the complex with subsequent production of NO at the neuronal cell membrane (Eugenin et al., 2007). In this macro molecular complex, the NMDAR, especially the NR2A receptor subunit, play a crucial role in the process of Tat-induced apoptosis in human primary neurons (Eugenin et al., 2003; King et al., 2010). Application of the LRP antagonist, receptor-associated protein (RAP) completely blocked the Tat-dependent potentiation, which suggests that Tat potentiates NMDAR function via LRP (Krogh et al., 2014b). Application of Tat into human fetal neuron cell culture followed by calcium efflux mediated by excitatory amino acid receptors induced the calcium release via stimulation of IP_3 receptor and activation of neuronal nitric oxide synthetase (nNOS). This process was abolished by inhibition of IP_3 receptors, also had a neuroprotective effect from Tat toxicity (Haughey et al., 1999). Moreover, Tat potentiates the effect of NMDA on calcium release, by neuronal NMDAR sensitization so that the physiological level of Glu causes significant excitotoxicity and increases the intracellular calcium level. Our data indicates that blocking LRP, NMDAR, or nNOS activation reduces HIV-1 Tat internalization into neurons and prevents HIV-1 Tat-mediated apoptosis (Eugenin et al., 2007).

Also, HIV-1 protein gp120 acts synergistically with Tat and potentiates each protein's neurotoxic actions, promoting neuronal cell death (Nath et al., 2000). Most studies concerning the effect of Tat on NMDAR report that the changes in receptors function are acute (minutes to hours) (Haughey et al., 2001; Green and Thayer, 2016), while the neurotoxic properties of Tat occur after hours to days (Popescu, 2014). Tat-induced potentiation of the calcium release induced by NMDAR by 8 h then adapted, as later it was reported that it returned to the baseline by 24 h and dropped below control by 48 h (Krogh et al., 2014a).

Oligodendrocytes (OLs) are myelinating CNS cells. Their dysfunction leads to abnormal myelination, impairment of cell–cell signaling, and axon degeneration. OLs express iGluRs, including NMDA and AMPA receptors, and the expression of these receptors is highly heterogeneous (Salter and Fern, 2005). The NMDARs are mainly present on the myelin sheath, while AMPARs are equally distributed on the cell body (Micu et al., 2006). Also, the pattern of iGluRs distribution is different on immature and mature OLs; immature OLs represent a higher level of AMPARs and mature NMDARs, respectively. OL cells are also very sensitive to the toxic actions of Glu since the OL NMDARs are less susceptible to magnesium blockage; the level of Glu necessary to induce toxic effect in OLs may be lower than required for neurons (Karadottir et al., 2005). Activation of iGluR induced the accumulation of calcium in myelin cells in response to chemical ischemia. This effect was completely reversed by AMPA/KA antagonist (NBOX) in the oligodendroglial cell body but only moderately in myelin. In contrast, the broad spectrum of NMDAR antagonists (MK-801, 7-chloro-kynurenic acid, or d-AP5) decreased the level of calcium in myelin.

The massive release of Glu stimulated the NMDAR located on myelin and the accumulation of calcium inside myelin cells. The toxic action of Tat was also present in OL cells. In studies using transgenic mice expressing HIV-1 Tat, the primary corpus callosum and anterior commissure cell culture represent the increased level of OLs with aberrant morphology. Moreover, in the caudate-putamen, the myelin protein expression was abnormal. Tat protein caused the death of immature OLs and reduced myelin-like membrane production by mature OLs in a

dose-dependent manner. Both toxic effects of Tat were reversed by the blocking of NMDARs by specific antagonist MK-801, while CNQX (AMPAR/KAR antagonist) only blocked the effect on immature OLs (Zou et al., 2015). This data confirms the results from Karadottir et al. (2005) that, depending on the stage of development (mature vs. immature), the pattern of iGluR is different.

In the current ART era, it still is a matter of debate whether viral proteins are produced within the brain. However, data from several laboratories including ours indicates that HIV-1 Tat, Nef, and gp120 are still produced and patients are developing antibodies to these proteins (Nath et al., 1987; Re et al., 1996; Rao, 2003; Eugenin et al., 2011b; Bachani et al., 2013; Nicoli et al., 2016). Besides, antiretroviral drugs do not affect the Tat protein level in HIV-1-infected patients, even when the viral blood load is low (Dickens et al., 2017). Thus, bystander damage by viral protein secretion is still a major concern mainly because ART blocks HIV-1 replication but is unable to reduce viral protein synthesis and secretion as well as associated inflammation and damage.

HIV-1 and Glutamate Transporters

The major function of astrocytes is to support neurons by maintaining the balance of local ion concentration and pH homeostasis, regulating neurotransmitters level in extracellular space and clearing the metabolic waste (He and Sun, 2007). The dysfunction of the astrocyte network is a serious reason for the decline in neurons' survival. One of the main causes of neuron death is a massive release of Glu and NMDARs overactivation (Petralia, 2012; Wroge et al., 2012). Astrocytes prevent the neuronal loss by removing excess Glu from extracellular space by two Na^+-dependent glutamate transporters (EAATs): GLAST/EAAT1 and GLT-1/EAAT2 (Rothstein et al., 1996). Loss of astrocytic Glu uptake and metabolism is one of the main factors responsible for neurotoxicity associated with neurodegenerative diseases, including HAND. It was shown that the most important factor in HIV-1-induced neurotoxicity is dysregulation of astrocytes Glu clearance (Potter et al., 2013). In monocyte-derived macrophages (MDM) HIV-1-infection up-regulate the EAAT-2 gene expression, but the transporter activity was decreased (Porcheray et al., 2006). Moreover, GS gene expression was decreased but the GS activity was increased (Porcheray et al., 2006). In HIV-1-positive people with HAND the astrocytic level of EAAT2 was reduced at the same time the extracellular level of Glu was increased (Xing et al., 2009; Borjabad et al., 2010). In the CNS a multiple factors cause the reduction of Glu transporters expression and in consequence astrocyte-dependent Glu clearance such as the pro-inflammatory mediators—tumor necrosis factor (TNF-α), interleukin (IL)-1β (Lindl et al., 2010) and HIV-1 viral protein—envelope glycoprotein (gp120), transactivator of transcription (Tat), and viral protein R (Vpr). The number of studies using cell cultures models has shown that gp120 significantly reduced the expression and function of Glu transporters, resulting in excessive accumulation of extracellular Glu in the synaptic cleft, overstimulation of Glu receptors and neurotoxicity (Nath et al., 2000; Wang et al., 2003; Visalli et al., 2007; Xu et al., 2011; Yang et al., 2013; Melendez et al., 2016; Zhou et al., 2017). HIV-1 protein, gp120 alters the neuronal function homeostasis and often cause synaptodendritic injury (Kaul et al., 2001; Visalli et al., 2007; Melendez et al., 2016; Zhou et al., 2017). In primary human astrocytes HIV-1 and gp120 by reducing the expression of EAAT2, impaired the clearance of Glu (Wang et al., 2003). In pharmacology studies of enzymes kinetics, the reduction in V_{max} of both glutamate systems (X_{AG} and X_{c-}) was observed in striatal glial and neuronal cells from gp120 mice, with no effect on K_m for Glu (Melendez et al., 2016). These results suggest that gp120 reduces the density of Glu transporters in the mouse striatum without effect on Glu affinity to the transporters. Moreover, the total expression of GLT-1 protein was also altered in gp120 mice, which is involved in the development of HAND (Melendez et al., 2016). In Tat-transgenic mice the stimulus-evoked Glu release in the hippocampus and cortex was increased as compared to control animals, but GABA exocytosis was unaltered in the hippocampus and decreased in the cortex (Zucchini et al., 2013).

Protease inhibitors—Amprenavir (APV) and Lopinavir (LPV) caused the reduction of EAAT2 expression and diminished the intracellular concentration of Glu. LPV induced monocytes/microglia and astrocytes activation (Gupta et al., 2012), also in astrocytes LPV and APV increased Glu-dependent calcium efflux (Vivithanaporn et al., 2016). Moreover, the *in vivo* studies show a reduction in EAAT2 protein and mRNA level followed by the LPV treatment and suppression of the cortical level of L-glutamate and L-aspartate in conjunction with L-serine level reduction. The reduction of EAAT2 expression induced by LPV and APV on astrocytes reduces Glu extracellular clearance resulting in Glu overstimulation and calcium increase in glial cells (Vivithanaporn et al., 2016). Thus, a combination of HIV-1 infection and ART could also be additive negative effects in the glutamatergic system within the brain.

Tat specifically induced the macrophage/microglia activation and microglia-mediate neurotoxicity (Minghetti et al., 2004). Further it was shown that this process is caused by Tat-dependent activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Turchan-Cholewo et al., 2009). NADPH oxidase is a key component of ROS production for oxidative burst and cell signaling pathways. The activation of NADPH oxidase in microglia induce the release of Glu (Barger et al., 2007). It was shown that, Tat induced the dose-dependent Glu release from microglial which is associated with increased expression of the X_{c-} glutamate-cystine antiporter, and this effect was blocked by NADPH oxidase and glutamate-cystine inhibitors (Gupta et al., 2010). Furthermore, the extracellular cystine is transported inside of the cell by the counter-transporter cystine and then transformed to GSH. In HIV-1-infection GSH levels are decreased enhancing further oxidative damage induced by HIV-1 (Wanchu et al., 2009; Ferrucci et al., 2012). GSH function is not only antioxidant but is also very important for a conjunction of various drugs and xenobiotics elimination (Rieder et al., 1995; Singh et al., 2017). Also the proper function of X_{c-} system can be interrupted by the upregulation of activity of nuclear factor erythroid 2-releated factor 2 (Nrf2), the main activator of the antioxidant response by neutralization of ROS accumulation

and GSH depletion (Zhang et al., 2013). In astrocytes, the upregulation of xCT – X_{c-} system subunit altered by Nrf2 could be a potential source of excitotoxicity and neuron death induced by massive Glu release (Fogal et al., 2007; Bridges et al., 2012a,b). In astrocytes Tat protein induced the Nrf2 activation and X_{c-} system up-regulation (Mastrantonio et al., 2019). Moreover, it reduced the neuron viability in a mechanism dependent on Nrf2 activation and Glu release from astrocytes. This effect was blocked by X_{c-} system inhibitor—sulfasalazine, thus blockade of the transporter confirms the mechanism of neurotoxicity dependent on astrocytes activation (Mastrantonio et al., 2019). During oxidative stress conditions, cells can up-regulate the activity of Nrf2 to neutralize the ROS accumulation and not lead to GSH depletion (Niture et al., 2014). Astrocyte more resistant to oxidative stress in comparison to neurons which can strongly up-regulate the Nrf2 expression (Baxter and Hardingham, 2016). In response for cellular redox stare, Nrf2 migrates to the nucleus and binds to promoter region—antioxidant response element (ARE) of many antioxidant genes such as system X_{c-} subunit xCT, superoxide dismutase (SOD), glutathione peroxidase (GPX) (Niture et al., 2014). Treatment of human U373 astroglial cells with recombinant Tat increased the mRNA expression of several antioxidant enzymes such as X_{c-}, Quinone Dehydrogenase 1 (NQO1), catalyze (CAT), Cu/Zn superoxide dismutase (SOD1 and SOD2), glutamate-cystine ligase (GCLC), and GPX (Mastrantonio et al., 2019). Also, U373 astroglial cells treated with Tat increased the nuclear Nrf2 level which was compatible with the transcriptional induction of antioxidant ARE genes, as well as decreased cell viability, suggesting that Tat-associated neurodegeneration could be caused by the X_{c-} system (Mastrantonio et al., 2019).

The peroxisome proliferator-activated receptors (PPARs) ligand-activated transcription factors belonging to the nuclear receptors for steroids, thyroid hormones, and retinoids. These receptors play major roles in cell differentiation, lipid homeostasis, and glucose regulation. Nowadays, the agonist of PPAR gamma (PPARγ) is promising treatment for neuroinflammation-related condition eg. Alzheimer's disease, Parkinson's disease, and stroke (Govindarajulu et al., 2018; Wen et al., 2018; Lee et al., 2019). It was demonstrated that PPAR attenuated the release of pro-inflammatory cytokines and oxidative stress promotors. Several laboratories demonstrated that PPAR agonist can be neuroprotective against HIV-1-associated neuroinflammation (Potula et al., 2008; Huang et al., 2014, 2015). In astrocytes and microglial PPARγ agonists, rosiglitazone and pioglitazone, prevented oxidative stress induced by cytokines and NO as well as GLT-1 expression induced by HIV-1 and gp120 exposure (Omeragic et al., 2017).

The astrocyte elevated gene-1 (AEG-1) is a novel protein involved in HAND development. AEG-1 is a multifunctional oncogene, identify as an HIV-1- and TNF-α-inducible transcript in astrocytes. Its higher expression has been demonstrated in HIV-1-infected astrocytes, as well as in astrocytes treated with gp120 and TNF-α (Kang et al., 2005). AEG-1 was studied in the cancer field, describe as oncogene implicated in the initiation of tumorigenesis, proliferation, metastasis, angiogenesis, and chemotherapy resistance of malignancies (Emdad et al., 2009, 2010; Lee et al., 2009; Chen et al., 2017). In malignant gliomas, expression of AEG-1 was high and associated with necrosis and neurodegeneration in correlation with reduced expression of EAAT2 (Lee et al., 2011). Moreover, the AEG-1 overexpression increased the expression of YY1, a transcriptional repressor of EAAT2 in astrocytes. The same effect was presented in the glioma cell line and is connected to glioma-associated necrosis (Lee et al., 2011; Vartak-Sharma et al., 2014). Present that inflammation-induced the expression of AEG-1 in astrocytes and loss of EAAT2 could contribute to neurodegeneration that has a neuroinflammatory manifestation, such as HAND. HIV-1-associated neuroinflammation is caused by cytokines and chemokines such as IL-1β, TNF-α which is followed by an imbalance in Glu homeostasis caused by EAAT2 and increased CCL2 production (Vartak-Sharma et al., 2014). Moreover, all the inflammation stimulus significantly increased AEG-1 expression in astrocytes and nuclear translocation induced by the nuclear factor (NF)-κB pathway which increases expression of NF- κB-responsive chemokine such as CCL2 (Vartak-Sharma et al., 2014).

HIV-1 and Glutamate Metabolism Pathways

The main source of Glu overproduction in HIV-1 infection, includes the release of Glu from dying cells, disturbance in neurotransmitter clearance *via* impaired enzyme activity, and activated macrophages/microglia that also can release significant amounts of Glu into the extracellular space. Glu neurotoxicity is mainly caused by the up-regulation of Glu-generated enzyme—GLS isoform C in HIV-1-infected microglia. Also, the level of GLS isoform C was elevated in HIV-1-positive *post-mortem* brains which may be a compensatory effect induced by chronic exacerbate levels of Glu (Huang et al., 2011). The GLS C overexpression is also correlated with several neurocognitive and neurodegenerative disorders (Wang et al., 2017). The overproduction of Glu during HIV-1 infection in monocyte-derived macrophage (MDM) is caused by the release of GLS from the mitochondria followed by the generation of oxidative stress (Erdmann et al., 2009; Tian et al., 2012). In HIV-1-infected human MDM the Gln-dependent production of Glu was blocked by a GLS inhibitor (6-diazo-5-oxo-l-norleucine) and by the antiretroviral drug (zidovudine) (Zhao et al., 2004). Moreover, the excessive release of Glu from HIV-1-infected MDMs was inhibited by using *a* drugs that target N-acetylaspartylglutamate (NAAG) to specifically inhibit GLS (Erdmann et al., 2007). It was shown that Glu antagonist 6-diazo-5-oxo-L-norleucin (DON), a compound with GLS inhibition activity significantly decreased the Glu overproduction induced by HIV-1 and Glu-dependent neurotoxicity (Conti and Minelli, 1994; Zhao et al., 2004; Thomas et al., 2014). The Pro-drug of DON that is metabolized by carboxylesterases increasing the levels of the active drug mainly in the brain showing a higher CSF/plasma ratio (Nedelcovych et al., 2017). Furthermore, the new DON prodrug, JHU083, in a mouse model of HAND, reversed the deficits in spatial learning and working memory induced by HIV-1 infection. In biochemical tests JHU083 reduced extracellular level of Glu in CNS of mice infected with Eco-HIV, and decreased overstimulation of GLS in microglial cells (Nedelcovych et al., 2019).

HIV-1 viral protein R (Vpr) is known to promote virus replication in monocytes and differentiated macrophages (Connor et al., 1995). It was shown that soluble Vpr is detected in the CSF of HIV-1-positive patients, and it is a source of neurotoxicity and antioxidant system dysfunction (Patel et al., 2000; Ferrucci et al., 2012). Vpr transduced human derived macrophages and subsequent SILAC analysis indicates that around 50% of the proteins identified are involved in glycolytic and citrate pathways (Barrero et al., 2013). Next, labeling of glucose with Carbon-13 (^{13}C) and subsequent treatment of macrophages transduced with Vpr indicates that labeled glucose was uptaked faster than untransduced cells. Measure of release of Gln, Glu, and α-KG in Vpr transduced cells indicate a faster glycolytic and TCA metabolism (Datta et al., 2016). Imbalance in glutaminolysis (deamination of Gln by GLS to Glu with subsequent conversion of Glu to α-KG) affects the production of antioxidants such as GSH and NADPH. Moreover, α-KG an intermediate product in Glu metabolism is an ROS homeostasis stabilizer. Thus maintenance of ROS homeostasis in HIV-1-infected macrophages is sufficient for cell survival (Datta et al., 2016). Vpr also up-regulates the GLS isoform C expression, resulting in an increased level of Glu in a media of HIV-1-infected macrophages (Erdmann et al., 2009; Datta et al., 2016).

HIV-1 Metabolomics and Reservoir Survival

The analysis of circulating lipids of two HIV-1-infected populations, responding and non-responding to ART for 36 months, detected higher levels of HDL in the responding group and a high VLDL and low LDL in the non-responding group, suggesting that healthy lipids also are associated with better ART response (Palmer et al., 2014; Rodriguez-Gallego et al., 2018). Furthermore, examination of different groups of HIV-1-infected individuals separated by neurocognitive status (normal: stable neurocognitive impairments; worsening group: progression of neurocognitive impairments; and improving group: improving of their cognitive status) were analyzed by NMR. In the worsening group, the CSF levels of citrate and pyroglutamate were decreased, and the levels of glutamine and lactate were increased as compared to the normal cognition group. In contrast, stable and improved cognition showed decreased glutamine and glucose and increased citrate, pyroglutamate, and lactate as compared to the worsening group. This pattern suggests that the worsening group was associated with a higher glucose metabolism based on higher levels of creatine, suggesting higher energy demand; in contrast, the improving group was associated with a decrease in glycolysis and TCA cycle intermediates, suggesting a shift from aerobic to anaerobic metabolism as observed in the worsening group (Dickens et al., 2015). In HIV-1, it is known and well-described that the virus compromises most of the synaptic control of Glu as well as glial regulation of the glutamatergic system; however, the interpretation of these data was to increase synaptic compromise by overactivation of Glu receptors. Now, our data also indicate that high levels of extracellular Glu and Gln that are abound in HIV-1-conditons are a major source of energy to viral reservoirs (Castellano et al., 2019).

Overall, significant changes in brain volume have been observed, which may be related to synaptic compromise. In the pre-ART era, people with advanced disease had a significant reduction of cortical volume (Heindel et al., 1994; Peavy et al., 1994; Aylward et al., 1995) as compared to age-matched seronegative controls (Tate et al., 2010). The introduction of ART stopped the progression of brain volume loss in the thalamus, caudate, and cerebellum and caused cortical thinning in the frontal and temporal lobes and cingulate cortex (Sanford et al., 2018). Using MRS, the most common metabolic changes observed in HIV-1-positive patients is a decreased level of creatine (Cr); an increased level of choline (Cho) and myo-inositole (MI), which reflect neuroinflammation and microglial proliferation; and a decreased level of N-acetyl aspartate (NAA) and Glu as a markers of neuronal dysfunction and injury (Young et al., 2014). In patients before ART, the cortical level of inflammatory biomarkers Cho/Cr and MI/Cr was increased. In the same patients, the level of Glu/Cr in the basal ganglia was elevated and significantly declined after therapy initiation, suggesting a massive release of Glu from HIV-1-infected macrophages and microglia, as activated astrocytes function dysregulation. Introduction of ART within 5 years after infection attenuated the increase of these inflammatory markers (Young et al., 2014). Furthermore, in HIV-1-individuals under ART who manifest HAND, a lower ratio of NAA/Cr in frontal white matter, post cingulate, and precuneus was observed. Also, a decrease in the Glu/Cr ratio correlated with worse performance on verbal recall, psychomotor speed, and reaction time (Mohamed et al., 2018). In agreement with the previous data, a decreased level of NAA and Glu-Gln was also observed in the cortical gray matter during early HIV-1 infection, suggesting that HIV-1 causes neuronal and astroglial dysfunction soon after infection (Lentz et al., 2009).

In other studies, it has been demonstrated that in CSF of HIV-1-positive individuals who manifest HAND, the level of Glu was elevated, which suggests astrocyte activation—impairment of BBB integrity and Glu clearance. Moreover, in CSF, of all the examined individuals had an increased level of ketone bodies (BHBA and 1,2 propenodiol), which suggests the dysregulation of lipid metabolism (Cassol et al., 2014). This result is in line with our and other studies showing that HIV-1-infected cells can rapidly adapt and use an alternative sources of energy. In several pathologies, including cancer, glucose deficit results in the use of alternative sources of energy such as ketone bodies or amino acids (Laffel, 1999; Castellano et al., 2017).

$CD4^+$ T lymphocytes are characterized as a circulating viral reservoir, which maintains survival by increased glucose transporter-Glut-1 expression and elevated glycolysis (Loisel-Meyer et al., 2012). It was shown that Glut-1 expression on $CD4^+$ T lymphocytes is increased in HIV-1-infected individuals, and expression of Glut-1 could be a biomarker of CD4+ T lymphocyte activation in infected individuals (Palmer et al., 2014). Also, increased pyruvate delivery may be supported via the Glu-Gln cycle to cope with the high metabolic activity of

infected cells. Delivery of a higher amount of ATP can enhance the proliferation of infected cells and induce viral replication (Loftus and Finlay, 2016).

Next to the $CD4^+$ T lymphocytes, tissue macrophages can also establish HIV-1 reservoirs. First, HIV-1 replication occurs intracellularly in specific compartments described as a virus-containing compartments (VCCs), in which newly created virus particles sustain their infectious potential for extended periods of time (Gaudin et al., 2013), and, second, HIV-1-infected macrophages can survive the apoptotic response of the host cells against viral infection (Swingler et al., 2007; Castellano et al., 2017). It was shown that HIV-1-infected macrophages increase GSH synthesis to limit excitotoxicity and neutralize generated oxygen free radicals (Rimaniol et al., 2001). Furthermore, in latently infected macrophages, Bim, a highly pro-apoptotic negative regulator of Bcl-2, was upregulated and prevented Bcl-2-dependent sequencing of pro-apoptotic proteins in mitochondria. Furthermore, HIV-1 infection prevents the release of apoptosis-inducing factor (AIF) or cytochrome C from the mitochondria into the cytoplasm and apoptosome formation. Thus, HIV-1 infection and latency prevent apoptosis by compromising the formation of the transition pore in the mitochondria and by preventing the formation of the apoptosome (Castellano et al., 2017).

Previously we demonstrated that latent HIV-1-infected macrophages used unusual pathways to block apoptosis of infected cells by a Bim-mediated mechanism (Castellano et al., 2017). We characterized three different stages of HIV-1 infection in macrophages: an early stage (1–3 days post-infection) with increased HIV-1 replication; a middle stage (7–14 days post-infection) characterized by higher viral replication and high cell death, which affects mainly uninfected macrophages; and a late state (14–21 days post-infection) with minimal viral replication and cell death similar to the middle stage, resulting in the survival of a small population of infected macrophages. The stage of infection was associated with changes in mitochondrial metabolism where the basal oxygen consumption rate (OCR) was reduced in the early stage in contrast to middle stage where OCR was reduced and correlated with increased cell death. In last stage, where most surviving cells were infected, no future changes of OCR were observed. Moreover, blocking of mitochondrial complex V, which is responsible for ATP production, induced different cell responses in the early and late stages, but not in the middle stage where the level of apoptosis was the highest; this suggests that during minimal apoptosis respiration, ATP is reduced by the virus. Next, we established the metabolic changes in latently infected macrophages, which in contrast to $CD4^+$ T lymphocytes, did not rely on glucose in the HIV-1 condition but used alternative sources of energy coming from the TCA cycle and accumulated lipids. Observed changes in macrophages metabolism suggest that compromised TCA is an additional source of carbon to accumulate lipids, which can be used as an alternative source of energy. The role of the TCA cycle was established by treatment of human macrophages with succinate, an intermediate in TCA that plays a crucial role in ATP production in mitochondria. Succinate induced lipid accumulation in HIV-1-infected macrophages in contrast to uninfected cells. Furthermore, we characterized that under HIV-1 conditions, latently infected macrophages are using Glu/Gln to provide α-KG and succinate for TCA cycle, whereas in uninfected macrophages, ATP is produced from fatty acids and glucose. Moreover, in the HIV-1 condition, blockade delivery of substrate for energy production (fatty acids, Gln, and glucose) induce a shift for use of alternative sources of energy. HIV-1-infected macrophages, in contrast to uninfected macrophages, cannot use alternative sources of energy, opening a unique opportunity to kill this type of reservoir. Furthermore, we demonstrated that latently HIV-1-infected macrophages used Gln, Glu, and α-KG to survive because the blocking of GLS or an amino acid transporter required for amino acid importation into the mitochondria induced a specific killing of the surviving HIV-1-infected macrophages (Castellano et al., 2019). Moreover, proinflammatory molecules, such as IFNγ and LPS, stimulate macrophage activation inducing glucose uptake with concomitant suppression of fatty acid uptake and oxidation (Vats et al., 2006). It was shown that released ROS and treatment with LPS increased the Glut-1 expression (Freemerman et al., 2014).

In this review, we presented the current knowledge about the role of Glu in NeuroHIV pathogenesis. Excessive release of Glu with concomitant blockade of reuptake and metabolism induce excitotoxicity and constant inflammation leads to HAND development. In a successful ART era, HIV-1 cannot yet be cured, due to viral reservoirs that are established in peripheral and CNS compartments. However, recent clinical observations have hypothesized that an early initiation of ART is crucial to a progressive contraction of the latent HIV-1 reservoir ("shrink"). This could possibly be accomplished with simultaneous strategies that activate ("kick" or "shock") the latent reservoir and increase the clearance of virus-infected cells ("kill"), known as a "kick-kill" or "shock-kill" strategy (Chun et al., 1999; Archin et al., 2012; Van Lint et al., 2013; Pace and Frater, 2019; Edara et al., 2020). Although ART suppresses viremia in HIV-1 infected individuals, infected cells used Glu to maintain their survival and latent virus reservoirs. Moreover, Glu as a toxic molecule can induce viral rebound in latent infected cells. Thus, suppressing the overproduction of Glu shortly after infection may prevent neuroinflammation and successfully reduce the size of viral reservoirs that relay a significant amount of Glu to survive.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was funded by the National Institute of Mental Health grant, MH096625, the National Institute of Neurological Disorders and Stroke, NS105584, and UTMB funding (to EE).

REFERENCES

Abbate, I., Cappiello, G., Longo, R., Ursitti, A., Spano, A., Calcaterra, S., et al. (2005). Cell membrane proteins and quasispecies compartmentalization of CSF and plasma HIV-1 from aids patients with neurological disorders. *Infect. Genet. Evol.* 5, 247–253. doi: 10.1016/j.meegid.2004.08.006

Abrahams, M. R., Joseph, S. B., Garrett, N., Tyers, L., Moeser, M., Archin, N., et al. (2019). The replication-competent HIV-1 latent reservoir is primarily established near the time of therapy initiation. *Sci. Transl. Med.* 11:aaw5589. doi: 10.1126/scitranslmed.aaw5589

Abreu, C. M., Veenhuis, R. T., Avalos, C. R., Graham, S., Parrilla, D. R., Ferreira, E. A., et al. (2019). Myeloid and CD4 T cells comprise the latent reservoir in antiretroviral therapy-suppressed SIVmac251-infected macaques. *MBio* 10:e01659-19. doi: 10.1128/mBio.01659-19

Albrecht, J., and Norenberg, M. D. (2006). Glutamine: a Trojan horse in ammonia neurotoxicity. *Hepatology* 44, 788–794. doi: 10.1002/hep.21357

Alexaki, A., Liu, Y., and Wigdahl, B. (2008). Cellular reservoirs of HIV-1 and their role in viral persistence. *Curr. HIV Res.* 6, 388–400. doi: 10.2174/157016208785861195

Al-Harti, L., Joseph, J., and Nath, A. (2018). Astrocytes as an HIV CNS reservoir: highlights and reflections of an NIMH-sponsored symposium. *J. Neurovirol.* 24, 665–669. doi: 10.1007/s13365-018-0691-8

Allen, N. J., Karadottir, R., and Attwell, D. (2004). Reversal or reduction of glutamate and GABA transport in CNS pathology and therapy. *Pflugers Arch.* 449, 132–142. doi: 10.1007/s00424-004-1318-x

Anderson, A. M., Harezlak, J., Bharti, A., Mi, D., Taylor, M. J., Daar, E. S., et al. (2015). Plasma and cerebrospinal fluid biomarkers predict cerebral injury in HIV-infected individuals on stable combination antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.* 69, 29–35. doi: 10.1097/QAI.0000000000000532

Angelovich, T. A., Churchill, M. J., Wright, E. J., Brew, B. J. (2020). "New Potential Axes of HIV Neuropathogenesis with Relevance to Biomarkers and Treatment," in *Current Topics in Behavioral Neurosciences* (Berlin; Heidelberg: Springer). doi: 10.1007/7854_2019_126

Archin, N. M., Liberty, A. L., Kashuba, A. D., Choudhary, S. K., Kuruc, J. D., Crooks, A. M., et al. (2012). Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* 487, 482–485. doi: 10.1038/nature11286

Ariazi, J., Benowitz, A., De Biasi, V., Den Boer, M. L., Cherqui, S., Cui, H., et al. (2017). Tunneling nanotubes and gap junctions-their role in long-range intercellular communication during development, health, and disease conditions. *Front. Mol. Neurosci.* 10:333. doi: 10.3389/fnmol.2017.00333

Avdoshina, V., Fields, J. A., Castellano, P., Dedoni, S., Palchik, G., Trejo, M., et al. (2016). The HIV protein gp120 alters mitochondrial dynamics in neurons. *Neurotox. Res.* 29, 583–593. doi: 10.1007/s12640-016-9608-6

Aylward, E. H., Brettschneider, P. D., McArthur, J. C., Harris, G. J., Schlaepfer, T. E., Henderer, J. D., et al. (1995). Magnetic resonance imaging measurement of gray matter volume reductions in HIV dementia. *Am. J. Psychiatry* 152, 987–994. doi: 10.1176/ajp.152.7.987

Bachani, M., Sacktor, N., McArthur, J. C., Nath, A., and Rumbaugh, J. (2013). Detection of anti-tat antibodies in CSF of individuals with HIV-associated neurocognitive disorders. *J. Neurovirol.* 19, 82–88. doi: 10.1007/s13365-012-0144-8

Bachis, A., Cruz, M. I., and Mocchetti, I. (2010). M-tropic HIV envelope protein gp120 exhibits a different neuropathological profile than T-tropic gp120 in rat striatum. *Eur. J. Neurosci.* 32, 570–578. doi: 10.1111/j.1460-9568.2010.07325.x

Bak, L. K., Schousboe, A., and Waagepetersen, H. S. (2006). The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J. Neurochem.* 98, 641–653. doi: 10.1111/j.1471-4159.2006.03913.x

Bandera, A., Taramasso, L., Bozzi, G., Muscatello, A., Robinson, J. A., Burdo, T. H., et al. (2019). HIV-associated neurocognitive impairment in the modern ART era: are we close to discovering reliable biomarkers in the setting of virological suppression? *Front. Aging Neurosci.* 11:187. doi: 10.3389/fnagi.2019.00187

Barger, S. W., Goodwin, M. E., Porter, M. M., and Beggs, M. L. (2007). Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *J. Neurochem* 101, 1205–1213. doi: 10.1111/j.1471-4159.2007.04487.x

Barrero, C. A., Datta, P. K., Sen, S., Deshmane, S., Amini, S., Khalili, K., et al. (2013). HIV-1 Vpr modulates macrophage metabolic pathways: a SILAC-based quantitative analysis. *PLoS ONE* 8:e68376. doi: 10.1371/journal.pone.0068376

Baxter, P. S., and Hardingham, G. E. (2016). Adaptive regulation of the brain's antioxidant defences by neurons and astrocytes. *Free Radic. Biol. Med.* 100, 147–152. doi: 10.1016/j.freeradbiomed.2016.06.027

Berman, J. W., Carvallo, L., Buckner, C. M., Luers, A., Prevedel, L., Bennett, M. V., et al. (2016). HIV-tat alters Connexin43 expression and trafficking in human astrocytes: role in NeuroAIDS. *J. Neuroinflamm.* 13:54. doi: 10.1186/s12974-016-0510-1

Bingham, R., Ahmed, N., Rangi, P., Johnson, M., Tyrer, M., and Green, J. (2011). HIV encephalitis despite suppressed viraemia: a case of compartmentalized viral escape. *Int. J. STD AIDS* 22, 608–609. doi: 10.1258/ijsa.2011.010507

Blankson, J. N., Persaud, D., and Siliciano, R. F. (2002). The challenge of viral reservoirs in HIV-1 infection. *Annu. Rev. Med.* 53, 557–593. doi: 10.1146/annurev.med.53.082901.104024

Bonansco, C., and Fuenzalida, M. (2016). Plasticity of hippocampal excitatory-inhibitory balance: missing the synaptic control in the epileptic brain. *Neural Plast.* 2016:8607038. doi: 10.1155/2016/8607038

Borderia, A. V., Codoner, F. M., and Sanjuan, R. (2007). Selection promotes organ compartmentalization in HIV-1: evidence from gag and pol genes. *Evolution* 61, 272–279. doi: 10.1111/j.1558-5646.2007.00025.x

Borjabad, A., Brooks, A. I., and Volsky, D. J. (2010). Gene expression profiles of HIV-1-infected glia and brain: toward better understanding of the role of astrocytes in HIV-1-associated neurocognitive disorders. *J. Neuroimmune Pharmacol.* 5, 44–62. doi: 10.1007/s11481-009-9167-1

Bridges, R., Lutgen, V., Lobner, D., and Baker, D. A. (2012a). Thinking outside the cleft to understand synaptic activity: contribution of the cystine-glutamate antiporter (System xc-) to normal and pathological glutamatergic signaling. *Pharmacol. Rev.* 64, 780–802. doi: 10.1124/pr.110.003889

Bridges, R. J., Natale, N. R., and Patel, S. A. (2012b). System xc(-) cystine/glutamate antiporter: an update on molecular pharmacology and roles within the CNS. *Br. J. Pharmacol.* 165, 20–34. doi: 10.1111/j.1476-5381.2011.01480.x

Brier, M. R., Wu, Q., Tanenbaum, A. B., Westerhaus, E. T., Kharasch, E. D., and Ances, B. M. (2015). Effect of HAART on brain organization and function in HIV-negative subjects. *J. Neuroimmune Pharmacol.* 10, 517–521. doi: 10.1007/s11481-015-9634-9

Capone, C., Cervelli, M., Angelucci, E., Colasanti, M., Macone, A., Mariottini, P., et al. (2013). A role for spermine oxidase as a mediator of reactive oxygen species production in HIV-Tat-induced neuronal toxicity. *Free Radic. Biol. Med.* 63, 99–107. doi: 10.1016/j.freeradbiomed.2013.05.007

Cardona, C., Sanchez-Mejias, E., Davila, J. C., Martin-Rufian, M., Campos-Sandoval, J. A., Vitorica, J., et al. (2015). Expression of Gls and Gls2 glutaminase isoforms in astrocytes. *Glia* 63, 365–382. doi: 10.1002/glia.22758

Cassol, E., Misra, V., Dutta, A., Morgello, S., and Gabuzda, D. (2014). Cerebrospinal fluid metabolomics reveals altered waste clearance and accelerated aging in HIV patients with neurocognitive impairment. *AIDS* 28, 1579–1591. doi: 10.1097/QAD.0000000000000303

Castellano, P., Prevedel, L., and Eugenin, E. A. (2017). HIV-infected macrophages and microglia that survive acute infection become viral reservoirs by a mechanism involving Bim. *Sci. Rep.* 7:12866. doi: 10.1038/s41598-017-12758-w

Castellano, P., Prevedel, L., Valdebenito, S., and Eugenin, E. A. (2019). HIV infection and latency induce a unique metabolic signature in human macrophages. *Sci. Rep.* 9:3941. doi: 10.1038/s41598-019-39898-5

Chen, B. S., and Roche, K. W. (2007). Regulation of NMDA receptors by phosphorylation. *Neuropharmacology* 53, 362–368. doi: 10.1016/j.neuropharm.2007.05.018

Chen, C. Y., Chen, Y. Y., Chen, J. J. W., Chen, K. Y., Ho, C. C., Shih, J. Y., et al. (2017). Astrocyte-elevated gene-1 confers resistance to pemetrexed in non-small cell lung cancer by upregulating thymidylate synthase expression. *Oncotarget* 8, 61901–61916. doi: 10.18632/oncotarget.18717

Chomont, N., El-Far, M., Ancuta, P., Trautmann, L., Procopio, F. A., Yassine-Diab, B., et al. (2009). HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat. Med.* 15, 893–900. doi: 10.1038/nm.1972

Chun, T. W., Engel, D., Mizell, S. B., Hallahan, C. W., Fischette, M., Park, S., et al. (1999). Effect of interleukin-2 on the pool of latently infected, resting CD4+ T

cells in HIV-1-infected patients receiving highly active anti-retroviral therapy. *Nat. Med.* 5, 651–655. doi: 10.1038/9498

Churchill, M. J., Gorry, P. R., Cowley, D., Lal, L., Sonza, S., Purcell, D. F., et al. (2006). Use of laser capture microdissection to detect integrated HIV-1 DNA in macrophages and astrocytes from autopsy brain tissues. *J. Neurovirol.* 12, 146–152. doi: 10.1080/13550280600748946

Churchill, M. J., Wesselingh, S. L., Cowley, D., Pardo, C. A., McArthur, J. C., Brew, B. J., et al. (2009). Extensive astrocyte infection is prominent in human immunodeficiency virus-associated dementia. *Ann. Neurol.* 66, 253–258. doi: 10.1002/ana.21697

Cohen, R. A., Harezlak, J., Gongvatana, A., Buchthal, S., Schifitto, G., Clark, U., et al. (2010). Cerebral metabolite abnormalities in human immunodeficiency virus are associated with cortical and subcortical volumes. *J. Neurovirol.* 16, 435–444. doi: 10.1007/BF03210849

Conn, P. J., and Pin, J. P. (1997). Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.* 37, 205–237. doi: 10.1146/annurev.pharmtox.37.1.205

Connor, R. I., Chen, B. K., Choe, S., and Landau, N. R. (1995). Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. *Virology* 206, 935–944. doi: 10.1006/viro.1995.1016

Conti, F., and Minelli, A. (1994). Glutamate immunoreactivity in rat cerebral cortex is reversibly abolished by 6-diazo-5-oxo-L-norleucine (DON), an inhibitor of phosphate-activated glutaminase. *J. Histochem. Cytochem.* 42, 717–726. doi: 10.1177/42.6.7910617

Contractor, A., Mulle, C., and Swanson, G. T. (2011). Kainate receptors coming of age: milestones of two decades of research. *Trends Neurosci* 34, 154–163. doi: 10.1016/j.tins.2010.12.002

Cysique, L. A., Juge, L., Lennon, M. J., Gates, T. M., Jones, S. P., Lovelace, M. D., et al. (2019). HIV brain latency as measured by CSF BcL11b relates to disrupted brain cellular energy in virally suppressed HIV infection. *AIDS* 33, 433–441. doi: 10.1097/QAD.0000000000002076

D'Antoni, S., Berretta, A., Bonaccorso, C. M., Bruno, V., Aronica, E., Nicoletti, F., et al. (2008). Metabotropic glutamate receptors in glial cells. *Neurochem. Res.* 33, 2436–2443. doi: 10.1007/s11064-008-9694-9

Datta, P. K., Deshmane, S., Khalili, K., Merali, S., Gordon, J. C., Fecchio, C., et al. (2016). Glutamate metabolism in HIV-1 infected macrophages: role of HIV-1 Vpr. *Cell Cycle* 15, 2288–2298. doi: 10.1080/15384101.2016.1190054

de Almeida, S. M., Rotta, I., Ribeiro, C. E., Oliveira, M. F., Chaillon, A., de Pereira, A. P., et al. (2017). Dynamic of CSF and serum biomarkers in HIV-1 subtype C encephalitis with CNS genetic compartmentalization-case study. *J. Neurovirol.* 23, 460–473. doi: 10.1007/s13365-017-0518-z

Dickens, A. M., Anthony, D. C., Deutsch, R., Mielke, M. M., Claridge, T. D., Grant, I., et al. (2015). Cerebrospinal fluid metabolomics implicate bioenergetic adaptation as a neural mechanism regulating shifts in cognitive states of HIV-infected patients. *AIDS* 29, 559–569. doi: 10.1097/QAD.0000000000000580

Dickens, A. M., Yoo, S. W., Chin, A. C., Xu, J., Johnson, T. P., Trout, A. L., et al. (2017). Chronic low-level expression of HIV-1 Tat promotes a neurodegenerative phenotype with aging. *Sci. Rep.* 7:7748. doi: 10.1038/s41598-017-07570-5

Duguid, I. C., and Smart, T. G. (2004). Retrograde activation of presynaptic NMDA receptors enhances GABA release at cerebellar interneuron-Purkinje cell synapses. *Nat. Neurosci.* 7, 525–533. doi: 10.1038/nn1227

Eck, H. P., Frey, H., and Droge, W. (1989). Elevated plasma glutamate concentrations in HIV-1-infected patients may contribute to loss of macrophage and lymphocyte functions. *Int. Immunol.* 1, 367–372. doi: 10.1093/intimm/1.4.367

Edara, V. V., Ghorpade, A., and Borgmann, K. (2020). Insights into the gene expression profiles of active and restricted red/green-HIV(+) human astrocytes: implications for shock or lock therapies in the brain. *J. Virol.* 94:e01563-19. doi: 10.1128/JVI.01563-19

Eggers, C., Arendt, G., Hahn, K., Husstedt, I. W., Maschke, M., Neuen-Jacob, E., et al. (2017). HIV-1-associated neurocognitive disorder: epidemiology, pathogenesis, diagnosis, and treatment. *J. Neurol.* 264, 1715–1727. doi: 10.1007/s00415-017-8503-2

Emdad, L., Lee, S. G., Su, Z. Z., Jeon, H. Y., Boukerche, H., Sarkar, D., et al. (2009). Astrocyte elevated gene-1 (AEG-1) functions as an oncogene and regulates angiogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21300–21305. doi: 10.1073/pnas.0910936106

Emdad, L., Sarkar, D., Lee, S. G., Su, Z. Z., Yoo, B. K., Dash, R., et al. (2010). Astrocyte elevated gene-1: a novel target for human glioma therapy. *Mol. Cancer Ther.* 9, 79–88. doi: 10.1158/1535-7163.MCT-09-0752

Erdmann, N., Tian, C., Huang, Y., Zhao, J., Herek, S., Curthoys, N., et al. (2009). *In vitro* glutaminase regulation and mechanisms of glutamate generation in HIV-1-infected macrophage. *J. Neurochem.* 109, 551–561. doi: 10.1111/j.1471-4159.2009.05989.x

Erdmann, N., Zhao, J., Lopez, A. L., Herek, S., Curthoys, N., Hexum, T. D., et al. (2007). Glutamate production by HIV-1 infected human macrophage is blocked by the inhibition of glutaminase. *J. Neurochem.* 102, 539–549. doi: 10.1111/j.1471-4159.2007.04594.x

Erickson, J. W., and Cerione, R. A. (2010). Glutaminase: a hot spot for regulation of cancer cell metabolism? *Oncotarget* 1, 734–740. doi: 10.18632/oncotarget.208

Ernst, T., Jiang, C. S., Nakama, H., Buchthal, S., and Chang, L. (2010). Lower brain glutamate is associated with cognitive deficits in HIV patients: a new mechanism for HIV-associated neurocognitive disorder. *J. Magn. Reson. Imaging* 32, 1045–1053. doi: 10.1002/jmri.22366

Eugenin, E. A., Clements, J. E., Zink, M. C., and Berman, J. W. (2011a). Human immunodeficiency virus infection of human astrocytes disrupts blood-brain barrier integrity by a gap junction-dependent mechanism. *J. Neurosci.* 31, 9456–9465. doi: 10.1523/JNEUROSCI.1460-11.2011

Eugenin, E. A., D'Aversa, T. G., Lopez, L., Calderon, T. M., and Berman, J. W. (2003). MCP-1 (CCL2) protects human neurons and astrocytes from NMDA or HIV-tat-induced apoptosis. *J. Neurochem.* 85, 1299–1311. doi: 10.1046/j.1471-4159.2003.01775.x

Eugenin, E. A., Gaskill, P. J., and Berman, J. W. (2009a). Tunneling nanotubes (TNT) are induced by HIV-infection of macrophages: a potential mechanism for intercellular HIV trafficking. *Cell. Immunol.* 254, 142–148. doi: 10.1016/j.cellimm.2008.08.005

Eugenin, E. A., Gaskill, P. J., and Berman, J. W. (2009b). Tunneling nanotubes (TNT): a potential mechanism for intercellular HIV trafficking. *Commun. Integr. Biol.* 2, 243–244. doi: 10.4161/cib.2.3.8165

Eugenin, E. A., King, J. E., Hazleton, J. E., Major, E. O., Bennett, M. V., Zukin, R. S., et al. (2011b). Differences in NMDA receptor expression during human development determine the response of neurons to HIV-tat-mediated neurotoxicity. *Neurotox. Res.* 19, 138–148. doi: 10.1007/s12640-010-9150-x

Eugenin, E. A., King, J. E., Nath, A., Calderon, T. M., Zukin, R. S., Bennett, M. V., et al. (2007). HIV-tat induces formation of an LRP-PSD-95- NMDAR-nNOS complex that promotes apoptosis in neurons and astrocytes. *Proc. Natl. Acad. Sci. U.S.A.* 104, 3438–3443. doi: 10.1073/pnas.0611699104

Eugenin, E. A., Osiecki, K., Lopez, L., Goldstein, H., Calderon, T. M., and Berman, J. W. (2006). CCL2/monocyte chemoattractant protein-1 mediates enhanced transmigration of human immunodeficiency virus (HIV)-infected leukocytes across the blood-brain barrier: a potential mechanism of HIV-CNS invasion and NeuroAIDS. *J. Neurosci.* 26, 1098–1106. doi: 10.1523/JNEUROSCI.3863-05.2006

Fernandes, N., and Pulliam, L. (2019). "Inflammatory Mechanisms and Cascades Contributing to Neurocognitive Impairment in HIV/AIDS," in *Current Topics in Behavioral Neurosciences* (Berlin; Heidelberg: Springer). doi: 10.1007/7854_2019_100

Ferrarese, C., Aliprandi, A., Tremolizzo, L., Stanzani, L., De Micheli, A., Dolara, A., et al. (2001). Increased glutamate in CSF and plasma of patients with HIV dementia. *Neurology* 57, 671–675. doi: 10.1212/WNL.57.4.671

Ferrucci, A., Nonnemacher, M. R., Cohen, E. A., and Wigdahl, B. (2012). Extracellular human immunodeficiency virus type 1 viral protein R causes reductions in astrocytic ATP and glutathione levels compromising the antioxidant reservoir. *Virus Res.* 167, 358–369. doi: 10.1016/j.virusres.2012.06.002

Finzi, D., Blankson, J., Siliciano, J. D., Margolick, J. B., Chadwick, K., Pierson, T., et al. (1999). Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat. Med.* 5, 512–517. doi: 10.1038/8394

Fischer-Smith, T., Croul, S., Sverstiuk, A. E., Capini, C., L'Heureux, D., Regulier, E. G., et al. (2001). CNS invasion by CD14+/CD16+ peripheral blood-derived monocytes in HIV dementia: perivascular accumulation and reservoir of HIV infection. *J. Neurovirol.* 7, 528–541. doi: 10.1080/135502801753248114

Fiszman, M. L., Barberis, A., Lu, C., Fu, Z., Erdelyi, F., Szabo, G., et al. (2005). NMDA receptors increase the size of GABAergic terminals and enhance GABA release. *J. Neurosci.* 25, 2024–2031. doi: 10.1523/JNEUROSCI.4980-04.2005

Fogal, B., Li, J., Lobner, D., McCullough, L. D., and Hewett, S. J. (2007). System x(c)- activity and astrocytes are necessary for interleukin-1 beta-mediated hypoxic neuronal injury. *J. Neurosci.* 27, 10094–10105. doi: 10.1523/JNEUROSCI.2459-07.2007

Ford, T. C., Nibbs, R., and Crewther, D. P. (2017). Increased glutamate/GABA+ ratio in a shared autistic and schizotypal trait phenotype termed social disorganisation. *Neuroimage Clin.* 16, 125–131. doi: 10.1016/j.nicl.2017.07.009

Freemerman, A. J., Johnson, A. R., Sacks, G. N., Milner, J. J., Kirk, E. L., Troester, M. A., et al. (2014). Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. *J. Biol. Chem.* 289, 7884–7896. doi: 10.1074/jbc.M113.522037

Ganor, Y., Real, F., Sennepin, A., Dutertre, C. A., Prevedel, L., Xu, L., et al. (2019). HIV-1 reservoirs in urethral macrophages of patients under suppressive antiretroviral therapy. *Nat Microbiol.* 4, 633–644. doi: 10.1038/s41564-018-0335-z

Garcia, F., Plana, M., Vidal, C., Cruceta, A., O'Brien, W. A., Pantaleo, G., et al. (1999). Dynamics of viral load rebound and immunological changes after stopping effective antiretroviral therapy. *AIDS* 13, F79–86. doi: 10.1097/00002030-199907300-00002

Gatanaga, H., Oka, S., Ida, S., Wakabayashi, T., Shioda, T., and Iwamoto, A. (1999). Active HIV-1 redistribution and replication in the brain with HIV encephalitis. *Arch. Virol.* 144, 29–43. doi: 10.1007/s007050050483

Gaudin, R., Berre, S., Cunha de Alencar, B., Decalf, J., Schindler, M., Gobert, F. X., et al. (2013). Dynamics of HIV-containing compartments in macrophages reveal sequestration of virions and transient surface connections. *PLoS One* 8:e69450. doi: 10.1371/journal.pone.0069450

Gelman, B. B. (2015). Neuropathology of HAND with suppressive antiretroviral therapy: encephalitis and neurodegeneration reconsidered. *Curr. HIV/AIDS Rep.* 12, 272–279. doi: 10.1007/s11904-015-0266-8

Gelman, B. B., Lisinicchia, J. G., Morgello, S., Masliah, E., Commins, D., Achim, C. L., et al. (2013). Neurovirological correlation with HIV-associated neurocognitive disorders and encephalitis in a HAART-era cohort. *J. Acquir. Immune Defic Syndr.* 62, 487–495. doi: 10.1097/QAI.0b013e31827f1bdb

Gianella, S., Kosakovsky Pond, S. L., Oliveira, M. F., Scheffler, K., Strain, M. C., De la Torre, A., et al. (2016). Compartmentalized HIV rebound in the central nervous system after interruption of antiretroviral therapy. *Virus Evol.* 2:vew020. doi: 10.1093/ve/vew020

Gibb, A. J., Ogden, K. K., McDaniel, M. J., Vance, K. M., Kell, S. A., Butch, C., et al. (2018). A structurally derived model of subunit-dependent NMDA receptor function. *J. Physiol.* 596, 4057–4089. doi: 10.1113/JP276093

Goldstein, G. (1996). HIV-1 Tat protein as a potential AIDS vaccine. *Nat. Med.* 2, 960–964. doi: 10.1038/nm0996-960

Gonzalez-Perez, M. P., O'Connell, O., Lin, R., Sullivan, W. M., Bell, J., Simmonds, P., et al. (2012). Independent evolution of macrophage-tropism and increased charge between HIV-1 R5 envelopes present in brain and immune tissue. *Retrovirology.* 9:20. doi: 10.1186/1742-4690-9-20

Govindarajulu, M., Pinky, P. D., Bloemer, J., Ghanei, N., Suppiramaniam, V., and Amin, R. (2018). Signaling mechanisms of selective PPARgamma modulators in alzheimer's disease. *PPAR Res.* 2018:2010675. doi: 10.1155/2018/2010675

Green, M. V., and Thayer, S. A. (2016). NMDARs adapt to neurotoxic HIV protein tat downstream of a GluN2A-ubiquitin ligase signaling pathway. *J. Neurosci.* 36, 12640–12649. doi: 10.1523/JNEUROSCI.2980-16.2016

Grewer, C., Gameiro, A., Zhang, Z., Tao, Z., Braams, S., and Rauen, T. (2008). Glutamate forward and reverse transport: from molecular mechanism to transporter-mediated release after ischemia. *IUBMB Life* 60, 609–619. doi: 10.1002/iub.98

Guo, H., Camargo, L. M., Yeboah, F., Digan, M. E., Niu, H., Pan, Y., et al. (2017). A NMDA-receptor calcium influx assay sensitive to stimulation by glutamate and glycine/D-serine. *Sci. Rep.* 7:11608. doi: 10.1038/s41598-017-11947-x

Gupta, S., Knight, A. G., Knapp, P. E., Hauser, K. F., Keller, J. N., and Bruce-Keller, A. J. (2010). HIV-Tat elicits microglial glutamate release: role of NAPDH oxidase and the cystine-glutamate antiporter. *Neurosci. Lett* 485, 233–236. doi: 10.1016/j.neulet.2010.09.019

Gupta, S., Knight, A. G., Losso, B. Y., Ingram, D. K., Keller, J. N., and Bruce-Keller, A. J. (2012). Brain injury caused by HIV protease inhibitors: role of lipodystrophy and insulin resistance. *Antiviral Res.* 95, 19–29. doi: 10.1016/j.antiviral.2012.04.010

Haase, A. T. (1999). Population biology of HIV-1 infection: viral and CD4+ T cell demographics and dynamics in lymphatic tissues. *Annu. Rev. Immunol.* 17, 625–656. doi: 10.1146/annurev.immunol.17.1.625

Hara, N., Yamada, K., Terashima, M., Osago, H., Shimoyama, M., and Tsuchiya, M. (2003). Molecular identification of human glutamine- and ammonia-dependent NAD synthetases. Carbon-nitrogen hydrolase domain confers glutamine dependency. *J. Biol. Chem.* 278, 10914–10921. doi: 10.1074/jbc.M209203200

Harris-Warrick, R. (2005). Synaptic chemistry in single neurons: GABA is identified as an inhibitory neurotransmitter. *J. Neurophysiol.* 93, 3029–3031. doi: 10.1152/classicessays.00026.2005

Haughey, N. J., Holden, C. P., Nath, A., and Geiger, J. D. (1999). Involvement of inositol 1,4,5-trisphosphate-regulated stores of intracellular calcium in calcium dysregulation and neuron cell death caused by HIV-1 protein tat. *J. Neurochem.* 73, 1363–1374. doi: 10.1046/j.1471-4159.1999.0731363.x

Haughey, N. J., Nath, A., Mattson, M. P., Slevin, J. T., and Geiger, J. D. (2001). HIV-1 Tat through phosphorylation of NMDA receptors potentiates glutamate excitotoxicity. *J. Neurochem.* 78, 457–467. doi: 10.1046/j.1471-4159.2001.00396.x

He, F., and Sun, Y. E. (2007). Glial cells more than support cells? *Int. J. Biochem. Cell Biol.* 39, 661–665. doi: 10.1016/j.biocel.2006.10.022

Heindel, W. C., Jernigan, T. L., Archibald, S. L., Achim, C. L., Masliah, E., and Wiley, C. A. (1994). The relationship of quantitative brain magnetic resonance imaging measures to neuropathologic indexes of human immunodeficiency virus infection. *Arch. Neurol.* 51, 1129–1135. doi: 10.1001/archneur.1994.00540230067015

Hertz, L., and Chen, Y. (2017). Integration between glycolysis and glutamate-glutamine cycle flux may explain preferential glycolytic increase during brain activation, requiring glutamate. *Front. Integr. Neurosci.* 11:18. doi: 10.3389/fnint.2017.00018

Hill, A. L. (2018). Mathematical models of HIV latency. *Curr. Top. Microbiol. Immunol.* 417, 131–156. doi: 10.1007/82_2017_77

Hill, A. L., Rosenbloom, D. I., Fu, F., Nowak, M. A., and Siliciano, R. F. (2014). Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1. *Proc. Natl. Acad. Sci. U.S.A.* 111, 13475–13480. doi: 10.1073/pnas.1406663111

Hill, A. L., Rosenbloom, D. I., Siliciano, J. D., and Siliciano, R. F. (2016). Insufficient evidence for rare activation of latent HIV in the absence of reservoir-reducing interventions. *PLoS Pathog.* 12:e1005679. doi: 10.1371/journal.ppat.1005679

Ho, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M., and Markowitz, M. (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 373, 123–126. doi: 10.1038/373123a0

Holcomb, T., Taylor, L., Trohkimoinen, J., and Curthoys, N. P. (2000). Isolation, characterization and expression of a human brain mitochondrial glutaminase cDNA. *Brain Res. Mol. Brain Res.* 76, 56–63. doi: 10.1016/S0169-328X(99)00331-9

Huang, W., Chen, L., Zhang, B., Park, M., and Toborek, M. (2014). PPAR agonist-mediated protection against HIV Tat-induced cerebrovascular toxicity is enhanced in MMP-9-deficient mice. *J. Cereb. Blood Flow Metab.* 34, 646–653. doi: 10.1038/jcbfm.2013.240

Huang, W., Mo, X., Wu, X., Luo, W., and Chen, Y. (2015). Rosiglitazone suppresses HIV-1 Tat-induced vascular inflammation via Akt signaling. *Mol. Cell. Biochem.* 407, 173–179. doi: 10.1007/s11010-015-2467-2

Huang, Y., Zhao, L., Jia, B., Wu, L., Li, Y., Curthoys, N., et al. (2011). Glutaminase dysregulation in HIV-1-infected human microglia mediates neurotoxicity: relevant to HIV-1-associated neurocognitive disorders. *J. Neurosci.* 31, 15195–15204. doi: 10.1523/JNEUROSCI.2051-11.2011

Jain, V., Hartogensis, W., Bacchetti, P., Hunt, P. W., Hatano, H., Sinclair, E., et al. (2013). Antiretroviral therapy initiated within 6 months of HIV infection is associated with lower T-cell activation and smaller HIV reservoir size. *J. Infect. Dis.* 208, 1202–1211. doi: 10.1093/infdis/jit311

Josefsson, L., Palmer, S., Faria, N. R., Lemey, P., Casazza, J., Ambrozak, D., et al. (2013). Single cell analysis of lymph node tissue from HIV-1 infected patients reveals that the majority of CD4+ T-cells contain one HIV-1 DNA molecule. *PLoS Pathog.* 9:e1003432. doi: 10.1371/journal.ppat.1003432

Kanai, Y., and Hediger, M. A. (2004). The glutamate/neutral amino acid transporter family SLC1: molecular, physiological and pharmacological aspects. *Pflugers Arch.* 447, 469–479. doi: 10.1007/s00424-003-1146-4

Kandathil, A. J., Sugawara, S., and Balagopal, A. (2016). Are T cells the only HIV-1 reservoir? *Retrovirology* 13:86. doi: 10.1186/s12977-016-0323-4

Kang, D. C., Su, Z. Z., Sarkar, D., Emdad, L., Volsky, D. J., and Fisher, P. B. (2005). Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. *Gene* 353, 8–15. doi: 10.1016/j.gene.2005.04.006

Karadottir, R., Cavelier, P., Bergersen, L. H., and Attwell, D. (2005). NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. *Nature* 438, 1162–1166. doi: 10.1038/nature04302

Kaul, M., Garden, G. A., and Lipton, S. A. (2001). Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature* 410, 988–994. doi: 10.1038/35073667

Kaul, M., Ma, Q., Medders, K. E., Desai, M. K., and Lipton, S. A. (2007). HIV-1 coreceptors CCR5 and CXCR4 both mediate neuronal cell death but CCR5 paradoxically can also contribute to protection. *Cell Death Differ.* 14, 296–305. doi: 10.1038/sj.cdd.4402006

Kim-Chang, J. J., Donovan, K., Loop, M. S., Hong, S., Fischer, B., Venturi, G., et al. (2019). Higher soluble CD14 levels are associated with lower visuospatial memory performance in youth with HIV. *AIDS* 33, 2363–2374. doi: 10.1097/QAD.0000000000002371

King, J. E., Eugenin, E. A., Hazleton, J. E., Morgello, S., and Berman, J. W. (2010). Mechanisms of HIV-tat-induced phosphorylation of N-methyl-D-aspartate receptor subunit 2A in human primary neurons: implications for neuroAIDS pathogenesis. *Am. J. Pathol.* 176, 2819–2830. doi: 10.2353/ajpath.2010.090642

Ko, A., Kang, G., Hattler, J. B., Galadima, H. I., Zhang, J., Li, Q., et al. (2019). Macrophages but not astrocytes harbor HIV DNA in the brains of HIV-1-infected aviremic individuals on suppressive antiretroviral therapy. *J. Neuroimmune Pharmacol.* 14, 110–119. doi: 10.1007/s11481-018-9809-2

Koller, H., Schaal, H., Freund, M., Garrido, S. R., von Giesen, H. J., Ott, M., et al. (2001). HIV-1 protein Tat reduces the glutamate-induced intracellular Ca2+ increase in cultured cortical astrocytes. *Eur. J. Neurosci.* 14, 1793–1799. doi: 10.1046/j.0953-816x.2001.01808.x

Kovalevich, J., and Langford, D. (2012). Neuronal toxicity in HIV CNS disease. *Future Virol.* 7, 687–698. doi: 10.2217/fvl.12.57

Krogh, K. A., Green, M. V., and Thayer, S. A. (2014a). HIV-1 Tat-induced changes in synaptically-driven network activity adapt during prolonged exposure. *Curr. HIV Res.* 12, 406–414. doi: 10.2174/1570162X13666150121110402

Krogh, K. A., Wydeven, N., Wickman, K., and Thayer, S. A. (2014b). HIV-1 protein Tat produces biphasic changes in NMDA-evoked increases in intracellular Ca2+ concentration via activation of Src kinase and nitric oxide signaling pathways. *J. Neurochem.* 130, 642–656. doi: 10.1111/jnc.12724

Kumar, A. (2015). NMDA receptor function during senescence: implication on cognitive performance. *Front. Neurosci.* 9:473. doi: 10.3389/fnins.2015.00473

Laffel, L. (1999). Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. *Diabetes Metab. Res. Rev.* 15, 412–426. doi: 10.1002/(SICI)1520-7560(199911/12)15:6<;412::AID-DMRR72>;3.0.CO;2-8

Latronico, T., Pati, I., Ciavarella, R., Fasano, A., Mengoni, F., Lichtner, M., et al. (2018). *In vitro* effect of antiretroviral drugs on cultured primary astrocytes: analysis of neurotoxicity and matrix metalloproteinase inhibition. *J. Neurochem.* 144, 271–284. doi: 10.1111/jnc.14269

Lau, A., and Tymianski, M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Arch.* 460, 525–542. doi: 10.1007/s00424-010-0809-1

Lee, G. Q., and Lichterfeld, M. (2016). Diversity of HIV-1 reservoirs in CD4+ T-cell subpopulations. *Curr. Opin. HIV AIDS* 11, 383–387. doi: 10.1097/COH.0000000000000281

Lee, S. G., Jeon, H. Y., Su, Z. Z., Richards, J. E., Vozhilla, N., Sarkar, D., et al. (2009). Astrocyte elevated gene-1 contributes to the pathogenesis of neuroblastoma. *Oncogene* 28, 2476–2484. doi: 10.1038/onc.2009.93

Lee, S. G., Kim, K., Kegelman, T. P., Dash, R., Das, S. K., Choi, J. K., et al. (2011). Oncogene AEG-1 promotes glioma-induced neurodegeneration by increasing glutamate excitotoxicity. *Cancer Res.* 71, 6514–6523. doi: 10.1158/0008-5472.CAN-11-0782

Lee, Y., Cho, J. H., Lee, S., Lee, W., Chang, S. C., Chung, H. Y., et al. (2019). Neuroprotective effects of MHY908, a PPAR alpha/gamma dual agonist, in a MPTP-induced Parkinson's disease model. *Brain Res.* 1704, 47–58. doi: 10.1016/j.brainres.2018.09.036

Lentz, M. R., Kim, W. K., Lee, V., Bazner, S., Halpern, E. F., Venna, N., et al. (2009). Changes in MRS neuronal markers and T cell phenotypes observed during early HIV infection. *Neurology* 72, 1465–1472. doi: 10.1212/WNL.0b013e3181a2e90a

Lewerenz, J., Hewett, S. J., Huang, Y., Lambros, M., Gout, P. W., Kalivas, P. W., et al. (2013). The cystine/glutamate antiporter system x(c)(-) in health and disease: from molecular mechanisms to novel therapeutic opportunities. *Antioxid. Redox Signal* 18, 522–555. doi: 10.1089/ars.2011.4391

Li, T., Giaume, C., and Xiao, L. (2014). Connexins-mediated glia networking impacts myelination and remyelination in the central nervous system. *Mol. Neurobiol.* 49, 1460–1471. doi: 10.1007/s12035-013-8625-1

Li, W., Huang, Y., Reid, R., Steiner, J., Malpica-Llanos, T., Darden, T. A., et al. (2008). NMDA receptor activation by HIV-Tat protein is clade dependent. *J. Neurosci.* 28, 12190–12198. doi: 10.1523/JNEUROSCI.3019-08.2008

Lindl, K. A., Marks, D. R., Kolson, D. L., and Jordan-Sciutto, K. L. (2010). HIV-associated neurocognitive disorder: pathogenesis and therapeutic opportunities. *J. Neuroimmune Pharmacol.* 5, 294–309. doi: 10.1007/s11481-010-9205-z

Little, S. J., McLean, A. R., Spina, C. A., Richman, D. D., and Havlir, D. V. (1999). Viral dynamics of acute HIV-1 infection. *J. Exp. Med.* 190, 841–850. doi: 10.1084/jem.190.6.841

Liu, Y., Jones, M., Hingtgen, C. M., Bu, G., Laribee, N., Tanzi, R. E., et al. (2000). Uptake of HIV-1 tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nat. Med.* 6, 1380–1387. doi: 10.1038/82199

Loftus, R. M., and Finlay, D. K. (2016). Immunometabolism: cellular metabolism turns immune regulator. *J. Biol. Chem.* 291, 1–10. doi: 10.1074/jbc.R115.693903

Loisel-Meyer, S., Swainson, L., Craveiro, M., Oburoglu, L., Mongellaz, C., Costa, C., et al. (2012). Glut1-mediated glucose transport regulates HIV infection. *Proc. Natl. Acad. Sci. U.S.A.* 109, 2549–2554. doi: 10.1073/pnas.1121427109

Lomeli, H., Mosbacher, J., Melcher, T., Hoger, T., Geiger, J. R., Kuner, T., et al. (1994). Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. *Science* 266, 1709–1713. doi: 10.1126/science.7992055

Lu, S. C. (2013). Glutathione synthesis. *Biochim. Biophys. Acta* 1830, 3143–3153. doi: 10.1016/j.bbagen.2012.09.008

Malik, S., and Eugenin, E. A. (2016). Mechanisms of HIV neuropathogenesis: role of cellular communication systems. *Curr. HIV Res.* 14, 400–411. doi: 10.2174/1570162X14666160324124558

Malik, S., and Eugenin, E. A. (2019). Role of connexin and pannexin containing channels in HIV infection and NeuroAIDS. *Neurosci. Lett.* 695, 86–90. doi: 10.1016/j.neulet.2017.09.005

Mangus, L. M., Beck, S. E., Queen, S. E., Brill, S. A., Shirk, E. N., Metcalf Pate, K. A., et al. (2018). Lymphocyte-dominant encephalitis and meningitis in simian immunodeficiency virus-infected macaques receiving antiretroviral therapy. *Am. J. Pathol.* 188, 125–134. doi: 10.1016/j.ajpath.2017.08.035

Mastrantonio, R., D'Ezio, V., Colasanti, M., and Persichini, T. (2019). Nrf2-mediated system xc(-) activation in astroglial cells is involved in HIV-1 tat-induced neurotoxicity. *Mol. Neurobiol.* 56, 3796–3806. doi: 10.1007/s12035-018-1343-y

McCormick, D. A. (1989). GABA as an inhibitory neurotransmitter in human cerebral cortex. *J. Neurophysiol.* 62, 1018–1027. doi: 10.1152/jn.1989.62.5.1018

Melendez, R. I., Roman, C., Capo-Velez, C. M., and Lasalde-Dominicci, J. A. (2016). Decreased glial and synaptic glutamate uptake in the striatum of HIV-1 gp120 transgenic mice. *J. Neurovirol.* 22, 358–365. doi: 10.1007/s13365-015-0403-6

Micu, I., Jiang, Q., Coderre, E., Ridsdale, A., Zhang, L., Woulfe, J., et al. (2006). NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. *Nature* 439, 988–992. doi: 10.1038/nature04474

Minghetti, L., Visentin, S., Patrizio, M., Franchini, L., Ajmone-Cat, M. A., and Levi, G. (2004). Multiple actions of the human immunodeficiency virus type-1 Tat protein on microglial cell functions. *Neurochem. Res.* 29, 965–978. doi: 10.1023/B:NERE.0000021241.90133.89

Mohamed, M., Barker, P. B., Skolasky, R. L., and Sacktor, N. (2018). 7T brain MRS in HIV infection: correlation with cognitive impairment and

performance on neuropsychological tests. *AJNR Am. J. Neuroradiol.* 39, 704–712. doi: 10.3174/ajnr.A5547

Moroni, F., Cozzi, A., Sili, M., and Mannaioni, G. (2012). Kynurenic acid: a metabolite with multiple actions and multiple targets in brain and periphery. *J. Neural. Transm.* 119, 133–139. doi: 10.1007/s00702-011-0763-x

Moses, A. V., Ibanez, C., Gaynor, R., Ghazal, P., and Nelson, J. A. (1994). Differential role of long terminal repeat control elements for the regulation of basal and Tat-mediated transcription of the human immunodeficiency virus in stimulated and unstimulated primary human macrophages. *J. Virol.* 68, 298–307 doi: 10.1128/JVI.68.1.298-307.1994

Mukerji, S. S., Misra, V., Lorenz, D. R., Uno, H., Morgello, S., Franklin, D., et al. (2018). Impact of antiretroviral regimens on cerebrospinal fluid viral escape in a prospective multicohort study of antiretroviral therapy-experienced human immunodeficiency virus-1-infected adults in the United States. *Clin. Infect. Dis.* 67, 1182–1190. doi: 10.1093/cid/ciy267

Nakanishi, N., Kang, Y. J., Tu, S., McKercher, S. R., Masliah, E., and Lipton, S. A. (2016). Differential effects of pharmacologic and genetic modulation of NMDA receptor activity on HIV/gp120-induced neuronal damage in an *in vivo* mouse model. *J. Mol. Neurosci.* 58, 59–65. doi: 10.1007/s12031-015-0651-1

Nath, A. (2002). Human immunodeficiency virus (HIV) proteins in neuropathogenesis of HIV dementia. *J. Infect. Dis.* 186(Suppl. 2), S193–198. doi: 10.1086/344528

Nath, A., Haughey, N. J., Jones, M., Anderson, C., Bell, J. E., and Geiger, J. D. (2000). Synergistic neurotoxicity by human immunodeficiency virus proteins Tat and gp120: protection by memantine. *Ann. Neurol.* 47, 186–1943. doi: 10.1002/1531-8249(200002)47:2<;186::AID-ANA8>;3.0.CO;2-3

Nath, A., Psooy, K., Martin, C., Knudsen, B., Magnuson, D. S., Haughey, N., et al. (1996). Identification of a human immunodeficiency virus type 1 Tat epitope that is neuroexcitatory and neurotoxic. *J. Virol.* 70, 1475–1480 doi: 10.1128/JVI.70.3.1475-1480.1996

Nath, N., Wunderlich, C., Darr, F. W. 2nd, Douglas, D. K., and Dodd, R. Y. (1987). Immunoglobulin level in donor blood reactive for antibodies to human immunodeficiency virus. *J. Clin. Microbiol.* 25, 364–369 doi: 10.1128/JCM.25.2.364-369.1987

Nedelcovych, M. T., Kim, B. H., Zhu, X., Lovell, L. E., Manning, A. A., Kelschenbach, J., et al. (2019). Glutamine antagonist JHU083 normalizes aberrant glutamate production and cognitive deficits in the EcoHIV murine model of HIV-associated neurocognitive disorders. *J. Neuroimmune Pharmacol.* 14, 391–400. doi: 10.1007/s11481-019-09859-w

Nedelcovych, M. T., Tenora, L., Kim, B. H., Kelschenbach, J., Chao, W., Hadas, E., et al. (2017). N-(Pivaloyloxy)alkoxy-carbonyl prodrugs of the glutamine antagonist 6-Diazo-5-oxo-l-norleucine (DON) as a potential treatment for HIV associated neurocognitive disorders. *J. Med. Chem.* 60, 7186–7198. doi: 10.1021/acs.jmedchem.7b00966

Nedergaard, M., Takano, T., and Hansen, A. J. (2002). Beyond the role of glutamate as a neurotransmitter. *Nat. Rev. Neurosci.* 3, 748–755. doi: 10.1038/nrn916

Nicoli, F., Chachage, M., Clowes, P., Bauer, A., Kowour, D., Ensoli, B., et al. (2016). Association between different anti-Tat antibody isotypes and HIV disease progression: data from an African cohort. *BMC Infect. Dis.* 16:344. doi: 10.1186/s12879-016-1647-3

Niswender, C. M., and Conn, P. J. (2010). Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.* 50, 295–322. doi: 10.1146/annurev.pharmtox.011008.145533

Niture, S. K., Khatri, R., and Jaiswal, A. K. (2014). Regulation of Nrf2-an update. *Free Radic. Biol. Med.* 66, 36–44. doi: 10.1016/j.freeradbiomed.2013.02.008

Niu, F., Yao, H., Zhang, W., Sutliff, R. L., and Buch, S. (2014). Tat 101-mediated enhancement of brain pericyte migration involves platelet-derived growth factor subunit B homodimer: implications for human immunodeficiency virus-associated neurocognitive disorders. *J. Neurosci.* 34, 11812–11825. doi: 10.1523/JNEUROSCI.1139-14.2014

Nowak, M. A., Lloyd, A. L., Vasquez, G. M., Wiltrout, T. A., Wahl, L. M., Bischofberger, N., et al. (1997). Viral dynamics of primary viremia and antiretroviral therapy in simian immunodeficiency virus infection. *J. Virol.* 71, 7518–7525 doi: 10.1128/JVI.71.10.7518-7525.1997

Ohagen, A., Devitt, A., Kunstman, K. J., Gorry, P. R., Rose, P. P., Korber, B., et al. (2003). Genetic and functional analysis of full-length human immunodeficiency virus type 1 env genes derived from brain and blood of patients with AIDS. *J. Virol.* 77, 12336–12345. doi: 10.1128/JVI.77.22.12336-12345.2003

Okafo, G., Prevedel, L., and Eugenin, E. (2017). Tunneling nanotubes (TNT) mediate long-range gap junctional communication: Implications for HIV cell to cell spread. *Sci. Rep.* 7:16660. doi: 10.1038/s41598-017-16600-1

Omeragic, A., Hoque, M. T., Choi, U. Y., and Bendayan, R. (2017). Peroxisome proliferator-activated receptor-gamma: potential molecular therapeutic target for HIV-1-associated brain inflammation. *J. Neuroinflammation* 14:183. doi: 10.1186/s12974-017-0957-8

Orellana, J. A., Saez, J. C., Bennett, M. V., Berman, J. W., Morgello, S., and Eugenin, E. A. (2014). HIV increases the release of dickkopf-1 protein from human astrocytes by a Cx43 hemichannel-dependent mechanism. *J. Neurochem.* 128, 752–763. doi: 10.1111/jnc.12492

Pace, M., and Frater, J. (2019). Curing HIV by 'kick and kill': from theory to practice? *Expert Rev. Anti Infect. Ther.* 17, 383–386. doi: 10.1080/14787210.2019.1617697

Pacheco, R., Gallart, T., Lluis, C., and Franco, R. (2007). Role of glutamate on T-cell mediated immunity. *J. Neuroimmunol.* 185, 9–19. doi: 10.1016/j.jneuroim.2007.01.003

Palmer, C. S., Ostrowski, M., Gouillou, M., Tsai, L., Yu, D., Zhou, J., et al. (2014). Increased glucose metabolic activity is associated with CD4+ T-cell activation and depletion during chronic HIV infection. *AIDS* 28, 297–309. doi: 10.1097/QAD.0000000000000128

Pardo, B., Rodrigues, T. B., Contreras, L., Garzon, M., Llorente-Folch, I., Kobayashi, K., et al. (2011). Brain glutamine synthesis requires neuronal-born aspartate as amino donor for glial glutamate formation. *J. Cereb. Blood Flow Metab.* 31, 90–101. doi: 10.1038/jcbfm.2010.146

Patel, C. A., Mukhtar, M., and Pomerantz, R. J. (2000). Human immunodeficiency virus type 1 Vpr induces apoptosis in human neuronal cells. *J. Virol.* 74, 9717–9726. doi: 10.1128/JVI.74.20.9717-9726.2000

Paul, R. (2019). Neurocognitive phenotyping of HIV in the era of antiretroviral therapy. *Curr. HIV/AIDS Rep.* 16, 230–235. doi: 10.1007/s11904-019-00426-9

Peavy, G., Jacobs, D., Salmon, D. P., Butters, N., Delis, D. C., Taylor, M., et al. (1994). Verbal memory performance of patients with human immunodeficiency virus infection: evidence of subcortical dysfunction. The HNRC Group. *J. Clin. Exp. Neuropsychol.* 16, 508–523. doi: 10.1080/01688639408402662

Peng, L., Hertz, L., Huang, R., Sonnewald, U., Petersen, S. B., Westergaard, N., et al. (1993). Utilization of glutamine and of TCA cycle constituents as precursors for transmitter glutamate and GABA. *Dev. Neurosci.* 15, 367–377. doi: 10.1159/000111357

Perelson, A. S., Essunger, P., Cao, Y., Vesanen, M., Hurley, A., Saksela, K., et al. (1997). Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* 387, 188–191. doi: 10.1038/387188a0

Persidsky, Y., Hill, J., Zhang, M., Dykstra, H., Winfield, M., Reichenbach, N. L., et al. (2016). Dysfunction of brain pericytes in chronic neuroinflammation. *J. Cereb. Blood Flow Metab.* 36, 794–807. doi: 10.1177/0271678X15606149

Petralia, R. S. (2012). Distribution of extrasynaptic NMDA receptors on neurons. *Sci. World J.* 2012:267120. doi: 10.1100/2012/267120

Petroff, O. A. (2002). GABA and glutamate in the human brain. *Neuroscientist* 8, 562–573. doi: 10.1177/1073858402238515

Pilc, A., Chaki, S., Nowak, G., and Witkin, J. M. (2008). Mood disorders: regulation by metabotropic glutamate receptors. *Biochem. Pharmacol.* 75, 997–1006. doi: 10.1016/j.bcp.2007.09.021

Pilcher, C. D., Shugars, D. C., Fiscus, S. A., Miller, W. C., Menezes, P., Giner, J., et al. (2001). HIV in body fluids during primary HIV infection: implications for pathogenesis, treatment and public health. *AIDS* 15, 837–845. doi: 10.1097/00002030-200105040-00004

Pinkevych, M., Cromer, D., Tolstrup, M., Grimm, A. J., Cooper, D. A., Lewin, S. R., et al. (2015). HIV reactivation from latency after treatment interruption occurs on average every 5-8 days–implications for HIV remission. *PLoS Pathog.* 11:e1005000. doi: 10.1371/journal.ppat.1005000

Pinkevych, M., Cromer, D., Tolstrup, M., Grimm, A. J., Cooper, D. A., Lewin, S. R., et al. (2016). Correction: HIV reactivation from latency after treatment interruption occurs on average every 5-8 days-implications for HIV remission. *PLoS Pathog.* 12:e1005745. doi: 10.1371/journal.ppat.1005745

Popescu, G. K. (2014). Dynamic plasticity of NMDA receptor-mediated calcium entry in neurons exposed to HIV-tat. *J. Neurochem.* 130, 609–611. doi: 10.1111/jnc.12737

Porcheray, F., Leone, C., Samah, B., Rimaniol, A. C., Dereuddre-Bosquet, N., and Gras, G. (2006). Glutamate metabolism in HIV-infected macrophages: implications for the CNS. *Am. J. Physiol. Cell Physiol.* 291, C618–626. doi: 10.1152/ajpcell.00021.2006

Portilla, I., Reus, S., Leon, R., van-der Hofstadt, C., Sanchez, J., Lopez, N., et al. (2019). Neurocognitive impairment in well-controlled HIV-infected patients: a cross-sectional study. *AIDS Res. Hum. Retroviruses* 35, 634–641. doi: 10.1089/aid.2018.0279

Potter, M. C., Figuera-Losada, M., Rojas, C., and Slusher, B. S. (2013). Targeting the glutamatergic system for the treatment of HIV-associated neurocognitive disorders. *J. Neuroimmune Pharmacol.* 8, 594–607. doi: 10.1007/s11481-013-9442-z

Potula, R., Ramirez, S. H., Knipe, B., Leibhart, J., Schall, K., Heilman, D., et al. (2008). Peroxisome proliferator-activated receptor-gamma activation suppresses HIV-1 replication in an animal model of encephalitis. *AIDS* 22, 1539–1549. doi: 10.1097/QAD.0b013e3283081e08

Prendergast, M. A., Rogers, D. T., Mulholland, P. J., Littleton, J. M., Wilkins, L. H. Jr., Self, R. L., et al. (2002). Neurotoxic effects of the human immunodeficiency virus type-1 transcription factor Tat require function of a polyamine sensitive-site on the N-methyl-D-aspartate receptor. *Brain Res.* 954, 300–307. doi: 10.1016/S0006-8993(02)03360-7

Rao, J. S. (2003). Molecular mechanisms of glioma invasiveness: the role of proteases. *Nat. Rev. Cancer* 3, 489–501. doi: 10.1038/nrc1121

Re, M. C., Furlini, G., Vignoli, M., Ramazzotti, E., Zauli, G., and La Placa, M. (1996). Antibody against human immunodeficiency virus type 1 (HIV-1) Tat protein may have influenced the progression of AIDS in HIV-1-infected hemophiliac patients. *Clin. Diagn. Lab. Immunol.* 3, 230–232 doi: 10.1128/CDLI.3.2.230-232.1996

Rieder, M. J., Krause, R., Bird, I. A., and Dekaban, G. A. (1995). Toxicity of sulfonamide-reactive metabolites in HIV-infected, HTLV-infected, and noninfected cells. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 8, 134–140 doi: 10.1097/00042560-199502000-00004

Rimaniol, A. C., Mialocq, P., Clayette, P., Dormont, D., and Gras, G. (2001). Role of glutamate transporters in the regulation of glutathione levels in human macrophages. *Am. J. Physiol. Cell Physiol.* 281, C1964–1970. doi: 10.1152/ajpcell.2001.281.6.C1964

Ritola, K., Robertson, K., Fiscus, S. A., Hall, C., and Swanstrom, R. (2005). Increased human immunodeficiency virus type 1 (HIV-1) env compartmentalization in the presence of HIV-1-associated dementia. *J. Virol.* 79, 10830–10834. doi: 10.1128/JVI.79.16.10830-10834.2005

Rodriguez-Gallego, E., Gomez, J., Pacheco, Y. M., Peraire, J., Vilades, C., Beltran-Debon, R., et al. (2018). A baseline metabolomic signature is associated with immunological CD4+ T-cell recovery after 36 months of antiretroviral therapy in HIV-infected patients. *AIDS* 32, 565–573. doi: 10.1097/QAD.0000000000001730

Rothstein, J. D., Dykes-Hoberg, M., Pardo, C. A., Bristol, L. A., Jin, L., Kuncl, R. W., et al. (1996). Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* 16, 675–686. doi: 10.1016/S0896-6273(00)80086-0

Rozzi, S. J., Avdoshina, V., Fields, J. A., Trejo, M., Ton, H. T., Ahern, G. P., et al. (2017). Human immunodeficiency virus promotes mitochondrial toxicity. *Neurotox. Res.* 32, 723–733. doi: 10.1007/s12640-017-9776-z

Ru, W., and Tang, S. J. (2016). HIV-1 gp120Bal down-regulates phosphorylated NMDA receptor subunit 1 in cortical neurons via activation of glutamate and chemokine receptors. *J. Neuroimmune Pharmacol.* 11, 182–191. doi: 10.1007/s11481-015-9644-7

Ru, W., and Tang, S. J. (2017). HIV-associated synaptic degeneration. *Mol. Brain* 10:40. doi: 10.1186/s13041-017-0321-z

Rumbaugh, J. A., Bachani, M., Li, W., Butler, T. R., Smith, K. J., Bianchet, M. A., et al. (2013). HIV immune complexes prevent excitotoxicity by interaction with NMDA receptors. *Neurobiol. Dis.* 49, 169–176. doi: 10.1016/j.nbd.2012.08.013

Russell, R. A., Chojnacki, J., Jones, D. M., Johnson, E., Do, T., Eggeling, C., et al. (2017). Astrocytes resist HIV-1 fusion but engulf infected macrophage material. *Cell Rep.* 18, 1473–1483. doi: 10.1016/j.celrep.2017.01.027

Sailasuta, N., Shriner, K., and Ross, B. (2009). Evidence of reduced glutamate in the frontal lobe of HIV-seropositive patients. *NMR Biomed.* 22, 326–331. doi: 10.1002/nbm.1329

Salter, M. G., and Fern, R. (2005). NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. *Nature* 438, 1167–1171. doi: 10.1038/nature04301

Sami Saribas, A., Cicalese, S., Ahooyi, T. M., Khalili, K., Amini, S., and Sariyer, I. K. (2017). HIV-1 Nef is released in extracellular vesicles derived from astrocytes: evidence for Nef-mediated neurotoxicity. *Cell Death Dis.* 8:e2542. doi: 10.1038/cddis.2016.467

Sanford, R., Ances, B. M., Meyerhoff, D. J., Price, R. W., Fuchs, D., Zetterberg, H., et al. (2018). Longitudinal trajectories of brain volume and cortical thickness in treated and untreated primary human immunodeficiency virus infection. *Clin. Infect. Dis.* 67, 1697–1704. doi: 10.1093/cid/ciy362

Santello, M., Cali, C., and Bezzi, P. (2012). Gliotransmission and the tripartite synapse. *Adv. Exp. Med. Biol.* 970, 307–331. doi: 10.1007/978-3-7091-0932-8_14

Schousboe, A., Bak, L. K., and Waagepetersen, H. S. (2013). Astrocytic control of biosynthesis and turnover of the neurotransmitters glutamate and GABA. *Front. Endocrinol.* 4:102. doi: 10.3389/fendo.2013.00102

Scott, D. B., Blanpied, T. A., and Ehlers, M. D. (2003). Coordinated PKA and PKC phosphorylation suppresses RXR-mediated ER retention and regulates the surface delivery of NMDA receptors. *Neuropharmacology* 45, 755–767. doi: 10.1016/S0028-3908(03)00250-8

Sengillo, J. D., Winkler, E. A., Walker, C. T., Sullivan, J. S., Johnson, M., and Zlokovic, B. V. (2013). Deficiency in mural vascular cells coincides with blood-brain barrier disruption in Alzheimer's disease. *Brain Pathol.* 23, 303–310. doi: 10.1111/bpa.12004

Shan, L., and Siliciano, R. F. (2013). From reactivation of latent HIV-1 to elimination of the latent reservoir: the presence of multiple barriers to viral eradication. *Bioessays* 35, 544–552. doi: 10.1002/bies.201200170

Siliciano, J. D., Kajdas, J., Finzi, D., Quinn, T. C., Chadwick, K., Margolick, J. B., et al. (2003). Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat. Med.* 9, 727–728. doi: 10.1038/nm880

Singh, H. O., Lata, S., Angadi, M., Bapat, S., Pawar, J., Nema, V., et al. (2017). Impact of GSTM1, GSTT1 and GSTP1 gene polymorphism and risk of ARV-associated hepatotoxicity in HIV-infected individuals and its modulation. *Pharmacogenomics J.* 17, 53–60. doi: 10.1038/tpj.2015.88

Skowronska, K., Obara-Michlewska, M., Zielinska, M., and Albrecht, J. (2019). NMDA receptors in astrocytes: in search for roles in neurotransmission and astrocytic homeostasis. *Int. J. Mol. Sci.* 20:309. doi: 10.3390/ijms20020309

Smit, T. K., Wang, B., Ng, T., Osborne, R., Brew, B., and Saksena, N. K. (2001). Varied tropism of HIV-1 isolates derived from different regions of adult brain cortex discriminate between patients with and without AIDS dementia complex (ADC): evidence for neurotropic HIV variants. *Virology* 279, 509–526. doi: 10.1006/viro.2000.0681

Soriano-Sarabia, N., Bateson, R. E., Dahl, N. P., Crooks, A. M., Kuruc, J. D., Margolis, D. M., et al. (2014). Quantitation of replication-competent HIV-1 in populations of resting CD4+ T cells. *J. Virol.* 88, 14070–14077. doi: 10.1128/JVI.01900-14

Swingler, S., Mann, A. M., Zhou, J., Swingler, C., and Stevenson, M. (2007). Apoptotic killing of HIV-1-infected macrophages is subverted by the viral envelope glycoprotein. *PLoS Pathog.* 3, 1281–1290. doi: 10.1371/journal.ppat.0030134

Swinton, M. K., Carson, A., Telese, F., Sanchez, A. B., Soontornniyomkij, B., Rad, L., et al. (2019). Mitochondrial biogenesis is altered in HIV+ brains exposed to ART: implications for therapeutic targeting of astroglia. *Neurobiol. Dis.* 130:104502. doi: 10.1016/j.nbd.2019.104502

Tate, D. F., Conley, J., Paul, R. H., Coop, K., Zhang, S., Zhou, W., et al. (2010). Quantitative diffusion tensor imaging tractography metrics are associated with cognitive performance among HIV-infected patients. *Brain Imaging Behav.* 4, 68–79. doi: 10.1007/s11682-009-9086-z

Taube, R., Fujinaga, K., Wimmer, J., Barboric, M., and Peterlin, B. M. (1999). Tat transactivation: a model for the regulation of eukaryotic transcriptional elongation. *Virology* 264, 245–253. doi: 10.1006/viro.1999.9944

Thomas, A. G., O'Driscoll, C. M., Bressler, J., Kaufmann, W., Rojas, C. J., and Slusher, B. S. (2014). Small molecule glutaminase inhibitors block glutamate

release from stimulated microglia. *Biochem. Biophys. Res. Commun.* 443, 32–36. doi: 10.1016/j.bbrc.2013.11.043

Tian, C., Sun, L., Jia, B., Ma, K., Curthoys, N., Ding, J., et al. (2012). Mitochondrial glutaminase release contributes to glutamate-mediated neurotoxicity during human immunodeficiency virus-1 infection. *J. Neuroimmune Pharmacol.* 7, 619–628. doi: 10.1007/s11481-012-9364-1

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., et al. (2010). Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol. Rev.* 62, 405–496. doi: 10.1124/pr.109.002451

Turchan-Cholewo, J., Dimayuga, V. M., Gupta, S., Gorospe, R. M., Keller, J. N., and Bruce-Keller, A. J. (2009). NADPH oxidase drives cytokine and neurotoxin release from microglia and macrophages in response to HIV-Tat. *Antioxid. Redox Signal* 11, 193–204. doi: 10.1089/ars.2008.2097

Underwood, J., Robertson, K. R., and Winston, A. (2015). Could antiretroviral neurotoxicity play a role in the pathogenesis of cognitive impairment in treated HIV disease? *AIDS* 29, 253–261. doi: 10.1097/QAD.0000000000000538

Valcour, V., Chalermchai, T., Sailasuta, N., Marovich, M., Lerdlum, S., Suttichom, D., et al. (2012). Central nervous system viral invasion and inflammation during acute HIV infection. *J. Infect. Dis.* 206, 275–282. doi: 10.1093/infdis/jis326

Valdebenito, S., Lou, E., Baldoni, J., Okafo, G., and Eugenin, E. (2018). The novel roles of connexin channels and tunneling nanotubes in cancer pathogenesis. *Int. J. Mol. Sci.* 19:1270. doi: 10.3390/ijms19051270

Van Lint, C., Bouchat, S., and Marcello, A. (2013). HIV-1 transcription and latency: an update. *Retrovirology* 10:67. doi: 10.1186/1742-4690-10-67

Vartak-Sharma, N., Gelman, B. B., Joshi, C., Borgamann, K., and Ghorpade, A. (2014). Astrocyte elevated gene-1 is a novel modulator of HIV-1-associated neuroinflammation via regulation of nuclear factor-kappaB signaling and excitatory amino acid transporter-2 repression. *J. Biol. Chem.* 289, 19599–19612. doi: 10.1074/jbc.M114.567644

Vats, D., Mukundan, L., Odegaard, J. I., Zhang, L., Smith, K. L., Morel, C. R., et al. (2006). Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab.* 4, 13–24. doi: 10.1016/j.cmet.2006.05.011

Vazquez-Santiago, F. J., Noel, R. J. Jr., Porter, J. T., and Rivera-Amill, V. (2014). Glutamate metabolism and HIV-associated neurocognitive disorders. *J. Neurovirol.* 20, 315–331. doi: 10.1007/s13365-014-0258-2

Veenstra, M., Byrd, D. A., Inglese, M., Buyukturkoglu, K., Williams, D. W., Fleysher, L., et al. (2019). CCR2 on peripheral blood CD14(+)CD16(+) monocytes correlates with neuronal damage, hiv-associated neurocognitive disorders, and peripheral HIV DNA: reseeding of CNS reservoirs? *J. Neuroimmune Pharmacol.* 14, 120–133. doi: 10.1007/s11481-018-9792-7

Veenstra, M., Leon-Rivera, R., Li, M., Gama, L., Clements, J. E., and Berman, J. W. (2017). Mechanisms of CNS viral seeding by HIV(+) CD14(+) CD16(+) monocytes: establishment and reseeding of viral reservoirs contributing to HIV-associated neurocognitive disorders. *MBio* 8: 01280-17. doi: 10.1128/mBio.01280-17

Velasquez, S., Prevedel, L., Valdebenito, S., Gorska, A. M., Golovko, M., Khan, N., et al. (2020). Circulating levels of ATP is a biomarker of HIV cognitive impairment. *EBiomedicine* 51:102503. doi: 10.1016/j.ebiom.2019.10.029

Visalli, V., Muscoli, C., Sacco, I., Sculco, F., Palma, E., Costa, N., et al. (2007). N-acetylcysteine prevents HIV gp 120-related damage of human cultured astrocytes: correlation with glutamine synthase dysfunction. *BMC Neurosci.* 8:106. doi: 10.1186/1471-2202-8-106

Vivithanaporn, P., Asahchop, E. L., Acharjee, S., Baker, G. B., and Power, C. (2016). HIV protease inhibitors disrupt astrocytic glutamate transporter function and neurobehavioral performance. *AIDS* 30, 543–552. doi: 10.1097/QAD.0000000000000955

Wallet, C., De Rovere, M., Van Assche, J., Daouad, F., De Wit, S., Gautier, V., et al. (2019). Microglial cells: the main HIV-1 reservoir in the brain. *Front. Cell. Infect. Microbiol.* 9:362. doi: 10.3389/fcimb.2019.00362

Wanchu, A., Rana, S. V., Pallikkuth, S., and Sachdeva, R. K. (2009). Short communication: oxidative stress in HIV-infected individuals: a cross-sectional study. *AIDS Res. Hum. Retroviruses* 25, 1307–1311. doi: 10.1089/aid.2009.0062

Wang, T. H., Donaldson, Y. K., Brettle, R. P., Bell, J. E., and Simmonds, P. (2001). Identification of shared populations of human immunodeficiency virus type 1 infecting microglia and tissue macrophages outside the central nervous system. *J. Virol.* 75, 11686–11699. doi: 10.1128/JVI.75.23.11686-11699.2001

Wang, Y., Li, Y., Zhao, R., Wu, B., Lanoha, B., Tong, Z., et al. (2017). Glutaminase C overexpression in the brain induces learning deficits, synaptic dysfunctions, and neuroinflammation in mice. *Brain Behav. Immun.* 66, 135–145. doi: 10.1016/j.bbi.2017.06.007

Wang, Z., Pekarskaya, O., Bencheikh, M., Chao, W., Gelbard, H. A., Ghorpade, A., et al. (2003). Reduced expression of glutamate transporter EAAT2 and impaired glutamate transport in human primary astrocytes exposed to HIV-1 or gp120. *Virology* 312, 60–73. doi: 10.1016/S0042-6822(03)00181-8

Wei, X., Ghosh, S. K., Taylor, M. E., Johnson, V. A., Emini, E. A., Deutsch, P., et al. (1995). Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 373, 117–122. doi: 10.1038/373117a0

Wen, L., You, W., Wang, H., Meng, Y., Feng, J., and Yang, X. (2018). Polarization of microglia to the M2 phenotype in a peroxisome proliferator-activated receptor gamma-dependent manner attenuates axonal injury induced by traumatic brain injury in Mice. *J. Neurotrauma* 35, 2330–2340. doi: 10.1089/neu.2017.5540

Westendorp, M. O., Shatrov, V. A., Schulze-Osthoff, K., Frank, R., Kraft, M., Los, M., et al. (1995). HIV-1 Tat potentiates TNF-induced NF-kappa B activation and cytotoxicity by altering the cellular redox state. *EMBO J.* 14, 546–554 doi: 10.1002/j.1460-2075.1995.tb07030.x

Westmoreland, S. V., Alvarez, X., deBakker, C., Aye, P., Wilson, M. L., Williams, K. C., et al. (2002). Developmental expression patterns of CCR5 and CXCR4 in the rhesus macaque brain. *J. Neuroimmunol.* 122, 146–158. doi: 10.1016/S0165-5728(01)00457-X

Wieronska, J. M., and Pilc, A. (2009). Metabotropic glutamate receptors in the tripartite synapse as a target for new psychotropic drugs. *Neurochem. Int.* 55, 85–97. doi: 10.1016/j.neuint.2009.02.019

Williams, D. W., Byrd, D., Rubin, L. H., Anastos, K., Morgello, S., and Berman, J. W. (2014). CCR2 on CD14(+)CD16(+) monocytes is a biomarker of HIV-associated neurocognitive disorders. *Neurol. Neuroimmunol. Neuroinflamm.* 1:e36. doi: 10.1212/NXI.0000000000000036

Williams, D. W., Calderon, T. M., Lopez, L., Carvallo-Torres, L., Gaskill, P. J., Eugenin, E. A., et al. (2013). Mechanisms of HIV entry into the CNS: increased sensitivity of HIV infected CD14+CD16+ monocytes to CCL2 and key roles of CCR2, JAM-A, and ALCAM in diapedesis. *PLoS ONE* 8:e69270. doi: 10.1371/journal.pone.0069270

Williams, D. W., Eugenin, E. A., Calderon, T. M., and Berman, J. W. (2012). Monocyte maturation, HIV susceptibility, and transmigration across the blood brain barrier are critical in HIV neuropathogenesis. *J. Leukoc. Biol* 91, 401–415. doi: 10.1189/jlb.0811394

Winkler, E. A., Sengillo, J. D., Sullivan, J. S., Henkel, J. S., Appel, S. H., and Zlokovic, B. V. (2013). Blood-spinal cord barrier breakdown and pericyte reductions in amyotrophic lateral sclerosis. *Acta Neuropathol.* 125, 111–120. doi: 10.1007/s00401-012-1039-8

Wong, J. K., and Yukl, S. A. (2016). Tissue reservoirs of HIV. *Curr. Opin. HIV AIDS* 11, 362–370. doi: 10.1097/COH.0000000000000293

Wroge, C. M., Hogins, J., Eisenman, L., and Mennerick, S. (2012). Synaptic NMDA receptors mediate hypoxic excitotoxic death. *J. Neurosci.* 32, 6732–6742. doi: 10.1523/JNEUROSCI.6371-11.2012

Wu, B., Huang, Y., Braun, A. L., Tong, Z., Zhao, R., Li, Y., et al. (2015). Glutaminase-containing microvesicles from HIV-1-infected macrophages and immune-activated microglia induce neurotoxicity. *Mol. Neurodegener.* 10:61. doi: 10.1186/s13024-015-0058-z

Xiao, H., Neuveut, C., Tiffany, H. L., Benkirane, M., Rich, E. A., Murphy, P. M., et al. (2000). Selective CXCR4 antagonism by Tat: implications for *in vivo* expansion of coreceptor use by HIV-1. *Proc. Natl. Acad. Sci. U.S.A.* 97, 11466–11471. doi: 10.1073/pnas.97.21.11466

Xing, H. Q., Hayakawa, H., Gelpi, E., Kubota, R., Budka, H., and Izumo, S. (2009). Reduced expression of excitatory amino acid transporter 2 and diffuse microglial activation in the cerebral cortex in AIDS cases with or without HIV encephalitis. *J. Neuropathol. Exp. Neurol.* 68, 199–209. doi: 10.1097/NEN.0b013e31819715df

Xing, L., Yang, T., Cui, S., and Chen, G. (2019). Connexin hemichannels in astrocytes: role in CNS disorders. *Front. Mol. Neurosci.* 12:23. doi: 10.3389/fnmol.2019.00023

Xu, H., Bae, M., Tovar-y-Romo, L. B., Patel, N., Bandaru, V. V., Pomerantz, D., et al. (2011). The human immunodeficiency virus coat protein gp120 promotes forward trafficking and surface clustering of NMDA

receptors in membrane microdomains. *J. Neurosci.* 31, 17074–17090. doi: 10.1523/JNEUROSCI.4072-11.2011

Yang, J., Hu, D., Xia, J., Liu, J., Zhang, G., Gendelman, H. E., et al. (2013). Enhancement of NMDA receptor-mediated excitatory postsynaptic currents by gp120-treated macrophages: implications for HIV-1-associated neuropathology. *J. Neuroimmune Pharmacol.* 8, 921–933. doi: 10.1007/s11481-013-9468-2

Yoshimura, K. (2017). Current status of HIV/AIDS in the ART era. *J. Infect. Chemother.* 23, 12–16. doi: 10.1016/j.jiac.2016.10.002

Young, A. C., Yiannoutsos, C. T., Hegde, M., Lee, E., Peterson, J., Walter, R., et al. (2014). Cerebral metabolite changes prior to and after antiretroviral therapy in primary HIV infection. *Neurology* 83, 1592–1600. doi: 10.1212/WNL.0000000000000932

Yukl, S. A., Shergill, A. K., Ho, T., Killian, M., Girling, V., Epling, L., et al. (2013). The distribution of HIV DNA and RNA in cell subsets differs in gut and blood of HIV-positive patients on ART: implications for viral persistence. *J. Infect. Dis.* 208, 1212–1220. doi: 10.1093/infdis/jit308

Zarate, S., Pond, S. L., Shapshak, P., and Frost, S. D. (2007). Comparative study of methods for detecting sequence compartmentalization in human immunodeficiency virus type 1. *J. Virol.* 81, 6643–6651. doi: 10.1128/JVI.02268-06

Zhang, M., An, C., Gao, Y., Leak, R. K., Chen, J., and Zhang, F. (2013). Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. *Prog. Neurobiol.* 100, 30–47. doi: 10.1016/j.pneurobio.2012.09.003

Zhao, J., Lopez, A. L., Erichsen, D., Herek, S., Cotter, R. L., Curthoys, N. P., et al. (2004). Mitochondrial glutaminase enhances extracellular glutamate production in HIV-1-infected macrophages: linkage to HIV-1 associated dementia. *J. Neurochem.* 88, 169–180. doi: 10.1046/j.1471-4159.2003.02146.x

Zhou, Y., and Danbolt, N. C. (2014). Glutamate as a neurotransmitter in the healthy brain. *J Neural Transm.*121, 799–817. doi: 10.1007/s00702-014-1180-8

Zhou, Y., Liu, J., and Xiong, H. (2017). HIV-1 Glycoprotein 120 enhancement of N-methyl-D-aspartate NMDA receptor-mediated excitatory postsynaptic currents: implications for HIV-1-associated neural injury. *J. Neuroimmune Pharmacol.* 12, 314–326. doi: 10.1007/s11481-016-9719-0

Zoratti, M., Szabo, I., and De Marchi, U. (2005). Mitochondrial permeability transitions: how many doors to the house? *Biochim. Biophys. Acta* 1706, 40–52. doi: 10.1016/j.bbabio.2004.10.006

Zou, S., Fuss, B., Fitting, S., Hahn, Y. K., Hauser, K. F., and Knapp, P. E. (2015). Oligodendrocytes are targets of HIV-1 Tat: NMDA and AMPA receptor-mediated effects on survival and development. *J. Neurosci.* 35, 11384–11398. doi: 10.1523/JNEUROSCI.4740-14.2015

Zucchini, S., Pittaluga, A., Brocca-Cofano, E., Summa, M., Fabris, M., De Michele, R., et al. (2013). Increased excitability in tat-transgenic mice: role of tat in HIV-related neurological disorders. *Neurobiol. Dis.* 55, 110–119. doi: 10.1016/j.nbd.2013.02.004

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

BRIEF RESEARCH REPORT
published: 16 July 2020
doi: 10.3389/fcimb.2020.00347

Early ART in Acute HIV-1 Infection: Impact on the B-Cell Compartment

Robert Badura[1,2]*, *Russell B. Foxall*[1], *Dario Ligeiro*[3], *Miguel Rocha*[4], *Ana Godinho-Santos*[1], *Amelia C. Trombetta*[1] *and Ana E. Sousa*[1]

[1] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal, [2] Serviço de Doenças Infecciosas, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal, [3] Centro de Sangue e Transplantação de Lisboa, Instituto Português de Sangue e Transplantação, IP, Lisbon, Portugal, [4] Grupo de Ativistas em Tratamentos, Community Based Center for HIV and STD, CheckpointLX, Lisbon, Portugal

HIV-1 infection induces B cell defects, not fully recovered upon antiretroviral therapy (ART). Acute infection and the early start of ART provide unique settings to address the impact of HIV on the B cell compartment. We took advantage of a cohort of 21 seroconverters, grouped according to the presence of severe manifestations likely mediated by antibodies or immune complexes, such as Guillain-Barré syndrome and autoimmune thrombocytopenic purpura, with a follow-up of 8 weeks upon effective ART. We combined B and T cell phenotyping with serum immunoglobulin level measurement and quantification of sj-KRECs and ΔB to estimate bone marrow output and peripheral proliferative history of B cells, respectively. We observed marked B cell disturbances, notably a significant expansion of cells expressing low levels of CD21, in parallel with markers of both impaired bone marrow output and increased peripheral B cell proliferation. This B cell dysregulation is likely to contribute to the severe immune-mediated conditions, as attested by the higher serum IgG and the reduced levels of sj-KRECs with increased ΔB in these individuals as compared to those patients with mild disease. Nevertheless, upon starting ART, the dynamic of B cell recovery was not distinct in the two groups, featuring both persistent alterations by week 8. Overall, we showed for the first time that acute HIV-1 infection is associated with decreased bone marrow B cell output assessed by sj-KRECs. Our study emphasizes the need to intervene in both bone marrow and peripheral responses to facilitate B cell recovery during acute HIV-1 infection.

Keywords: B cells, HIV, severe acute HIV-1 infection, antiretroviral therapy, KRECs

OPEN ACCESS

Edited by:
Santhi Gorantla, University of Nebraska Medical Center, United States

Reviewed by:
Vijayakumar Velu, Emory University, United States
Ilhem Messaoudi, University of California, Irvine, United States

***Correspondence:**
Robert Badura
rbadura@medicina.ulisboa.pt

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *14 January 2020*
Accepted: *05 June 2020*
Published: *16 July 2020*

Citation:
Badura R, Foxall RB, Ligeiro D, Rocha M, Godinho-Santos A, Trombetta AC and Sousa AE (2020) Early ART in Acute HIV-1 Infection: Impact on the B-Cell Compartment. Front. Cell. Infect. Microbiol. 10:347. doi: 10.3389/fcimb.2020.00347

INTRODUCTION

HIV has become a controllable disease since the introduction of antiretroviral therapy (ART), due to the very efficient viral suppression and consequent recovery from the CD4 T lymphocyte depletion. Nonetheless non-AIDS related diseases persisted in long-term treated subjects, in association with increased levels of immune-activation and dysregulation. The initial viral dynamics, as much as the immediate HIV impact upon the immune system, is known to determine, by and large, the ensuing progression rate of the disease (Mellors et al., 1997). The early start of therapy in the acute HIV-1 infection is likely to reduce viral set point reservoirs and immune-activation (Jain et al., 2013). Therefore, there is an increasing interest in the study of these patients.

The fact that acute HIV infection (AHI) largely goes unnoticed has clearly hampered the investigation during this initial stage (Cohen et al., 2010), and justifies the relatively reduced number of studies. More than 50% of patients with acute HIV-1 infection have very few or no symptoms (Vanhems et al., 1998). Up to a third may present a more severe primary HIV-1 infection (Nicolás et al., 2019), such as opportunistic infections, neurological involvement, thrombocytopenic purpura and other serious conditions, with an increased risk of disease progression (Hoenigl et al., 2017). The pathophysiologic mechanisms leading to these manifestations are not fully clarified. Autoimmune diseases have been related to HIV-1 infection and the immune dysregulation observed in these patients (Virot et al., 2017). Among the postulated causes has been cited a direct viral pathologic effect of HIV linked to an aberrant immune activation which may lead to a serum sickness like syndrome (Lumsden and Bloomfield, 2016). Although the nadir of CD4 T cell count is predictive of the course of the disease after the initiation of ART (Pedersen et al., 1990; Hogg et al., 2001), it hardly describes the complete picture of immune dysregulation observed in acute HIV-1 infection (Ipp et al., 2014). Some acute phase severe clinical presentations with autoimmune-like characteristics are possibly mediated by antibodies or immune-complexes and might be related to B cell alterations.

Chronic HIV-1 infection is known to have an impact on the B cell compartment, resulting in non-specific polyclonal activation (Nagase et al., 2001), decreased B cell proliferative responses (Hart et al., 2007), loss of naïve and resting memory B cells (Carrillo et al., 2018), and higher percentages of atypical or exhausted B-cells (Moir et al., 2008), which are not fully recovered by ART (Richard et al., 2010). It has been shown that ART is less able to recover the depressed bone-marrow B cell output in chronic HIV-1 infections the later the treatment is started (Quiros-Roldan et al., 2012). The impact of a very early treatment approach in the acute HIV-1 infection upon bone-marrow output is so far unknown.

We have been gathering longitudinal data of seroconverters with severe clinical manifestations, like Guillain Barré syndrome, immune thrombocytopenic purpura, pericarditis, pneumonitis, rhabdomyolysis, along with data from mild or asymptomatic patients. This cohort represents a unique opportunity to investigate the B cell compartment in the early stages of the disease, and evaluate the impact that ART may have upon the putative B cell disturbances. We combined the study of the T and B cell subsets, with strategies to evaluate the B cell dynamics by the estimation of bone marrow output and peripheral proliferative history.

METHODS

Patients and Study Design

An established network between the Department of Infectious Disease at the University Hospital of Santa Maria (Lisbon, Portugal) and the community based center for HIV testing in the same area (CheckpointLX) allowed the recruitment of 21 HIV-1 seroconverters with a very short interval between diagnosis and the suspected time of infection (33 days [26, 48]), who were studied prospectively from diagnosis up to 8 weeks after starting ART (**Table 1**). These patients were stratified at presentation, according to Fiebig stages (Fiebig et al., 2003), which subdivides acute infection into 5 stages according to the detection of HIV-RNA and the degree of Western blot's positivity, aiming to differentiate the successive phases of the acute infection. These stages are defined as follows: Fiebig 1: only viral RNA is detectable; Fiebig 2: both viral RNA and p24 antigen are detectable; Fiebig 3: one or two positive bands on a WB but not enough to declare positivity; Fiebig 4: all bands except p31 positive on a WB; Fiebig 5: is a fully positive WB. In agreement to this classification 11 of our patients had a Fiebig stage of 3 (see **Table 1** and **Figure 1**). Patients were further grouped according to the clinical manifestations at presentation, namely: "Severe," including 8 patients with organ specific or systemic disease requiring hospitalization; and "Mild," the 13 subjects with the typical acute presentation being either asymptomatic or featuring self-limiting fever (for <5 days) with or without a mononucleosis like syndrome. All patients started ART, with the drug combinations listed in **Table 1**. Immunological and virological studies were performed immediately before starting ART (T0), and at 2 (T2), 4 (T4), and 8 (T8) weeks of treatment. Difference in sample numbers, in some time points was due to the necessity of having comparable timings among all patients, therefore some follow-ups were needed to be excluded if the patient did not appear for clinical evaluation and sample collection in the assumed week. This study was approved by the Ethical Board of the University Hospital of Santa Maria and the Faculty of Medicine of the University of Lisbon. All subjects provided written informed consent before data collection and blood sampling.

Cell Isolation and Flow Cytometry

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation (Gibco, Grand Island, New York, USA) washed and re-suspended at 1×10^6 cells/ml in RPMI 1640 (Gibco). Samples were surface stained for 20 min at room temperature as previously described (Blanco et al., 2018, 2019), using the following anti-human monoclonal antibodies, with clone and fluorochrome specified in brackets: CD3 (OKT3, PerPC-Cy5.5); CD4 (SK3; PerCP), CD8 (SK1; allophycocyanin (APC-Cy7); CD19 (HIB19; PerCP-Cy5.5), CD38 (HB7; PE), CD45RA (HI100; APC), IgD (IA6-2; PE), HLA-DR (L243; FITC), CD27 (O323; APC), CD21 (BL13; FITC); Ki67 (B56; FITC), Samples were acquired on a 6-parameter FACSCalibur flow cytometer (BD Biosciences, San Jose, California, USA), with a minimum of 20,000 events analyzed for each parameter, and analyzed using FlowJo software (TreeStar, Inc., Ashland, Oregon, USA). Cells were successively gated on lymphocytes, according to forward/side scatter characteristics and B cells, as illustrated in **Figure 1**. T cell phenotype was assessed as previously described (Sousa et al., 2002). The absolute numbers of lymphocyte subsets were calculated by multiplying their frequency by the absolute lymphocyte counts obtained at the clinical laboratory at the same day of sampling.

TABLE 1 | Clinical and epidemiological data at baseline.

		Age	Sex	Route	Symptoms	Fiebig	Days to ART	ART: TFV+FTC+	VL log10 cp/ml	Viral DNA cp/10^6 cells	CD4 cells/μl	CD8 cells/μl	CD19 cells/μl
Severe		29*	m	H	Severe hypoxic pneumonia	2	21	EFV	5.26	1,713	591	3,861	81
		65	m	H	Severe hypoxic pneumonitis	4	15	EFV	5.51	340	556	2,791	150
		31**	f	H	Rhabdomyolysis	3	18	EFV	7.04	43	220	623	125
		63	m	H	Guillain-Barre	4	48	DRV/r	4.94	1,310	919	2,280	54
		29	m	MSM	Pericarditis	4	17	EFV	6.55	NA	566	750	197
		57	m	H	Immune thrombocytopenic purpura	3	32	EFV	6.00	132	280	504	40
		29	m	MSM	Persistent fever and diarrhea	3	85	***+ RGV	6.26	NA	225	1,156	33
		39	m	H	Persistent fever	3	37	RGV	6.76	3176	425	343	190
	median	35	–	–	–	–	26.5	–	6.13	825	490	953	103
Mild		31	m	MSM	Fever and myalgia	4	20	EFV	5.49	778	417	1,737	67
		47	m	MSM	Mononucleosis-like syndrome	3	14	EFV	6.83	83,885	459	1,190	117
		32	m	MSM	Mononucleosis-like syndrome	4	32	RGV	4.37	12,163	448	916	54
		25	m	MSM	Adenopathies and asthenia	3	21	RGV	4.92	4,400	711	1,538	125
		40	m	MSM	Mononucleosis-like syndrome	3	18	RGV	5.45	321	329	529	60
		24	m	MSM	Fever and exanthema	3	31	RGV	5.41	1,260	619	2,292	104
		57	m	MSM	Fever and exanthema	2	14	RGV	7.00	2,097	397	561	88
		51	f	H	Fever and myalgia	2	14	RGV	7.00	106	513	344	32
		32	m	H	Fever and odynophagia	3	24	RGV	5.84	132	1,078	1,797	117
		23	f	H	No symptoms	1	/	RGV	6.39	113	474	374	58
		26	m	MSM	Adenopathies	3	32	RGV	6.01	9,454	835	1,185	74
		32	m	MSM	Fever and aphtosis	3	22	RGV	5.43	1,498	641	1,270	73
		35	m	H	Fever and exanthema	2	15	RGV	7.18	NA	470	607	110
	median	32	–	–	–	–	20.5	–	5.84	1,379	474	1,185	74

*Additional therapies applied: *Streptococcal pneumonia treated with ceftriaxone and **Guillain-Barre syndrome treated with high dose Immunoglobulin. No statistical differences were found between the two groups. NA, not available; med, median; H, Heterosexual; MSM, Men having sex with men. Treatment was initiated in all patients as indicated in ART (antiretroviral therapy) with RGV, raltegravir or EFV, efavirenze or DRV/r, darunavir with ritonavir together with a backbone with TFV+FTC, tenofovir e emtricitabina, except as indicated in one patient ***were the backbone was constituted by ABV+3TC: abacavir and lamivudine; VL, viral load in Log10; DNA, for proviral DNA as copies of Gag/10^6 cells.*

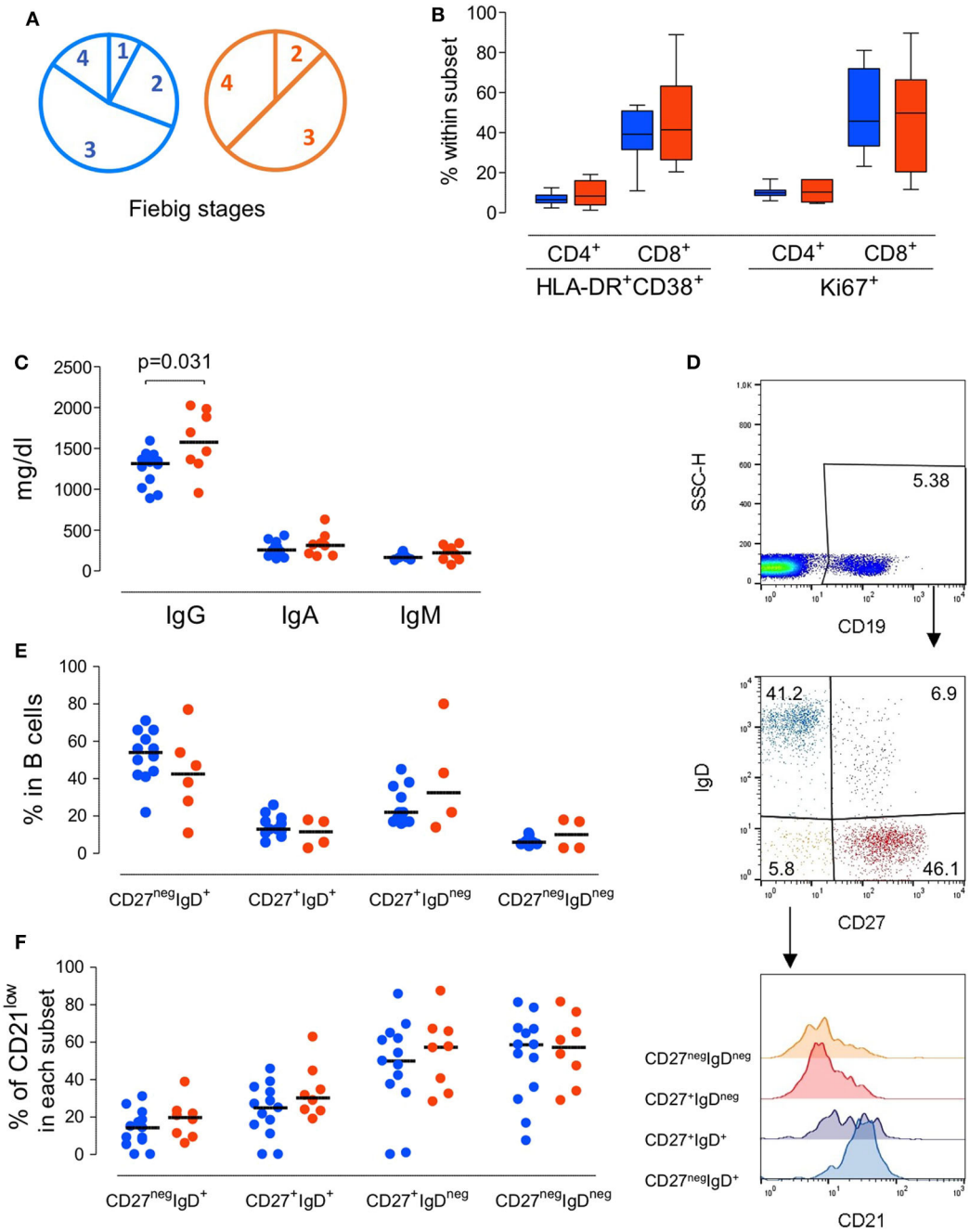


FIGURE 1 | *Immunological profile of the patients at presentation grouped according to the severity of the clinical manifestations.* **(A)** Pie-charts of the patient distribution according to Fiebig scale. **(B)** Proportion of CD4 T cells and CD8 T cells expressing activation and/or cycling markers, namely concomitant expression of HLA-DR and CD38 or KI-67. **(C)** Serum Immunoglobulin levels. **(D)** Illustrative dot-plots of the B cell analysis by flow cytometry. **(E)** Frequency of subsets defined according to the expression of surface IgD and CD27 within total B cells. **(F)** Proportion of cells featuring low CD21 expression within each B cell subset. Each dot represents one individual and blue refers to subjects with mild symptoms and orange to those with severe manifestations. Bars represent median and $P < 0.05$ are shown.

DNA Extraction and Total Viral DNA Quantification

DNA was extracted from PBMC (at least 0.5×10^6), with DNAzol reagent (Life Technologies, Glasgow, UK). Digital PCR was performed using ddPCR Probe Supermix (Bio-Rad, California, USA) with 900 nM primers (2 pairs were used: set 1 – Primer Fw1: CGAGAGCGTCAGTATTAAGC; Primer Rv1: AGCTCC CTGCTTGCCCATAC; set 2 – Primer Fw2: CGAGAGCGTCGG TATTAAGC; Primer Rv2: AACAGGCCAGGATTAAGTGC), 250 nM probe (5′-FAM-CCCTGGCCTTAACCGAATT-MGB),

and template DNA. Positive controls were generated from serial dilutions of plasmids containing the amplicons of HIV-1 gag and CD3 (a kind gift from Rémi Cheynier) (Fabre-Mersseman et al., 2011). Each 20 μL PCR reaction mixture was loaded into the Bio-Rad QX-200 emulsification device and droplets were formed following the manufacturer's instructions. The contents were transferred to a 96-well reaction plate and sealed with a pre-heated Eppendorf 96-well heat sealer for 5 s, as recommended by Bio-Rad. Total DNA was amplified separately in a T100TM Bio-Rad thermal cycler with the following cycling conditions: 10 min at 95°C, 45 cycles each consisting of a 30 s denaturation at 94°C followed by a 58°C extension for 60 s, and a final 10 min at 98°C. After cycling droplets were analyzed immediately.

Quantification of sj-KRECs and Estimation of Peripheral B Cell Proliferation

A qPCR assay was conducted to determine the single joint (sj)-Kappa deleting recombination excision circles (KRECs) sequences in peripheral blood. The primers and probes used have been previously described (Serana et al., 2013). Briefly, 125 ng DNA was used for PCR amplification with 1x Taqman Universal Master Mix II (Applied Biosystems, Foster City, CA), 900 nM of 3'/5' outer primers and 250 nM of probes (FAM-TAMRA for TRAC and JOE-TAMRA for sj-KRECs). Sequence copy numbers were extrapolated from standard curves obtained by 10-fold serial dilutions of a plasmid, which contains KREC and TRAC fragments in a 1:1 ratio (a kind gift from L. Imberti, Spedali Civili of Brescia, Italy) (Serana et al., 2013). sj-KREC copies per μl of blood were calculated from the number of genome normalized sj-KREC molecules corrected for the number of white blood cells in peripheral blood (Chen et al., 2005). Finally, the number of B cell divisions was estimated as reported: somatic coding joints sequences cj-RssKde resulting from kappa-deleting rearrangement were quantified by qPCR, the difference of signal joint cycle threshold (Ct) to the coding joint Ct represents an estimate of divisions B cells had undergone (van Zelm et al., 2007, 2013). Threshold lines for Ct determination were positioned at the same level.

Serum Immunoglobulin Levels

Total immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) were quantified by immunonephelometry (Beckman-Coulter, Brea, California, USA) at the clinical laboratory of the Hospital de Santa Maria.

Statistics

Statistical analyses were performed using SPSS version 24 and GraphPad/Prism version 7.0 (GraphPad Software, San Diego, California, USA). Mann-Withney U and Wilcoxon tests were used to compare unpaired and paired data, respectively. Anova and Mixed Anova tests were used for total and between group variance calculations in the follow-ups. Simple linear regression was performed to estimate associations of two numeric variables. Results are expressed as median, with interquartile range in [brackets], and $p < 0.05$ were considered to be significant.

RESULTS

We first evaluated whether patients with atypical/severe manifestations requiring hospitalization during acute HIV-1 infection present distinct immunological and/or virological features, as compared to those with the typical/mild presentation. As shown in **Table 1**, the severe group included 8 patients with clinical manifestations that are likely to be immune-mediated, namely Guillain-Barré syndrome, pneumonitis, pericarditis and autoimmune thrombocytopenic purpura. Notably, no opportunistic infections were reported. At enrolment the patients in the mild group ($n = 13$) were asymptomatic or had limiting symptoms, such as fever or a short mononucleosis-like syndrome. Distribution of Fiebig stages was dominated by stage 3 in both groups (**Figure 1A**), with no significant difference in distribution ($p = 0.8$), indicating that the differences in clinical manifestations were not related to the time since infection. Moreover, there were no differences in respect to age ($p = 0.41$), gender distribution ($p = 0.85$), or route of transmission between the two groups.

Strikingly, no significant differences were found in the levels of plasma viremia (5.84 and 6.13 Log, respectively, for mild and severe diseased patients groups, $p = 0.86$) or the cell associated total viral DNA (**Table 1**), suggesting that the degree of viral load was not the main determinant of the severity of the clinical manifestations.

Regarding the immune parameters, although there were three patients in the severe group with a profound CD4 depletion (<300 cells/μl), the described decline of CD4 T cell counts during the acute infection was observed in both groups (**Table 1**), with a respective median of 474 and 490/μl ($p = 0.37$). Moreover, the degree of the CD8 expansion was also comparable in the two groups (**Table 1**), leading to similar alteration of the CD4/CD8 ratio (0.60 [0.42–0.74] and 0.38 [0.19–0.70] for seroconverters with mild or severe symptoms, respectively, $p = 0.16$). In agreement, the markedly increased levels of CD4 and CD8 T cell activation were comparable between the two patient groups, despite their distinct clinical features (**Figure 1B**).

Concerning the B cell compartment, the "severe" group featured significantly higher levels of serum IgG (**Figure 1C**), mainly due to IgG_1 (796 mg/dl [644–820] and 1,060 mg/dl [749–1,520] for mild and severe disease, respectively). This is particularly interesting because the documented clinical manifestations are thought to be largely immune-complex or antibody-mediated. This led us to analyse the B cell compartment in detail (**Figure 1D**). Both, the mild and severe group, featured reduced B cell counts. In fact, previously published data from the EuroFlow PID group, to which our lab contributed, reported in the 18 to 39 age group (the one most closely comparable to our patients age) a total number of 220 B cells/μl (median, range 41–470 and 54–438, 5th−95th percentile) (Blanco et al., 2018, 2019). These values, even taking in consideration the high variability of B cell count, are significantly different ($p < 0.0001$) compared to our patients' total B cell counts at baseline (median 80, range 32–197). Moreover, a similar distribution of the B cell subsets according to the expression of the memory marker CD27 and the surface levels of IgD was observed in the

two groups of patients (**Figure 1E**), namely for $IgD^{+}CD27^{neg}$ or naive, $IgD^{neg}CD27^{+}$ or memory switched, $IgD^{+}CD27^{+}$ or memory unswitched, and $IgD^{neg}CD27^{neg}$ or other memory switched cells. Additionally, the percentage of cells expressing reduced levels of CD21 were expanded in all B cell subsets at baseline compared to the end of the follow up, irrespective of the clinical manifestations (**Figure 1F**). The increase in the $CD21^{low}$ population percentage was particularly marked at baseline in switched B cells (median 50% in $CD27^{+}IgD^{neg}CD21^{low}$ and 60% in $CD27^{neg}IgD^{neg}CD21^{low}$) indicating high levels of B cell activation in both patient groups. Of note, the global analysis of the patients revealed a direct relationship between the frequency of $CD21^{low}$ cells within switched memory ($IgD^{neg}CD27^{+}$) B cells and viremia (r^2: 0.21/$p = 0.04$/slope: 0.11). Therefore, the B cell disturbances were not significantly different in the two groups of patients and appeared to be more closely related to viral load.

Importantly, upon starting ART there was a progressive improvement in all the clinical conditions paralleled by increased CD4 T cell count ($p < 0.0001$) and the effective control of viral load (with a median of 0 RNA copies/ml [0–65] and 18 RNA copies/ml [0-119] upon eight weeks of treatment for the mild and severe groups, respectively), $p < 0.0001$ (**Supplementary Table 1**).

The variation of the B-cell frequency and absolute numbers where not different between the two groups (**Figure 2**). There was a progressive increase in the B cell counts, though they did not return to normal levels (Blanco et al., 2018, 2019), during the follow-up period (**Figure 2**).

It is known that immune activation is a key determinant of the B cell disturbances that characterize HIV infection (Moir and Fauci, 2014). In agreement, we observed a direct correlation between CD4 and CD8 T cell activation and the expansion of switched memory B cells (correlation between the frequency of $CD27^{+}IgD^{neg}$ within B cells and the proportion of $HLA\text{-}DR^{+}CD38^{+}$ cells within CD4 and CD8 T cells; r^2: 0.2/p: 0.04/slope: 0.13, and r^2: 0.45/p: 0.0016/slope: 0.7, respectively), as well as a negative correlation with frequency of naive B cells (correlation between the frequency of $CD27^{neg}IgD^{+}$ within B cells and the proportion of $HLA\text{-}DR^{+}CD38^{+}$ cells within CD4 and CD8 T cells; r^2: 0.4/p: 0.007/slope: -0.15, and r^2: 0.3/p: 0.02/slope: -0.55, respectively) (**Figure 3A**). Of note, no significant correlations were observed after 8 weeks of ART (**Figure 3B**), suggesting distinct dynamics of the recovery of the T and the B cell activation.

In order to further investigate the relative contributions of peripheral B cell proliferation induced by the immune stimulation and the possible impairment in B cell production in the bone marrow associated with the HIV infection, KRECs were quantified. Sj-KRECs are generated by the rearrangement of the Jκ-Cκ intron recombination signal sequence (intronRSS) to the kappa-deleting element which renders the IGK locus non-functional and precludes any further rearrangements in the IGK locus. Therefore, the coding joint of this rearrangement remains stably present in the genome. When a B lymphocyte with an intronRSS–Kde rearrangement divides, both daughter cells inherit the intronRSS–Kde coding joint in the genome. Conversely, the signal joint, which is on the episomal KREC, will be inherited by only one of the two daughter cells. As a consequence the difference between the kappa-deleting rearrangement and the corresponding excision circle (Delta B, ΔB) can be used as a measure for the *in vivo* replication history of an isolated B cell subset, assuming that the excision circle is a stable DNA structure, which is diluted 2-fold in every cell division. Since these rearrangements are terminated in the bone marrow, the quantification of sj-KRECs corresponds to B cell output from the bone marrow, and the calculated ΔB expresses their relative proliferation within the periphery. Before starting ART, the patients with severe manifestations featured significantly lower levels of sj-KRECs and higher levels of ΔB than those with mild disease (**Figure 3C**). These data suggest that severe disease was associated with both a more compromised bone marrow output and increased peripheral proliferation. Strikingly, upon ART there was a trend to a recovery of bone marrow output, as assessed by the sj-KRECS, as well as a significant decline in ΔB, supporting a reduction in peripheral proliferation in both groups of patients (**Figure 3D**).

Overall, the study of this unique cohort of acute HIV-1 infection suggests that severe disease is associated with an impairment in bone marrow output and more marked polyclonal B cell activation, as defined by higher IgG levels and higher ΔB. Notably, reduced B cell counts were a feature of acute HIV-1 infection irrespectively of disease severity. ART led to a recovery of total circulating B cells mainly due to an increase of the naïve B cell population. Moreover, 8 weeks of suppressive ART were unable to induce a full recovery of the memory switched B cell subset despite the contraction of the $CD21^{low}$ population.

DISCUSSION

Our study confirms the importance of B cell disturbances in acute HIV-1 infection. The marked disruption of the B cell compartment was associated with impaired bone marrow output coupled with increased peripheral cell proliferation, as demonstrated via quantification of sj-KRECSs and ΔB for the first time in acute HIV-1 infected individuals. B cell dysregulation was likely contributed to the severe immune-mediated conditions observed in a subset of our patients, which featured lower sj-KRECs and increased ΔB and serum IgG levels, when compared to patients with mild disease.

B cell disturbances are well-recognized in untreated chronic HIV-1 infection (Cagigi et al., 2008; Amu et al., 2013). They are thought to be mainly linked to persistent immune activation (De Milito, 2004) and only partially recover upon ART initiation (Younas et al., 2016). The hallmarks of B cell perturbations are polyclonal hypergammaglobulinemia and expansion of B cells expressing low levels of CD21 (Fogli et al., 2012). Interestingly, these aberrant B cells are also increased in other conditions of chronic inflammation, such as autoimmune diseases (Thorarinsdottir et al., 2016; Das et al., 2018). Overall B-cells expressing low CD21 have a decreased proliferative capacity and effector function, as measured by decreased immunoglobulin diversification leading to the assumption that they do represent an exhausted B cell subpopulation (Moir et al., 2008). Moir et al. have further shown that they fail to respond to B cell stimuli

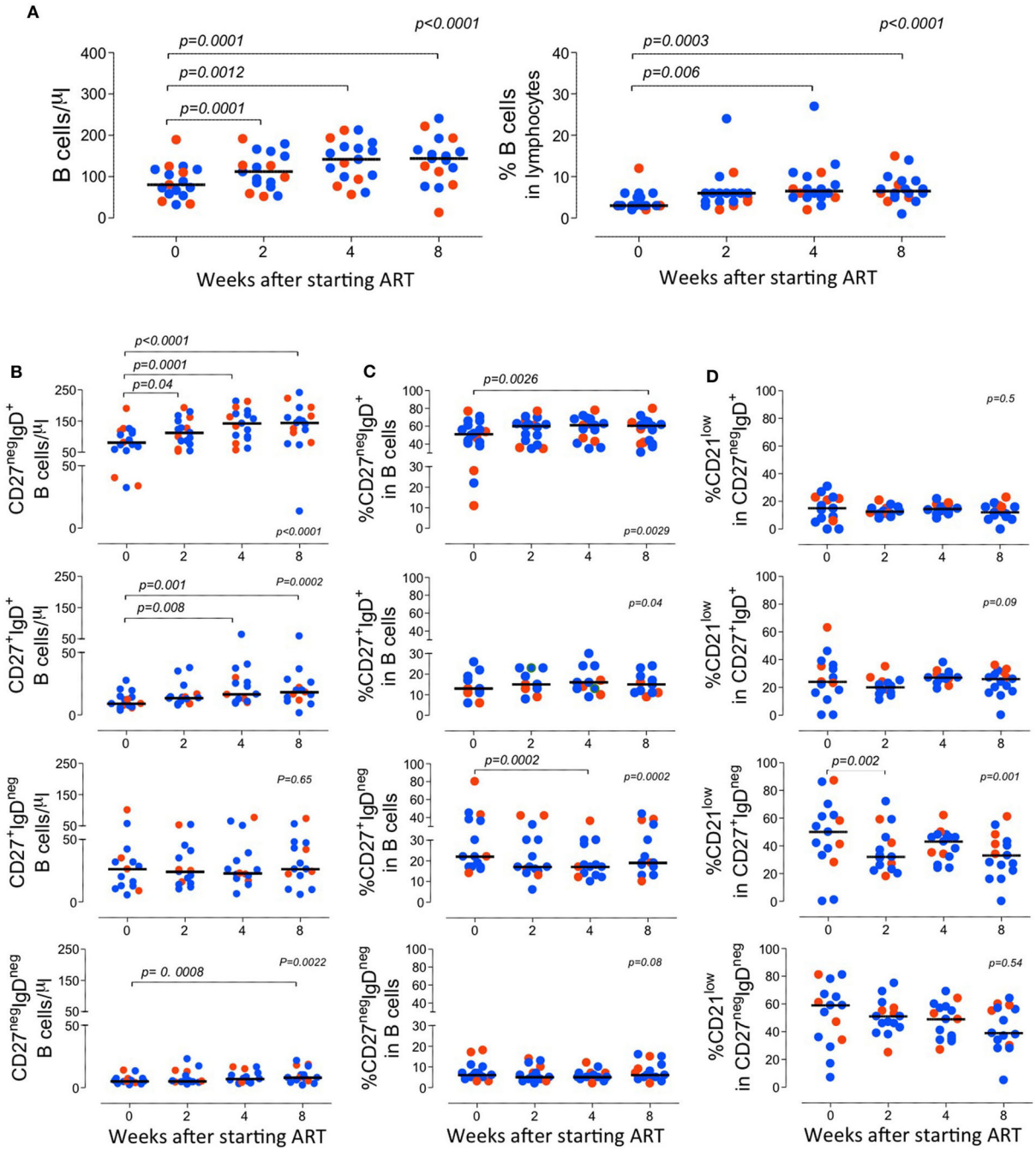


FIGURE 2 | *Longitudinal analysis of the B cell compartment before and after starting ART*. **(A)** Total B cell counts (left graph) and frequency of B cells within lymphocytes (right graph). **(B–D)** B cell subsets were defined according to the expression of CD27 and IgD and graphs show the absolute counts of circulating subsets **(B)**; the proportion of each subset within total B cells **(C)**; and the frequency of B cells expressing low levels of CD21 within the subset **(D)**. Each dot represents one individual and blue refers to subjects with mild symptoms and orange to those with severe manifestations. Time 0 refers to evaluation immediately before starting ART and sub sequential analyses were performed at 2, 4, and 8 weeks upon therapy. Bars represent median. Anova for repeated measures and Dunn's Test were calculated. $P < 0.05$ are shown.

in HIV-1- infected individuals (Moir et al., 2001). We showed that there is a linear association between viral load and memory switched B cells expressing low CD21 before therapy in acute HIV-1 infection.

Although part of the B cell responses are HIV specific, many indirect effects of the HIV infection that have been shown to contribute to the B cell stimulation (Schnittman et al., 1986; Kacani et al., 2000; Moir et al., 2000; Malaspina

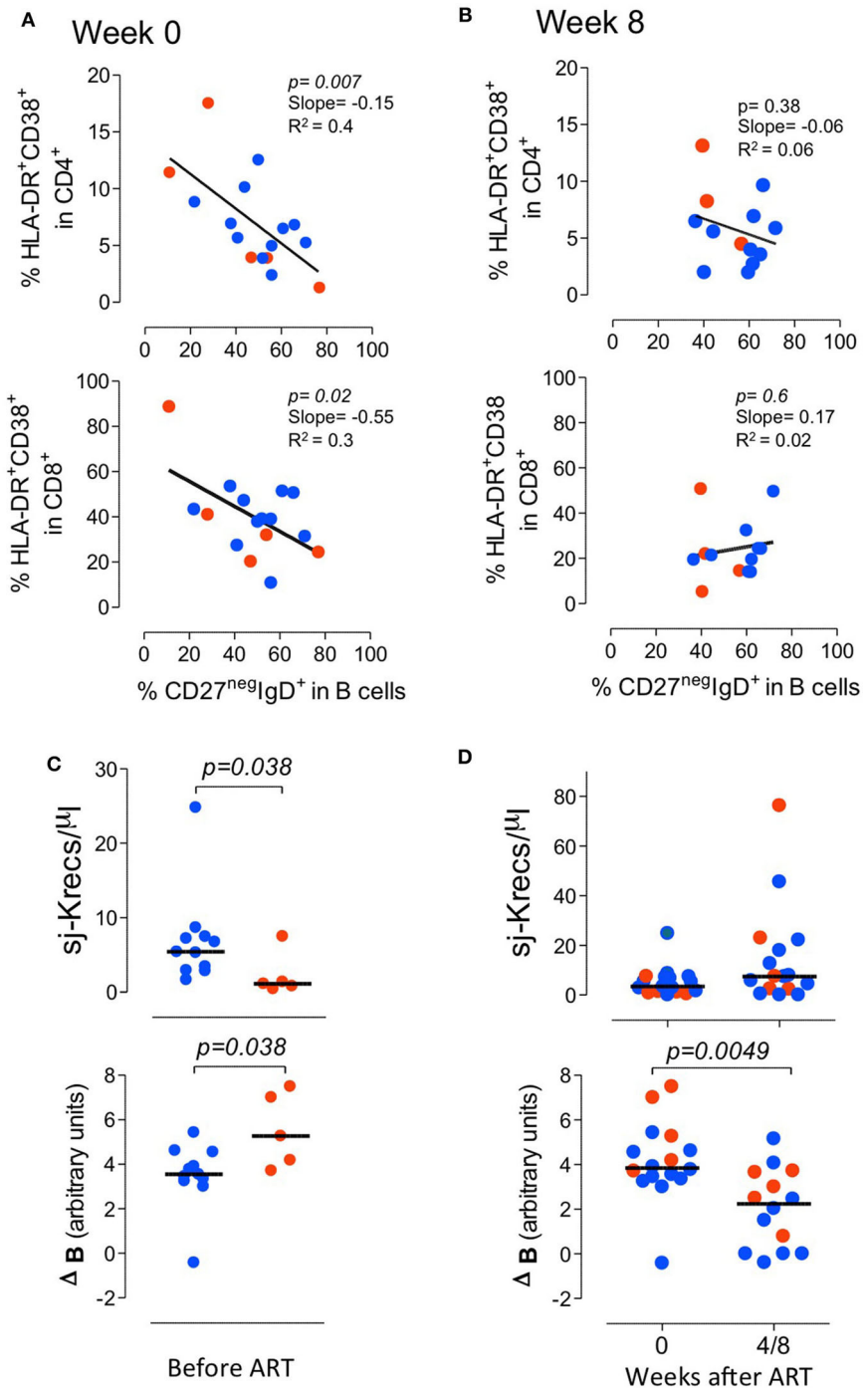


FIGURE 3 | *Impact of immune activation and bone marrow output evaluated by KRECs in the B cell imbalances.* **(A)** Correlation between the frequency of HLA-DR+CD38+ cells within CD4 (top graph) and CD8 (bottom graph) T cells and the proportion of naive cells ($CD27^{+}IgD^{neg}$) within total B cells before treatment. **(B)** The same correlation after 8 weeks of ART. **(C)** Comparison of the levels of sj-KRECs and Delta B between patients with severe and mild disease before treatment. **(D)** Changes of sj-KREC and Delta B levels upon 4 or 8 weeks of ART. Each dot represents one individual and blue refers to subjects with mild symptoms and orange to those with severe manifestations. Bars represent median and $P < 0.05$ are shown.

et al., 2006; Swingler et al., 2008; Haas et al., 2011; Ruffin et al., 2012; Sokoya et al., 2017). In fact, neither virema nor viral DNA level significantly differed between the two clinical groups, suggesting a contribution of other factors for the B cell dysregulation associated with severe manifestations. Curiously, HIV-2 infection, which is associated with low to

undetectable viremia, and slow progressive CD4 decline in direct correlation with immune activation markers (Sousa et al., 2002), provides an example of profound B cell disturbances, namely marked depletion of both switched and unswitched memory B cells when compared to HIV-1 or seronegative controls (Tendeiro et al., 2012). This memory B cell reduction was mainly due to depletion of the class-switched subsets. CD27+IgD- depletion correlated with CD4+ T cell count in HIV-2 but not in HIV-1. A comparable loss of IgD+ memory B cell, correlating with markers of disease progression, like CD4 T-cell count and detectable viremia, was observed in both HIV-2 and HIV-1 chronically infected patients. Of note, we have also recently shown that follicular helper T cells (Tfh), the CD4 subset specialized in promoting B cell responses, supports productive HIV-2 infection and are an important reservoir in HIV-2-infected individuals (Godinho-Santos et al., 2020), suggesting that Tfh infection may contribute to the memory B cell imbalances.

An important finding of our study is the lack of full recovery of the switched memory B cell compartment upon 8 weeks of effective ART. Interestingly, Muir et al. suggested that HIV-1 induces disturbances in circulating Tfh function during the acute infection that may prevent the full recovery of the memory B cells upon treatment (Muir et al., 2016). Thus, preservation of Tfh function and B cell memory would require a very early start of ART, which would only be feasible with active prospective testing to identify seroconverters within the first week of infection (Ananworanich et al., 2016). Detailed functional studies of both the Tfh and the B cell compartments, ideally complemented with lymph node biopsies, are critically needed to understand the early HIV impact (Suresh et al., 2019).

The studies on the acute infection are always limited by the reduced number of subjects, though all the collected data are of high relevance. Given the rarity of acutely infected patients, we proritized the longitudinal analysis of the clinical and immunological data of seroconverters that consecutively presented to our clinic during a period of 90 months. Therefore, our study did not include the study of a group with chronic HIV infection in parallel and used the available data previously generated by us and others as a reference (Tendeiro et al., 2012; Blanco et al., 2018, 2019). Furthermore, differences in treatment regimens could have influenced the result. However, all patients showed decreased viremia and increased $CD4^+$ counts at the end of 8 weeks observation, irrespective of which regimen they received. Given the relatively small number of patients, in this type of study it is difficult to investigate the influence of treatment regimens on the results. Even though all patients showed decreased viremia and increased CD4+ counts at the end of 8 weeks observation, irrespective of which regimen they received it is known that patients with full CD4 recovery and undetectable viremia may maintain some other immune disturbances after long-time ART. We believe that longer follow-up studies of acutely infected HIV patients therefore would be desirable. However, we report that several alterations related to B cell functions improved significantly, such as hyper-gammaglobulinemia and peripheral proliferation, as signified by the ΔB values. The profile of B cell recovery was not different in the two groups of patients, despite the association of severe disease with more pronounced B cell imbalances at presentation. Severe acute presentation has historically been linked to a more progressive disease (Hoenigl et al., 2017), nonetheless, larger follow up studies will be needed to demonstrate the impact of early treatment on improvement of prognosis.

Our study calls attention to the impact of HIV-1 acute infection in the bone marrow, although other factors, such as increased cell death, could have contributed to the observed levels of sjKRECs and ΔB, a question, which should be addressed in future studies. Bone marrow abnormalities are common in chronic HIV-1 infection and characterized by erythroid dysplasia and perturbations of myelopoiesis along with an increased plasma cell population (Tripathi et al., 2005; Alexaki and Wigdahl, 2008; Dhurve and Dhurve, 2013). The evaluation of sj-KRECs has previously revealed a decreased B cell output during chronic HIV-1 infection, that declines in direct association with the length of disease (Quiros-Roldan et al., 2012). Quantification of sj-KRECs represents, therefore, an important tool to address this aspect using peripheral blood (Verstegen et al., 2019).

Overall, we showed for the first time that acute HIV-1 infection is associated with decreased bone marrow B cell output assessed by sj-KRECs. This impairment is significantly more marked in patients with severe clinical manifestations, in parallel with increased peripheral B cell proliferation and higher levels of polyclonal IgG levels. Thus, bone marrow in addition to peripheral responses should be targeted to correct B cell dynamics in acute HIV-1 infection.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Board of the Centro Hospitalar Universitário Lisboa Norte (CHULN) and of the Faculty of Medicine of the University of Lisbon. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RB, RF, and AS designed the study. RB, RF, and MR collected the clinical data. RF, DL, AG-S, and AT performed the immunological studies. AT, AG-S, RF, and RB analyzed data and investigated the selected variants. RB, AT, RF, DL, AG-S, and AS discussed the results. RB and AS supervised the study and wrote the paper. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by unconditional research grants provided by Gilead Sciences Portugal and GlaxoSmithKline foundation to RB.

ACKNOWLEDGMENTS

The authors acknowledge the participants in the HIV seroconverter study, the staff of CheckpointLX, and the health care providers from the Serviço de Doenças Infecciosas, Centro Hospitalar Universitário Lisboa Norte, for their collaboration.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2020.00347/full#supplementary-material

REFERENCES

Alexaki, A., and Wigdahl, B. (2008). HIV-1 infection of bone marrow hematopoietic progenitor cells and their role in trafficking and viral dissemination. *PLoS Pathog.* 4:e1000215. doi: 10.1371/journal.ppat.1000215

Amu, S., Ruffin, N., Rethi, B., and Chiodi, F. (2013). Impairment of B-cell functions during HIV-1 infection. *AIDS*. 44427, 2323–2334.doi: 10.1097/QAD.0b013e328361a427

Ananworanich, J., Chomont, N., Eller, L. A., Kroon, E., Tovanabutra, S., Bose, M., et al. (2016). HIV DNA set point is rapidly established in acute HIV infection and dramatically reduced by early, ART. *EBioMedicine* 11, 68–72. doi: 10.1016/j.ebiom.2016.07.024

Blanco, E., Pérez-Andrés, M., Arriba-Méndez, S., Contreras-Sanfeliciano, T., Criado, I., Pelak, O., et al. (2018). Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. *J. Allergy Clin. Immunol.* 141, 2208–2219.e16. doi: 10.1016/j.jaci.2018.02.017

Blanco, E., Pérez-Andrés, M., Arriba-Méndez, S., Serrano, C., Criado, I., Del Pino-Molina, L., et al. (2019). Defects in memory B-cell and plasma cell subsets expressing different immunoglobulin-subclasses in patients with CVID and immunoglobulin subclass deficiencies. *J. Allergy Clin. Immunol.* 144, 809–824. doi: 10.1016/j.jaci.2019.02.017

Cagigi, A., Nilsson, A., De Milito, A., and Chiodi, F. (2008). B cell immunopathology during HIV-1 infection: lessons to learn for HIV-1 vaccine design. *Vaccine* 26, 3016–3025. doi: 10.1016/j.vaccine.2007.11.063

Carrillo, J., Negredo, E., Puig, J., Molinos-Albert, L. M., Rodríguez de la Concepción, M. L., Curriu, M., et al. (2018). Memory B cell dysregulation in HIV-1-infected individuals. *AIDS* 32, 149–160. doi: 10.1097/QAD.0000000000001686

Chen, X., Barfield, R., Benaim, E., Leung, W., Knowles, J., Lawrence, D., et al. (2005). Prediction of T-cell reconstitution by assessment of T-cell receptor excision circle before allogeneic hematopoietic stem cell transplantation in pediatric patients. *Blood* 105, 886–893. doi: 10.1182/blood-2004-04-1405

Cohen, M. S., Gay, C. L., Busch, M. P., and Hecht, F. M. (2010). The detection of acute HIV infection. *JID* 202, S270–S277. doi: 10.1086/655651

Das, R., Bar, N., Ferreira, M., Newman, A. M., Zhang, L., Bailur, J. K., et al. (2018). Early B cell changes predict autoimmunity following combination immune checkpoint blockade. *J. Clin. Invest.* 128, 715–720. doi: 10.1172/JCI96798

De Milito, A. (2004). B lymphocyte dysfunctions in HIV infection. *Curr. HIV Res.* 2, 11–21. doi: 10.2174/1570162043485068

Dhurve, S. A., and Dhurve, A. S. (2013). Bone marrow abnormalities in HIV disease. *Mediterr. J. Hematol. Infect. Dis*. 5:e2013033. doi: 10.4084/MJHID.2013.033

Fabre-Mersseman, V., Dutrieux, J., Louise, A., Rozlan, S., Lamine, A., Parker, R., et al. (2011). CD4+ recent thymic emigrants are infected by HIV *in vivo*, implication for pathogenesis. *AIDS* 25, 1153–1162. doi: 10.1097/QAD.0b013e3283471e89

Fiebig, E. W., Wright, D. J., Rawal, B. D., Garrett, P. E., Schumacher, R. T., Peddada, L., et al. (2003). Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS* 17, 1871–1879. doi: 10.1097/00002030-200309050-00005

Fogli, M., Torti, C., Malacarne, F., Fiorentini, S., Albani, M., and Izzo, I. (2012). Emergence of exhausted B cells in asymptomatic HIV-1-infected patients naïve for HAART is related to reduced immune surveillance. *Clin. Dev. Immunol.*. 2012:829584. doi: 10.1155/2012/829584

Godinho-Santos, A., Foxall, R. B., AntÃeo, A. V., Tavares, B., Ferreira, T., and Serra-Caetano, A., et al. (2020). Follicular helper T cells are major human immunode ciency virus-2 reservoirs and support productive infection. *J. Infect. Dis.* 221, 122–126. doi: 10.1093/infdis/jiz431

Haas, A., Zimmermann, K., and Oxenius, A. (2011). Antigen-dependent and -independent mechanisms of T and B cell hyperactivation during chronic HIV-1 infection. *J. Virol.* 85, 12102–12113. doi: 10.1128/JVI.05607-11

Hart, M., Steel, A., Clark, S. A., Moyle, G., Nelson, M., Henderson, D. C., et al. (2007). Loss of discrete memory B cell subsets is associated with impaired immunization responses in HIV-1 infection and may be a risk factor for invasive pneumococcal disease. *J. Immunol.* 178, 8212–8220. doi: 10.4049/jimmunol.178.12.8212

Hoenigl, M., Braun, D. L., Kouyos, R., Günthard, H. F., Little, S. J., (2017). Evaluation of the predictive potential of the short acute retroviral syndrome severity score for, HIV-1 disease progression in individuals with acute HIV infection. *J. Acq. Immune. Defic. Synd.* 74, e114–e117. doi: 10.1097/QAI.0000000000001263

Hogg, R. S., Yip, B., Chan, K. J., Wood, E., Craib, K. J., O'Shaughnessy, M. V., et al. (2001). Rates of disease progression by baseline CD4 cell count and viral load after initiating triple-drug therapy. *JAMA* 286, 2568–2577. doi: 10.1001/jama.286.20.2568

Ipp, H., Zemlin, A. E., Erasmus, R. T., and Glashoff, R. H. (2014). Role of inflammation in HIV-1 disease progression and prognosis. *Crit. Rev. Clin. Lab. Sci.* 51, 98–111. doi: 10.3109/10408363.2013.865702

Jain, V., Hartogensis, W., Bacchetti, P., Hunt, P. W., Hatano, H., Sinclair, E., et al. (2013). Antiretroviral therapy initiated within 6 months of HIV infection is associated with lower T-cell activation and smaller HIV reservoir size. *J. Infect. Dis.* 208, 1202–1211. doi: 10.1093/infdis/jit311

Kacani, L., Prodinger, W. M., Sprinzl, G. M., Schwendinger, M. G., Spruth, M., Stoiber, H., et al. (2000). Detachment of human immunodeficiency virus type 1 from germinal centers by blocking complement receptor type 2. *J. Virol.* 74, 7997–8002. doi: 10.1128/jvi.74.17.7997-8002.2000

Lumsden, R. H., and Bloomfield, G. S. (2016). The causes of HIV-associated cardiomyopathy: a tale of two worlds. *Biomed. Res. Int*. 2016:8196560. doi: 10.1155/2016/8196560

Malaspina, A., Moir, S., Ho, J., Wang, W., Melissa, L., Howell, M. L., et al. (2006). Appearance of immature transitional B cells in HIV-infected individuals with advanced disease: correlation with increased IL-7. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2262–2267. doi: 10.1073/pnas.0511094103

Mellors, J. W., Muñoz, A., Giorgi, J. V., Margolick, J. B., Tassoni, C. J., Gupta, P., et al. (1997). Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann. Intern. Med.* 126, 946–954. doi: 10.7326/0003-4819-126-12-199706150-00003

Moir, S., and Fauci, A. S. (2014). B-cell exhaustion in HIV infection: the role of immune activa-tion. *Curr. Opin. HIVAIDS* 9, 472–477. doi: 10.1097/COH.0000000000000092

Moir, S., Ho, J., Malaspina, A., Wang, W., DiPoto, A. C., O'Shea, M. A., et al. (2008). Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J. Exp. Med.* 205, 1797–1805. doi: 10.1084/jem.20072683

Moir, S., Malaspina, A., Li, Y., Chun, T. W., Lowe, T., Adelsberger, J., et al. (2000). B cells of HIV-1-infected patients bind virions through CD21-complement interactions and transmit infectious virus to activated T cells. *J. Exp. Med.* 192, 637–646. doi: 10.1084/jem.192.5.637

Moir, S., Malaspina, A., Ogwaro, K. M., Donoghue, E. T., Hallahan, C. W., Ehler, L. A., et al. (2001). HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals. *Proc. Natl. Acad. Sci. U.S.A.* 98, 10362–10367. doi: 10.1073 pnas.181347898

Muir, R., Metcalf, T., Tardif, V., Takata, H., Phanuphak, N., Kroon, E., et al. (2016). Altered memory circulating T follicular helper-B cell interaction in early acute HIV infection. *PLoS Pathog.* 12:e1005777. doi: 10.1371/journal.ppat.1005777

Nagase, H., Agematsu, K., Kitano, K., Takamoto, M., Okubo, Y., Komiyama, A., et al. (2001). Mechanism of hypergammaglobulinemia by HIV infection: circulating memory B-cell reduction with plasmacytosis. *Clin. Immunol.* 100, 250–259. doi: 10.1006/clim.2001.5054

Nicolás, D., Suárez, A., Ambrosioni, J., Manzardo, C., Ligero, C., Costa, J., et al. (2019). Prevalence, clinical characteristics and outcome of severe primary HIV-1 infection: a prospective cohort study. *Int. J. Infect. Dis.* 88, 73–79. doi: 10.1016/j.ijid.2019.08.001

Pedersen, C., Gerstoft, J., Tauris, P., Lundgren, J. D., Gøtzsche, P. C., Buhl, M., et al. (1990). Trends in survival of danish AIDS patients from 1981 to 1989. *AIDS* 4, 1111–1116. doi: 10.1097/00002030-199011000-00009

Quiros-Roldan, E., Serana, F., Chiarini, M., Zanotti, C., Sottini, A., Gotti, D., et al. (2012). Effects of combined antiretroviral therapy on B- and T-cell release from production sites in long-term treated HIV-1+ patients. *J. Transl. Med.* 10:94. doi: 10.1186/1479-5876-10-94

Richard, Y., Amiel, C., Jeantils, V., Mestivier, D., Portier, A., Dhello, G., et al. (2010). Changes in blood B cell phenotypes and Epstein-Barr virus load in chronically human immunodeficiency virus–infected patients before and after antiretroviral therapy. *J. Infect. Dis.* 202, 1424–1434. doi: 10.1086/656479

Ruffin, N., Thang, P. H., Rethi, B., Nilsson, A., and Chiodi, F. (2012). The impact of inflammation and immune activation on B cell differentiation during HIV-1 infection. *Front. Immunol.* 2:90. doi: 10.3389/fimmu.2011.00090

Schnittman, S. M., Lane, H. C., Higgins, S. E., Folks, T., and Fauci, A. S. (1986). Direct polyclonal activation of human B lymphocytes by the acquired immune deficiency syndrome virus. *Science* 233, 1084–1086. doi: 10.1126/science.3016902

Serana, F., Chiarini, M., Zanotti, C., Sottini, A., and Bertoli, D. (2013). Use of V (D) J recombination excision circles to identify T-and B-cell defects and to monitor the treatment in primary and acquired immunodeficiencies. *J. Transl. Med.* 11:119. doi: 10.1186/1479-5876-11-119

Sokoya, T., Steel, H. C., Nieuwoudt, M., and Rossouw, T. M. (2017). HIV as a cause of immune activation and immunosenescence. *Mediat. Inflamm.* 2017:6825493. doi: 10.1155/2017/6825493

Sousa, A. E., Carneiro, J., Meier-Schellersheim, M., Grossman, Z., and Victorino, R. M. (2002). CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J. Immunol.* 169, 3400–3406. doi: 10.4049/jimmunol.169.6.3400

Suresh, P. K., Poojary, S., Basavaiah, S. H., Kini, J. R., Lobo, F. D., and Sahu, K. K. (2019). Utility of fine-needle aspiration cytology in the diagnosis of HIV lymphadenopathy. *Diagn. Cytopathol.* 47, 1011–1017. doi: 10.1002/dc.24255

Swingler, S., Zhou, J., Swingler, C., Dauphin, A., Greenough, T., Jolicoeur, P., et al. (2008). Evidence for a pathogenic determinant in HIV-1 Nef involved in B cell dysfunction in HIV/AIDS. *Cell Host Microbe* 4, 63–76. doi: 10.1016/j.chom.2008.05.015

Tendeiro, R., Fernandes, S., Foxall, R. B., Marcelino, J. M., Taveira, N., Soares, R. S., et al. (2012). Memory B-cell depletion is a feature of HIV-2 infection even in the absence of detectable viremia. *AIDS* 26, 1607–1617. doi: 10.1097/QAD.0b013e3283568849

Thorarinsdottir, K., Camponeschi, A., Cavallini, N., Grimsholm, O., Jacobsson, L., Gjertsson, I., et al. (2016). CD21(-/low) B cells in human blood are memory cells. *Clin. Exp. Immunol.* 185, 252–262. doi: 10.1111/cei.12795

Tripathi, A. K., Misra, R., Kalra, P., Gupta, N., and Ahmad, R. (2005). Bone marrow abnormalities in HIV disease. *J. Assoc. Phys. Ind.* 53, 705–710.

van Zelm, M. C., Berkowska, M. A., van der Burg, M., and van Dongen, J. J. M. (2013). Real-time quantitative (RQ-)PCR approach to quantify the contribution of proliferation to B lymphocyte homeostasis. *Methods Mol. Biol.* 979, 133–145. doi: 10.1007/978-1-62703-290-2_11

van Zelm, M. C., Szczepanski, T., van der Burg, M., and van Dongen, J. J. M. (2007). Replication history of B lymphocytes reveals homeostatic proliferation and extensive antigen-induced B cell expansion. *J. Exp. Med.* 204, 645–655. doi: 10.1084/jem.20060964

Vanhems, P., Lambert, J., Cooper, D. A., Perrin, L., Carr, A., Hirschel, B., et al. (1998). Severity and prognosis of acute human immunodeficiency virus type 1 illness: a dose-response relationship. *Clin. Infect. Dis.* 26, 323–329. doi: 10.1086/516289

Verstegen, R. H. J., Aui, P. M., Watson, E., De Jong, S., Bartol, S. J. W., Bosco, J. J., et al. (2019). Quantification of T-cell and B-cell replication history in aging, immunodeficiency, and newborn screening. *Front. Immunol.* 10:2084. doi: 10.3389/fimmu.2019.02084

Virot, E., Duclos, A., Adelaide, L., Miailhes, P., Hot, A., Ferry, T., et al. (2017). Autoimmune diseases and HIV infection diseases and HIV infection. *Medicine* 96:4.e5769. doi: 10.1097/MD.0000000000005769

Younas, M., Psomas, C., Reynes, J., and Corbeau, P. (2016). Immune activation in the course of HIV-1 infection: causes, phenotypes and persistence under therapy. *HIV Med.* 17, 89–105. doi: 10.1111/hiv.12310

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: 21 July 2020
doi: 10.3389/fcimb.2020.00362

Per2 Upregulation in Circulating Hematopoietic Progenitor Cells During Chronic HIV Infection

Veronica Bordoni[1], Eleonora Tartaglia[1], Giulia Refolo[2], Alessandra Sacchi[1], Germana Grassi[1], Andrea Antinori[3], Gian Maria Fimia[2,4] and Chiara Agrati[1]*

[1] Laboratory of Cellular Immunology, National Institute for Infectious Diseases "L. Spallanzani" IRCCS, Rome, Italy, [2] Laboratory of Cell Biology and Electron Microscopy, National Institute for Infectious Diseases "L. Spallanzani" IRCCS, Rome, Italy, [3] Clinical Department, National Institute for Infectious Diseases "L. Spallanzani" IRCCS, Rome, Italy, [4] Department of Molecular Medicine, University of Rome "Sapienza", Rome, Italy

OPEN ACCESS

Edited by:
Santhi Gorantla,
University of Nebraska Medical Center, United States

Reviewed by:
Fatah Kashanchi,
George Mason University, United States
Agustin Valenzuela-Fernandez,
University of La Laguna, Spain
Larisa Y. Poluektova,
University of Nebraska Medical Center, United States

***Correspondence:**
Veronica Bordoni
veronica.bordoni@inmi.it

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: 15 November 2019
Accepted: 11 June 2020
Published: 21 July 2020

Citation:
Bordoni V, Tartaglia E, Refolo G, Sacchi A, Grassi G, Antinori A, Fimia GM and Agrati C (2020) Per2 Upregulation in Circulating Hematopoietic Progenitor Cells During Chronic HIV Infection. Front. Cell. Infect. Microbiol. 10:362. doi: 10.3389/fcimb.2020.00362

Chronic HIV infection accelerates immune aging and is associated with abnormal hemato-lymphopoiesis, but the relationship between HIV-induced aging and Hematopoietic Progenitor Cells (HPC) function is not well-defined. In the context of aging, it has been demonstrated using a murine model that Per2 (Period circadian clock 2) is a negative regulator of HPC survival and lineage potential. A possible involvement of Per2 modulation on hematopoietic failure during HIV infection has not yet been investigated. The aim of this study was to analyze whether Per2 is differently expressed and regulated on HPC during HIV infection, possibly providing a therapeutic target to restore lymphoid potential in the HPC compartment. To this purpose, Per2 expression in circulating HPC was compared in 69 chronic HIV infected patients under successful ART and in matched 30 uninfected healthy donors (HD). HPC aging was assessed by measuring relative telomere length (RTL), and HPC functionality was evaluated by Colony Forming Cell (CFC) assay from both *ex vivo* HIV+ patients and *in vitro* Per2 overexpressing donors. Our results showed a lower RTL in HPC and a decrease of white progenitor colonies from HIV+ patients with lower CD4 respect to those with higher CD4 T cell count (<500 respect to >500 CD4 T cell/mmc). Interestingly, we found that the frequency of Per2-expressing HPC is higher in HIV+ patients than in HD and correlated to RTL of CFC derived cells, highlighting a relationship between low proliferative rate and Per2 expression. Indeed, the *in vitro* overexpression of Per2 resulted in a significant decrease of white progenitor colonies respect to control cells. Finally, we showed that the deacetylase Sirtuin 1, a negative regulator of Per2, was downregulated in HPC from HIV+ patients, and the peripheral blood treatment with resveratrol (Sirtuin 1 inducer) determined a decrease of Per2 expressing HPC. Altogether, these results suggest that during HIV infection, Per2 is involved in the regulation of HPC expansion and differentiation and its overexpression may impair the immune reconstitution. These data support the rationale to explore the role of this regulatory mechanism during aged-associated hemato-lymphopoiesis impairment in HIV infection.

Keywords: hematopoietic progenitor cells, HIV, senescence, telomere length, period circadian clock 2, Sirtuin 1

INTRODUCTION

It is generally accepted that stem cell aging is the primary factor driving the aging of tissues characterized by high cell turnover such as the immune system (Rossi et al., 2008; Geiger et al., 2013). Indeed, hematopoiesis declines with age, resulting in a reduced production of immune cells (a process termed immune senescence) and in an increased frequency of myeloid malignancies (Beerman et al., 2010; Pietras et al., 2011). Aged hematopoietic progenitors cells (HPC) exhibit an impairment in lymphoid and erythroid lineage differentiation, while maintain or increase myeloid lineage differentiation potential (Geiger et al., 2013). This decline is thought to contribute to the evolution of immune defects, limiting overall fitness, and organismal survival during aging (Su et al., 2013). A robust indicator of the proliferative history of a cell, and how close this cell is to reaching senescence, somehow reflecting its "age," is represented by telomere length (Hodes et al., 2002). Indeed, telomeres (long nucleotide repeats at the end of the chromosomes) shorten with every cell division, and thus are markers for cellular aging, senescence, and replicative capacity.

Even if life span is getting closer to that of the general population, HIV+ patients, and mainly the older ones, present an increased prevalence of age-related comorbidities (Pathai et al., 2014; Lagathu et al., 2017). An important matter is whether aging mechanisms associated with HIV-infection are similar or not to those observed in the general population. In HIV infected subjects the immune senescence has been associated with negative immune outcomes, such as thymic involution and poor antigen responsiveness (Deeks, 2011; Lagathu et al., 2017). Moreover, the leukocytes telomere length was lower in viremic or ART-controlled HIV-infected patients than in uninfected individuals (Effros, 2000; Zanet et al., 2014; Liu et al., 2015) and was associated with poor immune recovery.

In a murine model of aging, it has been demonstrated that Per2 (circadian rhythm gene) is a negative regulator of HPC survival and lineage potential, suggesting a strict relationship between Per2 expression and hematopoiesis effectiveness (Wang J. et al., 2016). Per2 is a transcription factor that binds E-boxes and is mainly studied in the mammalian central nervous system in the context of circadian rhythms. However, E-boxes have been demonstrated to play a crucial role in lymphopoiesis (Ephrussi et al., 1985) and Wang J. et al. (2016) identified Per2 as a gene regulating HSC potential in the context of critically short telomeres. Moreover, several studies reported that Per2 is regulated at multiple layers by Sirtuin 1, a protein deacetylase that negatively regulates Per2 expression in aging process (Asher et al., 2008; Chang and Guarente, 2013; Wang R. H. et al., 2016). Moreover, Sirtuin 1 is considered a multitask molecule as it may act on co-activators, transcription factors, and signaling molecules (p53), forkhead box O1 (FOXO, and RelA/p65) (Rajendrasozhan et al., 2009) with anti-apoptotic, anti-aging, and anti-inflammatory properties (Rahman et al., 2012; Hwang et al., 2013; Mendes et al., 2017; Chadha et al., 2019). Interestingly, Kwon et al. (2008) reported that HIV transactivator Tat inhibits the Sirt1 deacetylase, resulting in increased acetylation of NF-kB and subsequently in T cell hyperactivation.

Whether Per2 induction in HPC could be involved in hematopoietic failure during HIV infection and the possible Sirtuin 1 implication in this process have not been investigated yet. The aim of this study was to analyze Per2 expression in circulating HPC during chronic HIV infection and its relationship with HIV-associated aging.

MATERIALS AND METHODS

Study Population

The study was performed on 69 peripheral blood samples from HIV+ patients and 36 from healthy individuals (HD) afferent to National Institute of Infectious Diseases (INMI) "Lazzaro Spallanzani" (Rome, Italy). Sixty-nine HIV+ patients on antiretroviral therapy with a CD4 T cell counts ranging from 130 to 2,001 cells/mm^3 (median 471 cells/mm^3), and the viral loads undetected at least from 1 year, were recruited for the purpose of this study. All patients were selected by excluding any co-morbidity as HCV, HBV, EBV, MTB co-infections, and malignancies. The HIV-seronegative donors were used as a control and were processed under the same conditions used for samples obtained from HIV-infected donors. The study was approved by the Institutional Review board of INMI "Lazzaro Spallanzani" (Approval No. 70 dated December 17, 2018). Residual blood samples obtained from CD4 and CD8 routine analysis were completely anonymized. The characteristics of the study population are presented in **Table 1**.

Peripheral Blood Mononuclear Cells Isolation and CD34+ Cells Purification

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood by density gradient centrifugation (Lympholyte-H; Cedarlane). Freshly isolated CD34+ Hematopoietic Progenitor Cells were purified from PBMC by a two-step magnetic procedure involving a lineage depletion, followed by a CD34 isolation (CD34 Diamond isolation kit, Miltenyi Biotec). For RTL quantification, CD34+Lin- cells were purified from pooled samples and verified the purity by flow cytometry (>85%).

Reagents and Flow Cytometry

The levels of *ex vivo* Per2 expression was evaluated using whole blood 24 h after draw. Two hundred microliter of whole blood was stained for 20 min at 4°C with surface antibody

TABLE 1 | Characteristics of the study population.

Parameter	HIV+ CD4>500 subset (n = 33)	HIV+ CD4<500 subset (n = 36)	HD (n = 36)
Age (year)	44 (38–49.5)	46 (38.5–50.75)	43 (39–56)
Sex (F/M)	14/19	12/24	21/15
CD4 T cell count	857 (753.5–1,175)	374 (296–430)	
CD4/CD8 T cell ratio	1.3 (0.76–1.86)	0.5 (0.33–0.86)	

All data are presented as median (IQR).

anti-CD34 APC and anti-CD45 V500 (Becton-Dickinson BD); following monoclonal staining, BD Pharm Lyse (BD Biosciences) lyse solution was used to lyse red blood cells according to standard procedures and fixed with 4% paraformaldehyde. After red cell lysing, cells were incubated with anti-PER2 antibody (ab179813 Abcam) or anti-Sirt1 Alexa Fluor 488 (ab196368 Abcam) for intracellular staining 20 min at room temperature using a permeabilizing buffer containing 0.5% saponin (PBS1X /BSA 1%/NaN3 0.1%/Saponin 0.5%) and then washed with 0.1% saponin buffer. Alexa Fluor 488-coniugated goat anti rabbit (Invitrogen) diluted in staining buffer containing 0.1% saponin was used as secondary antibody. To evaluate cell viability, we used the 7AAD staining. In some experiments 1 mL of whole blood of HIV infected patients was stimulated with different concentrations of Resveratrol (R5010- Sigma-Aldrich) and YK-3-237 (Tocris) overnight at room temperature (see section results for details). After stimulation, the frequency of HPC expressing Per2 was evaluated by flow cytometry. At least 300,000 total cell events were acquired on FACSCanto II (Becton-Dickinson BD) flow cytometer and analyzed using DIVA software.

DNA Extraction and RTL Quantification

Genomic DNA was extracted from PBMCs with Quick-gDNA miniprep Kit (Zymo Research) according to manufacturer's instruction. The relative telomere length was measured using the Absolute Human Telomere Length Quantification qPCR Assay Kit (AHTLQ; ScienCell Research Laboratories) according to manufacturer's instruction. Briefly, quantitative real-time PCR (qRT-PCR) was performed with 10 ng of total DNA, FastStart Essential DNA Green Master (Roche Life Science), and two primer set, telomere specific primer set (TEL) and single copy reference primer set (SCR). qRT-PCR reactions were always performed in duplicates. The relative telomere length was calculated using the Comparative ΔΔCq (Quantification Cycle Value) method. The value of ΔΔCq was calculated by the difference of ΔCq (TEL) and ΔCq (SCR), the relative telomere length was calculated by 2-ΔΔCq. The run was performed on Corbett Research Rotor-Gene RG-6000.

Colony-Forming Cell (CFC) Assay

PBMC from subjects were not purified according to CD34 cell expression because of a limited concentration of cells available (Redd et al., 2007). PBMC were seeded at 10^6 cells/mL into complete methylcellulose medium (MethoCult complete medium with necessary cytokines and growth factors; StemCell Technologies) following the manufacturer's instructions. Each experiment was performed in duplicate (35 mm Petri dish format) and after 14 days of incubation, erythroid colonies (BFU-E, CFU-E) and white colonies (GM-CFU, GEMM-CFU) were counted using an inverted light microscope. Colonies obtained from Methylcellulose Assay (CFC assay) of HIV and HD were selected, washed twice in PBS and pooled for real time PCR analysis of RTL.

Sirt1 Expression

Total RNA was extracted from PBMC after whole blood Resveratrol stimulation using TRIzol reagent (Thermo Fisher Scientific), and cDNA synthesis was generated from 1 μg of RNA using the reverse transcription kit (Promega) according to manufacturer's recommendations. Real time PCR reactions were performed with the Corbett Research Rotor Gene 6000 analyzer using FastStart Essential DNA Green Master (Roche Life Science) according to the manufacturer's instructions. Two microliter of cDNA was used as template and cycling parameters were 95°C for 10 min, followed by 40 cycles of 92°C 10 s, 62°C 10 s, 72°C 10s. Levels of RNAs were normalized to the GAPDH level using the equation $2^{-\Delta Ct}$. Primers Sirt1 forward: F: GCAGATTAGTAGGCGGCTTG, Sirt1 reverse: TCTGGCAT GTCCCACTATCA; GAPDH_F: GTGGAATCATATTGGAACA TGT GAPDH_R: CTCTCTGCTCCTCCTGTTCGACAG.

Lentiviral Production and Viral Infection

For lentiviral production, HEK293T cells were cotransfected with 10 μg of lentiviral vectors, 2.5 μg pVSV-G plasmid, and 7.5 μg of psPAX2 plasmid using the calcium phosphate method, as previously described (Refolo et al., 2019). Per2 lentiviral vector specific for human Per2 (pLenti-GIII-CMV-C-term-HA, LV260156) and GFP lentiviral vector used as a control of infection (pLenti-CMV-GFP-2A-Puro) were purchased from ABMGood. Lentiviral productions were tested by infecting HuH7 cells with serial dilutions and counting the number of cells resistant to puromycin selection upon 3 days. In addition, they were tested for the levels of Per2 expression upon 293T infection by western blotting by using anti-HA antibody. 1×10^6 freshly isolated PBMC were infected with 100 μl of viral suspension in RPMI medium supplemented with 4 μg/ml polybrene for 18 h and after collected for flow cytometry and CFC assay.

Statistics

Quantitative variables were compared with non-parametric Mann–Whitney or Kruskal-Wallis test. Spearman rank test was used to determine correlations. Wilcoxon test was used for matched samples. A $p < 0.05$ was considered statistically significant. Statistical analyses were performed using GraphPad Prism v7.0 (GraphPad Software, Inc.).

RESULTS

Telomere Length and Differentiation Capability of HPC From HIV-Infected Patients

To assess the relationship between aging and immune reconstitution during chronic HIV infection, we first evaluated the level of aging and functional properties of circulating Hematopoietic Progenitor Cells (HPC) in comparison to those in age-matched uninfected adult. To this purpose, the non-viremic ART-treated HIV infected patients were separated into two distinct groups according to their CD4 T-cell count, as a marker of progression state and immunological reconstitution: (i) low immunological responder (CD4 T cells <500/mmc, LowIR); (ii) immunological responder (CD4 T cells >500/mmc, IR).

To evaluate a possible association between the immune reconstitution in HIV patients and the expansion/differentiation of HPC, we performed the Colony-Forming Cell (CFC)

assay. Specifically, the generation of white [i.e., granulocytes-macrophages (CFU-GM) and granulocyte-erythroid-macrophage-megakaryocyte (CFU-GEMM)] or red (i.e., erythroid CFU and erythroid burst-forming unit) progenitor colonies was analyzed in LowIR and in IR and in HD. We showed that the capacity of circulating HPC to produce white progenitor colonies appeared to be preferentially impaired during HIV disease progression (LowIR vs. IR, $p < 0.05$, and LowIR vs. HD, $p < 0.05$, **Figure 1A** left panel) in comparison to HD and IR, while the erythroid lineage was not significantly affected (**Figure 1A** right panel).

To evaluate the impact of HPC senescence, the analysis of relative telomere length (RTL) was performed after CFC assay both in HD and in HIV subjects (**Figure 1B**). Interestingly, we found an inverse correlation between the telomere length after culture and the number of white CFC (r: −0.52, $p = 0.04$), suggesting a block in telomere shortening in patients with an impairment in white cells differentiation.

No impact of different cART regimens (NRTI, nucleoside reverse-transcriptase inhibitors; PI, protease inhibitors; NNRTI, non-nucleoside reverse-transcriptase inhibitors; INSTI, Integrase Strand Transfer Inhibitor) on CD34 frequency was observed [(NRTI+PI: median 0.031%, IQR: 0.025–0.10) vs. (NRTI+NNRTI: median 0.053%, IQR: 0.03–0.08) vs. (NRTI+INSTI: median 0.037, IQR 0.03–0.2)]. Altogether the results highlight a relationship between HPC senescence and dysfunction in ART-treated HIV-infected patients.

Increase in the Frequency of HPC Expressing Per2 During Chronic HIV Infection

To verify whether the hematopoietic failure observed in HIV infected patients may be associated to a higher Per2 expression, we analyzed the percentage of HPC expressing Per2 in chronically HIV+ patients. Interestingly, we found that the frequency of Per2-expressing HPC is higher in HIV+ patients than in HD [median lowIR = 5% (IQR 1.4–22), median IR = 5% (IQR = 2.7–11.4) vs. median HD = 3% (IQR 0.75–5.25) $p < 0.05$, **Figures 2A,B**]. No statistically differences in Per2 expression in HPC between lowIR e IR were found.

To assess a possible relationship between Per2 expression and HPC aging, we analyzed the RTL after HPC expansion and differentiation from HIV+ patients. Interestingly, we found that the frequency of Per2-expressing HPC is directly correlated to RTL of CFC derived cells, underlining a low proliferative rate in relationship with Per2 expression ($r = 0.6$, $p = 0.04$, **Figure 2C**).

Per2 Overexpression Induces a Decrease in CFC White Colonies Expansion

The above data indicated that Per2 is higher in HIV-infected subjects under successful ART. To finally demonstrate that Per2 is directly associate with the impairment in circulating HPC expansion and differentiation, we overexpressed Per2 in PBMC from HD and performed CFC assay. The lentiviral infection did not affect CD34 frequency. When PBMC were infected with Per2 expressing lentiviral vector, the percentage of CD34+ cells expressing Per2 increase over 60% (**Figure 3**). We found a significant decrease of white colonies, but not red colonies, when Per2 is overexpressed with respect to GFP infection that was used as control ($p < 0.05$, **Figure 3B**).

Sirt1 Expression on HPC and Downregulation of the Expression of Per2 in Response to Resveratrol Stimulation

To investigate how Per2 is regulated in HPC from HIV+ patients, we analyze the expression of the deacetylase Sirtuin 1 (Sirt1), a negative regulator of Per2 previously associated to aging modulation (Chang and Guarente, 2013). As showed in

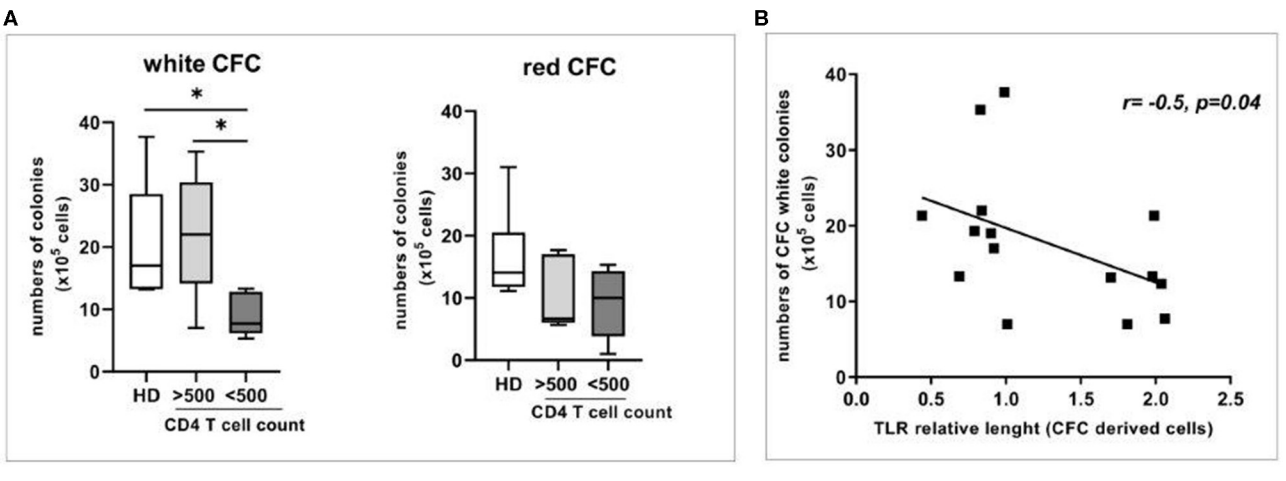


FIGURE 1 | Relative telomere length (RTL) and expansion/differentiation of circulating Hematopoietic Progenitor Cells (HPC) from HD, and HIV-infected patients (<500 and >500 CD4 T cell count). **(A)** Colony forming cell assay (CFC) showing the numbers of white and red colonies (left and right panel) generated from circulating HPC from HD ($n = 8$) and from HIV-infected patients ($n = 15$). **(B)** Correlation between relative telomere length and numbers of CFC white colonies in HD and HIV-infected subjects. Mann–Whitney test, $^*p < 0.05$.

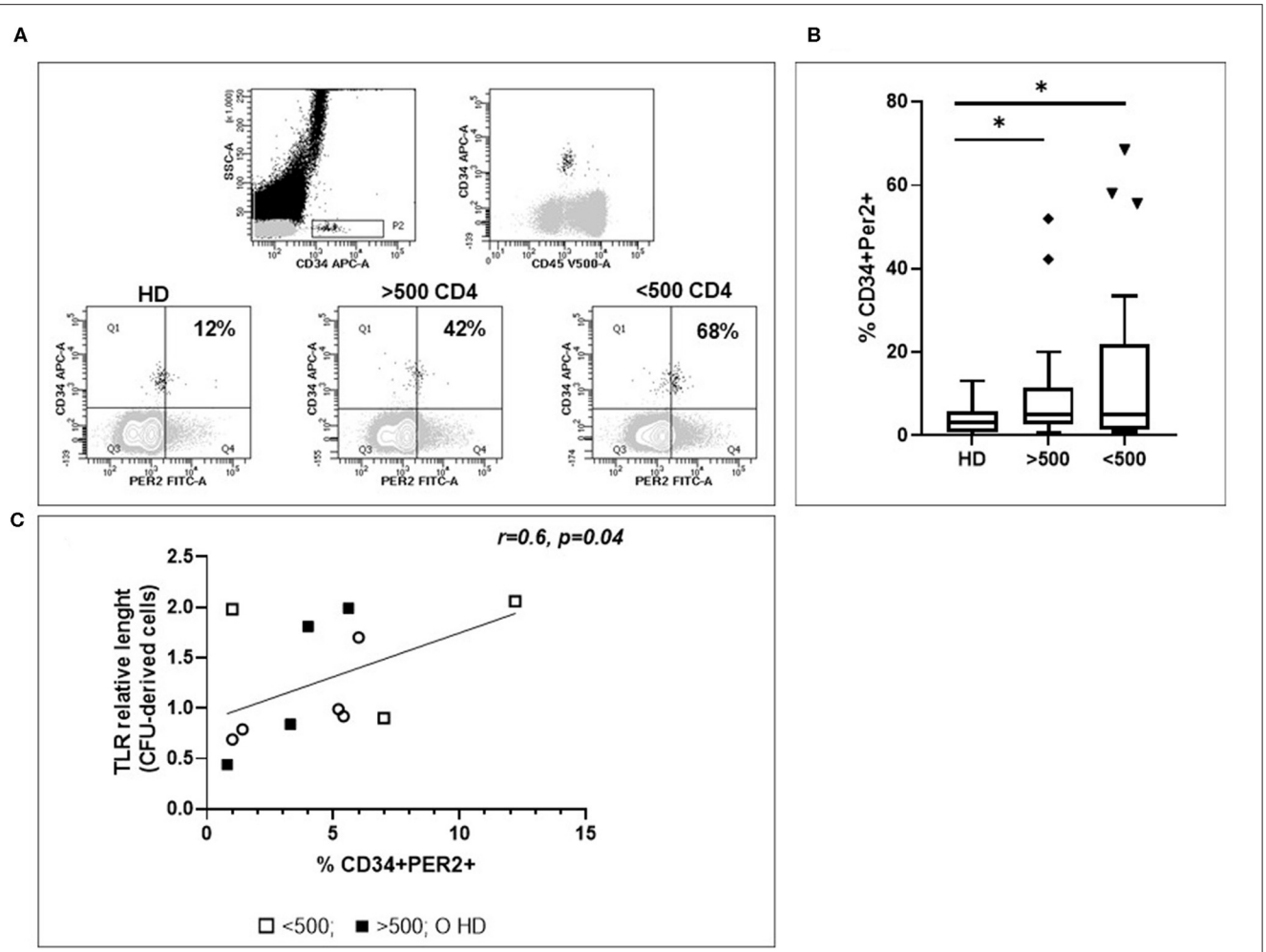


FIGURE 2 | Evaluation of Per2 expression in circulating HPC. **(A)** Representative flow cytometry contour plots of CD34+CD45+/low cells from whole blood of HIV+ patients. In the square the percentage of CD34+Per2+ cells is shown. To set Per-2 positive/negative gate, we used a mix of antibodies containing CD34–APC and the secondary antibody Alexa 488 without Per2 primary antibody. **(B)** Histograms show the median and range percentage of CD34+Per2+ cells of HD and HIV-infected patients. Mann–Whitney test, $^*p < 0.05$; HD, healthy donors ($n = 32$), HIV + patients with CD4 T cells count <500 ($n = 27$), HIV + patients with CD4 T cells count >500 ($n = 26$). **(C)** Linear regression analysis between RTL of CFC derived cells and % CD34+Per2+ cells ($r = 0.6$, $p = 0.04$).

Figure 4A, we observed a significant lower expression of Sirt1 on circulating HPC from HIV+ patients respect to HD ($p < 0.05$), suggesting a dysregulation in the Per2/Sirt1 pathway during HIV infection. To better elucidate the Per2-Sirt1 axis, we wondered whether the stimulation of Sirt1 expression could modulate the percentage of Per2-expressing HPC. To this purpose, we treated whole blood of HIV+ patients with resveratrol, a compound known to induce the expression and activity of Sirt1, and the percentage of CD34+Per2+ cells was analyzed by flow cytometry. This treatment did not affect the cell viability as measured by 7AAD staining (% 7–AAD+ cells: unstimulated 7.2% vs. Resveratrol 9.8%, $p = 0.5$). As reported in **Figures 4B,C**, we found that resveratrol induces Sirt1-mRNA in PBMC from HIV+ patients and a significant dose dependent decrease of Per2-expressing HPC ($p < 0.05$). Finally, we tested the efficacy of YK-3-237, a more specific Sirt-1 targeting drug, to reduce Per2 expression on CD34 cells [fold of decrease: YK-3-237 0.01 μM: 0.99 (0.97–1.02) vs. 1 μM:1.04 (1.02–1.07, $p < 0.03$)], confirming the involvement of the Sirt1 in the regulation of Per2 in HPC during chronic HIV infection.

DISCUSSION

In HIV infected patients, several mechanisms influencing the immune restoration has been described, such as inflammation and immune activation (Sauce et al., 2011; Bordoni et al., 2017), upregulation of apoptosis of CD34+ cells (Isgro et al., 2008), and the reduction of CD34 + CD7 + CXCR4 + T cell progenitors (Tsukamoto, 2019), but the relationship between aging and HPC in ART-treated HIV patients has not been clarified.

Wang and co-authors identifies period circadian clock 2 (Per2) as a critical factor limiting the maintenance of hematopoietic stem cells in response to DNA damage and aging in mice (Wang J. et al., 2016). The authors show that the selective

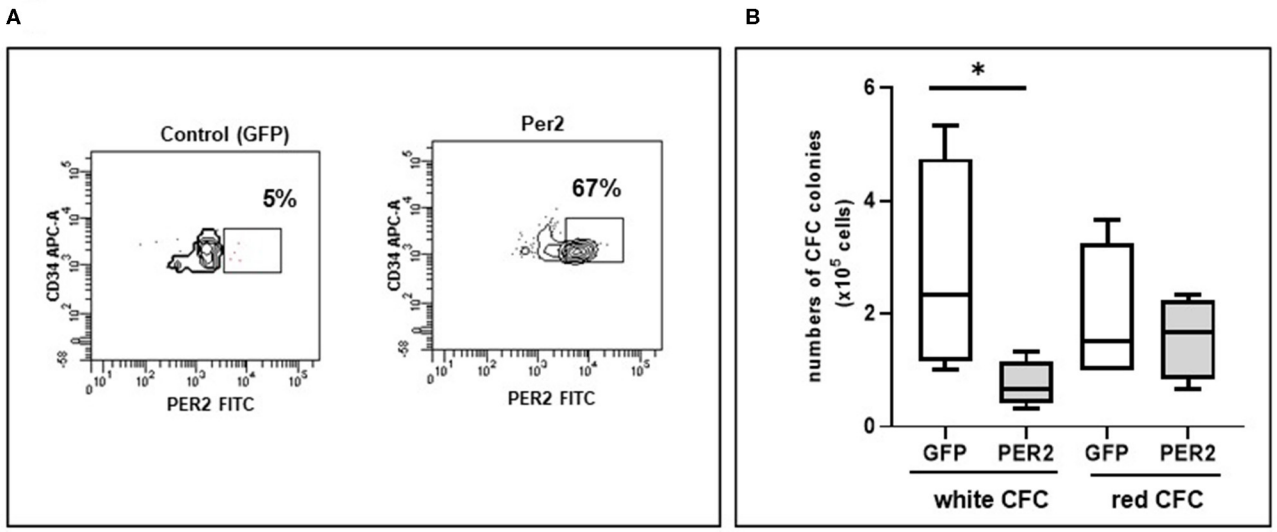


FIGURE 3 | Circulating Per2 expressing-HPC differentiation features. **(A)** Representative flow cytometry contour plots of Per2 expression in HPC after lentiviral infection: in the square is indicated the percentage of CD34+Per2+ cells obtained after infection of PBMC with control lentiviral vector (GFP, left panel) or with Per2 specific lentiviral vector (right panel). **(B)** Colony Forming Cell assay (CFC) showing the absolute value of white and red colonies generated from circulating HPC of HD infected with Per2 specific lentiviral vector or control GFP lentiviral vector. Mann-Whitney test, $^*p < 0.05$.

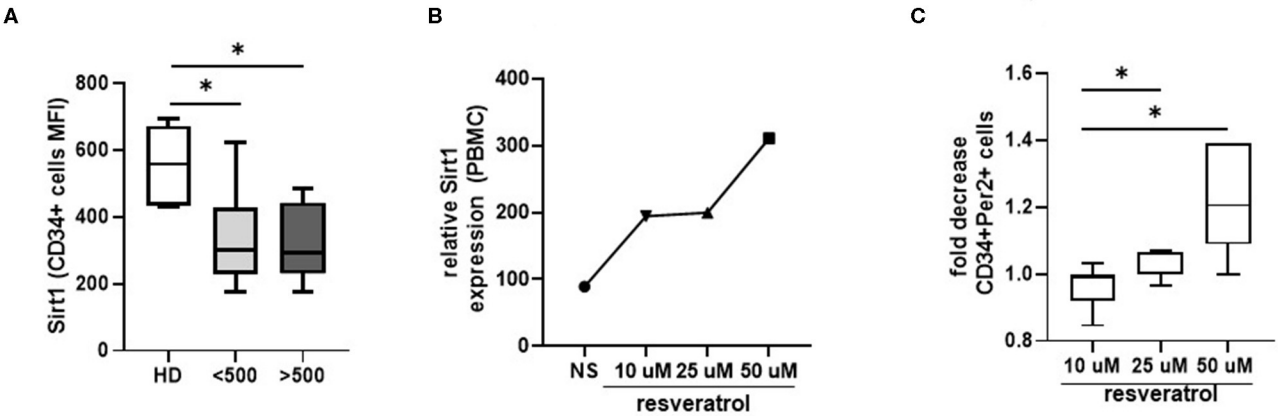


FIGURE 4 | Sirt1 modulation. **(A)** Histograms show the median and range of Sirt1 MFI in CD34+ cells from HD and HIV-infected patients. Mann–Whitney test, $^*p < 0.05$; HD, healthy donors ($n = 7$), <500, HIV+ patients with CD4 T cells count <500 ($n = 8$), >500, HIV+ patients with CD4 T cells count >500 ($n = 7$). **(B)** Relative Sirt1 expression after 24 h of whole blood stimulation with resveratrol (50, 25, and 10 μM concentration). The data refer to the mean of 2 pool (each pool consists of 4 samples). **(C)** Histograms show the median and range of the fold of decrease of CD34+Per2+ cells percentage after 24 h of whole blood stimulation with resveratrol (50, 25, and 10 μM concentration). Wilcoxon matched-pairs signed rank test, $^*p < 0.05$.

induction of Per2 in lymphoid precursors is likely to contribute to the reduction of lymphopoiesis, and in the maintenance of immune functions in aging mice. In this paper we found that Per2 is overexpressed in circulating HPC of ART treated HIV infected patients respect to healthy donors. Moreover, Per2 expression on HPC is inversely correlated to telomeres length of progenitors, indicating the low replicative potential or growth arrest when Per2 is induced.

In human hepatocytes it was reported that Per 2 expression is negatively regulated by the Sirt1 deacetylase (Wang R. H. et al., 2016). In this study, we found a lower expression of Sirtuin 1 in circulating HPC from HIV patients when compared to healthy controls, suggesting a possible relationship between the low Sirtuin expression and the high Per2 expression on HPC from HIV infected patients. Accordingly, when inducing *in vitro* Sirtuin expression by treating whole blood with resveratrol, the percentage of Per2 expressing HPC significantly decrease. These data showed for the first time the axis sirtuin1-Per2 in HPC in HIV-infected patients and highlight new interesting pathways regulating HPC potential.

A number of *in vivo* studies that utilize Sirt1$^{-/-}$ mice have demonstrated that Sirt1 positively regulates stemness in HSCs, highlighting the involvement of ROS elimination, FOXO activation, and inhibition of p53 (Matsui et al., 2012; O'Callaghan and Vassilopoulos, 2017). However, the involvement Sirt1 down regulation in HPC function during HIV infection has not been elucidated. Furthermore, some sirtuins seem to be involved in aging and cell senescence, although data from different experimental models are controversial (Lin et al., 2000; Mostoslavsky et al., 2006; Rodriguez et al., 2013).

An important role of Sirtuin family has been described in the regulation of inflammatory response (Hwang et al., 2013; Mendes et al., 2017; Chadha et al., 2019). Sirt1 has been shown to suppress NF-κb activity, the regulator of cellular inflammatory response and increase antioxidant gene expression that suppressed inflammation (Mendes et al., 2017). During senescence, the level of Sirt1 mRNA is finely regulated by HuR (Hu antigen R) and Checkpoint kinase (Chk). Decreased levels of HuR and increased levels of CHk2 synergize to decrease Sirt1 mRNA and protein levels during senescence (Kwon and Ott, 2008). Therefore, it is possible that the mechanism of senescence triggered by immune-activation and inflammation lead to a decrease of Sirt1 protein levels.

Finally, Sirt1 can be also inhibited by HIV–Tat protein resulting in increased acetylation of the NF-kB p65 subunit and subsequently in T cell hyperactivation (Kwon et al., 2008). Although this mechanisms can play a role in viremic phase of HIV infection, the patients enrolled in this study were under successful ART (undetectable virus in the circulating blood). Therefore, is unlikely that Sirt1 activity in HPC was inhibited by HIV–Tat protein. Nevertheless, the effect of *in vitro* HIV−1 infection (X4- and R5-tropic strains) on Sirt-1 expression and function could be useful to depict the role of viral components on Sirt-1/Per2 axis.

Our results showed an altered differentiation capacity of circulating HPC from ART-treated HIV infected patients, in line with previous observations obtained in bone marrow samples from viremic HIV subjects (Moses et al., 1998; Sauce et al., 2011). The HPC impairment was associated to a block in telomere shortening after long term culture, suggesting a relationship between HPC senescence and functional impairment in HIV disease. Of note, we showed that the overexpression of Per2 may modulate HPC expansion and differentiation, as confirmed by *in vitro* overexpression. Further analysis are mandatory to understand the factors driving Sirt-1/Per2 dysregulation observed in HIV infected patients and their possible impact on lymphopoiesis and immune reconstitution efficacy. Moreover, we have to take into account a possible involvement of different drugs of ART regimens on Per2 expression. In fact, it was reported that the ART regimens containing the nucleoside analog reverse transcriptase inhibitors can alter telomerase reverse transcriptase activity, resulting in telomere length shortening in leukocytes (Hukezalie et al., 2012; Leeansyah et al., 2013; Lagathu et al., 2017).

Altogether, these results suggest that in viral suppressed HIV-infected patients the axis Sirt1/Per2 is unbalanced: Per2 increase in HPC and its overexpression may contribute to the impairment in immune reconstitution and accelerate the aging process.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was approved by the Institutional Review board of INMI Lazzaro Spallanzani (Approval No. 70 dated December 17, 2018). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

VB conceived the study, performed the experiments, analyzed the data, and prepared the manuscript. AA was charge of patients. ET performed the experiments and analyzed the data. AS analyzed the data and provided comments to the manuscript. GR performed the lentiviral infection experiments. GG performed flow cytometry experiments. CA and GF analyzed the data and prepared the manuscript. All authors read, reviewed, and approved the final manuscript.

FUNDING

This work was supported by grants from Italian Ministry of Health (Ricerca Corrente) to INMI L. Spallanzani, IRCCS.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the patients who made this study possible.

REFERENCES

Asher, G., Gatfield, D., Stratmann, M., Reinke, H., Dibner, C., Kreppel, F., et al. (2008). SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell* 134, 317–328. doi: 10.1016/j.cell.2008.06.050

Beerman, I., Maloney, W. J., Weissmann, I. L., and Rossi, D. J. (2010). Stem cells and the aging hematopoietic system. *Curr. Opin. Immunol.* 22, 500–506. doi: 10.1016/j.coi.2010.06.007

Bordoni, V., Bibas, M., Viola, D., Sacchi, A., Cimini, E., Tumino, N., et al. (2017). Bone marrow CD34(+) progenitor cells from HIV-infected patients show an impaired T cell differentiation potential related to proinflammatory cytokines. *AIDS Res. Hum. Retroviruses* 33, 590–596. doi: 10.1089/aid.2016.0195

Chadha, S., Wang, L., Hancock, W. W., and Beier, U. H. (2019). Sirtuin-1 in immunotherapy: a Janus-headed target. *J. Leukoc. Biol.* 106, 337–343. doi: 10.1002/JLB.2RU1118-422R

Chang, H. C., and Guarente, L. (2013). SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. *Cell* 153, 1448–1460. doi: 10.1016/j.cell.2013.05.027

Deeks, S. G. (2011). HIV infection, inflammation, immunosenescence, and aging. *Annu Rev Med*. 62, 141–155. doi: 10.1146/annurev-med-042909-093756

Effros, R. B. (2000). Telomeres and HIV disease. *Microbes Infect*. 2, 69–76. doi: 10.1016/S1286-4579(00)00283-5

Ephrussi, A., Church, G. M., Tonegawa, S., and Gilbert, W. (1985). B lineage–specific interactions of an immunoglobulin enhancer with cellular factors *in vivo*. *Science* 227, 134–140. doi: 10.1126/science.3917574

Geiger, H., de Haan, G., and Florian, M. C. (2013). The ageing haematopoietic stem cell compartment. *Nat. Rev. Immunol*. 13, 376–389. doi: 10.1038/nri3433

Hodes, R. J., Hathcock, K. S., and Weng, N. P. (2002). Telomeres in T and B cells. *Nat. Rev. Immunol*. 2, 699–706. doi: 10.1038/nri890

Hukezalie, K. R., Thumati, N. R., Cote, H. C., and Wong, J. M. (2012). *In vitro* and *ex vivo* inhibition of human telomerase by anti-HIV nucleoside reverse transcriptase inhibitors (NRTIs) but not by non-NRTIs. *PLoS ONE* 7:e47505. doi: 10.1371/journal.pone.0047505

Hwang, J. W., Yao, H., Caito, S., Sundar, I. K., and Rahman, I. (2013). Redox regulation of SIRT1 in inflammation and cellular senescence. *Free Radic. Biol. Med*. 61, 95–110. doi: 10.1016/j.freeradbiomed.2013.03.015

Isgro, A., Leti, W., De Santis, W., Marziali, M., Esposito, A., Fimiani, C., et al. (2008). Altered clonogenic capability and stromal cell function characterize bone marrow of HIV-infected subjects with low CD4+ T cell counts despite viral suppression during HAART. *Clin. Infect. Dis*. 46, 1902–1910. doi: 10.1086/588480

Kwon, H. S., Brent, M. M., Getachew, R., Jayakumar, P., Chen, L. F., Schnolzer, M., et al. (2008). Human immunodeficiency virus type 1 Tat protein inhibits the SIRT1 deacetylase and induces T cell hyperactivation. *Cell Host Microbe* 3, 158–167. doi: 10.1016/j.chom.2008.02.002

Kwon, H. S., and Ott, M. (2008). The ups and downs of SIRT1. *Trends Biochem. Sci*. 33, 517–525. doi: 10.1016/j.tibs.2008.08.001

Lagathu, C., Cossarizza, A., Bereziat, V., Nasi, M., Capeau, J., and Pinti, M. (2017). Basic science and pathogenesis of ageing with HIV: potential mechanisms and biomarkers. *AIDS* 31(Suppl. 2), S105–S119. doi: 10.1097/QAD.0000000000001441

Leeansyah, E., Cameron, P. U., Solomon, A., Tennakoon, S., Velayudham, P., Gouillou, M., et al. (2013). Inhibition of telomerase activity by human immunodeficiency virus (HIV) nucleos(t)ide reverse transcriptase inhibitors: a potential factor contributing to HIV-associated accelerated aging. *J. Infect. Dis*. 207, 1157–1165. doi: 10.1093/infdis/jit006

Lin, S. J., Defossez, P. A., and Guarente, L. (2000). Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* 289, 2126–2128. doi: 10.1126/science.289.5487.2126

Liu, J. C., Leung, J. M., Ngan, D. A., Nashta, N. F., Guillemi, S., Harris, M., et al. (2015). Absolute leukocyte telomere length in HIV-infected and uninfected individuals: evidence of accelerated cell senescence in HIV-associated chronic obstructive pulmonary disease. *PLoS ONE* 10:e0124426. doi: 10.1371/journal.pone.0124426

Matsui, K., Ezoe, S., Oritani, K., Shibata, M., Tokunaga, M., Fujita, N., et al. (2012). NAD-dependent histone deacetylase, SIRT1, plays essential roles in the maintenance of hematopoietic stem cells. *Biochem. Biophys. Res. Commun*. 418, 811–817. doi: 10.1016/j.bbrc.2012.01.109

Mendes, K. L., Lelis, D. F., and Santos, S. H. S. (2017). Nuclear sirtuins and inflammatory signaling pathways. *Cytokine Growth Factor Rev*. 38, 98–105. doi: 10.1016/j.cytogfr.2017.11.001

Moses, A., Nelson, J., and Bagby, G. C. Jr. (1998). The influence of human immunodeficiency virus-1 on hematopoiesis. *Blood* 91, 1479–1495. doi: 10.1182/blood.V91.5.1479

Mostoslavsky, R., Chua, K. F., Lombard, D. B., Pang, W. W., Fischer, M. R., Gellon, L., et al. (2006). Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* 124, 315–329. doi: 10.1016/j.cell.2005.11.044

O'Callaghan, C., and Vassilopoulos, A. (2017). Sirtuins at the crossroads of stemness, aging, and cancer. *Aging Cell* 16, 1208–1218. doi: 10.1111/acel.12685

Pathai, S., Bajillan, H., Landay, A. L., and High, K. P. (2014). Is HIV a model of accelerated or accentuated aging? *J. Gerontol. A Biol. Sci. Med. Sci*. 69, 833–842. doi: 10.1093/gerona/glt168

Pietras, E. M., Warr, M. R., and Passegue, E. (2011). Cell cycle regulation in hematopoietic stem cells. *J. Cell Biol*. 195, 709–720. doi: 10.1083/jcb.201102131

Rahman, I., Kinnula, V. L., Gorbunova, V., and Yao, H. (2012). SIRT1 as a therapeutic target in inflammaging of the pulmonary disease. *Prev. Med*. 54(Suppl.), S20–S28. doi: 10.1016/j.ypmed.2011.11.014

Rajendrasozhan, S., Yao, H., and Rahman, I. (2009). Current perspectives on role of chromatin modifications and deacetylases in lung inflammation in COPD. *COPD* 6, 291–297. doi: 10.1080/15412550903049132

Redd, A. D., Avalos, A., and Essex, M. (2007). Infection of hematopoietic progenitor cells by HIV-1 subtype C, and its association with anemia in southern Africa. *Blood* 110, 3143–3149. doi: 10.1182/blood-2007-04-086314

Refolo, G., Ciccosanti, F., Di Rienzo, M., Basulto Perdomo, A., Romani, M., Alonzi, T., et al. (2019). Negative regulation of mitochondrial antiviral signaling protein-mediated antiviral signaling by the mitochondrial protein LRPPRC during hepatitis C virus infection. *Hepatology* 69, 34–50. doi: 10.1002/hep.30149

Rodriguez, R. M., Fernandez, A. F., and Fraga, M. F. (2013). Role of sirtuins in stem cell differentiation. *Genes Cancer* 4, 105–111. doi: 10.1177/1947601913479798

Rossi, D. J., Jamieson, C. H., and Weissman, I. L. (2008). Stems cells and the pathways to aging and cancer. *Cell* 132, 681–696. doi: 10.1016/j.cell.2008.01.036

Sauce, D., Larsen, M., Fastenackels, S., Pauchard, M., Ait-Mohand, H., Schneider, L., et al. (2011). HIV disease progression despite suppression of viral replication is associated with exhaustion of lymphopoiesis. *Blood* 117, 5142–5151. doi: 10.1182/blood-2011-01-331306

Su, D. M., Aw, D., and Palmer, D. B. (2013). Immunosenescence: a product of the environment? *Curr. Opin. Immunol*. 25, 498–503. doi: 10.1016/j.coi.2013.05.018

Tsukamoto, T. (2019). HIV impacts CD34(+) progenitors involved in T-cell differentiation during coculture with mouse stromal OP9-DL1 cells. *Front. Immunol*. 10:81. doi: 10.3389/fimmu.2019.00081

Wang, J., Morita, Y., Han, B., Niemann, S., Loffler, B., and Rudolph, K. L. (2016). Per2 induction limits lymphoid-biased haematopoietic stem cells and lymphopoiesis in the context of DNA damage and ageing. *Nat. Cell. Biol*. 18, 480–490. doi: 10.1038/ncb3342

Wang, R. H., Zhao, T., Cui, K., Hu, G., Chen, Q., Chen, W., et al. (2016). Negative reciprocal regulation between Sirt1 and Per2 modulates the circadian clock and aging. *Sci. Rep*. 6:28633. doi: 10.1038/srep28633

Zanet, D. L., Thorne, A., Singer, J., Maan, E. J., Sattha, B., Le Campion, A., et al. (2014). Association between short leukocyte telomere length and HIV infection in a cohort study: No evidence of a relationship with antiretroviral therapy. *Clin. Infect. Dis*. 58, 1322–1332. doi: 10.1093/cid/ciu051

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

BRIEF RESEARCH REPORT
published: 24 September 2020
doi: 10.3389/fcimb.2020.546189

The CD133$^+$ Stem/Progenitor-Like Cell Subset Is Increased in Human Milk and Peripheral Blood of HIV-Positive Women

Jacqueline María Valverde-Villegas[1], Mar Naranjo-Gomez[1,2], Mélusine Durand[1], David Rutagwera[3], Anne-Sophie Bedin[1], Chipepo Kankasa[3], Ségolène Debiesse[1], Nicolas Nagot[1,4], Edouard Tuaillon[1,4], Philippe Van de Perre[1,4] and Jean-Pierre Molès[1]*

[1] Pathogenesis and Control of Chronic Infections (PCCI), INSERM, University of Montpellier, Établissement Français du Sang, Montpellier, France, [2] IRMB, University of Montpellier, INSERM, CHU Montpellier, Montpellier, France, [3] Department of Paediatrics and Child Health, University Teaching Hospital, School of Medicine University of Zambia, Lusaka, Zambia, [4] CHU Montpellier, Department of Bacteriology-Virology and Department of Medical Information, Montpellier, France

OPEN ACCESS

Edited by:
Tetsuo Tsukamoto, Kindai University, Japan

Reviewed by:
Prasad S. Koka, Research and Testing, India
Veronica Bordoni, Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani, (IRCCS), Italy

***Correspondence:**
Jacqueline María Valverde-Villegas
jacquelin0203@gmail.com

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *27 March 2020*
Accepted: *20 August 2020*
Published: *24 September 2020*

Citation:
Valverde-Villegas JM, Naranjo-Gomez M, Durand M, Rutagwera D, Bedin A-S, Kankasa C, Debiesse S, Nagot N, Tuaillon E, Van de Perre P and Molès J-P (2020) The CD133$^+$ Stem/Progenitor-Like Cell Subset Is Increased in Human Milk and Peripheral Blood of HIV-Positive Women. Front. Cell. Infect. Microbiol. 10:546189. doi: 10.3389/fcimb.2020.546189

Human milk is a significant source of different CD133$^+$ and/or CD34$^+$ stem/progenitor-like cell subsets in healthy women but their cell distribution and percentages in this compartment of HIV-positive women have not been explored. To date, a decrease of CD34$^+$ hematopoietic stem and progenitor cell frequencies in peripheral blood and bone marrow of HIV-positive patients has been reported. Herein, human milk and peripheral blood samples were collected between day 2–15 post-partum from HIV-positive and HIV-negative women, and cells were stained with stem cell markers and analyzed by flow cytometry. We report that the median percentage of CD45$^{+/high}$CD34$^-$CD133$^+$ cell subset from milk and blood was significantly higher in HIV-positive than in HIV-negative women. The percentage of CD45^{dim}CD34$^-$CD133$^+$ cell subset from blood was significantly higher in HIV-positive than HIV-negative women. Moreover, percentages of CD45^{dim}CD34$^+$, CD45^{dim}CD34$^+$CD133$^-$, and CD45^{+high}CD34$^+$CD133$^-$ cell subsets from blood were significantly lower in HIV-positive than HIV-negative women. The CD133$^+$ stem/progenitor-like cell subsets are increased in early human milk and blood of HIV-positive women and are differentially distributed to CD34$^+$ cell subset frequencies which are decreased in blood.

Keywords: CD133, CD34, CD45, HIV-1, human milk

INTRODUCTION

The percentage of activated CD4$^+$ T cells productively infected by HIV-1 in the peripheral blood of asymptomatic individuals is relatively low (Simmonds et al., 1990). Thereby, the susceptibility to HIV infection and AIDS progression cannot be explained only by the consequence of a direct perturbation on mature activated immunological cells, but through non-immunologic cells including stem and progenitor cells (Re et al., 1994).

Different phenotypes of stem/progenitor cells have been described using the CD34 marker in different compartments. More recently, CD133 has taken considerable importance because it allows characterization of different stem/progenitor cell subsets when used alone or in combination

with CD34, and because it describes more precisely different cell subsets which vary between organs (Handgretinger and Kuçi, 2013). For example, human cord blood (CB) or bone marrow (BM)-derived $CD133^+$ cells have characteristics of primitive hematopoietic cells (Handgretinger and Kuçi, 2013), $CD34^+CD133^+$ cells of BM were enriched in primitive and myeloid progenitor cells, whereas $CD34^+CD133^-$ cells from $CD45^+$ population contained B cells and late erythroid progenitors and $CD34^-CD133^+$ cells could commit to T, B, and natural killer cells lineage (Bühring et al., 1999).

In the HIV infection context, $CD34^+$ hematopoietic and progenitor cells (HSPCs) were intensely investigated. Overall, $CD34^+$ stem/progenitor cells are susceptible to HIV infection, an infection that impairs the differentiation and proliferation capacities of these cells (Zauli et al., 1992a,b). As a consequence, their numbers were decreased in BM and peripheral blood (PB) of HIV-positive individuals compared to HIV-negative donors (Zauli et al., 1992a,b). It was suggested that the impairment could contribute to the HIV-1/AIDS outcome by inhibiting the production of mature blood cells or BM-accessory cell populations (macrophages, fibroblasts and T cells) (Zauli et al., 1992a; Davis and Zauli, 1995; Guo et al., 2016; Bordoni et al., 2017). Subsequent studies observed that CCR5 and CXCR4, the main receptors used by HIV to infect $CD4^+$ T cells, are also expressed at the surface of $CD34^+$ HSPCs (Carter et al., 2011). Of note, CXCR4 was more widely expressed on multipotent $CD34^+$ cells than CCR5, and its sole expression renders these cells permissive to HIV-1 infection when the sole expression of CCR5 could not (Carter et al., 2011). Furthermore, $CD34^+$ cells, as well as $CD133^+$ cells from BM and PB, harbored latent HIV provirus, and some authors have suggested that these cells could be potential HIV reservoirs (McNamara et al., 2013; Sebastian et al., 2017). However, there are very few studies unveiling stem/progenitor cell phenotypes and their cell frequencies assessed by $CD133^+$ stem cell marker upon HIV infection. It was reported that the percentage of $CD133^+$ cells from peripheral blood characterized as endothelial progenitor cells (EPCs) was increased in HIV-positive individuals with suppressed VL when compared to healthy controls (Papasavvas et al., 2012; Vecchiet et al., 2013).

In human milk (HM), stem/progenitor cells have been previously identified (Hassiotou et al., 2012; Indumathi et al., 2013). We recently identified the presence of a large and heterogeneous proportion of $CD133^+$ and/or $CD34^+$ stem/progenitor-like cell phenotypes from the mononuclear cell population in HM from healthy women (Valverde-Villegas et al., 2019). Also, we reported that some $CD133^+$ cell subset phenotypes were far more frequent in HM than in PB. The reasons for having such a high number of stem cells in HM are unclear and it cannot be overlooked since it was hypothesized that these cells are transferred to the offspring during breastfeeding and are likely to integrate into different organs and have an active role in the development of the neonate (Molès et al., 2017, 2018). Furthermore, HIV-infected women shed both cell-associated HIV and cell-free HIV in HM which can be responsible for HIV-1 mother-to-child transmission during the breastfeeding period (Van de Perre et al., 2012; Rutagwera et al., 2019).

Taken together, our aims are to compare the distributions and percentages of $CD133^+$ and/or $CD34^+$ stem/progenitor-like cell subsets from HM in HIV-positive women to HIV-negative women and to its counterpart peripheral blood.

METHODS

Design, Population, and Sample Preparation

The design of this study was a prospective observational cross-sectional study. Twenty-four HIV-infected mothers and 10 healthy mothers were recruited during pregnancy or after delivery at Mother and Newborn Hospital of the University Teaching Hospitals in Lusaka, Zambia. All participants fulfilling the inclusion criteria (aged of 18-year or more and do not suffer from serious medical events) signed an informed consent form before enrolment. Samples were collected for a consecutive period of 3 months in 2014. This study was approved by the ERES Converge institutional review board (00005948 IRB number, Lusaka) and data collection, as well as samples and, all experiments followed ethical procedures according to the Declaration of Helsinki.

Approximately, between 8–50 mL of HM from each breast and 10mL of PB samples were collected between day 2 to day 15 post-partum. Samples were centrifuged at 1,200 g for 15 min at 4°C within the next 4 h after collection to separate lactoserum and cell pellets of HM. Plasma was isolated after centrifugation of peripheral blood. Peripheral blood mononuclear cells (PBMCs) from PB were recovered from the Ficoll-plasma interface, washed three times in PBS/2% Fetal Bovine Serum. Samples were immediately cryopreserved and stored at −80°C until further laboratory analysis.

Flow Cytometry Analyses

Cell pellets from each breast milk were thawed and pooled. Cells were stained using CD34-FITC, CD133-PE, CD45-APC Alexa-fluor700, CD38-PerCP-Cy5.5, and, a cocktail of lineage-committed antibodies conjugated with APC: CD4, CD8, CD11c, CD14, CD16, CD19, CD20 along with Live/Dead™ Fixable Near-IR viability marker. Fluorescence Minus One (FMO) negative controls for CD34 and CD133 expression were included in each experiment. The cellular acquisition was performed on LSRFortessa™ cell analyzer (Becton Dickinson) and analyses were performed using FlowJo® software v10.0 (FlowJo LLC, Ashland, USA). Of note, we used the CD34-FITC, clone 581, which is recommended by the International Society of Hematotherapy and Graft Engineering (ISHAGE) for $CD34^+$ cell quantification by flow cytometry (Sutherland et al., 1996), as this clone recognizes a class type III epitope and avidly binds to all glycoforms of CD34. Stem/progenitor-like cells were identified through CD34, CD133, and CD45. Details of fluorochrome-conjugated antibodies as well as

the gating strategy were previously described (Valverde-Villegas et al., 2019 and see **Supplementary Figure 1**). Also, progenitors using lineage negative and CD38 were identified as $CD34^+CD38^-Lin^-$ and $CD34^+CD38^+Lin^-$ as well as $CD34^+CD133^+Lin^-$ and $CD34^+CD133^+Lin^-$, each one in the $CD45^{dim}$ and $CD45^{+/high}$ populations.

HIV Viral Load Quantification

Viral RNAs from 560 μL of lactoserum or 200 μL of plasma were manually extracted with QIAmp® Viral RNA Mini Kit (Qiagen®, Hilden, Germany), following the manufacturer's instructions. An internal extraction control was added to the lysis buffer prior to extraction. RNAs from five HIV standards (3–7 log_{10} copies/mL), from one positive and one negative controls, were also extracted in the same batch. The qRT-PCR was performed using Generic HIV Charge Virale kit (Biocentric®, Bandol, France) following manufacturer's instructions. Amplification reaction was done using LightCycler® 480 (Roche, Indianapolis, USA) and quantification against the standard curve. The viral load in plasma and lactoserum samples were qualified as undetectable when it was below 1,000 copies/mL. Mean values of the viral load from right and left breast samples were used for analyses.

Subclinical Mastitis (SCM): Ratio [Na^+/K^+] Quantification

Na^+ and K^+ lactoserum concentrations were measured by Compact K^+ and Na^+ meters according to manufacturer's recommendations (Horiba Ltd., Kyoto, Japan). Before each measurement, the calibration was done with low (150 ppm) and high (2,000 ppm) standards. The [Na^+/K^+] ratio >1 was indicative of SCM as suggested in a previous study (Tuaillon et al., 2017).

Cytokines/Chemokines Measurement

Seven cytokines/chemokines, namely IL-1β, IL6, IL8, TNF-α, CXCL10, CXCL12, and IFN-γ were measured independently in lactoserum and plasma using ELISA method according to manufacturer's recommendations (PeproTech, Stockholm, Sweden). A Multiskan™ FC microplate photometer (Thermo Fisher Scientific, Vantaa, Finland) was used to measure absorbance according to manufacturer's recommendations. Mean values of cytokines/chemokines levels from the right and left breast samples were used for analyses.

Statistical Analyses

Quantitative variables were reported as median and interquartile range (IQR). Group comparisons were performed using Mann Whitney *U*-test for non-Gaussian variables or Student's *t*-test for Gaussian variables. Percentages of $CD133^+$ and $CD34^+$ cell subsets of HM and its PB counterpart samples were compared between HIV-positive and HIV-negative women. Sub-analyses considering viral load were done for the HIV-positive women. In addition, correlation analyses regarding percentages of cell subsets and clinical data such as SCM, $CD4^+$ T cell counts, white blood cells (WBCs), body mass index (BMI), and cytokines/chemokines measurements from mothers regarding were performed by Spearman's non-parametric correlation tests. The significance level was set at $p < 0.05$. Analyses were done using SPSS V20.0 (IBM Corp., 2011) and graphs were plotted using the GraphPad Prism 5.01 software (GraphPad Software Inc., San Diego, USA). The proportions represented in the pie chart figures was performed over the sum of median values for $CD34^+$ and $CD133^+$ cell subsets from mononuclear cells ($CD45^-CD34^+$, $CD45^{dim}CD34^+$, $CD45^{+/high}CD34^+$ and $CD45^-CD133^+$, $CD45^{dim}CD133^+$, $CD45^{+/high}CD133^+$). Of note, percentages of progenitor cells characterized with lineage negative (Lin^-) and/or CD38 expression were excluded from analyses because were lower and undetectable in some samples, in both HIV-positive and HIV-negative women groups.

RESULTS

Characteristics of Study Participants

Twenty-four HIV-positive participants were included in this study; 16/24 (67%) had a detectable viral load in lactoserum (right or left breast) or plasma, 11/19 (58%) received antiretroviral treatment (ART) with Atripla, and 13/24 (54%) presented subclinical mastitis (SCM). Amongst the 10 HIV-negative participants, 5/10 (50%) presented a unilateral or bilateral SCM. Demographic and clinical data of participants are shown in **Table 1**. There were no significant differences when HIV-negative and HIV-positive mothers were compared, except for $CD4^+$ T cells counts at sampling (**Table 1**).

$CD133^+$ Stem/Progenitor-Like Cell Percentages in HM and PB of HIV-Positive Women

Distribution of $CD133^+$ cell subsets according CD45 expression from HM and PB compartments are represented in **Figure 1**. When CD133 positive expression was combined with CD34 and CD45 markers, the median percentage of $CD45^{+/high}CD34^-CD133^+$ cell subset phenotype from the mononuclear cell population of HM was significantly higher in HIV-positive women than HIV-negative women (2.59% [IQR:1.02–6.13] vs. 0.92% [IQR:0.24–2.96], $p = 0.04$; **Table 2**). Of note, the $CD45^-CD34^-CD133^+$ and $CD45^{dim}CD34^-CD133^+$ cell subsets were also increased in HIV-positive women vs. HIV-negative women (5.1% [1.9–8.4] vs. 1.5% [1.0–8.4], $p = 0.238$ and 5.2% [2.4–9.7] vs. 2.5% [1.5–6.8], $p = 0.179$, respectively), but the difference was not statistically significant (**Table 2**). In PB counterpart, the $CD45^{+/high}CD34^-CD133^+$ cell subset and $CD45^{dim}CD34^-CD133^+$ cell subset were significantly higher in HIV-positive women vs. HIV-negative women (1.56% [0.44–5.39] vs. 0.22% [0.13–0.36], $p = 0.004$ and 0.68% [0.12–2.25] vs. 0.06% [0.02–0.38], $p = 0.01$, respectively; **Table 2**). Indeed, when analyzed in the $CD45^-$ cell population, the median percentage of $CD45^-CD34^-CD133^+$ cell subset was detectable in HIV-positive women while in HIV-negative women it was undetectable in some samples (0.03% [0.01–0.6] vs. 0.0% [0.0–0.1]).

TABLE 1 | Demographic and clinical characteristics HIV-positive and HIV-negative women and their infants.

	HIV-positive (n = 24) Median(IQR)	HIV-negative (n = 10) Median(IQR)	p-value[§] HIV+ vs. HIV–
Women			
Age, years	27.0 (23.2–32.7)	23.5 (21.7–26.0)	0.07
Parity	1.0 (1.0–2.0)*	1.0 (1.0–2.2)	0.91
Body Mass Index (kg/m²)	22.6 (20.1–24.2)*	22.3 (21.5–24.2)	0.63
White cell counts (cells/mm^3)[a]	7,275 (5,185–9,480)*	8,120 (1,374–9,870)*	0.84
CD4$^+$ T cell counts (cells/mm^3)[a]	479 (357–556)*	914 (638–1,156)*	**0.004**
Hemoglobin (g/dL)[a]	11.1 (10.7–12.7)*	10.3 (8.7–11.9)*	0.59
VL plasma (log)[a,b]	4.9 (4.0–5.7)	NA	NA
VL lactoserum (log)[a,c]	4.3 (3.8–5.4)	NA	NA
Infants			
Birth weight (g)	2,900 (1,900–3,200)*	2,350 (1,825–2,460)*	0.06

IQR, interquartile range (25–75); VL, viral load; NA, not applicable. Bold values reached signficance.
[§]Student's t-test ($p < 0.05$).
[a]Counts at sampling.
[b]From 14 detectable women.
[c]From 13 detectable women.
[*]Missed information from 1 or 2 participants.

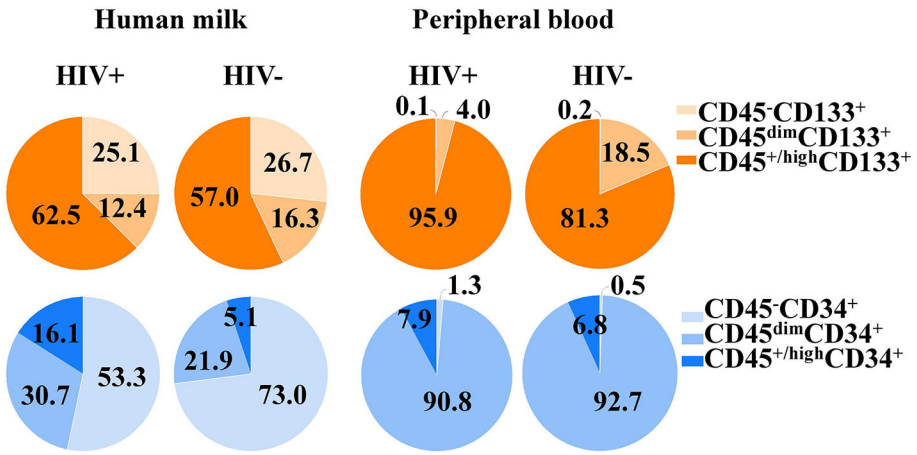


FIGURE 1 | Proportions of CD34$^+$ and CD133$^+$ cell subsets in human milk and its peripheral blood counterpart of HIV-positive and HIV-negative women. The CD34$^+$ and CD133$^+$ cell subsets and cell populations according CD45 expression from both compartments.

CD34$^+$ Stem/Progenitor-Like Cell Percentages in HM and PB of HIV-Positive Women

Distribution of CD34$^+$ cell subsets according CD45 expression from HM and PB compartments are represented in **Figure 1**. When CD34 positive expression was combined with CD45 differential expression in PB, the CD45^{dim}CD34$^+$ cell subset was predominant and the median percentage was significantly lower in HIV-positive women than HIV-negative women (0.11% [0.1–0.2] vs. 0.26% [0.1–0.4], p = 0.018). When the stem/progenitor cell markers were analyzed together, the CD45^{dim}CD34$^+$CD133$^-$ and CD45$^{+/high}$CD34$^+$CD133$^-$ cell subset phenotypes were significantly lower in PB of HIV-positive women than HIV-negative women (2.61% [1.4–4.6] vs. 11.0% [7.4–17.5], $p < 0.001$ and, 0.004% [0.002–0.02] vs. 0.03% [0.01–0.05], p = 0.01; **Table 2**). On the other hand, the median percentages of these CD34$^+$ cell subsets of HM were similar between HIV-positive and HIV-negative women (**Table 2**).

The CD133$^+$ and CD34$^-$CD133$^+$ Cell Subsets Are Increased in HM of Undetectable VL HIV-Positive Women

To investigate if the percentages of cell subsets could be associated with viremia, we stratified the HIV-positive individuals as undetectable VL or detectable VL (plasma or lactoserum, threshold at 1,000 copies/mL). In HM, CD45$^{+/high}$CD133$^+$, CD45^{dim}CD34$^-$CD133$^+$, and CD45$^{+/high}$CD34$^-$CD133$^+$ cell subsets were increased

TABLE 2 | Percentages with interquartile range of cell subsets in HM and PB in HIV-positive and HIV-negative women.

Cell subsets	HM		PB	
	HIV+ ($n = 24$)	**HIV- ($n = 9$)**	**HIV+ ($n = 18$)**	**HIV- ($n = 10$)**
$CD133^+$	1.94 (1.1–6.3)	2.96 (0.5–4.1)	1.5 (0.2–4.9)	0.24 (0.1–1.0)
$CD45^-CD133^+$	0.46 (0.1–1.1)	0.23 (0.1–2.4)	0.001 (0.0–0.02)	0.001 (0.0–0.003)
$CD45^{dim}CD133^+$	0.23 (0.1–0.4)	0.14 (0.1–0.4)	0.06 (0.03–0.1)	0.05 (0.02–0.1)
$CD45^{+/high}CD133^+$	1.16 (0.2–5.2)	0.49 (0.3–0.8)	1.37 (0.1–4.5)	0.22 (0.06–0.9)
$CD34^+$	0.09 (0.03–0.3)	0.17 (0.05–0.7)	0.16 (0.1–0.3)	0.28 (0.2–0.5)
$CD45^-CD34^+$	0.04 (0.01–0.1)	0.09 (0.04–0.6)	0.002 (0.001–0.004)	0.002 (0.0–0.004)
$CD45^{dim}CD34^+$	0.02 (0.001–0.05)	0.03 (0.001–0.1)	**0.11 (0.1–0.2)[b]**	**0.26 (0.1–0.4)[b]**
$CD45^{+/high}CD34^+$	0.01 (0.0–0.03)	0.01 (0.005–0.02)	0.01 (0.004–0.02)	0.02 (0.01–0.03)
Combined markers				
$CD45^-CD34^+CD133^+$	0.08 (0.0–0.7)	0.57 (0.003–0.8)	0.00 (0.0–0.01)	0.00 (0.0–0.04)
$CD45^-CD34^+CD133^-$	0.13 (0.02–0.5)	0.64 (0.1–1.0)	0.05 (0.02–0.1)	0.15 (0.02–0.8)
$CD45^-CD34^-CD133^+$	5.07 (1.9–8.4)	1.50 (1.0–8.4)	0.03 (0.01–0.6)	0.00 (0.0–0.1)
$CD45^{dim}CD34^+CD133^+$	0.12 (0.0–0.6)	0.17 (0.0–0.7)	1.38 (1.0–3.2)	1.07 (0.3–5.7)
$CD45^{dim}CD34^+CD133^-$	0.09 (0.0–0.3)	0.14 (0.0–0.9)	**2.61 (1.4–4.6)[c]**	**11.0 (7.4–17.5)[c]**
$CD45^{dim}CD34^-CD133^+$	5.18 (2.4–9.7)	2.50 (1.5–6.8)	**0.68 (0.1–2.2)[d]**	**0.06 (0.02–0.4)[d]**
$CD45^{+/high}CD34^+CD133^+$	0.0 (0.0–0.0)	0.004 (0.0–0.04)	0.001 (0.0–0.01)	0.004 (0.001–0.02)
$CD45^{+/high}CD34^+CD133^-$	0.01 (0.0–0.03)	0.02 (0.0–0.02)	**0.004 (0.002–0.02)[e]**	**0.03 (0.01–0.05)[e]**
$CD45^{+/high}CD34^-CD133^+$	**2.59 (1.0–6.1)[a]**	**0.92 (0.2–2.9)[a]**	**1.56 (0.4–5.4)[f]**	**0.22 (0.1–0.4)[f]**

HM, human milk; PB, peripheral blood.
[a,b,c,d,e,f] Comparisons were statistically significant different (in bold) using Mann-Whitney U non-parametric unpaired tests ($p < 0.05$).

in undetectable VL group when compared to detectable VL group, but no significant differences were observed (**Supplementary Table 1**). Also, these cell subsets from PB were increased in undetectable VL group when compared to detectable VL group (**Supplementary Table 1**).

When these viremic or non-viremic groups were compared to HIV-negative individuals in HM, the $CD45^{+/high}CD133^+$ and $CD45^{+/high}CD34^-CD133^+$ cell subsets were significantly higher in the undetectable VL group when compared to HIV-negative individuals (3.62% [0.64–8.84] vs. 0.49% [0.3–0.8], $p = 0.027$, and 3.97% [1.65–12.3] vs. 0.92% [0.24–2.96], $p = 0.015$; **Supplementary Table 1** and **Supplementary Figures 2A,B**). Of note, the $CD45^{dim}CD34^-CD133^+$ cell subset tended to be higher in undetectable VL group than HIV-negative individuals, but the difference was not statistically significant (7.48% [2.9–22.1] vs. 2.5% [1.5–6.8], $p = 0.059$).

The $CD133^+$ and $CD34^-CD133^+$ Cell Subsets Are Increased in PB of HIV-Positive Women With Detectable VL

In PB, the $CD45^{dim}CD34^-CD133^+$ and $CD45^{+/high}CD34^-CD133^+$ cell subsets were significantly increased in detectable VL group than HIV-negative individuals (0.52% [0.14–2.7] vs. 0.06% [0.02–0.38], $p = 0.02$ and 1.0% [0.45–4.0] vs. 0.22% [0.13–0.36], $p = 0.006$). Of note, these two cell subsets were also increased among the undetectable VL group when compared to HIV-negative individuals, but no significant difference was observed ($CD45^{dim}CD34^-CD133^+$: 1.0% [0.08–4.6] vs. 0.06% [0.02–0.38], $p = 0.056$, and $CD45^{+/high}CD34^-CD133^+$: 3.6% [0.34–7.74] vs. 0.22% [0.13–0.36], $p = 0.056$]; **Supplementary Table 1**). In the same way, the percentage of $CD45^{+/high}CD133^+$ cell subset was increased in the undetectable VL group when compared to HIV-negative group, without significant difference (3.1% [0.23–5.71] vs. 0.2% [0.06–0.9], $p = 0.12$; **Supplementary Table 1**).

The $CD34^+$ and $CD34^+CD133^-$ Cell Subsets Are Decreased in PB of HIV-Positive Women Independently of the HIV Viremia

In HM, no significant differences were observed when percentages of $CD34^+$ cell subsets were compared between detectable and undetectable VL positive groups. The percentage of $CD45^{dim}CD34^+CD133^-$ cell subset was lower in both HIV-positive groups than their counterpart PB HIV-positive groups (**Supplementary Table 1**). When comparisons were done against HIV-negative individuals, the $CD45^{dim}CD34^+$ cell subset from PB of detectable VL group was significantly decreased (0.11% [0.07–0.2] vs. 0.26% [0.14–0.45], $p = 0.017$). Furthermore, the $CD45^{dim}CD34^+CD133^-$ cell subset were significantly decreased in both detectable VL group when compared to HIV-negative individuals (2.61% [1.7–4.3] vs. 11.0% [7.4–17.5], $p < 0.001$) and in undetectable VL group compared to HIV-negative individuals (2.34% [1.2–6.8] vs. 11.0% [7.4–17.5], $p = 0.008$). Finally, the $CD45^{+/high}CD34^+CD133^-$ cell subset was significantly decreased in detectable VL group when compared to HIV-negative individuals (0.004%

[0.002–0.01] vs. 0.03% [0.01–0.05], $p = 0.012$). All data is shown in **Supplementary Table 1**.

SCM Does Not Affect the Percentages of Stem/Progenitor-Like Cell Subsets

To investigate if cell subset percentages in HM could be associated with SCM, we stratified women with or without SCM. HIV-negative women with SCM and HIV-negative women without SCM women showed no significant differences when median percentages of different cell subsets were compared (data not shown). However, the median percentages of $CD45^{+/high}CD133^{+}$ and $CD45^{+/high}CD34^{-}CD133^{+}$ cell subsets were significantly higher in HIV-positive women with SCM than in HIV-negative women with SCM (**Table 3**). These differences were not observed when HIV-positive women without SCM were compared to HIV-negative women without SCM (**Table 3**). The same analysis was done for HIV VL groups and no difference was observed between the groups (**Table 3**). Of note, other variables such as CD4 T cell counts, white blood cells and, body mass index were analyzed but no significant association was observed with stem/progenitor-like cell subsets.

Correlation of Cytokines and Chemokines From HM and PB and $CD133^{+}$ Cell Subsets

To investigate if inflammation can influence the frequencies of $CD133^{+}$ cell subsets, levels of TNF-α, CXCL10, CXCL12, IL-8, IL-6, IL-1β, and IFN-γ were quantified in lactoserum and plasma samples. CXCL10 and CXCL12 levels from HM were significantly increased in HIV-positive compared to HIV-negative women (148.5 [87.14–181.4] pg/mL vs. 87.90 [64.97–104.5] pg/mL, $p = 0.008$ and; 544.6 [420.6–987.8] pg/mL vs. 296.3 [276.4–365.1] pg/mL, $p < 0.001$, respectively; **Supplementary Table 2**). Furthermore, CXCL10 and IL-1β levels were increased in the HIV positive mothers with detectable VL compared to mothers with undetectable VL (162.6 [146.6–196.2] pg/mL vs. 90.89 [55.03–118.1] pg/mL, $p = 0.003$ and; 21.65 [12.57–30.63] vs. 11.24 [7.59–15.80] pg/mL, $p = 0.02$; **Supplementary Table 2**). In PB, the TNF-α, and CXCL10 levels were significantly increased in HIV-positive compared to HIV-negative women (43.08 [26.05–46.88] pg/mL vs. 22.28 [18.04–28.41] pg/mL, $p = 0.04$ and; 52.67 [15.70–67.05] pg/mL vs. 7.59 [4.80–8.55] pg/mL, $p = 0.004$, respectively; **Supplementary Table 3**). No significant correlation was observed between cytokine/chemokine levels and an increase of $CD133^{+}$ cell subsets in PB of HIV-positive women (data not shown). However, IL-8 levels were significantly correlated with $CD45^{+/high}CD133^{+}$ and $CD45^{+/high}CD34^{-}CD133^{+}$ cell subsets in HM of mothers with detectable VL (**Supplementary Figures 2C,D**).

DISCUSSION

Upon HIV-1 infection, cell distributions of immune cells but also of $CD34^{+}$ stem cells are profoundly modified in PB and BM. In this study, we reported that that the progenitor/stem-like cell subsets characterized by the expression of CD133 was increased in HM of HIV-positive women as well as in their PB counterpart.

Our observations are in line with previous works, showing an increased level of peripheral circulating endothelial progenitor cells (EPCs), characterized as $CD133^{+}KDR^{+}$ cells, in HIV-positive individuals compared to healthy controls (Papasavvas et al., 2012; Vecchiet et al., 2013), but we extend the observation to other $CD133^{+}$ cell phenotypes, less differentiated cells. It was previously observed that HIV provirus can be detected in $CD133^{+}$ HSPCs from BM of subjects on successful ART (undetectable viral load) up to 8 years, suggesting this cell population was permissive to HIV infection (McNamara et al., 2013). Furthermore, CCR5 and CXCR4, the receptors for HIV infection, are expressed at the surface $CD34^{+}CD133^{+}$ HSPCs from umbilical cord blood and that CXCR4 was necessary for the infection by HIV-1 (Carter et al., 2011). Recently, Zaikos et al. (2018) observed proviral sequences in $CD133^{+}$ and $CD34^{+}CD133^{-}$ HSPCs of BM and PB. They further demonstrated that these cells were the main sources of residual plasma virus as compared to other cells from BM and PB, suggesting that these HSPCs are putative reservoir of persistent HIV infection (Zaikos et al., 2018). Finally, the expression of chemokine receptors on these cells could prompt the infection by HIV and also the trafficking of HSPCs, which was observed in response to tissue damage or infections (Massberg et al., 2007). Thus, the high levels of $CD45^{+/high}CD133^{+}$ and $CD34^{-}CD133^{+}$ cell subsets in HM and PB of HIV-positive women reported in this study could be due by three mechanisms: (i) the $CD133^{+}$ cell subsets are not (productively) infected by HIV-1 and are likely resistant to the virus; (ii) to an active self-renewing of these cells in response to the infection itself, or (iii) by active recruitment from reservoir territories through CCR5 and CXCR4 receptors toward these compartments. By contrast, the $CD34^{+}$ cell subsets are decreased in PB of HIV-positive individuals suggesting that the mechanisms upon HIV-1 infection on $CD34^{+}$ and $CD133^{+}$ cells are different but are concurrent since both cell subsets seem to be resistant to HIV-1 infection.

Regarding progenitor cells through Lin^{-} and/or CD38, the cell subsets were excluded from the analyses because of the lower number of cells from both compartments. This observation suggests that most of the cell subsets reported in the $CD45^{dim}$ and $CD45^{+/high}$ population are lineage positive. Indeed, the $CD38^{+}Lin^{-}$ number cell subset were higher regard to $CD38^{-}Lin^{-}$ cell subset (data not shown). It was also suggested that inflammatory signals are also important for HSPCs biology, even in homeostatic conditions, thus the number of HSPCs or their proliferative condition can be influenced by inflammatory or microbial signaling, even in the absence of active infection (King and Goodell, 2011). Indeed, SCM is common in HIV-positive women during breastfeeding and pro-inflammatory chemokines/cytokines are associated with SCM in HIV-positive women (Tuaillon et al., 2017). Herein, we observed that the percentages of $CD45^{+/high}CD133^{+}$ and $CD45^{+/high}CD34^{-}CD133^{+}$ cell subsets were significantly increased only in HIV-positive women with undetectable VL in HM and in HIV-positive women with SCM when compared

TABLE 3 | Median percentages with interquartile of stem/progenitor cell subsets in HIV-positive women and HIV-negative women with SCM and non-SCM and between detectable VL and undetectable VL HIV-positive women.

Compartment	Cell subset	SCM		*p*-value	Non-SCM		*p*-value
		HIV+	HIV–		HIV+	HIV–	
		(*n* = 13)	(*n* = 4)		(*n* = 11)	(*n* = 5)	
HM	$CD45^{+/high}CD133^{+}$	1.6 (0.8–5.1)	0.4 (0.2–0.6)	**0.044**	0.6 (0.1–5.4)	0.5 (0.4–2.4)	1.000
	$CD45^{+/high}CD34^{-}CD133^{+}$	2.7 (1.5–7.3)	0.5 (0.1–1.9)	**0.032**	2.1 (0.8–5.8)	0.9 (0.5–4.1)	0.510
		(*n* = 10)	(*n* = 5)		(*n* = 8)	(*n* = 5)	
PB	$CD45^{dim}CD34^{+}$	0.09 (0.1–0.1)	0.2 (0.1–0.6)	**0.019**	0.2 (0.07–0.3)	0.3 (0.1–0.5)	0.284
	$CD45^{dim}CD34^{+}CD133^{-}$	2.3 (1.3–4.0)	10.3 (6.8–13.1)	**0.003**	3.2 (1.6–5.0)	17.2 (8.2–22.4)	**0.006**
		SCM HIV+			Non-SCM HIV+		
		Und VL	Det VL		Und VL	Det VL	
		(*n* = 4)	(*n* = 9)		(*n* = 4)	(*n* = 7)	
HM	$CD45^{+/high}CD133^{+}$	3.6 (0.9–7.7)	1.3 (0.4–3.5)	0.330	4.8 (0.4–11.8)	0.2 (0.04–4.1)	0.164
	$CD45^{+/high}CD34^{-}CD133^{+}$	3.9 (1.7–7.7)	2.7 (1.0–7.3)	0.825	7.9 (1.5–19.4)	0.9 (0.2–5.7)	0.164
		(*n* = 3)	(*n* = 7)		(*n* = 3)	(*n* = 5)	
PB	$CD45^{dim}CD34^{+}$	0.1 (0.1–0.0)	0.1 (0.04–0.1)	0.383	0.1 (0.03–0.0)	0.2 (0.1–0.4)	0.571
	$CD45^{dim}CD34^{+}CD133^{-}$	3.3 (1.4–0.0)	2.2 (1.0–3.7)	0.667	1.4 (0.8–1.4)	3.7 (2.5–4.9)	0.571

SCM, subclinical mastitis; VL, viral load; Und, undetectable VL; Det, detectable VL; HM, human milk; PB, peripheral blood. Mann-Whitney U non-parametric test was applied for unpaired comparisons. Significant $p < 0.05$ are shown in bold.

to HIV-negative women with SCM. Because this increase of $CD133^{+}$ cell subsets could be related to one or the other process, seven cytokines/chemokines were quantified. Only IL-8 levels from HM were positively correlated with an increase of $CD45^{+high}CD133^{+}$ and $CD45^{+high}CD34^{-}CD133^{+}$ cell subsets in mothers with detectable HIV VL. On the other hand, cytokines/chemokines known to regulate the proliferation of HSPC such as IL-1β, IL-6, CXCL10, IFN-γ, TNF-α (King and Goodell, 2011; Dickinson-Copeland et al., 2016; Chavakis et al., 2019) or to control HSPC migration such as CXCL12 (Liesveld et al., 2001; Sahin and Buitenhuis, 2016; Ganuza and McKinney-Freeman, 2017) did not associate with this increase.

This study has some limitations. It is an exploratory cross-sectional study with small sample size. In a previous study, we reported differences in percentages of these cell subsets between lactation stages (Valverde-Villegas et al., 2019), however, due to the low number of samples we did not stratify the analyses according to this variable. Frozen breast milk samples were used in this study and thawing procedure has been reported to damage different cells, including $CD34^{+}$ stem/progenitor cells. Finally, the full characterization of cell subsets regarding their stemness nature has not been done yet as well as their permissivity to HIV-1 infection assay.

In conclusion, in this study, we reported that the $CD133^{+}$ cell subsets from early HM and PB, specifically the $CD45^{+/high}CD133^{+}$ cell subset and $CD34^{-}CD133^{+}$ cell subsets were increased in HIV-positive women. By contrast, the $CD34^{+}$ cell subsets, specifically the $CD45^{dim}CD34^{+}$ and the $CD45^{dim/+high}CD34^{+}CD133^{-}$ cell subsets were decreased in PB of HIV-positive women. Stem cell compartments as defined by one or the other marker show opposite behaviors in HM and PB in HIV infected individuals.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ERES Converge, institutional review board, Lusaka, Zambia. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JV-V performed the experiments, provided the intellectual content and participated in the design of this work, analyzed and interpreted data, and wrote the manuscript. MN-G provided the intellectual content and analyzed and interpreted the data. MD performed some experiments and participated in analysis of data. DR enrolled participants, collected samples, recorded clinical data, and revised the manuscript. A-SB and SD performed some experiments. CK, NN, ET, and PV participated in the interpretation of data. J-PM provided intellectual content, supervised the study, participated in the design of this work, interpretation of data, and writing of the manuscript. All authors reviewed the manuscript.

FUNDING

This work was funded by Pierre Bergé endowment fund in collaboration with SIDACTION and was sponsored by the French National Agency for Research on AIDS and Viral

Hepatitis [ANRS#12341]. It was also supported by Labex EpiGenMed, an Investissements d'avenir program, ANR-10-LABX-12-01 and scholarship from the Norwegian government [Quota scheme].

ACKNOWLEDGMENTS

We acknowledge the imaging facility MRI, member of the national infrastructure France-BioImaging supported by the French National Research Agency (ANR-10-INBS-04, Investments for the future) and Marianne Peries and Roselyne Vallo for their statistic support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2020.546189/full#supplementary-material

REFERENCES

Bordoni, V., Bibas, M., Viola, D., Sacchi, A., Cimini, E., Tumino, N., et al. (2017). Bone marrow CD34$^+$ progenitor cells from HIV-infected patients show an impaired T cell differentiation potential related to proinflammatory cytokines. *AIDS Res. Hum. Retroviruses* 33, 590–596. doi: 10.1089/aid.2016.0195

Bühring, H. J., Seiffert, M., Bock, T. A., Scheding, S., Thiel, A., Scheffold, A., et al. (1999). Expression of novel surface antigens on early hematopoietic cells. *Ann. N. Y. Acad. Sci.* 872, 25–38. doi: 10.1111/j.1749-6632.1999.tb08450.x

Carter, C. C., McNamara, L. A., Onafuwa-Nuga, A., Shackleton, M., Riddell, J., Bixby, D., et al. (2011). HIV-1 utilizes the CXCR4 chemokine receptor to infect multipotent hematopoietic stem and progenitor cells. *Cell Host Microbe* 9, 223–234. doi: 10.1016/j.chom.2011.02.005

Chavakis, T., Mitroulis, I., and Hajishengallis, G. (2019). Hematopoietic progenitor cells as integrative hubs for adaptation to and fine-tuning of inflammation. *Nat. Immunol.* 20, 802–811. doi: 10.1038/s41590-019-0402-5

Davis, B. R., and Zauli, G. (1995). Effect of human immunodeficiency virus infection on haematopoiesis. *Baillieres. Clin. Haematol.* 8, 113–130. doi: 10.1016/S0950-3536(05)80234-3

Dickinson-Copeland, C. M., Wilson, N. O., Liu, M., Driss, A., Salifu, H., Adjei, A. A., et al. (2016). Correction: heme-mediated induction of CXCL10 and depletion of CD34$^+$ progenitor cells is toll-like receptor 4 dependent. *PLoS ONE* 11:e0147460. doi: 10.1371/journal.pone.0147460

Ganuza, M., and McKinney-Freeman, S. (2017). Hematopoietic stem cells under pressure. *Curr. Opin. Hematol.* 24, 314–321. doi: 10.1097/MOH.0000000000000347

Guo, X., He, S., Lv, X., Ding, H., Li, S., Kang, J., et al. (2016). The role of HIV-1 in affecting the proliferation ability of HPCs derived from BM. *J. Acquir. Immune Defic. Syndr.* 71, 467–473. doi: 10.1097/QAI.0000000000000892

Handgretinger, R., and Kuçi, S. (2013). "CD133-positive hematopoietic stem cells: from biology to medicine," in *Advances in Experimental Medicine and Biology Advances in Experimental Medicine and Biology*, ed D. Corbeil (New York, NY: Springer New York), 99–111. doi: 10.1007/978-1-4614-5894-4_7

Hassiotou, F., Beltran, A., Chetwynd, E., Stuebe, A. M., Twigger, A.-J., Metzger, P., et al. (2012). Breastmilk is a novel source of stem cells with multilineage differentiation potential. *Stem Cells* 30, 2164–2174. doi: 10.1002/stem.1188

IBM Corp. (2011). *IBM SPSS Statistics for Windows*, *Version 20.0* (Bois-Colombes).

Indumathi, S., Dhanasekaran, M., Rajkumar, J. S., and Sudarsanam, D. (2013). Exploring the stem cell and non-stem cell constituents of human breast milk. *Cytotechnology* 65, 385–393. doi: 10.1007/s10616-012-9492-8

King, K. Y., and Goodell, M. A. (2011). Inflammatory modulation of HSCs: viewing the HSC as a foundation for the immune response. *Nat. Rev. Immunol.* 11, 685–692. doi: 10.1038/nri3062

Liesveld, J. L., Rosell, K., Panoskaltsis, N., Belanger, T., Harbol, A., and Abboud, C. N. (2001). Response of human CD34$^+$ cells to CXC, CC, and CX3C chemokines: implications for cell migration and activation. *J. Hematother. Stem Cell Res.* 10, 643–655. doi: 10.1089/152581601753193850

Massberg, S., Schaerli, P., Knezevic-Maramica, I., Köllnberger, M., Tubo, N., Moseman, E. A., et al. (2007). Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. *Cell* 131, 994–1008. doi: 10.1016/j.cell.2007.09.047

McNamara, L. A., Onafuwa-Nuga, A., Sebastian, N. T., Riddell, J., Bixby, D., and Collins, K. L. (2013). CD133$^+$ hematopoietic progenitor cells harbor HIV genomes in a subset of optimally treated people with long-term viral suppression. *J. Infect. Dis.* 207, 1807–1816. doi: 10.1093/infdis/jit118

Molès, J.-P., Tuaillon, E., Kankasa, C., Bedin, A.-S., Nagot, N., Marchant, A., et al. (2017). Breastfeeding-related maternal microchimerism. *Nat. Rev. Immunol.* 17, 729–721. doi: 10.1038/nri.2017.115

Molès, J.-P., Tuaillon, E., Kankasa, C., Bedin, A.-S., Nagot, N., Marchant, A., et al. (2018). Breastmilk cell trafficking induces microchimerism-mediated immune system maturation in the infant. *Pediatr. Allergy Immunol.* 29, 133–143. doi: 10.1111/pai.12841

Papasavvas, E., Hsue, P., Reynolds, G., Pistilli, M., Hancock, A., Martin, J. N., et al. (2012). Increased CD34$^+$/KDR$^+$ cells are not associated with carotid artery intima-media thickness progression in chronic HIV-positive subjects. *Antivir. Ther.* 17, 557–563. doi: 10.3851/IMP2013

Re, M. C., Furlini, G., Zauli, G., and La Placa, M. (1994). Human immunodeficiency virus type 1 (HIV-1) and human hematopoietic progenitor cells. *Arch. Virol.* 137, 1–23. doi: 10.1007/BF01311169

Rutagwera, D. G., Molès, J.-P., Kankasa, C., Mwiya, M., Tuaillon, E., Peries, M., et al. (2019). Prevalence and determinants of HIV shedding in breast milk during continued breastfeeding among Zambian mothers not on antiretroviral treatment (ART): a cross-sectional study. *Medicine* 98:e17383. doi: 10.1097/MD.0000000000017383

Sahin, A. O., and Buitenhuis, M. (2016). Molecular mechanisms underlying adhesion and migration of hematopoietic stem cells. *Cell Adh. Migr.* 6, 39–48. doi: 10.4161/cam.18975

Sebastian, N. T., Zaikos, T. D., Terry, V., Taschuk, F., McNamara, L. A., Onafuwa-Nuga, A., et al. (2017). CD4 is expressed on a heterogeneous subset of hematopoietic progenitors, which persistently harbor CXCR4 and CCR5-tropic HIV proviral genomes *in vivo*. *PLoS Pathog.* 13:e1006509. doi: 10.1371/journal.ppat.1006509

Simmonds, P., Balfe, P., Peutherer, J. F., Ludlam, C. A., Bishop, J. O., and Brown, A. J. (1990). Human immunodeficiency virus-infected individuals contain provirus in small numbers of peripheral mononuclear cells and at low copy numbers. *J. Virol.* 64, 864–72.

Sutherland, D. R., Anderson, L., Keeney, M., Nayar, R., and Chin-Yee, I. (1996). The ISHAGE guidelines for CD34$^+$ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. *J. Hematother.* 5, 213–226. doi: 10.1089/scd.1.1996.5.213

Tuaillon, E., Viljoen, J., Dujols, P., Cambonie, G., Rubbo, P.-A., Nagot, N., et al. (2017). Subclinical mastitis occurs frequently in association with dramatic changes in inflammatory/anti-inflammatory breast milk components. *Pediatr. Res.* 81, 556–564. doi: 10.1038/pr.2016.220

Valverde-Villegas, J. M., Durand, M., Bedin, A.-S., Rutagwera, D., Kankasa, C., Tuaillon, E., et al. (2019). Large stem/progenitor-like cell subsets can also be identified in the CD45− and CD45$^+$/high populations in early human milk. *J. Hum. Lact.* 36, 303–309. doi: 10.1177/0890334419885315

Van de Perre, P., Rubbo, P.-A., Viljoen, J., Nagot, N., Tylleskar, T., Lepage, P., et al. (2012). HIV-1 reservoirs in breast milk and challenges to elimination of breast-feeding transmission of HIV-1. *Sci. Transl. Med.* 4:143sr3. doi: 10.1126/scitranslmed.3003327

Vecchiet, J., Iachininoto, M. G., Capodimonti, S., Nuzzolo, E. R., Falasca, K., Martini, M., et al. (2013). Effect of antiviral therapy on pro-angiogenic hematopoietic and endothelial progenitor cells in HIV-infected people. *Thromb. Res.* 131, 238–243. doi: 10.1016/j.thromres.2012.12.007

Zaikos, T. D., Terry, V. H., Sebastian Kettinger, N. T., Lubow, J., Painter, M. M., Virgilio, M. C., et al. (2018). Hematopoietic stem and progenitor cells are a distinct HIV reservoir that contributes to persistent viremia in suppressed patients. *Cell Rep*. 25, 3759–3773.e9. doi: 10.1016/j.celrep.2018.11.104

Zauli, G., Re, M. C., Davis, B., Sen, L., Visani, G., Gugliotta, L., et al. (1992a). Impaired *in vitro* growth of purified ($CD34^+$) hematopoietic progenitors in human immunodeficiency virus-1 seropositive thrombocytopenic individuals. *Blood* 79, 2680–2687. doi: 10.1182/blood.V79.10.2680.bloodjournal79102680

Zauli, G., Re, M. C., Visani, G., Furlini, G., Mazza, P., Vignoli, M., et al. (1992b). Evidence for a human immunodeficiency virus type 1-mediated suppression of uninfected hematopoietic ($CD34^+$) cells in AIDS patients. *J. Infect. Dis*. 166, 710–716. doi: 10.1093/infdis/166.4.710

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

REVIEW
published: 29 September 2020
doi: 10.3389/fcimb.2020.523379

Astrocytes, HIV and the Glymphatic System: A Disease of Disrupted Waste Management?

Caitlin Tice[1], *Jane McDevitt*[2] *and Dianne Langford*[1]*

[1] Department of Neuroscience, Lewis Katz School of Medicine, Philadelphia, PA, United States, [2] Department of Kinesiology, College of Public Health at Temple University, Philadelphia, PA, United States

The discovery of the glial-lymphatic or glymphatic fluid clearance pathway in the rodent brain led researchers to search for a parallel system in humans and to question the implications of this pathway in neurodegenerative diseases. Magnetic resonance imaging studies revealed that several features of the glymphatic system may be present in humans. In both rodents and humans, this pathway promotes the exchange of interstitial fluid (ISF) and cerebrospinal fluid (CSF) through the arterial perivascular spaces into the brain parenchyma. This process is facilitated in part by aquaporin-4 (AQP4) water channels located primarily on astrocytic end feet that abut cerebral endothelial cells of the blood brain barrier. Decreased expression or mislocalization of AQP4 from astrocytic end feet results in decreased interstitial flow, thereby, promoting accumulation of extracellular waste products like hyperphosphorylated Tau (pTau). Accumulation of pTau is a neuropathological hallmark in Alzheimer's disease (AD) and is accompanied by mislocalization of APQ4 from astrocyte end feet to the cell body. HIV infection shares many neuropathological characteristics with AD. Similar to AD, HIV infection of the CNS contributes to abnormal aging with altered AQP4 localization, accumulation of pTau and chronic neuroinflammation. Up to 30% of people with HIV (PWH) suffer from HIV-associated neurocognitive disorders (HAND), and changes in AQP4 may be clinically important as a contributor to cognitive disturbances. In this review, we provide an overview and discussion of the potential contributions of NeuroHIV to glymphatic system functions by focusing on astrocytes and AQP4. Although HAND encompasses a wide range of neurocognitive impairments and levels of neuroinflammation vary among and within PWH, the potential contribution of disruption in AQP4 may be clinically important in some cases. In this review we discuss implications for possible AQP4 disruption on NeuroHIV disease trajectory and how HIV may influence AQP4 function.

Keywords: NeuroHIV, glymphatic system, brain, astrocyte, neuroinflammation

OPEN ACCESS

Edited by:
Santhi Gorantla, University of Nebraska Medical Center, United States

Reviewed by:
Siddappa N. Byrareddy, University of Nebraska Omaha, United States
Kathleen Ruth Borgmann, University of North Texas Health Science Center, United States

***Correspondence:**
Dianne Langford
tdl@temple.edu

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *28 December 2019*
Accepted: *19 August 2020*
Published: *29 September 2020*

Citation:
Tice C, McDevitt J and Langford D (2020) Astrocytes, HIV and the Glymphatic System: A Disease of Disrupted Waste Management? Front. Cell. Infect. Microbiol. 10:523379. doi: 10.3389/fcimb.2020.523379

INTRODUCTION

Maintaining homeostasis within tissues is dependent on the clearance of excess fluid and interstitial solutes. In the periphery, excess fluid and soluble waste products that include abnormal proteins are removed from the interstitial space and transferred to the lymphatic system that deposits waste products into the systemic circulation for processing by the liver, kidneys, and small intestine for

excretion (Liao and Padera, 2013). The density of lymphatic vessels within a tissue is proportional to the metabolic rate of that particular tissue. Despite having a high metabolic rate, the brain is not incorporated into the lymphatic system (Jessen et al., 2015). Waste management in the central nervous system (CNS) is vital for maintaining neuronal connectivity, fitness and crosstalk with other cell types including astrocytes. Since many neurocognitive disorders include accumulation of aberrant proteins and other cellular waste products (Benveniste et al., 2017), understanding mechanisms underlying waste clearance in the brain are critical.

A brain-specific pathway for fluid transport in mice was discovered in 2012 and included the para-arterial influx of subarachnoid cerebrospinal fluid (CSF) into the brain interstitium. This pathway allowed for clearance of interstitial fluid (ISF) along large draining veins (Iliff et al., 2012). In 2015, studies in rodents provided evidence of functional lymphatic-like vessels lining the cranial dural sinuses (Aspelund et al., 2015). This finding challenged the previous notion that the CNS had no lymphatic or lymphatic-like system. In this model, CSF and ISF drain into the glial-lymphatic or glymphatic fluid clearance pathway carrying with it any waste products that have accumulated in the brain (Jessen et al., 2015; Eide et al., 2018). These findings in rodents led researchers to search for a similar pathway in humans. An MRI study in patients with various CSF disorders reported that a CSF tracer drained into cervical lymph nodes (Eide et al., 2018), providing evidence for a glymphatic system in humans. Other studies indicated that the glymphatic system is most active during sleep and plays numerous roles in many biological processes. *In vivo* 2-photon imaging studies in mice have shown that glymphatic activity is greatly increased in a sleep state compared to an awake state due to an influx of CSF that results in a 10% increase in the interstitial space (Sweeney et al., 2019). It is speculated that the increase in glymphatic activity during sleep functions to remove neurotoxic waste that builds up during waking periods (Xie et al., 2013). Moreover, glymphatic function is has been reported to be significantly decreased in experimental rodent models of hemorrhagic and ischemic stroke (Gaberel et al., 2014; Goulay et al., 2017; Schain et al., 2017; Golanov et al., 2018; Rasmussen et al., 2018; Zbesko et al., 2018) and traumatic brain injury (Plog et al., 2015; Lundgaard et al., 2017).

Glymphatic function is proposed to be reduced with aging (Kress et al., 2014), and studies report an 80% decrease in glymphatic function in older mice (18 months) compared to younger mice (2–3 months) (Kress et al., 2014). Thus, it stands to reason that aging is a major risk factor for developing a neurodegenerative disease, many of which are characterized by accumulation of aberrant proteins, such as hyperphosphorylated Tau (pTau). In this context, decreased glymphatic clearance may increase the risk of neurodegenerative changes contributing to cognitive decline in the aging population or in age-related CNS diseases (Rainey-Smith et al., 2018). For example, during aging, cerebral arterial walls begin to stiffen and prevent smooth arterial pulses needed for CSF influx and ISF exchange (Badaut et al., 2014; Sweeney et al., 2019). Given the abnormal aging trajectory in people with HIV (PWH), it is possible that HIV may also negatively impact glymphatic clearance. PWH have normal life expectancies due to the success of combination anti-retroviral therapy (cART) (Cassol et al., 2014) thus, supporting a scenario whereby chronic inflammation associated with viral infection promotes a more rapid or abnormal aging processes. In this review, we will provide an overview and discussion of potential contributions of NeuroHIV to glymphatic system dysfunction and implications for NeuroHIV disease trajectory.

THE GLYMPHATIC SYSTEM

Since the discovery of the glymphatic system in both rodents and humans, researchers have focused on understanding the mechanisms of action for CSF and ISF exchange (Mestre et al., 2018). The brain has four fluid compartments including CSF, ISF, intracellular fluid and blood. The blood-brain barrier (BBB) and blood-CSF barrier separate blood from the brain parenchyma and CSF, respectively (Redzic, 2011) to maintain ionic and biochemical composition of the different fluid compartments for proper cellular signaling and brain function (Damkier et al., 2013). The neurovascular unit (NVU) supports brain homeostasis and is composed of neurons, astrocytes, cerebral endothelial cells, myocytes, pericytes and extracellular matrix components (**Figure 1**; Harder et al., 2002). Cellular components of the NVU detect the changes in CNS cells and signal for necessary responses such as vasodilation and/or vasoconstriction (Koehler et al., 2006).

CSF and ISF are constantly exchanged in the brain due to the convective influx of CSF along the periarterial spaces located in the subarachnoid space (Iliff et al., 2012). CSF is then transported to the parenchyma through the NVU by a combination of arterial pulses, respiration, CSF pressure gradients and the loose fibrous matrix of the perivascular space (**Figure 2**). In addition, water channels called aquaporin-4 (AQP4) located on the perivascular end feet of astrocytes facilitate the transfer of CSF into the parenchyma (Nakada et al., 2017). AQP4 water channels are normally highly concentrated on the perivascular end feet of astrocytes and are critical for the brain's vascular and glymphatic systems. Within the parenchyma, CSF influx promotes CSF/ISF exchange within the peri-venous space that surrounds the deep veins within the brain. ISF then collects in the peri-venous space that is transported out of brain toward the cervical lymphatic system (**Figure 2**; Iliff et al., 2013).

MENINGEAL LYMPHATICS

The meninges consist of three layers (dura mater, arachnoid, and pia mater) which protect the CNS by lining the brain and spinal cord through forming the various CSF-filled compartments. As with the discovery of the glymphatic system, the CNS and its meningeal components were long believed to lack a traditional lymphatic vascular system since the only lymphatic vessels were identified around the cranial nerves and dural blood vessels (Iliff et al., 2015). Recent studies have described the extensive meningeal lymphatic vessel network that functions in macromolecular clearance and immune cell trafficking in the brain (Aspelund et al., 2015; Louveau et al., 2015). These

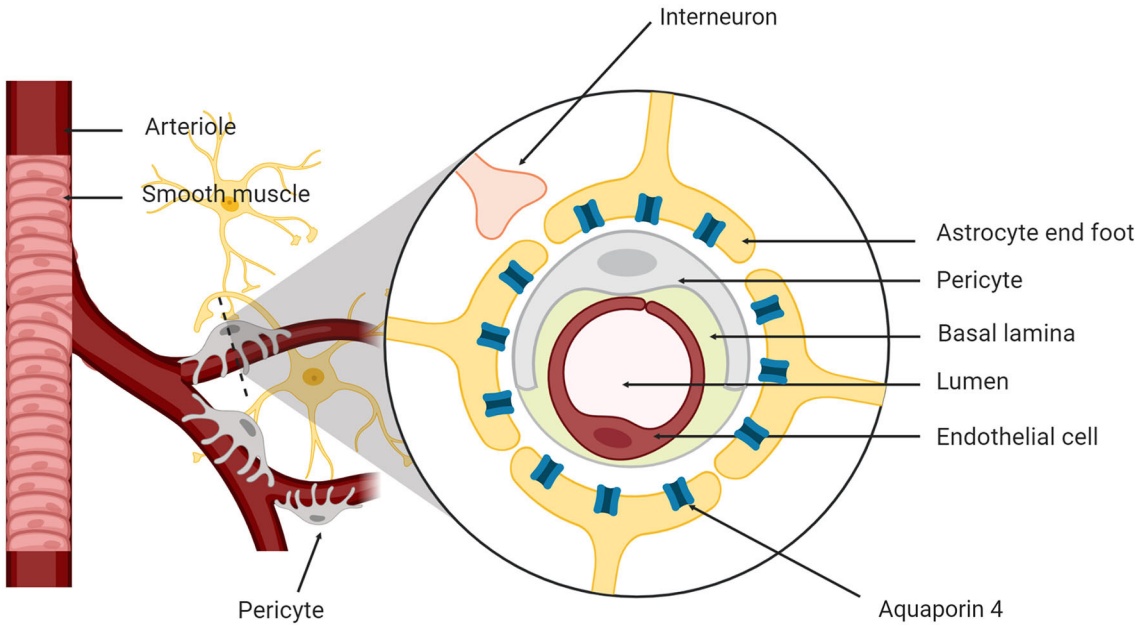


FIGURE 1 | The neurovascular unit. The neurovascular unit is composed of neurons, astrocytes, cerebral endothelial cells, smooth muscle myocytes, pericytes, and matrix components.

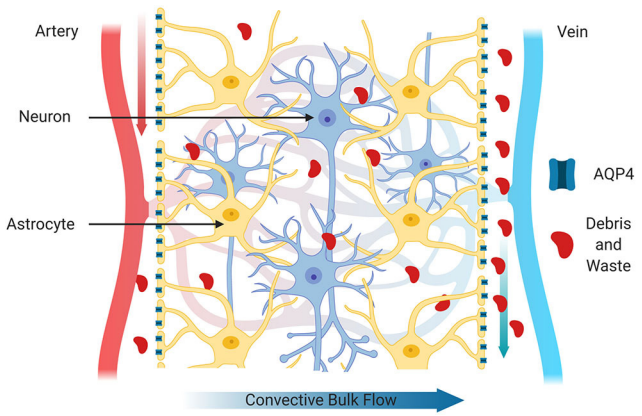


FIGURE 2 | Glymphatic system. The glymphatic system is comprised of astrocytes, aquaporin 4 water channels and cerebral perivascular spaces. Aquaporin 4 water channels, located on astrocytic end feet facilitate movement of water to for the exchange of interstitial fluid and cerebrospinal fluid and to propel extracellular contents such as cellular debris, abnormal proteins and waste products through brain parenchyma for clearance. The glymphatic system also transports glucose, lipids, amino acids, growth factors and neuromodulators through-out the brain. In health, these components can be cleared more efficiently primarily during sleep.

vessels were found to have immunohistological and structural characteristics of lymphatic vessels, and to express traditional markers of tissue lymphatic endothelial cells, such as Prox1, CD31, Lyve-1, Podoplanin, VEGFR3, and CCL2 (Aspelund et al., 2015; Louveau et al., 2015). Interestingly, meningeal lymphatic vessels are evolutionarily conserved in fish, rats, non-human primates and humans (Absinta et al., 2017; Bower et al., 2017; Jung et al., 2017). Compared to peripheral lymphatics, meningeal lymphatics are composed of a less complex network of thin-walled initial lymphatic vessels that converge and exit the cranium along the retroglenoid vein, sigmoid sinus, and meningeal portions of the pterygopalatine artery (Aspelund et al., 2015; Louveau et al., 2015). Functional studies have shown that meningeal lymphatic vessels can carry numerous immune cells under physiological conditions, suggesting a role in normal immune surveillance of the brain (Aspelund et al., 2015).

Aging is a major risk factor for many neurodegenerative diseases, including Alzheimer's disease (AD) which has a detrimental effect on brain CSF/ISF paravascular recirculation that is thought to be caused by the deterioration of meningeal lymphatic vessels that occurs with aging (Da Mesquita et al., 2018a). Studies have shown that meningeal lymphatic dysfunction in young-adult mice results in impaired brain perfusion by CSF and deficits in learning and memory (Da Mesquita et al., 2018b). Aged mice demonstrated significant disruption of meningeal lymphatic function leading to cognitive decline. Interestingly, augmentation of meningeal lymphatic drainage in aged mice facilitated the clearance of CSF/ISF macromolecules from the brain, resulting in improved cognitive function (Da Mesquita et al., 2018b; Stower, 2018). These data demonstrate that impairment of meningeal lymphatics decreases brain perfusion and leads to diminished cognitive ability accompanied by increased amyloid-beta (Aβ) accumulation. These data suggest that disruption in meningeal lymphatics is at least one of the mechanisms that contribute to the buildup

of aberrant proteins observed in AD and other neurocognitive disorders (Da Mesquita et al., 2018b; Stower, 2018).

GLYMPHATIC AND MENINGEAL LYMPHATIC SYSTEMS CONNECTIONS

Recent discoveries of the glymphatic and meningeal lymphatic systems have changed the previous idea that the CNS was devoid of lymphatic vessels (Aspelund et al., 2015; Plog and Nedergaard, 2018; Rasmussen et al., 2018). Both the glymphatic and meningeal lymphatic systems allow for the clearance of aberrant proteins such as Aβ and pTau. Thus, both systems are critical for healthy aging as the buildup of aberrant proteins can contribute to AD or other age-related neurocognitive disorders (Rasmussen et al., 2018). The connections between the glymphatic and meningeal lymphatic systems have been demonstrated through studies following tracers injected into the CSF of rodents (Aspelund et al., 2015). Both systems use the water channel, AQP4 to facilitate the movement of CSF throughout the CNS (Filippidis et al., 2016; Cao et al., 2018). The continuous flow of CSF that perfuses the brain parenchyma and is drained to cervical lymph nodes through the meningeal lymphatic system. As previously mentioned, CSF and ISF are constantly exchanged in the brain due to the convective influx of CSF along the peri-arterial spaces located in the subarachnoid space (Iliff et al., 2012). The glymphatic system is responsible for the movement of the CSF into the parenchyma. Within the parenchyma, CSF influx forces connective ISF exchange within the peri-venous space that surrounds the deep veins within the brain (Iliff et al., 2013). The meningeal lymphatic system has been shown to be necessary for the efficient clearance of ISF collected in the subarachnoid space that is transported out of brain toward the deep cervical lymph nodes through dural meningeal lymphatics (Louveau et al., 2015). The deep cervical lymph nodes can connect to the inner carotid and empty the waste metabolites into the general circulation (Aspelund et al., 2015; Louveau et al., 2015). This link provides a plausible route to study CNS-immune connection (Bower and Hogan, 2018). Dysfunction of meningeal lymphatic vessels and their connection to the glymphatic system should be addressed by future studies.

AQUAPORINS

Aquaporins (AQPs) are hydrophobic transmembrane proteins that facilitate the passive transport of water depending on the osmotic pressure on both sides of membrane. The functional AQP unit is a homo-tetramer and is comprised of six α-helix transmembrane domains with two conserved asparagine–proline–alanine (NPA) motifs, five loops (A-E) and intracellular N- and C-termini (Potokar et al., 2016). AQPs are subdivided into two subfamilies, classical AQPs that are water selective and aquaglyceroporins that act as glycerol channels. However, this characterization was challenged by recent evidence revealing that both subfamilies overlap functionally, for example, some classical AQPs transport water and other small solutes such as glycerol (Papadopoulos and Verkman, 2013). AQPs are found in all species ranging from simple prokaryotes to complex multicellular organisms. Thirteen AQPs have been identified in mammalian cells with eleven of these in humans. The distribution of AQPs varies among different tissue types (Ma et al., 2011), with AQPs 1, 4, and 9 representing the key aquaporins expressed in the CNS.

AQUAPORINS IN THE NERVOUS SYSTEM

AQPs are present in the CNS, spinal cord, enteric nervous system and sensory organs. AQP expression has been studied more extensively in the CNS and sensory organs than in other systems (Rosu et al., 2019). From the three main aquaporins in the CNS, AQP4 has the highest expression levels in the brain and is expressed by astrocytes (Chen et al., 2008). AQP1 and AQP4 are true aquaporins and transport only water, while AQP9 is an aquaglyceroporin that transports both water and glycerol (Papadopoulos and Verkman, 2013). AQPs in the CNS have diverse functions including bidirectional water exchange between the brain and cerebrovascular circulation and CSF for osmoregulation of in the brain. AQPs are also involved in neural cell signal transduction (Badaut et al., 2014; Filippidis et al., 2016).

AQP1 is located on astrocytic cell membrane where it is co-expressed with AQP4. AQP1 is also expressed on the apical surface of the epithelium of the choroid plexus aiding in CSF production and flux (Rosu et al., 2019). AQP1 is not found in normal brain capillary endothelium, however it is highly expressed by peripheral endothelial cells (Rosu et al., 2019). Importantly, AQP1 expression is upregulated in many neurodegenerative disorders including choroid plexus tumors, spinal cord injury, and AD (Filippidis et al., 2016). A recent study showed that AQP1, but not AQP4 expression levels were increased in astrocytes in brains from AD patients (Misawa et al., 2008). Increased AQP1 levels were observed at early stages of AD, and remained elevated throughout more advanced stages of AD (Pérez et al., 2007).

AQP9 is located in the ependymal cells surrounding the cerebral ventricles, brain stem catecholaminergic neurons, dopaminergic neurons of the substantia nigra and ventral tegmental area and astrocytes (Xu et al., 2017). AQP9 is permeable to water and to small solutes including glycerol, urea and monocarboxylates which may contribute to energy metabolism in the CNS (Potokar et al., 2016). AQP9 and AQP4 have similar distribution patterns in mice and rats, suggesting that these two channels may act together to facilitate the movement of water or small solutes between CSF and the brain parenchyma (Yool, 2007; Lv et al., 2018; Shi et al., 2019).

AQP4 is the most abundant AQP in the brain, spinal cord and optic nerve (Potokar et al., 2016), is widely expressed on the plasma membranes of astrocytes with the highest concentration on perivascular end feet of astrocytes (Rosu et al., 2019). In the brain, AQP4 is primarily localized to the sub-pial astrocyte processes that form the glial-limiting membrane, the basolateral membrane of ependymal cells throughout the brain and spinal cord, and the perivascular astrocyte end feet that constitute a main component of the NVU (Hubbard et al., 2018; Ciappelloni

et al., 2019; Dasdelen et al., 2019). AQP4 facilitates the flow of water into and out of the brain due its expression on cells that form barriers between the brain and major fluid compartments (Dasdelen et al., 2019).

AQUAPORIN 4 VARIANTS

There are six isoforms of AQP4 in astrocytes and some other organ specific cells designated AQP4a-f. The distribution of these isoforms varies among cell types and their cellular location. All of the AQP4 isoforms are expressed on astrocytes either on the plasma membrane or on cellular organelles. AQP4c is also expressed in the kidney and the sarcolemma of fast-twitch fibers of skeletal muscle (Frigeri et al., 1998; Potokar et al., 2016). AQP4a and AQP4c are most commonly located in the plasma membranes of perivascular end feet of astrocytes, along with AQP4e that is expressed at much lower levels. AQP4c is the predominant isoform located on the plasma membrane compared to AQP4a (Hubbard et al., 2018).

Interestingly, a recent study reported that a single nucleotide polymorphism (SNP) in AQP4, rs72878776, is associated with altered overall sleep quality according to the Pittsburgh Sleep Quality Index (PSQI) sleep parameters such as sleep latency, comfort, and difficulty falling asleep (Rainey-Smith et al., 2018). Individuals that are homozygous for the AQP4 rs72878776-A (0.001% of the population) allele reported worse overall sleep compared to those with a different genotype (Rainey-Smith et al., 2018). This SNP is in the 5-prime untranslated region (5′UTR) of the AQP4 gene and may be of functional relevance through potentially influencing gene transcription, via modification (creation or deletion) of transcription factor binding sites (Rainey-Smith et al., 2018). Individuals with AQP4 variant rs72878776 may be at greater risk for neurodegenerative diseases due to impaired glymphatic function accompanied by poor sleep quality (Rainey-Smith et al., 2018). In addition, individuals homozygous for either AQP4 SNPS rs3763040-A or rs3763043-A have been reported to experience more rapid cognitive decline after AD diagnosis (Burfeind et al., 2017). Other noncoding AQP4 SNPs were associated with altered rates of cognitive decline after AD diagnosis. Interestingly, individuals homozygous for either AQP4 SNPS rs9951307-A and rs3875089-A have been reported to experience slower cognitive decline (Burfeind et al., 2017). These studies indicate the importance of AQP4 in maintaining proper glymphatic clearance in age-related neurodegenerative diseases in both protective and detrimental aspects.

AQUAPORINS AND NEUROINFLAMMATION

Astrocytes express all of the AQPs (1, 4, and 9) found in the CNS. Astrocytes are the primary homeostatic cells of the brain and are the only cell type in the CNS that can undergo rapid volume changes (Thrane et al., 2014). The CNS must tightly regulate water homeostasis as the brain is confined within the skull allowing limited space for expansion. AQP4 is the main water channel in the brain thus, it is critical that AQP4 functions properly (Papadopoulos and Verkman, 2013). This is increasingly important given that injury can decrease expression and induce mislocalization of AQP4 from end feet to cell body, thereby reducing fluid exchange. As extracellular volume changes, ion concentration is also changed to alter neuronal excitability (Dalkara et al., 2011). Disturbances in water homeostasis in the CNS are typically observed in CNS diseases/disorders including HIV infection, AD, PD, meningitis, stroke, brain abscesses, tumors or neurotrauma, all of which have a neuroinflammatory component. It has been established that expression of AQP4 is pro-inflammatory (Chmelova et al., 2019). For example, studies demonstrate increased neuroinflammation in wild type mice administered LPS compared to APQ4 knockout mice (Wu et al., 2019). Astrocytes from wild type mice produced higher levels of the pro-inflammatory cytokines TNFα and IL-6 in response to LPS than AQP4 knockout mice. These data suggest that neuroinflammation is dependent, in part on AQP4 (Zhang et al., 2019). AQP4 is also the target antigen in the neurological autoimmune disease neuromyelitis spectrum disorder, which is an autoimmune astrocyte-specific cytopathy characterized by astrocyte loss, demyelination in the spinal cord, optic nerve and brain (Hinson et al., 2008; Sakalauskaite-Juodeikiene et al., 2018; Ciappelloni et al., 2019; Mader and Brimberg, 2019). Neuromyelitis spectrum disorder patients have chronic neuroinflammation leading to the classic presentations of this disease which include long segments of spinal cord inflammation, severe optic nerve inflammation, and area postrema syndrome (Huda et al., 2019). Area postrema syndrome results from inflammation in the emetic reflex center located in the rhomboid fossa of the 4th ventricle leading to nausea, vomiting, and hiccups in patients (Duvernoy and Risold, 2007). All neuromyelitis spectrum disorder patients are treated with AQP4 antibodies at their first attack with long-term immunosuppression (Mealy et al., 2014).

AQP4 DYSREGULATION, ADENOSINE A2A RECEPTOR AND NEURODEGENERATION

Adenosine A2a receptor (A2aR) is highly expressed by astrocytes and microglia and contributes significantly to neuroinflammatory and neuromodulator processes (Orr et al., 2015; Zhao et al., 2017; Masjedi et al., 2019). Many neurodegenerative diseases including HIV, AD and TBI involve chronic neuroinflammation thereby increasing the risk for regional accumulation of pTau, tau oligomers and neurofibrillary tangles contributing to neurocognitive dysfunction. The substantial neuroinflammatory response may contribute to disruption of the glymphatic system in part, through dysregulation of AQP4 (Iliff et al., 2014; Rasmussen et al., 2018). In fact, in a rodent model of TBI, disruption of perivascular polarization of AQP4 from astrocyte end feet to the soma promoted tau pathology (Xu et al., 2017; Zhao et al., 2017). Widespread reactive astrogliosis was observed 7 days after TBI in multiple brain regions, and impaired AQP4 polarity correlated with regions of pTau accumulation and reactive astrogliosis

(Zhao et al., 2017). In this context, a significant increase in A2aR expression was detected 1 day, 3 days, 7 days, and 4 weeks post-TBI (Zhao et al., 2017). This is important because A2aR activates the adenylate cycle to generate cAMP that may contribute to AQP4 mislocalization (Borroto-Escuela et al., 2018). A2aR knockout alleviated the disruption of AQP4 polarity, pTau accumulation and neuronal damage post-injury (Zhao et al., 2017). However, the mechanism(s) of how A2aR/cAMP mediate AQP4 mislocalization is not clear and further studies are warranted in this direction.

A recent study showed increased expression of A2aR in the hippocampus of AD patients compared to aged-matched controls (Orr et al., 2015). Moreover, in an AD-model, mice expressing the human amyloid precursor protein (hAPP), increased expression of A2aR was observed in astrocytes (Orr et al., 2015). However, A2aR receptor ablation enhanced memory in older hAPP mice even when there was a significant abundance of amyloid plaques. In studies of mouse models of TBI, localization of AQP4 to the cell soma and was accompanied by increased accumulation of Tau in this region. However, 1 month post-TBI, pTau accumulated in the brain parenchyma with continued deterioration of AQP4 polarity (Zhao et al., 2017). In these studies, increased levels of A2aR was accompanied by the mislocalization of AQP4 (Zhao et al., 2017) and possibly increased accumulation of aberrant proteins in TBI. These data were supported by further data showing that inactivation of A2aR prevented TBI induced AQP4 mislocalization and pTau accumulation.

Although no literature exists that investigate expression of A2aR in PWH, there are numerous overlapping neuropathological hallmarks between HIV and age-related neurodegenerative diseases that may involve A2aR. For example, progressive accumulation of aberrant proteins such as pTau, Aβ and α-synuclein are accompanied by damage to selective neural circuits, neuroinflammation, gliosis and vascular alterations are common in both HIV and AD (Mackiewicz et al., 2019). In fact, the HIV protein Tat that may be released from infected astrocytes, even during effective anti-retroviral therapy, is believed to be a major factor in promoting pTau and Aβ formation and accumulation (Hategan et al., 2019). Taken together, even though limited, current evidence indicates that changes in AQP4 localization on astrocytes and decreased waste clearance may involve increased A2aR activity. Potential contributions of HIV infection of the CNS to APQ4 mislocalization in astrocytes, astrocyte phenotype and impaired waste clearance and clearly need to be explored. In this context, it is important to understand interactions among HIV, astrocytes and other cells in the brain.

ASTROCYTES AND HIV

HIV-infected monocytes/macrophages or CD4+ T-cells can cross the BBB and release virions or viral proteins that may negatively impact other cells types in the brain (Valcour et al., 2011). Perivascular macrophages and microglia are the primary cell types in the CNS that are infected by HIV-1 and support productive viral replication (Rojas-Celis et al., 2019). Neurons and oligodendrocytes are not believed to be infected by HIV-1. HIV infection of the CNS occurs early after infection and macrophages and microglia are responsible in large part for productive infection within the CNS. HIV has been shown to infect astrocytes to a lesser degree, and productive infection and release of infectious virions by astrocytes has not been observed consistently (Brack-Werner et al., 1992; Conant et al., 1994; Brack-Werner, 1999; Canki et al., 2001; Olivier et al., 2018). Astrocytes are not infected with HIV through classical gp120 and CD4 binding, but possibly through cell-to cell contact or receptor mediated endocytosis (Chauhan et al., 2014; Do et al., 2014; Luo and He, 2015; Russell et al., 2017), allowing for reverse transcription and incorporation of viral DNA into the host genome (Narasipura et al., 2012). Taken together, most data support that astrocytes can be infected by HIV, release high levels of inflammatory factors and produce several viral proteins including Tat, Nef, and Rev, which promote inflammation and damage to surrounding cells (Ferrell and Giunta, 2014; Hong and Banks, 2015). In fact, recent studies propose that astrocytes infected with HIV in addition to releasing viral proteins, may also release infectious virions that can infect T-cells in the brain that can then be trafficked to the periphery (Lutgen et al., 2020).

ASTROCYTE PHENOTYPE AND GLYMPHATICS

Astrocytes make up about 30% of the cells in the CNS (Churchill et al., 2015) and in health, play vital roles in many homeostatic mechanisms to maintain proper CNS functioning and to provide trophic support of neurons (Sun et al., 2019). Thus, it stands to reason that during HIV infection of the CNS, the proper functioning of astrocytes whether infected by the virus as described above, or activated by chronic neuroinflammation would be impacted. Rationale for this prediction is supported by studies from other neurodegenerative diseases with chronic inflammation and reactive glial cell populations. Upon stressful stimuli, astrocytes become activated from a quiescent state, become reactive and undergo structural and functional changes (Ferrer, 2017; Hinkle et al., 2019). In this context, astrocytes can be classified into numerous subtypes based on morphology, activation state and anatomical location. For the purpose of this review, we will consider two reactive subtypes, A1 and A2 that are induced by inflammation or ischemia, respectively (Zamanian et al., 2012; Liddelow et al., 2017; Joshi et al., 2019; **Figure 3**). The A1 subtype has increased complement 3 (Liddelow et al., 2017; Cohen and Torres, 2019), a neuroinflammatory profile, and releases NFκB-related inflammatory cytokines including TNF-α and IL-1β. Transcriptome analyses of reactive astrocytes show that A1 neuroinflammatory reactive astrocytes upregulate many genes that are destructive to synapses (Shijo et al., 2019). A1 astrocytes are more common in CNS HIV infection and neurodegenerative disease including AD (Cohen and Torres, 2019) and are specifically associated with neurotoxicity. For example, studies demonstrate that amyloid plaques and pTau in the brains of AD patients are surrounded by A1 astrocytes (Li et al., 2011).

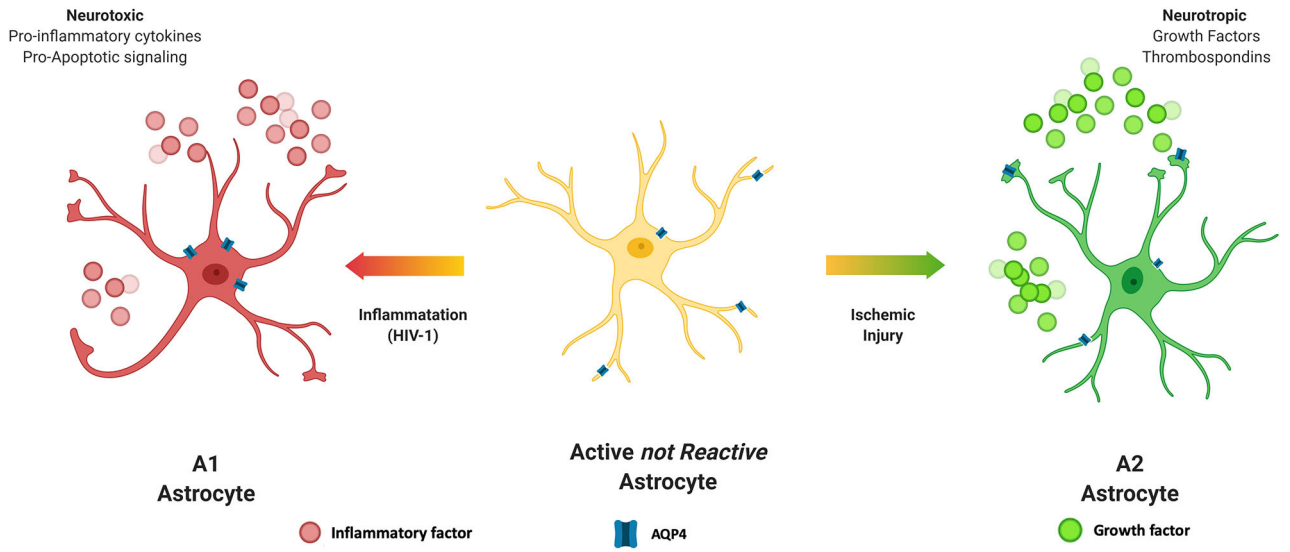


FIGURE 3 | Astrocyte subtypes. Astrocytes can be classified into numerous subtypes based on morphology, active state and anatomical location. Astrocytes are stimulated to become reactive by challenges such as inflammation or ischemia. The A1 subtype is induced by inflammation, is generally considered neurotoxic and produces inflammatory cytokines and pro-apoptotic factors. On the other hand, the A2 subtype is induced by ischemic conditions, such as stroke and releases neurotrophic factors and is considered to be neuroprotective.

The A2 subtype on the other hand, is induced by ischemic event. Ischemia-induced A2 reactive astrocytes upregulate many neurotrophic factors that promote survival and growth of neurons. The A2 subtype is associated with a neurotrophic profile with increased levels of BDNF, FGF2 and VEGF to promote CNS repair and regeneration (Gao et al., 2005; Zador et al., 2009; Hayakawa et al., 2014). A2 astrocytes also produce thrombospondins that aid in synapse repair (Chan et al., 2019).

Since A1 and A2 phenotypes may impact glymphatic function differently, consideration should be given to neurodegenerative events that are accompanied by increased accumulation of aberrant proteins or waste products that are characterized by one phenotype or the other. For example, the A1 phenotype is most commonly observed in HIV, AD, PD, epilepsy, and TBI, all of which include increased accumulation of aberrant proteins or waste products, as well as chronic inflammation (Liddelow and Barres, 2017; Clark et al., 2019; Matias et al., 2019). Interestingly, normal aging as well as age-related neurodegeneration are accompanied by the increased presence of A1 astrocytes (Clarke et al., 2018). Given that AQP4 is reported to be mislocalized in the A1 phenotype, it may be surmised that this could lead to glymphatic dysregulation. On the other hand, the A2 phenotype is generally reported to be protective, however recent studies report that brain and BBB ischemia, and reperfusion can promote changes in Tau that include both increased and decreased phosphorylation (Pluta et al., 2018a,b). Given that astrocytic phenotype has been described as existing in a heterogeneous continuum of mixed populations, more research is needed to determine how glymphatic function in the context of a specific clinical setting is impacted.

As discussed in previous sections of the review, some astrocytes are infected by HIV and can produce and release several viral proteins such as Tat, Nef, and Rev due to integrated virus, thereby inducing an A1 phenotype and production of inflammatory factors that damage surrounding cells (Hinkle et al., 2019; Shijo et al., 2019). Thus, the A1 astrocyte phenotype likely contributes in part to the development HAND (Ances and Clifford, 2008).

HIV-ASSOCIATED NEUROCOGNITIVE DISORDER (HAND)

With the development of cART, PWH typically have normal life spans (Bandera et al., 2019). While cART has dramatically increased the life expectancy of PWH, it has also brought attention to an array of neurocognitive issues, collectively known as HAND. HAND has been described as a spectrum disorder that is typically diagnosed by neurocognitive testing to assess attention, information processing, language, executive functioning, sensory-perceptual skills, simple motor skills, complex perceptual-motor skills and memory (Olivier et al., 2018; Robinson-Papp et al., 2019; Saloner et al., 2019). In earlier efforts, the National Institute of Mental Health and National Institute of Neurological Diseases and Stroke charged a working group to assess HAND criteria and develop an updated consensus for the definition (Antinori et al., 2007). According to this criteria in what is commonly called the "Frascati criteria," the following three conditions are recognized: asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD) (Antinori et al., 2007). ANI is currently the most prevalent manifestation of HAND in the developing world (Métral et al., 2020) and can be difficult for clinicians to diagnose as the deterioration of cognitive

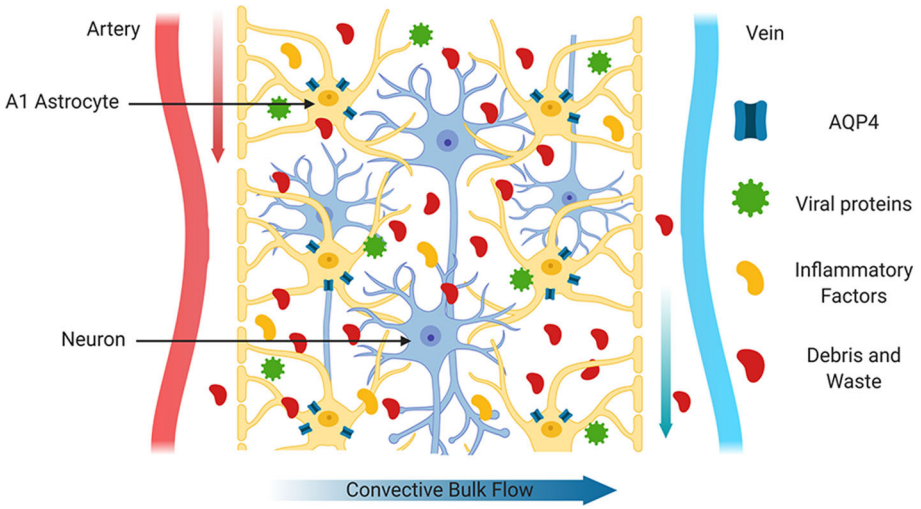


FIGURE 4 | Impaired glymphatic system. After injury or during CNS diseases with a neuroinflammatory component such as HIV infection, the glymphatic system functions less efficiently. This may be due in part to increased inflammatory factors or to the production of viral proteins by HIV infected cells. In this scenario, mislocalization of AQP4 from astrocyte end feet to the cell body and/or reduced expression of AQP4 may lead to accumulation of toxic waste products such as hyperphosphorylated Tau.

functioning is undetectable by some assessments. Importantly, research indicates that ANI is clinically important because these individuals are at risk for transitioning to the more severe forms of HAND (Ciccarelli, 2019). Participants of the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study who had ANI at baseline were two to six times more likely to develop symptomatic HAND during several years of follow-up than those who had no impairment at baseline (Olivier et al., 2018; Ciccarelli, 2019). MND is similar to that of ANI also may causes difficulty for diagnosis (Haddow et al., 2013). Like ANI, MND may also be difficult diagnose in PWH when they are one standard deviation below mean in two cognitive domains based from the Frascati Criteria. HAD is the most severe of the form of HAND and according tot he Frascati Criteria is diagnosed in PWH when they are two standard deviations below the mean in at least two cognitive domains (Olivier et al., 2018; Cysique and Brew, 2019; Matchanova et al., 2019). Despite the evolution of defining and diagnosing HAND over the past several decades, debate over screening tools and management plans continue (Winston and Spudich, 2020). In fact, numerous contributing factors to cognitive impairment in PWH must be considered and include, nearly normal life spans with advanced age, age-related co-morbidities, effects of initial infection on the CNS, lifestyle, medications other than cART, cART-induced toxicities, and chronic neuroinflammation (Winston and Spudich, 2020), all of which my impact astrocyte reactivity and waste clearance from the brain.

ALTERED WASTE CLEARANCE AND ABNORMAL AGING IN HAND

The prevalence of PWH over 50 years of age is increasing rapidly each year, and is predicted to increase from 28 to 73% by 2030 (Yang et al., 2019). There is growing evidence that the prevalence of comorbidities and other age-related conditions including geriatric syndromes, functional or neurocognitive problems, polypharmacy or social difficulties are higher in PWH than in their uninfected counterparts (Guaraldi et al., 2019; Winston and Spudich, 2020). Limited information is available regarding the optimal clinical management of older PWH (Chen et al., 2019; Guaraldi et al., 2019; Guo and Buch, 2019). Chronic neuroinflammation has been associated with altered synaptic connectivity and BBB function, and with neuronal injury (Bandera et al., 2019; Guaraldi et al., 2019). In the CNS, age-associated neuroinflammation is also linked with impaired waste clearance (Cassol et al., 2014). In a larger metabolomic profiling study comparing CSF from PWH on cART to CSF from age matched HIV negative individuals, alterations in the HIV CSF metabolome indicated persistent inflammation, glial reactivity, and glutamate neurotoxicity (Cassol et al., 2014). Moreover, abnormal aging in PWH impacts brain waste disposal systems and likely contribute to mechanisms involved in HAND (Cassol et al., 2014). Importantly, these alterations were not directly associated with cART or with ongoing HIV replication in CSF or plasma (Cassol et al., 2014). Although specific mechanisms that could be directly associated with HAND were not identified, it has been speculated that HIV-mediated disruption of AQP4 may contribute to altered glymphatic function (Xing et al., 2017; **Figure 4**).

CERVICAL LYMPH NODES AS A RESERVOIR FOR HIV

As described above, CSF and ISF exchange occurs in the perivenous space and ISF is then transported from the brain to the cervical lymph nodes (**Figure 2**; Iliff et al., 2013). Cervical

lymph nodes (CLNs) are a group of lymph nodes in the neck region that are located adjacent to the cervical region of the spinal cord near the sternocleidomastoid muscle (Dave et al., 2018a). The glymphatic and meningeal systems function as a waste removal pathway by connecting the CNS to the CLNs (Benveniste et al., 2017). T cells and antigen-presenting cells (APCs) that are present within the CNS drain via the lymphatic path into the CLNs (Benveniste et al., 2017). CLNs contain follicular dendritic cells (FDCs) which can retain antigens for a prolonged duration allowing them to potentially act as a reservoir for HIV (Sabri et al., 2016; Dave et al., 2018a). FDCs are important for immune function as they allow for germinal center formation, long term immune recovery (Dave et al., 2018a,b) and may contribute significantly to immune system response to the virus, the viral reservoir and escape. Importantly, given the intimate connection between glymphatic drainage and CLNs, the potential role of CLNs in glymphatic impairment and HIV infection should be considered. In addition to well-studied viral reservoirs including macrophages, microglia and astrocytes (Churchill et al., 2015), virion-immune complexes may become trapped within the interconnected processes of FDCs, and may represent a significant reservoir for the virus (Fletcher et al., 2014; Dave et al., 2018a). Studies also show that infectious virus can be recovered from the FDCs PWH who are cART experienced, indicating that FDCs (Ho et al., 2007; Fletcher et al., 2014; Heesters et al., 2015). FDCs have been also been shown to retain infectious virus inside endosomes even in the presence of neutralizing antibodies (Heesters et al., 2015). On the other hand, in several species of non-human primates (NHP) infected with simian immunodeficiency virus (SIV), progression to disease including chronic inflammation, usually does not occur (Rey-Cuillé et al., 1998; Diop et al., 2000; Goldstein et al., 2000; Broussard et al., 2001; Onanga et al., 2002). Additionally, other studies have shown that FDCs do not serve as reservoirs for SIV in NHP (Paiardini and Muller-Trutwin, 2013; Huot et al., 2016, 2018). NHP hosts control viral replication in lymph nodes and resolve the inflammatory response despite high viremia. Although no studies have investigated potential glymphatic impairment in SIV-infected NHP, evidence for a similar system has been reported whereby subarachnoid stroke resulted in impaired CSF flux into periarterial regions (Goulay et al., 2017). Given the striking differences between SIV-infected NHP and HIV infection of humans in viral reservoirs, viral and inflammation control in the context of CLN response, studies aimed at AQP4 and astrocytic involvement in SIV are warranted.

AQUAPORIN 4 AND NEUROHIV

Few studies have investigated changes in AQP4 and HIV CNS infection. In one study, expression levels of AQP4 in brain homogenates from the mid frontal gyrus of HAD patients and to a lesser extent HIV patients without HAD, were significantly increased (St Hillaire et al., 2005). Immunolabeling of brain tissue indicated patchy AQP4 in perivascular regions as well as in morphologically characterized reactive astrocytes not necessarily associated with brain vasculature.

A single study in the SIV-infected macaque model using three different viruses, SIVmac239, simian-human immunodeficiency virus (SHIV)-RT and SIVsm543-3 assessed changes in levels and patterns of AQP4 in the frontal cortex (Xing et al., 2017). In uninfected control brains, AQP4 was diffusely and evenly expressed in subpial and perivascular areas of the cortex, suggesting expression on astrocytic cell bodies as well as processes. On the other hand, in the brains of infected animals, AQP4 labeling was largely limited to astrocyte-like cells with loss of even distribution. Areas showing loss of AQP4 labeling showed intense GFAP labeling with intracellular dense glial fibrils and increased pro-apoptotic caspase-3 expression (Xing et al., 2017). Thus, these findings support the concept that viral infection does in fact alter patterns and levels of AQP4 in the brain (Xing et al., 2017). Importantly, patterns of AQP4 in SIV brains were reminiscent of those observed in HIV patients' brains (St Hillaire et al., 2005; Xing et al., 2017). Studies by Aoki-Yoshino *et al* report that HIV encephalitis and HAD are not the only neuroinflammatory diseases with altered AQP4 expression and report changes in progressive multifocal leukoencephalopathy and multiple sclerosis (Aoki-Yoshino et al., 2005).

SUMMARY

As mentioned previously, the brain was initially thought to be devoid of typical lymphatic system, but in the past few years, studies have indicated that there is a system whereby CSF and ISF interchange to clear waste products, solutes and maintain homeostatic conditions within the brain parenchyma. The glymphatic system has been shown to clear aberrant proteins including pTau, Aβ and other macromolecules that tend to increase and accumulate in neurodegenerative diseases. Proper functioning of the glymphatic system is therefore particularly important in age-related CNS diseases including AD and HAND, both of which are characterized by glial cell activation, chronic inflammation and accumulation of toxic waste products. It is also important in recovery from TBI, whereby secondary injury cascades lead to accumulation of pTau, chronic inflammation, reactive gliosis in addition to structural damage. Astrocyte expression of the water channel AQP4 is intimately involved in normal glymphatic clearance and its function is impaired in AD, TBI and HIV infection of the brain. The proper localization of AQP4 in end foot processes of astrocytes is essential to its function (**Figure 2**), and in neurodegenerative diseases with chronic inflammation, increased ROS and glial cell reactivity, AQP4 becomes localized to the cell body (**Figure 4**).

Thus far, most studies of glymphatic impairment and AQP4 mislocalization have been conducted in AD and TBI patients or animal models. Given the neuropathological similarities among AD, TBI and HIV infection of the CNS, investigations into the potential impact of viral latency and reactivation in the brain are particularly timely. For example, AQP4 mislocalization on astrocytes and decreased waste clearance may involve increased A2aR-mediated cAMP generation, especially since blocking A2aR activity preserved AQP4 localization to astrocyte end feet accompanied by decreased pTau accumulation.

Among the important considerations in proposing that HIV infection of the brain may be impacted by glymphatic disruption are the changing status and spectrum of HAND within a person with HIV during their lifetime. In this context, astrocyte reactivity, phenotype and neuroinflammatory status can fluctuate. Another critical aspect of potential glymphatic involvement in HIV infection is the role played by CLNs in human infection compared to SIV infected NHP. Given that LN FDCs may serve as a reservoir in human infection with limited immune control, and peri-venous collection of ISF drainage into CLNs that may have lost their normal architecture and be harboring virus, another level of complexity exists within studies aimed at understanding if and how HIV impacts glymphatic and lymphatic functioning.

Approaches aimed at improving AQP4 functioning in the brains of PWH may include modulating activation levels of A2aR and assessing flux of fluid through the brain parenchyma. Studies to investigate molecular changes and neurobiological consequences of regulating A2aR are necessary both *in vitro* and *in vivo*. Positron emission tomography (PET) imaging studies have shown reduced clearance of CSF in AD patients and in patients with normal pressure hydrocephalus (De Leon et al., 2017; Ringstad et al., 2017, 2018). In this context, early detection of changes in clearance using PET imaging may be possible. Consideration must also be given to interactions among the dural lymphatic network, CLN and glymphatic clearance can be linked. Only after increased understanding of disease-specific alterations in these pathways can potential new therapeutic directions be realized.

AUTHOR CONTRIBUTIONS

CT wrote manuscript and created figures. JM wrote manuscript. DL wrote manuscript and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

R01 MH107340-01A1, P01 DA037830-01A1, Pennsylvania Department of Health (SAP# 4100083099), T32MH079785 and the Lemole Integrated Center for Lymphatics Research at LKSOM.

REFERENCES

Absinta, M., Ha, S. K., Nair, G., Sati, P., Luciano, N. J., Palisoc, M., et al. (2017). Human and nonhuman primate meninges harbor lymphatic vessels that can be visualized noninvasively by MRI. *Elife* 6:e29738. doi: 10.7554/eLife.29738

Ances, B. M., and Clifford, D. B. (2008). HIV-associated neurocognitive disorders and the impact of combination antiretroviral therapies. *Curr. Neurol. Neurosci. Rep.* 8, 455–461. doi: 10.1007/s11910-008-0073-3

Antinori, A., Arendt, G., Becker, J. T., Brew, B. J., Byrd, D. A., Cherner, M., et al. (2007). Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* 69, 1789–1799. doi: 10.1212/01.WNL.0000287431.88658.8b

Aoki-Yoshino, K., Uchihara, T., Duyckaerts, C., Nakamura, A., Hauw, J. J., and Wakayama, Y. (2005). Enhanced expression of aquaporin 4 in human brain with inflammatory diseases. *Acta Neuropathol.* 110, 281–288. doi: 10.1007/s00401-005-1052-2

Aspelund, A., Antila, S., Proulx, S. T., Karlsen, T. V., Karaman, S., Detmar, M., et al. (2015). A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. *J. Exp. Med.* 212, 991–999. doi: 10.1084/jem.20142290

Badaut, J., Fukuda, A. M., Jullienne, A., and Petry, K. G. (2014). Aquaporin and brain diseases. *Biochim. Biophys. Acta* 1840, 1554–1565. doi: 10.1016/j.bbagen.2013.10.032

Bandera, A., Taramasso, L., Bozzi, G., Muscatello, A., and Gori, A. (2019). HIV-associated neurocognitive impairment in the modern ART Era: are we close to discovering reliable biomarkers in the setting of virological suppression? *Front. Aging. Neurosci.* 11:187. doi: 10.3389/fnagi.2019.00187

Benveniste, H., Lee, H., and Volkow, N. D. (2017). The glymphatic pathway: waste removal from the CNS via cerebrospinal fluid transport. *Neuroscientist* 23, 454–465. doi: 10.1177/1073858417691030

Borroto-Escuela, D. O., Hinz, S., Navarro, G., Franco, R., Müller, C. E., and Fuxe, K. (2018). Understanding the role of adenosine A2AR heteroreceptor complexes in neurodegeneration and neuroinflammation. *Front. Neurosci.* 12:43. doi: 10.3389/fnins.2018.00043

Bower, N. I., and Hogan, B. M. (2018). Brain drains: new insights into brain clearance pathways from lymphatic biology. *J. Mol. Med.* 96, 383–390. doi: 10.1007/s00109-018-1634-9

Bower, N. I., Koltowska, K., Pichol-Thievend, C., Virshup, I., Paterson, S., Lagendijk, A., et al. (2017). Mural lymphatic endothelial cells regulate meningeal angiogenesis in the zebrafish. *Nat. Neurosci.* 20, 774–783. doi: 10.1038/nn.4558

Brack-Werner, R. (1999). Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis. *AIDS* 13, 1–22. doi: 10.1097/00002030-199901140-00003

Brack-Werner, R., Kleinschmidt, A., Ludvigsen, A., Mellert, W., Neumann, M., Herrmann, R., et al. (1992). Infection of human brain cells by HIV-1: restricted virus production in chronically infected human glial cell lines. *AIDS* 6, 273–285. doi: 10.1097/00002030-199203000-00004

Broussard, S. R., Staprans, S. I., White, R., Whitehead, E. M., Feinberg, M. B., and Allan, J. S. (2001). Simian immunodeficiency virus replicates to high levels in naturally infected African green monkeys without inducing immunologic or neurologic disease. *J. Virol.* 75, 2262–2275. doi: 10.1128/JVI.75.5.2262-2275.2001

Burfeind, K. G., Murchison, C. F., Westaway, S. K., Simon, M. J., Erten-Lyons, D., Kaye, J. A., et al. (2017). The effects of noncoding aquaporin-4 single-nucleotide polymorphisms on cognition and functional progression of Alzheimer's disease. *Alzheimers Dement.* 3, 348–359. doi: 10.1016/j.trci.2017.05.001

Canki, M., Thai, J. N. F., Chao, W., Ghorpade, A., Potash, M. J., and Volsky, D. J. (2001). Highly productive infection with pseudotyped human immunodeficiency virus type 1 (HIV-1) indicates no intracellular restrictions to HIV-1 replication in primary human astrocytes. *J. Virol.* 75, 7925–7933. doi: 10.1128/JVI.75.17.7925-7933.2001

Cao, X., Xu, H., Feng, W., Su, D., and Xiao, M. (2018). Deletion of aquaporin-4 aggravates brain pathology after blocking of the meningeal lymphatic drainage. *Brain Res. Bull.* 143, 83–96. doi: 10.1016/j.brainresbull.2018.10.007

Cassol, E., Misra, V., Dutta, A., Morgello, S., and Gabuzda, D. (2014). Cerebrospinal fluid metabolomics reveals altered waste clearance and accelerated aging in HIV patients with neurocognitive impairment. *AIDS* 28, 1579–1591. doi: 10.1097/QAD.0000000000000303

Chan, S. J., Niu, W., Hayakawa, K., Hamanaka, G., Wang, X., Cheah, P., et al. (2019). Promoting neuro-supportive properties of astrocytes with epidermal growth factor hydrogels. *Stem Cells Transl. Med.* 8, 1242–1248. doi: 10.1002/sctm.19-0159

Chauhan, A., Mehla, R., Vijayakumar, T. S., and Handy, I. (2014). Endocytosis-mediated HIV-1 entry and its significance in the elusive behavior of the virus in astrocytes. *Virology* 45, 1–19. doi: 10.1016/j.virol.2014.03.002

Chen, L., Khodr, C. E., Al-Harthi, L., and Hu, X. T. (2019). Aging and HIV-1 alter the function of specific K+ channels in prefrontal cortex pyramidal neurons. *Neurosci. Lett.* 708:134341. doi: 10.1016/j.neulet.2019.134341

Chen, M. J., Sepramaniam, S., Armugam, A., Shyan Choy, M., Manikandan, J., Melendez, A., et al. (2008). Water and ion channels: crucial in the initiation and

progression of apoptosis in central nervous system? *Curr. Neuropharmacol.* 6, 102–116. doi: 10.2174/157015908784533879

Chmelova, M., Sucha, P., Bochin, M., Vorisek, I., Pivonkova, H., Hermanova, Z., et al. (2019). The role of aquaporin-4 and transient receptor potential vaniloid isoform 4 channels in the development of cytotoxic edema and associated extracellular diffusion parameter changes. *Eur. J. Neurosci.* 50, 1685–1699. doi: 10.1111/ejn.14338

Churchill, M. J., Cowley, D. J., Wesselingh, S. L., Gorry, P. R., and Gray, L. R. (2015). HIV-1 transcriptional regulation in the central nervous system and implications for HIV cure research. *J Neurovirol.* 21, 290–300. doi: 10.1007/s13365-014-0271-5

Ciappelloni, S., Bouchet, D., Dubourdieu, N., Boué-Grabot, E., Kellermayer, B., Manso, C., et al. (2019). Aquaporin-4 surface trafficking regulates astrocytic process motility and synaptic activity in health and autoimmune disease. *Cell Rep.* 27, 3860. e4–3872.e4. doi: 10.1016/j.celrep.2019.05.097

Ciccarelli, N. (2019). Considerations on nosology for HIV-associated neurocognitive disorders: it is time to update? *Infection* 48, 1–6. doi: 10.1007/s15010-019-01373-8

Clark, D. P., Perreau, V. M., Shultz, S. R., Brady, R. D., Lei, E., Dixit, S., et al. (2019). Inflammation in traumatic brain injury: roles for toxic A1 astrocytes and microglial-astrocytic crosstalk. *Neurochem. Res.* 44, 1410–1424. doi: 10.1007/s11064-019-02721-8

Clarke, L. E., Liddelow, S. A., Chakraborty, C., Münch, A. E., Heiman, M., and Barres, B. A. (2018). Normal aging induces A1-like astrocyte reactivity. *Proc. Natl. Acad. Sci. U.S.A.* 115, E1896–E1905. doi: 10.1073/pnas.1800165115

Cohen, J., and Torres, C. (2019). Astrocyte senescence: evidence and significance. *Aging Cell.* 18:e12937. doi: 10.1111/acel.12937

Conant, K., Tornatore, C., Atwood, W., Meyers, K., Traub, R., Major, E., et al. (1994). *In vivo* and *in vitro* infection of the astrocyte by HIV-1. *Adv. Neuroimmunol.* 4, 287–289. doi: 10.1016/S0960-5428(06)80269-X

Cysique, L. A., and Brew, B. J. (2019). Comorbid depression and apathy in HIV-associated neurocognitive disorders in the era of chronic HIV infection. *Handb. Clin. Neurol.* 165, 71–82. doi: 10.1016/B978-0-444-64012-3.00006-X

Da Mesquita, S., Fu, Z., and Kipnis, J. (2018a). The meningeal lymphatic system: a new player in neurophysiology. *Neuron* 100, 375–388. doi: 10.1016/j.neuron.2018.09.022

Da Mesquita, S., Louveau, A., Vaccari, A., Smirnov, I., Cornelison, R. C., Kingsmore, K. M., et al. (2018b). Functional aspects of meningeal lymphatics in ageing and Alzheimer's disease. *Nature* 560, 185–191. doi: 10.1038/s41586-018-0368-8

Dalkara, T., Gursoy-Ozdemir, Y., and Yemisci, M. (2011). Brain microvascular pericytes in health and disease. *Acta Neuropathol.* 122:1. doi: 10.1007/s00401-011-0847-6

Damkier, H. H., Brown, P. D., and Praetorius, J. (2013). Cerebrospinal fluid secretion by the choroid plexus. *Physiol. Rev.* 93, 1847–1892. doi: 10.1152/physrev.00004.2013

Dasdelen, D., Mogulkoc, R., and Baltaci, A. K. (2019). Aquaporins in brain health and brain injury. *Mini. Rev. Med. Chem.* 20, 498–512. doi: 10.2174/1389557519666191018142007

Dave, R. S., Jain, P., and Byrareddy, S. N. (2018a). Follicular dendritic cells of lymph nodes as human immunodeficiency virus/simian immunodeficiency virus reservoirs and insights on cervical lymph node. *Front. Immunol.* 9:805. doi: 10.3389/fimmu.2018.00805

Dave, R. S., Sharma, R. K., Muir, R. R., Haddad, E., Gumber, S., Villinger, F., et al. (2018b). FDC:TFH interactions within cervical lymph nodes of SIV-infected rhesus macaques. *J. Neuroimmune Pharmacol.* 13, 204–218. doi: 10.1007/s11481-017-9775-0

De Leon, M. J., Li, Y., Okamura, N., Tsui, W. H., Saint-Louis, L. A., Glodzik, L., et al. (2017). Cerebrospinal fluid clearance in Alzheimer disease measured with dynamic PET. *J. Nucl. Med.* 58, 1471–1476. doi: 10.2967/jnumed.116.187211

Diop, O. M., Gueye, A., Dias-Tavares, M., Kornfeld, C., Faye, A., Ave, P., et al. (2000). High levels of viral replication during primary simian immunodeficiency virus SIVagm infection are rapidly and strongly controlled in African green monkeys. *J. Virol.* 74, 7538–7547. doi: 10.1128/JVI.74.16.7538-7547.2000

Do, T., Murphy, G., Earl, L. A., Del Prete, G. Q., Grandinetti, G., Li, G. H., et al. (2014). Three-dimensional imaging of HIV-1 virological synapses reveals membrane architectures involved in virus transmission. *J. Virol.* 88, 10327–10339. doi: 10.1128/JVI.00788-14

Duvernoy, H. M., and Risold, P. Y. (2007). The circumventricular organs: an atlas of comparative anatomy and vascularization. *Brain Res. Rev.* 56, 119–147. doi: 10.1016/j.brainresrev.2007.06.002

Eide, P. K., Vatnehol, S. A. S., Emblem, K. E., and Ringstad, G. (2018). Magnetic resonance imaging provides evidence of glymphatic drainage from human brain to cervical lymph nodes. *Sci. Rep.* 8, 7194–7194. doi: 10.1038/s41598-018-25666-4

Ferrell, D., and Giunta, B. (2014). The impact of HIV-1 on neurogenesis: implications for HAND. *Cell Mol. Life Sci.* 71, 4387–4392. doi: 10.1007/s00018-014-1702-4

Ferrer, I. (2017). Diversity of astroglial responses across human neurodegenerative disorders and brain aging. *Brain Pathol.* 27, 645–674. doi: 10.1111/bpa.12538

Filippidis, A. S., Carozza, R. B., and Rekate, H. L. (2016). Aquaporins in brain Edema and neuropathological conditions. *Int. J. Mol. Sci.* 18:55. doi: 10.3390/ijms18010055

Fletcher, C. V., Staskus, K., Wietgrefe, S. W., Rothenberger, M., Reilly, C., Chipman, J. G., et al. (2014). Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proc. Natl. Acad. Sci. U.S.A.* 111, 2307–2312. doi: 10.1073/pnas.1318249111

Frigeri, A., Nicchia, G. P., Verbavatz, J. M., Valenti, G., and Svelto, M. (1998). Expression of aquaporin-4 in fast-twitch fibers of mammalian skeletal muscle. *J. Clin. Invest.* 102, 695–703. doi: 10.1172/JCI2545

Gaberel, T., Gakuba, C., Goulay, R., de Lizarrondo, S. M., Hanouz, J. L., Emery, E., et al. (2014). Impaired glymphatic perfusion after strokes revealed by contrast-enhanced MRI: a new target for fibrinolysis? *Stroke* 45, 3092–3096. doi: 10.1161/STROKEAHA.114.006617

Gao, Q., Li, Y., and Chopp, M. (2005). Bone marrow stromal cells increase astrocyte survival via upregulation of phosphoinositide 3-kinase/threonine protein kinase and mitogen-activated protein kinase kinase/extracellular signal-regulated kinase pathways and stimulate astrocyte trophic factor gene expression after anaerobic insult. *Neuroscience* 136, 123–134. doi: 10.1016/j.neuroscience.2005.06.091

Golanov, E. V., Bovshik, E. I., Wong, K. K., Pautler, R. G., Foster, C. H., Federley, R. G., et al. (2018). Subarachnoid hemorrhage - induced block of cerebrospinal fluid flow: role of brain coagulation factor III (tissue factor). *J. Cereb. Blood Flow Metab.* 38, 793–808. doi: 10.1177/0271678X17701157

Goldstein, S., Ourmanov, I., Brown, C. R., Beer, B. E., Elkins, W. R., Plishka, R., et al. (2000). Wide range of viral load in healthy african green monkeys naturally infected with simian immunodeficiency virus. *J. Virol.* 74, 11744–11753. doi: 10.1128/JVI.74.24.11744-11753.2000

Goulay, R., Flament, J., Gauberti, M., Naveau, M., Pasquet, N., Gakuba, C., et al. (2017). Subarachnoid hemorrhage severely impairs brain parenchymal cerebrospinal fluid circulation in nonhuman primate. *Stroke* 48, 2301–2305. doi: 10.1161/STROKEAHA.117.017014

Guaraldi, G., Milic, J., and Mussini, C. (2019). Aging with HIV. *Curr HIV/AIDS Rep.* 16, 475–481. doi: 10.1007/s11904-019-00464-3

Guo, M. L., and Buch, S. (2019). Neuroinflammation & pre-mature aging in the context of chronic HIV infection and drug abuse: role of dysregulated autophagy. *Brain Res.* 1724:146446. doi: 10.1016/j.brainres.2019.146446

Haddow, L. J., Floyd, S. A., Copas, and Gilson, R. J. (2013). A systematic review of the screening accuracy of the HIV dementia scale and International HIV dementia scale. *PLoS ONE* 8:e61826. doi: 10.1371/journal.pone.0061826

Harder, D. R., Zhang, C., and Gebremedhin, D. (2002). Astrocytes function in matching blood flow to metabolic activity. *News Physiol. Sci.* 17, 27–31. doi: 10.1152/physiologyonline.2002.17.1.27

Hategan, A., Masliah, E., and Nath, A. (2019). HIV and Alzheimer's disease: complex interactions of HIV-Tat with amyloid beta peptide and Tau protein. *J. Neurovirol.* 25, 648–660. doi: 10.1007/s13365-019-00736-z

Hayakawa, K., Pham, L. D., Arai, K., and Lo, E. H. (2014). Reactive astrocytes promote adhesive interactions between brain endothelium and endothelial progenitor cells via HMGB1 and beta-2 integrin signaling. *Stem Cell Res.* 12, 531–538. doi: 10.1016/j.scr.2013.12.008

Heesters, B. A., Lindqvist, M., Vagefi, P. A., and Scully, E. P. (2015). Follicular dendritic cells retain infectious HIV in cycling endosomes. *PLoS Pathog.* 11:e1005285. doi: 10.1371/journal.ppat.1005285

Hinkle, J. T., Dawson, V. L., and Dawson, T. M. (2019). The A1 astrocyte paradigm: new avenues for pharmacological intervention in neurodegeneration. *Mov. Disord.* 34, 959–969. doi: 10.1002/mds.27718

Hinson, S. R., Roemer, S. F., Lucchinetti, C. F., Fryer, J. P., Kryzer, T. J., Chamberlain, J. L., et al. (2008). Aquaporin-4-binding autoantibodies in patients with neuromyelitis optica impair glutamate transport by down-regulating EAAT2. *J. Exp. Med.* 205, 2473–2481. doi: 10.1084/jem.20081241

Ho, J., Moir, S., Kulik, L., Malaspina, A., Donoghue, E. T., Miller, N. J., et al. (2007). Role for CD21 in the establishment of an extracellular HIV reservoir in lymphoid tissues. *J. Immunol.* 178, 6968–6974. doi: 10.4049/jimmunol.178.11.6968

Hong, S., and Banks, W. A. (2015). Role of the immune system in HIV-associated neuroinflammation and neurocognitive implications. *Brain Behav. Immun.* 45, 1–12. doi: 10.1016/j.bbi.2014.10.008

Hubbard, J. A., Szu, J. I., and Binder, D., K. (2018). The role of aquaporin-4 in synaptic plasticity, memory and disease. *Brain Res. Bull.* 136, 118–129. doi: 10.1016/j.brainresbull.2017.02.011

Huda, S., Whittam, D., Bhojak, M., Chamberlain, J., Noonan, C., and Jacob, A. (2019). Neuromyelitis optica spectrum disorders. *Clin. Med.* 19, 169–176. doi: 10.7861/clinmedicine.19-2-169

Huot, N., Bosinger, S. E., Paiardini, M., Reeves, R. K., and Muller-Trutwin, M. (2018). Lymph node cellular and viral dynamics in natural hosts and impact for HIV cure strategies. *Front. Immunol.* 9:780. doi: 10.3389/fimmu.2018.00780

Huot, N., Rascle, P., Garcia-Tellez, T., Jacquelin, B., and Muller-Trutwin, M. (2016). Innate immune cell responses in non pathogenic versus pathogenic SIV infections. *Curr. Opin. Virol.* 19, 37–44. doi: 10.1016/j.coviro.2016.06.011

Iliff, J. J., Chen, M. J., Plog, B. A., Zeppenfeld, D. M., Soltero, M., Yang, L., et al. (2014). Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. *J. Neurosci.* 34, 16180–16193. doi: 10.1523/JNEUROSCI.3020-14.2014

Iliff, J. J., Goldman, S. A., and Nedergaard, M. (2015). Implications of the discovery of brain lymphatic pathways. *Lancet Neurol.* 14, 977–979. doi: 10.1016/S1474-4422(15)00221-5

Iliff, J. J., Lee, H., Yu, M., Feng, T., Logan, J., Nedergaard, M., et al. (2013). Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. *J. Clin. Invest.* 123, 1299–1309. doi: 10.1172/JCI67677

Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., et al. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci. Transl. Med.* 4:147ra111. doi: 10.1126/scitranslmed.3003748

Jessen, N. A., Munk, A., Lundgaard, S. I., and Nedergaard, M. (2015). The glymphatic system: a beginner's guide. *Neurochem. Res.* 40, 2583–2599. doi: 10.1007/s11064-015-1581-6

Joshi, A. U., Minhas, P. S., Liddelow, S. A., Haileselassie, B., Andreasson, K. I., Dorn 2nd, G. W., et al. (2019). Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. *Nat. Neurosci.* 22, 1635–1648. doi: 10.1038/s41593-019-0486-0

Jung, E., Gardner, D., Choi, D., Park, E., Jin Seong, Y., Yang, S., et al. (2017). Development and characterization of a novel Prox1-EGFP lymphatic and schlemm's canal reporter rat. *Sci. Rep.* 7:5577. doi: 10.1038/s41598-017-06031-3

Koehler, R. C., Gebremedhin, D., and Harder, D., R. (2006). Role of astrocytes in cerebrovascular regulation. *J. Appl. Physiol.* 100, 307–317. doi: 10.1152/japplphysiol.00938.2005

Kress, B. T., Iliff, J. J., Xia, M., Wang, M., Wei, H. S., Zeppenfeld, D., et al. (2014). Impairment of paravascular clearance pathways in the aging brain. *Ann. Neurol.* 76, 845–861. doi: 10.1002/ana.24271

Li, C., Zhao, R., Gao, K., Wei, Z., Yaoyao Yin, M., Ting Lau, L., et al. (2011). Astrocytes: implications for neuroinflammatory pathogenesis of Alzheimer's disease. *Curr. Alzheimer Res.* 8, 67–80. doi: 10.2174/156720511794604543

Liao, S., and Padera, T. P. (2013). Lymphatic function and immune regulation in health and disease. *Lymphat. Res. Biol.* 11, 136–143. doi: 10.1089/lrb.2013.0012

Liddelow, S. A., and Barres, B. A. (2017). Reactive astrocytes: production, function, and therapeutic potential. *Immunity* 46, 957–967. doi: 10.1016/j.immuni.2017.06.006

Liddelow, S. A., Guttenplan, K. A., Clarke, L. E., Bennett, F. C., Bohlen, C. J., Schirmer, L., et al. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 541, 481–487. doi: 10.1038/nature21029

Louveau, A., Smirnov, I., Keyes, T. J., Eccles, J. D., Rouhani, S. J., Peske, J. D., et al. (2015). Structural and functional features of central nervous system lymphatic vessels. *Nature* 523, 337–341. doi: 10.1038/nature14432

Lundgaard, I., Lu, M. L., Yang, E., Peng, W., Mestre, H., Hitomi, E., et al. (2017). Glymphatic clearance controls state-dependent changes in brain lactate concentration. *J. Cereb. Blood Flow Metab.* 37, 2112–2124. doi: 10.1177/0271678X16661202

Luo, X., and He, J. J. (2015). Cell-cell contact viral transfer contributes to HIV infection and persistence in astrocytes. *J. Neurovirol.* 21, 66–80. doi: 10.1007/s13365-014-0304-0

Lutgen, V., Narasipura, S. D., Barbian, H. J., Richards, M., Wallace, J., Razmpour, R., et al. (2020). HIV infects astrocytes *in vivo* and egresses from the brain to the periphery. *PLoS Pathog.* 16:e1008381. doi: 10.1371/journal.ppat.1008381

Lv, Y., Huang, Q., Dai, W., Jie, Y., Yu, G., Fan, X., et al. (2018). AQP9 promotes astrocytoma cell invasion and motility via the AKT pathway. *Oncol. Lett.* 16, 6059–6064. doi: 10.3892/ol.2018.9361

Ma, T. H., Gao, H. W., Fang, X. D., and Yang, H. (2011). Expression and function of aquaporins in peripheral nervous system. *Acta Pharmacol. Sin.* 32, 711–715. doi: 10.1038/aps.2011.63

Mackiewicz, M. M., Overk, C., Achim, C. L., and Masliah, E. (2019). Pathogenesis of age-related HIV neurodegeneration. *J. Neurovirol.* 25, 622–633. doi: 10.1007/s13365-019-00728-z

Mader, S., and Brimberg, L. (2019). Aquaporin-4 water channel in the brain and its implication for health and disease. *Cells* 8:90. doi: 10.3390/cells8020090

Masjedi, A., Hassannia, H., Atyabi, F., Rastegari, A., Hojjat-Farsangi, M., Namdar, A., et al. (2019). Downregulation of A2AR by siRNA loaded PEG-chitosan-lactate nanoparticles restores the T cell mediated anti-tumor responses through blockage of PKA/CREB signaling pathway. *Int J. Biol. Macromol.* 133, 436–445. doi: 10.1016/j.ijbiomac.2019.03.223

Matchanova, A., Woods, S. P., and Kordovski, V. M. (2019). Operationalizing and evaluating the Frascati criteria for functional decline in diagnosing HIV-associated neurocognitive disorders in adults. *J. Neurovirol.* 26, 1–13. doi: 10.1007/s13365-019-00809-z

Matias, I., Morgado, J., and Gomes, F. C. A. (2019). Astrocyte heterogeneity: impact to brain aging and disease. *Front. Aging Neurosci.* 11:59. doi: 10.3389/fnagi.2019.00059

Mealy, M. A., Wingerchuk, D. M., Palace, J., Greenberg, B. M., and Levy, M. (2014). Comparison of relapse and treatment failure rates among patients with neuromyelitis optica: multicenter study of treatment efficacy. *JAMA Neurol.* 71, 324–330. doi: 10.1001/jamaneurol.2013.5699

Mestre, H., Hablitz, L., M., Xavier, A., L., Feng, W., et al. (2018). Aquaporin-4-dependent glymphatic solute transport in the rodent brain. *Elife* 7:e40070. doi: 10.7554/eLife.40070.022

Métral, M., Darling, K., Locatelli, I., Nadin, I., Santos, G., Brugger, P., et al. (2020). The neurocognitive assessment in the metabolic and aging cohort (NAMACO) study: baseline participant profile. *HIV Med.* 21, 30–42. doi: 10.1111/hiv.12795

Misawa, T., Arima, K., Mizusawa, H., and Satoh, J. (2008). Close association of water channel AQP1 with amyloid-beta deposition in Alzheimer disease brains. *Acta Neuropathol.* 116, 247–260. doi: 10.1007/s00401-008-0387-x

Nakada, T., Kwee, I. L., Igarashi, H., and Suzuki, Y. (2017). Aquaporin-4 functionality and virchow-robin space water dynamics: physiological model for neurovascular coupling and glymphatic flow. *Int. J. Mol. Sci.* 18:1798. doi: 10.3390/ijms18081798

Narasipura, S. D., Henderson, L. J., Fu, S. W., Chen, L., Kashanchi, F., and Al-Harthi, L. (2012). Role of beta-catenin and TCF/LEF family members in transcriptional activity of HIV in astrocytes. *J. Virol.* 86, 1911–1921. doi: 10.1128/JVI.06266-11

Olivier, I. S., Cacabelos, R., and Naidoo, V. (2018). Risk factors and pathogenesis of HIV-associated neurocognitive disorder: the role of host genetics. *Int. J. Mol. Sci.* 19:3594. doi: 10.3390/ijms19113594

Onanga, R., Kornfeld, C., Pandrea, I., Estaquier, J., Souquiere, S., Rouquet, P., et al. (2002). High levels of viral replication contrast with only transient changes in CD4(+) and CD8(+) cell numbers during the early phase of experimental infection with simian immunodeficiency virus SIVmnd-1 in Mandrillus sphinx. *J. Virol.* 76, 10256–10263. doi: 10.1128/JVI.76.20.10256-10263.2002

Orr, A. G., Hsiao, E. C., Wang, M. M., Ho, K., Kim, D. H., Wang, X., et al. (2015). Astrocytic adenosine receptor A2A and Gs-coupled signaling regulate memory. *Nat. Neurosci.* 18, 423–434. doi: 10.1038/nn.3930

Paiardini, M., and Muller-Trutwin, M. (2013). HIV-associated chronic immune activation. *Immunol. Rev.* 254, 78–101. doi: 10.1111/imr.12079

Papadopoulos, M. C., and Verkman, A. S. (2013). Aquaporin water channels in the nervous system. *Nat. Rev. Neurosci.* 14, 265–277. doi: 10.1038/nrn3468

Pérez, E., Barrachina, M., Rodríguez, A., Torrejón-Escribano, B., Boada, M., Hernández, I., et al. (2007). Aquaporin expression in the cerebral cortex is increased at early stages of Alzheimer disease. *Brain Res.* 1128, 164–174. doi: 10.1016/j.brainres.2006.09.109

Plog, B. A., Dashnaw, M. L., Hitomi, E., Peng, W., Liao, Y., Lou, N., et al. (2015). Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. *J. Neurosci.* 35, 518–526. doi: 10.1523/JNEUROSCI.3742-14.2015

Plog, B. A., and Nedergaard, M. (2018). The glymphatic system in central nervous system health and disease: past, present, and future. *Annu. Rev. Pathol.* 13, 379–394. doi: 10.1146/annurev-pathol-051217-111018

Pluta, R., Bogucka-Kocka, A., Ułamek-Kozioł, M., Bogucki, J., Januszewski, S., Kocki, J., et al. (2018a). Ischemic tau protein gene induction as an additional key factor driving development of Alzheimer's phenotype changes in CA1 area of hippocampus in an ischemic model of Alzheimer's disease. *Pharmacol. Rep.* 70, 881–884. doi: 10.1016/j.pharep.2018.03.004

Pluta, R., Ulamek-Koziol, M.., Januszewski, S., and Czuczwar, S. J. (2018b). Tau protein dysfunction after brain Ischemia. *J. Alzheimers Dis.* 66, 429–437. doi: 10.3233/JAD-180772

Potokar, M., J., Jorgačevski, and Zorec, R. (2016). Astrocyte aquaporin dynamics in health and disease. *Int. J. Mol. Sci.* 17:1121. doi: 10.3390/ijms17071121

Rainey-Smith, S. R., Mazzucchelli, G. N., Villemagne, V. L., Brown, B. M., Porter, T., Weinborn, M., et al. (2018). Genetic variation in Aquaporin-4 moderates the relationship between sleep and brain Aβ-amyloid burden. *Transl. Psychiatr.* 8:47. doi: 10.1038/s41398-018-0094-x

Rasmussen, M. K., Mestre, H., and Nedergaard, M. (2018). The glymphatic pathway in neurological disorders. *Lancet Neurol.* 17, 1016–1024. doi: 10.1016/S1474-4422(18)30318-1

Redzic, Z. (2011). Molecular biology of the blood-brain and the blood-cerebrospinal fluid barriers: similarities and differences. *Fluids Barriers CNS* 8:3. doi: 10.1186/2045-8118-8-3

Rey-Cuillé, M. A., Berthier, J. L., Bomsel-Demontoy, M. C., Chaduc, Y., Montagnier, L., Hovanessian, A. G., et al. (1998). Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. *J. Virol.* 72, 3872–3886. doi: 10.1128/JVI.72.5.3872-3886.1998

Ringstad, G., Valnes, L. M., Dale, A. M., Pripp, A. H., Vatnehol, S. A. S., Emblem, K. E., et al. (2018). Brain-wide glymphatic enhancement and clearance in humans assessed with MRI. *JCI Insight* 3:e121537. doi: 10.1172/jci.insight.121537

Ringstad, G., Vatnehol, S. A. S., and Eide, P. K. (2017). Glymphatic MRI in idiopathic normal pressure hydrocephalus. *Brain* 140, 2691–2705. doi: 10.1093/brain/awx191

Robinson-Papp, J., Gensler, G., Navis, A., Sherman, S., Ellis, R. J., Gelman, B. B., et al. (2019). Characteristics of motor dysfunction in longstanding HIV. *Clin. Infect. Dis.* doi: 10.1093/cid/ciz986. [Epub ahead of print].

Rojas-Celis, V., Valiente-Echeverría, F., Soto-Rifo, R., and Toro-Ascuy, D. (2019). New challenges of HIV-1 Infection: how HIV-1 attacks and resides in the central nervous system. *Cells* 8:1245. doi: 10.3390/cells8101245

Rosu, G. C., Pirici, I., Grigorie, A. A., Istrate-Ofiteru, A. M., Iovan, L., Tudorica, V., et al. (2019). Distribution of Aquaporins 1 and 4 in the central nervous system. *Curr. Health Sci. J.* 45, 218–226. doi: 10.12865/CHSJ.45.02.14

Russell, R. A., Chojnacki, J., Jones, D. M., Johnson, E., Do, T., Eggeling, C., et al. (2017). Astrocytes resist HIV-1 fusion but engulf infected macrophage material. *Cell Rep.* 18, 1473–1483. doi: 10.1016/j.celrep.2017.01.027

Sabri, F., Prados, A., Muñoz-Fernández, R., Lantto, R., Fernandez-Rubio, P., Nasi, A., et al. (2016). Impaired B cells survival upon production of inflammatory cytokines by HIV-1 exposed follicular dendritic cells. *Retrovirology* 13:61. doi: 10.1186/s12977-016-0295-4

Sakalauskaite-Juodeikiene, E., Armaliene, G., Kizlaitiene, R., Bagdonaite, L., Giedraitiene, N., Mickevičiene, D., et al. (2018). Detection of aquaporin-4 antibodies for patients with CNS inflammatory demyelinating diseases other than typical MS in Lithuania. *Brain Behav* 8:e01129. doi: 10.1002/brb3.1129

Saloner, R., Campbell, L. M., Serrano, V., Montoya, J. L., Pasipanodya, E., Paolillo, E. W., et al. (2019). Neurocognitive superaging in older adults living with HIV: demographic, neuromedical and everyday functioning correlates. *J. Int. Neuropsychol. Soc.* 25, 507–519. doi: 10.1017/S1355617719000018

Schain, A. J., Melo-Carrillo, A., Strassman, A. M., and Burstein, R. (2017). Cortical spreading depression closes paravascular space and impairs glymphatic flow: implications for migraine headache. *J. Neurosci.* 37, 2904–2915. doi: 10.1523/JNEUROSCI.3390-16.2017

Shi, Q., Wu, Y. Z., Yang, X., and Xiao, K. (2019). Significant enhanced expressions of aquaporin-1,−4 and−9 in the brains of various prion diseases. *Prion* 13, 173–184. doi: 10.1080/19336896.2019.1660487

Shijo, M., Hamasaki, H., Honda, H., Suzuki, S. O., Tachibana, M., Ago, T., et al. (2019). Upregulation of annexin A1 in reactive astrocytes and its subtle induction in microglia at the boundaries of human brain infarcts. *J. Neuropathol. Exp. Neurol.* 78, 961–970. doi: 10.1093/jnen/nlz079

St Hillaire, C., Vargas, D., Pardo, C. A., Gincel, D., Mann, J., Rothstein, J. D., et al. (2005). Aquaporin 4 is increased in association with human immunodeficiency virus dementia: implications for disease pathogenesis. *J. Neurovirol.* 11, 535–543. doi: 10.1080/13550280500385203

Stower, H. (2018). Meningeal lymphatics in aging and Alzheimer's disease. *Nat. Med.* 24:1781. doi: 10.1038/s41591-018-0281-6

Sun, L., Zhang, Y., Liu, E., Ma, Q., Anatol, M., Han, H., and Yan, J. (2019). The roles of astrocyte in the brain pathologies following ischemic stroke. *Brain Inj* 33, 712–716. doi: 10.1080/02699052.2018.1531311

Sweeney, A. M., Pla, V., Du, T., Liu, G., Sun, Q., Peng, S., et al. (2019). *In vivo* imaging of cerebrospinal fluid transport through the intact mouse skull using fluorescence macroscopy. *J. Vis. Exp.* doi: 10.3791/59774

Thrane, A. S., Rangroo Thrane, V., and Nedergaard, M. (2014). Drowning stars: reassessing the role of astrocytes in brain edema. *Trends Neurosci.* 37, 620–628. doi: 10.1016/j.tins.2014.08.010

Valcour, V., Sithinamsuwan, P., Letendre, S., and Ances, B. (2011). Pathogenesis of HIV in the central nervous system. *Curr HIV/AIDS Rep.* 8, 54–61. doi: 10.1007/s11904-010-0070-4

Winston, A., and Spudich, S. (2020). Cognitive disorders in people living with HIV. *Lancet HIV* 7, e504–e513. doi: 10.1016/S2352-3018(20)30107-7

Wu, J., Ding, D., Wang, X., Li, Q., Sun, Y., Li, L., and Wang, Y. et al. (2019). Regulation of aquaporin 4 expression by lipoxin A4 in astrocytes stimulated by lipopolysaccharide. *Cell Immunol.* 344:103959. doi: 10.1016/j.cellimm.2019.103959

Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., et al. (2013). Sleep drives metabolite clearance from the adult brain. *Science* 342, 373–377. doi: 10.1126/science.1241224

Xing, H. Q., Zhang, Y., Izumo, K., Arishima, S., Kubota, R., Ye, X., et al. (2017). Decrease of aquaporin-4 and excitatory amino acid transporter-2 indicate astrocyte dysfunction for pathogenesis of cortical degeneration in HIV-associated neurocognitive disorders. *Neuropathology* 37, 25–34. doi: 10.1111/neup.12321

Xu, M., Xiao, M., Li, S., and Yang, B. (2017). Aquaporins in nervous system. *Adv. Exp. Med. Biol.* 969, 81–103. doi: 10.1007/978-94-024-1057-0_5

Yang, H. Y., Beymer, M. R., and Suen, S., C. (2019). Chronic disease onset among people living with HIV and AIDS in a large private insurance claims dataset. *Sci. Rep.* 9:18514. doi: 10.1038/s41598-019-54969-3

Yool, A. J. (2007). Aquaporins: multiple roles in the central nervous system. *Neuroscientist* 13, 470–485. doi: 10.1177/1073858407303081

Zador, Z., Stiver, S., Wang, V., and Manley, G. T. (2009). Role of aquaporin-4 in cerebral edema and stroke. *Handb. Exp. Pharmacol.* 2009:159–170. doi: 10.1007/978-3-540-79885-9_7

Zamanian, J. L., Xu, L., Foo, L. C., Nouri, N., Zhou, L., Giffard, R. G., et al. (2012). Genomic analysis of reactive astrogliosis. *J. Neurosci.* 32, 6391–6410. doi: 10.1523/JNEUROSCI.6221-11.2012

Zbesko, J. C., Nguyen, T. V. V., Yang, T., Frye, J. B., Hussain, O., Hayes, M., et al. (2018). Glial scars are permeable to the neurotoxic environment of

chronic stroke infarcts. *Neurobiol. Dis.* 112, 63–78. doi: 10.1016/j.nbd.2018.01.007

Zhang, Y., Xu, K., Liu, Y., Erokwu, B. O., Zhao, P., Flask, C. A., et al. (2019). Increased cerebral vascularization and decreased water exchange across the blood-brain barrier in aquaporin-4 knockout mice. *PLoS ONE* 14:e0218415. doi: 10.1371/journal.pone.0218415

Zhao, Z. A., Li, P., Ye, S. Y., Ning, Y. L., Wang, H., Peng, Y., et al. (2017). Perivascular AQP4 dysregulation in the hippocampal CA1 area after traumatic brain injury is alleviated by adenosine A2A receptor inactivation. *Sci. Rep.* 7:2254. doi: 10.1038/s41598-017-02505-6

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: 01 July 2021
doi: 10.3389/fcimb.2021.535352

Human Beta-Defensin 2 and 3 Inhibit HIV-1 Replication in Macrophages

Jennifer P. Bharucha[1], *Lingling Sun*[1], *Wuyuan Lu*[1,2], *Suzanne Gartner*[1,3] *and Alfredo Garzino-Demo*[1,4,5*]

[1] Division of Virology, Pathogenesis, and Cancer, Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, United States, [2] Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD, United States, [3] Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, United States, [4] Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States, [5] Department of Molecular Medicine, University of Padova, Padova, Italy

Human beta-defensins (hBDs) are broad-spectrum antimicrobial peptides, secreted by epithelial cells of the skin and mucosae, and astrocytes, which we and others have shown to inhibit HIV-1 in primary $CD4^+$ T cells. Although loss of $CD4^+$ T cells contributes to mucosal immune dysfunction, macrophages are a major source of persistence and spread of HIV and also contribute to the development of various HIV-associated complications. We hypothesized that, besides T cells, hBDs could protect macrophages from HIV. Our data in primary human monocyte-derived macrophages (MDM) *in vitro* show that hBD2 and hBD3 inhibit HIV replication in a dose-dependent manner. We determined that hBD2 neither alters surface expression of HIV receptors nor induces expression of anti-HIV cytokines or beta-chemokines in MDM. Studies using a G-protein signaling antagonist in a single-cycle reporter virus system showed that hBD2 suppresses HIV at an early post-entry stage *via* G-protein coupled receptor (GPCR)-mediated signaling. We find that MDM express the shared chemokine-hBD receptors CCR2 and CCR6, albeit at variable levels among donors. However, cell surface expression analyses show that neither of these receptors is necessary for hBD2-mediated HIV inhibition, suggesting that hBD2 can signal *via* additional receptor(s). Our data also illustrate that hBD2 treatment was associated with increased expression of APOBEC3A and 3G antiretroviral restriction factors in MDM. These findings suggest that hBD2 inhibits HIV in MDM *via* more than one CCR thus adding to the potential of using β-defensins in preventive and therapeutic approaches.

Keywords: macrophages, HIV-1, human β-defensin 2, CCRs, APOBEC3G, APOBEC3A

OPEN ACCESS

Edited by:
John Hiscott,
Istituto Pasteur Italia Cenci Bolognetti Foundation, Italy

Reviewed by:
Sharof M. Tugizov,
University of California, San Francisco, United States
Ge Jin,
Case Western Reserve University, United States

****Correspondence:***
Alfredo Garzino-Demo
agarzinodemo@ihv.umaryland.edu;
alfredo.garzinodemo@unipd.it

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *15 February 2020*
Accepted: *17 June 2021*
Published: *01 July 2021*

Citation:
Bharucha JP, Sun L, Lu W, Gartner S and Garzino-Demo A (2021) Human Beta-Defensin 2 and 3 Inhibit HIV-1 Replication in Macrophages. Front. Cell. Infect. Microbiol. 11:535352. doi: 10.3389/fcimb.2021.535352

INTRODUCTION

Mucosal surfaces, especially of the genital and gastrointestinal tracts, are the primary sites of initial transmission of HIV-1. The virus can reach target cells across both intact and damaged mucosal surfaces *via* different mechanisms [reviewed in (Moutsopoulos et al., 2006)]. Virus may also enter *via* damaged mucosal surfaces to infect susceptible dendritic cells (DCs), macrophages and T cells.

Regardless of the mode of entry, once the virus has breached the mucosal barrier and entered susceptible target cells, including macrophages, it is subsequently transported *via* the lymphatic system and blood stream to other sites in the body.

Macrophages are versatile cells of the immune system. They can independently recognize and attack foreign antigens, activate various aspects of the innate immune response, as well as interact with and activate cells of the adaptive immune response (Dobrovolskaia and Vogel, 2002; Mantovani et al., 2004; Gordon and Taylor, 2005; Gordon and Mantovani, 2011; Sica and Mantovani, 2012; Wynn et al., 2013). Macrophages are susceptible to infection by HIV and are in mucosae, potentially exposing them to infection during heterosexual transmission (Greenhead et al., 2000). Several studies have shown that cells of the monocyte/macrophage lineage serve as both, an active site for virus replication and dissemination through the body (Gartner et al., 1986b), especially to protected sites such as perivascular macrophages (Williams et al., 2001) and microglia in the central nervous system (Gartner et al., 1986a; Koenig et al., 1986), and as a reservoir of latent virus (Gendelman et al., 1989; Brown et al., 2006; Li et al., 2010; Honeycutt et al., 2016; Honeycutt et al., 2017; Ganor et al., 2019; Ko et al., 2019). Additionally, infected macrophages alter the innate immune response, making the host more vulnerable to other infections. Thus, by virtue of their importance in the regulation of the immune response, their relatively long life span, and their susceptibility to infection, macrophages contribute to the persistence and amplification of HIV infection [reviewed in (Alexaki et al., 2008; Koppensteiner et al., 2012; Churchill and Nath, 2013)].

Current antiretroviral therapy (ART) for HIV infection has evolved tremendously over the past thirty years and has resulted in significant reductions in morbidity and mortality. Despite these advances, toxicity, multi-drug resistance, lack of response to drugs, failure to restore immune competence and to eradicate latent virus reservoirs are some of the common problems associated with ART. The problem is further compounded by the high cost, lack of compliance, and/or unavailability of treatment and patients also remain susceptible to the serious complications of AIDS. In particular, while the introduction of cART has significantly decreased the occurrence of HIV-associated dementia, and the incidence of AIDS, the prevalence of HIV-Associated Neurocognitive Disorders (HAND) has increased despite long standing viremia suppression [reviewed in (Broder, 2010; Deeks, 2013; Nath and Tyler, 2013)]. Hence, there is an urgent need to develop strategies that serve as complementary or alternative therapies.

Components of innate immunity participate to control HIV infection and studying their mechanisms of action may contribute to the development of new treatments. Our studies highlight potential therapeutic application of human defensins and the pathways that they induce in cells susceptible to HIV infection.

Defensins are a heterogeneous group of small molecular weight peptides that exhibit potent antimicrobial properties against a broad variety of pathogens, including bacteria, fungi, and viruses [reviewed in (Lehrer and Lu, 2012; Jarczak et al., 2013; Wilson et al., 2013; Holly et al., 2017; Brice and Diamond, 2020)]. In addition, they are involved in stimulation, proliferation, differentiation, morphogenesis, motility and function of immune cells, hence, playing significant roles in both innate and adaptive immunity [reviewed in (Yang et al., 2007)]. Several studies have also identified defensins as potential immunotherapies for different cancers (Papo and Shai, 2005; Kesting et al., 2012; Mei et al., 2012), auto-immune (Badr, 2013) and inflammatory disorders (Niyonsaba et al., 2001; Poiraud et al., 2012). In humans, two major subclasses, α- and ß-defensins, are produced. Human β-defensins are produced primarily by epithelial cells of diverse mucosal tissues and by monocytes, macrophages, astrocytes and DCs [reviewed in (Yang et al., 2007)]. Immunohistochemical studies show that hBD2 is constitutively expressed in the oral mucosa of normal healthy individuals, producing a barrier across the epithelium. In contrast, a previous study from our group showed that hBD2 levels were not detectable in HIV-1-positive individuals (Sun et al., 2005), which may predispose them to oral complications of AIDS.

Studies by various groups over the years show that both α- and ß-defensins exhibit anti-HIV activity *in vitro*. α-defensins can inhibit HIV-1 replication by direct interaction and inactivation of the virions or by affecting target cells (Nakashima et al., 1993; Mackewicz et al., 2003; Wang et al., 2004; Chang et al., 2005). Previous studies from our laboratory (Sun et al., 2005; Lafferty et al., 2010; Lafferty et al., 2017) and from others (Quinones-Mateu et al., 2003; Feng et al., 2006) show that hBD2 and hBD3 elicit anti-HIV activity in peripheral blood mononuclear cells (PBMCs) and $CD4^+$ T cells. It was further shown by our group (Lafferty et al., 2010; Lafferty et al., 2017) that hBD2 inhibits HIV at an early stage post-entry and the intracellular mechanism involves induction of the host anti-viral restriction factor apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G) *via* the CC chemokine receptor 6 (CCR6). CCR6 is expressed on cells that are highly permissive to HIV infection, including memory T cells, Th_{17} cells, α4ß7 cells, and microglia (Liao et al., 1999; Flynn et al., 2003; Acosta-Rodriguez et al., 2007; Singh et al., 2008; El Hed et al., 2010; Gosselin et al., 2010; Monteiro et al., 2011; Christensen-Quick et al., 2016; Lafferty et al., 2017). It has been shown that chemokine receptors can be functionally bound by non-chemokine ligands such as defensins (Yang et al., 1999; Jin et al., 2010; Lafferty et al., 2010; Rohrl et al., 2010).

Due to the lack of studies on macrophages, we tested the ability of hBD2 to inhibit HIV-1 infection in primary human monocyte-derived macrophages (MDM) and elucidate its mechanism(s) of action. We demonstrate that in MDM, hBD2 inhibits HIV post-entry *via* more than one mechanism. It inhibits virus at an early stage in the life cycle *via* binding to and signaling through more than one CCR type that results in induction of anti-retroviral restriction factors of the APOBEC3 family i.e. 3G and 3A. This suggests that the mechanism(s) of hBD2-mediated HIV-1 inhibition in primary human macrophages is quite different from that in primary human $CD4^+$ T cells.

MATERIALS AND METHODS

Ethics Statement

Human PBMCs were isolated from healthy blood donor's leukopaks obtained from New York Blood Center, Long Island, NY, in accordance with their guidelines. Donors were anonymous; hence, patient consent was not required.

Reagents

Recombinant human IFN-α was obtained from R&D Systems, Inc. The HIV-1 reverse transcriptase (RT) inhibitor azidothymidine (AZT); the chemical antagonist of CCR2, RS102895, Cytochalasin D (CytD) and paraformaldehyde were purchased from Sigma-Aldrich. Pertussis toxin (Bordetella pertussis, glycerol solution) was from Calbiochem. TURBO DNase I was from Ambion. The HIV-1 fusion inhibitor T20 peptide was a kind gift from Dr. Lai-Xi Wang at the Institute of Human Virology.

Isolation and Culture of Primary Cells

Human PBMCs were isolated from leukopaks from healthy human subjects with the use of Histopaque-1077 (Sigma-Aldrich). Monocyte-derived macrophages (MDM) were prepared by adherence method. Cells were plated at ~$2x10^6$ cells/ml in 100mm petri dishes (Corning) and left to differentiate for 5–7 days in Roswell Park Memorial Institute (RPMI)-1640 (Cellgro, Mediatech, Inc.) complete medium (which is supplemented with 1% penicillin/streptomycin, 2 mM L-glutamine [Quality Biochemical, Gaithersburg, MD], 20% heat-inactivated Fetal Bovine Serum [Gemini Bio-Products]) in the presence of 10% human AB serum (Gemini Bio-Products). Non-adherent cells were removed by thorough washing and differentiated macrophages were cultured in RPMI-1640 complete medium only (no human serum here onwards). In this study, differentiated macrophages were detached with StemPro Accutase (GIBCO by Life Technologies) following manufacturer's recommendations and gentle scraping when needed. Flow cytometry analyses confirmed that more than 95% of the adherent cells were macrophages. Cell viability was determined using trypan blue exclusion method.

Virus Production

HIV-1_{BaL} (R5 isolate) virus stocks were prepared in MDM, while the transmitted-founder isolate HIV-1AD17 (kindly provided by George Shaw was produced in PM1 cells (Li et al., 2010). Virus p24 levels were measured using a commercial ELISA kit (Perkin Elmer, Foster City, CA). $TCID_{50}$ of virus stocks were determined in PBMCs using the protocol of ACTG Laboratory Technologist Committee. Luciferase reporter pseudotyped virus was generated by cotransfection of 293T cells with pNL4-3.Luc.R^-E^- plasmid which has the firefly luciferase gene inserted into the *nef* gene, and an Amphotropic Murine Leukemia Virus (AMLV) envelope-expressing plasmid, using Fugene 6 (Roche Diagnostics and Promega) and plasmids kindly provided by Dan R. Littman (New York University) (Page et al., 1990). Supernatants from 293T cells were harvested 72 hours after cotransfection and p24 levels measured as described above. Virus was concentrated, if needed, by ultra-centrifugation on a sucrose cushion. Virus was titrated in primary MDM.

Total Chemical Synthesis of Human β-Defensins

hBDs 1, 2 and 3 were chemically synthesized by solid phase peptide synthesis with a custom-modified procedure tailored from the published *in situ* neutralization protocol developed for Boc chemistry (Schnolzer et al., 1992). The syntheses, purification, folding, and characterizations were published previously (Wu et al., 2003). The beta connectivity of three disulfide bonds (Cys1-Cys5, Cys2-Cys4, and Cys3-Cys6) in highly pure synthetic hBDs 1 to 3 was independently verified by mass mapping of peptide fragments generated by enzymatic digestion and Edman degradation (Wu et al., 2003). Protein concentrations were determined by absorbance measurements at 280 nm using molar extinction coefficients calculated according to a published algorithm (Pace et al., 1995).

Cell Metabolism Assay

Cell metabolism was determined by using the MTS [3,4-(5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt] assay (Promega, Madison, WI), which measures conversion of MTS tetrazolium into formazan by cellular dehydrogenase enzymes in metabolically active cells. For this, MDM (10^5 cells/well) were cultured in triplicate in 96-well plates with media alone as a control or hBDs for 3-4 days and then the MTS/PMS mixture was added as per manufacturer's protocol and incubated for 1 to 4 hours before spectrophotometric absorbance reading at 490 nm. Triplicate readings were averaged and OD ratios of treated/control cells were calculated as percentages.

Infectivity Assays

MDM ($2x10^6$ cells/ml) were infected with 6-$8x10^4$ $TCID_{50}$/ml of HIV-1_{BaL} for 2 hours followed by three washes, and then cultured at 10^5 cells/well of 96-well plate in RPMI complete medium with the appropriate treatment(s) up to 14 days at 37°C. Half of the culture supernatants were changed with fresh medium on days 4, 7 and 10. Each culture was performed in triplicate. As a positive control, cells were pretreated with AZT (10 μM) prior to infection. Infection/HIV-1 replication was determined by measuring the amount of p24 antigen in the culture supernatants using a commercially available p24 antigen capture assay kit (Perkin Elmer, Foster City, CA) following manufacturer's protocol. For experiments with the chemical antagonist of CCR2, RS102895, after infection of MDM with HIV-1BaL, cells were pretreated with the inhibitor (15 μM) at 37°C for 2 hours, followed by addition of hBD2, and cultured as described above. Percentage of inhibition in treated cells was calculated with the use of the following formula: % Inhibition= [1 – ($p24_{treated}/p24_{control}$)] x 100, where $p24_{treated}$ and $p24_{control}$ are concentrations of HIV-1 p24 measured in supernatants of treated and untreated cells, respectively.

Single-round infections were performed with the use of AMLV envelope-pseudotyped HIV-1. MDM were incubated

with pseudotyped virus for 3 hours and subsequently washed with PBS and then cultured ($2x10^5$ cells/well of 48-well plate) in RPMI complete medium in the presence or absence of hBD2 (4.7 μM) for 3 days at 37°C. Following this, infected cells were washed with PBS and lysed with Reporter Lysis Buffer (SteadyGlo kit, Promega), and luciferase activity was measured in a Turner Luminometer. For inhibition of G_i-protein signaling with pertussis toxin (PTx), cells were infected with pseudotyped virus as described above followed by treatment with PTx (100 ng/ml) for at least 3 hours and then cultured in the presence or absence of hBD2.

Flow Cytometry

Effect of hBD2 on cell surface expression of HIV-1 receptor and co-receptors, CD4, CCR5 and CXCR4 was analyzed by flow cytometry. For this, MDM were treated with hBD2 and hBD3 (4.7 μM) for time periods described in the *Results* section below, after which the cells were harvested, processed and stained. Briefly, the cells were washed with cold FACS buffer (PBS containing 2% FBS and 0.1% Na-azide), and then blocked with either 5% human AB serum or human FcR blocking reagent (Miltenyi Biotec) in FACS buffer for up to 30 minutes followed by washing and staining with mouse monoclonal anti-human antibodies (mAbs) for 30 minutes at 4°C in the dark. After incubation, cells were washed and fixed in 1% paraformaldehyde in FACS buffer. The cell preparations were analyzed with a FACS Calibur flow cytometer (BD Biosciences, CA). Macrophage surface markers including CD14, CD11b, CD36, and HLA-DR were used to identify and gate on macrophages. Live cells were gated to exclude all nonviable cells and debris according to forward and scatter profiles. These gated events were further analyzed for expression of CD4, CCR5 and CXCR4. Positives and negatives were determined by comparison with matching isotype controls. The following mAbs were used: PE-conjugated anti-CD4, APC-conjugated anti-CCR5, and PE-conjugated anti-CXCR4 (all from BD Pharmingen, San Jose, CA). In all cases, isotype-matched control mAbs were used. For flow cytometry analysis of cell surface expression of CCR2 and CCR6 on MDM, similar FACS staining method was used with following mAbs: APC-conjugated anti-CCR2 (R&D Systems, Inc.) and PE-conjugated anti-CCR6 (BD Pharmingen). All data were analyzed using FlowJo software (Tree Star Inc., San Carlos, CA).

Quantitation of Interferons and β-Chemokines in hBD2 Treated MDM Supernatants

Supernatants derived from MDM incubated for different times up to 24 hours with hBD2 (4.7 μM) were tested for the presence of interferon-α and –β and the β-chemokines RANTES, MIP-1α and MIP-1β by commercial ELISA kits following the manufacturer's protocols (R&D Systems, Inc.).

Real-Time Quantitative PCR of HIV-1 DNA

MDM (10^6 cells/time point) were untreated or pretreated with AZT (10 μM) or fusion inhibitor T20 (2.5 μM) and infected for 2 hours at 37°C with 10^5 $TCID_{50}$/ml of DNase I–treated $HIV\text{-}1_{BaL}$. Cells were washed to remove extracellular virus, and resuspended in RPMI complete media, and infected untreated cells were incubated in the presence or absence of hBD2 (4.7 μM). Total cellular DNA was extracted with the use of the DNeasy Blood and Tissue Kit (QIAGEN) at 6, 12, 24 and 48 hours post-infection. DNA was analyzed by real-time quantitative PCR to determine the number copies of early (negative strand strong stop) reverse transcripts present with iQSYBR green supermix (Bio-Rad) and primers F: 5'-GGCTAACTAGGGAACCCACTG-3' and R: 5'-CTGCTAGAGATTTTCCACACTGAC-3' (Lafferty et al., 2010). Transcript levels were normalized using endogenous *albumin* gene as a reference. Primers used for *albumin* are F: 5'-TGTTGCATGAGAAAACGCCA -3' and R: 5'-GTCGCCTGTTCACCAAGGAT -3' (Lafferty et al., 2010). Annealing temperatures of 60°C and 62°C were used for strong stop and *albumin*, respectively. Reactions were performed in triplicate with the use of a Bio-Rad iQ5 Real-Time PCR machine. A standard curve for number of HIV-1 DNA copies was set up with dilutions of HxB2 plasmid DNA. Data was analyzed with Bio-Rad iQ5 and Microsoft Excel software.

Real-Time Quantitative RT-PCR Analysis of APOBEC3G and APOBEC3A

Cells (10^6 cells per time point) were untreated or treated with hBD2 (4.7 μM) or IFN-α (1000 U/ml). RNA was extracted with the Rneasy Kit (Qiagen) at the indicated time points. First strand cDNA was synthesized from 500 ng total RNA with iScript cDNA Synthesis Kit (Bio-Rad). cDNA was analyzed by real-time quantitative PCR with iQSYBR green supermix (Bio-Rad) with the use of primers specific for APOBEC3G; F: 5'-CGCAGCCTG TGTCAGAAAAG-3' and R: 5'-CCAACAGTGCTGA AATTCGTCATA-3' (Jin et al., 2005), and for 18S ribosomal RNA; F: 5'-ATCAACTTTCGATGGTAGTCG-3' and R: 5'-TCCTTGGATGTGGTAGCCG-3' (Lafferty et al., 2010) and annealing temperature of 60°C. For APOBEC3A, cDNA was synthesized as described above and analyzed by real-time quantitative PCR with iQ Supermix (Bio-Rad) and Taqman primer-probe sets Hs00377444_m1 APOBEC3A-FAM and Hs03928985_g1 RN18S1-VIC_PL (Applied Biosystems) and annealing temperature of 60°C. In both cases, all reactions were performed in triplicate with the use of a Bio-Rad iQ5 Real-Time PCR machine. Data was analyzed with Bio-Rad iQ5 and Microsoft Excel software. The ΔΔCt method was used to calculate fold change between untreated and treated cells normalized to 18S ribosomal RNA.

Immunoblotting

For CCR6 western blots, untreated MDM and JKT-FT7 cells were lysed with RIPA buffer (Sigma) containing 0.1 mM PMSF, 1X EDTA-free protease inhibitor cocktail (Sigma). Total protein concentration was determined with the BCA Protein Assay kit (Pierce, Thermo Scientific, Inc.), and equal amounts of total protein were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis. Immunoblots were probed with either mouse monoclonal anti-human CCR6

antibody (MAB195, R&D Systems, Inc.) or rabbit polyclonal anti-human CCR6 antibody (ab78429, Abcam Inc.) followed by horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibodies (Santa Cruz Biotechnology) detected with the ECL-plus kit (GE Healthcare, Bucks, UK) on a PharosFX Plus Molecular Imager (Bio-Rad). Immunoblotting for CCR6 in $CD4^+$ T $CCR6^+$ and $CCR6^-$ sorted cell lysates was performed on a separate blot that was previously used for another experiment in our lab. The low frequency of these subsets of cells in total human PBMCs and the high cost involved limits the availability of whole cell lysates from these populations.

For APOBEC3 induction experiments, MDM were incubated in the presence or absence of increasing concentrations of hBD2 or IFN-α (1000 U/ml) for 0, 4, 8, and 24 hours. Cells were lysed as described above. Immunoblots were probed with either rabbit polyclonal anti-hAPOBEC3G antisera or rabbit anti-hAPOBEC3G-C antisera for APOBEC3A and mouse anti-hβ-actin antibody (Abcam Inc.) or rabbit anti-GAPDH monoclonal antibody (Cell Signaling Technology, Inc.) as load control followed by horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies (Santa Cruz Biotechnology) detected with the ECL-plus kit (GE Healthcare, Bucks, UK). The polyclonal anti-APOBEC3G antibodies from Drs. Warner C. Greene (#9968), Klaus Strebel (anti-ApoC17 #10082), and Jaisri Lingappa (#10201) and, rabbit anti-APOBEC3G-C antibody from Dr. Klaus Strebel (#9906) (Kao et al., 2003) used to detect APOBEC3A were obtained through the National Institutes of Health AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Densitometric quantification of protein levels was done with Quantity One software, version 4.6.9 (Bio-Rad Laboratories, Inc., CA, USA). Each lane/sample was normalized to its respective load control, and background signal for the blot was subtracted from all lanes.

Replicates

With the exception of FACS analyses and pseudotyped HIV infections, every experiment was performed in triplicate with cells from a particular donor and was performed independently in cells from 3 or more donors. The number of donors used is noted in each figure legend as *n*.

Data analyses was performed using Microsoft Excel and GraphPad Prism 5. Images and figures were prepared using Adobe Photoshop 7.0 or CS software (Adobe Systems, San Jose, CA).

RESULTS

hBD2 Inhibits HIV-1 Replication in Macrophages in a Dose-Dependent Manner

To evaluate the effect of hBDs 1, 2 and 3 on replication of R5 virus in primary human MDM, cells were infected with $1.24x10^3$ $TCID_{50}$ of $HIV\text{-}1_{BaL}$ and then cultured in the absence or presence of hBD 1, 2 or 3, at a final concentration of 4.7 μM, for up to 14 days. AZT was used as a positive control in all infection experiments. Release of HIV-1 p24 was quantified in supernatants every 3 days starting at day 7 after infection. **Figure 1A** shows p24 amounts in the culture supernatants. Both hBD2 (40-57%) and hBD3 (70-80%) inhibited HIV-1 replication, in contrast to hBD1 which, at the concentration tested, appears to increase HIV-1 replication in MDM. To test for toxicity of hBDs on MDM, cell metabolism was measured by the MTS assay on cells treated with hBD 1, 2 or 3 for 3 to 4 days. As shown in **Figure 1C**, at the concentrations used, these ß-defensins have a marginal positive effect on cell metabolism in MDM. Although hBD3 shows more potent inhibition of HIV-1 infection in macrophages as compared to hBD2 and it does not appear to be affecting cell metabolism, it was shown to decrease proliferation of PBMCs (Sun et al., 2005), thereby making it a less desirable candidate as compared to hBD2 for further studies. Hence, from here onward, studies to determine and characterize the mechanism(s) of ß-defensin-mediated suppression of HIV-1 infection in macrophages were limited to hBD2. To evaluate the effect of different concentrations of hBD2 on HIV infection in MDM, cells were infected and cultured as described above in the presence or absence of hBD2 concentrations ranging from 0.9 to 23.3 μM (corresponds to 4 to 100 μg/ml). As shown in **Figure 1B**, hBD2 significantly inhibited R5 virus replication in a dose-dependent manner. We also tested the effect of hBD2 in a transmitted/founder virus, AD17 (Li et al., 2010). As seen with BaL, 4.7 μM concentration shows inhibition ranging from 45-65% over time, and the higher concentration of 23.3 μM shows inhibition ranging from 70-80% over time (**Figure 1D**). These results, taken together with published reports in PBMCs and $CD4^+$T cells (Quinones-Mateu et al., 2003; Sun et al., 2005), suggest that the effect of hBD2 on HIV-1 replication extends to macrophages. Since the concentration of 4.7 μM hBD2 is well within the range of secreted hBD2 measured in oral mucosa and epidermal tissues (Liu et al., 2002), we used this concentration in further studies.

hBD2 Does Not Alter Surface Expression of HIV Receptors on Macrophages

Previous studies have shown that β-defensins alter HIV-1 coreceptor CXCR4 expression on PBMCs and $CD4^+$ T cell lines (Quinones-Mateu et al., 2003; Feng et al., 2006). This raises the possibility that interaction of hBD2 with macrophages may affect the expression pattern of HIV-1 receptor and coreceptors. To test this hypothesis, we measured cell surface expression of CD4, CCR5 and CXCR4 on cells after treatment with hBD2 for different time periods. To this end, we treated uninfected MDM with hBD2 for 1, 2, 3, and 24 hours followed by staining with PE-CD4 or PerCP-CD4, APC-CCR5, PE-CXCR4, and isotype-matched control antibodies and flow cytometric analyses. hBD2 had no significant effect on the surface expression of CD4, CXCR4 or CCR5 on MDM. **Figure 2** shows the results at 1 and 24 hours post-treatment with hBD2; similar results were obtained with 2 and 3 hours of treatment (data not shown). **Table 1** shows the median fluorescence intensity (MFI) values for each surface marker at the different times tested. These results indicate that treatment

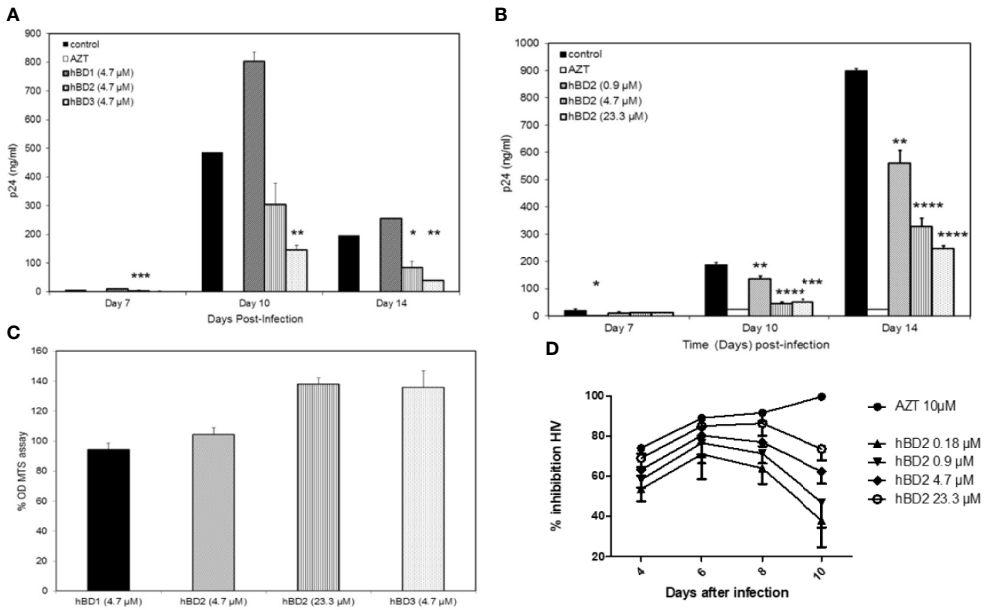


FIGURE 1 | Human β-defensins inhibit HIV-1 replication in macrophages *in vitro*. MDM were infected with HIV-1_{BaL}. After virus removal and washing, hBD1, hBD2, and hBD3 (4.7 µM) **(A)** or increasing concentrations of hBD2 (0-23.3 µM) **(B)** were added to cultures. Cells were pretreated with AZT as control. Infection was monitored by assaying supernatants for HIV p24 production by ELISA at the times indicated. Data are presented as mean ± SEM of triplicates. Representative experiment, *n*=3. *P < 0.05, **P < 0.005, ***P < 0.0005, ****P < 0.0001 between treatment and control groups determined with unpaired two-tailed *t* test. **(C)** ß-defensins are not toxic to macrophages at the concentrations that inhibit HIV-1. Cells treated with hBDs were tested using MTS assay. Cells were cultured in triplicate in 96-well plates for 3 days in the presence or absence of β-defensins; MTS mix was added and incubated 1 to 4 hrs prior to spectrophotometric absorbance readings at 490 nm. Triplicate readings were averaged (± SEM) and percentage OD ratios of treated/control cells were calculated. **(D)** hBD2 inhibit infection of MDM with a transmitted-founder HIV strain. MDM were infected with transmitter-founder virus AD17. After virus removal and washing, hBD2 at concentrations indicated above were added to cultures. HIV p24 release in supernatants was monitored by ELISA at the time indicated, and % inhibition was calculated as % of HIV p24 production from untreated MDM. Data are presented as mean ± SEM of triplicates, N=3.

with hBD2 does not modulate HIV receptors. We observed similar results treating MDMs with hBD3 (**Figure S1**).

hBD2 Does Not Reproducibly Induce Expression of Anti-Viral Cytokines or β-Chemokines in Macrophages

Type I interferons (IFN) -α and -ß are induced in response to viral infections and are the cytokines that suppress HIV replication both *in vitro* and *in vivo* [reviewed in (Shankar et al., 2012)]. Several studies have also demonstrated the significance of ß-chemokines in restriction of, and protection from, HIV infection both *in vitro* and *in vivo* [reviewed in (Garzino-Demo et al., 2000; DeVico and Gallo, 2004)]. Previous studies have shown that α-defensins can inhibit HIV-1 replication in macrophages by triggering release of HIV-1 inhibitory ß-chemokines (Guo et al., 2004) and that treatment of DCs with hBD2 upregulated the expression and release of ß-chemokines (MIP-1α and MIP-1ß) in culture supernatants (Biragyn et al., 2002). Therefore, we tested whether hBD2 inhibition of HIV-1 in MDM is mediated *via* up-regulation of the expression of (anti-R5 tropic) ß-chemokines- MIP-1α, MIP-1ß and RANTES and/or Type I IFNs. Culture supernatants obtained from MDM treated with hBD2 up to 24 hours were used to quantify these molecules by commercial ELISA kits. As shown in **Figure S2**, treatment with hBD-2 induced an increase in production of all three ß-chemokines at the 24 hour time point in Donor 1, and an increase in production of RANTES at the 4 and 8 hour time points in Donor 3 as compared to levels in untreated cultures. It is unlikely; however, that the modest increase in β-chemokines observed at different time points post-treatment significantly contribute to the anti-HIV-1 activity of hBD2 in macrophages as the concentrations measured in the hBD2 treated culture supernatants are well below the concentrations needed to efficiently inhibit HIV replication (Cocchi et al., 1995). Boniotto et al. had made similar observations with hBD2 in PBMCs (Boniotto et al., 2006). Further, IFN-α and -ß were not detected in supernatants of both untreated control cells as well as hBD2 treated cells at any time points in any of the donors (data not shown). These data suggest that the anti-HIV-1 effect of hBD2 in macrophages is not mediated through induction and release of these anti-viral cytokines and ß-chemokines.

hBD2 Suppresses HIV-1 at an Early Post-Entry Stage

We and others have shown that hBD2 and hBD3 interact directly with HIV-1 decreasing infectivity irreversibly (Quinones-Mateu et al., 2003; Sun et al., 2005; Lafferty et al., 2010). In the current

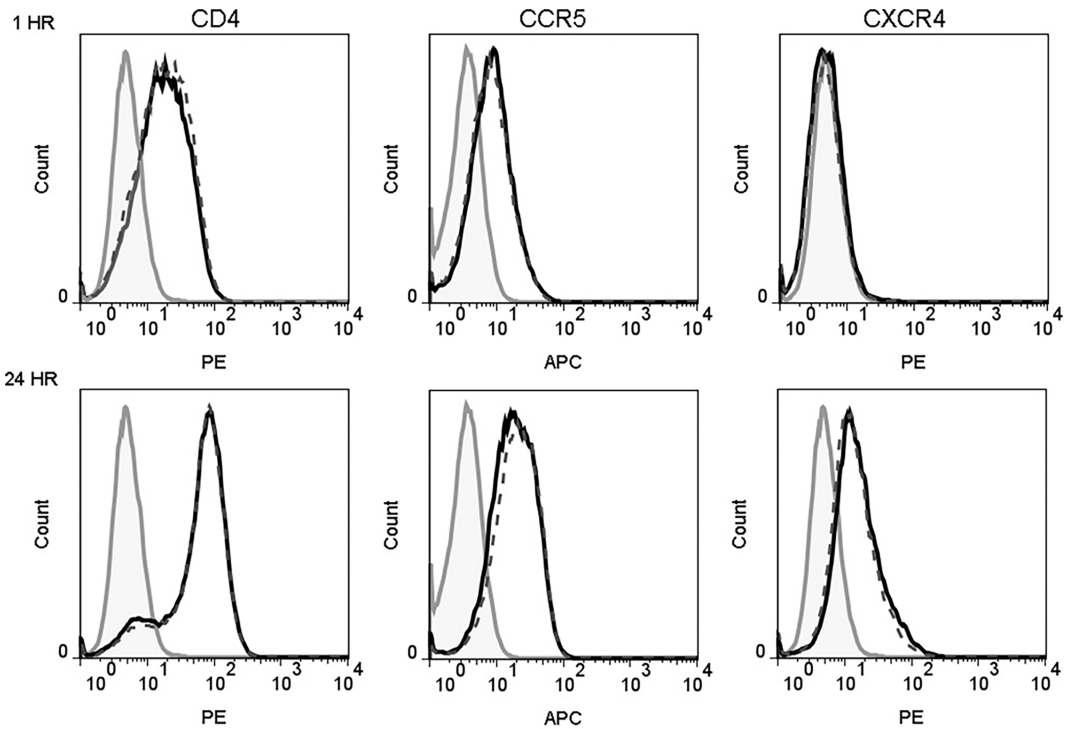


FIGURE 2 | hBD2 does not alter surface expression of HIV receptors on macrophages. MDM were cultured in the absence (black solid lines) or presence (black dotted lines) of hBD2 for different times. The surface expression of CD4, CCR5, and CXCR4 was assessed by flow cytometry as described in *Materials and Methods*. Data analyzed using FlowJo software. Left, middle, and right panels show staining of CD4, CCR5, and CXCR4, respectively. Isotype-matched control antibodies are shown in grey. The x-axis and y-axis show fluorescence intensity and cell count, respectively. Representative experiment, *n*=2.

TABLE 1 | Median Fluorescence Intensity (MFI) values for surface receptor expression on MDM.

Time (hr) post treatment	Condition	Donor 1			Donor 2		
		CD4-PE	CCR5-APC	CXCR4-PE	CD4-PE	CCR5-APC	CXCR4-PE
1	untreated	17.30	8.27	4.71	11.35	6.82	2.97
	hBD2	17.72	7.77	4.58	10.76	6.53	2.83
2	untreated	19.04	8.82	5.25	11.29	7.35	3.28
	hBD2	20.07	9.12	5.00	11.17	7.27	2.94
3	untreated	21.89	9.60	5.34	13.67	8.42	3.39
	hBD2	21.61	9.53	5.12	12.53	8.67	3.32
24	untreated	68.72	18.12	14.04	45.86	14.40	5.50
	hBD2	70.00	20.26	12.51	48.33	20.86	5.57

study, hBD2 was always added to macrophage cultures post-infection, decreasing the direct impact of hBD2 on HIV. To investigate whether hBD2 inhibits HIV-1 in macrophages post-entry, a single-cycle infection assay was employed with an HIV luciferase reporter virus pseudotyped with the AMLV envelope that does not use either CXCR4 or CCR5 for host entry. MDM were infected for 3 hours and incubated for 3 days in presence or absence of hBD2. AZT was used as a positive control. Subsequently, luciferase activity was measured and the percentage of inhibition compared with infected untreated cells was calculated. As shown in **Figure 3A**, hBD2 treatment resulted in significant inhibition (>70%) of luciferase expression in 3 of 4 donors tested. Treatment with AZT under the same conditions resulted in inhibitory activity greater than 80% in all donors. We observed donor-to-donor variability exemplified by the lack of inhibition with hBD2 in Donor 1 which is not unusual in studies with primary cells from a random pool of human subjects. This may be due to lack of hBD2 receptor(s) on the cell surface, or other parameters. Overall, these results show that hBD2-mediated restriction of HIV-1 is not dependent on the Env-host cell interaction, and occurs post-entry.

To probe the intracellular mechanism of inhibition, the presence of early (negative strand strong stop, -sss) products of HIV-1 reverse transcription were quantified in MDM infected

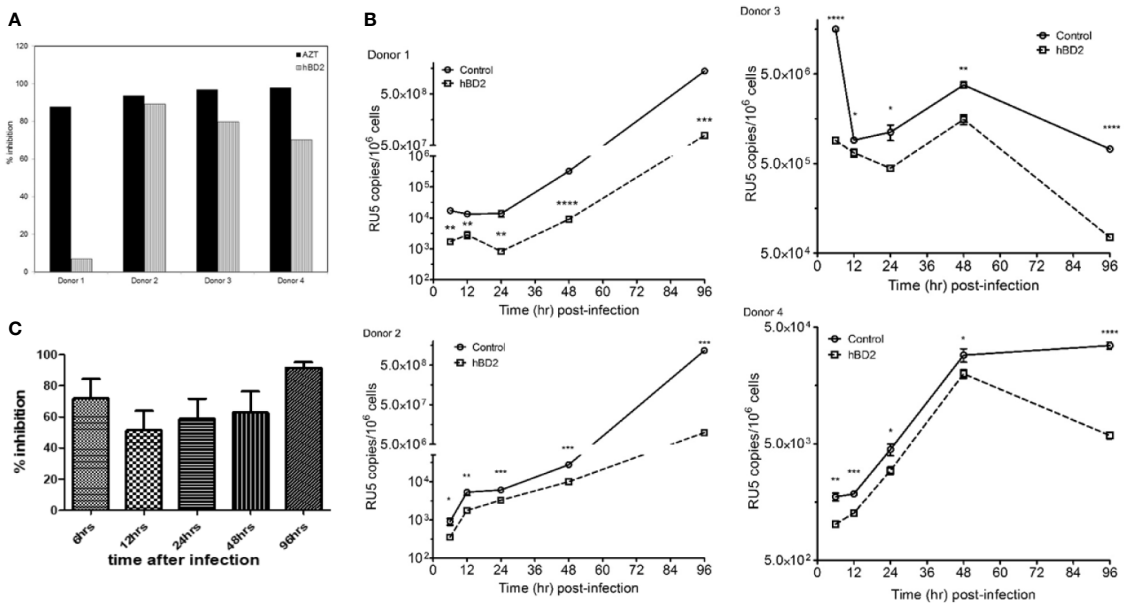


FIGURE 3 | hBD2 suppresses HIV-1 at an early post-entry stage. **(A)** Single-cycle infection of MDM. Cells were infected with HIV-luciferase pseudotyped with AMLV envelope. After infection, cells were incubated 3 days in presence or absence of hBD2. Subsequently, cells were lysed and luciferase activity was measured. Percentage of inhibition was calculated for treated infected cells in reference to untreated infected cells. Data are presented for independent experiments from 4 different donors. **(B)** hBD2 inhibits accumulation of early reverse transcription products of HIV-1. MDM were challenged with DNase I-treated HIV-1_{BaL}. Post-infection, hBD2 was added to the cultures. Total cellular DNA was isolated at the indicated time points and copies of LTR/RU5 products of reverse transcription were measured in triplicate by real-time PCR. Readings were averaged ± SEM and are presented as copies per million cells; log-scale graph. *P < 0.05, **P < 0.005, ***P < 0.0005, ****P < 0.0001 between treatment and control groups determined with unpaired two-tailed *t* test. Data are presented for independent experiments from 4 different donors and **(C)** summary graph of the average inhibition for the four donors shown in **3B** was determined as the percentage of HIV-1 DNA copies in treated infected cells in reference to untreated infected cells.

with HIV-1_{BaL} by real-time quantitative PCR at various time points post-infection, using α-tubulin as the reference gene. Cells were infected, treated after infection with hBD2 (4.7 μM) and harvested for processing as described in *Materials and Methods*. For positive controls, cells were pretreated with either AZT or T20. Percent inhibition was calculated for ratio of HIV infected treated cells to infected untreated cells. The results in **Figures 3B, C** and **Figure S3** confirm that inhibition occurs post-entry, as HIV-1 DNA products are detected in the presence and absence of hBD2. After 6 hours post-infection, hBD2 treated samples showed significant inhibition of accumulation of LTR/RU5 products (**Figures 3C**, **S3**). Pretreatment with AZT also inhibited accrual of these products (data not shown). Some variability in the level of inhibition was observed in cells from different donors, ranging from 40-90%, but overall they all showed inhibition of early reverse transcription products. Thus, hBD2 restriction acts after the initiation of reverse transcription, preventing the completion of full length viral DNA products.

hBD2 Inhibits HIV-1 in Macrophages Post-Entry *via* Gi-Protein Mediated Signaling

hBD2 is known to use C-C chemokine receptor 6 (CCR6) and C-C chemokine receptor 2 (CCR2) to induce cell migration in immature DCs, memory T (Yang et al., 1999) and mast cells (Niyonsaba et al., 2002), respectively, although there is evidence that additional receptors might be involved in the migration of cells of myeloid origin (Soruri et al., 2007). Preincubation of cells with pertussis toxin (PTx) abrogated migration towards hBD2, indicating that hBD2 signals *via* a receptor(s) coupled to PTx-sensitive $G_{\alpha i}$ proteins (Soruri et al., 2007). To elucidate whether the inhibition of HIV by hBD2 in macrophages is mediated by $G_{\alpha i}$-proteins signaling, we used PTx in our assays. MDM were infected with the AMLV pseudotyped HIV luciferase reporter virus and pretreated with PTx (100 ng/ml) and incubated for 3 days in presence or absence of hBD2. AZT was used as a positive control. Treatment of infections with PTx alone resulted in some inhibition of HIV LTR driven luciferase gene expression. Both inhibition (Alfano et al., 1999; Copeland et al., 1999; Alfano et al., 2000; Alfano et al., 2001; Iordanskiy et al., 2002; Lapenta et al., 2005; Hu et al., 2006) and enhancement (Momoi et al., 2000) of HIV infection in response to varying concentrations of PTx have been previously reported by different groups. However, in our experiments pretreatment with PTx resulted in abrogation of hBD2 inhibition of HIV (**Figure 4**), although inhibition was not completely reversed in all donors tested, which is indicative of more than one mechanism for hBD2 inhibition of HIV in macrophages. This result suggests

that, at least in part, hBD2 binds GPCRs on the surface of macrophages and activates intracellular G_i-protein signaling pathways to mediate its HIV inhibitory activity.

Expression Pattern of Known hBD2 Receptors on the Surface of Macrophages

CCR6 is responsible for both hBD1 and hBD2 binding and chemotaxis of memory T cells, immature DCs (Yang et al., 1999), and TNF-α-treated neutrophils (Niyonsaba et al., 2004). Several studies have reported that hBD3 and hBD4 are chemotactic for peripheral blood monocytes (Wu et al., 2003; Soruri et al., 2007) and mast cells which do not express CCR6 (Soruri et al., 2007) implying the existence of an unidentified receptor. Two independent studies implicated CCR2 as the GPCR responsible for hBD2 and hBD3-mediated signaling and chemotaxis of monocytes (Jin et al., 2010; Rohrl et al., 2010). CCR2 is largely expressed on myeloid cells, such as monocytes (Katschke et al., 2001), DCs, macrophage subsets (Lin et al., 2008), and neutrophils (Iida et al., 2005).

In order to identify the receptor(s) used by hBD2 on macrophages for intracellular signaling that results in restriction of HIV-1 replication, we analyzed the surface expression patterns of the known hBD2 receptors i.e. CCR2 and CCR6 on seven to ten days old MDM from healthy human donors (n=53) by flow cytometry. Based on the percentage of cells expressing the CCR, MDM were classified as follows: ≥50% cells positive= + (moderate to high levels); 10-49% cells positive= +/- (low to moderate); <10% cells positive= - (negative). Results are summarized in **Tables 2**, **3** representative MDM of each type is shown in **Figures 5A–C**. **Figure 5D** shows the MFI of CCR6 signal, as compared to its respective isotype-matched control, on MDM from these different donors. As shown in **Table 2**, only 4% of the donors were $CCR2^+$ and 9% were $CCR2^{+/-}$ compared to 87% that were $CCR2^-$. In contrast, 15% of the same donors were $CCR6^+$ and 28% were $CCR6^{+/-}$ while 57% of the donors were $CCR6^-$. However, as shown in **Table 3**, MDM from 51% of the donors were negative for both CCR2 and CCR6. These data indicate that hBD2 may be using additional receptors on primary human MDM.

Since this is the first report of CCR6 surface expression on macrophages, we used additional biochemical methods to confirm CCR6 protein expression in MDM. To this end, we performed immunoblot analysis for CCR6 on whole cell lysates of MDM and used JKT-FT7 cell line ($CCR6^-$) and primary human $CD4^+$ T cells that were sorted into $CCR6^+$ and $CCR6^-$ populations as controls. As shown in **Figure 5E**, MDM do synthesize CCR6 protein at levels sufficient to be detected in immunoblots.

hBD2 Can Signal *via* More Than One Receptor on Macrophages to Inhibit HIV-1

Although several studies show that CCR2 acts as the receptor for hBD2 and hBD3 on monocytes, Phillips et al. (Phillips et al., 2005) determined that CCR2 expression is down regulated as human monocytes gradually differentiate into macrophages. To determine whether CCR2, when expressed on MDM, is involved in hBD2-mediated intracellular inhibition of HIV-1, we used $CCR2^+$ MDM. Cells were infected, pretreated with the potent and selective CCR2 pharmacological antagonist RS102895 (Jin et al., 2010) and subsequently cultured in the presence or absence of hBD2. As expected, hBD2 showed 60 to 75% inhibition of R5 virus replication over time in cells that were not treated with the inhibitor. The CCR2 antagonist, or DMSO control, by itself did not have a significant effect on infection. In contrast, blocking CCR2 resulted in complete reversal of HIV-1 inhibition by hBD2 (**Figure 6A**) suggesting a role for CCR2, when present, in mediating intracellular inhibition of the virus.

Since all MDM (from different donors) tested were not positive for surface expression of CCR2, CCR6 or both, we hypothesized that hBD2 signals in macrophages *via* additional receptors. To examine this possibility, we tested the ability of hBD2 to inhibit HIV-1 in $CCR2^-$ $CCR6^-$ MDM. Unexpectedly, we found that hBD2, at the same concentration used in previous infectivity experiments, completely abrogated HIV-1 replication in these cells up to 14 days post-infection, similar to the AZT control (**Figure 6B**). This is not attributable to low or no infection as all cells were infected in the same tube and split post-infection into infected untreated and infected hBD2-treated. These data suggest that hBD2 can use receptor(s) other than CCR2 and CCR6 on MDM. We decided to follow the infection over a longer time period, replenishing hBD2 in the culture every 3 days. As shown in **Figure 6B**, after 14 days, we observed virus replication gradually in the hBD2 treated cells, although inhibition (ranging from 92% to 64% on days 17 and 24, respectively) was still present. The MDM were also analyzed at the same time points for CCR2 and CCR6 surface expression. As seen in the scatter plots in **Figure 6C**, compared to no expression of either CCR2 or CCR6 at Day 0 (gray), 49% of the cells expressed CCR2 and 83% expressed CCR6 at Day 23 (black overlay on Day 0). Collectively, these data lead us to hypothesize that hBD2 uses different CCRs on macrophages with varying affinities.

hBD2 Upregulates APOBEC3G and/or APOBEC3A in Macrophages

Our results suggest that the post-entry inhibition occurs during early reverse transcription. To explore the mechanism(s) by which hBD2 suppresses HIV-1, we examined its ability to affect host restriction factors, specifically members of the APOBEC3 family of cytidine deaminases, namely APOBEC3G (A3G) and APOBEC3A (A3A) which are known intracellular inhibitors of HIV-1 in macrophages (Dong et al., 2006; Peng et al., 2007; Hou et al., 2009; Thielen et al., 2010; Chaipan et al., 2013; Mashiba and Collins, 2013). In addition, data from our lab demonstrated that hBD2 induced A3G expression *via* CCR6 resulting in HIV inhibition in $CD4^+$ T cells (Lafferty et al., 2010). Also, recent reports show that A3A expression is significantly upregulated and is the major cytidine deaminase in myeloid cells in response to IFN-α treatment (Peng et al., 2007; Koning et al., 2009; Thielen et al., 2010).

To determine whether hBD2 influences A3G and/or A3A expression, macrophages were treated with hBD2 and kinetics of message levels were determined by real-time RT-PCR.

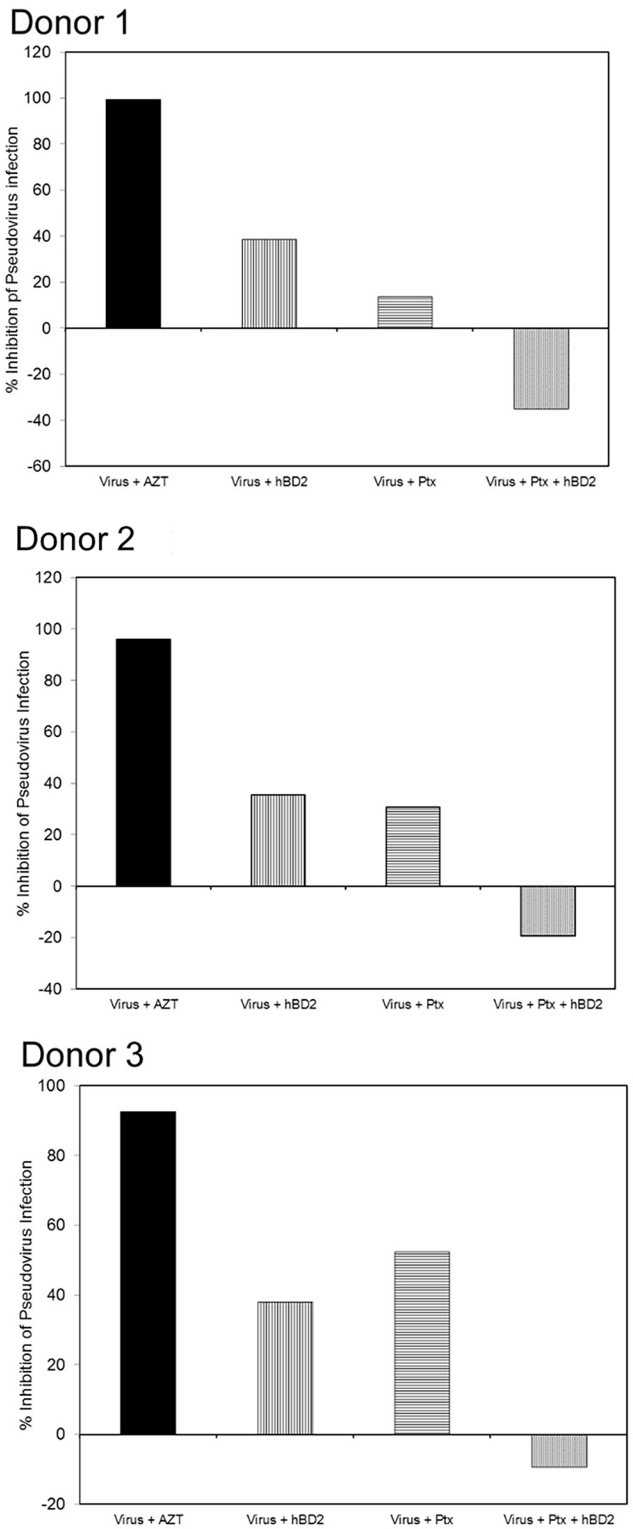


FIGURE 4 | hBD2 inhibits HIV-1 in macrophages post-entry via G_i-protein-mediated signaling. MDM were infected with single-cycle HIV-luciferase virus pseudotyped with AMLV envelope followed by pretreatment with or without PTx (100 ng/ml). Cells were then incubated 3 days in presence or absence of hBD2. Subsequently, cells were lysed and luciferase activity was measured. Percentage of inhibition was calculated for treated infected cells in reference to untreated infected cells. Data are presented for independent experiments from 3 different donors.

TABLE 2 | Expression of chemokine receptors from blood donors.

CCR Phenotype	%of Donors
CCR2+	4
CCR2 +/-	9
CCR2-	87
CCR6+	15
CCR6 +/-	28
CCR6-	57

key: - = negative; + = 50% cells positive; +/- = 10-49% cells positive.

TABLE 3 | Patterns of co-expression of chemokine receptors from blood donors.

CCR Phenotype	No. of Donors	%of Donors
CCR2+ CCR6+	4	8
CCR2- CCR6-	27	51
CCR2 OR CCR6	22	42
Total (n)	53	

N, no. of donors.

Treatment of cells with hBD2 induced a 2 to 3-fold increase in A3G mRNA signal in five of six donors tested, with one donor showing an 8-fold increase as compared to untreated cells, although we observed differences in the kinetics of induction among different donors (**Figure 7A**). In contrast, A3A levels increase from 1.5 to 6-fold within the first hour post-treatment with hBD2 compared with untreated cells, with variable kinetics between different donors (**Figure 7A**). To determine whether this increase in expression translated into increase in protein, cells were treated with hBD2 and lysates were prepared at 4, 8, and 24 hours. Equal amounts of total protein were subjected to immunoblotting with anti–human A3G antibodies. hBD2 enhanced A3G protein levels (**Figure 7B**) but high levels of endogenous A3G in untreated MDM made it difficult to decipher a clear increase in treated cell lysates from different donors. In case of A3A, we observed 1.5- to 2-fold increase in protein 24 to 48 hours post-treatment compared with endogenous levels in untreated MDM (**Figure 7C**). Peak protein signal was delayed compared to peak RNA signal for A3A, which may be due to differences in the kinetics of RNA and protein expression of this protein, and donor-to donor variability. Overall, gene expression and protein production analyses reveal that hBD2 induced both A3G and A3A in MDM with stronger induction of A3A.

DISCUSSION

We and others previously demonstrated that hBD2 and hBD3 inhibit HIV-1 replication in primary human PBMCs and $CD4^+$

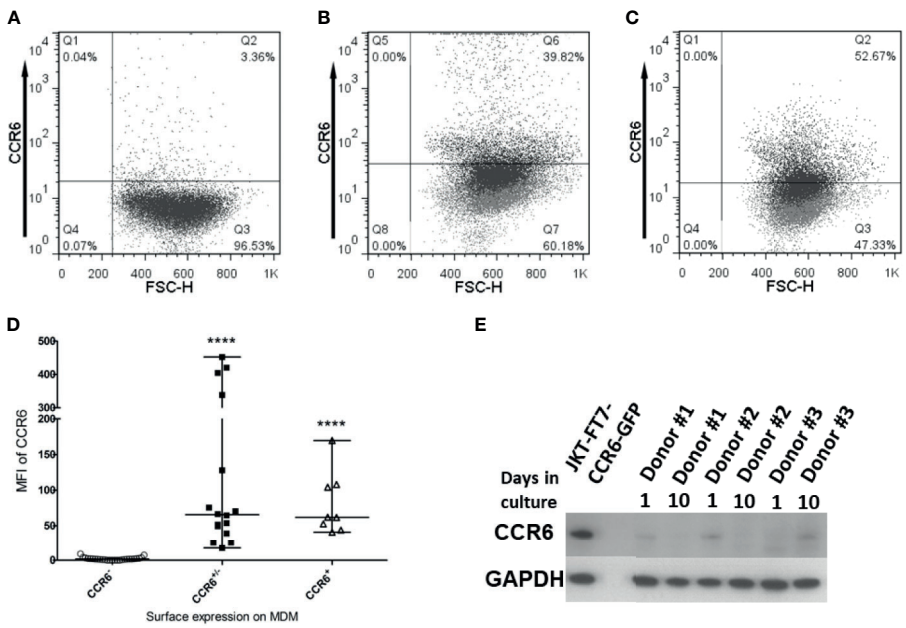


FIGURE 5 | CCR6 is expressed on macrophages. Untreated, uninfected MDM were harvested and stained for flow cytometry analysis of CCR6 as described in *Materials and Methods*. Data analyses were performed using FlowJo software. Forward scatter dot plots show the fluorescence and percentage of cells positive for CCR6 as compared to the respective isotype-matched control. A representative of each type **(A)** $CCR6^-$, **(B)** $CCR6^{+/-}$, and **(C)** $CCR6^+$ is shown. **(D)** Median Fluorescence Intensity (MFI) values for CCR6 surface expression (as compared to MFI for isotype control) on MDM from different donors designated as either $CCR6^-$, $CCR6^{+/-}$, or $CCR6^+$. Data are presented as median and range of MFI values. Each dot represents one donor. ****$P < 0.0001$ between $CCR6^{+/-}$ and $CCR6^-$ or $CCR6^+$ and $CCR6^-$ determined with unpaired two-tailed *t* test. **(E)** Immunoblot analysis of CCR6 on MDM from 3 donors, 1 day and 10 days of tissue culture without treatment. JKT-FT CCR6 GFP cell line lysate in first lane from left is used as a positive control; the second lane is empty; subsequent lanes are donors #1-3, at day 1 and day 10 of tissue culture.

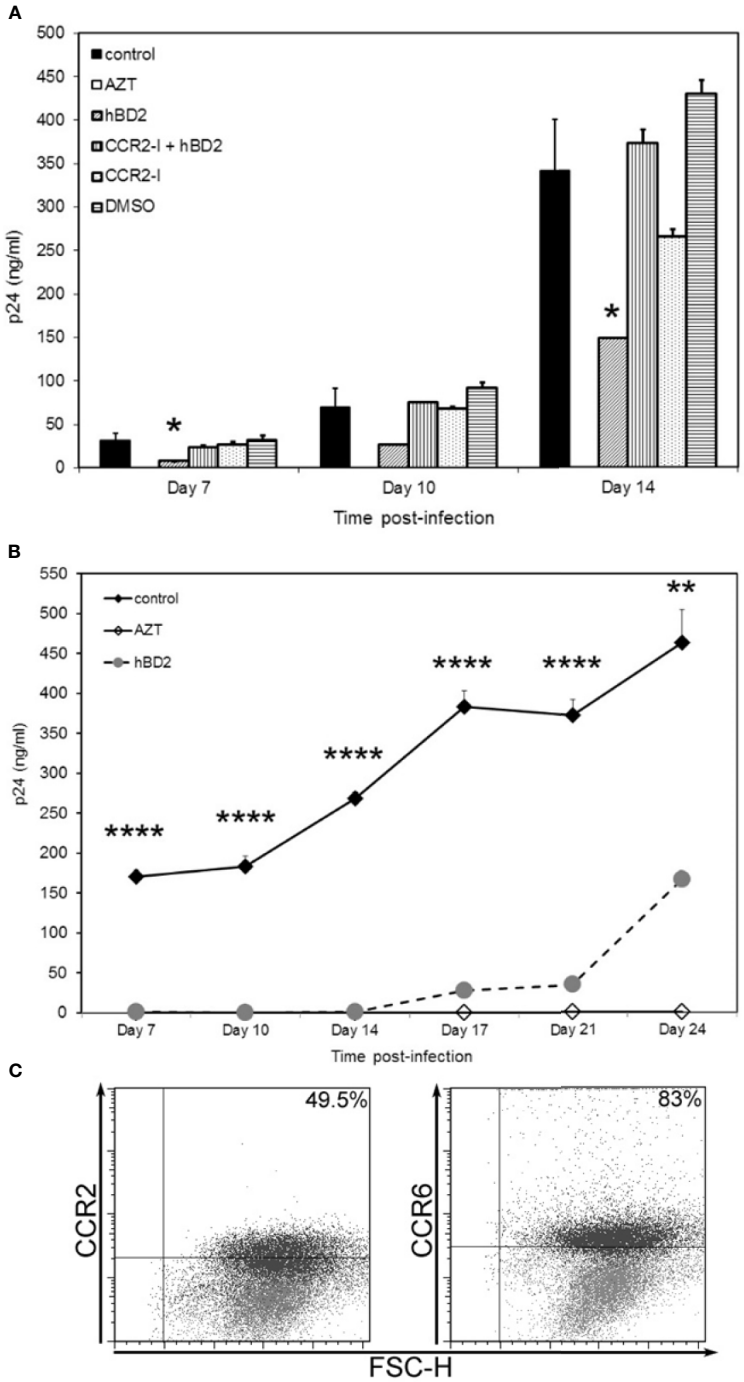
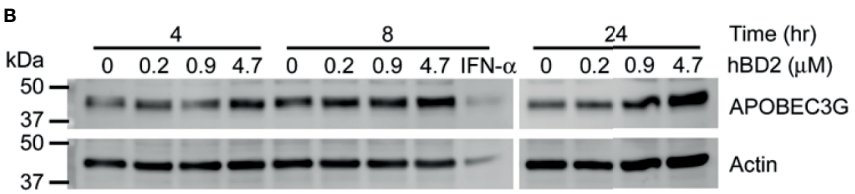


FIGURE 6 | hBD2 can signal via more than one receptor type on macrophages. **(A)** Neutralization of CCR2 rescues HIV-1 infection. MDM were infected with HIV-1_{BaL}. Cells were pretreated with AZT as control. Post-infection, infected untreated cells were pretreated with pharmacological antagonist RS102895 or DMSO control for 2 hrs followed by culture in presence or absence of hBD2 to the cultures. Infection was monitored by assaying supernatants for HIV p24 production by ELISA at the times indicated. Data are presented as mean ± SEM of triplicates. *$P < 0.05$ between treatment and control infection determined with unpaired two-tailed *t* test. Representative experiment, *n*=2. **(B)** hBD2 signals via an as yet unidentified receptor. MDM, that were CCR2- CCR6- (by FACS staining), prior to start of infection, were infected with HIV-1_{BaL}. Cells were pretreated with AZT as control. Post-infection, cells were cultured in presence or absence of hBD2. Infection was monitored by p24 ELISA. Data are presented as mean ± SEM of triplicates. **$P < 0.005$, ****$P < 0.0001$ between treatment and control infection determined with unpaired two-tailed *t* test. **(C)** CCR2 and CCR6 surface expression levels vary with time. Uninfected, untreated cells from the donor used in **(B)** were harvested and stained for flow cytometry analysis as described in *Materials and Methods*. Data analyzed using FlowJo software. Forward scatter dot plots show the fluorescence and percentage of cells positive for CCR2 and CCR6 at Day 0 (grey) and Day 23 (black) as compared to the respective isotype-matched controls.

A

Time (hr) post-treatment	APOBEC3G 1	2	3	4	5	6	APOBEC3A 4	5	6
0.25				0.5	1.0	0.7	0.4	1.5	2.6
0.5				1.2	1.1	0.8	4.0	1.3	4.6
0.75				2.3	1.0	0.8	5.7	1.5	4.5
1				1.0	0.9	2.0	2.0	1.0	5.5
2		0.5	1.3			2.1			2.7
4	8.4	1.4	0.9	0.3	0.5	1.0	0.7	1.0	3.3
8	0.0	0.9	1.9	2.5	1.3	2.1	0.7	1.3	1.3
24	8.4	2.6	1.1	5.0	0.9	0.7	1.8	0.8	1.31



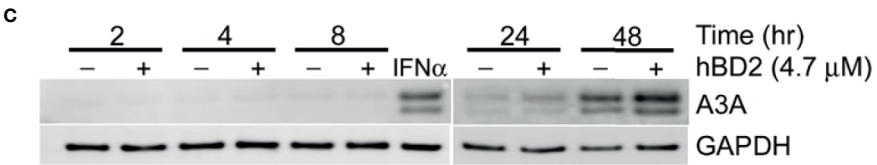


FIGURE 7 | hBD2 upregulates APOBEC3G and/or APOBEC3A in macrophages. **(A)** APOBEC3G and APOBEC3A expression in response to hBD2. MDM were treated with hBD2 for indicated times and mRNA levels were assessed by quantitative real-time RT-PCR. The data was normalized to 18S ribosomal RNA. Triplicate measurements were used to calculate fold change as described in *Materials and Methods*. Data are presented as fold change in treated samples compared to untreated samples at matched time points. Data are for independent experiments from different donors. Analyses of APOBEC3G **(B)** and APOBEC3A **(C)** protein levels in response to hBD2. MDM were treated with hBD2 for various times and cell lysates were used to detect APOBEC3G and APOBEC3A proteins by western blotting. ß-actin and GAPDH serve as load controls. Representative experiment, n=3.

T cells (Quinones-Mateu et al., 2003; Sun et al., 2005; Lafferty et al., 2010; Lafferty et al., 2017). To the best of our knowledge, we demonstrate here for the first time that hBD2 and hBD3, but not hBD1, inhibit HIV-1 in human MDM. This inhibition occurs in the micromolar range of concentrations similarly to our previous observations on T cells, without effects on cell metabolism that we previously observed in $CD4^+$ T cells treated with hBD3 (Sun et al., 2005). The dose-response profile of hBD2 that we observed was probably on primary cells was probably affected by donor-to-donor variability in expression of CCR6 and CCR2 (see below). The enhancement of HIV replication we observed by hBD1 was not observed in PBMC, where hBD1 has low inhibitory activity (Quinones-Mateu et al., 2003; Sun et al., 2005). The earlier studies on the effects of hBDs on HIV replication showed that the inhibitory activity was due to both a virucidal component, and an intracellular component (Sun et al., 2005). In this study, we addressed mostly intracellular mechanisms of inhibition in MDM. We further show that hBD2 acts *via* more than one receptor type to inhibit the virus post-entry, and not by altering surface expression levels of HIV receptor-co-receptors or by enhancing expression of anti-viral cytokines and β-chemokines. One mechanism is *via* binding to and signaling through different CCRs (GPCRs) on the cell surface. The known hBD2 receptor on monocytes, CCR2, is expressed on the surface of human MDM in 4% of donors we tested. We found CCR6 expression on MDM from more than 40% of the donors tested, albeit at varying levels. To our best knowledge, this is the first report of CCR6 expression on peripheral blood monocyte-derived macrophages. Our data differs from published data that CCR6 is not expressed on peripheral blood monocytes (Ruth et al., 2003; Rohrl et al., 2010) or macrophages (Soruri et al., 2007). However, low level of CCR6 expression has been reported on myeloid blasts from the peripheral blood of AML patients (Cignetti et al., 2003), and on $CD14^+$ monocytes from peripheral blood and synovial fluid of rheumatoid arthritis patients (Ruth et al., 2003). Also, studies have reported surface expression of CCR6 on microglial cells (Flynn et al., 2003), the resident macrophages of the brain, astrocytes (Flynn et al., 2003), and spinal cord infiltrate macrophages (Mony et al., 2014). The broader biological significance of this discovery warrants further exploration because of the role of CCR6 in both pro- and anti-inflammatory immune responses. The lack of a good CCR6 specific neutralizing antibody, antagonist, or a human CCR6 expressing monocyte cell line at this time restricts our ability to test the role of CCR6 in hBD2-mediated inhibition of HIV in

MDM, and/or whether some of the activity of hBD2 may be due to penetration of the peptide in cells.

Further support for our hypothesis that hBD2 signals in macrophages *via* more than one CCR comes from our experiments with PTx. MDM used from different donors for these experiments (**Figure 4**) expressed no CCR2 and either no CCR6 or low levels of CCR6 suggesting that these are not the only CCRs used by hBD2 on macrophages. Further, unexpectedly, maximum hBD2 inhibition of HIV was observed in MDM that were $CCR2^{-}$ $CCR6^{-}$ (**Figure 6B**). Therefore, we hypothesized that hBD2 may inhibit HIV in macrophages *via* additional receptors. This is consistent with a study which demonstrated that hBD2 mediates chemotaxis of mast cells *via* signaling through more than one receptor, and identified both high and low affinity receptors for hBD2 on this cell type (Niyonsaba et al., 2002). Our results on putative receptors for defensins open up more avenues of investigation as there may be more such β-defensin-CCR interactions on other cell types as well. Our results with PTx show that regardless of receptor usage, hBD2 requires Gαi signaling pathway(s) for HIV inhibition in macrophages. When Ptx was used in conjuction with hBD2 treatment, HIV replication was not just restored, but it appeared to be enhanced. While this finding does not invalidate the role of Gi-mediated signaling in the inhibitory effects of hBD2, It open the possibility that hBD2 may also induce activation of other intracellular pathways that increase HIV replication.

Similar to previous findings from our laboratory in $CD4^{+}$ T cells (Lafferty et al., 2010; Lafferty et al., 2017), we found that post-entry hBD2 blocks virus replication at an early stage of the life cycle after the initiation of reverse transcription as evidenced by inhibition of the accumulation of early reverse transcription products. The intracellular inhibition is further mediated *via* upregulation of the innate anti-viral restriction factors APOBEC3G (A3G) and APOBEC3A (A3A) to different levels. We detected increased A3G RNA signal in most donors tested with a corresponding increase in protein levels at 24 hours in macrophage lysates. As mentioned in the *Results*, high signal for endogenous A3G detected in untreated MDM lysates complicates our ability to accurately determine the effect of hBD2 on the level of A3G protein synthesized in MDM. The level of variability we observed is not unexpected in primary cells, so that it is possible that the effects of hBD2 may vary *in vivo*. We observed A3A induction, although while RNA levels increased within the first hour following addition of hBD2, either very weak or no signal was observed for A3A protein in both untreated and treated cells until 48 hours post-treatment. This is similar to studies of IFN-α-induction of A3A in monocytes (Thielen et al., 2010) and macrophages (Goujon et al., 2013) that showed low levels of A3A protein 8-10 hours post-treatment, and more robust signal 24 hours post-treatment, hence, our results may be due to the kinetics of translation and/or the half-life of this protein in myeloid cells. Our data that hBD2 enhanced A3G and A3A expression support our model that elevated levels of APOBEC3 proteins contribute to hBD2-mediated anti-HIV-1 activity in macrophages.

Taken together, our results provide evidence that hBD2 has the ability to suppress HIV-1 infection of primary macrophages *in vitro*. While the role of macrophages in the initial stages of infection is still debated (Collins et al., 2000; Greenhead et al., 2000; Gupta et al., 2002; Hladik et al., 2007; Bouschbacher et al., 2008; Shen et al., 2011), it is widely accepted that macrophages are not only a major target of HIV during both the acute and chronic phases of disease-they produce and spread infectious virus-but also a major reservoir of latent virus that, especially in the CNS, eludes eradication by existing therapies. Since hBD2 is expressed by epithelial cells in mucosae and in the CNS by astrocytes (Hao et al., 2001), our findings could be relevant to both systemic infection and neurological complications of the disease. Indeed, a protective role for human ß-defensins against HIV acquisition in high risk exposed individuals has been suggested by Rugeles and colleagues (Zapata et al., 2008; Aguilar-Jimenez et al., 2013), and hBD2 has been shown to be correlated with ant- HIV activity in cervical-vaginal secretions (Ghosh et al., 2010; Patel et al., 2014). Several studies have also demonstrated an association between different ß-defensin gene polymorphisms and HIV infection in adults as well as children of diverse ethnicities (Braida et al., 2004; Milanese et al., 2006; Baroncelli et al., 2008; Milanese et al., 2009; Ricci et al., 2009; Aguilar-Jimenez et al., 2011; Freguja et al., 2012; Hardwick et al., 2012). Human ß-defensins, especially hBD2, therefore merit further consideration as both an immunotherapeutic and topical microbicide to prevent spread through sexual contact. While we did not address the effects of hBDs in a model of HIV-latency in MDM in this study, the possibility that hBDs can influence HIV reservoirs deserves to be addressed in further studies.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

JB designed and performed experiments, wrote the article. LS performed experiment. WL contributed key reagents and edited the article. SG contributed key reagents, and edited the manuscript. AG-D conceived and supervised the study and edited the article. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Institute of Neurological Disorders And Stroke award R01NS066842 to AG-D and the National Institute of Allergy and Infectious Diseases Research award R01AI061482 to WL.

ACKNOWLEDGMENTS

The authors would like to express gratitude to Dr. Lingling Sun for her continued support, to Drs. Mark Lafferty and Mohammad A. Khan for useful suggestions and, to Drs. Anthony L. DeVico and Marvin S. Reitz Jr. for generous use of their lab facilities and equipment. We thank Dr. Lai-Xi Wang for the gift of T20 peptide. We also thank Dr. Brian Taylor for Core Facility Lab services, Drs. Yutaka Tagaya and Cristiana Cairo for advice with trouble-shooting FACS staining, and James Foulke for technical advice on making pseudo-typed reporter viruses. The anti-APOBEC3G antibodies from Drs. Warner C. Greene, Klaus Strebel, and Jaisri Lingappa were obtained through the National Institutes of Health AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases, the National Institute of Neurological Disorders And Stroke, or the National Institutes of Health.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2021.535352/full#supplementary-material

REFERENCES

Acosta-Rodriguez, E. V., Rivino, L., Geginat, J., Jarrossay, D., Gattorno, M., Lanzavecchia, A., et al. (2007). Surface Phenotype and Antigenic Specificity of Human Interleukin 17-Producing T Helper Memory Cells. *Nat. Immunol.* 8, 639–646. doi: 10.1038/ni1467

Aguilar-Jimenez, W., Zapata, W., Caruz, A., and Rugeles, M. T. (2013). High Transcript Levels of Vitamin D Receptor are Correlated With Higher mRNA Expression of Human Beta Defensins and IL-10 in Mucosa of HIV-1-Exposed Seronegative Individuals. *PloS One* 8, e82717. doi: 10.1371/journal.pone.0082717

Aguilar-Jimenez, W., Zapata, W., and Rugeles, M. T. (2011). Differential Expression of Human Beta Defensins in Placenta and Detection of Allelic Variants in the DEFB1 Gene From HIV-1 Positive Mothers. *Biomedica* 31, 44–54. doi: 10.7705/biomedica.v31i1.335

Alexaki, A., Liu, Y., and Wigdahl, B. (2008). Cellular Reservoirs of HIV-1 and Their Role in Viral Persistence. *Curr. HIV Res.* 6, 388–400. doi: 10.2174/157016208785861195

Alfano, M., Pushkarsky, T., Poli, G., and Bukrinsky, M. (2000). The B-Oligomer of Pertussis Toxin Inhibits Human Immunodeficiency Virus Type 1 Replication at Multiple Stages. *J. Virol.* 74, 8767–8770. doi: 10.1128/JVI.74.18.8767-8770.2000

Alfano, M., Schmidtmayerova, H., Amella, C. A., Pushkarsky, T., and Bukrinsky, M. (1999). The B-Oligomer of Pertussis Toxin Deactivates CC Chemokine Receptor 5 and Blocks Entry of M-Tropic HIV-1 Strains. *J. Exp. Med.* 190, 597–605. doi: 10.1084/jem.190.5.597

Alfano, M., Vallanti, G., Biswas, P., Bovolenta, C., Vicenzi, E., Mantelli, B., et al. (2001). The Binding Subunit of Pertussis Toxin Inhibits HIV Replication in Human Macrophages and Virus Expression in Chronically Infected Promonocytic U1 Cells. *J. Immunol.* 166, 1863–1870. doi: 10.4049/jimmunol.166.3.1863

Badr, G. (2013). Camel Whey Protein Enhances Diabetic Wound Healing in a Streptozotocin-Induced Diabetic Mouse Model: The Critical Role of beta-Defensin-1, -2 and -3. *Lipids Health Dis.* 12, 46. doi: 10.1186/1476-511X-12-46

Baroncelli, S., Ricci, E., Andreotti, M., Guidotti, G., Germano, P., Marazzi, M. C., et al. (2008). Single-Nucleotide Polymorphisms in Human Beta-Defensin-1 Gene in Mozambican HIV-1-Infected Women and Correlation With Virologic Parameters. *Aids* 22, 1515–1517. doi: 10.1097/QAD.0b013e3282fd6e0c

Biragyn, A., Ruffini, P. A., Leifer, C. A., Klyushnenkova, E., Shakhov, A., Chertov, O., et al. (2002). Toll-Like Receptor 4-Dependent Activation of Dendritic Cells by Beta-Defensin 2. *Science* 298, 1025–1029. doi: 10.1126/science.1075565

Boniotto, M., Jordan, W. J., Eskdale, J., Tossi, A., Antcheva, N., Crovella, S., et al. (2006). Human Beta-Defensin 2 Induces a Vigorous Cytokine Response in Peripheral Blood Mononuclear Cells. *Antimicrob. Agents Chemother.* 50, 1433–1441. doi: 10.1128/AAC.50.4.1433-1441.2006

Bouschbacher, M., Bomsel, M., Verronese, E., Gofflo, S., Ganor, Y., Dezutter-Dambuyant, C., et al. (2008). Early Events in HIV Transmission Through a Human Reconstructed Vaginal Mucosa. *Aids* 22, 1257–1266. doi: 10.1097/QAD.0b013e3282f736f4

Braida, L., Boniotto, M., Pontillo, A., Tovo, P. A., Amoroso, A., and Crovella, S. (2004). A Single-Nucleotide Polymorphism in the Human Beta-Defensin 1 Gene Is Associated With HIV-1 Infection in Italian Children. *Aids* 18, 1598–1600. doi: 10.1097/01.aids.0000131363.82951.fb

Brice, D. C., and Diamond, G. (2020). Antiviral Activities of Human Host Defense Peptides. *Curr. Med. Chem.* 27, 1420–1443. doi: 10.2174/0929867326666190805151654

Broder, S. (2010). The Development of Antiretroviral Therapy and its Impact on the HIV-1/AIDS Pandemic. *Antiviral Res.* 85, 1–18. doi: 10.1016/j.antiviral.2009.10.002

Brown, A., Zhang, H., Lopez, P., Pardo, C. A., and Gartner, S. (2006). In Vitro Modeling of the HIV-Macrophage Reservoir. *J. Leukoc. Biol.* 80, 1127–1135. doi: 10.1189/jlb.0206126

Chaipan, C., Smith, J. L., Hu, W. S., and Pathak, V. K. (2013). APOBEC3G Restricts HIV-1 to a Greater Extent Than APOBEC3F and APOBEC3DE in Human Primary CD4+ T Cells and Macrophages. *J. Virol.* 87, 444–453. doi: 10.1128/JVI.00676-12

Chang, T. L., Vargas, J. Jr., DelPortillo, A., and Klotman, M. E. (2005). Dual Role of Alpha-Defensin-1 in Anti-HIV-1 Innate Immunity. *J. Clin. Invest.* 115, 765–773. doi: 10.1172/JCI21948

Christensen-Quick, A., Lafferty, M., Sun, L., Marchionni, L., DeVico, A., and Garzino-Demo, A. (2016). Human Th17 Cells Lack HIV-Inhibitory RNases and Are Highly Permissive to Productive Hiv Infection. *J. Virol.* 90, 7833–7847. doi: 10.1128/JVI.02869-15

Churchill, M., and Nath, A. (2013). Where Does HIV Hide? A Focus on the Central Nervous System. *Curr. Opin. HIV AIDS* 8, 165–169. doi: 10.1097/COH.0b013e32835fc601

Cignetti, A., Vallario, A., Roato, I., Circosta, P., Strola, G., Scielzo, C., et al. (2003). The Characterization of Chemokine Production and Chemokine Receptor Expression Reveals Possible Functional Cross-Talks in AML Blasts With Monocytic Differentiation. *Exp. Hematol.* 31, 495–503. doi: 10.1016/S0301-472X(03)00066-3

Cocchi, F., DeVico, A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C., and Lusso, P. (1995). Identification of RANTES, MIP-1 Alpha, and MIP-1 Beta as the Major HIV-Suppressive Factors Produced by CD8+ T Cells. *Science* 270, 1811–1815. doi: 10.1126/science.270.5243.1811

Collins, K. B., Patterson, B. K., Naus, G. J., Landers, D. V., and Gupta, P. (2000). Development of an In Vitro Organ Culture Model to Study Transmission of HIV-1 in the Female Genital Tract. *Nat. Med.* 6, 475–479. doi: 10.1038/74743

Copeland, K. F., McKay, P. J., Newton, J., and Rosenthal, K. L. (1999). Cd8+ T Cell-Mediated Enhancement of Tumour Necrosis Factor-Alpha (TNF-Alpha) Production and HIV-1 LTR-Driven Gene Expression in Human Monocytic Cells Is Pertussis Toxin-Sensitive. *Clin. Exp. Immunol.* 116, 479–485. doi: 10.1046/j.1365-2249.1999.00897.x

Deeks, S. G. (2013). Towards an HIV Cure: A Global Scientific Strategy. *Nat. Rev. Immunol.* 12, 607–614. doi: 10.1038/nri3262

DeVico, A. L., and Gallo, R. C. (2004). Control of HIV-1 Infection by Soluble Factors of the Immune Response. *Nat. Rev. Microbiol.* 2, 401–413. doi: 10.1038/nrmicro878

Dobrovolskaia, M. A., and Vogel, S. N. (2002). Toll Receptors, CD14, and Macrophage Activation and Deactivation by LPS. *Microbes Infect. Institut. Pasteur.* 4, 903–914. doi: 10.1016/S1286-4579(02)01613-1

Dong, W., Liu, Y., Peng, J., Chen, L., Zou, T., Xiao, H., et al. (2006). The IRAK-1-BCL10-MALT1-TRAF6-TAK1 Cascade Mediates Signaling to NF-KappaB From Toll-Like Receptor 4. *J. Biol. Chem.* 281, 26029–26040. doi: 10.1074/jbc.M513057200

El Hed, A., Khaitan, A., Kozhaya, L., Manel, N., Daskalakis, D., Borkowsky, W., et al. (2010). Susceptibility of Human Th17 Cells to Human Immunodeficiency Virus and Their Perturbation During Infection. *J. Infect. Dis.* 201, 843–854. doi: 10.1086/651021

Feng, Z., Dubyak, G. R., Lederman, M. M., and Weinberg, A. (2006). Cutting Edge: Human Beta Defensin 3–A Novel Antagonist of the HIV-1 Coreceptor CXCR4. *J. Immunol.* 177, 782–786. doi: 10.4049/jimmunol.177.2.782

Flynn, G., Maru, S., Loughlin, J., Romero, I. A., and Male, D. (2003). Regulation of Chemokine Receptor Expression in Human Microglia and Astrocytes. *J. Neuroimmunol.* 136, 84–93. doi: 10.1016/S0165-5728(03)00009-2

Freguja, R., Gianesin, K., Del Bianco, P., Malacrida, S., Rampon, O., Zanchetta, M., et al. (2012). Polymorphisms of Innate Immunity Genes Influence Disease Progression in HIV-1-Infected Children. *Aids* 26, 765–768. doi: 10.1097/QAD.0b013e3283514350

Ganor, Y., Real, F., Sennepin, A., Dutertre, C. A., Prevedel, L., Xu, L., et al. (2019). HIV-1 Reservoirs in Urethral Macrophages of Patients Under Suppressive Antiretroviral Therapy. *Nat. Microbiol.* 4, 633–644. doi: 10.1038/s41564-018-0335-z

Gartner, S., Markovits, P., Markovitz, D. M., Betts, R. F., and Popovic, M. (1986a). Virus Isolation From and Identification of HTLV-III/LAV-Producing Cells in Brain Tissue From a Patient With AIDS. *JAMA* 256, 2365–2371. doi: 10.1001/jama.256.17.2365

Gartner, S., Markovits, P., Markovitz, D. M., Kaplan, M. H., Gallo, R. C., and Popovic, M. (1986b). The Role of Mononuclear Phagocytes in HTLV-III/LAV Infection. *Science* 233, 215–219. doi: 10.1126/science.3014648

Garzino-Demo, A., DeVico, A. L., Conant, K. E., and Gallo, R. C. (2000). The Role of Chemokines in Human Immunodeficiency Virus Infection. *Immunol. Rev.* 177, 79–87. doi: 10.1034/j.1600-065X.2000.17711.x

Gendelman, H. E., Orenstein, J. M., Baca, L. M., Weiser, B., Burger, H., Kalter, D. C., et al. (1989). The Macrophage in the Persistence and Pathogenesis of HIV Infection. *Aids* 3, 475–495. doi: 10.1097/00002030-198908000-00001

Ghosh, M., Fahey, J. V., Shen, Z., Lahey, T., Cu-Uvin, S., Wu, Z., et al. (2010). Anti-HIV Activity in Cervical-Vaginal Secretions From HIV-Positive and -Negative Women Correlate With Innate Antimicrobial Levels and IgG Antibodies. *PloS One* 5, e11366. doi: 10.1371/journal.pone.0011366

Gordon, S., and Mantovani, A. (2011). Diversity and Plasticity of Mononuclear Phagocytes. *Eur. J. Immunol.* 41, 2470–2472. doi: 10.1002/eji.201141988

Gordon, S., and Taylor, P. R. (2005). Monocyte and Macrophage Heterogeneity. *Nat. Rev. Immunol.* 5, 953–964. doi: 10.1038/nri1733

Gosselin, A., Monteiro, P., Chomont, N., Diaz-Griffero, F., Said, E. A., Fonseca, S., et al. (2010). Peripheral Blood CCR4+CCR6+ and CXCR3+CCR6+CD4+ T Cells are Highly Permissive to HIV-1 Infection. *J. Immunol.* 184, 1604–1616. doi: 10.4049/jimmunol.0903058

Goujon, C., Schaller, T., Galao, R. P., Amie, S. M., Kim, B., Olivieri, K., et al. (2013). Evidence for IFNalpha-induced, SAMHD1-Independent Inhibitors of Early HIV-1 Infection. *Retrovirology* 10, 23. doi: 10.1186/1742-4690-10-23

Greenhead, P., Hayes, P., Watts, P. S., Laing, K. G., Griffin, G. E., and Shattock, R. J. (2000). Parameters of Human Immunodeficiency Virus Infection of Human Cervical Tissue and Inhibition by Vaginal Virucides. *J. Virol.* 74, 5577–5586. doi: 10.1128/JVI.74.12.5577-5586.2000

Guo, C. J., Tan, N., Song, L., Douglas, S. D., and Ho, W. Z. (2004). Alpha-Defensins Inhibit HIV Infection of Macrophages Through Upregulation of CC-Chemokines. *Aids* 18, 1217–1218. doi: 10.1097/00002030-200405210-00020

Gupta, P., Collins, K. B., Ratner, D., Watkins, S., Naus, G. J., Landers, D. V., et al. (2002). Memory Cd4(+) T Cells Are the Earliest Detectable Human Immunodeficiency Virus Type 1 (HIV-1)-Infected Cells in the Female Genital Mucosal Tissue During HIV-1 Transmission in an Organ Culture System. *J. Virol.* 76, 9868–9876. doi: 10.1128/JVI.76.19.9868-9876.2002

Hao, H. N., Zhao, J., Lotoczky, G., Grever, W. E., and Lyman, W. D. (2001). Induction of Human Beta-Defensin-2 Expression in Human Astrocytes by Lipopolysaccharide and Cytokines. *J. Neurochem.* 77, 1027–1035. doi: 10.1046/j.1471-4159.2001.00305.x

Hardwick, R. J., Amogne, W., Mugusi, S., Yimer, G., Ngaimisi, E., Habtewold, A., et al. (2012). Beta-Defensin Genomic Copy Number Is Associated With HIV Load and Immune Reconstitution in Sub-Saharan Africans. *J. Infect. Dis.* 206, 1012–1019. doi: 10.1093/infdis/jis448

Hladik, F., Sakchalathorn, P., Ballweber, L., Lentz, G., Fialkow, M., Eschenbach, D., et al. (2007). Initial Events in Establishing Vaginal Entry and Infection by Human Immunodeficiency Virus Type-1. *Immunity* 26, 257–270. doi: 10.1016/j.immuni.2007.01.007

Holly, M. K., Diaz, K., and Smith, J. G. (2017). Defensins in Viral Infection and Pathogenesis. *Annu. Rev. Virol.* 4, 369–391. doi: 10.1146/annurev-virology-101416-041734

Honeycutt, J. B., Thayer, W. O., Baker, C. E., Ribeiro, R. M., Lada, S. M., Cao, Y., et al. (2017). HIV Persistence in Tissue Macrophages of Humanized Myeloid-Only Mice During Antiretroviral Therapy. *Nat. Med.* 23, 638–643. doi: 10.1038/nm.4319

Honeycutt, J. B., Wahl, A., Baker, C., Spagnuolo, R. A., Foster, J., Zakharova, O., et al. (2016). Macrophages Sustain HIV Replication In Vivo Independently of T Cells. *J. Clin. Invest.* 126, 1353–1366. doi: 10.1172/JCI84456

Hou, W., Wang, X., Ye, L., Zhou, L., Yang, Z. Q., Riedel, E., et al. (2009). Lambda Interferon Inhibits Human Immunodeficiency Virus Type 1 Infection of Macrophages. *J. Virol.* 83 (8), 3834–3842. doi: 10.1128/JVI.01773-08

Hu, Q., Younson, J., Griffin, G. E., Kelly, C., and Shattock, R. J. (2006). Pertussis Toxin and Its Binding Unit Inhibit HIV-1 Infection of Human Cervical Tissue and Macrophages Involving a CD14 Pathway. *J. Infect. Dis.* 194, 1547–1556. doi: 10.1086/508898

Iida, S., Kohro, T., Kodama, T., Nagata, S., and Fukunaga, R. (2005). Identification of CCR2, Flotillin, and gp49B Genes as New G-CSF Targets During Neutrophilic Differentiation. *J. Leukoc. Biol.* 78, 481–490. doi: 10.1189/jlb.0904515

Iordanskiy, S., Iordanskaya, T., Quivy, V., Van Lint, C., and Bukrinsky, M. (2002). B-Oligomer of Pertussis Toxin Inhibits HIV-1 LTR-Driven Transcription Through Suppression of NF-kappaB p65 Subunit Activity. *Virology* 302, 195–206. doi: 10.1006/viro.2002.1618

Jarczak, J., Kosciuczuk, E. M., Lisowski, P., Strzalkowska, N., Jozwik, A., Horbanczuk, J., et al. (2013). Defensins: Natural Component of Human Innate Immunity. *Hum. Immunol.* 74, 1069–1079. doi: 10.1016/j.humimm.2013.05.008

Jin, X., Brooks, A., Chen, H., Bennett, R., Reichman, R., and Smith, H. (2005). Apobec3g/Cem15 (hA3G) mRNA Levels Associate Inversely With Human Immunodeficiency Virus Viremia. *J. Virol.* 79, 11513–11516. doi: 10.1128/JVI.79.17.11513-11516.2005

Jin, G., Kawsar, H. I., Hirsch, S. A., Zeng, C., Jia, X., Feng, Z., et al. (2010). An Antimicrobial Peptide Regulates Tumor-Associated Macrophage Trafficking Via the Chemokine Receptor CCR2, a Model for Tumorigenesis. *PloS One* 5, e10993. doi: 10.1371/journal.pone.0010993

Kao, S., Khan, M. A., Miyagi, E., Plishka, R., Buckler-White, A., and Strebel, K. (2003). The Human Immunodeficiency Virus Type 1 Vif Protein Reduces Intracellular Expression and Inhibits Packaging of APOBEC3G (CEM15), a Cellular Inhibitor of Virus Infectivity. *J. Virol.* 77, 11398–11407. doi: 10.1128/JVI.77.21.11398-11407.2003

Katschke, K. J.Jr., Rottman, J. B., Ruth, J. H., Qin, S., Wu, L., LaRosa, G., et al. (2001). Differential Expression of Chemokine Receptors on Peripheral Blood, Synovial Fluid, and Synovial Tissue Monocytes/Macrophages in Rheumatoid Arthritis. *Arthritis Rheumatol.* 44, 1022–1032. doi: 10.1002/1529-0131(200105)44:5<1022::AID-ANR181>3.0.CO;2-N

Kesting, M. R., Stoeckelhuber, M., Kuppek, A., Hasler, R., Rohleder, N., Wolff, K. D., et al. (2012). Human Beta-Defensins and Psoriasin/S100A7 Expression in Salivary Glands: Anti-Oncogenic Molecules for Potential Therapeutic Approaches. *BioDrugs* 26, 33–42. doi: 10.2165/11597570-000000000-00000

Koenig, S., Gendelman, H. E., Orenstein, J. M., Dal Canto, M. C., Pezeshkpour, G. H., Yungbluth, M., et al. (1986). Detection of AIDS Virus in Macrophages in

Brain Tissue From AIDS Patients With Encephalopathy. *Science* 233, 1089–1093. doi: 10.1126/science.3016903

Ko, A., Kang, G., Hattler, J. B., Galadima, H. I., Zhang, J., Li, Q., et al. (2019). Macrophages But Not Astrocytes Harbor HIV DNA in the Brains of HIV-1-Infected Aviremic Individuals on Suppressive Antiretroviral Therapy. *J. Neuroimmune Pharmacol.* 14, 110–119. doi: 10.1007/s11481-018-9809-2

Koning, F. A., Newman, E. N., Kim, E. Y., Kunstman, K. J., Wolinsky, S. M., and Malim, M. H. (2009). Defining APOBEC3 Expression Patterns in Human Tissues and Hematopoietic Cell Subsets. *J. Virol.* 83, 9474–9485. doi: 10.1128/JVI.01089-09

Koppensteiner, H., Brack-Werner, R., and Schindler, M. (2012). Macrophages and Their Relevance in Human Immunodeficiency Virus Type I Infection. *Retrovirology* 9, 82. doi: 10.1186/1742-4690-9-82

Lafferty, M. K., Sun, L., Christensen-Quick, A., Lu, W., and Garzino-Demo, A. (2017). Human Beta Defensin 2 Selectively Inhibits HIV-1 in Highly Permissive Ccr6(+)Cd4(+) T Cells. *Viruses May* 16, 9. doi: 10.3390/v9050111

Lafferty, M. K., Sun, L., DeMasi, L., Lu, W., and Garzino-Demo, A. (2010). CCR6 Ligands Inhibit HIV by Inducing APOBEC3G. *Blood* 115, 1564–1571. doi: 10.1182/blood-2009-06-226423

Lapenta, C., Spada, M., Santini, S. M., Racca, S., Dorigatti, F., Poli, G., et al. (2005). Pertussis Toxin B-Oligomer Inhibits HIV Infection and Replication in hu-PBL-SCID Mice. *Int. Immunol.* 17, 469–475. doi: 10.1093/intimm/dxh226

Lehrer, R. I., and Lu, W. (2012). alpha-Defensins in Human Innate Immunity. *Immunol. Rev.* 245, 84–112. doi: 10.1111/j.1600-065X.2011.01082.x

Liao, F., Rabin, R. L., Smith, C. S., Sharma, G., Nutman, T. B., and Farber, J. M. (1999). CC-Chemokine Receptor 6 is Expressed on Diverse Memory Subsets of T Cells and Determines Responsiveness to Macrophage Inflammatory Protein 3 Alpha. *J. Immunol.* 162, 186–194.

Li, H., Bar, K. J., Wang, S., Decker, J. M., Chen, Y., Sun, C., et al. (2010). High Multiplicity Infection by HIV-1 in Men Who Have Sex With Men. *PloS Pathog.* 6, e1000890. doi: 10.1371/journal.ppat.1000890

Lin, K. L., Suzuki, Y., Nakano, H., Ramsburg, E., and Gunn, M. D. (2008). CCR2+ Monocyte-Derived Dendritic Cells and Exudate Macrophages Produce Influenza-Induced Pulmonary Immune Pathology and Mortality. *J. Immunol.* 180, 2562–2572. doi: 10.4049/jimmunol.180.4.2562

Liu, A. Y., Destoumieux, D., Wong, A. V., Park, C. H., Valore, E. V., Liu, L., et al. (2002). Human Beta-Defensin-2 Production in Keratinocytes Is Regulated by interleukin-1, Bacteria, and the State of Differentiation. *J. Invest. Dermatol.* 118, 275–281. doi: 10.1046/j.0022-202x.2001.01651.x

Mackewicz, C. E., Yuan, J., Tran, P., Diaz, L., Mack, E., Selsted, M. E., et al. (2003). alpha-Defensins Can Have Anti-HIV Activity But Are Not CD8 Cell Anti-HIV Factors. *Aids* 17, F23–F32. doi: 10.1097/00002030-200309260-00001

Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., and Locati, M. (2004). The Chemokine System in Diverse Forms of Macrophage Activation and Polarization. *Trends Immunol.* 25, 677–686. doi: 10.1016/j.it.2004.09.015

Mashiba, M., and Collins, K. L. (2013). Molecular Mechanisms of HIV Immune Evasion of the Innate Immune Response in Myeloid Cells. *Viruses* 5, 1–14. doi: 10.3390/v5010001

Mei, H. F., Jin, X. B., Zhu, J. Y., Zeng, A. H., Wu, Q., Lu, X. M., et al. (2012). Beta-Defensin 2 as an Adjuvant Promotes Anti-Melanoma Immune Responses and Inhibits the Growth of Implanted Murine Melanoma In Vivo. *PloS One* 7, e31328. doi: 10.1371/journal.pone.0031328

Milanese, M., Segat, L., Arraes, L. C., Garzino-Demo, A., and Crovella, S. (2009). Copy Number Variation of Defensin Genes and HIV Infection in Brazilian Children. *J. Acquir. Immune Defic. Syndr.* 50, 331–333. doi: 10.1097/QAI.0b013e3181945f39

Milanese, M., Segat, L., Pontillo, A., Arraes, L. C., de Lima Filho, J. L., and Crovella, S. (2006). DEFB1 Gene Polymorphisms and Increased Risk of HIV-1 Infection in Brazilian Children. *Aids* 20, 1673–1675. doi: 10.1097/01.aids.0000238417.05819.40

Momoi, Y., Ichiyama, K., Chowdhury, I. H., Koyanagi, Y., and Yamamoto, N. (2000). Pertussis Toxin Enhances Human Immunodeficiency Virus Type 1 Replication. *AIDS Res. Hum. Retroviruses* 16, 373–379. doi: 10.1089/088922200309250

Monteiro, P., Gosselin, A., Wacleche, V. S., El-Far, M., Said, E. A., Kared, H., et al. (2011). Memory CCR6+CD4+ T Cells Are Preferential Targets for Productive HIV Type 1 Infection Regardless of Their Expression of Integrin Beta7. *J. Immunol.* 186, 4618–4630. doi: 10.4049/jimmunol.1004151

Mony, J. T., Khorooshi, R., and Owens, T. (2014). Chemokine Receptor Expression by Inflammatory T Cells in EAE. *Front. Cell. Neurosci.* 8, 187. doi: 10.3389/fncel.2014.00187

Moutsopoulos, N. M., Greenwell-Wild, T., and Wahl, S. M. (2006). Differential Mucosal Susceptibility in HIV-1 Transmission and Infection. *Adv. Dent. Res.* 19, 52–56. doi: 10.1177/154407370601900111

Nakashima, H., Yamamoto, N., Masuda, M., and Fujii, N. (1993). Defensins Inhibit HIV Replication In Vitro. *Aids* 7, 1129. doi: 10.1097/00002030-199308000-00019

Nath, A., and Tyler, K. L. (2013). Novel Approaches and Challenges to Treatment of Central Nervous System Viral Infections. *Ann. Neurol.* 74, 412–422. doi: 10.1002/ana.23988

Niyonsaba, F., Iwabuchi, K., Matsuda, H., Ogawa, H., and Nagaoka, I. (2002). Epithelial Cell-Derived Human Beta-Defensin-2 Acts as a Chemotaxin for Mast Cells Through a Pertussis Toxin-Sensitive and Phospholipase C-Dependent Pathway. *Int. Immunol.* 14, 421–426. doi: 10.1093/intimm/14.4.421

Niyonsaba, F., Ogawa, H., and Nagaoka, I. (2004). Human Beta-Defensin-2 Functions as a Chemotactic Agent for Tumour Necrosis Factor-Alpha-Treated Human Neutrophils. *Immunology* 111, 273–281. doi: 10.1111/j.0019-2805.2004.01816.x

Niyonsaba, F., Someya, A., Hirata, M., Ogawa, H., and Nagaoka, I. (2001). Evaluation of the Effects of Peptide Antibiotics Human Beta-Defensins-1/-2 and LL-37 on Histamine Release and Prostaglandin D(2) Production From Mast Cells. *Eur. J. Immunol.* 31, 1066–1075. doi: 10.1002/1521-4141(200104)31:4<1066::AID-IMMU1066>3.0.CO;2-#

Pace, C. N., Vajdos, F., Fee, L., Grimsley, G., and Gray, T. (1995). How to Measure and Predict the Molar Absorption Coefficient of a Protein. *Protein Sci.* 4, 2411–2423. doi: 10.1002/pro.5560041120

Page, K. A., Landau, N. R., and Littman, D. R. (1990). Construction and Use of a Human Immunodeficiency Virus Vector for Analysis of Virus Infectivity. *J. Virol.* 64, 5270–5276. doi: 10.1128/jvi.64.11.5270-5276.1990

Papo, N., and Shai, Y. (2005). Host Defense Peptides as New Weapons in Cancer Treatment. *Cell Mol. Life Sci.* 62, 784–790. doi: 10.1007/s00018-005-4560-2

Patel, M. V., Ghosh, M., Fahey, J. V., Ochsenbauer, C., Rossoll, R. M., and Wira, C. R. (2014). Innate Immunity in the Vagina (Part II): Anti-HIV Activity and Antiviral Content of Human Vaginal Secretions. *Am. J. Reprod. Immunol.* 72, 22–33. doi: 10.1111/aji.12218

Peng, G., Greenwell-Wild, T., Nares, S., Jin, W., Lei, K. J., Rangel, Z. G., et al. (2007). Myeloid Differentiation and Susceptibility to HIV-1 Are Linked to APOBEC3 Expression. *Blood* 110, 393–400. doi: 10.1182/blood-2006-10-051763

Phillips, R. J., Lutz, M., and Premack, B. (2005). Differential Signaling Mechanisms Regulate Expression of CC Chemokine Receptor-2 During Monocyte Maturation. *J. Inflamm. (Lond)* 2, 14. doi: 10.1186/1476-9255-2-14

Poiraud, C., Quereux, G., Knol, A. C., Zuliani, T., Allix, R., Khammari, A., et al. (2012). Human Beta-Defensin-2 and Psoriasin, Two New Innate Immunity Targets of Zinc Gluconate. *Eur. J. Dermatol. EJD* 22, 634–639. doi: 10.1684/ejd.2012.1800

Quinones-Mateu, M. E., Lederman, M. M., Feng, Z., Chakraborty, B., Weber, J., Rangel, H. R., et al. (2003). Human Epithelial Beta-Defensins 2 and 3 Inhibit HIV-1 Replication. *Aids* 17, F39–F48. doi: 10.1097/00002030-200311070-00001

Ricci, E., Malacrida, S., Zanchetta, M., Montagna, M., Giaquinto, C., and De Rossi, A. (2009). Role of Beta-Defensin-1 Polymorphisms in Mother-to-Child Transmission of HIV-1. *J. Acquir. Immune Defic. Syndr.* 51, 13–19. doi: 10.1097/QAI.0b013e31819df249

Rohrl, J., Yang, D., Oppenheim, J. J., and Hehlgans, T. (2010). Human Beta-Defensin 2 and 3 and Their Mouse Orthologs Induce Chemotaxis Through Interaction With CCR2. *J. Immunol.* 184, 6688–6694. doi: 10.4049/jimmunol.0903984

Ruth, J. H., Shahrara, S., Park, C. C., Morel, J. C., Kumar, P., Qin, S., et al. (2003). Role of Macrophage Inflammatory Protein-3alpha and Its Ligand CCR6 in Rheumatoid Arthritis. *Lab. Investigation J. Tech. Methods Pathol* 83, 579–588. doi: 10.1097/01.lab.0000062854.30195.52

Schnolzer, M., Alewood, P., Jones, A., Alewood, D., and Kent, S. B. (1992). In Situ Neutralization in Boc-Chemistry Solid Phase Peptide Synthesis. Rapid, High Yield Assembly of Difficult Sequences. *Int. J. Pept. Protein Res.* 40, 180–193. doi: 10.1111/j.1399-3011.1992.tb00291.x

Shankar, E. M., Velu, V., Vignesh, R., Vijayaraghavalu, S., Rukumani, D. V., and Sabet, N. S. (2012). Recent Advances Targeting Innate Immunity-Mediated Therapies Against HIV-1 Infection. *Microbiol. Immunol.* 56, 497–505. doi: 10.1111/j.1348-0421.2012.00485.x

Shen, R., Richter, H. E., and Smith, P. D. (2011). Early HIV-1 Target Cells in Human Vaginal and Ectocervical Mucosa. *Am. J. Reprod. Immunol.* 65, 261–267. doi: 10.1111/j.1600-0897.2010.00939.x

Sica, A., and Mantovani, A. (2012). Macrophage Plasticity and Polarization: In Vivo Veritas. *J. Clin. Invest.* 122, 787–795. doi: 10.1172/JCI59643

Singh, S. P., Zhang, H. H., Foley, J. F., Hedrick, M. N., and Farber, J. M. (2008). Human T Cells That Are Able to Produce IL-17 Express the Chemokine Receptor CCR6. *J. Immunol.* 180, 214–221. doi: 10.4049/jimmunol.180.1.214

Soruri, A., Grigat, J., Forssmann, U., Riggert, J., and Zwirner, J. (2007). beta-Defensins Chemoattract Macrophages and Mast Cells But Not Lymphocytes and Dendritic Cells: CCR6 Is Not Involved. *Eur. J. Immunol.* 37, 2474–2486. doi: 10.1002/eji.200737292

Sun, L., Finnegan, C. M., Kish-Catalone, T., Blumenthal, R., Garzino-Demo, P., La Terra Maggiore, G. M., et al. (2005). Human Beta-Defensins Suppress Human Immunodeficiency Virus Infection: Potential Role in Mucosal Protection. *J. Virol.* 79, 4318–14329. doi: 10.1128/JVI.79.22.14318-14329.2005

Thielen, B. K., McNevin, J. P., McElrath, M. J., Hunt, B. V., Klein, K. C., and Lingappa, J. R. (2010). Innate Immune Signaling Induces High Levels of TC-Specific Deaminase Activity in Primary Monocyte-Derived Cells Through Expression of APOBEC3A Isoforms. *J. Biol. Chem.* 285, 27753–27766. doi: 10.1074/jbc.M110.102822

Wang, W., Owen, S. M., Rudolph, D. L., Cole, A. M., Hong, T., Waring, A. J., et al. (2004). Activity of Alpha- and Theta-Defensins Against Primary Isolates of HIV-1. *J. Immunol.* 173, 515–520. doi: 10.4049/jimmunol.173.1.515

Williams, K. C., Corey, S., Westmoreland, S. V., Pauley, D., Knight, H., deBakker, C., et al. (2001). Perivascular Macrophages Are the Primary Cell Type Productively Infected by Simian Immunodeficiency Virus in the Brains of Macaques: Implications for the Neuropathogenesis of AIDS. *J. Exp. Med.* 193, 905–915. doi: 10.1084/jem.193.8.905

Wilson, S. S., Wiens, M. E., and Smith, J. G. (2013). Antiviral Mechanisms of Human Defensins. *J. Mol. Biol.* 425, 4965–4980. doi: 10.1016/j.jmb.2013.09.038

Wu, Z., Hoover, D. M., Yang, D., Boulegue, C., Santamaria, F., Oppenheim, J. J., et al. (2003). Engineering Disulfide Bridges to Dissect Antimicrobial and Chemotactic Activities of Human Beta-Defensin 3. *Proc. Natl. Acad. Sci. U. S. A.* 100, 8880–8885. doi: 10.1073/pnas.1533186100

Wynn, T. A., Chawla, A., and Pollard, J. W. (2013). Macrophage Biology in Development, Homeostasis and Disease. *Nature* 496, 445–455. doi: 10.1038/nature12034

Yang, D., Chertov, O., Bykovskaia, S. N., Chen, Q., Buffo, M. J., Shogan, J., et al. (1999). Beta-Defensins: Linking Innate and Adaptive Immunity Through Dendritic and T Cell CCR6. *Science* 286, 525–528. doi: 10.1126/science.286.5439.525

Yang, D., Liu, Z. H., Tewary, P., Chen, Q., de la Rosa, G., and Oppenheim, J. J. (2007). Defensin Participation in Innate and Adaptive Immunity. *Curr. Pharm. Design* 13, 3131–3139. doi: 10.2174/138161207782110453

Zapata, W., Rodriguez, B., Weber, J., Estrada, H., Quinones-Mateu, M. E., Zimermman, P. A., et al. (2008). Increased Levels of Human Beta-Defensins mRNA in Sexually HIV-1 Exposed But Uninfected Individuals. *Curr. HIV Res.* 6, 531–538. doi: 10.2174/157016208786501463

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership