Hiding in plain sight – Platelets, the silent carriers of HIV-1

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Abstract

There are approximately 38 million people globally living with Human immunodeficiency virus 1 (HIV-1) and given the tremendous success of combination antiretroviral therapy (cART) this has dramatically reduced mortality and morbidity with prevention benefits. However, HIV-1 persists during cART within the human body and re-appears upon cART interruption. This HIV-1 reservoir remains a barrier to cure with cellular sites of viral persistence not fully understood. In this study we provide evidence corroborating a recently published article in STM demonstrating the role of platelets as a novel cellular disseminator of HIV-1 particles in the setting of viral suppression. Using classical transmission electron microscopy with and without immunogold labeling, we visualize HIV-1 in both platelets and monocytes in cART suppressed HIV donors. Our study

suggests that due to the close proximity of platelets and monocytes an alternative life cycle of HIV-1 cycling within monocytes and platelets without the need of active replication under cART occurs. Our findings are supported by the lack of detectable HIV-1 particles in platelets derived from HIV uninfected donors or the 'Berlin' patient suggesting that platelets may serve as an underappreciated hidden bearer for HIV-1 and should be considered in HIV remission studies and trials.

To the editor

Combination antiretroviral therapy (cART) suppresses HIV-1 replication in people living with HIV (PLWH), which ultimately restricts HIV-1 transmission and the progression of AIDS-related disease. Virally suppressed PLWH, however, still exhibit resurgence of HIV when cART is interrupted [1]. The two 'Boston patients', after being diagnosed and treated for hematological tumors, underwent bone marrow transplantation with cells derived from HIV-uninfected donors [2]. Both individuals had a resurgence of blood viral loads within 12 or 32 weeks, indicating the existence of an active virus-producing reservoir separate from the bone marrow-derived cell lineages [2]. In view of the recently published article by Real et al. [3] demonstrating that human platelets can harbor replication-competent HIV-1 even in the context of cART, we wish to extend these findings here by illustrating the existence of monocyte-platelet-HIV particle interactions.

To identify HIV-1 particles we imaged immune cells, including monocytes and monocyte-derived macrophages, from PLWH on suppressive cART alongside healthy, HIV-uninfected individuals, and the 'Berlin' patient known to be the first patient cured from HIV-1 infection by both scanning and transmission electron microscopy (SEM and TEM; Donor characteristics in Supplementary Table 1). Our data revealed that platelets were present in every monocyte preparation, both in the proximity of and attached to monocytes (Figure 1A). Interestingly, adherent or 'hitchhiking' platelets were also present on monocyte-derived macrophages after being in culture for more than 7 days, a phenomenon we recently reported in a study independent from HIV-1 [4]. However, we were unable to detect major differences of platelet adherence to immune cells by visual examination between control and HIV-1 infected study participants, indicating that perhaps the limitations of SEM for these quantitative purposes, especially since monocyte-platelet aggregates are shown to be more frequent in PLWH compared to healthy controls [5]. When examining monocytes or macrophages by TEM we found a large number of platelets which were identified by their specific ultrastructural characteristics (glycogen pockets, the open canalicular system (OCS), and dense granules). We observed the appearance of virus-like particles present within the platelet population (Figure 1B), even though study participants were on cART. It is known that platelets possess the capability to capture and engulf HIV [6–8]. We also identified virus-like particles (VLP) within endosomal structures and in the OCS of platelets, as previously described [7]. Several observed VLPs displayed a dense core, which could indicate these particles are still undergoing maturation [9]. However, VLP ultrastructure imaged is in accordance with previous finding [3, 9–13]. Extension of pseudopods, a sign of platelet activation (Figure 1B, I arrowhead), and an OCS displaying dilation were also observed.

We next examined monocytes and macrophages from the 'Berlin patient', the first person to be cured of HIV-1 infection. The 'Berlin' patient received a bone marrow transplant for treatment of leukemia from a donor with a CCR5 32 mutation and has since remained free of active viral infection in spite of not being on cART [14]. Monocyte-platelet aggregates in the 'Berlin' patient sample preparations, detected by SEM, were similar to aggregates observed in HIV-infected and control samples (Figure 1A), but more importantly, viral particles were not detected within platelets or monocytes/macrophages, further substantiating our observation that particles identified by TEM are HIV (Figure 1B).

Additionally, we incubated monocytes from PLWH on suppressive cART with agents that reverse HIV latency, histone deacetylase (HDAC) inhibitors, and noted the appearance of virus-like particles on the surface of monocytes (Figure 1C), although this needs to be confirmed. Additionally, the frequency of virus-like particles within platelets increased significantly after HDAC inhibitor treatment (Figure 1D, E). Several virus-like particles were found within a single platelet, whereas under control conditions only a few platelets each contained a single virus-like particle (Figure 1E). Additionally, we were able to detect VLP in the supernatant of PLWH on cART by negative staining and TEM imaging (Figure 1F). Our data suggest that HDAC inhibition increases virus-like particle release within both monocytes and their 'hitchhiking' platelets, potentially indicating that monocytes may harbor HIV-1 and particles released by monocytes can be rapidly engulfed by platelets, presumably due to their close symbiotic contact. We also showed in our TEM preparations evidence of monocytes phagocytosing platelets (Figure 1G), a phenomenon previously described [15].

To further substantiate whether the observed particles were indeed HIV-1, we employed immunogold labeling with several antibodies from the NIH AIDS Reagents Program that target HIV-1-specific proteins. Platelets of PLWH on cART showed positive staining for p24 (size of 70–100 nm; Figure 1H) within monocyte/platelet cultures, as indicated by at least two gold particles being present and potentially forming a semi-circle. In contrast, secondary antibody control staining and p24 staining of HIV negative cells did not reveal p24 staining associated with VLPs.

Based on the findings of Real et al. publication and our group, we propose the following mechanisms regarding platelet-monocyte complexes in viral persistence (Figure 2): In untreated HIV-1, HIV-1 particles are taken up by various cell types like monocytes or megakaryocytes where HIV-1 will integrate into the cellular DNA and persist after cART [16, 17]. Additionally, HIV-1 is engulfed by platelets where it is not able to integrate into cellular DNA and remains sequestered therefore persisting in circulation evading immune surveillance. Due to the close contact of platelets with other cell types, including monocytes HIV-1 virus particle transfer between platelets and other cell types is possible. Once cART is initiated, active proliferation of HIV-1 is inhibited; however, the close proximity of HIV-1-containing platelets with monocytes may provide a suitable environment for phagocytosis of the platelets or through bridging conduits [18, 19] or nanotubes allowing for viral particle exchange potentially reseeding the virus in monocytes and monocyte-derived tissue macrophages, which allows for persistence of HIV-1 within circulating cells and tissues. Additionally, we cannot exclude the possibility that HIV-1 found in platelets might be

derived from megakaryocytes, a second potential way of transmission as megakaryocytes have recently been found to be carrying replication competent HIV-1 even under cART [3]. We therefore highlight the importance of further research investigating HIV-1 presence in platelets and circulating immune cells in PLWH on cART. We would also like to advocate using multiple techniques, especially 3D electron microscopy techniques (e.g., FIB-SEM) with correlative immunofluorescence in timeline experiments to determine whether phagocytosis of HIV-1 into platelets or from platelets into monocytes actually occurs. Presented here are images using classical TEM, which does not allow for verification of whether the location of the particles/platelets is due to phagocytosis or merely being surrounded by the monocyte/platelet. We further highlight the importance of displaying results from presumably negative internal assay controls for immunogold labeling to clearly verify and validate the presence of HIV-1 particles.

The HIV-1 cell associated reservoir persists as resurgence has been observed in single-copy experiments even after 3–5 years of continuous cART [20]; therefore, further investigation to determine whether the HIV-1 particles we observed are replication competent and contribute to viral recrudescence. Recent literature have demonstrated that monocytes can serve as a HIV-1 reservoir capable of active viral replication [21–23] and these observations were more prominent in a subset of HIV infected donors [17]. Further research is therefore warranted to gain further insight into the 'lifecycle' of HIV latency and reservoir formation in monocytes, but also the role of platelets in the pathogenesis of comorbidities in PLWH on cART [24]. Platelets have been shown to drive inflammation and to potentially accelerate progression of various diseases [25, 26] as well as of HIV-1 [27]. Platelets engulfed by monocytes/macrophages have been reported to alter the phenotype of macrophage differentiation which can have multiple effects on many diseases [15]. Although our data are by no means complete and need to be confirmed using more powerful and specific imaging techniques, our findings demonstrate platelets could be an underappreciated bearer and conveyer of HIV-1 to myeloid cells which certainly needs to be considered and investigated in the global fight against HIV-1 infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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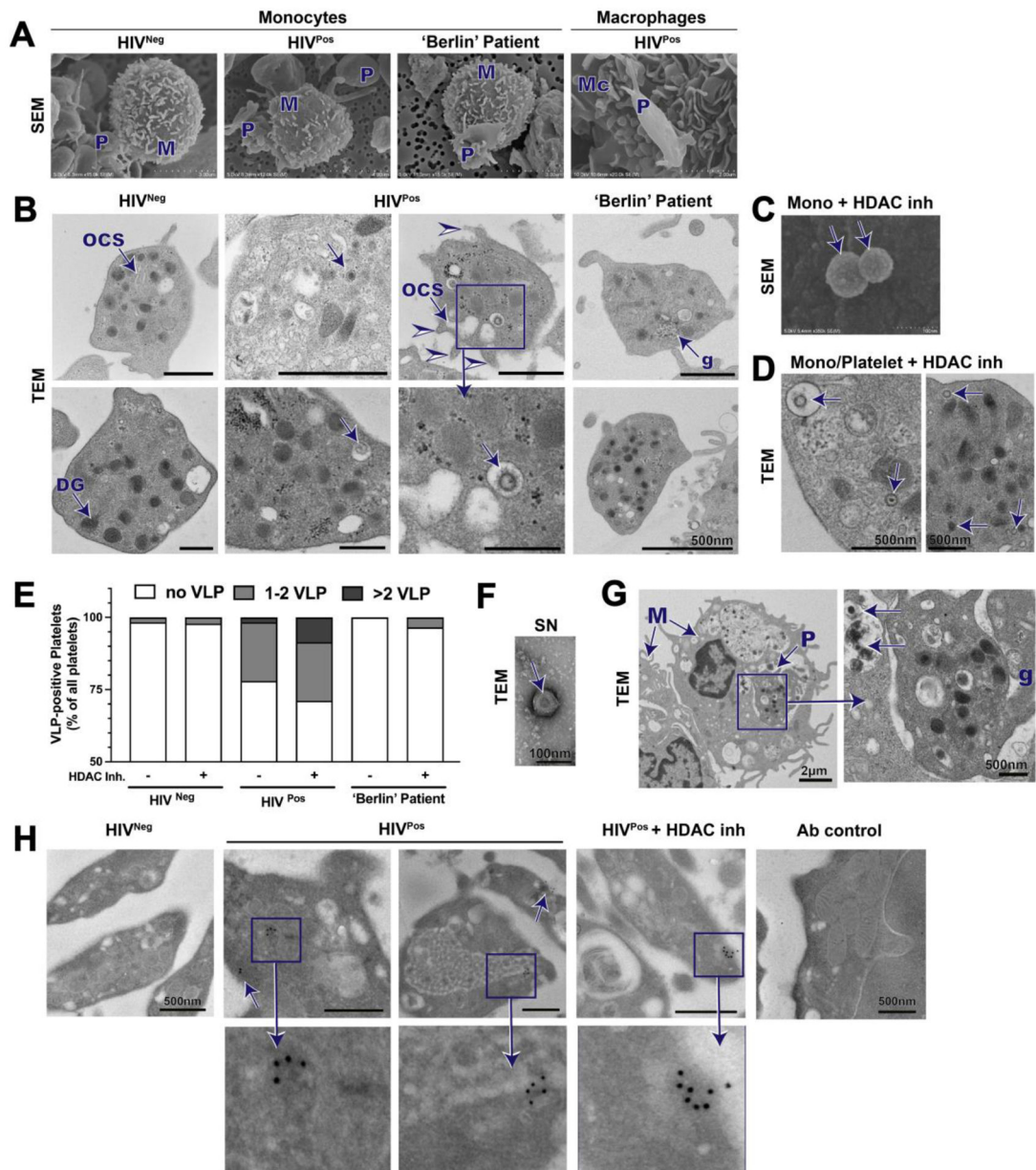
Figure 1: Hiding in plain sight – Platelets as carriers of HIV-1.

Platelets (P) adhering to monocytes (M) and macrophages (Mc) from healthy (HIV^neg),
suppressed HIV-1-infected individuals (HIV^pos), and the 'Berlin' patient by SEM (**A**).
TEM sections imaging monocyte preparations and platelets surrounding monocytes (**B**);
platelet histological features: glycogen pockets (g), open canalicular system (OCS), and
electron dense granules (DG). Viral particles (**arrows**) are detectable in platelets of HIV^pos.
Pseudopods on platelets (**arrowheads**) were apparent, indicative of an increased activated
state. Virus-like particles detected on surface by SEM of HIV^pos monocytes treated with
HDAC inhibitors (inh) (**C**) and intracellularly as detected by TEM (**D**). (**E**) Quantification of
platelets with visual VLPs from TEM images of monocyte/platelet preparations with control
or HDAC inhibitor treatment. Platelets with VLP were counted and separated into two

groups; platelets with 1–2 VLPs or with >2 VLPs. (**F**)Visualization of VLPs in supernatant (SN) of HDAC inh. treated monocyte/platelet preparations. (**G**) TEM imaging reveals signs of monocytes phagocytosing platelets. (**H**) p24 immunogold labeling of monocyte/platelet preparations. Positive p24 staining is indicated by arrows.
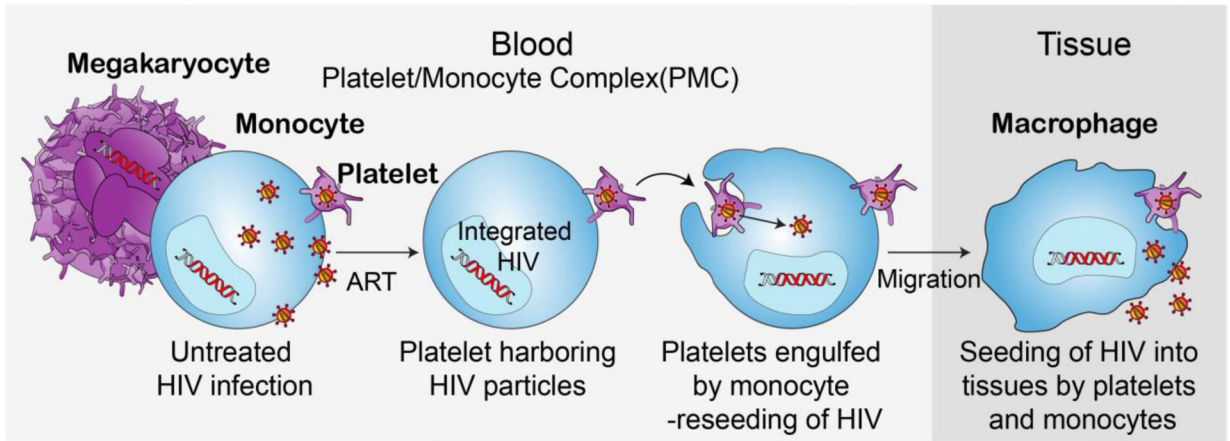
Figure 2: Hiding in plain sight – Platelets as carriers of HIV-1.
Proposed 'life cycle' of HIV-1 utilizing platelets as a carrier: Upon HIV-1 infection of a human body several cell types will be infected with HIV-1. HIV-1 integrates rather rapidly in the DNA of various cell types including megakaryocytes and monocytes. Additionally, in the scenario of untreated HIV-1 infection platelets can engulf HIV-1 particles. Due to the close contact of platelets and monocytes HIV-1 transfer between these two cell types, especially upon phagocytosis of dying platelets will occur. Upon start of cART treatment no active HIV-1 replication can occur, however, few platelets will still harbor HVI-1 particles and allow for an alternative 'life' cycle of HIV-1 under cART therapy allowing for circulation of active HIV-1 particles within monocytes, which subsequently can enter tissues, differentiate into macrophages carrying HIV-1 particles. It is likely that upon cell death within the tissue HIV-1 can either be distributed within the tissue or phagocytosed by other cell types entering another life cycle without the need of active replication, allowing HIV-1 lingering within cells and tissues able to infect various cell types.