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Erythrocyte Lysate from HIV-1-Infected Individuals Contains HIV-1 RNA

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Abstract

Background: Many studies have reported the presence of HIV in association with erythrocytes. This pool of the virus is implicated in the pathogenesis and progression of disease. This association has been attributed to erythrocyte membrane lipids. We previously reported varying risks for HIV infection associated with C, Lu^b and P_1 blood groups. This study sought to confirm such claims and further determine if this pool extends into the interior of the erythrocyte.

Methods: Qualitative PCR (n=27) to detect erythrocyte-associated HIV-1 RNA was carried on purified erythrocyte stroma and lysates. The plasma viral load (pVL) was also determined for these samples.

Results: Viral RNA was detected in both lysate and membranous fractions of purified erythrocytes, including those with undetectable pVL. Erythrocyte-associated HIV was detectable in 22/27 (81%) stroma 11/27 lysates. HIV RNA was undetectable in 4/5 subjects with detectable or high pVL. On the other hand, E-RNA was demonstrable in 6 patients who had undetectable pVL. Only one sample yielded RNA from the lysate but not from the stroma.

Conclusion: HIV RNA is demonstrable in patients with undetectable pVL. Furthermore, the presence of this pool is independent of plasma viral load. We conclude that the erythrocyte membrane possesses properties that not only enable viral attachment, but entry as well.

Keywords: HIV-1; RNA; Viral load; Erythrocytes

Introduction

In 2002, Hess and colleagues reported some HIV-1 to be associated with erythrocyte membranous ghosts in about 98% of HIV-1-infected patients [1]. The erythrocyte-associated HIV was also detectable in one third of the patients in whom plasma viral load was undetectable. The erythrocyte-associated HIV in some of the patients exceeded that associated with leukocytes and was associated with advanced clinical stages of the disease. Other investigators confirmed that erythrocyte-binding to the virus was sufficiently effective to reduce viral load [2] and that HIV-infected serum adsorbed with erythrocytes was rendered non-infectious by removal of virtually all infective virions [3].

In response to viral infection, the body produces antibodies that form complexes with the various HIV antigens and whole virions. Some of the antibodies activate and fix complement to the immune complex. Erythrocytes, as part of the innate immune system, bind the immune complexes via complement receptors (CR1) for elimination through the reticuloendothelial system [4]. Thus, complement fixation has been identified as one mechanism by which erythrocytes bind HIV [2]. It has also been observed that the virus binds to erythrocytes via non-complement receptor pathways, suggesting that molecules other than complement may be operational in this association [4].

Erythrocyte membranes are constituted with lipids, some of which are also known to promote viral fusion in T-cells. These lipids include sphingolipids and their glycosylated derivatives [5-7] that form part of the blood group milieu of the red blood cell. In other reports, lipid rafts comprising of glycosphingolipids and cholesterol were considered to be sufficient for viral fusion without the need for co-receptors [8,9]. In particular, the P^k blood group antigen (also called globotriaosyl ceramide, or Gb3) was shown to enhance fusion with X4 viruses while P blood group enhanced R5 strains [6]. We have previously reported reduced risk for individuals expressing blood group C, as well as double and triple risks for P_1 and Lu^b-positive individuals, respectively [10].

These reports prompted us to investigate whether erythrocyte cytosol may contain viral RNA. In the current study, purified erythrocyte suspensions were tested for the presence of HIV RNA in both membranous and lysate fractions. If found inside the erythrocytes, we also sought to find out if this was dependent on plasma viral load.

Material and Methods

The study enrolled 27 residual blood samples from HIV-1-in-

fected individuals, (9 males and 18 females) prior to initiation of highly active anti-retroviral therapy in Gaborone.

The study was approved by the University of Botswana's Office of Research and Development, Human Research and Development Committee of the Ministry of Health and Wellness, Gaborone District Health Management Team and the Research Ethics Committee, of the faculty of health and Wellness Sciences, Cape Peninsula University of Technology. Individual consent was not needed since the study used anonymized, residual samples [11].

The viral load was determined by use of the Cobas® Taqman 48 analyser (Pleasanton, CA., USA), while the RNA qualitative test for HIV-1 was performed using the COBAS® Ampliprep/COBAS® Taqman HIV-1 qualitative test, version 2.0 (Pleasanton, CA., USA).

Viral RNA detection was performed on purified erythrocytes. To achieve this, 1ml of the packed red cells was diluted with an equal volume of normal saline and then overlaid on 14ml of ficoll, followed by centrifugation at 400xG for 30 minutes. The suspended leukocyte layer was removed by aspiration together with all the saline and ficoll above the erythrocytes. The remaining cells were again diluted in 1 ml normal saline and subjected to a second round of ficoll gradient centrifugation. The cells were then washed three times with 14ml of normal saline. The supernatant of the last wash was tested for viral nucleic acids using the COBAS® AmpliPrep/COBAS® Taqman® (Pleasanton, CA., USA) and none was detectable, indicating a successful removal of unbound viral nucleic acids.

A sample was reconstituted for CD45 testing by adding equal volumes of the purified packed red cells and normal saline to bring the haematocrit to 41.3±1.8%. The CD45 determination was repeated to assess white cell depletion and a reduction of more than 97% after the first and over 99% after the second purification was achieved.

Subsequently, the purified cells were lysed with 1ml of distilled water and allowed to lyse on a rotary mixer for 5 minutes before being centrifuged at 1000xG for 3 minutes to pellet erythrocytic ghosts and any virus that might have been attached to them. The top 500µL of the RBC lysate was removed and tested for viral nucleic acids as described above, to demonstrate the presence, if any, of intra-erythrocytic viral RNA in the lysate.

The remainder of the sample was mixed by vortexing and viral RNA was also determined, which would represent the membrane-bound RNA plus that from the residual hemolysate. A positive test was recorded if both the sample and internal standard showed PCR products and a negative test recorded if only the internal standard was amplified. The results were analysed using the IBM SPSS version 24 statistical software.

Results

The mean plasma viral load (pVL), though higher in subjects who tested positive for Erythrocyte-associated RNA (E-RNA), did not reach statistical significance (mean±sd= 4.90±0.17 versus 4.06±0.81, p=0.111). E-RNA was estimated in 27 patients and was detectable in 22 (81%) and undetectable in 5. Of these 5, 4 had a detectable plasma viral load (pVL) ranging from 1.8 – 5.7 Logs. Only one subject tested negative for pVL, and E-RNA. On the other hand, E-RNA was demonstrable in 6 patients who had undetectable pVL. Of the 22 with demonstrable E-RNA, 10/22 (45%) demonstrated the virus on both the stroma and lysate. E-RNA was demonstrated in 21/27 stromal preparations compared to 11/27 lysate preparations. Only one sample yielded RNA from the lysate but not from the stroma.

Discussion

The presence of HIV virions on erythrocytes and erythrocyte-bound platelets has been documented by various reports [1,12,13]. However, some investigators have refuted the presence of erythrocyte-bound HIV in virally suppressed patients [14]. The current study confirms the presence of viral RNA from purified erythrocytes in patients with undetectable plasma viral load. Our results corroborate previous studies on the observation that erythrocyte-associated HIV (E-HIV) is not dependent on plasma viral load levels. Our results further demonstrate that while E-HIV is present in most HIV-infected samples, it is not demonstrable in others, even those with viral loads way above the viral set-point determined for this population [15]. The extreme opposite observation of this phenomenon is that E-RNA was detectable in patients with undetectable viral load, suggesting that the association of the virus with erythrocytes is not simply a function of plasma viral load. Our observations here are in concordance with those Beck et al. [3] Garcia et al. [13] & Hess et al. [1], but in contradiction to those of Fierer [14], who reported no such evidence in virally suppressed individuals.

Most of the E-HIV was associated with erythrocyte membrane and only half of the samples were associated with the lysate. The presence of viral RNA is a novel finding whose significance is yet to be elucidated. Our experiments were limited in the sense that the viability or infectivity of the viral RNA in the lysate could not be determined. However, the results provide further insight into a potential reservoir of erythrocyte associated HIV, which may be explored in future projects. Although the sample size was small, the presence of the E-HIV was consistently and satisfactorily demonstrated.

Conclusion

We report for the first time, the occurrence of HIV RNA in the lysate of erythrocytes. This phenomenon is independent of plasma viral load and potentially explains a GSL-based, receptor-independent entry of the virus into those cells possessing adequate levels of membrane GSL

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