Transfusion of red blood cells from an HIV-RNA-positive/anti-HIV-negative donor without HIV infection in the recipient

More than 90 percent of recipients of HIV-1-containing blood components acquire HIV infection.[1] We report the case of a recipient of red blood cells (RBCs), collected from an HIV-1-infected donor in the HIV-RNA positive/anti-HIV negative window phase, who did not develop HIV infection.

In September 2004, HIV-1-specific antibodies (HIV1-2 Ab-Capture EIA, Ortho Diagnostic System, Raritan, NJ) and p24 antigen (Genescreen Plus HIV Ag-Ab, Bio-Rad Laboratories, Hercules, CA) were detected in a 44-year-old male blood donor who denied HIV-related risk behavior. The HIV-RNA assay (Cobas Amplicor HIV-1 Monitor Test, v. 1.5, Roche Diagnostics, Indianapolis, IA) confirmed HIV infection (56,000 copies/ml). Viral genotyping (Abbott Viroseq™ HIV1 Genotyping System Version 2.0, Abbott Molecular, Des Plaines, IL) showed an HIV-1 subtype B virus, not resistant to anti-retroviral medications. A routine blood donation 6 months earlier tested anti-HIV-negative. At that time, nucleic acid testing (NAT) for HIV was not performed in our Blood Service, and the components were released for transfusion. The RBCs were transfused 7 days after collection to the patient of this report. The plasma was transferred for processing (Kedrion, Lucca, Italy). Retrospectively, a sample that had been retained from this donation tested positive for HIV-RNA (98 copies/mL). However, a sample retained from a blood donation on December 22, 2003, tested repeatedly HIV-RNA negative. Notice of the HIV-infected plasma unit was communicated immediately to Kedrion and to the recipient, a 54-year-old woman who had laparoscopic surgery. She has remained negative for HIV–RNA, p24Ag and anti-HIV when tested at 6, 12, and 14 months after transfusion.

In vitro studies showed that the recipient's peripheral blood mononuclear cells (PBMCs) could be infected with the donor's HIV isolate. The recipient was genotyped by PCR as wild-type CCR5. Because exposure to HIV in the absence of seroconversion can be inferred by the detection of HIV-specific T lymphocytes,[2,3] an in-depth immunologic analysis on the recipient was performed 14 months after the RBCs transfusion.

Recipient's PBMCs were stimulated with pools of antigens from *env* and *gag* regions of HIV. A higher than normal percentage of *env*-specific, IL-2-expressing CD4+ T lymphocytes (0.9%) was observed, whereas neither *env*-specific CD8+ nor *gag*-specific CD4+ or CD8+ T lymphocytes were detected. Naïve (CCR7+/CD45RA+), as well as central (CCR7+/CD45RA-) and effector (CCR7-/CD45RA-) memory CD4+ and CD8+ T lymphocytes, were measured. Both CD4+ and CD8+ naïve lymphocytes were reduced (11.3% vs. 23.4% and 10.1% vs. 22.2%) whereas CD8+ terminally-differentiated lymphocytes were augmented (22.7% vs. 7.0%) compared to results obtained in sex- and age-matched controls.

These results strongly suggest that exposure to HIV antigens in the absence of replicating virus occurred in this patient. Thus, only actual infection with live and replicating virus would result in presentation of viral antigens in association with HLA class I molecules, and elicitation of a CD8-mediated immune response. Additionally, responses to *env*, but not to *gag*, would be detected in the case of exposure to viral antigens in the absence of viral replication within the host cells. Finally, the observation that naïve cells were decreased in this patient is suggestive of a massive antigenic exposure, possibly HIV antigen-driven.

Of importance, the donor's HIV isolate was able to infect the recipient's PBMCs and a CCR5Δ32 deletion was not present in either the donor or the recipient.

Other investigators have reported cases illustrating that blood components may differ in their ability to transmit HIV infection.[1,4] In one report, platelets from an HIV-positive donor transmitted infection, while RBCs from the same blood collection did not.[5] There are several possible explanations for exposure to HIV without infection, including: 1) the presence of a low viral load at the time of donation; 2) a sub-infective volume of plasma in the RBCs; 3) loss of infectivity during storage of the component before transfusion; and 4) recipient's resistance related to genetic background (other than the CCR5Δ32 deletion, which was not present in our patient).

We believe that our results indicate that the presence of a strong cell-mediated immune response, possibly secondary to the exposure to a low amount of HIV, plays a role in resistance to HIV infection.

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All formulations of hydroxyethyl starch are not the same

Auwerda et al. recently reported that patients undergoing repeated plasma exchange procedures, using hydroxyethyl starch (HES) as replacement fluid, exhibited increased levels of chitotriosidase—a finding presumed to indicate accumulation of HES molecules within activated foamy macrophages. These findings occurred primarily in patients with impaired renal function, who received relatively large amounts of HES.[1]

It is extremely important to note that the HES formulation used as replacement fluid for the Auwerda patients is quite different biochemically from HES preparations available in the United States, and I suspect that clinically significant foamy macrophages and lysosomal storage disorders will NOT occur in patients or in granulocyte donors receiving HES approved for use in the United States. My rationale is as follows.

In the Auwerda study, patients received EloHAES® (also spelled Elohes in other papers) manufactured by Fresenius-Kabi.[1] This HES formulation has a molecular weight of 200 kDa, a degree of hydroxyethyl substitution of 0.5, and a C2:C6 hydroxyethylation ratio of approximately 13.[2,3] The C2:C6 hydroxyethylation ratio of 13 is not mentioned in the paper of Auwerda et al., but is extremely critical in terms of the metabolism of HES because the higher the C2:C6 ratio the slower and more difficult is HES metabolism—leading to prolonged circulation in the bloodstream and, presumably, to the accumulation of HES molecules within tissue macrophages.[2-4] Briefly, starch without attached hydroxyethyl groups is rapidly broken down by plasma amylase and eliminated so quickly from the bloodstream into the urine that it is not an effective plasma volume expander. Attaching hydroxyethyl groups to the glucose units comprising the starch molecules at carbon positions 2, 3 and/or 6 retards the action of amylase and permits longer circulation time in the blood—hence, mediating plasma volume expansion.[2]

Metabolism of HES molecules by plasma amylase depends not only on the number of hydroxyethyl groups attached to each glucose molecule comprising the starch—the larger the number of hydroxyethyl groups attached to each glucose, the slower the metabolism—but, also, on the specific carbons to which the hydroxyethyl groups are attached—the more attached to carbon 2, the slower the metabolism.[2-4] Thus, formulations of HES that, at first glance, might be predicted to be metabolized and cleared from the bloodstream fairly quickly because of relatively low molecular weight and extent of hydroxyethylation, actually have a very slow clearance due to a high C2:C6 hydroxyethylation ratio.[2-4] This slow and difficult clearance mimics that of HES formulations with high molecular weight and high hydroxyethyl substitution and results in prolonged circulation of HES within the bloodstream and to adverse effects. For example, EloHAES (Elohes) has been reported to cause a substantial reduction in the plasma concentration of von Willebrand factor with clinically significant bleeding.[5]

In contrast to EloHAES (Elohes) with its C2:C6 hydroxyethylation ratio of 13, HES formulations available in the United States have a C2-C6 ratio of only 4 to 5 and are metabolized much more quickly.[2,4] One of these formulations (hetastarch) has a relatively high molecular weight of approximately 450 kDa and a high molar hydroxyethyl substitution of 0.7 that results in a fairly prolonged plasma half-life with modest lowering of plasma von Willebrand factor.[6] The other preparation (pentastarch) has an average molecular weight of 264 kDa and a lower molar hydroxyethyl substitution of 0.45, permitting a much more rapid clearance from the bloodstream with minimal effects on plasma levels of von Willebrand factor.[6]