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Effect of mucosal fluid from women with bacterial vaginosis on HIV *trans*-infection mediated by dendritic cells

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ABSTRACT

Women with bacterial vaginosis (BV) have a higher risk of HIV transmission but the cause of risk is unknown. Dendritic cells (DC) are implicated in transmission of HIV and we previously observed that DC mature when exposed to mucosal fluid from women with BV. We hypothesized that maturation of DC by BV mucosal fluid would enhance DC-mediated *trans*-infection of HIV.

Monocyte-derived DC (MDDC) were treated with mucosal fluid, incubated with HIV_{Bal}, and HIV *trans*-infection was evaluated. While LPS-treated MDDC increased HIV_{Bal} *trans*-infection, BV fluid reduced *trans*-infection. HIV_{Bal} DNA levels in MDDC were not affected by BV fluid or LPS but productive infection of MDDC was decreased by LPS and BV fluid.

Mucosal fluid from women with BV does not increase MDDC-mediated *trans*-infection suggesting that BV does not increase HIV susceptibility by increasing DC-mediated *trans*-infection. However, indirect effects of DC maturation on HIV transmission cannot be ruled out.

Introduction

Dendritic cells are believed to be an important target for HIV during sexual transmission due to their presence at mucosal surfaces, their function as antigen capturing cells and their role in initiating adaptive immune responses by interacting with T cells (Piguet and Steinman, 2007). Experimental models of HIV sexual transmission show that DC are one of the first cells infected. Thus, 18 h after intravaginal inoculation of macaques with SIV, SIV RNA can be detected in DC isolated from the vaginal epithelium (Hu, Gardner, and Miller, 2000). In an ex vivo human vaginal tissue culture system, HIV rapidly enters Langerhans cells (Hladik et al., 2007) and DC in ex vivo cervical explants take up HIV (Hu et al., 2004). DC can be directly infected by HIV but also pass HIV to T cells (*trans*-infection) and maturation of DC induces an increase in *trans*-infection (Granelli-Piperno et al., 1998; Izquierdo-Useros et al., 2007; McDonald et al., 2003; Sanders et al., 2002).

Bacterial vaginosis (BV) is highly prevalent worldwide and is associated with an increased risk for HIV acquisition (Cohen et al., 1995; Martin et al., 1999; Myer et al., 2005; Sewankambo et al., 1997; Taha et al., 1998). BV consists of a shift in vaginal microbiota from predominantly lactobacilli in healthy women to other bacteria that include both gram-positive and gram-negative bacteria (Eschenbach et al., 1989; Fredricks, Fiedler, and Marrazzo, 2005). The frequency of BV in women of child bearing age in North American populations is between 5–30% and can be much higher in groups from some African nations (Greenblatt et al., 1999; Schwebke, 2003; Sewankambo et al., 1997).

We previously observed that monocyte-derived DC (MDDC) are activated and matured when exposed to the mucosal fluid from women with BV (St John et al., 2007) and there have been several studies that show that MDDC exposed to lipopolysaccharide (LPS) causes them to mature and concomitantly increase their ability to mediate HIV *trans*-infection (Granelli-Piperno et al., 1998; McDonald et al., 2003). We therefore hypothesized that the association between BV and increased sexual transmission of HIV could be due to increased *trans*-infection of HIV to T cells caused by the activating/maturing effect that BV has on DC. This study determined whether mucosal fluid from women with BV increased in vitro HIV *trans*-infection of T cells mediated by MDDC.

Results

Mucosal fluid from women with BV activate MDDC

To determine the effect of mucosal secretions on MDDC activation and maturation, MDDCs were cultured with stimuli for 48 h and

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expression of cell-surface CD83 and HLA-DR was determined. Expression of CD83 and HLA-DR were significantly increased due to BV CVL when compared to medium (control) treated MDDC ($p=0.0286$ and $p<0.001$ respectively, Mann Whiney test of eight experiments) (Fig. 1). LPS-treated MDDC also had increased levels of CD83 and HLA-DR compared to control, but these levels were not significantly different from BV CVL-treated MDDC. Normal CVL (CVL from women with normal flora — no BV) did not significantly increase CD83 or HLA-DR on MDDC compared to medium-treated MDDC.

AnnexinV/PI staining of MDDC was also analyzed following treatments to determine if the MDDC were undergoing apoptosis or cell death. Medium-treated MDDC were 17.3% positive for Annexin plus PI dual staining. LPS, BV CVL or Normal CVL treatment did not significantly increase this level of staining (data not shown).

Mucosal fluid from women with BV reduces MDDC-mediated HIV-1_{Bal} trans-infection of PBMC

Previous studies showed that LPS treatment of MDDCs increased HIV *trans*-infection (Granelli-Piperno et al., 1998; McDonald et al., 2003; Sanders et al., 2002). Since LPS and BV CVL both induced activation and maturation of MDDC (Fig. 1), we hypothesized that BV CVL would also increase *trans*-infection.

To test this, MDDC from 8 different donors were treated with either Normal CVL or BV CVL were incubated with HIV for 2 h and then cultured with PHA-stimulated PBMC for 5 days. Cultures with medium-treated MDDC had median p24 levels of 55,703 pg/ml whereas LPS-treated MDDC had significantly increased levels of p24 at 91,181 pg/ml ($p=0.0068$) (Fig. 2). Unexpectedly, p24 levels from cultures with BV CVL-treated MDDC were significantly lower than medium-treated MDDC (21,835 pg/ml, $p=0.001$). Cultures with Normal CVL-treated MDDC (44,344 pg/ml) had significantly lower p24 than LPS-treated MDDC ($p=0.0068$) but were not significantly different than medium-treated MDDC (Fig. 2). AZT treatment of cultures reduced *trans*-infection to near background levels with median value of 80 pg/ml ($p<0.0001$).

Experiments were also performed to determine if CVL samples had a direct effect on HIV infection of CD4 T cells. Positively selected CD4 T cells were incubated with CVL, infected with HIV and cultured. Neither BV CVL or Normal CVL had a significant effect on the amount of p24 measured in cultures after 5 days of culture (data not shown).

To determine if higher amounts of HIV_{Bal} added to BV CVL-treated MDDC would lead to increased *trans*-infection, 2× or 4× more HIV_{Bal} than were used in the Fig. 2 experiments were incubated with MDDC before addition to PBMC. However, increasing the viral inoculum added to BV CVL-treated MDDC did not increase *trans*-infection over 1× virus (data not shown).

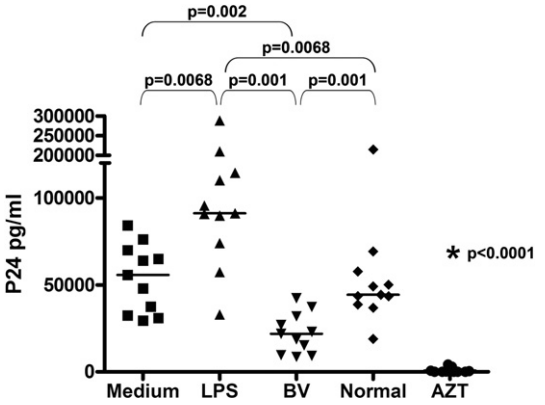

Fig. 2. Effect of mucosal fluids on MDDC-mediated *trans*-infection. MDDCs were incubated with medium, LPS or CVL for 48 h, washed and followed by 2 h exposure to HIV-1_{Bal}. Unbound virus was removed by washing and PHA-stimulated PBMCs were then added to MDDC in wells of a 96 well plate at a ratio of 1:3 (MDDC: PBMC). AZT was added to some wells containing medium-treated MDDC and PHA-stimulated PBMC. Supernatants were removed after 5 days of culture and analyzed for p24 by ELISA. *p* values were determined by Wilcoxon matched-pairs signed-ranks test. Each data point represents the average of triplicate wells from one independent experiment. Eight different MDDC donors were used.

BV mucosal fluid decreased HIV infection of MDDC with HIV-1_{Bal}

To investigate why BV CVL decreased MDDC-mediated *trans*-infection while LPS increased *trans*-infection, the level of infection of MDDC was analyzed. Previous reports showed that LPS decreased MDDC infection (Cavrois et al., 2006; Granelli-Piperno et al., 1998; McDyer et al., 1999). Both the level of productive infection and HIV DNA formation were measured in MDDC after exposure to CVL.

Medium-treated MDDC from five different donors produced 2764 pg p24/ml (median) 5 days after infection (Fig. 3a). Exposure to either BV CVL or LPS decreased p24 production to 395 and 609 respectively ($p<0.001$ and $p<0.01$ Repeated Measures ANOVA). In contrast, p24 production from MDDC exposed to Normal CVL (2005 pg/ml) was not significantly different than medium control. MDDC cultured with AZT was significantly lower than all other treatments at 107 pg/ml ($p<0.001$).

When HIV DNA formation in MDDC from the same five donors was measured, medium-treated MDDC yielded 675 HIV DNA copies/50 ng

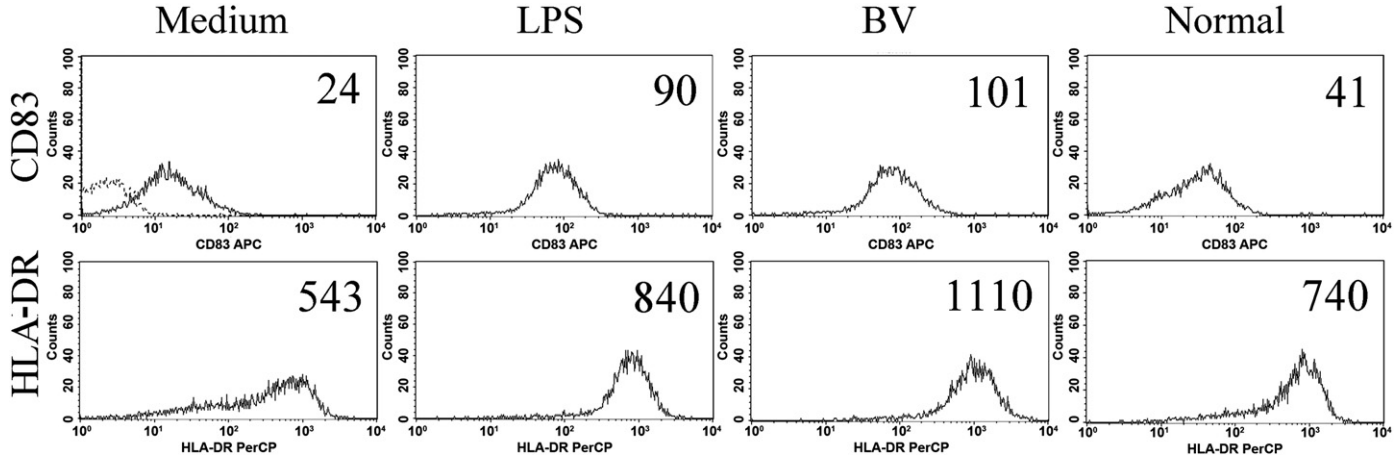

Fig. 1. Effect of mucosal fluid on MDDC expression of HLA-DR and CD83. MDDCs were incubated for 48 h with either medium, LPS or CVL from women with BV or normal flora. MDDC were harvested and assessed by flow cytometry for expression (Mean Fluorescence Intensity) of HLA-DR and CD83. Isotype control is shown in the Medium panel. One representative experiment of eight independent experiments is shown using MDDC from eight different donors.