



Combination of antibodies to CD18 and ICAM-1 reduces transmission of cell-associated HIV-1

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Abstract

Background: A better understanding of the interactions between HIV-1 positive cells and the cervical epithelium is essential for development of microbicides to prevent sexual transmission of HIV-1 to women. Our lab has previously demonstrated that cell-associated transmission of HIV-1 by monocytes is the most efficient route of transmission across a model cervical epithelial monolayer and in a Hu-PBL-SCID model of vaginal HIV-1 transmission. In addition, antibody to ICAM-1 has been shown to block transmission of cell-associated HIV-1 in both of these models. Use of antibodies to produce antibody-like single-chain Fv (scFv) offers many advantages over traditional microbicides, but the available concentration of antibody is limited by the amount of scFv that can be secreted *in vivo*. In order to determine whether a significant reduction in the amount of antibody required to block HIV-1 transmission could be achieved, we evaluated the blocking capabilities of antibodies to CD18, the beta-chain of the ICAM-1 ligands LFA-1 and Mac-1, both separately and in combination with anti-ICAM-1.

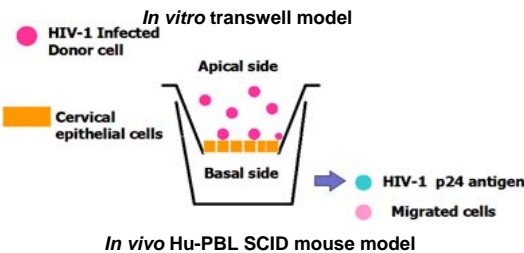
Methods: Peripheral blood mononuclear cells (PBMC) infected with HIV-1 Bal, were added to the apical side of confluent monolayers of HT-3 cervical epithelial cells grown on permeable transwell supports. After 24 hours, PBMC in the basal compartment were counted, and HIV transmission was measured by p24 ELISA on the basal supernatant. Data were analyzed using one-way ANOVA with Bonferroni correction by the STATA statistical package.

Results: Two different anti-CD18 antibodies, H52 and 7E4, were able to reduce transmission of cell-associated HIV-1 by 90±1% and 67±6%, respectively. Anti-CD18 and anti-ICAM-1 used in a 50:50 combination at a total concentration of 5 µg/ml reduced migration of PBMC from HIV-1 infected cultures significantly better (p<0.05) than 20 µg/ml total of either antibody alone.

Conclusions: These findings indicate that a combination of antibodies to the adhesion receptor pair ICAM-1 and CD18 offers better protection against cell-associated HIV-1 transmission than either antibody alone, and that this combination can protect at a concentration which is feasible for use in a lactobacillus-based microbicide delivery system.

Background

A variety of potential anti-HIV-1 microbicides are currently in advanced stages of development and clinical testing. However, many chemically-based microbicides currently being tested present several drawbacks including reduced effectiveness against some CCR5-utilizing isolates, disruption of the normal flora of the genitourinary tract, toxicity for the genital epithelium, carcinogenic potential, and undemonstrated efficacy against cell-associated virus (reviewed in D'Cruz and Uckun, 2004, *Curr Pharm Des*). Use of antibodies as microbicides may avoid the toxicity problems associated with many chemical compounds. One difficulty in designing chemically- or antibody-based microbicides targeting cell-free transmission of HIV-1 is the high degree of variability of viral surface epitopes. This difficulty can be avoided by targeting the host cell protein epitopes involved in cell-associated and/or cell-free virus transmission. Despite the prominence of sexual transmission in the continued spread of HIV-1, the cellular interactions involved in this mode of transmission are poorly understood. For example, it is not known if cell-free virus, cell-associated virus, or both, are essential for HIV-1 transmission in humans. Currently, our laboratory utilizes a cervical epithelial transwell culture system for *in vitro* studies, and a Hu-PBL-SCID mouse model for *in vivo* studies of HIV-1 sexual transmission. Prior studies in the laboratory have demonstrated that cell-associated virus is transmitted more efficiently than cell-free virus *in vitro*, that antibody to ICAM-1 on the cervical epithelium can reduce transmission of cell-associated virus both *in vitro* and *in vivo*, that anti-ICAM-1 single chain antibodies produced in lactobacilli can block transmission in our *in vitro* model, and that antibody to CD18, the beta-chain of the ICAM-1 ligands Mac-1 and LFA-1, can also reduce transmission of cell-associated HIV-1 *in vitro*. In these studies, we demonstrate that a combination of antibody to ICAM-1 and CD18 blocks cell-associated HIV-1 transmission *in vitro* more effectively than either antibody alone and that anti-human CD18, targeting ligands on the transmitting cells, effectively blocks transmission *in vivo*. Additionally, the combination of anti-ICAM-1 and anti-CD18 antibodies at half the concentration of either antibody used alone could also block transmission *in vivo*.



Day -7
Depo-Provera Treatment of SCID mice to render vaginal epithelium more cervix-like; uninfected human PBMC transplanted intraperitoneally into the mice

Day 0
Intravaginal anti-ICAM-1 and/or anti-CD18 antibody or PBS, then intravaginally inoculate HIV-1-infected human PBMC

Day 14
Euthanize and harvest PBMC from peritoneum, place cells in co-culture to look for presence of HIV-1

Days 21 - 28
HIV-1 p24 from cultured human PBMC

Methods

PBMC Culture: Peripheral blood mononuclear cells (PBMC) were isolated from Leuko-pacs (Johns Hopkins Hemapheresis Center) or whole blood using Ficoll Plus (GE Bioscience) and cultured at 2×10^6 cells/ml in RPMI-1640 supplemented with L-glutamine, sodium bicarbonate, penicillin-streptomycin, gentamicin (referred to as cRPMI); 10% heat-inactivated fetal bovine serum, and 5 µg/ml phytohemagglutinin (PHA-P, Sigma). After 48 hours, PHA-blasts were infected with 10⁷ TCID₅₀ HIV-1 Bal (AB) per flask for a period of 24 hours and fed with fresh cRPMI-10% FBS supplemented with 10U/ml IL-2 (Roche) on days 3 and 6 post-infection. Cells were used for transmission assays on Day 7 PI.

Cervical Epithelial cells: HT-3 cells, a cervical epithelial carcinoma line (ATCC HTB-32), were seeded at a density of 2×10^6 cells per well in cMcCoy's 55a-10% FBS (supplemented as with cRPMI above) on the apical side of a 1.1 cm² transwell insert, 3.0 µm pore size (Millipore PTF 01250) and grown for 7 days. Confluence was confirmed by assaying permeability of monolayers to horseradish peroxidase.

Antibodies: Anti-ICAM-1 MT-M5 and anti-CD18 H52 were obtained from J.E.K. Hildreth, Johns Hopkins School of Medicine. Hamster anti-mouse anti-ICAM-1 and hamster isotype control were purchased from BD Biosciences/Pharmingen, and the mouse IgG1 isotype control was purchased from Zymed.

In Vitro Transmission Assays: 1×10^6 HIV-1-infected PBMC and the indicated antibody treatment were added to the apical surface of confluent monolayers of HT-3 cervical epithelial cells grown on permeable transwell supports as described above and incubated at 37°. After 24 hours, supernatants were collected from both apical and basal surfaces for HIV-1 p24 ELISA (Perkin-Elmer), and the number and viability of PBMC on the basal side were determined by trypan blue exclusion.

In Vivo Transmission Studies: Hu-PBL SCID mice were administered 5x10⁷ PHA blasts intraperitoneally (I.P.) and treated with 2.5 mg progesterin (Depo-Provera®, Upjohn Pharmaceuticals) on day 14 and day 7 prior to use in transmission assays, respectively. 40 µg/ml total concentration of designated antibody, mix, or isotype controls were administered intravaginally 5 min before intravaginal inoculation of 1×10^6 HIV-1-Bal, infected PBMC. 14 days post-inoculation, mice were sacrificed and cells were collected by IP lavage with PBS. Recovered cells were put into co-culture with fresh PHA blasts, and p24 ELISA was performed after 7 days of coculture to determine which mice were infected. PCR for human b-globin was performed to determine the success rate of the IP human cell engraftments and mice that were both p24 and b-globin negative were excluded from further analysis.

Statistical Analysis: Data were analyzed with the STATA statistical package using one-way ANOVA with Bonferroni correction for *in vitro* studies, and a chi-squared analysis for *in vivo* studies.

Results

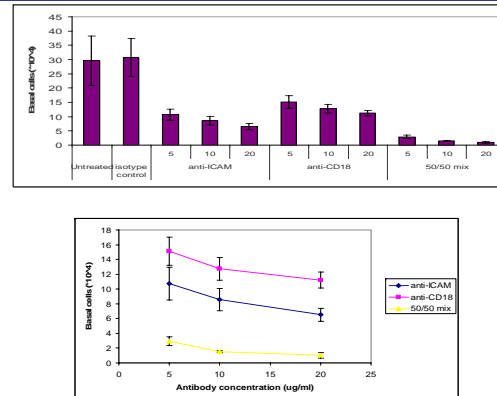


Figure 1. A 50:50 mix of anti-ICAM-1 and anti-CD18 reduces migration of cells from HIV-1 infected cultures to a greater extent than either antibody alone. 1×10^6 HIV-1 infected PBMC were added with designated antibodies (anti-ICAM-1 MT-M5, anti-CD18 7E4, or isotype control) or mix at a total amount of 20, 10 or 5 µg/ml to the apical side of HT-3 monolayers grown on permeable transwell supports, and allowed to transigrate for 24 hours. Error bars represent ± 1 standard deviation. (A) p<0.05 for all antibody treatments compared to untreated and isotype controls. (B) p<0.05 between each treatment at the same concentration.

Results continued

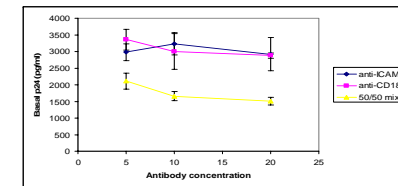
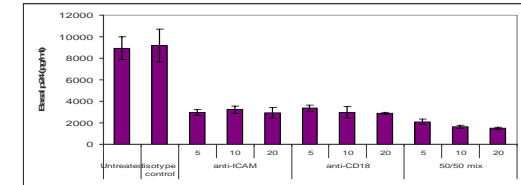


Figure 2. A 50:50 mix of anti-ICAM-1 and anti-CD18 reduces transmission of HIV-1 p24 to a greater extent than either antibody alone. 1×10^6 HIV-1 infected PBMC were added with designated antibodies (anti-ICAM-1 MT-M5, anti-CD18 7E4, or isotype control) or mix at a total amount of 20, 10 or 5 µg/ml to the apical side of HT-3 monolayers grown on permeable transwell supports, and allowed to transigrate for 24 hours. Error bars represent ± 1 standard deviation. (A) p<0.05 for all antibody treatments compared to untreated and isotype controls. (B) p<0.05 between mix and other treatments at the same concentration.

Table 1. Effect of anti-CD18 alone and in combination with anti-ICAM-1 *in vivo*.

Treatment	HIV-positive mice/total*
Anti-mouse-ICAM-1	0/8, (0%) p<0.01
Anti-human-CD18	0/8, (0%) p<0.01
Anti-mouse-ICAM-1 + anti-human-CD18	1/6, (17%) p<0.05
Isotype control	6/7 (86%)

*Positivity of mice assayed by p24 positivity from co-culture of peritoneal cells and activated PBMC after 7 days of culture. Mice that were p24 negative and PCR negative for human beta globin were excluded from analysis due to a presumption of poor human cell engraftment.

Conclusions and Acknowledgments

•Anti-CD18 (7E4) used in combination with anti-ICAM-1 reduces both migration of PBMC from HIV-1 infected cultures and transmission of HIV-1 p24 significantly more than corresponding concentrations of either antibody alone. This is important because the concentrations which block significantly when using a mix of antibodies should be achievable when using lactobacilli as the *in vivo* antibody delivery system.

•Treatment with anti-CD18 alone and with the combination of anti-ICAM-1 and anti-CD18 (each antibody at 50% of the concentration of that used with single antibodies alone) significantly reduced HIV-1 transmission *in vivo*.

•Further *in vivo* studies to determine the minimum concentration of antibodies required to provide protection are warranted.

*We would like to thank Dr. James Hildreth for providing anti-ICAM-1 hybridoma MT-M5 and for helpful discussions on the use of anti-CD18 antibody in this model system.

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