Poster #530

Development of a Lactobacillus-based Anti-ICAM-1 Antibody Delivery System for Use as an Anti-HIV Microbicide

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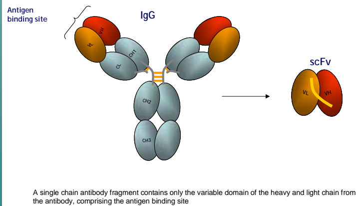
Abstract

Background: The relative importance of cell-associated and cell-free virus in sexual transmission of HIV-1 is unknown. 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. Antibody to ICAM-1 has been shown to block transmission of cell-associated HIV-1 in both of these systems; in addition, it has been shown that the presence of ICAM-1 on the virion surface increases infectivity, and that antibody to ICAM-1 can reduce the efficiency of virus entry 100-fold. Because of antibody to ICAM-1's potential use as a microbicide, we have been developing a lactobacillus-based delivery system for in situ secretion of this antibody in the female genitourinary tract. Bacteria can only express antibodies as single-chain variable regions (scFv), which are analogous in function to Fab molecules. In order to determine the likely effectiveness of scFv, we compared the transmission- and cell-migration-blocking capabilities of monoclonal anti-ICAM-1 to the corresponding Fabs and examined the ability of recently obtained scFv from lactobacilli to efficiently bind ICAM-1 on cell surfaces.
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. scFv secreted into culture medium by growing transformed lactobacilli were evaluated for their ability to bind ICAM-1 expressing cells, as determined by flow cytometry. Data were analyzed using one-way ANOVA with Bonferroni correction by the STATA statistical package.
Results: Anti-ICAM Fab were capable of blocking HIV-1 transmission and migration of PBMC from infected cultures ($p<0.05$ compared to untreated and isotype-control treated cells). scFv, both in purified form and in bacterial culture supernatants, efficiently bound cell-associated ICAM-1 on expressing cells even at a 10-fold dilution of the crude supernatant.
Conclusions: These findings indicate that lactobacilli expressing scFv specific for ICAM-1 have potential application as a microbicide to block cell-mediated HIV-1 sexual transmission.

Background

HIV-1 infections are acquired primarily through sexual contact, with the majority of sexual transmission of HIV-1 worldwide occurring as a result of heterosexual contact. Women of childbearing age are at the greatest risk for HIV-1 infection, which has resulted in a corresponding increase in HIV-1 infection of women, newborns and infants worldwide. The relative importance of cell-associated vs. cell-free virus in sexual transmission of HIV-1 is still unclear. Utilizing both *in vivo* and *in vitro* systems to model HIV-1 transmission, previous studies in this laboratory have demonstrated increased efficiency of transmission of cell-associated virus when compared with free virus (Khanna, Whaley et al., J Clin Invest, 2002) and that antibody to ICAM-1 can inhibit transmission of cell-associated virus (Khanna et al, submitted for publication). Antibody to ICAM-1 has also been shown to inhibit infection of cells by free virus (Rizzuto and Sodroski, J Virol 1997, and Bounou et al, J Virol 2002) .



A single chain antibody fragment contains only the variable domain of the heavy and light chain from the antibody, comprising the antigen binding site

Since direct application of antibody for use as a microbicide could be prohibitively expensive, another method of production and delivery must be found. Lactobacilli, a component of the normal flora of the vagina, can be engineered to express proteins that fold to resemble the variable region of an antibody molecule, known as single-chain Fv, or scFv. This type of delivery system offers a number of advantages over both direct application of antibody and "traditional" microbicides.

As a commensal organism, lactobacilli offer high efficacy with minimal toxicity, and would not require application within the immediate pre-coital timeframe. In addition, since lactobacilli do not require refrigeration, are inexpensive to produce, and are transparent to users, they offer an opportunity to create a female-controlled microbicide feasible for use in diverse cultural settings.
In this study, we wished to assess the potential capability of anti-ICAM scFv to block transmission of cell-associated HIV-1 by comparing the transmission- and cell-migration-blocking capabilities of monoclonal anti-ICAM-1 to the corresponding Fabs. We then examined the ability of recently obtained scFv from lactobacilli to efficiently bind ICAM-1 on cell surfaces, and tested whether the anti-ICAM scFv could then block transmission of HIV-1-infected PBMC and p24 antigen.

Methods

Methods:
PBMC Culture: Peripheral blood mononuclear cells (PBMC) were isolated from Leuko-pacs or whole blood using Ficoll Plus ()and cultured at 2 x 10^6 cells/ml in RPMI-1640 (Cellgro) supplemented with L-glutamine, sodium bicarbonate, penicillin-streptomycin, geneticin (referred to as cRPMI); 10% heat-inactivated fetal bovine serum (Atlanta Biologicals), and mg/ml phytohemagglutinin (PHA-P, Sigma). After 48 hours, PHA-blasts were infected with 10^4 TCID50 HIV-1 BaL (ABI) per flask for a period of 24 hours and maintained in cRPMI-10% FBS supplemented with 10U/ml IL-2 (Roche). Cells were used for transmission assays on Day 7 post-infection.
Cervical Epithelial cells: HT-3 cells, a cervical epithelial carcinoma line (ATCC), were seeded at a density of 2 x 10^5 cells per well in cMcCoy's5a-10% FBS (supplemented as cRPMI above) on the apical side of a 1.1 cm2 transwell insert (Millipore PITP 012 50) and grown for 7 days. Confluence was confirmed by assaying permeability of monolayers to horseradish peroxidase.
Antibodies and Fabs: All antibodies used were mouse IgG1 isotype. Anti-ICAM HA58 was obtained from Becton Dickenson Pharmingen, anti-ICAM MT-M5 was obtained from the Hildreth Lab at the Johns Hopkins School of Medicine, and the isotype control used was a mouse myeloma IgG1 (Zymed). Fabs were generated from MT-M5 and the myeloma isotype control using the Immunopure IgG1 Fab and $F(ab')_2$ Preparation Kit (Pierce, cat #44880).
Transmission Assays: 1 x 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, and the number and viability of PBMC on the basal side were determined by trypan blue exclusion.
Lactobacillus Culture and ScFv Production: The Vh and Vl regions from anit-ICAM hybridoma were cloned into the vector pSCN112, illustrated below. Transfected lactobacilli were grown in MRS medium at 37, and secreted scFv were purified using the Etag HiTrap purification system.
ScFv Binding Assay: The ability of scFv both in culture supernatant and purified form to bind CHO cells overexpressing full length ICAM-1 was assessed by flow cytometry and compared to binding abliity of intact MT-M5 antibody.
Statistical Analysis: Data were analyzed using one-way ANOVA with Bonferroni correction by the STATA statistical package.

Results

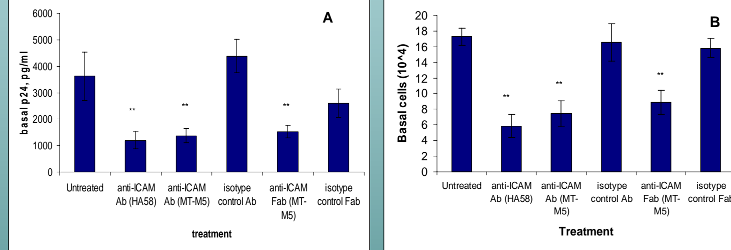


Fig. 1. Anti-ICAM Fab block transmission of HIV-1-infected PBMC (A) and p24 (B) across an HT-3 cell monolayer. 1 x 10^6 HIV-1 infected PBMC were added with designated treatment to apical side of HT-3 monolayers grown on permeable transwell supports and allowed to transmigrate for 24 hours. All intact antibodies were used at a concentration of 100 μg/ml and all Fab were used at 67 μg/ml to equalize available binding sites. Error bars represent +/- 1 standard deviation. *, $p<0.05$, ** $p<0.01$.

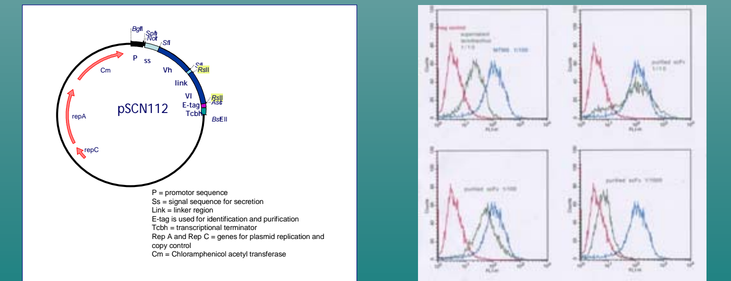


Fig. 2. Cloning vector used for expression of the hIcam-I single chain antibody. Vector allows for direct cloning of PCR amplified fragments of the variable domains of the heavy (Vh) and light (Vl) chain.

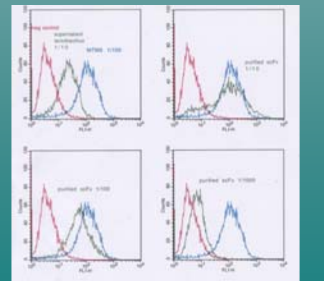


Fig. 3. Anti-ICAM scFv from lactobacillus culture supernatants specifically bind ICAM-expressing cells.

Results continued

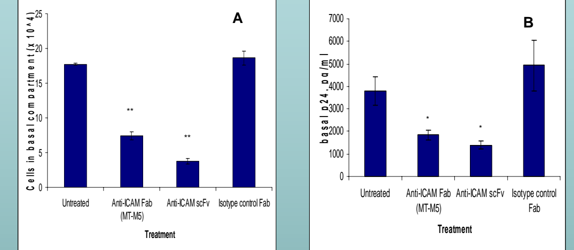


Fig. 5. Anti-ICAM scFv block transmission of HIV-1-infected PBMC (A) and p24 (B) across an HT-3 cell monolayer. 1 x 10^6 HIV-1 infected PBMC were added with designated treatment to apical side of HT-3 monolayers grown on permeable transwell supports and allowed to transmigrate for 24 hours. All intact antibodies were used at a concentration of 100 μg/ml and all Fab were used at 67 μg/ml to equalize available binding sites. Error bars represent +/- 1 standard deviation. *, $p<0.05$, ** $p<0.01$.

Conclusions and Discussion

- Anti-ICAM Fab significantly block transmission of both HIV-1-infected PBMC and HIV-1 p24 across a model cervical epithelial monolayer at approximately the same level as intact antibody.
- Lactobacilli were successfully engineered to produce anti-ICAM scFv which are capable of binding CHO cells transfected to overexpress ICAM-1.
- Anti-ICAM scFv are capable of blocking transmission of both HIV-1-infected PBMC and HIV-1 p24 across a model cervical epithelial monolayer.

These findings indicate that lactobacilli expressing scFv specific for ICAM-1 have potential application as a microbicide to block cell-mediated HIV-1 sexual transmission.

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