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Short communication

Infection with feline immunodeficiency virus alters intestinal epithelial transport and mucosal immune responses to probiotics

Laura L. Stoeker[a], Elizabeth L. Overman[a], Shila K. Nordone[a], Adam J. Moeser[a], Rita D. Simões[a], Gregg A. Dean[b,*]

[a] Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University, 1060 William Moore Dr., Raleigh, NC 27607, United States
[b] Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523, United States

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ABSTRACT

HIV infection is associated with intestinal mucosal dysfunction and probiotics offer the therapeutic potential to enhance the mucosal barrier in HIV+ patients. To evaluate the response of immunocompromised hosts to probiotics, we orally administered *Lactobacillus acidophilus* to cats with chronic feline immunodeficiency virus (FIV) infection. FIV infection significantly affected transcellular, but not paracellular, transport of small molecules across the intestinal epithelium. Additionally, probiotic treatment of FIV+ cats resulted in changes in cytokine release and mucosal leukocyte percentages that were not paralleled in FIV- cats. These results suggest a novel role for FIV in upregulating transcellular transport across the gastrointestinal epithelial barrier and demonstrate the potential therapeutic use of probiotic bacteria to restore intestinal homeostasis.

1. Introduction

The intestinal mucosal epithelium and associated mucosal immune system provide a first line of defense against invading pathogens in healthy individuals. Mucosal damage caused by human immunodeficiency virus (HIV) or feline immunodeficiency virus (FIV) infection leads to compromise in both the mucosal barrier and its associated immune system. Probiotics and prebiotics are attractive options for enhancement of epithelial barrier function and modulation of mucosal immune function in HIV infected individuals (Hummelen et al., 2010) and may hold promise for FIV+ patients as well.

Beyond therapeutic applications, probiotic bacteria are also emerging as vaccine vector candidates due to their recognized safety and their interaction with key mucosal immune cells, particularly dendritic cells. Though oral administration of probiotics to HIV patients has been studied as a means to decrease diarrhea and supplement immune function (Anukam et al., 2008; Hummelen et al., 2011), the physiological effects of these bacteria on the mucosal barrier in HIV+ patients, and immunocompromised patients in general, is relatively unknown. Many studies have examined the effect of probiotic bacteria on the gastrointestinal immune system and mucosal barrier of healthy patients and have shown the beneficial effects of therapeutic probiotic usage. We sought to determine whether probiotics may be beneficial or potentially harmful in a patient where the mucosal barrier is modified or damaged. Using the well-established feline immunodeficiency virus (FIV) model for HIV infection (Burkhard

* Corresponding author. Tel.: +1 970 491 6144/6136.
E-mail address: Gregg.Dean@colostate.edu (G.A. Dean).

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and Dean, 2003; Elder et al., 2010; Kenyon and Lever, 2011; Yamamoto et al., 2010), we studied the effect of genetically modified *Lactobacillus acidophilus* on epithelial integrity and transport functions in the small intestine of chronically FIV-infected cats. Additionally, we explored FIV-related differences in cytokine release and mucosal immune cell populations following bacterial treatment. In the future, such genetically modified bacteria may be used to carry immunomodulators or vaccine antigens to the site of infection. Therefore, the physiological and immunological effects of *L. acidophilus* carrying an empty expression plasmid were explored in these studies.

2. Materials and methods

2.1. Animal care

Random source cats euthanized independently of the current study were used to develop the feline Ussing model ($N = 5$). After assay development, uninfected and chronically FIV infected (>12 months) laboratory cats were treated orally for three days with vehicle-control PBS or 1×10^8 CFU of NCK1895, *L. acidophilus* carrying an empty expression plasmid (described in (Duong et al., 2011)) ($N = 3$ cats/group or 12 total cats). FIV infection was diagnosed by quantitative RT-PCR to determine plasma viremia (Mikkelsen et al., 2010) and by qPCR for pro-viral load in lymph nodes and peripheral blood mononuclear cells (Assogba et al., 2007; Pedersen et al., 2001). Cats were housed and cared for in accordance with Association for the Assessment of Laboratory Animal Care standards and the North Carolina State University Institutional Animal Care and Use Committee guidelines.

2.2. Bacterial preparation

Lactobacilli cultures were grown overnight to mid-log phase in MRS broth (BD Biosciences, San Jose, CA) supplemented with erythromycin (5 μg/mL). 1×10^8 CFU of each bacterial treatment were centrifuged at $5250 \times g$ for 10 min and resuspended in 100 μL PBS before addition to the Ussing chamber.

2.3. Ussing chamber experiments

Distal small intestine was harvested and the seromuscular layer removed. The remaining mucosal layer was mounted on Ussing chambers, as described in previous studies (Smith et al., 2010). Tissues were bathed in 10 mL of Ringer solution containing erythromycin (5 μg/mL) and 10 mM glucose (serosal side) or 10 mM mannitol (mucosal side). Solutions were oxygenated (95% O_2–5% CO_2) and circulated in water-jacketed reservoirs at 37 °C. The spontaneous potential difference (PD) was measured using Ringer-agar bridges connected to calomel electrodes, and the PD was short-circuited through Ag–AgCl electrodes using a voltage clamp that corrected for fluid resistance. Tissues were maintained in the short-circuit state, except for brief intervals to record the open-circuit PD.

Transepithelial electrical resistance (TER; $\Omega\,cm^2$) was calculated from the spontaneous PD and short-circuit current (I_{sc}), as previously described (Argenzio and Liacos, 1990). After a 30-min equilibration period on Ussing chambers, TER was recorded at 15-min intervals over a 4 h period and percent change in TER was calculated and averaged to derive baseline TER values for a given animal. Percent change was calculated by comparing final TER to TER 30 min post-tissue mounting.

2.4. Measurement of mucosal-to-serosal flux of FITC-labeled dextran and albumin

Mucosal-to-serosal flux of FITC-dextran (4 kDa; Sigma–Aldrich, St. Louis, MO) or FITC-albumin (66 kDA; Sigma–Aldrich) was measured at 30 min intervals in conjunction with TER measurements. FITC-dextran (FD4) or FITC-albumin was added after equilibration (15 min) to the mucosal side of chamber-mounted tissues (0.25 mM), and then standards were taken from the mucosal side of each chamber. 100 μL samples from the serosal compartment of each chamber were collected in triplicate at 30-min intervals over 4 h and the relative abundance of FITC-dextran or albumin was measured by fluorescence intensity (485 excitation/520 emission) on an fMax Fluorescence Microplate Reader (Molecular Devices, Sunnyvale, CA).

2.5. Cytokine collection

After harvesting distal feline small intestine, the serosal layer was removed and approximately one square inch of the remaining epithelial layer was placed in 25 mL PBS and agitated for 30 min at 210 RPM. After shaking, 0.05 g/well of tissue was added to 24-well tissue culture plates (Advangene, Lake Bluff, IL) containing 400 μL of complete RPMI medium. Complete RPMI medium consisted of RPMI 1640 (Invitrogen, Carlsbad, CA), heat-inactivated FBS (Invitrogen, 10%, v/v), sodium pyruvate (Invitrogen, 1%, v/v), MEM non-essential amino acids (Invitrogen, 1%, v/v), HEPES (Invitrogen, 1%, v/v), and erythromycin (5 μg/mL). Samples were diced into smaller pieces and incubated with bacterial treatments (1×10^8 CFU) for 24 h at 37 °C. Supernatants were then removed and frozen at −80 °C until downstream analysis.

2.6. ELISA for cytokine detection

Feline IL-10, IL-12, and TNF-α (R&D Systems, Minneapolis, MN) were measured by ELISA according to manufacturer's instructions with modifications. Capture antibody was diluted in filtered PBS and used to coat Chromalux HB 96-well plates (Dynex, Chantilly, VA) overnight at 4 °C. Plates were flicked but not washed, then incubated with blocking buffer (1% bovine serum albumin (BSA) (Equitech-Bio, Kerrville, TX), 1% dry milk, and filtered PBS) for 1 h at 37 °C, then washed three times (0.05% Tween 20 in PBS) before primary antibody (diluted in 1% BSA/PBS) was added. Plates were incubated 2 h at 37 °C, washed, and then detection antibody (diluted in PBS with 5% mouse serum, 5% goat serum, 5% FBS, 0.1% Tween 20) was added for 2 h at 37 °C. Streptavidin–HRP was added according to manufacturer's instructions and plates were developed with Pierce