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Short communication

Differential effects of clathrin and actin inhibitors on internalization of *Escherichia coli* and *Salmonella choleraesuis* in porcine jejunal Peyer's patches

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Abstract

Peyer's patches constitute both an inductive immune site and an enteropathogen invasion route. Peyer's patch mucosae from porcine jejunum were mounted in Ussing chambers, and either *Salmonella choleraesuis* vaccine strain SC-54 or non-pathogenic rodent and porcine *Escherichia coli* strains contacted the Peyer's patch mucosa for 90 min. Internalized bacteria were quantified by a gentamicin resistance assay. Monodansylcadaverine (300 μM, luminal addition), an inhibitor of clathrin-mediated endocytosis, significantly inhibited internalization of both *E. coli* strains relative to tissues untreated with the inhibitor; internalization of SC-54 was unaffected. The actin-disrupting agent cytochalasin D (10 μM, luminal addition), inhibited internalization of pig-adapted *E. coli* but not that of rodent-adapted *E. coli* or SC-54. Internalization of SC-54 and non-pathogenic *E. coli* in Peyer's patches appears to occur through different cellular routes.

Keywords: *Salmonella choleraesuis*; *Escherichia coli*; Mucosal immunity; Enteropathogen; Endocytosis; Clathrin; Intracellular internalization; Cytochalasin D; Monodansylcadaverine

1. Introduction

By disseminating immunological information from the gut lumen to mucosal surfaces throughout the body, discrete Peyer's patch follicles in the small intestine constitute an inductive site for mucosal immunity. The uptake of antigens and other macromolecules involves the adherence of luminal material to the follicle-associated epithelium (FAE), and its subsequent capture and transcellular transport by endosomes (Neutra, 1998). In addition to their role in mucosal immunity, Peyer's patches serve as portals for rapid intestinal invasion by a number of pathogenic microorganisms (Vazquez-Torres and Fang, 2000). *Salmonella* (Jepson and Clark, 2001), *Yersinia* (Autenrieth and Firsching, 1996) and *Shigella*

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(Sansonetti et al., 1996) are among the bacterial species that have been shown to gain access to the intestinal submucosa through macropinocytosis into the FAE. On the other hand, *Listeria monocytogenes* internalizes in Peyer's patches through clathrin-mediated endocytosis (Velge et al., 1997).

Jejunal Peyer's patches from swine internalize significant amounts of yeast (Beier and Gebert, 1998) as well as macromolecules such as ferritin (Liebler et al., 1995) and horseradish peroxidase (Keljo and Hamilton, 1983). They also internalize *Salmonella choleraesuis* and *Escherichia coli* O157:H7 (Green et al., 2003). The present study was designed to test the hypothesis that the internalization processes for pathogenic salmonellae and non-pathogenic *E. coli* in Peyer's patches are different. Therefore, we examined and compared the effects of the actin-disrupting drug cytochalasin D and monodansylcadaverine, an inhibitor of clathrin-mediated endocytosis, on the internalization of the enteropathogenic *S. choleraesuis* vaccine strain SC-54 and both rodent- and swine-adapted commensal *E. coli* strains in jejunal Peyer's patches from juvenile pigs.

2. Materials and methods

2.1. Drugs

Cytochalasin D was obtained from Calbiochem (San Diego, CA), and initially solubilized in chloroform. Monodansylcadaverine (MDC) was obtained from Sigma Chemical Co. (St. Louis, MO) and initially dissolved in glacial acetic acid. Serial dilutions of both stock solutions were made in distilled water.

2.2. Animals and tissue preparation

Jejunal Peyer's patches were isolated from outbred Yorkshire-Landrace pigs of either sex that were 5–9-week-old and weighed between 10 and 18 kg. Animals had continuous access to water and non-medicated pig feed and were not fasted prior to sacrifice. They were anesthetized with tiletamine hydrochloride-zolazepam (Telazol®; 8 mg/kg, i.m. injection; Fort Dodge Laboratories, Fort Dodge, IA) in combination with xylazine (8 mg/kg), and subsequently euthanized with Beuthanasia®-D Special (0.5 ml/kg, i.v. injection; Schering-Plough Animal Health, Union, NJ) in accordance with approved University of Minnesota IACUC protocols. Each jejunal Peyer's patch was stripped of its underlying smooth muscle coats and the remaining mucosa with attached submucosa was mounted in Ussing flux chambers (2 cm^2 area). Mucosal sheets were bathed on their luminal and contraluminal aspects with a physiological saline solution similar in composition to porcine extracellular fluid (composition in mM: NaCl, 130; KC1, 6; $CaCl_2$, 3; $MgCl_2$, 0.7; $NaHCO_3$, 20; NaH_2PO_4, 0.29; Na_2HPO_4, 1.3); this buffer solution was maintained at porcine core temperature (39 °C) and aerated with 95% O_2/5% CO_2 by gas lift.

2.3. Bacterial cultures and assessment of intracellular bacterial uptake

A mucosa-associated strain (lab code #3) of porcine non-0157 *E. coli* was obtained by plating homogenized colonic mucosa from normal pigs onto Fluorocult agar (EM Science, Gibbstown, NJ) supplemented with 100 μg/ml streptomycin sulfate to isolate *E. coli* strains that were resistant to this antibiotic drug. The selective isolation and differentiation capabilities of Fluorocult medium for *Enterobacteriaceae*, especially *E. coli* O157:H7, which are achieved by a combination of fluorogenic and chromogenic substrates, have been well described to identify relevant bacteria from a variety of sources (Heizmann et al., 1988). Presumptive colonies of *E. coli* that did not have the appearance of *E. coli* O157:H7 were randomly chosen from Fluorocult plates following overnight incubation and were streaked onto Luria-Bertani (LB) agar plates supplemented with 100 μg/ml streptomycin. Following 24 h incubation at 37 °C, individual colonies were picked from these plates and their identities confirmed as *E. coli* using the API-20E Enteric Identification System (BioMerieux, Hazelwood, MO). Colonies were further determined to represent non-0157 *E. coli* with the use of an *E. coli* 0157 latex agglutination-based diagnostic test kit (Oxoid, Ogdensburg, NY).

On the day of each experiment, an inoculum of the porcine commensal *E. coli* strain, a non-pathogenic, streptomycin-resistant *E. coli* strain M-21 (Wells