Anaerobe 17 (2011) 137–141

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Molecular Biology, Genetics and Biotechnology

The human commensal *Bacteroides fragilis* binds intestinal mucin

Julie Y. Huang [1], S. Melanie Lee, Sarkis K. Mazmanian*

Division of Biology, California Institute of Technology, 1200 E. California Bl., Mail Code 156-29, Pasadena, CA 91125, USA

ARTICLE INFO

Article history:
Received 28 October 2010
Received in revised form
14 May 2011
Accepted 16 May 2011
Available online 2 June 2011

Keywords:
Bacteroides fragilis
Gut commensal
Mucin
Adhesion
Microbiota

ABSTRACT

The mammalian gastrointestinal tract harbors a vast microbial ecosystem, known as the microbiota, which benefits host biology. *Bacteroides fragilis* is an important anaerobic gut commensal of humans that prevents and cures intestinal inflammation. We wished to elucidate aspects of gut colonization employed by *B. fragilis*. Fluorescence in situ hybridization was performed on colonic tissue sections from *B. fragilis* and *Escherichia coli* dual-colonized gnotobiotic mice. Epifluorescence imaging reveals that both *E. coli* and *B. fragilis* are found in the lumen of the colon, but only *B. fragilis* is found in the mucosal layer. This observation suggests that physical association with intestinal mucus could be a possible mechanism of gut colonization by *B. fragilis*. We investigated this potential interaction using an *in vitro* mucus binding assay and show here that *B. fragilis* binds to murine colonic mucus. We further demonstrate that *B. fragilis* specifically and quantitatively binds to highly purified mucins (the major constituent in intestinal mucus) using flow cytometry analysis of fluorescently labeled purified murine and porcine mucins. These results suggest that interactions between *B. fragilis* and intestinal mucin may play a critical role during host-bacterial symbiosis.

Following a sterile birth, the gastrointestinal (GI) tracts of humans and all mammals coordinately assemble a diverse multitude of microorganisms, collectively known as the microbiota. It has been acknowledged for decades that many of these microorganisms live symbiotically with their hosts, performing beneficial functions such as metabolizing complex carbohydrates and providing essential nutrients [1]. Recent studies have shown that the microbiota critically augments the development and function of the immune system (reviewed in [2] and [3]). Although the microbial diversity in the mammalian gut is vast (with an estimated 500–1000 species of microorganisms present in the human), organisms belonging to the genus *Bacteroides* represent one of the most abundant microbial taxa in both mice and humans [4]. *Bacteroides fragilis* is a ubiquitous Gram-negative anaerobic bacterium that inhabits the lower GI tract of most mammals [5]. Recent findings have revealed that this organism possesses the ability to direct the cellular and physical maturation of the host immune system and to protect its host from experimental colitis [6–8]. Therefore, the contributions of the microbiota to human health appear to be profound.

We wanted to understand how *B. fragilis* colonizes the mammalian gut. *B. fragilis* expresses at least eight distinct surface capsular polysaccharides (CPS), and previous studies have shown that CPS expression by the bacterium is required for successful intestinal colonization [9,10]. How these molecules mediate the initial interactions with the host, and whether they are involved in long-term persistence in the gut are currently unknown. Several mechanisms of gut colonization by symbiotic bacteria have been studied. Some organisms form biofilms, composed of a polymeric matrix secreted by the bacteria, which adhere to the epithelial layer. Others interact with components of the mucosal layer (reviewed in [11]). Mucus is a viscous stratum which separates epithelial cells from the lumen of the gut and acts as a crucial barrier against infection by pathogens. Various membrane-bound or secreted glycoproteins called mucins associate with one another to form the gel-like mucus. Interactions between gut bacteria and mucus have been hypothesized to be important for the assembly and stability of the microbiota [12]. Accordingly, we sought to determine whether or not *B. fragilis* binds intestinal mucus and purified mucin.

Initially, we visualized the spatial localization in the colon of 2 different commensal bacteria to determine potential differences in association with the mucus layer *in vivo*. Wild-type *B. fragilis* NCTC9343 was grown anaerobically in brain-heart infusion (BHI) supplemented with hemin (5 μg/ml) and vitamin K (0.5 μg/ml), and *Escherichia coli* BL21 was grown aerobically in BHI at 37 °C. Bacteria were grown to OD_{600} of 0.7–0.8, and 1×10^8 colony forming units (CFUs) were orally inoculated into germ-free Swiss Webster mice by gavage. Following 1 week of colonization, mice were sacrificed and colon tissue was fixed in Carnoy's solution and embedded in

* Corresponding author. Tel.: +1 626 395 2356.
E-mail address: sarkis@caltech.edu (S.K. Mazmanian).
[1] Present address: Dept. of Microbiology and Immunology, Fairchild Science Building D300, 299 Campus Drive, Stanford University, Stanford, CA 94305, USA.

1075-9964/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.anaerobe.2011.05.017

paraffin wax for sectioning. Fluorescence in situ hybridization was performed on tissue sections mounted on glass slides using a 6-carboxyfluorescein (6-FAM)-labeled oligonucleotide probe for *E. coli* (EnterbactB [AAGCCACGCCTCAAGGGCACAA]) and a Cy3-labeled oligonucleotide probe for *B. fragilis* (Bfra602 [GAGCCG-CAAACTTTCACAA]) (Integrated DNA Technologies, Inc.). Briefly, slides were deparaffinized, dried, and hybridized with both probes at 5 ng/μl concentration each for 2 h at 46 °C in hybridization buffer (0.9 M NaCl, 15% formamide, 20 mM Tris–HCl (pH 7.4), and 0.01% sodium dodecyl sulfate (SDS)). Slides were washed for 15 min at 48 °C in wash buffer (20 mM Tris–HCl (pH 7.4), 318 mM NaCl, and 0.01% SDS). For visualization of the colon epithelial cell nuclei, the slides were counterstained with 4′,6′-diamidino-2-phenylindole (DAPI). The autofluorescence background allowed visualization of the tissue structures. The slides were examined with an Axioplan microscope (Zeiss, Oberkochen, Germany) using a 100× oil immersion objective. Epifluorescence images of a cross section through the colon of gnotobiotic mice that were dual-colonized with both *E. coli* and *B. fragilis* reveal that both bacteria are found in the lumen of the gut in high abundance (Fig. 1). Surprisingly however, only *B. fragilis* is found in the mucus layer that lies between the lumen and the gut epithelium tissue (Fig. 1). The spatial segregation of *B. fragilis* and *E. coli* across the colon mucus barrier suggests that *B. fragilis* may interact with mucus *in vivo* and this may be important for sustained colonization of commensal *B. fragilis*. Furthermore, these results reveal that not all bacteria are equally able to penetrate the mucus layer, suggesting dedicated mucus associating functions for *B. fragilis*.

To test the hypothesis that *B. fragilis* colonization of the distal gut is mediated by mucus binding, a standard mucus binding assay was used to determine if live bacteria are able to bind a crude, intestinal mucus preparation. Crude mucus was isolated from the colon and cecum of conventionally-colonized Swiss Webster mice as described in Cohen et al. [13]. Briefly, colonic and cecal mucus was scraped into HEPES-Hanks' Buffer (pH 7.4 with Calcium Chloride and Magnesium Chloride). Next, non-soluble material was removed by centrifuging once at 12,000 × g for 10 min at 4 °C, and then once at 26,500 × g for 15 min at 4 °C. The final concentration of the crude mucus solution was determined by the Bradford assay. The mucus was diluted with HEPES-Hanks' Buffer to 1 mg/ml. 0.2 ml of mucus was added into the wells of a 24-well tissue culture plate and incubated overnight at 4 °C. Controls included wells containing 0.2 ml of a 1 mg/ml solution of Bovine Serum Albumin (BSA, which served as a specificity control) or 0.2 ml of HEPES-Hanks' Buffer (which served as a negative control). The wells were washed with HEPES-Hanks' Buffer to remove non-immobilized proteins. The plate was UV-sterilized for 10 min and was then ready for use in the mucus binding assay. 1×10^8 CFUs of bacteria were added to both the immobilized mucus and the BSA control, and incubated at 37 °C for 1 h. Wells were washed with HEPES-Hanks' Buffer, treated with 0.05% trypsin for 10 min at room temperature to liberate bacteria. One milliliter of cold BHI was added to quench the trypsin activity. Samples were serially diluted and plated for CFUs. Fig. 2A shows that *B. fragilis* binds to crudely purified mucus *in vitro*, as determined by recovered CFUs. The BSA- and buffer-containing wells illustrate low background binding. A mutant strain of *B. fragilis* (CPM1), which only expresses one of the eight CPS [9], is able to bind mucus as effectively as wild-type *B. fragilis*, suggesting that CPS expression does not mediate mucus binding. Therefore, *B. fragilis* specifically binds intestinal mucus via a mechanism that appears not to involve expression of multiple surface polysaccharides.

Next, a mucus binding competition assay was performed to determine if the interaction between *B. fragilis* and mucus is saturable. We reasoned that as *B. fragilis* is pre-coated with higher concentrations of excess mucus, fewer putative receptors would be available to bind immobilized mucus in the well. Briefly, 1×10^8 CFUs of *B. fragilis* were incubated with excess mucus at 37 °C for 2 h under aerobic conditions with shaking. Bacteria were washed and added to

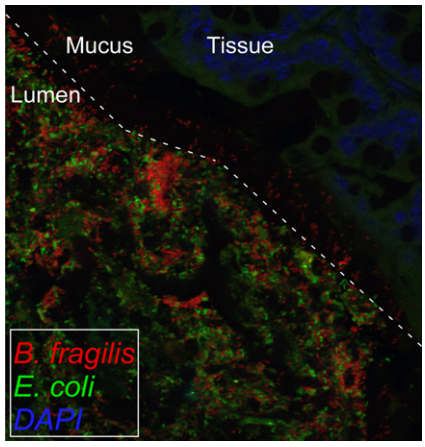

Fig. 1. Colon tissue section from a *B. fragilis* and *E. coli* dual-colonized Swiss Webster mouse. Epifluorescence image of bacteria visualized by FISH, and the epithelial cells counterstained with DAPI (blue) to visualize DNA. Both *E. coli* (green) and *B. fragilis* (red) are found in the lumen but only *B. fragilis* is found in the mucus layer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

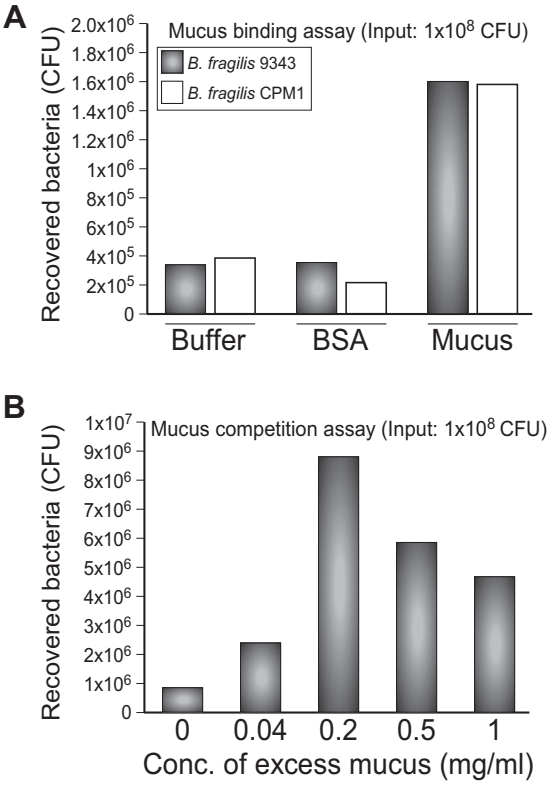

Fig. 2. *B. fragilis* binds intestinal mucus. (A) Number of *B. fragilis* (in CFUs) recovered after 1 h incubation in wells with an immobilized mucus layer, an immobilized BSA layer, or buffer only. Of the 1×10^8 CFUs incubated, 1.6×10^6 (1.6%) bound to immobilized mucus. The CPM1 mutant binds mucus similarly to wild-type bacteria. These data are representative of four independent trials. (B) Number of bacteria recovered from mucus binding assay after a 2 h pre-incubation with different concentrations of excess mucus. These data are representative of three independent trials.