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Biofilms in the large bowel suggest an apparent function of the human vermiform appendix

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Abstract

The human vermiform (“worm-like”) appendix is a 5 to 10 cm long and 0.5 to 1 cm wide pouch that extends from the cecum of the large bowel. The architecture of the human appendix is unique among mammals, and few mammals other than humans have an appendix at all. The function of the human appendix has long been a matter of debate, with the structure often considered to be a vestige of evolutionary development despite evidence to the contrary based on comparative primate anatomy. The appendix is thought to have some immune function based on its association with substantial lymphatic tissue, although the specific nature of that putative function is unknown. Based (a) on a recently acquired understanding of immune-mediated biofilm formation by commensal bacteria in the mammalian gut, (b) on biofilm distribution in the large bowel, (c) the association of lymphoid tissue with the appendix, (d) the potential for biofilms to protect and support colonization by commensal bacteria, and (e) on the architecture of the human bowel, we propose that the human appendix is well suited as a “safe house” for commensal bacteria, providing support for bacterial growth and potentially facilitating re-inoculation of the colon in the event that the contents of the intestinal tract are purged following exposure to a pathogen.

Keywords: mucus, Immunoglobulin A, commensal, safe house, bioreactor

1. Introduction: the human appendix.

The tendency of the human appendix to become painfully inflamed and send many otherwise healthy individuals to the hospital for surgery has made the structure well known. The function of the 5 to 10 cm long and 0.5 to 1 cm wide pouch that extends from the cecum of the human large bowel has long been a matter of debate. Appendix-like structures are relatively rare in phylogeny, being found in humans, rabbits, and two species of marsupials (opossums and wombats), but not in other marsupials or in a vast host of other animal species. The occurrence of the appendix sporadically throughout phylogeny might suggest that the structure is evolutionarily derived for a specific function rather than merely a vestige of a once important digestive organ. This idea was confirmed by Scott, who performed a detailed comparative analysis of primate anatomy and demonstrated conclusively that the appendix is derived for some unidentified function and is not a vestige. (Scott, 1980) The appendix is thought to play a role in immune function because the structure is associated with substantial lymphatic tissue (Gorgollon, 1978). However, the specific nature of the putative function of the appendix has never been identified, and, as a result, the idea has lingered that the appendix is a vestige.

2. Host-mediated biofilm formation by colonizing bacteria.

A specific function for which the human appendix is well suited is suggested by studies that have recently redefined how we think of the relationship between the mammalian host and bacteria that typically colonize the lumen of the large bowel (Everett et al., 2004; Sonnenburg et al., 2004). These studies indicate that biofilms, or adherent colonies of microbes growing within an extracellular matrix, are formed in the

mammalian large bowel and are associated with and dependent on the mucus that lines the epithelium of the bowel. This particular mutually beneficial relationship between microbes and the more complex life forms they colonize is apparently ubiquitous. For example, release of mucoid organic material from plant roots as they grow facilitates biofilm formation by commensal bacteria on the root surface, and in turn enhances bacterial growth into the area surrounding the root. This process is estimated to utilize approximately 20% of a plant's overall organic synthesis capacity, suggesting the importance of this process for the plant. As another example, coral, a relatively simple aquatic animal, secretes mucus that provides a matrix in which bacterial biofilms grow on the coral's surface (Ritchie, 2006). The microbes associated with the coral surface benefit the host in a number of ways, providing nutritional supplementation and protection against microbial pathogens (Reshef et al., 2006; Rosenberg et al., 2007). The microbes, in turn, derive nutritional support and a protected environment from their more complex hosts.

3. Host mediated biofilm formation in the mammalian gut.

Studies pointing at the importance of biofilms in the mammalian gut derive from a number of fields. For example, Costerton et al. evaluated hundreds of aquatic systems and considered the evaluations of others, concluding that biofilms predominate in virtually all nutrient-sufficient aquatic systems, independent of the system-specific dynamics. Further, Costerton concluded that biofilms reflect the most common steady state for bacterial growth (Costerton et al., 1995). Further, microbiologists working from a perspective of cell surface marker expression by normal gut bacteria and others

working independently on factors that direct establishment and maintenance of a spatially diversified gut microflora postulated that biofilms should be found as a part of the normal gut flora. Further, plasmid transfer rates in the gut are consistent with that of biofilms (Licht et al., 1999) rather than the alternative planktonic (non-adherent) growth. In addition, Jeffrey Gordon and colleagues (Sonnenburg et al., 2004), evaluating data from immunologists, environmental engineers, and glycobiologists, proposed that “symbionts inhabiting the polysaccharide-rich mucus gel layer overlying the gut epithelium constitute a biofilm-like community and that retention in such a matrix benefits the host by promoting functions served by the microbiota, including digestion of luminal contents and fortification of host defenses.” Examination of a variety of data from microbiologists and of the medical literature also point toward the same conclusions (Everett et al., 2004).

Although biofilms lining the normal large bowel are expected from a variety of viewpoints, direct empirical observations of biofilms in the mammalian bowel were lacking as recently as five years ago, and indeed initial efforts to assess the presence of biofilms in the large bowel proved difficult. We observed that techniques commonly used for preparing gut tissues for staining, such as washing with saline or fixation with aldehydes, tended to disrupt biofilms from the gut surface (Palestrant et al., 2004). This observation is probably not surprising, since disruption of predominantly carbohydrate cell surface coats by fixatives has been observed frequently, and is known to be a factor in preserving the adherent mucus layer of the gastrointestinal epithelium (Allen and Pearson, 2000) and the extracellular matrix of bacterial biofilms (Fassel and Edmiston, 1999).

Avoiding the pitfalls associated with preservation of the mucus layer adjacent to the epithelium, biofilms have been observed on the normal large bowel of mice, rats, baboons, and humans. (Palestrant et al., 2004; Swidsinski et al., 2005). Previously published work by our laboratory, using human appendices removed from recipients during incidental appendectomies conducted during kidney-pancreas transplants, revealed biofilms on the epithelial lining of the mucosal epithelium (Palestrant et al., 2004). These biofilms grow in the mucus layer covalently attached (Allen and Pearson, 2000; Atuma et al., 2001) to the epithelial surface that is immediately adjacent to the microvilli of the gut epithelium. Such biofilms were found to be associated with secretory IgA (Palestrant et al., 2004), and it is supposed that these biofilms are in a continuous state of shedding and regeneration (Bollinger et al., 2005; Bollinger et al., 2003; Sonnenburg et al., 2004), in a manner similar to that of the epithelial lining of the bowel. Further, it was proposed (Everett et al., 2004; Sonnenburg et al., 2004) that such biofilm formation not only enhances survival of normal enteric bacteria in the gut, but also aids in the exclusion of pathogens. Recapitulating our previously published observations of biofilms in the human appendix (Palestrant et al., 2004), we examined samples taken from a fresh, normal, intact, unprepped (containing fecal material) human colon of a deceased organ donor. Blinded evaluation of the samples revealed that biofilms were most prominent in the appendix, both in terms of bacterial density and biofilm continuity along the epithelial surface (Figure 1). The prominence of biofilms decreased progressively from the appendix to the distal end of the human large bowel, with substantially greater biofilm formation in the cecum compared to the ascending colon and transverse colon, and little or no biofilm formation in the descending and sigmoid colon (Figure 1).

Observations made from the study of animal colons also point toward the proximal end of the large bowel as being particularly important for biofilm formation. In rats (Palestrant et al., 2004), for example, biofilms lining the gut epithelium are most prominent in the cecum, observed less frequently in the ascending and transverse large bowel, and are least prominent in the distal colon, near the rectum. Similar observations have been made in mice (Swidsinski et al., 2005).

4. The appendix as a “safe house” for beneficial bacteria.

The observations described above, in conjunction with the survival advantages afforded to bacteria by biofilms (Costerton, 1995; Costerton, 1999; Costerton et al., 1995) and the architecture of the human large bowel, give rise to the idea that the appendix is a compartment well suited for maintaining beneficial or commensal microorganisms, being well positioned to avoid contamination by pathogenic organisms present transiently in the fecal stream. Indeed, the narrow lumen of the appendix as well as its location at the lower end of the cecum are both factors that afford relative protection from the fecal stream as it is propelled by peristalsis. Given the metabolic advantages (Bradshaw et al., 1994; Bradshaw et al., 1997) and other advantages (Costerton, 1995; Costerton, 1999; Costerton et al., 1995) that biofilms are known to afford bacteria, biofilm formation in the appendix is expected to be a relatively effective means of preserving and protecting commensal bacteria. In essence, the structure of the appendix is expected to enhance the protective effect of biofilm formation for commensal bacteria. Effective biofilm formation by commensal bacteria in the appendix is expected to facilitate not only the exclusion of pathogens, but also the adherence of the non-pathogenic commensal organisms within

that cavity. Regular shedding and regeneration of biofilms within the appendix would be expected to re-inoculate the large bowel with commensal organisms in the event that the large bowel became infected by a pathogen and was flushed out as a defensive response to that infection. (**Figure 2**)

Given the association of the human appendix with immune tissue and the function of the human appendix proposed above, examination of the possible role of the immune system in the proposed function of the appendix is warranted: A number of recent studies point strongly toward the idea that the immune system supports biofilm formation in the gut. For example, mucin and secretory IgA, two of the most abundant effector molecules produced by the immune system, mediate rapid biofilm formation by enteric bacteria *in vitro* (Bollinger et al., 2005; Bollinger et al., 2003; Orndorff et al., 2004), and both are associated with biofilms *in vivo* (Palestrant et al., 2004). Further, receptors for secretory IgA that are important for biofilm formation are up-regulated by the presence of secretory IgA *in vivo* (Friman et al., 1996; Wold and Adlerberth, 2000). Perhaps most compelling is the observation that increased immunoglobulin production observed in patients with inflammatory bowel disease is associated with increased biofilm formation in those patients compared to healthy individuals. (**Table 1**) Given the idea that the immune system supports biofilm formation in the gut, the observation that the appendix is associated with substantial lymphoid tissue (Gorgollon, 1978) lends further support to the idea that the appendix is a structure which maintains a reserve of normal enteric bacteria within biofilms associated with its epithelial mucosa.

If, in contrast to recent findings, the immune system were completely antagonistic to the commensal flora, then the function of the human appendix proposed herein does

not seem likely. Indeed, it seems unreasonable to propose that the appendix, a structure with apparent immune function, would protect bacterial growth if the immune system worked in the intestine for the primary purpose of thwarting bacterial growth. Thus, it is only a recently acquired understanding of the agonistic relationship between the immune system and the commensal flora (Everett et al., 2004; Sonnenburg et al., 2004) that makes evident the apparent function of the human appendix.

5. The human appendix: useless in the face of modern medicine and sanitation practices?

If indeed the appendix has an important function, the fact that the human appendix is frequently removed during surgery might be of concern. However, to the extent that the primary function of the appendix is the one proposed herein, it might be argued that the human appendix is not important in industrialized countries with modern medical care and sanitation practices. Indeed, maintenance of a reserve supply of commensal bacteria in the event of infection by pathogens may be unnecessary in areas where outbreaks of enteric pathogens do not affect the vast majority of the population at any one time. Certainly this idea is consistent with the well-known observation that appendectomy is without currently discernable long-term side effects in societies with modern medical and sanitation practices.

The identification of a potential function for the human appendix well suited to its location and architecture lends credence to the idea that the structure is not a vestige, but rather is derived for a specific function, consistent with conclusions drawn from evaluation of a comparative analysis of primate anatomy (Scott, 1980). However,

absolute proof of such a function may be difficult to obtain since the unique nature of the human appendix may preclude the use of animals to study the issue. Further, it is anticipated that the biological function of the appendix may be observed only under conditions in which modern medical care and sanitation practices are absent, adding difficulty to any potential studies aimed at demonstrating directly the role of the appendix in humans.

In as much as 6 % of the population in industrialized countries, the appendix becomes inflamed and must be surgically removed to avoid a potentially life-threatening infection. The widespread “dysfunction” of the appendix in industrialized countries is of interest in the light of the present discussion. The typical diet in countries with widespread appendicitis is substantially different than in non-industrialized countries where appendicitis is rare, and thus diet may indeed account at least in part for the incidence of appendicitis (Adamidis et al., 2000). However, the typical immune system of people in countries with modern healthcare and sanitation practices is profoundly different than that of people in non-industrialized countries without modern healthcare (Kemp and Bjorksten, 2003; Wills-Karp et al., 2001; Yazdanbakhsh et al., 2002). Indeed, the widely accepted “hygiene hypothesis” suggests that modern medicine and sanitation may give rise to an under-stimulated and subsequently overactive immune system that is responsible for high incidences of immune-related ailments such as allergy and autoimmune disease. Such over-reactivity of the immune system may lead to inflammation or other immune processes associated with appendicitis, and could hypothetically lead directly to obstruction of the lumen of the appendix with subsequent suppuration and acute appendicitis. Thus, given the apparent immune-related function of

the appendix, it seems possible that the same hygiene-associated changes in the immune system that give rise to allergy (~15% incidence) and autoimmune disease (~2.5% incidence) may contribute, at least in part, to an increased rate of appendicitis (~6% incidence) in industrialized countries.

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Figure captions

Figure 1. Biofilms adjacent to epithelium in a normal human bowel obtained from a deceased organ donor were evaluated using a confocal laser microscope following cryosectioning and staining of the tissue with acridine orange as previously described (Palestrant et al., 2004). Photos were taken of the areas at the border between the epithelium and the lumen. The smaller fluorescent points are bacteria within the mucus layer stained with acridine orange, and the larger brightly stained areas are the nuclei of the epithelial cells that also stain with acridine orange. Images on the right show an enlarged section of the images on the left. Sections taken from the appendix (A), cecum (B), transverse colon (C), and descending colon (D) are shown. Images taken from the ascending colon, not shown, appear similar in terms of biofilm density to images taken from the transverse colon. Likewise, images taken from the sigmoid colon, not shown, appear similar to images from the descending colon. The images of the appendix tissue (A) display the most dense and confluent biofilms, with less dense biofilms observed in the cecum (B), and less still in the transverse colon (C). The descending colon (D) shows staining of the nuclei of the epithelium, with diffuse staining of the adjacent mucus and no apparent bacterial biofilm. The conclusion that biofilm density and continuity decreased from the proximal to the distal end of the colon was confirmed by independent, blinded evaluations of the samples using a subjective grading scale. The bars = 30 microns (panels on left) and 15 microns (panels on right).

Figure 2. Schematic diagram, illustrating the proposed mechanism by which the human immune system facilitates the maintenance of a relatively protected reservoir of normal gut flora in the human appendix. Host-mediated biofilm formation is most prominent in the proximal part of the colon, particularly in the appendix, with declining levels of biofilm formation toward the distal end (rectum) of the colon.

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Population	Mean Fecal IgA concentration	Percent patients with 10³ to 10⁶ cfu/μl mucosal bacteria (associated with a wash-resistant biofilm)	Percent patients with 10⁴ to 10⁶ cfu/μl mucosal bacteria (associated with a “thick” wash-resistant biofilm)
Normal	73 mg/l	10%	5%
Ulcerative colitis	486 mg/l	61%	16%
Crohns colitis	1194 mg/l	80%	36%

Table 1. Fecal IgA concentrations and gut mucosa-associated biofilms in normal individuals and in patients with ulcerative colitis and with Crohns disease. In contrast to gut mucosa-associated biofilms in normal individuals and animals (Palestrant et al., 2004), the gut mucosa-associated biofilms in patients with ulcerative colitis and especially patients with Crohns colitis are resistant to washing with saline (Swidsinski et al., 2002). The degree of wash resistant biofilms correlates with the average concentration of SIgA in those patient populations. The fecal IgA concentrations are those determined by Ellen Schoof and colleagues (Schoof et al., 1997). The number (colony forming units; cfu) of wash-resistant gut mucosal-associated bacteria was determined by Alexander Swidsinski and colleagues (Swidsinski et al., 2002). The association of particular concentrations of mucosal-associated bacteria with biofilm formation was also described by Swidsinski (Swidsinski et al., 2002).

Fig 1

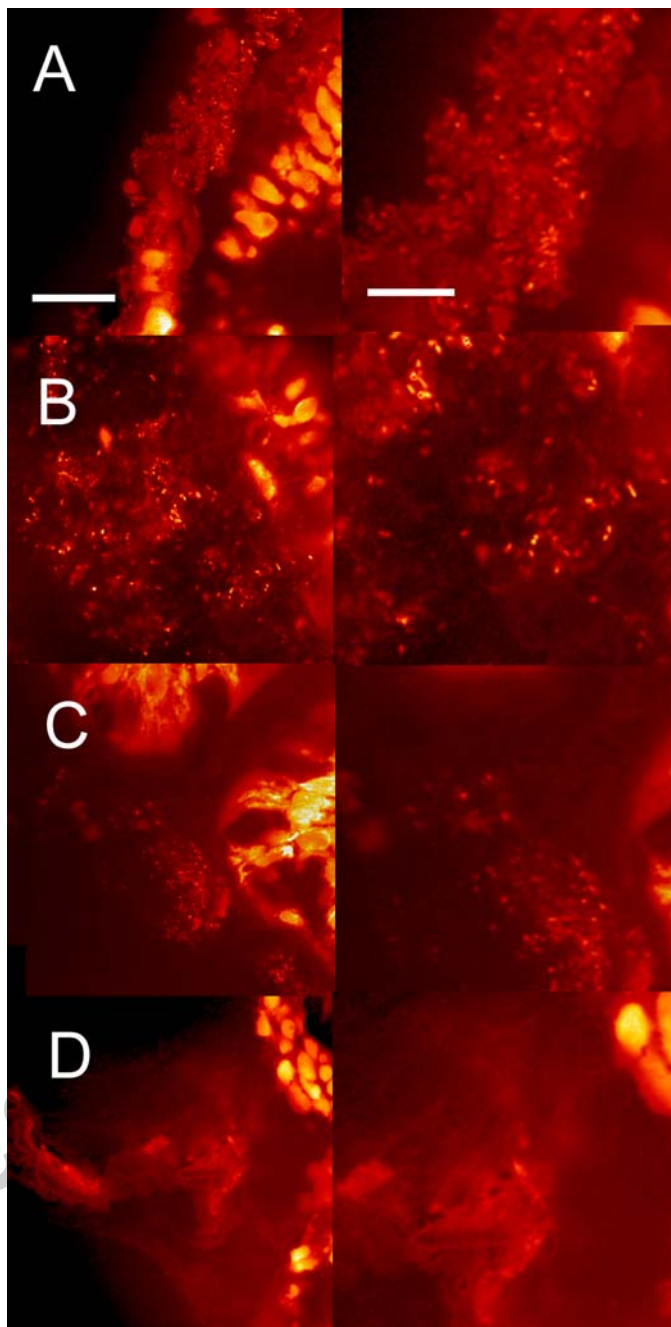
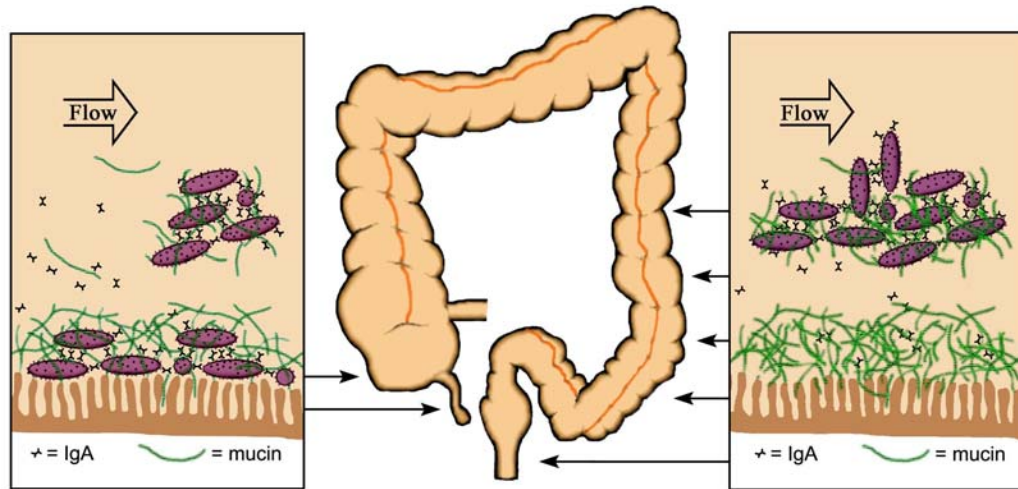


Fig 2



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