Published in final edited form as:

Neurogastroenterol Motil. 2014 June ; 26(6): 745–748. doi:10.1111/nmo.12366.

Germ free mice as a model to study effect of gut microbiota on host physiology

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Abstract

The alterations in resident gut microbiota seen in chronic gastrointestinal disorders has led to an increasing interest in the role of gut bacteria in maintaining intestinal barrier function. While acute alterations in colonic secretomotor function in response to pathogens have been well described, the effect of commensal bacteria on intestinal barrier function and colonic secretomotor function still remains poorly understood. Germ free mice represent a model system to study effect of gut microbes on host gastrointestinal physiology. The study by Lomasney et al. represents an important step in this direction by demonstrating that the colonic secretomotor function is largely preserved in germ free mice, hence making them a suitable model to study effect of gut microbiota on host function.

Keywords

Germ free; microbiota; Ussing chamber; barrier function; transepithelial resistance

In this issue of *Neurogastroenterology and Motility*, Lomasney et al.¹ describe responsiveness of colonic mucosa-submucosa preparations from germ free and conventionally housed mice to neural, epithelial and bacterial stimulation in Ussing chambers. Interestingly the authors saw no differences between germ free and conventionally raised colon in the baseline short circuit current, transepithelial resistance, response to cholinergic stimulation using bethanechol or neural stimulation using capsaicin or veratridine. The germ free tissue did however demonstrate heightened response to cAMP stimulation induced by 10 μ M of forskolin. Overall the findings reported in the study suggest that the colonic secretomotor function is largely preserved in germ free mice. The similar response of germ free and conventional mouse colon to probiotic strains *Bifidobacterium infantis* 35624 or *Lactobacillus salivarius* further highlights the preserved function of germ free colon though a more comprehensive evaluation of barrier function is warranted. The study highlights the future utility of gnotobiotic mouse models to understand effect of gut microbiota on host physiology such as intestinal barrier function and secretion. The finding of increased responsiveness to forskolin is intriguing and merits further investigation in different segments of the colon using an incremental dose response.

The gut microbiome represents the aggregate genomes of the trillion of microbes residing in the gastrointestinal tract which exist in a mutualistic relationship with the host.² The host gut in turn has evolved with physiological adaptation to allow for co-existence with the commensal gut bacteria while at the same time maintaining constant vigilance against pathogenic bacteria. The acute alteration in colonic secretion and barrier function by pathogens has been known for a long time. Cholera toxin causes activation of adenylate cyclase in intestinal epithelial cells which results in elevation of intracellular cAMP.³ This leads to increased chloride secretion by crypt cells and reduced absorption of sodium and chloride ions by villous cells resulting in diarrhea. *E. coli* heat-stable enterotoxin activates guanylate cyclase C receptors to induce chloride secretion.⁴ *Yersinia enterocolitica* is associated with altered tight junction proteins and induction of cell necrosis, both resulting in a decrease in transepithelial resistance in colonic HT-29/B6 cell monolayers.⁵ *Entamoeba histolytica* infection causes constitutive production and secretion of prostaglandin E₂ which results in altered paracellular permeability of T84 monolayers resulting in increased sodium permeability and chloride secretion by activation of cystic fibrosis transmembrane conductance regulator.⁶ *Campylobacter* infection disrupts barrier function, with decreased transepithelial electrical resistance and a change in the distribution of the tight junction protein occludin within Caco-2 cell monolayers.⁷ Epithelial tight junction changes have been reported with *Giardia* infection, which causes caspase-3 dependent disruption of epithelial tight junction and enterocyte apoptosis.⁸

While specific mechanisms underlying the effect of pathogens on intestinal barrier function are well studied the role of commensal bacteria in maintaining or enhancing the barrier are still not well understood. Alteration in composition of resident bacterial composition and function in chronic gastrointestinal disorders such as irritable bowel syndrome (IBS)⁹⁻¹¹ and inflammatory bowel disease (IBD)¹² has led to an increasing interest in the role of commensal bacteria in regulating the intestinal barrier over the past decade.¹³ The majority of these data relate to probiotics (live nonpathogenic microorganisms with putative beneficial effects on the host) which have shown some degree of benefit in diseases such as IBS¹⁴ though the effect is inconsistent and highly strain dependent.¹³ In order to better understand the molecular mechanisms by which probiotics exert a beneficial effect, several studies have now focused on the effect of probiotic microbial strains on intestinal barrier function and shown increased mucus production, antimicrobial peptides and tight junction integrity of intestinal epithelial cells.¹³

The mucus layer overlying the intestinal epithelium provides the first layer of defense against microbes and consists primarily of glycoproteins secreted by goblet cells. Pathogenic bacteria such as *Helicobacter pylori*, *Entamoeba histolytica* and *Pseudomonas aeruginosa* have mechanisms that allow them to invade or utilize mucus associated nutrients by reduction of mucin disulfide bonds or utilizing proteases.¹⁵⁻¹⁷ On the other hand commensal microorganisms are thought to fortify the intestinal barrier. In order to understand microbial regulation of the host physiology it is imperative to understand both the effect of normal gut resident bacteria which the host evolves with as well as probiotic strains administered to correct potential abnormalities within the resident bacteria. Probiotic *Lactobacillus* strains have been shown to increase expression of mucin glycoproteins MUC2 and 3 *in vitro* in

human intestinal cell lines such as Caco-2 and HT29^{18, 19} however convincing data from *in vivo* studies is lacking. Probiotics have also been shown to augment secretory IgA in the mucus layer¹³, though this effect again varies based on the probiotic strain used. Interestingly colonization of germ free mice with commensal bacterium *B. thetaiotaomicro*n significantly increases the influx of IgA producing B cells in the ileal mucosa with increased expression of polymeric immunoglobulin receptor which transports IgA across the epithelium suggesting an important role for commensal gut bacteria normally found in the human gut in modulating intestinal barrier function.²⁰

The enterocyte barrier underneath the mucus layer is composed of several intercellular junctional complexes; the best characterized being tight junctions composed of transmembrane proteins occludin and claudins. Disruption of tight junctions can be seen in IBD, a disease associated with intestinal inflammation and alteration in resident gut microbiota (dysbiosis) but it remains unclear if disruption of the barrier is a cause or effect of dysbiosis/inflammation seen in IBD.¹³ The majority of the evidence showing an effect of bacteria on tight junction alteration is based on *in vitro* cell culture data. Bacteria or bacterial products can alter tight junctions as evidenced by change in transepithelial resistance across cell monolayers. Probiotic species such as *S. thermophilus* and *L. acidophilus* enhance transepithelial resistance in HT-29 and Caco-2 cell lines²¹ whereas conditioned medium from *B. infantis* is sufficient to decrease permeability²² though the two groups appear to act through different pathways. Colonization of germ free mice with a common gut resident *B. thetaiotaomicro*n is associated with a 280 fold increase in villous epithelial expression of small proline-rich protein-w2 which plays an important role in fortifying the intestinal epithelial barrier function.²⁰

The above data highlights some of the mechanisms by which probiotics can alter gut barrier function. However the data is primarily from *in vitro* studies. It is hard to delineate the effect of probiotics on host function in *in vivo* studies using conventionally raised mice as, while probiotics can alter host physiological functions they can also affect host immune system and have secondary impact on the resident microbial community and its functionality.²³ In order to better understand how individual microbial species, either resident members of the gut or probiotic species, affect host physiology we need a simplified model system that can help control off target effects of these bacteria. Gnotobiotic mice represent such a system where we can introduce microbes individually or sequentially in germ free mice and study effects of a single bacterium or known consortia of bacteria on host function.²⁴ Gnotobiotic mice also allow study of microbe-microbe interaction to better understand how introduction of a new microbial member affects community membership and function. However, the altered physiology seen in germ free mice raises issues regarding the use of these mice as a model to study effects of gut microbes on host physiology.²⁵

Early studies have characterized differences in host physiology in germ free and colonized mice, the most striking being the enlarged cecum. *Ex vivo* preparations demonstrate reduced smooth muscle tone in germ free rat cecal muscle strips. The muscle however is responsive showing a restoration of tone to conventional values when treated with bacteria free filtrates *ex vivo*.²⁵ Cecal contents are also more liquid in germ free mice compared to conventionally raised mice in part due to decreased sodium and chloride ion concentrations in the cecal

contents and impaired water absorption in the colon. The germ free cecal tissue however maintains the capacity to absorb water similar to conventional mouse cecal tissue as observed in *in vitro* studies.²⁵ These studies highlight that while germ free mice have an altered gastrointestinal physiology, this represents an adaptation to the absence of microbes and introduction of gut microbiota can reverse some of the changes and help provide insights into the role of gut microbes on host function.

The current study by Lomasney et al.¹ further highlights that the colonic secretomotor function is preserved in germ free mice making it a model system for *in vivo* studies of host microbial interaction. The increased secretory response to cAMP in germ free mice also generates future questions regarding the underlying mechanisms and potential implications. Overall, the study is a welcome step in an increasingly relevant area targeted to explore mechanistic underpinnings of gut microbiome in modulating host physiology.

Acknowledgments

We thank Kristy Zodrow for her secretarial assistance. This work was supported by NIH K08DK100638 (PK) and P30DK84567 (PK and MG) at Mayo Clinic Rochester, MN.

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