Old Herborn University Seminar Monograph 27:
Persisting consequences of intestinal infection.
Editors: Peter J. Heidt, Dennis Lang, Mark Riddle, Richard Walker, and Volker Rusch.
Old Herborn University Foundation, Herborn-Dill, Germany: 61-70 (2014).

AN ENTERIC TRIANGLE: PROTOZOAN INFECTIONS, LINKS TO ENVIRONMENTAL ENTEROPATHY AND THE POTENTIAL INFLUENCE OF THE INTESTINAL BACTERIAL MICROBIOME ON THIS INTERACTION AND OVERALL HEALTH IN THE DEVELOPING WORLD

STACEY L. BURGESS and WILLIAM A. PETRI

University of Virginia School of Medicine, Carter Harrison Research Building, Charlottesville, VA, USA

SUMMARY

Lasting changes to the host intestinal mucosal surface may be caused by faecal enteropathogens such as *Entamoeba histolytica,* one of the causes of host diarrhoea, which when it repeatedly and frequently occurs instigates a subclinical condition, environmental enteropathy (EE), characterized by blunting of intestinal villi and intestinal inflammation. Amoebiasis and EE occur in the context of the host's intestinal bacterial microbiome and the persistent changes driven by enteropathogens could be modulated by the composition of these intestinal bacterial communities. In this work, we will explore the long term changes to the host by protozoan infections such as *E. histolytica* in man and model organisms such as mice, how the intestinal bacterial microbiota and probiotic organisms might influence overall health and infections with these protozoa, and finally the role these interactions might have in health and wellness in the developing world where enteric diarrheal disease is endemic. We hypothesize that interactions between the host's immune system, protozoan infections and the intestinal microbiome might influence EE and in turn vaccine failure and perhaps exacerbate nutritional deficits, increasing the risk of malnutrition in a food insecure household.

ENTERIC INFECTIONS AND ENVIRONMENTAL ENTEROPATHY

For many years our laboratory has tracked the medical histories of a large cohort of children living in the Mirpur slums of Dhaka, Bangladesh. Here, colonization with parasites is a major cause of diarrheal illness, which is a significant source of morbidity and mortality in the developing world. Colonization with *Giardia lamblia*, *Cryptosporidium parvum*, and *Entamoeba histolytica* likely underlie 58 million cases of childhood diarrhoea (*den Hartog* et al., 2013). Furthermore, diarrheal disease is a primary cause of mortality in children less than five years of age in these nations and accounts for nearly two million deaths annually (WHO). However, in tracking these children, it has also become apparent that these infections in early life may have lasting influences on health, particularly growth, later nutritional status, susceptibility to further infection and perhaps even influence vaccine

efficacy (*Korpe* and *Petri*, 2012; *Mondal* et al., 2012; *Kotloff* et al., 2013). This cohort, and many others in developing tropical nations, has a high incidence of environmental enteropathy (EE), a subclinical condition caused by constant faecal–oral contamination that is characterized by blunting of intestinal villi and other histopathological abnormalities of the intestine such as increased crypt lengthening and intestinal inflammation, including lymphocyte and monocyte infiltration. EE is believed to significantly contribute to malnutrition and stunting in these populations by preventing normal development of the intestine. Much nutrient absorption occurs at the tips of intestinal villi and this is not possible in severely damaged and inflamed intestines (*Fagundes-Neto* et al., 1984, 1994; *Korpe* and *Petri*, 2012). Our laboratory has shown a correlation between *E. histolytica* driven childhood diarrhoea and later stunting (*Mondal* et al., 2006). In turn, malnourished children have higher rates of infection by *E. histolytica* and *Cryptosporidium* (*Korpe* and *Petri*, 2012; *den Hartog* et al., 2013a). Thus repeated colonization with enteropathogens, including these protozoa, likely instigates a feedback loop of poor nutrition and stunting. While E. *histolytica* and *Cryptosporidium* do influence diarrheal diseases, they have not specifically been shown to cause EE in man. However, *E. histolytica* has been shown to instigate many of the characteristics of EE in murine models of amoebiasis (*Houpt* et al., 2002a; *Mondal* et al. 2012; *Verkerke* et al. 2012; *den Hartog* et al., 2013a). *Houpt* et al. (2002b) have shown, for instance, that injection of *E. histolytica* trophozoites into C3H/HeJ mice leads to chronic caecal infection in the majority of mice. They demonstrated that infected mice had histological changes including crypt hyperplasia, epithelial ulceration, and submucosal inflammatory infiltration that were reminiscent not only of human amoebiasis but also human EE. Thus, we have hypothesized that repeated infections with *E. histolytica* and other protozoans in children might contribute to EE by eliciting long lasting changes to their intestinal mucosa and to the infiltrating populations of immune cells present. Our laboratory is currently exploring how this organism and other enteropathogens might influence the long term sequelae of EE. However, while entertaining this notion, it is useful to understand the context of intestinal *E. histolytica* infection.

Initial infection occurs after ingestion of faecally contaminated water or food containing *E. histolytica* cysts which then undergo excystation in the lumen of the small intestine. The amoeba trophozoite then feeds on resident bacteria and possibly the intestinal epithelium and in rare cases, it may cause systemic amoebiasis by invading the intestinal mucosa and traveling to the blood stream, liver or brain (*Petri* and *Singh*, 1999; *Haque* et al., 2003; *Verkerke* et al., 2012). However the intestinal lumen is densely populated by a community of bacteria that may have a significant influence on the host's immune response at baseline, and during amoeba infection, as well as the virulence of the amoeba itself (*Mirelman* et al., 1983; *Noverr* and *Huffnagle*, 2004; *Frederick* and *Petri*, 2005; *Maslowski* and *Mackay*, 2010; *Cho* and *Blaser*, 2012). These interactions may in turn also influence the hosts nutritional status and ability to mount a successful immune response to later infections (*Mondal* et al., 2012). Thus when considering the persistent effects of protozoan infection and EE, it is also pertinent to examine the contribution of the intestinal bacterial microbiota to human health.

INFLUENCE OF COMMENSAL BACTERIA ON MAN AND MICE

The normal flora of the human gastrointestinal tract is a large, complex community of bacteria that is composed of at least several hundred species and consists of 10^{12} bacteria per gram of large bowel content. There are far more bacterial cells than there are eukaryotic cells in the human body and these organisms form a symbiosis that influences many aspects of human physiology including the composition of the metabolome, which is the complete set of body wide small-molecule metabolites including hormones, chemokines, cytokines and other signalling molecules (*Arumugam* et al., 2011; *Siezen* and *Kleerebezem*, 2011; *Cho* and *Blaser*, 2012). However, understanding the mechanisms by which the human microbiota influences the metabolome, immune system and nutrition is an emerging science. In studying the composition of the microbiota it has become clear that, while there is some stability on a phyla and genera level within human populations, there is much variability in the milieu of specific bacterial species between individual humans. Enterotypes are classification units based on the bacterial composition of the gut microbiome and have been utilized to describe these shared groups of bacterial phyla and genera in humans and to study correlations with diseases that might be influenced by the microbiome. These enterotypes are independent of ethnic background but appear to be influenced to some degree by the composition of diet (*Arumugam* et al., 2011; *Wu* et al., 2011). How many enterotypes are present is yet to be determined, however there are at least three (*Arumugam* et al., 2011). They are defined largely by the variation in the levels of three genera, *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2), and *Ruminococcus* (enterotype 3) but have contributions from other genera (*Arumugam* et al., 2011). These enterotypes can further be represented by analysing clusters of bacterial families and this analysis method shows that these first two enterotypes are primarily characterized by the presence of *Bacteroides* and *Prevotella*, whereas the third cluster is mostly characterized by related groups of the family *Clostridiaceae*, and unclassified *Lachnospiraceae*. Several studies have suggested that specific enterotypes might be associated with inflammatory responses in the intestine and deregulation of normal metabolic controls. Specifically, intestinal autoimmunity, colitis, and obesity, have been associated with enterotypes 2 and 3 above, however, which specific organisms might influence these diseases is still a point of contention (*Arumugam* et al., 2011; *Giongo* et al., 2011; *Siezen* and *Kleerebezem*, 2011). Studies of type 1 diabetes have further demonstrated that there are distinct differences in the microbiome of infants that developed the disease compared to those that did not, with decreased microbial diversity and a higher proportion of the *Bacteroidetes* phyla compared to *Firmicutes* in the group with the disease (*Giongo* et al., 2011). It has also recently been shown that members of the *Bacteroidetes* and *Firmicutes* are heavily involved in metabolism of complex carbohydrates in the intestine (*Flint* et al., 2012). Thus, the composition of the intestinal bacterial microbiota might significantly influence intestinal inflammation, metabolism and nutrition in man. However, the bulk of these studies have taken place in the developed world. Changes in the microbiome that might underlie the development of EE are not well described.

Mouse models have demonstrated

more directly that the microbiome can have a significant influence on the function, structure and composition of the immune system and intestine as well as malnutrition. Gnotobiotic, or germ free, mice have structural defects in peyers patch formation in the small intestine, decreased or absent IgA production, few intra epithelial lymphocytes and a systemic defect in T regulatory cell induction, all of which is reversed once the animals are recolonized with a normal murine faecal microbiota (*Umesaki* and *Setoyama*, 2000; *Jiang* et al., 2004). Components of the intestinal microbiota such as *Lactobacillus casei* and bacteria present on vegetables and in the soil such as *Lactobacillus plantarum* and *Bifidobacterium bifidum* can also drive Th1 and T regulatory helper cell induction which may be antagonistic to inflammatory Th2 helper cell driven pathologies such as asthma and eczema and have been shown to be protective in colitis models (*Feleszko* et al., 2007; *Schwarzer* et al., 2011). The microbiome can also have a significant influence on nutritional status in murine models. Smith et al. have recently shown that kwashiorkor, a type of severe acute malnutrition, may be influenced by the gut microbiome. They observed that when the microbiome from malnourished versus healthy Malawian twins was transplanted into gnotobiotic mice the kwashiorkor microbiome instigated marked weight loss in recipient mice, as well as altered the metabolism of carbohydrates and amino acids, when compared to the microbiome from the healthy twins (*Smith* et al., 2013).

Another striking example of a specific component of the bacterial microbiota influencing physiology and the immune responses is the role segmented filamentous bacteria (SFB) play in immune maturation in the intestine of mice. SFB are commensal, uncultivable, obligate gut tropic, members of the *Clostridiaceae* that have been shown to drive potent IgA induction and Th17 helper cell induction in the intestine (*Davis* and *Savage*, 1974; *Ivanov II* et al., 2008; *Gaboriau-Routhiau* et al., 2009; *Kuwahara* et al., 2011). Colonization with SFB has been shown to exacerbate or influence a number of intestinal and extra intestinal models of human disease in mice, including colitis, autoimmune myelitis, arthritis and type 1 diabetes via Th17 cell induction (*Nutsch* and *Hsieh*, 2012). The bacteria, while uncultivable, are well described both genetically and morphologically in mice and a number of studies have suggested that they may be present in humans as well (*Davis* and *Savage*, 1974; *Child* et al., 2006; *Kuwahara* et al., 2011; *Yin* et al., 2012; *Caselli* et al., 2013; *Jonsson*, 2013). SFB colonization also strongly influences the composition of the intestinal microbiota through its unique kinetics of colonization and influence on the immune system. For a short time after weaning, the bacteria becomes the dominate species within the murine gut and has been shown to outcompete enteropathic bacteria species (*Chase* and *Erlandsen*, 1976; *Heczko* et al., 2000; *Stepankova* et al., 2007; *Kuwahara* et al., 2011). The ability of SFB, a component of the mouse, and possibly human, normal flora, to strongly influence the basic makeup of the immune system as well as colonization by other bacterial species illustrates the interconnectedness of individual bacterial species, the broader microbiome and health. However the influence of the intestinal bacterial microbiome is by no means limited to other bacterial species and may have profound influences on protozoa virulence and colonization and in turn amoebiasis, diarrheal disease and EE.

INTERACTIONS BETWEEN BACTERIA AND PROTOZOA

As mentioned previously, many protozoa inhabit the intestine for a significant portion of their lives, and in doing so interact intimately with other organisms present there (*Mirelman* et al., 1983). *Berrilli* et al. (2012) have recently highlighted some of the interactions between intestinal microbial communities, probiotics and bacterial pathogens and many types of parasite infections. Protozoa, including *E. histolytica* , are both influenced by the presence of other enteropathic and probiotic bacteria and influence the composition of the broader intestinal microbiota. *Galván-Moroyoqui* et al. (2008) have explored the effect of co-culture of trophozoites from *E. histolytica* and *E. dispar* with the enteropathogenic bacteria strains *Escherichia coli* (ETEC), *Shigella dysenteriae* and a commensal *Escherichia coli* on epithelial cell monolayers. In doing this they determined that phagocytosis of pathogenic bacteria augmented the cytopathic effect of *E. histolytica* on the cell monolayer as well as increased expression of the adherence lectin, Gal/GalNAc, on the amoeba's surface. Thus, interactions with enteropathic bacteria in humans might serve to increase the virulence of *E. histolytica* during amoebiasis. *E. histolytica* colonization in turn also influences the composition of the microbiome. *Verma* et al. (2012) have shown that during amoebiasis there is a significant decrease in absolute quantification of *Bacteroides*, *Clostridium coccoides*, *Clostridium leptum, Lactobacillus* and *Campylobacter* and an increase in *Bifdobacterium,* while there was no change in *Ruminococcus* compared to healthy patients. These works suggest that some of the pathology that results during amoebiasis might be driven by a disregulated microbiome or cross talk between enteropathic bacteria and protozoa and the intestinal immune system, particularly the intestinal epithelium. In fact, there are several works suggesting that probiotics, particularly *Lactobacillus* species, might be protective in the context of protozoan infections (*Preidis* et al., 2011; *Travers* et al., 2011). Thus a decrease in protective, commensal, lactobacillus species during *E. histolytica* infection might influence the severity of disease. One murine study has shown that daily administration of *Lactobacillus acidophilus*, a bacteria common in yogurt, for one week in *Giardia lamblia* infected BALB/c mice significantly reduced *G. lamblia* infection burden in those mice. Disease severity was also significantly decreased. Histological analysis of the intestine showed that probiotic administration protected mice against parasite induced mucosal damage and decreased intestinal villous atrophy (*Shukla* et al., 2010). Thus probiotic interventions might provide an attractive avenue to decrease intestinal damage in populations in which repeated intestinal protozoal infections occur (*Shukla* et al., 2010). Further understanding of how protozoa influence and are influenced by the intestinal microbiome, enteropathogens and probiotics would thus be informative in designing microbiome based interventions for diseases such as EE and malnutrition.

ENTERIC INFECTIONS, HEALTH AND EXPLORATION OF THE MICROBIOME IN THE DEVELOPING WORLD

The Petri laboratory has long sought to find connections between enteric infections, diarrheal disease and diseases with persistent effects such as EE and

malnutrition in order to develop targeted interventions that might decrease the burden of disease in the developing world. In our field site in Mipur, Dhaka, we followed children for the first year of life with every-other-day home visits and surveyed enteropathogens in diarrheal and monthly surveillance stool samples. We also measured intestinal barrier function by endocab antibodies, which correlate with translocation of bacterial LPS into blood, and measured nutritional status and stunting by anthropometry. In this study we found that diarrhoea co-occurred with infections caused by several organisms including enteric protozoa (amoebiasis, cryptosporidiosis, and giardiasis), rotavirus, astrovirus, and enterotoxigenic *Escherichia coli* (ETEC). We also observed that malnutrition was present in 16.3% of children at birth and 42.4% at 12 months of age and that children that were malnourished at birth had increased *Entamoeba histolytica*, *Cryptosporidium*, and ETEC infections as well as more severe diarrhoea. The children who became malnourished at 12 months of age were also much more likely to have prolonged diarrhoea and intestinal barrier dysfunction, a mother without education, and low family expenditure (*Korpe* and *Petri*, 2012; *Mondal* et al., 2012; *den Hartog* et al., 2013b).

Our laboratory, along with many other collaborators, has also recently begun exploring links between malnutrition, EE and oral polio vaccine failure. This is particularly important as vaccines for polio (OPV) and rotavirus are far less effective in poor children in the developing world and the underlying cause for this failure is largely unknown. Based on our previous experience we thus hypothesized that failure of oral vaccines such as OPV might be due to EE and be driven by inflammation from endotoxin exposure. We tested this hypothesis in the Mirpur cohort of children by measuring responses to oral poliovirus vaccine in children who received a minimum of three doses of OPV by age 6 months. We observed that diminished antibody responses to OPV were associated with malnutrition, increased serum endocab levels, and shorter breastfeeding duration. We also examined potential immune mechanisms that might underlie vaccine failure in a smaller subset of these children and found that children with OPV failure exhibited globally reduced cellular responsiveness to a range of cytokine stimulations, as well as elevated pro-inflammatory cytokine expression. These data indicated that oral vaccine failure in these children is influenced by a combination of malnutrition, gut barrier dysfunction in early childhood, and is associated phenotype (unpublished data/abstract). These studies further highlighted the complex interrelationship of malnutrition, protozoan and enteric infections, diarrhoea and vaccine failure in infants in low-income settings and the persistent effects of these problems. Thus, identification of probiotics, or particular enterotypes, that are protective during protozoan infection might help mitigate destruction of the intestinal barrier and significantly improve health outcomes in these populations.

Unfortunately, the contribution, if any, of specific components of the intestinal bacterial microbiota to EE and vaccine failure in the developing world is not currently well described. However given the profound influence that intestinal bacteria such as *Lactobacillus*, *Bifidobacterium,* SFB, and enteropathic E. *coli* (ETEC) can have on host immunity, parasite burden and virulence in mice and man, and the relative inexpensiveness of probiotic interven-

tions, it is certainly an area of study that should be pursued. Interestingly, one such recent study has shown a link between the composition of the bacterial microbiota and the effectiveness of an oral typhoid vaccine in a small group of individuals. In this study the composition of the microbiome did not influence the ability of responders to mount an immune response to oral typhoid vaccination, but those with a more complex microbiota mounted a more robust, multiphasic IFN-y response to oral vaccination. Many different organisms represented by operational taxonomic units (OTUs) were found to differ between individuals displaying robust responses and those with late, less robust responses. However the vast majority of these OTUs were classified within the order *Clostridiales* (*Eloe-Fadrosh* et al., 2013). Another recent paper has shown that the presence of some commensal *Clostridium* related species is decreased during autoimmune colitis in children (*Michail* et al., 2012). Thus the composition of the microbiota, and *Clostridia* related organisms, or commensals that induce immune responses similar to these bacteria, may very well be an important factor influencing homeostasis of the intestine and the success of vaccination in the developing world.

FINAL THOUGHTS

Infection with enteropathogens such as the protozoa *Entamoeba histolytica* likely contributes to the development of environmental enteropathy and induces lasting effects on the intestinal mucosa that may negatively impact nutritional outcomes and vaccine success in millions of children each year. These pathogenic organisms live in the context of the intestinal bacteria microbiota and interactions with these species may significantly influence the virulence, and infectivity, of those protozoa as well as the host's ability to mount protective responses against future infections. A better understanding of the interactions between these organisms and the intestinal microbiota, which might include probiotics commonly found in many foods, may lead to cost effective treatments that could significantly decrease the burden of enteropathogens in the developed world.

LITERATURE

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., Fernandes, G.R., Tap, J., Bruls, T., Batto, J.M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., Kurokawa, K., Leclerc, M., Levenez, F., Manichanh, C., Nielsen, H.B., Nielsen, T., Pons, N., Poulain, J., Qin, J., Sicheritz-Ponten, T., Tims, S., Torrents, D., Ugarte, E., Zoetendal, E.G., Wang, J., Guarner, F., Pedersen, O., de Vos, W.M., Brunak, S., Doré, J.; MetaHIT Consortium, Antolín, M., Artiguenave, F., Blottiere, H.M., Almeida, M., Brechot, C., Cara, C., Chervaux, C., Cultrone, A., Delorme, C., Denariaz, G., Dervyn, R., Foerstner, K.U., Friss, C., van de Guchte, M., Guedon, E., Haimet, F., Huber, W., van Hylckama-Vlieg, J., Jamet, A., Juste, C., Kaci, G., Knol, J., Lakhdari, O., Layec, S., Le Roux, K., Maguin, E., Mérieux, A., Melo Minardi, R., M'rini, C., Muller, J., Oozeer, R., Parkhill, J., Renault, P., Rescigno, M.,

Sanchez, N., Sunagawa, S., Torrejon, A., Turner, K., Vandemeulebrouck, G., Varela, E., Winogradsky, Y., Zeller, G., Weissenbach, J., Ehrlich, S.D., and Bork, P.: Enterotypes of the human gut microbiome. Nature 473,174-180 (2011).

Berrilli, F., Di Cave, D., Cavallero, S., and D'Amelio, S.: Interactions between parasites and microbial communities in the human gut. Front. Cell. Infect. Microbiol. 2,141 (2012).

Caselli, M., Tosini, D., Gafà, R., Gasbarrini, A., and Lanza, G.: Segmented filamentous bacteria-like organisms in histological slides of ileo-cecal valves in patients with ulcerative colitis. Am. J. Gastroenterol. 108, 860-861 (2013).

Chase, D.G. and Erlandsen, S.L.: Evidence for a complex life cycle and endospore formation in the attached, filamentous, segmented bacterium from murine ileum. J. Bacteriol. 127, 572-583 (1976).

Child, M.W., Kennedy, A., Walker, A.W., Bahrami, B., Macfarlane, S., and Macfarlane, G.T.: Studies on the effect of system retention time on bacterial populations colonizing a three-stage continuous culture model of the human large gut using FISH techniques. FEMS Microbiol. Ecol. 55, 299-310 (2006).

Cho, I. and Blaser, M.J.: The human microbiome: at the interface of health and disease. Nat. Rev. Genet. 13, 260-270 (2012).

Davis, C.P. and Savage, D.C.: Habitat, succession, attachment, and morphology of segmented, filamentous microbes indigenous to the murine gastrointestinal tract. Infect. Immun. 10, 948-956 (1974).

den Hartog, J., Rosenbaum, L., Wood, Z., Burt, D., and Petri, W.A.: Diagnosis of multiple enteric protozoan infections by enzyme-linked immunosorbent assay in the Guatemalan highlands. Am. J. Trop. Med. Hyg. 88, 167-71 (2013).

Eloe-Fadrosh, E.A., McArthur, M.A., Seekatz, A.M., Drabek, E.F., Rasko, D.A., Sztein, M.B., and Fraser, C.M.: Impact of oral typhoid vaccination on the human gut microbiota and correlations with s. Typhi-specific immunological responses. PloS One 8, e62026 (2013).

Fagundes-Neto, U., Viaro, T., Wehba, J., Patrício, F.R., and Machado, N.L.: Tropical enteropathy (environmental enteropathy) in early childhood: a syndrome caused by contaminated environment. J. Trop. Pediatr., 30, 204-209 (1984).

Fagundes-Neto, U., Martins, M.C., Lima, F.L Patricio, F.R, Toledo, M.R.: Asymptomatic environmental enteropathy among slum-dwelling infants. J. Am. Coll. Nutr. 13, 51-60 (1994).

Feleszko, W., Jaworska, J., Rha, R.D., Steinhausen, S., Avagyan, A., Jaudszus, A., Ahrens, B., Groneberg, D.A., Wahn, U., and Hamelmann, E.: Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. Clin. Exp. Allergy 37, 498-505 (2007).

Flint, H.J., Scott, K.P., Duncan, S.H., Louis, P., and Forano, E.: Microbial degradation of complex carbohydrates in the gut. Gut Microbes 3, 289-306 (2012).

Frederick, J.R. and Petri, W.A.: Roles for the galactose-/N-acetylgalactosamine-binding lectin of Entamoeba in parasite virulence and differentiation. Glycobiology 15, 53R–59R (2005).

Gaboriau-Routhiau, V., Rakotobe, S., Lecuyer, E., Mulder, I., Lan, A., Bridonneau, C., Rochet, V., Pisi, A., De Paepe, M., Brandi, G., Eberl, G., Snel, J., Kelly, D., Cerf-Bensussan, N.: The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity 31, 677-689 (2009).

Galván-Moroyoqui, J.M., Del Carmen Domínguez-Robles, M., Franco, E., and Meza, I.: The interplay between Entamoeba and enteropathogenic bacteria modulates epithelial cell damage. PLoS Negl. Trop. Dis. 2, e266 (2008).

Giongo, A., Gano, K.A., Crabb, D.B., Mukherjee, N., Novelo, L.L., Casella, G., Drew, J.C., Ilonen, J., Knip, M., Hyöty, H.,

Veijola, R., Simell, T., Simell, O., Neu, J., Wasserfall, C.H., Schatz, D., Atkinson, M.A., and Triplett, E.W.: Toward defining the autoimmune microbiome for type 1 diabetes. ISME J. 5, 82-91 (2011).

Haque, R., Huston, C.D., Hughes, M., Houpt, E., and Petri, W.A.: Amebiasis. N. Engl. J. Med. 348, 1565-1573 (2003).

Heczko, U., Abe, A., and Finlay, B.B.: Segmented filamentous bacteria prevent colonization of enteropathogenic Escherichia coli O103 in rabbits. J. Infect. Dis. 181, 1027–1033 (2000).

Houpt, E.R., Glembocki, D.J., Obrig, T.G., Moskaluk, C.A., Lockhart, L.A., Wright, R.L., Seaner, R.M., Keepers, T.R., Wilkins, T.D., and Petri, W.A. Jr.: The mouse model of amebic colitis reveals mouse strain susceptibility to infection and exacerbation of disease by CD4+ T cells. J. Immunol. 169, 4496-4503 (2002a).

Houpt, E.R., Glembocki, D.J., Obrig, T.G., Moskaluk, C.A., Lockhart, L.A., Wright, R.L., Seaner, R.M., Keepers, T.R., Wilkins, T.D., and Petri, W.A. Jr.: The mouse model of amebic colitis reveals mouse strain susceptibility to infection and exacerbation of disease by CD4+ T cells. J. Immunol. 169, 4496-4503 (2002b).

Ivanov, I.I., Frutos Rde, L., Manel, N., Yoshinaga, K., Rifkin, D.B., Sartor, R.B., Finlay, B.B., and Littman, D.R.: Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell. Host Microbe 4, 337-349 (2008).

Jiang, H.Q., Thurnheer, M.C., Zuercher, A.W., Boiko, N.V., Bos, N.A., and Cebra, J.J.: Interactions of commensal gut microbes with subsets of B- and T-cells in the murine host. Vaccine 22,805-811 (2004).

Jonsson, H.: Segmented filamentous bacteria in human ileostomy samples after high-fiber intake. FEMS Microbiol. Lett. 342, 24-29 (2013).

Korpe, P.S. and Petri, W.A.: Environmental enteropathy: critical implications of a poorly understood condition. Trends Mol. Med. 18, 328-336 (2012).

Kotloff, K.L., Nataro, J.P., Blackwelder, W.C., Nasrin, D., Farag, T.H., Panchalingam, S., Wu, Y., Sow, S.O., Su,r D., Breiman, R.F., Faruque, A.S., Zaidi, A.K., Saha, D., Alonso, P.L., Tamboura, B., Sanogo, D., Onwuchekwa, U., Manna, B., Ramamurthy, T., Kanungo, S., Ochieng, J.B., Omore, R., Oundo, J.O., Hossain, A., Das, S.K., Ahmed, S., Qureshi, S., Quadri, F., Adegbola, R.A., Antonio, M., Hossain, M.J., Akinsola, A., Mandomando, I., Nhampossa, T., Acácio, S., Biswas, K., O'Reilly, C.E., Mintz, E.D., Berkeley, L.Y., Muhsen, K., Sommerfelt, H., Robins-Browne, R.M., and Levine, M.M.: Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet 382, 209-222 (2013).

Kuwahara, T., Ogura, Y., Oshima, K., Kurokawa, K., Ooka, T., Hirakawa, H., Itoh, T., Ishifune, C., Maekawa, Y., Yasutomo, K., Hattori, M., and Hayashi, T.: The lifestyle of the segmented filamentous bacterium: a non-culturable gut-associated immunostimulating microbe inferred by whole-genome sequencing. DNA Res. 18, 291-303 (2011).

Maslowski, K.M. and Mackay, C.R.: Diet, gut microbiota and immune responses. Nat. Immunol. 12, 5-9 (2010).

Michail, S., M. Durbin, D. Turner, A.M. Griffiths, D.R. Mack, J. Hyams, N. Leleiko, Kenche, H., Stolfi, A., and Wine, E.: Alterations in the gut microbiome of children with severe ulcerative colitis. Inflamm. Bowel Dis. 18, 1799–1808 (2012).

Mirelman, D., Feingold, C., Wexler, A., and Bracha, R.: Interactions between Entamoeba histolytica, bacteria and intestinal cells. Ciba Found. Symp. 99, 2-30 (1983).

Mondal, D., Petri, W.A., Sack, R.B., Kirkpatrick, B.D., and Haque, R.: Entamoeba histolytica-associated diarrheal illness is negatively associated with the

growth of preschool children: evidence from a prospective study. Trans. R. Soc. Trop. Med. Hyg. 100, 1032-1038 (2006).

Mondal, D., Minak, J. Alam, M. Liu, Y. Dai, J. Korpe, P. Liu, L., Haque, R., and Petri, W.A. Jr.: Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. Clin. Infect. Dis. 54, 185-192 (2012).

Noverr, M.C. and Huffnagle, G.B.: Does the microbiota regulate immune responses outside the gut? Trends. Microbiol. 12, 562-568 (2004).

Nutsch, K.M. and Hsieh, C.S.: T cell tolerance and immunity to commensal bacteria. Curr. Opin. Immunol. 24, 385-391 (2012).

Petri, W.A. and Singh, U.: Diagnosis and management of amebiasis. Clin. Infect. Dis. 29, 1117-1125 (1999).

Preidis, G.A., Hill, C., Guerrant, R.L., Ramakrishna, B.S., Tannock, G.W., and Versalovic, J.: Probiotics, enteric and diarrheal diseases, and global health. Gastroenterology 140, 8-14 (2011).

Schwarzer, M., Repa, A., Daniel, C., Schabussova, I., Hrncir, T., Pot, B., Stepankova, R., Hudcovic T, Pollak A, Tlaskalova-Hogenova H, Wiedermann U, Kozakova H. Neonatal colonization of mice with Lactobacillus plantarum producing the aeroallergen Bet v 1 biases towards Th1 and T-regulatory responses upon systemic sensitization. Allergy 66, 368-375 (2011).

Shukla, G., Kaur, T., Sehgal, R., Rishi, P., and Prabha, V.: Protective potential of L. acidophilus in murine giardiasis. CEJ Med. 5, 456-463 (2010).

Siezen, R.J. and Kleerebezem, M.: The human gut microbiome: are we our enterotypes? Microb. Biotechnol. 4, 550-553 (2011).

Smith, M.I., Yatsunenko, T., Manary, M.J., Trehan, I., Mkakosya, R., Cheng, J., Kau, A.L., Rich, S.S., Concannon, P., Mychaleckyj, J.C., Liu, J., Houpt, E., Li, J.V., Holmes, E., Nicholson, J., Knights, D., Ursell, L.K., Knight, R., and Gordon, J.I.: Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science 339, 548-554 (2013).

Stepankova, R., Powrie, F., Kofronova, O., Kozakova, H., Hudcovic, T., Hrncir, T., Uhlig, H., Read S, Rehakova Z, Benada O, Heczko P, Strus M, Bland P, Tlaskalova-Hogenova H. Segmented filamentous bacteria in a defined bacterial cocktail induce intestinal inflammation in SCID mice reconstituted with CD45RBhigh CD4+ T cells. Inflamm. Bowel Dis. 13, 1202-1211 (2007).

Travers, M.A., Florent, I., Kohl, L., and Grellier, P.: Probiotics for the control of parasites: an overview. J. Parasitol. Res. 2011, 610769 (2011).

Umesaki, Y. and Setoyama, H.: Structure of the intestinal flora responsible for development of the gut immune system in a rodent model. Microbes Infect. 2, 1343-1351 (2000).

Verkerke, H.P., Petri, W.A., and Marie, C.S.: The dynamic interdependence of amebiasis, innate immunity, and undernutrition. Semin. Immunopathol. 34, 771-785 (2012).

Verma, A.K., Verma, R., Ahuja, V., and Paul, J.: Real-time analysis of gut flora in Entamoeba histolytica infected patients of Northern India. BMC Microbiol. 12, 183 (2012).

Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.Y., Keilbaugh, S.A., Bewtra, M., Knights, D., Walters, W.A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F.D., and Lewis, J.D.: Linking long-term dietary patterns with gut microbial enterotypes. Science 334, 105-108 (2011).

Yin, Y., Wang, Y., Zhu, L., Liu, W., Liao, N., Jiang, M., Zhu, B., Yu, H.D., Xiang, C., and Wang, X: Comparative analysis of the distribution of segmented filamentous bacteria in humans, mice and chickens. ISME J. 7, 615-621 (2012).