Intestinal Ecosystem: Interaction and Coexistence Between “Parasitome” and Microbial Communities

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Abstract

The vertebrate gut has been continuously populated with complex and dynamic microbial and eukaryotic communities, that over millions of years have coevolved both spatially and temporally (Kreisinger *et al.*, 2015). Due to the prolonged coexistence, intestinal parasites (protozoa and helminths) and resident microbiota have developed the ability to influence one another by several mechanisms: 1) produce changes at the level of intestinal mucus and epithelial barrier; 2) alter the host immune response or 3) direct interaction (Leung *et al.*, 2018). The uncontrolled use of anthelmintics can lead to the elimination of commensal organisms and alteration of host immunity and intestinal microbial community composition. Thus, the aim of this research is to highlight the complexity of interactions between intestinal bacteria and parasites and their importance for the host. The “parasitome”- microbiota relationship is a complex phenomenon that plays an essential role in host intestinal homeostasis, the absence or alteration of either of these organisms being able to cause a severe disruption of host immune system (Leung *et al.*, 2018). Is therefore essential to acquire a deeper understanding of the molecular mechanisms of interaction between these two communities.

Keywords: helminths, interactions, microbiota, parasitome, anthelmintics

Introduction

The gastrointestinal tract of animals serves as the home for a complex and dynamic community of bacteria, viruses, fungi, and protozoans. Outnumbering the host cells, bacterial cells alongside fungi and viruses form a unique microbial “fingerprint”, which starts to develop soon after birth and reaches stability a few years after that, as a result of constant interaction between the individual and the environment (Gilbert, 2015). Thanks to the development of new “omic” and “meta-omic” diagnostic techniques, the past decades have seen increasingly rapid advances in the understanding of the importance of microbiota for both human and animal health. In addition

to its known role in digestion and absorption of nutrients, the gut microbiota plays an important role in the “gut-brain axis”, disruptions in the microbial community being linked with diseases like inflammatory bowel disease, autism, allergies and obesity (Fattorusso *et al.*, 2019).

Gut is also home for other inhabitants – helminths. During millions of years, they cohabitated and coevolved with intestinal microbial community, developing the ability to modulate each other both directly and indirectly, forming a complex ecosystem (Berrilli *et al.*, 2012). Studies have shown that people infected by helminths had an increased diversity of intestinal microbiota, compared to uninfected individuals

(Lee *et al.*, 2014). In the macaques with colitis and following dysbiosis, helminth infection helped to restore the normal gut microbial community composition (Broadhurst *et al.*, 2012). At the same time, helminths continuously challenged the mammalian immune system, playing an important role in interleukin (IL) genes selection (Fumagalli *et al.*, 2009). However, humans associated them with a disease state and financial losses in the livestock industry, and continuously tried to eradicate gut parasites by improving hygiene and excessively using anthelmintic drugs. Loss of chronic helminth infection has led to an increased incidence of autoimmune diseases and the rapid onset of inflammatory responses (Elliott and Weinstock, 2012).

Nowadays the research community seems to be divided into ones that believe the exposure to the helminths is crucial for the host immune system development and the others that believe the helminth infections cause an alteration in the gut microbiota and as a result in the gut-brain axis (Guernier *et al.*, 2017). The development of metabolomics will allow the scientists to decode the functional activity of the gut community and understand the changes that helminths cause to the composition of gut microbiota and how the bacteria, in turn, influence the helminth virulence and survival rate. The aim of this research is to highlight the complexity of interactions between intestinal bacteria and parasites and their importance for the host. This can lead to the discovery of 1) new biomarkers of helminth infection, making the diagnostic process faster and easier; 2) new methods of treatment of helminth parasites or even the 3) safe way of using helminths in the treatment of diseases.

Interplay between gut “parasitome” and microbiota

Modifications of mucus and epithelial barrier

The epithelium of intestinal mucosa is the innermost barrier that contains seven different types of cells, from them Paneth cells, goblet cells, tuft cells and microfold (M) cells being an important part of nonspecific innate immunity (Gerbe *et al.*, 2012). At the level of colon, mucus secreted by goblet cells is organized into two layers, outer mucus layer serving as the home for

resident microbiota, at the same time preventing bacteria to reach the epithelium (Sharpe *et al.*, 2018).

Upon their arrival into the intestinal lumen, helminths trigger the activation of Th2 cells and cause goblet cells hyperplasia. This leads to excessive secretion of mucin and alterations of mucus barrier, therefore parasites are not able to interact with intestinal epithelium and can be easily eliminated by the so-called “mucus – trap”. In chronic cases, despite the increased mucus production, parasites manage to increase the porosity of mucus membrane and attach to the epithelium (Sharpe *et al.*, 2018). The excessive production of mucus can also positively impact the GI barrier, by not allowing the bacteria to pass the inner layer of mucus and associate with intestinal epithelium, therefore restoring the mucus layer integrity. During the infection with *Trichuris suis* in pigs, the increased quantity and viscosity of mucus creates a favourable medium for the bacteria that prefer mucosal surfaces and which use mucin as a source of carbon, such as *Mucispirillum* (Li *et al.*, 2012).

At the same time, interleukin (IL)-4 and IL-13 stimulate goblet cells to release a nonmucin protein, called resistin-like molecule (RELM) beta, which depending on the parasite either reduce its ability to feed or affects their motility, causing their expulsion (Herbert *et al.*, 2009). RELM- β together with intestinal trefoil factors (TFF) also stabilizes the mucin polymer and regulates the mucus barrier integrity, stimulating epithelial repair (Sharma *et al.*, 2010). Quick repair of epithelial barrier increases the survival chances of the bacteria with faster multiplication rate (Leung *et al.*, 2018).

Th2-stimulating gastrointestinal nematodes can affect the epithelial barrier integrity through several mechanisms. For example, *Heligmosomoides polygyrus* and *Trichinella spiralis* induce the lymphocyte and STAT6 (signal transducer and activator of transcription 6)-dependent alteration of the intestinal epithelium. *Nippostrongylus brasiliensis* affects the expression of E-cadherin, which leads to a loss of cellular adherence. Because of increased epithelial permeability, bacteria are able to move into deeper layers of the intestinal wall (Fernández-Blanco *et al.*, 2015). However, some helminth species are known to facilitate the mucosal healing by increasing the production of IL-

22, and therefore not allowing the translocation of bacteria into the epithelium and bloodstream (Broadhurst *et al.*, 2010).

Short-chain fatty acids (SCFAs), mainly butyrate, produced by intestinal bacteria that is responsible for fiber fermentation, have immunomodulatory effects. The main butyrate producing-bacteria belong to the phylum Firmicutes (Parada Venegas *et al.*, 2019). Butyrate is proven to inhibit TNF- α , IL-6 and IL-12, which can lead to a slower helminth expulsion from GI tract (Ierna *et al.*, 2009). In addition, butyrate is used as a main source of energy by colonocytes and increases epithelial cell proliferation. Constant regeneration of intestinal cells can impede helminth attachment to the epithelium and therefore leads to parasite expulsion (Leung *et al.*, 2018).

Modulation of the host immune system

The presence of helminths in the GI tract induces Th2-type immune response, which is characterised by the production of variable amounts of IL-4, IL-5, IL-9, IL-10, IL-13, IL-25 and IL-33 (Allen *et al.*, 2014; Cortés *et al.*, 2017; Gazzinelli-Guimaraes and Nutman, 2018).

IL-25, produced by intestinal tuft cells, together with IL-33 and thymic stromal lymphopoietin (TSLP) are alarmins that have a critical role in the activation of group 2 innate lymphoid cells (ILC2) and as a result – induction of Th2-type immunity (Cortés *et al.*, 2017). ILC2 produce IL-13, that cause hyperplasia of tuft cells and goblet cells, increasing the production of IL-25, secretion of mucus and smooth muscle contraction (Camelo *et al.*, 2017).

IL-5 is a chemoattractant for eosinophils and is responsible for eosinophilopoiesis. IL-4 and IL-13, produced by both eosinophils and Th2-cells, play an important role in the activation of macrophages, which collaborate together with neutrophils to kill helminths (Allen *et al.*, 2014).

IL-10, is an anti-inflammatory cytokine produced during helminth and bacterial infection, that plays an important role in the suppression of the immune response and as a result to the expansion of natural and induced Treg cells (Gazzinelli-Guimaraes and Nutman, 2018).

While Th2-type immunity is responsible for the alteration of the mucus barrier and the inhibition of Th1 response, leading to excessive

bacterial multiplication, Treg cell-mediated immune tolerance is responsible for the chronicity of helminth and bacterial infection, decreasing the damage produced by Th2 response (King and Li, 2018).

Direct interactions

Throughout the years of their cohabitation, intestinal helminths and gut microbiota have developed several mechanisms to influence one another in order to coexist peacefully within the host intestine. It is now proven that *Escherichia coli*, which is found in the composition of gut microbiota, helps *Trichuris muris* embryonated eggs to hatch. The mechanism of action is still unclear, studies have shown that for hatching eggs, a temperature of 37 °C is required and direct contact with an intact surface of bacteria, that gathers around the opercula at the pores of the egg and induces hatching. Once reaching the adult stage, *Trichuris muris* controls the composition of intestinal microbiota, creating an environment less favorable for hatching (Hayes *et al.*, 2010). At the same time adult forms of *Trichuris muris* have their own endosymbiotic bacteria, which are essential for their health, shortly after *in vitro* treatment with antibiotics, the adult form dies (White *et al.*, 2018). *E. coli* is also known for its properties to catabolize and inactivate benzimidazoles, using them as a source of carbon, therefore decreasing the efficacy of anthelmintic treatment (Whittaker *et al.*, 2016).

Another mechanism involved in direct regulation of gut microbiota composition is secretion of antibacterial and proteolytic substances. *Heligmosomoides polygyrus* is able to detect the composition of the surrounding bacteria and inhibit the excessive growth of both Gram negative and Gram positive bacteria by producing C-type lectin protein that can agglutinate bacteria (Rausch *et al.*, 2018). *Ascaris suum* impairs biofilm formation by production of lysozyme, c-type lectin domain-containing (CTLDD) proteins, cystatin, cecropins, lysozyme, beta-galactoside-binding lectin and others (Midha *et al.*, 2018).

A study involving newborn rats have demonstrated that helminth infection with *Hymenolepis diminuta* in pregnant females combined with post-weaning helminth infection diminished the brain cytokine response and alteration of the gut microbial composition produced as a result of ne-

onatal *E. coli* infection, therefore preventing neurodevelopmental and cognitive disorders in rats (Williamson *et al.*, 2015).

Helminths also secrete the so-called helminth defense molecules (HDM), they are peptides which possess antimicrobial activity and at the same time act as immunomodulators (Robinson *et al.*, 2013). Positively charged HDMs attracted by the negatively charged surface of bacteria produce disruption of the bacterial membrane and it results in direct lysis of the cell. HDMs does not affect the host cells, because the presence of cholesterol in eukaryotic membranes leaves the outer surface of the membrane with little or no electric charge (Cotton *et al.*, 2012).

During its migration from the intestinal lumen towards the bile ducts and liver, *Fasciola hepatica* creates an opening into the gut wall, which causes bacteria to pass into the blood stream. In order to protect the host from an excessive inflammatory response, the parasite secretes an immune modulatory peptide FhHDM-1 that attaches to *Escherichia coli* lipopolysaccharide (LPS) and does not allow the bacterial molecules to bind to the macrophage receptors, and therefore prevents septicemia (Robinson *et al.*, 2013).

Biome depletion theory

Around 200 years ago humans started the so-called “hygiene revolution”, trying by all the means possible, to reduce theirs and farm animals’ contact with helminths and bacteria, despite hundred millions of years of coevolution. This excessive cleanliness has led to a phenomenon known as “biome depletion”. In addition to the industrialization, the uncontrolled use of anthelmintics and antibiotics also contributed to the elimination of commensal organisms and alteration of intestinal microbial community composition.

As previously described, helminth – microbiota relationship plays an essential role in host intestinal homeostasis, the absence or alteration of either of these organisms being able to cause a severe disruption of host immune system (Leung *et al.*, 2018). This has resulted in an increased frequency of allergic and autoimmune diseases (Parker and Ollerton, 2013)

Nowadays, the aim of the scientific community is to find safe and cost-effective strategies of intestinal biome reconstruction and maintenance,

which is believed to be the key to a healthy and well-functioning individual (Gagliardi *et al.*, 2018).

Conclusion

Concurrent with the new diagnostic techniques comes a deeper understanding of the molecular mechanisms of interaction between microbiota and helminths. This raised a problem previously neglected – the lack of macrofauna in the animal (or human) gut. Although there is still not enough information regarding the mechanisms by which helminths regulate the composition of the bacterial community nor the host immune system, it is obvious that they are an indispensable part of gut ecosystem.

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