Conceptions in parasite-microbiota relationships

Review Article

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ABSTRACT

Abundant information refers to the role of microbiota in maintaining homeostasis by assisting, improving, and regulating immunity in humans. Yet, the effect exerted by parasitic infections on the developing microbiota, is so far not recognized. Various parasites were related to specific alterations in the essential loads of developing microbiota. Additionally, modulation of immune functions was reported. This review aimed to present examples for the interaction between parasitic infections and microbial gut imbalance dubbed dysbiosis. This form of parasite-microbiota interaction seems to influence both the metabolism and the acquired immunity of the host. Consideration of this data will direct future studies to focus on the altered microbiota during various parasitic infections and to highlight the benefits of its re-enrichment in the therapeutic trials.

Keywords: dysbiosis; flora; luminal parasites; microbiota; probiotics

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INTRODUCTION

Parasites are organisms that depend on their hosts, and typically in a parasitic relationship, one organism flourishes and proliferates at the expense of the other. Research on the normal microbiome communities during parasitic infections showed that the definition of 'intestinal parasitosis' requires re-assessment. It was postulated that intestinal dysbiosis as a state of disease induced by a parasite, is distinctive from the term intestinal parasitosis[1,2].

The word 'microbiota' is composed of two syllables; 'micro' that refers to the invisible size, and 'biota' which is the bacterial ecosystem that inhabits a specific site in the host. This microbiota plays a crucial role in early life to establish the immune system in health and disease[3-5]. During early life, a stable gut microbiome is completed by the age of 31 months[6,7]; and its balance continues to be crucial for the long-term well-being and health[8,9]. Elwakil[1] suggested that intimate factors and pathways governing the relationships between host, microbiota, and parasites are multifaceted. Although in several cases, the relation is not fully defined, a recent review has supported the role of microbiota in remedying the host immature immune system[10].

Dysbiosis due to bacterial destabilization has been associated with several diseases. For instance, recent studies revealed alterations in intestinal bowel diseases[11,12]. Additionally, a recent study conducted by Ramírez-Carrillo *et al*.[13] related the incidence of depression to the disturbance in the networks of the gut microbiota by *Ascaris lumbricoides*. However, modulation of this microbiota through consumption of live bacteria (probiotics), or non-digestible elements has been speculated in the inhibition or even treatment of some conditions[14]. The current review aimed to characterize some figures of alterations in microbiota associated with various parasitic infections.

[I] Intracellular parasites

(1) ***Toxoplasma gondii*** is an intracellular sporozoa that can cause infection in any nucleated cell. Toxoplasmosis has been shown to induce massive necrosis and extensive pathology in the distal intestinal mucosa and villi in murine models. These pathological changes were attributed to bacterial dysbiosis besides immune factors such as the CD-4 T cells and interferon gamma (IFN-γ) cytokine[15]. Dysbiosis had been proposed to be dependent on the destabilization of the intestinal barrier either by the lytic replication of the parasite or from the toxic by-products of the host immune reactions, such as nitrogen intermediates or reactive oxygen species. For instance, production of IFN-γ during toxoplasmosis leads to loss of Paneth cells resulting in reduced levels of the antimicrobial peptides and dysbiosis[16,17] dominated chiefly by *Enterobacteriaceae*[14]. In fact, the induced IFN-γ/signal transducer and activator of transcription-1/inducible nitric oxide synthase (IFN-γ/STAT-1/i-NOS) axis, was described as crucial for parasite resistance[18]. It was assumed to provide a pool of nitrate that acts as a source for anaerobic respiration and promotes overgrowth of *Enterobacteriaceae*.

Similarly, in experimentally induced colitis using dextran sulphate sodium (DSS), host-derived nitrate was found to directly promote the flush of *Enterobacteriaceae* bacteria in the colon[19]. This proves that local inflammation in the large intestine causes upgrade of the respiratory electron acceptors

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levels like N oxides and S oxides, that act as a substrate for the expansion of facultative anaerobic bacteria[20]. Hatter *et al*.[15] noted an ominous outgrowth of *Clostridia* spp. that was sustained in chronic infection with toxoplasmosis. Also, Egan *et al*.[21] suggested that the depletion of gut flora reduces mice resistance to *T. gondii*-triggered ileitis. Likewise, Couturier-Maillard *et al*.[22] suggested that the collaboration between intestinal commensals and *T. gondii* in the development of small intestine inflammation increases the capability of the parasite to approach the epithelial cells and promotes its invasion to the mucosa.

(2) *Cryptosporidium parvum* is another intracellular intestinal sporozoa that has been hypothesized to trigger dysbiosis indirectly by causing damage to the intestinal epithelium[23]. Fecal microbiota from mice infected with *C. parvum* were shown to differ significantly from that of non-infected controls. Amazingly, a recent study demonstrated that probiotics can significantly influence the intestinal microenvironment and the epithelial lining which promotes the proliferation of *C. parvum*[24].

(3) Leishmaniasis is caused by intracellular flagellates that were shown to trigger skin dysbiosis in humans and murine models. Cutaneous leishmaniasis is characterized by augmented *Staphylococcus* and/or *Streptococcus* infections and skin inflammation. Interestingly, in murine models this dysbiosis was found to be transferable to naive mice[25]. Similarly, visceral leishmaniasis (VL) patients are more susceptible to fatal nosocomial secondary bacterial infections (*Pseudomonas aeruginosa* and *Staphylococcus aureus*)[26]. This was attributed to the suppressed status of T-helper 1 due to alterations in antigen presentation, MHC/HLA, antigen processing, T cell receptor recognition, and the myeloid derived suppressor cells[27-29]. However, establishment of VL infection in murine models with previously induced intestinal dysbiosis showed late onset and development of weight loss[30].

[II] Luminal (intestinal and genitourinary) parasites

(1) *Giardia duodenalis* (*G. lamblia*) is an intestinal flagellate that inspires dysbiosis by overwhelming the physical mucus and epithelial barrier by several mechanisms. The trophozoite stage of the parasite is extremely motile *via* its flagella[31] and possesses proteolytic activity that disrupts the integrity of mucin-2 (MUC2) to yield a less viscous physical barrier[32-34]. In addition, it produces secretory/excretory cysteine proteases (CP)[35] (CP2, CP3, and CP16160[32,36]) that cause apoptosis of the epithelial lining of the intestine and disruption of the tight junctions[37]. Giardipain-1 is another virulent factor with proteolytic activity similar to cathepsin B-like protein. It induces the formation of pore-like defects and membrane blebs, reduces the trans-epithelial electrical resistance, and targets mainly the tight junction proteins (occludin and claudin-1). In addition, it stimulates the activation of caspase-3, the fragmentation of poly ADP ribose polymerase (PARP), and the exposure of phosphatidylserine causing apoptosis of the epithelial cells[38]. Consequently, the mucosal microbiota adjacent to the epithelial cells can penetrate the thin lining of the host-derived glycans, the cell surface glycocalyx and the extracellular secreted mucus[39]. The pathogenic dysbiotic microbiota associated with giardiasis in germ free mice, induces the signalling pathway of the toll-like receptor-4, and the production of the pro-inflammatory cytokine IL-1β[37].

Gerbaba *et al*.[40] showed that *G. lamblia* induces functional alterations in the commensal microbiota, possibly transforming them into opportunistic pathogens, and altering the host-microbe homeostatic interactions. Interestingly, Allain *et al*.[32] demonstrated that the high proteolytic activity by *G. lamblia* is protective to the host against concurrent bacterial entero-pathogens by promoting bacterial killing and alle*via*ting inflammation of the intestine. Bartelt *et al*.[41] assumed that in association with protein malnutrition the intestinal microbiota promoted persistent colonization by *G. lamblia* that led to growth impairment in experimentally infected mice.

(2) *Blastocystis* spp. are the most common eukaryotic intestinal protozoa in human[42]. As an anaerobe, it lacks several definitive mitochondrial features and misses the standard mitochondrial electron transport chain and oxidative phosphorylation[43]. Notably in a healthy gut, oxygen concentration is extremely low[44] to promote the growth of obligate anaerobic microbiota from the Bacteroides and Firmicutes phyla[45]. In a dysbiotic gut, when the intestinal microbiota get disrupted and luminal bioavailability of oxygen increases, the resulting alteration in intestinal biodiversity promotes the growth of the facultative anaerobic *Enterobacteriaceae*[12,46,47]. A strict anaerobe such as *Blastocystis* spp. cannot sustain itself in this medium that is not its ideal ecosystem. In this context, in a well-established irritable bowel syndrome (IBS), dysbiosis *Blastocystis* spp. may be compelled out of the gut since the early stages of the disease[48].

On the other hand, Tsaousis *et al*.[49] suggested that *Blastocystis* spp. has an alternative oxidase nature that allows it to deal with fluctuating oxygen concentrations in the gut and should be better termed as a microaerophilic. *Blastocystis* infection in rats involved colon hypersensitivity and showed alterations in the composition of the microbiota and hence their metabolic shifts[50].

Conversely, two studies[51,52] reported the presence of healthy microbiota rather than dysbiosis in patients infected with *Blastocystis* spp. Moreover, Scanlan *et al*.[53] considered *Blastocystis* spp. a member of the

healthy microbiota. However, in a previous study the genotype of the parasite appeared to play a hidden role in the capability of the parasite in contributing to disease[54]. In this context, the relationship between different genotypes of the parasite and dysbiosis seems to be a new point of research.

(3) ***Entamoeba histolytica*** was previously shown to modify the tight junction proteins and disturb the permeability of the epithelial barrier, allowing translocation of intestinal microbiota into the mucosal surfaces and spread to the other organs[55,56]. Iyer *et al*.[57] reported that *E. histolytica* favours the phagocytosis of bacterial species such as *Lactobacillus ruminus*. Similarly, Verma *et al*.[58] revealed that *E. histolytica* associated dysbiosis was characterized by increased *Bifidobacterium* spp. in faecal samples, while other bacteria that are essential for the maintenance of intestinal homeostasis such as *Bacteroides, Clostridia, Campylobacter, Lactobacillus*, and *Eubacterium* were reduced as compared to healthy subjects. Interestingly, Sicard *et al*.[59] demonstrated that colonic microbiota break down complex carbohydrates into glycans that can act as a nutrient source for *E. histolytica*. With the absence of bacteria, *E. histolytica* is forced to be in close contact with the epithelium to feed and search for alternative sources of energy in the lumen[60]. Moreover, in germ free mice the MUC2 layer was found to be thinner and penetrable[59] with diminutive O-glycan monomers[52].

Additionally, it was postulated that the ratio of bacterial phyla present in the host microbiota may alter or influence the colonization of *E. histolytica*. For instance, *Escherichia coli* was found to protect *E. histolytica* against oxidative stress in two patterns[61] described in figure (1). However, in murine models *Clostridium* is defensive during *E. histolytica* infection through the increased production of interleukin 17-A, dendritic cells, and neutrophils. Moreover, bone marrow-derived dendritic cells were reported to secrete elevated levels of IL-23[62]. Gilchrist *et al*.[63] showed that the expansion of *Prevotella copri* probiotic was associated with dysentery due to intestinal amoebiasis in infants from 600 to 800 days of age. Sarjapuram *et al*.[64] revealed that probiotics (specifically *Lactobacillus casei*) have an anti-proliferative effect on *E. histolytica*. Varet *et al*.[61] speculated this effect to be unrelated to alterations in the pH value of the media.

(4) ***Trichuris trichiura*** is a round worm reported in a Malaysian study to be associated with shifts in the composition of the gut microbiota of infected patients especially with *Paraprevotellaceae* bacteria concerned with the breakdown of proteins and carbohydrates in food[65]. In general, helminthic infections may promote regulatory immune cell reactions that may suppress harmful allergic and inflammatory diseases. They are supposed to elicit regulatory T cells by altering the gut microbiota or *via* secretion of bioactive molecules. Hence, worms and their secretory molecules may be innovative treatments for allergic inflammation[1,66].

(5) ***Trichomonas vaginalis*** is a flagellate that causes the most common sexually transmitted disease (STD) during the child bearing period in female patients[67]. In fact, trichomoniasis was shown to pave the way for bacterial dysbiosis owing to multiple virulence factors. This protozoon employs its cytopathic effect through the release of pore-forming proteins active at pH 5.8[68]. Garber *et al*.[69] isolated a specific soluble heat and acid labile cell-detaching factor from cultured *T. vaginalis* that possesses trypsin like action and can disrupt the monolayer cells at optimum pH 6.5. In another experimentally infected murine model, β-hemolysin was reported to be another virulence factor[70] that destructs the carbohydrate cell monolayers and was found to produce subcutaneous abscesses[71]. Invasion of the mucous layer entails efficient motility by the parasites and the production of adhesins and mucinases[72]. Additionally, to thrive the antibody response, it produces several cysteine proteinases that degrade IgG, IgM, and IgA[73].

Increased *T. vaginalis* colonization was associated with the bacterial *Gardenella vaginalis* species that cause bacterial vaginosis at the expense of the commensal *Lactobacillus* spp.[74]. This was attributed to the efficient

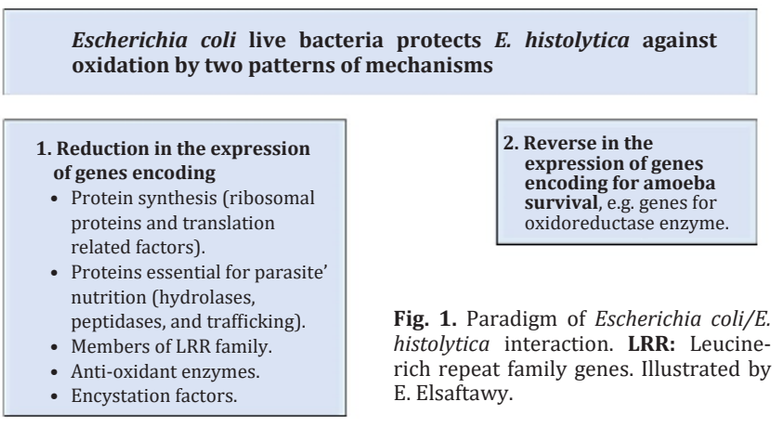

Fig. 1. Paradigm of *Escherichia coli/E. histolytica* interaction. **LRR:** Leucine-rich repeat family genes. Illustrated by E. Elsaftawy.

phagocytic action of the parasite by killing and ingesting lactobacilli[75]. Diminution in lactobacilli populations upgrades the vaginal pH that is normally fluctuating between 2.8 and 4.2[76] creating a milieu more promising for the proliferation of dysbiotic bacteria. Subsequently, the biofilm of the dysbiotic bacteria was found to provide adhesion for *T. vaginalis*[78]. Also, dysbiotic bacteria refine the cytoadhesion of the parasite to the vaginal cells and mucins. *Gardnerella vaginalis* is the most associating dysbiotic bacterium; however other genera, such as *Atopobium vaginae, Prevotella bivia, Bacteroides*, and *Mycoplasma hominis* can also flourish[67,73,78,79].

[III] Blood flukes (*Schistosoma* spp.)

S. haematobium is a urinary blood fluke (trematode) that inhabits the vesical plexus around the bladder. It has been related to a shift in the structure of the faecal microbiome community[80-82], in the form of reduced phylum Firmicutes bacteria and prevalence of phylum Proteobacteria bacteria. A number of alterations were also detected including reductions in Clostridiales family bacteria and surges in *Moraxellaceae, Veillonellaceae, Pasteurellaceae*, and *Desulfovibrionaceae*[81]. Enhancement of urease enzyme has been related to dysbiosis and inflammation. Owing to the habitat of the parasite in the vasculatures around the urinary bladder the indirect link between systemic immunity and microbiota can be suggested as a point of research[81].

S. mansoni is another blood fluke that inhabits the inferior mesenteric plexus of veins draining the colon. Using murine models, a partial relationship was proposed between the parasitic infection and changes in the configuration of the host microbiome[83]. Variances in the gut microbial paradigm involved enrichment in Proteobacteria phylum and the deficiency of the immunoregulatory bacteria (e.g. *Lactobacillus*). This disruption in the gut microbiota was directly associated with higher worm and egg burden, together with amplified immune responses to *Schistosoma* antigens[84]. Interestingly, higher abundance of *Fusobacterium* spp. was found to be accompanied with increased efficacy of praziquantel[85]. On the other hand, Jenkins *et al*.[83] and Schneeberger *et al*.[85] proposed that neither *S. mansoni* infection nor praziquantel administration promotes a significant alteration in the composition of gut microbiota.

Another interesting finding was published by Floudas *et al*.[86] who reported less susceptibility to induced colitis in mice infected with adult male *S. mansoni* as compared to infection with adult male and female worms. This was assumed to be related to the capability of the male worms to regulate the host's immune reactions and overcomes colitis through restricting gut dysbiosis.

[IV] Skin parasites

Sarcoptes scabiei is a mite that causes skin infestation and is thought to promote bacterial infections by breaching the skin barrier. It incapacitates the expression of many adhesion molecules of the epidermal cells, the endothelial cells and the dermal fibroblasts[87-89]. This is achieved by secretion of protease enzymes to facilitate tissue invasion and migration[90]. It evades immunity through the excretion of uncharacterized immune modulatory molecules[91] and complement inhibitors that are supposed to assist the propagation of *Streptococcus pyogenes*[92]. Moreover, it induces itching due to allergy and inflammatory responses against the arthropod and its secretory/excretory metabolic by-products that pave the way for bacterial infections. Abreu-Velez *et al*.[93] assumed that itching sensation may be aggravated as an auto-reactivity to sweat glands and nerves in close proximity to the sites of infestation.

Disruption of the skin barrier facilitates the invasion of opportunistic pathogens and permits secondary bacterial infection, most frequently by *Staphylococcus aureus* and S. *pyogenes* that belongs to group A *Streptococci* (GAS). These bacteria have been isolated from skin tunnels and faecal by products of the mite suggesting that the arthropod could contribute directly to the dissemination of the bacteria and increase risk of impetigo[94,95]. Moreover, Swe *et al*.[96] using a porcine model, assumed that scabies mites shift the growth of the skin microbiota from the commensal *Staphylococcus hominis* to *Staphylococcus chromogenes*. Further sequel of skin dysbiota is the real possibility of renal glomerular damage due to streptococcal infection that may occur many years afterwards[97] (Figure 2). Another study showed that control of scabies with ivermectin is also related to significant lower isolations of streptococci from skin lesions and haematuria[98].

CONCLUDING REMARKS

1. It is currently accepted that the gut microbiota interrelate with human health and that its disruption by a parasite has a hidden effect in the progression of the parasitic disease.
2. The real effect of dysbiosis in many parasitic diseases is still not clear and necessitates further research.
3. The current recommendations for probiotic therapies in parasitic diseases are still limited and requires further large cohorts' studies to determine the right probiotic strain for a given parasitic strain.
4. Parasites alter microbiota through different mechanism, as proposed in the following parasites:
   - ***T. gondii:*** Destabilization of the intestinal barrier, and increased production of nitrates.
   - ***Cryptosporidium* spp:** Damage to the intestinal epithelium.
   - ***Leishmania* spp.:** Alterations in antigen presentation, MHC/HLA, and antigen processing, disruption of the T cell receptor recognition, as well as modification in the myeloid derived suppressor cells.
   - ***G. lamblia:*** Disruption of the integrity of MUC2, reduction of the viscous physical barrier, and degradation of tight junction proteins and apoptosis

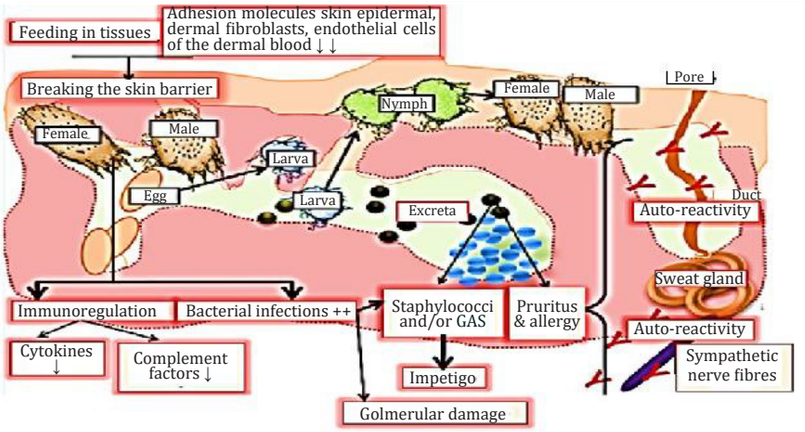

Fig. 2. Dysbiosis in scabies infection. **GAS:** Group A streptococci. Illustrated by E. Elsaftawy.

of the intestinal epithelial cells utilizing secretory-excretory cysteine proteases.

- ***Blastocystis* spp.:** Colon hypersensitivity, alterations of microbiota composition, and metabolic shifts of the intestinal microbiota
- ***E. histolytica:*** Several mechanisms were proposed: modification of goblet cell, reduction of mucus assembly (germ free murine models), modification of tight junction proteins, distortion of epithelial barrier permeability, translocation of intestinal mirobiota into mucosal surfaces, dissemination of microbiota to other organs, gene regulation and altered expression of the leucine-rich repeat (LRR) family genes (documented with enteric bacteria but not with probiotics), phagocytosis of beneficial bacteria, and increased *Bifidobacterium* species .
- ***T. trichura:*** Promotion of regulatory immune cell reactions, and secretion of bioactive molecules.
- ***T. vaginalis:*** Interaction with urogenital epithelium, B-hemolytic and mucinase activities, increasing pH for optimal activity of cell-detaching factor and pore-forming proteins, degradation of IgG, IgM, IgA, and phagocytosis of beneficial bacteria.
- ***Schistosoma* spp.:** Enhancement of urease enzyme, immunomodulation, and alterations of microbiota composition.
- ***S. scabiei:*** Breach skin barrier was proposed through skin scratching (auto reactivity), and protease enzymes. Besides, immunomodulation and inhibition of the adhesion molecules were reported.

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