Pathogenic, Commensal, or Beneficial Role of Intestinal Protozoa

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The human gastrointestinal microbiota contains a diverse consortium of microbes, including bacteria, protozoa, viruses, and fungi. Through millennia of co-evolution, the host–microbiota interactions have shaped the immune system to both tolerate and maintain the symbiotic relationship with commensal microbiota, while exerting protective responses against invading pathogens. Microbiome research is dominated by studies describing the impact of prokaryotic bacteria on gut immunity with a limited understanding of their relationship with other integral microbiota constituents. However, converging evidence shows that eukaryotic organisms, such as commensal protozoa, can play an important role in modulating intestinal immune responses as well as influencing the overall health of the host. The presence of several protozoa species has recently been shown to be a common occurrence in healthy populations worldwide, suggesting that many of these are commensals rather than invading pathogens.

bacteria Blastocystis Dientamoeba Entamoeba gut immunity intestinal protozoa microbiota

1. Introduction

The mammalian gut harbors a vast number of viruses, bacteria, fungi, and protozoa (single-celled eukaryotes), collectively referred to as the microbiota [1]. Dynamic interplay between these distinct microbiota constituents and the host contributes to many essential physiological processes, including development, metabolism, and immunity [2][3]. It is becoming increasingly evident that disruption of this complex ecosystem, referred to as gut dysbiosis, contributes to the development of various gastrointestinal as well as systemic diseases, such as inflammatory bowel disease (IBD), metabolic disorder, autoimmunity, and cancer [4][5][6]. Furthermore, emerging evidence of a bidirectional crosstalk between the intestinal microbiota and the brain has linked dysbiosis to various diseases of the central nervous system, such as depression, multiple sclerosis, and Parkinson's disease [7][8]. It is well recognized that the intestinal microbiota plays a pivotal role in the manifestations of IBD [9]. IBD is a chronic inflammatory disease of the intestinal lining that can be classified into two distinct conditions, Crohn's disease or ulcerative colitis [10]. Although usually not fatal, IBD is associated with lowered life expectancy and significantly decreased quality of life for patients that suffer from chronic symptoms, including persistent diarrhea, abdominal pain, and rectal bleeding. Moreover, chronic inflammation in IBD has been associated with serious, often fatal comorbidities, including cancer, cardiovascular diseases, and liver diseases [11][12]. The rate of incidence of IBD has been rising dramatically since the industrial revolution, with a current global burden of more than 6 million people [13]. Although the IBD etiology remains largely unknown, it has been hypothesized that the industrial

lifestyle, which includes increased use of antibiotics and a diet rich in highly processed foods, has resulted in detrimental changes to the intestinal microbiota that significantly contribute to disease risk [9][14]. Therefore, a detailed understanding of the biological roles of distinct intestinal microbial groups, their mutual interactions, as well as their impact on human disease is of paramount importance.

Since most studies have concentrated on gut-resident bacteria as the main component of the microbiota, the mechanisms and consequences of intestinal protozoa colonization have only recently begun to be clarified [3][15][16][17]. Protozoa are a diverse group of single-celled eukaryotic organisms that can be found in a variety of environments either as free-living or parasitic/symbiotic microbes. Historically, protozoa have been classified into four subgroups: amoebas, flagellates, coccidians, and ciliates, and their categorization depends on specific morphological features, such as internal structure and motility [18]. After the emergence of molecular phylogenetics, an updated classification has been proposed, integrating insights from the genomic studies with the structural and biochemical evidence. Thus, protozoa have been proposed to comprise two subkingdoms, with Choanozoa and Amoebozoa grouped together as the subkingdom Sarcomastigota, while Alveolata, Rhizaria, Excavata, and Apusozoa constitute the subkingdom Biciliata [19]. More recently, an even more updated classification has been proposed [20]. From an evolutionary point of view, eukaryotic microbes such as protozoa have co-evolved with humans and undoubtedly affected the dynamics of the gut microbiota [21]. Despite extensive strides in parasitic research, including studies of pathogenic protozoa, the role of commensal protozoa in shaping the immune landscape of the gut remains enigmatic and questioned [22][23][24]. One of the main challenges lies in the characteristic features and biological classification of commensal protozoa [25]. By definition, commensal microbes reside within the host without causing a negative health impact and are well tolerated by the immune system [26]. However, due to the highly dynamic nature of the host–microbiota interactions, a particular protozoa can be classified as commensal rather than parasitic, and vice-versa, often in a context-specific manner [21][27][28]. Furthermore, heterogeneity in experimental design, differences between protozoa species, and geographical changes in gut microbiota all result in a lack of consensus regarding the exact role of intestinal protozoa and their contribution to mucosal immune homeostasis [15][29]. It is well established that the bacterial compartment of human gut microbiota comprises a plethora of different species, ranging from beneficial to opportunistic and/or pathogenic [30].

2. Intestinal Protozoa—Pathogenic, Commensal, or Beneficial?

2.1. *Blastocystis* spp.

Blastocystis spp. is one of the most prevalent protozoa found in humans, with an estimated 1 billion colonized individuals worldwide [22][31]. Historically, *Blastocystis* was predominantly characterized as a parasitic protozoa [32], but conflicting results regarding its pathogenic potential and clinical significance have emerged in several studies [31]. *Blastocystis* has been associated with the etiology of irritable bowel syndrome (IBS) [33][34][35][36] and IBD [37]. In contrast, other cohort studies have found no correlation between gastrointestinal symptoms and the presence of *Blastocystis,* either in healthy subjects or IBS patients [38][39]. Similarly, the prevalence of *Blastocystis* infection has

been inconsistently reported to be higher in either immunocompetent or immunocompromised individuals depending on the study [40][41]. One possible explanation for this discrepancy is that *Blastocystis* has mainly been investigated as a causative agent in disease propagation, with limited information about its distribution in a healthy population. With recent advances in sequencing technologies and an increased number of epidemiological surveys, it has become evident that the presence of Blastocystis is a common occurrence in both healthy and symptomatic individuals, which inevitably questions its alleged pathogenicity [42][43]. To date, at least 17 *Blastocystis* subtypes (ST) have been identified, of which nine are found in humans (ST1-ST9), with ST1–ST4 accounting for up to 90% of all occurrences [31][44].

At the other end of the spectrum, beneficial roles for *Blastocystis* have also been proposed. Colonization with *Blastocystis* is associated with higher microbial diversity and richness, both of which are suggested to benefit intestinal health [22][45]. Additionally, it has been shown that body mass index is strongly negatively correlated with *Blastocystis* presence [46]. Several studies have reported that colonization with *Blastocystis* is more common in healthy subjects than in patients with active IBD, IBS, or CRC, supporting that *Blastocystis* might be considered a component of the healthy intestinal microbiota [37][39][46][47][48].

2.2. *Dientamoeba fragilis*

Like *Blastocystis* spp., colonization with *Dientamoeba fragilis* has been reported to exert conflicting roles in gut homeostasis. In contrast to other intestinal protozoa whose colonization prevalence is generally considered higher in the emerging nations, *D. fragilis* has been identified more frequently in the developed world [49][50]. However, due to differences in surveillance systems and diagnostic procedures, its prevalence might be underestimated in some regions [49]. The presence of *D. fragilis* has frequently been associated with disease [51], just as it has been commonly found in asymptomatic carriers [47][48][52].

2.3. *Entamoeba* spp.

Other common intestinal inhabitants with a worldwide distribution are *Entamoeba* species, the majority of which are generally accepted as commensal organisms [53]. Currently, eight species have been identified that are able to infect humans: *E. histolytica*, *E. bangladeshi*, *E. dispar*, *E. hartmanni*, *E. moshkovskii*, *E. coli*, and *E. polecki*, with *E. histolytica* as the only one with well-established pathogenicity [54]. The worldwide frequency of *Entamoeba* occurrence in humans is estimated at 3.5%. However, the prevalence of commensal *Entamoeba* spp. has largely been underestimated due to high morphological and genetic similarity with the invasive *E. histolytica* [53][54]. Microscopy, the most widely used method for the detection of *Entamoeba* organisms, is not always sufficient for differentiating between the invasive *E. histolytica* and non-pathogenic strains of *Entamoeba* [55]. Increased use of molecular diagnostic methods has recently revealed that colonization with commensal *Entamoeba* spp. is overall more common than infections with *E. histolytica* [55].

3. Protozoa–Microbiota Interactions

The various communities of intestinal bacteria play a fundamental role in determining human health. It is generally suggested that high intestinal microbial diversity is a hallmark of a healthy and resilient gut microbiota [56]. Emerging studies consistently report increased bacterial diversity as well as community compositional changes evident in protozoa-colonized individuals [21][22][45][57]. Among the characteristic features of *Blastocystis* colonization is a higher abundance of specific taxa within *Firmicutes*, especially those from the *Clostridia* class, such as *Ruminococcaceae* and *Prevotellaceae* families, and a general decrease of *Bacteroides* abundance [46][52][58]. Furthermore, *Blastocystis* carriers show a significant decrease of *Enterobacteriaceae* and *Proteobacteria* when compared to *Blastocystis*-free subjects [45][46]. Interestingly, *Proteobacteria* and several species within the *Enterobacteriaceae* family can be considered “pathogenic” and linked to microbial dysbiosis associated with the development and pathogenesis of IBD [59][60][61]. Moreover, the presence of *Blastocystis* is strongly associated with the abundance of archaeal organisms, primarily *Methanobrevibacter smithii* [45][46][52]. *M. smithii* has been shown to play an important role in human health, supporting the digestion of glycans through the removal of bacterial fermentation end products [62]. *M. smithii*, together with members of the *Faecalibacterium* and *Roseburia* genera that are also enriched in *Blastocystis*-colonized individuals [22][45], increase the production of the short-chain fatty acid butyrate [63]. Butyrate has well-established beneficial effects on gut health, serving as an important energy source for colonic epithelial cells and acting as an inhibitor of gut inflammation [63][64]. Butyrate-producing bacteria, specifically Faecalibacterium prausnitzii and Roseburia spp., appear to be significantly reduced in patients with Crohn’s disease and have emerged as potential therapeutics for IBD [63][65][66].

On the other hand, adverse associations between *Blastocystis* colonization and eubiotic microbial profile have also been described. Several studies have reported a decrease of *Bifidobacterium* in individuals colonized with *Blastocystis* [45][67]. *Bifidobacterium* spp. have been associated with homeostatic functions within the gut, including protection of the epithelial barrier and regulation of inflammation [68]. Accordingly, a study by Alzate et al. showed that children colonized with *Blastocystis* exhibited markedly reduced abundance of the highly beneficial *Akkermansia* spp. compared to children that were *Blastocystis*-free [69].

Research on microbiota composition associated with *D. fragilis* colonization is limited. However, a study conducted in Denmark investigating the microbial profile in *D. fragilis*-positive children revealed 16 bacterial genera that were significantly more abundant in colonized children [70]. Some of the most enriched bacterial genera in *D. fragilis* carriers were *Victivallis*, *Oscillibacter* and *Coprococcus*, whereas *Flavonifractor* was enriched in non-colonized children. After the removal of *D. fragilis* by metronidazole treatment, the abundance of *Flavonifractor* increased while other bacteria, such as *Coproccocus,* were reduced in previously colonized children that were cleared of *D. fragilis*. Evaluating microbiota composition after metronidazole treatment should be done cautiously since this drug is effective against most anaerobic bacteria [71].

Colonization with *Entamoeba* spp. results in increased microbiota diversity and compositional changes, characterized by an increase of *Firmicutes* taxa, such as *Ruminococcaceae,* coupled with a significant decrease of *Bacteroides* [21][72]. Interestingly, a reduced ratio of *Firmicutes* to *Bacteroides* causes loss of microbial diversity as well as dysbiosis linked to the progression of IBD, CRC, and type 2 diabetes [73][74].

Together, commensal gut protozoa significantly remodel the intestinal bacterial niche, potentially creating a favorable microenvironment beneficial for the host. A common observation across the different protozoa species seems to be an enrichment of SCFA-producing bacteria. Importantly, this is in contrast to what has been demonstrated for pathogenic protozoa, e.g., *Cryptosporidium*, where increased infection severity corresponded with a decreased level of fecal SCFA content [75].

4. The Impact of Commensal Gut Protozoa on the Host Immune System

Recently, it was shown that colonization with *Blastocystis* ST4 attenuates colonic inflammation in a dextran sulfate sodium (DSS)-induced colitis mouse model via induction of T helper (Th) 2 cells and T regulatory (Treg) cells [76]. Mice colonized with *Blastocystis* ST4 showed a decrease of tumor necrosis factor-α expressing (TNF) CD4+ T-cells and an upregulation of signature Th2 cytokines interleukin (IL)-4, IL-5, and IL-13, as well as the anti-inflammatory cytokine IL-10 [76]. Additionally, a marked increase of abundance of SCFA-producing bacteria, such as *Ruminococcaceae* and *Roseburia*, was observed following *Blastocystis* ST4 colonization. Analysis of the SCFA content in feces from colitic mice that had received fecal matter transplant from *Blastocystis* ST4-colonized mice revealed enrichment of 6 SCFAs (butyric, isobutyric, valeric, isovaleric, 2-methylbutyric, and caproic acid) compared to mice that received fecal matter transplant from *Blastocystis*-free mice [76]. Importantly, recent reports have repeatedly suggested a highly beneficial role of SCFAs on gut homeostasis and immune modulation [77]. SCFAs in the intestinal lumen are absorbed by colonocytes where they enter the citric acid cycle and are used for energy production. Unmetabolized SCFAs enter the systemic circulation and travel to different organs, serving as substrates or signaling molecules for various cellular processes such as chemotaxis, proliferation, and differentiation [78][79]. SCFAs achieve this by acting as histone deacetylase (HDAC) inhibitors as well as activators of cell surface receptors [78]. It has been demonstrated that butyrate, created by SCFA-producing microorganisms in the intestines, can facilitate generation of extrathymic Tregs via enhanced acetylation of *Foxp3* locus in $CD4^+$ T cells.

On the other hand, *Blastocystis* ST7 has been suggested to have immunocompromising functions, and several potential virulence factors have been identified that could support the notion of pathogenicity. Antigens from *Blastocystis* ST7 have been reported to induce the mitogen-activated protein kinase-dependent expression of pro-inflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor, in macrophages, mouse intestinal explants, and colonic tissue [80]. Furthermore, *Blastocystis* ST7 has a significantly higher activity of cysteine proteases compared to other *Blastocystis* subtypes. Cysteine proteases are a characteristic feature of parasitic protozoa (e.g., *Entamoeba histolytica* and *Cryptosporidium* spp.) that have been shown to facilitate invasion of host tissue, as well as immune evasion [81].

5. Conclusions

Intestinal protozoa have co-evolved with humans, and their interactions with the human host seem to be highly dynamic and variable, with some species and subtypes exhibiting beneficial properties, while others manifest adverse immunomodulatory effects. The fact that many of these protozoa species cause dormant persistent colonization that often leads to life-long affiliation with their host, points towards commensalism or even symbiosis rather than parasitism. In line with that, colonization with intestinal protozoa appears to significantly increase the diversity of the gut microbiota and selectively modulate the composition of different bacterial communities. Some outstanding questions remain as to whether a therapeutic impact might be achieved by diversification of the human gut via controlled colonization with commensal protozoa strains or by FMT from protozoa-colonized healthy donors to patients with IBD or other gastrointestinal diseases. FMT is an emerging therapy with a successful track record against severe intestinal bacterial infections and a potential therapeutic candidate against diseases associated with microbial dysbiosis [82].

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