Dual infective burden of *Helicobacter pylori* and intestinal parasites: Good or bad news for the host?

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Gastric cancer (GC) remains an important public health concern worldwide, and the best-known carcinogen is *Helicobacter pylori* (*H. pylori*), a Gram-negative helical shaped bacterium found in the stomach by Marshall and Warren [1, 2]. Although *H. pylori* infects nearly half of the world population including India [3] and Malaysia [4], GC rates vary in different populations [5]. Despite the high prevalence of *H. pylori*, India reported low rates of GC (the Indian Enigma) similar to the Zulia population reported in study by Fuenmayor-Boscán et al. [6, 7]. However, the Chinese in Malaysia have the highest reported rates of *H. pylori* and also GC among the three ethnic groups (the cohort effect) [8]. A similarity between India and Zulia is the high burden of unicellular parasites and multicellular worms typically found in these communities [6, 7]. From the above observations thus arise the following questions:

‘Why does *H. pylori* follow a benign course in certain individuals but malignant in others?’
‘Could the intestinal parasites co-infection with *H. pylori* explain the enigma?’

The bad news – overdrive effects of Th1 responses

H. pylori-infected hosts have been shown to display strong polarization of T helper 1 (Th1) cells [9]. The dominance of Th1 over Th2 responses promotes the secretion of proinflammatory cytokines including IL-2, IL-12, IL-18, TNF- α and INF- γ (Fig. 1) [10, 11]. However, the presence of intestinal protozoa (rather than helminth) together with *H. pylori* has been shown to exaggerate (rather than attenuate) the host immune responses [9]. Coinfection of parasites and *H. pylori* actually supplements the recruitment of Th1 cells, aggravates the responses against both microbial entities and intensifies gastric mucosal damage [9].

Another important player is the Th17 cells, which upon activation by *H. pylori* infection, release the cytokines including IL-17A, IL-17F, IL-21 and IL-22 [9–11] (Fig. 1). These intermediaries are involved in antimicrobial activity directed against extracellular bacteria and fungi [10, 12, 13]. Th17-mediated secretion of cytokines affects the production of the proinflammatory cytokine IL-8 and activates the intercellular adhesion molecules-1 (ICAM-1) [14]. The Th17 mediated events then stimulate the chemotaxis of neutrophils into the gastric tissue [14]. Neutrophil infiltration has damaging effects on the gastric mucosa due to reactive forms of oxygen and nitrogen generated by these white cells [11]. Mediators such as histamine and protease (tryptase) are also released by activated mast cells Th17 response, which then induce vasodilation and edema [11].

So, both the Th1 and Th17 responses are activated by the host to clear *H. pylori* (and protozoa or parasites) but often unexpectedly these responses may fail to eradicate *H. pylori* from the gastrointestinal (GI) tract. The reason for failure of clearance is unclear but mucosal recruitment of regulatory T lymphocytes (Treg) may be important. It has been shown that the over-activated Tregs in gastric mucosa could diminish the

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Fig. 1 The role of immunological cells in *Helicobacter pylori* (*H. pylori*) and helminths coinfection. *H. pylori* infection polarize T helper 1 (Th1), it stimulates the secretion of proinflammatory cytokines Interleukin-12 (IL-12), IL-18, IL-1β, Tumor necrosis factor-α (TNF-α) and Interferon-γ (INF-γ). INF-γ in turn activates macrophages, release TNF-α. *H. pylori* infection also activates Th17 and releases cytokines IL-17A, IL-17F, IL-21 and IL-22. These cytokines affect the production of proinflammatory cytokine IL-

8 and activates intercellular adhesion molecules-1 (ICAM-1) and stimulates chemotaxis of neutrophils. The combined effects of TNF-α, INF-γ and reactive species released by neutrophils damage gastric mucosa. *H. pylori* coinfection with helminths on the other hand polarize Th2 cells via IL-4 and secrete IL-4, IL-10 and IL-13 which suppress proinflammatory immune response of Th1 and Th17 and hence enhance enterocyte differentiation and regenerative process of damaged tissues

antibacterial host immune reaction towards *H. pylori* and initiate gastric mucosal damage [10]. The hygiene theory may be the basis for the over-activated Treg response observed in the stomach [15, 16], where early exposure to a diverse range of microbes including *H. pylori* might have activated protective immune processes that also include Treg cells [17]. In addition, the host's genetic polymorphism of different pro-inflammatory, anti-inflammatory, xenobiotic metabolizing enzyme and adhesion molecule gene polymorphisms might have influenced the outcome of *H. pylori* infection [18, 19].

The good news – enhanced Th2 responses with Helminth co-infection

Unlike protozoa, helminths are multicellular and capable of surviving in the host for decades, often with little damaging effects unless there is a high worm burden [20]. Also, unlike protozoa and *H. pylori*, helminths drive predominantly Th2 responses, and probably due to this reason, a host that is

infected by *trichuris* species is somewhat protected from inflammatory bowel disease [20, 21]. Activation of Th2 cells release cytokines including IL-4, IL-5, IL-10 and IL-13, and these cytokines suppress the proinflammatory immune response and enhance the regenerative processes of damaged tissues [9, 22]. Activation of Th2 is also favorable to the recruitment of eosinophils in the stomach, since eosinophils store many compounds that are involved in tissue repair and remodeling [23]. As a result, population with significant exposure to helminthic infection also have lower prevalence of allergic diseases [18, 19].

It is hypothesized that coinfection with intestinal helminths (rather than protozoa or parasites) may induce protective effects against *H. pylori* through alleviation of Th1 dependent responses and thus inflammation in the stomach [9, 24]. These findings have been demonstrated in animal models but not in humans. However, the recent study of Fuenmayor-Boscán et al. in Zulia state of Venezuela has replicated findings of animal models in humans [7]. Although, limited in sample size, they clearly showed overexpression of proinflammatory

cytokines (IL-1 β , TNF- α and IFN- γ), in addition to higher IFN- γ /IL-4 ratio in the *H. pylori* infected individuals as compared to *H. pylori* negative control group [7]. On the other hand, individuals with helminth infection exhibited decrease in the expression of proinflammatory cytokines and increase in the degree of expression of the cytokine IL-4 and lower IFN- γ /IL-4 ratio [7].

The good news – beneficial effects on the gut barrier from Helminth co-infection

The epithelial lining of the gut is the interface but also the barrier between the body proper (i.e. lamina propria and mucosa) and the gut lumen [25]. An essential function of the barrier is to prevent harmful elements (antigens or microbes including protozoa and helminths) in the lumen from gaining access below the mucosa and to the circulatory system. The main components of gut barrier include the thick mucus produced from goblet cells, trefoil factors, defensins or the other antimicrobial peptides from the Paneth cells, serotonin from the enterochromaffin (EC) cells, and secretory IgA [26]. Several studies have indicated that *H. pylori* was not only able to colonize the human gastric epithelium but have also evolved numerous strategies to perturb the integrity of the gastric barrier allowing its continued survival [27, 28].

On the other hand, helminth-induced changes on the epithelium may potentially compromise the survival strategy of *H. pylori*. These helminth-evoked changes have included enhanced epithelial cell turnover and goblet cell hyperplasia; both phenomena assist in expulsion of intestinal helminths [29–32] but also likely *H. pylori*. Helminths also induce Paneth cell hyperplasia, and therefore regulate the release of antimicrobial peptides [33, 34] although more work is required to identify which of these peptides are harmful to *H. pylori*. Helminths infection may also have effect on GI motility mediated through the enteric nervous system by serotonin, a critical signaling molecule involved in a wide range of physiological functions such as secretion, sensing and signaling [35, 36]. ECs within the enteric epithelium are the body's major source of serotonin [36]. However, there have been contradictory findings in which one study demonstrates an increase [37] while another showed a decrease [36] in intestinal ECs and serotonin levels with helminthic infection.

H. pylori are normally found adhering to gastric epithelial cells near the apical junctional complex [38], and apart from gastric surface, the bacterium has been identified within the lamina propria, gastric lymph nodes and within the intracellular canaliculi of parietal cells (Fig. 2) [39]. Disruption of the tight junction complex is assisted by genes related to *H. pylori* [37, 38]. In contrast, intestinal helminths are too big to reside within an epithelial cell, but helminths do cause increases in

gut permeability by ‘scratching’ the epithelium directly or from helminth-derived products [9]. For example, infection with nematodes has been found to increase epithelial permeability by compromising the paracellular spaces and tight junctions [40]. Furthermore, helminth infection promotes mast cell hyperplasia and activation, and the release of proteases from mast cells can affect tight junction proteins and gut permeability [41]. Helminths are also adept at manipulating the host immunity to serve their own needs; for example, it results in the release of alarmins from enterocytes which then promote Th2 type immunity [42, 43].

The controversy – role of Helminth co-infection on the gut microbiome

The gut microbiome plays a central homeostatic role in health and disease but this homeostasis may be disrupted by different intrinsic and extrinsic factors [44]. While the evidence is clear that *H. pylori* can disrupt the gut microbiome [45, 46], the same cannot be said about helminths in which the literature is sparse and at times contradictory [47]. A few animal studies evaluated the relationship between helminth infection with alterations of gut microbiota composition [48, 49] and there are reports of adverse changes. For example, Su et al. reported that co-infection with the intestinal helminth *heligmosomoides polygyrus* (*H. polygyrus*) exacerbated intestinal inflammation caused by the bacterial pathogen *Citrobacter rodentium* due to increase in abundance of Bacteroidetes and reductions in Firmicutes and Lactobacillales [50]. The effects of the helminth-altered microbiota appear to be detrimental based on the study by Su et al, presumably because the helminths interfere with immune responses that are required for *Citrobacter* clearance [50].

However, a few studies have shown beneficial effects of helminths on the gut microbiome [51–53]. For example, Lee et al. compared fecal samples of helminth-infected vs. non-infected individuals residing in rural Malaysia and reported a significant increase in bacterial diversity among individuals infected with any helminth species [51]. Similarly, Cantacessi et al. reported increased bacterial richness among patients with celiac disease infected with helminths [52]. In another study, Whary et al. reported that helminth infection may provide protection against *H. pylori*-induced gastric adenocarcinoma [53]. Helminth infection may also have prevented increased bacterial colonization of the stomach and therefore reduced number of neoplastic lesions [29]. However, the mechanism by which helminths alter the gut microbiome diversity is still not clear. Intact Th2 response and activation or polarization of regulatory T-cells may play a central role in helminth-induced variations in the gut microbiota [42, 50, 54]. Alternatively, helminth-induced effects on the gut microbiome may occur independent of the Th2 immune response [55].

Fig. 2 *Helicobacter pylori* (*H. pylori*) mediated disruption of epithelial barrier integrity. *H. pylori* adhere to gastric epithelial cells near apical junctional complex. *H. pylori* genes disrupt the tight junction complex. *H. pylori* infections polarize Th1 and stimulate the secretion of pro-inflammatory cytokines and damages gastric mucosa. Helminths

coinfection polarizes Th2 polarization and suppresses the damaging effects of Th1 and Th17. The mechanism how intestinal helminths would reverse the disruption of epithelial barrier integrity induced by *H. pylori* and prevents the colonization of pathogenic microbes is still unanswered

There are a number of environmental and lifestyle factors associated with gastric carcinogenesis and *H. pylori* (Table 1), one of which is dietary factor. Earlier studies showed that some foods were protective against carcinogenesis (e.g. onion, curcumin, fruits, vegetables etc.) whereas others promoted it (meat, fish, excess salt etc.) [56]. Diet may also have a direct influence on the pathogenesis and rates of *H. pylori* infection; for example, *centenella asiatica* and budu (fish sauce) have

been found to be associated with a lower prevalence of *H. pylori* among the Malays [57]. Diet is known to modulate the gut microbiota [58]; for example, vegetarians reported significant variations in their gut microbiota from the non-vegetarians [59]. Likewise food preservatives alter microbiota [60, 61]. It is probable that gastric carcinogenesis associated with diet may be mediated by gut dysbiosis [62–66].

Table 1 Environmental and lifestyle factors altering the risk of gastric cancer associated with *Helicobacter pylori* [56]

Factors associated with gastric cancer	Protective factors against gastric cancer
Genetic factors	Total vegetables, onion, garlic
Processed meat	Selenium
Artificial preservatives	Vitamin C
Smoking and other environmental factors	Zinc element in drinking water
Dysbiosis	Vitamin E, Carotenoid (α and β)
Geographical factors	Coffee and black tea
	Green tea

Summary and future direction

Interactions between *H. pylori*, parasites, and the host are under the influence of multiple factors including immune responses, gut barrier function and gut microbiome and hence subject to variable clinical consequences including development of GC. Indeed, *H. pylori* infection has been shown to have detrimental effects on the host gastric mucosa. Co-infection with protozoa may worsen gastric pathology but co-infection with helminths may be potentially beneficial, with gastric outcomes largely mediated by the balance between Th1 and Th2 responses. Epithelial barrier integrity, gut microbiota, diet and host genetic factors may have pivotal roles in determining the ultimate clinical outcomes of co-infection with parasites but at the present time, research is limited and sometimes contradictory.

Compliance with ethical standards

Conflict of interest ZH, EE-O and YYL declare that they have no conflicts of interest.

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