

Genetic and environmental factors shape the host response to *Helicobacter hepaticus*: Insights into IBD pathogenesis

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Abstract

Pathobionts are members of the gut microbiota with the capacity to cause disease when there is malfunctioning intestinal homeostasis. These organisms are thought to be major contributors to the pathogenesis of inflammatory bowel disease (IBD), a group of chronic inflammatory disorders driven by dysregulated responses towards the microbiota. Over two decades have passed since the discovery of *Helicobacter hepaticus*, a mouse pathobiont which causes colitis in the context of immune deficiency. During this time, we have developed a detailed understanding of the cellular players and cytokine networks which drive *H. hepaticus* immunopathology. However, we are just beginning to understand the microbial factors that enable *H. hepaticus* to interact with the host and influence colonic health and disease. Here we review key *H. hepaticus* host interactions, their relevance to other exemplar pathobionts and how when maladapted they drive colitis. Further understanding of these pathways may offer new therapeutic approaches for IBD.

Introduction

The intestine is uniquely home to a vast number of highly diverse microorganisms collectively termed the gut microbiome. Mutualism with commensal bacteria governs gut homeostasis and is upheld by barrier function, innate defence and the generation of commensal-specific adaptive immune responses (figure 1) [1]. Breakdown in gut homeostasis underpins the development of inflammatory bowel disease (IBD), a collection of chronic inflammatory disorders of the gastrointestinal (GI) tract including ulcerative colitis (UC), Crohn's disease (CD) and IBD unclassified (IBDU) [2]. Genome wide association studies (GWAS) and Mendelian disorders which present with IBD-like pathology (MD-IBD) have demonstrated the need for competency in key host pathways to maintain gut health. For example, genes involved in innate recognition of microbial products (NOD2), microbial clearance (ATG16L1) and T cell differentiation (IL23R, IL2RA and JAK2) have been linked to polygenic IBD, whilst

loss of anti-inflammatory interleukin-10 (IL-10) signalling (IL10, IL10RA, IL10RB) and tolerogenic T regulatory (T_{reg}) cell activity (FOXP3) underlie two major forms of MD-IBD [2].

There is increasing evidence to suggest a role for the microbiome in IBD pathogenesis. IBD is associated with alterations in microbiome composition (dysbiosis), which is recapitulated in mouse models [2]. Moreover, in many colitis models disease is ameliorated when animals are housed in germ-free conditions and therefore microbiota dependent [3]. Characterisation of the laboratory mouse microbiota has led to the identification of commensals with pathogenic potential when homeostasis is compromised, commonly referred to as pathobionts [3,4]. These bacteria exploit host-microbe maladaptation and demonstrate the need for both microbial and host factors in disease development. Thus, pathobionts and the susceptible hosts they operate in are highly physiologically relevant models to dissect human IBD pathogenesis.

Helicobacter hepaticus is a gram-negative *Proteobacteria* that colonises the murine large intestine. Since its discovery in immunodeficient mice with chronic hepatitis and colitis [5,6], numerous experiments have formally established *H. hepaticus* as colitogenic predominantly in the context of defective IL-10 and T cell function (table 1) [3,7–15] amongst other immune deficiencies [16]. Whilst *H. hepaticus* serves as the prototypical mouse pathobiont, there are now multiple examples within and extending beyond the *Helicobacter* genus (table 1) [17–25]. In addition to inducing inflammation in susceptible hosts, pathobionts exert potent effects on immunocompetent host physiology. Unsurprisingly, homeostatic and inflammatory influence is exerted over key pathways of intestinal homeostasis, through interactions with the intestinal barrier and functional alteration of innate and adaptive immune responses. Below, we summarise our current understanding of host-*H. hepaticus* interactions in health and disease, highlighting related host-microbe pathways that may shape future treatment strategies in IBD.

Table 1

Bacteria	Colitis model	Immune defect	References
<i>Helicobacter hepaticus</i>	<i>Il10</i> ^{-/-} or treatment with anti-IL-10 receptor (IL-10R)	Genetic or pharmacologically induced deficiency in anti-inflammatory IL-10 signalling	[7,8]
	129SvEv <i>Rag2</i> ^{-/-}	Lymphodeplete mice with increased susceptibility to <i>H. hepaticus</i> colitis conferred by the <i>Helicobacter hepaticus</i> -induced colitis and associated susceptibility cancer (<i>Hiccs</i>) genetic locus in the 129SvEv strain. Pathology is driven by an aberrant group 3 innate lymphoid cell (ILC3) response	[9,10]
	<i>H. hepaticus</i> infected <i>Rag1/2</i> ^{-/-} mice reconstituted with i) naïve T cells, ii) <i>Il10</i> ^{-/-} T cells, iii) <i>H. hepaticus</i> induced T helper type 1 (Th1), Th1/Th17, Th17 cells and iv) <i>H. hepaticus</i> -	T cell transfer into a lymphodeplete environment leading to inflammatory T effector (T _{eff}) cell expansion. Notably, pathology is ameliorated by the co-transfer of <i>H. hepaticus</i> induced T regulatory (T _{reg}) cells.	[3,8,11–14]

	specific T cell clones		
	DC-LMP1/CD40	Constitutive CD40 signalling in CD11c ⁺ cells leading to intestinal CD103 ⁺ dendritic cell (DC) depletion and an inability to generate induced T _{reg} cells.	[15]
<i>Helicobacter bilis</i>	Dextran sodium sulfate (DSS) treatment in C3H/HeN mice colonised with the altered Schaedler flora (ASF)	Low-level (1.5%) chemically induced epithelial barrier disruption in gnotobiotic C3H/HeN mice colonised with the ASF, which are susceptible to <i>H. bilis</i> -induced colitis.	[17,18]
<i>Helicobacter typhlonius</i>	<i>Tbx21</i> ^{-/-} <i>Rag2</i> ^{-/-} ulcerative colitis (TRUC)	Genetic ablation of the transcription factor T-bet in lymphodeplete mice leading to ILC3-driven colitis	[19]
	<i>Il10</i> ^{-/-}	Global ablation of IL-10 function. Notably, colitis severity is exacerbated in the context of co-infection with <i>Helicobacter rodentium</i>	[20]
	DSS + aIL-10R	Barrier disruption induced by	[21]
<i>Helicobacter apodemus</i>	DSS + aIL10R	1% DSS treatment coupled with pharmacological blockage of IL-10 signalling	
	<i>H. apodemus</i> infected B6 <i>Rag1</i> ^{-/-} mice reconstituted	Expansion of bacterial-specific T cells in a lymphodeplete environment	

	with <i>H. apodemus</i> -specific T cells	leading to inflammatory T _{eff} cell differentiation	
<i>Bacteroides thetaiotaomicron</i> (<i>B. theta</i>)	<i>CD4-dnTgfrβII</i> x <i>Il10r2^{-/-}</i> (dnKO)	Genetic CD4-specific deletion of transforming growth factor β receptor (TGFβR), an important cytokine for T _{reg} cell differentiation, and global ablation of IL-10 signalling	[22]
<i>Mucispirillum schaedleri</i>	<i>Nod2^{-/-}Cybb^{-/-}</i>	Double genetic deficiency in the innate pattern recognition receptor (PRR), nucleotide-binding oligomerization domain containing protein 2 (NOD2), and NADPH oxidase function, resulting in impaired anti-microbial function	[23]
<i>Akkermansia muciniphila</i>	<i>Il10^{-/-}Nlrp6^{-/-}</i>	Genetic ablation of IL-10 and Nod-like receptor family pyrin domain containing 6 (NLRP6)-mediated inflammasome function.	[24]
Adherent-invasive <i>Escherichia coli</i> (AIEC)	<i>T5KO</i>	Genetic deficiency in toll like receptor 5 (TLR5), an innate PRR	[25]

The intestinal barrier is a critical mediator of initial host-microbial contact

The intestinal barrier comprises a single layer of closely connected epithelial cells that is segregated from commensals by stratified mucus. Mucin 2 (MUC2) is the major component of mucus secreted by goblet cells in the proximal and distal colon. Notably, proximal-derived MUC2 is critical for epithelial-microbial segregation and is

induced by the microbiota to promote its own encapsulation and establishment of the mucus niche [26]. Defective MUC2 production leads to spontaneous colitis and renders the host susceptible to lethal infectious colitis driven by the murine pathogen *Citrobacter rodentium*, which is also seen following bacterial-mediated mucus degradation under fibre-restricted conditions [26–29]. Defective mucus function likely contributes to *H. hepaticus*-induced colitis. Goblet cell depletion is a histopathological feature of *H. hepaticus* colitis models and mucus derived from susceptible IL-10^{-/-} mice is penetrable by bacteria, both of which are features shared by UC patients [30–32].

Additional factors operate in colonic mucus to reinforce barrier function. Ly6/PLAUR domain containing 8 (LYPD8) protein is constitutively shed by intestinal epithelial cells (IECs) and binds to flagellated bacteria including *Helicobacter* species to inhibit motility and restrict epithelial contact [33]. In the presence of pathobionts including *Helicobacter* species, IECs regulate microbiome composition in-part through antimicrobial peptide (AMP) production, produced following engagement of the pattern recognition receptor (PRR) and inflammasome component nod-like receptor family pyrin domain containing 6 (NLRP6) [34,35]. B cell-derived immunoglobulin A (IgA) targets *Helicobacter* species in the colonic mucosa during homeostasis and *H. hepaticus* is bound by IgA and IgG in colitic mouse sera [15,36]. IBD patients display elevated intestinal IgA and IgG responses, which identify bacterial populations with elevated colitogenicity [37–39].

In addition to producing factors to reinforce mucus sterility, the epithelium modulates adaptive responses through various mediators, such as inflammasome-derived IL-18 [40], and acts as a second regulator of immune exposure to microbial antigen. *H. hepaticus* interacts with IECs through its cytolethal distending toxin (CDT), which is critical for colitis development. CDT induces STAT3 activation and DNA double strand breaks in IECs and epithelial dysplasia [31,41]. DNA damage leads to the induction of autophagy, which protects IECs from apoptosis through the removal of CDT-induced micronuclei structures [42]. Autophagy also protects IECs against tumour necrosis factor (TNF)-induced apoptosis, which limits the severity of *H. hepaticus*-induced colitis [43].

H. hepaticus, other *Helicobacter* species, *Mucispirillum schaedleri*, adherent-invasive *Escherichia coli* (AIEC) and *Akkermansia muciniphila* (table 1) are mucosa-associated or reside within the mucus layer, suggesting their pathobiont status is in-part conferred by their ability to closely associate with the host [5,17,21,23–25]. An exception to this is *Bacteroides thetaiotaomicron* (*B. theta*) which can also reside in the gut lumen, where it secretes antigen-loaded outer membrane vesicles (OMVs) that migrate across the intestinal barrier and into the colonic mesenchyme [44]. *H. hepaticus*-derived antigenic targets in colitis models include a flagellar hook protein, the uncharacterized protein HH_1713 and the chaperonin GroEL (heat shock protein 60 (HSP60)) [12,13,15]. Limited information is available regarding the methods employed by *H. hepaticus* to support its colonisation, notably of intestinal crypts [4], and subsequent targeting by the immune system. *H. hepaticus* motility, conferred by its bipolar flagella, is critical for colonisation in wild-type mice [45]. In contrast, the *H. hepaticus* type 6 secretion system (T6SS) limits colonisation in colitis models, with contradictory effects on inflammation development [4,46].

Innate immune cells set the tone for adaptive responses to *H. hepaticus*

Monocytes, macrophages, and dendritic cells (DCs) in the colonic lamina propria interact with commensals through PRR-mediated binding of pathogen-associated molecular patterns (PAMPs), active sampling of the microbiome or phagocytosis of bacteria that have breached the epithelial barrier. Under homeostasis, this creates a microenvironment that is conducive to tolerogenic immune responses. For example, through the PRR, toll-like receptor 2 (TLR2) and subsequent activation of the anti-inflammatory transcription factor CREB, a polysaccharide produced by *H. hepaticus* induces IL-10 production by caecal macrophages [47]. Similarly, TLR2-mediated recognition of whole *H. hepaticus* by bone marrow derived macrophages (BMDMs) limits pro-inflammatory IL-12 production. This signalling pathway is distinct to that mediated by *H. hepaticus*-derived polysaccharide and involves alpha-protein kinase 1 (ALPK1). ALPK1-mediated suppression of IL-12 limits T helper type 1 (Th1) cell expansion following *H. hepaticus* infection, which provides some protection against pathology in the *H. hepaticus* + anti-IL-10 receptor (aIL-10R) model [48]. It is of note that ALPK1 function in this setting is not clear, although it is most prominently described as a PRR for lipopolysaccharide intermediates in human epithelial cells

[49], and the ability of *H. hepaticus* to activate CREB and Alpk1 differs from that of canonical TLR2 agonists [47,48]. Moreover, TLR2-derived homeostatic signals are not sufficient to protect against *H. hepaticus*-induced inflammation [50].

Group 3 innate lymphoid cells (ILC3s) interpret cytokine signals derived from macrophages and DCs to uphold barrier function and promote tolerogenic responses during homeostasis. Antigen presentation by ILC3s to T follicular helper (Tfh) cells limits homeostatic IgA production, preventing excessive interaction with *Helicobacter* species and shedding into the gut lumen [36]. In *H. hepaticus*-induced colitis, ILC3s respond to macrophage-derived IL-23 and IL-1 β by producing various pro-inflammatory mediators [10,51–56] (figure 2). This inflammatory macrophage phenotype is orchestrated by interferon regulatory factor 5 (IRF5), which operates downstream of PRR engagement to direct monocyte differentiation [57]. A similar role for IL-23/IL-1-responsive ILC3s has been shown in the *Tbx21*^{-/-}*Rag2*^{-/-} ulcerative colitis (TRUC) model, in which mice lack T-bet function in the innate immune system. In TRUC mice, nucleotide-binding oligomerization domain containing protein 2 (NOD2) recognition of microbial components by DCs leads to the production of IL-23 and IL-1, in turn promoting pro-inflammatory ILC3 responses [58]. Importantly, *Helicobacter typhlonius* is a key driver of TRUC pathology [19].

***H. hepaticus* infection exerts context dependent control on the T_{reg}/T_{eff} cell axis**

The presence of commensal-reactive T cells and microbiota-bound IgA and IgG in the human intestine supports a fundamental role for T cell immunity in controlling commensal-specific responses [37,39,59]. *H. hepaticus* serves as a model for understanding microbe-T cell interplay and results show that commensal-specific T cell phenotype is context dependent. For example, when transferred into a colonised immunocompetent host, *H. hepaticus*-specific T cells differentiate into ROR γ t⁺ induced T_{reg} (iT_{reg}) and, to a lesser extent, Tfh cells [12]. iT_{reg} cell generation is dependent on intestinal CD103⁺ DCs, intrinsically controlled by the transcription factor c-MAF and plays a non-redundant role in mediating homeostasis [12,15]. By contrast, in colonised IL-10^{-/-} or lymphodeplete *Rag*^{-/-} mice, *H. hepaticus*-specific T cells deviate to a pathogenic Th1/Th17 phenotype, directed by macrophage-derived IL-23 and epithelial-, macrophage- and DC-derived serum amyloid A proteins (SAAs)

(figure 2) [11,12,60,61]. Thus, the T cell response to *H. hepaticus* can be pathogenic or homeostatic depending on environmental cues controlled by host genetics.

Context-dependent T cell differentiation is not exclusive to *H. hepaticus*. Indeed, T cell clones reactive with *H. typhlonius* and *Helicobacter apodemus* differentiate into T_{reg} cells in an immunocompetent host, and deviate to pathogenic T effector (T_{eff}) cells in Rag^{-/-} mice [62]. Similarly, *B. theta*-specific T cells readily differentiate into colonic T_{reg} cells during homeostasis and can suppress the generation of inflammatory T_{eff} responses with the same antigen specificity [63]. In addition to host genetics, the microbiota contributes to context-dependent T cell differentiation. For example, *A. muciniphila*-specific T cells differentiate exclusively into T_{fh} cells in monocolonized mice and adopt additional T_{eff} phenotypes in a specific pathogen free (SPF) host [64]. T cell responses are dependent on DCs which integrate bacterial- and host-derived signals to shape naïve T cell differentiation programmes during antigen presentation. Notably, colonic DCs derived from healthy individuals respond to *B. theta* OMVs by producing IL-10, a property that is defective in IBD patients [65]. To facilitate context dependent T cell differentiation, multiple migratory DC subsets have been shown to induce *Helicobacter*-specific T_{reg} cells in a default mechanism that is over-ridden during activation in T_{eff} polarising stimuli [66]. In addition to antigen-specificity, pathobionts also influence the T cell axis through metabolite production. For example, *B. theta* has been shown to promote T_{reg} cell induction through bile acid and tryptophan metabolism [67,68].

H. typhlonius and *H. apodemus*-specific T cell clones were isolated from a commensal reactive heterogeneous pool obtained from colitic mice in which the majority of TCRs recognised *Helicobacter* species [62]. Those bacteria are therefore adept manipulators of host immunity, although the reason for this is not completely understood. T cells reactive with Cbir1 flagellin expressed by luminal dwelling *Clostridia* species are colitogenic following epithelial barrier disruption but do not elicit pathology when transferred to Rag^{-/-} mice. In this lymphodeplete setting and due to endogenous T cell receptor (TCR) rearrangement, populations with alternate reactivities, including to *Helicobacter* species, dominate the colitic response [69]. This demonstrates the importance of antigen exposure in shaping T cell immunity. Indeed, *B. theta*-specific T_{reg} cell responses are inhibited by dietary glucose, which

directly represses antigen expression by *B. theta* [63]. Moreover, emergence of T cell populations that lack Cbir specificity reinforces the idea that mucosal association likely contributes to the modulatory potential of a particular bacterium in the face of an intact epithelium. This differential reactivity to microbiota constituents is likely relevant to different IBD pathologies, for example those arising predominantly from barrier breakdown versus dysregulated T_{reg} cell function [2].

Extrinsic microbial factors influence *H. hepaticus* infection outcome

Within the gut microbiota, competition for resources can alter bacterial localization, and cross-feeding between strains can influence the expression of virulence factors and metabolic output, all of which can alter host physiology. In GF IL-10^{-/-} mice, *H. hepaticus* colonises but fails to induce colitis, suggesting a requirement for other commensals in disease development [70]. Introduction of *Lactobacillus reuteri* prior to *H. hepaticus* infection results in pathology, although *L. reuteri* monocolonized animals also do not develop disease [71]. Susceptibility to *H. hepaticus*-induced colitis in IL-10^{-/-} mice has been shown to differ by vivarium, where mice with identical strain backgrounds display substantial differences in microbiome composition [72]. Differences in microbiome composition inevitably leads to differences in the metabolite milieu that is capable of shaping *H. hepaticus* growth and function. For example, commensal-derived short chain fatty acid (SCFA) signalling through free fatty acid receptor 2 (FFAR2) limits *H. hepaticus* abundance. Additionally, hydrogen sulfide (H₂S), produced by sulfate reducing bacteria such as *Desulfovibrio* species, suppresses pro-inflammatory responses in *H. hepaticus*-induced innate colitis [73,74]. *H. hepaticus* also alters host responses towards other microbes, as prior infection with *H. hepaticus* leads to impaired control of *Mycobacterium tuberculosis* and *H. hepaticus* + aIL-10R treatment leads to differential IgA binding of other commensals [75,76]. This draws similarities with *Helicobacter bilis*, which, while necessary for disease, elicits host reactivity to other microbiota members during sub-pathological chemically induced colitis [18].

Therapeutic outlook and conclusion

Immunological drivers of pathology in colitis models have long served as therapeutic targets in the clinic. Nevertheless, up to 30% of IBD patients do not respond to anti-

TNF- α , a first line treatment for IBD [77]. It is noteworthy that treatment of colitis in the *H. hepaticus* + aIL-10R model is refractory to anti-TNF- α and therefore may be used to understand the efficacy of alternative treatment strategies [78]. More recently, therapies aimed at manipulating gut microbiome composition have been trialled in IBD. In addition to faecal microbiome transplants (FMTs), the identification of beneficial strains has led to the development of prebiotics, probiotics, and designer consortia with more defined effects on the host. Advancing from this, our understanding of bacterial-host interactions which promote gut health can be used to engineer probiotics with advanced homeostatic properties [79].

Our understanding of *H. hepaticus* biology reflects this conceptual shift in therapeutic strategies developed for the clinic. *H. hepaticus* colitis models have largely been used to identify immunological drivers of pathology. Defining pathways which support colonisation, antigen delivery and polarisation of the T cell axis will complement our understanding of host-*H. hepaticus* interactions that underpin health and disease. Of note, elucidating how *H. hepaticus* can drive such potent T_{reg} cell responses has important therapeutic implications (figure 3). Whilst we have focused on mouse models in this review, bacteria with pathobiont status are highly relevant to human physiology. Most pertinent in relation to *H. hepaticus* is the gastric pathobiont *Helicobacter pylori*, which drives peptic ulcer disease and gastric cancer in only a subset of colonised individuals. Disease outcome is thought to be determined by a number of complex host-bacterial interactions including perturbations in the gastric ecosystem [80]. Thus, identifying interbacterial interactions which influence *H. hepaticus* colitogenicity will place this highly physiologically relevant model into the context of the ecological principles which operate in the gut microbiota.

Acknowledgments

RJ DPhil is funded by the Wellcome Trust. NEI and FP are supported by the Wellcome Trust (212240/Z/18/Z) and the Kennedy Trust for Rheumatology Research (KTRR).

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