Fakultät für Medizin
Institut für Medizinische Mikrobiologie, Immunologie und Hygiene
Technische Universität München

Co-infection of *H.pylori* and *S.mansoni*: Impact of immunological crosstalk on pathology

Sonakshi Bhattacharjee

Vollständiger Abdruck der von der Fakultät für Medizin der Technischen Universität München zur Erlangung des akademischen Grades eines

Doctor of Philosophy (Ph.D.)

genehmigten Dissertation.

Vorsitzende/r:
Prof. Dr. Jürgen Ruland

Betreuer/in:
Prof. Dr. med. Markus Gerhard

Prüfende der Dissertation:
1. Prof. Dr. med Clarissa Prazeres da Costa
2. Prof. Dr. Dirk Haller

Die Dissertation wurde am 30.11.2017 bei der Technischen Universität München eingereicht und durch die Fakultät für Medizin am 08.02.2018 angenommen.

Abstract

Helicobacter pylori chronically persists in the stomachs of infected individuals and is strongly associated with gastric cancer. This has been attributed to the response it generates via the continuous induction of various pro-inflammatory cytokines. Interestingly, certain countries with a high prevalence of *H.pylori* do not present with comparable high rates of gastric cancer. Epidemiological data has suggested that one of the reasons for this discrepancy may be due to highly prevalent co-infections with helminths, which suppress *H.pylori* associated pro-inflammatory responses. However, there is limited experimental evidence to support this hypothesis and none so far with *S.mansoni*, one of the most common helminths endemic in countries with the highest rate of *H.pylori* prevalence. On the other hand, there is even lesser known on how *H.pylori* may influence *S.mansoni* related hepatic disease. In this thesis, we fill in the basic gaps in the understanding of the immunological mechanisms employed by these pathogens upon co-infection. Importantly, we analyse these effects during the different immune phases of the helminth infection. Surprisingly, we observed increased colonization of *H.pylori* in the stomach of co-infected mice in the Th1 phase of schistosome-infection despite high levels of IFN-γ and the fact that this cytokine is known to be responsible for bacterial clearance. Further investigations revealed the occurrence of immune deviation of antigen experienced T cells, away from gastric tissue, due to strong Th1 chemokine gradients induced by the helminth in the liver. On the other hand, *H.pylori* co-infection altered the liver pathology associated with schistosome infection, resulting in smaller granulomas accompanied by decreased liver-specific alanine aminotransferase (ALT) and total collagen levels. This observation hints towards a "protective" role of *H.pylori* co-infection in *S.mansoni* associated liver disease. In summary, our data strongly point towards an immunological interaction of pathogens occupying two anatomically distant organs, eventually resulting in pathological changes that alters the course of both diseases.

Zusammenfassung

Die Infektion mit *Helicobacter pylori* führt zu einer chronischen Entzündung im Magen infizierter Individuen und ist stark mit der Entstehung von Magenkarzinomen assoziiert, dessen Entwicklung durch die kontinuierliche Freisetzung entzündungsfördernder Zytokine gefördert wird. Interessanterweise weisen bestimmte Länder mit hoher Prävalenz für *H.pylori* überraschenderweise eher niedrigere Raten von Magenkarzinomen auf. Epidemiologische Daten lassen vermuten, dass einer der Gründe für diese Diskrepanz auf Koinfektionen mit Helminthen zurückzuführen ist, welche die durch *H.pylori* verursachte chronische Entzündung unterdrücken. Jedoch gibt es hierfür nur eingeschränkt experimentelle Belege, die diese These unterstützen. Speziell für *S.mansoni*, einen der am weitesten verbreiteten Helminthen, der in Ländern mit der höchsten Prävalenz von *H.pylori* endemisch ist, fehlt bisher jeglicher Nachweis einer möglichen Interaktion. Darüber hinaus ist noch weit weniger darüber bekannt, ob und in welchem Maße *H.pylori* die mit *S.mansoni* assoziierten hepatischen Erkrankungen beeinflussen kann. In der hier vorliegenden Dissertation schließen wir grundlegende Lücken hinsichtlich des Verständnisses der immunologischen Interaktionsmechanismen beider ko-infizierender Krankheitserreger. Von besonderer Bedeutung ist hierbei, dass wir diese Effekte in den verschiedenen Immunphasen der Helmintheninfektion analysieren konnten. Wir beobachteten eine verstärkte Besiedlung von *H.pylori* im Magen von ko-infizierten Mäusen während der Th1 Phase der Schistosomeninfektion trotz hoher systemischer Ausschüttung von IFN-γ. Dies ist unerwartet, da dieses Zytokin bekanntermaßen verantwortlich für Kontrolle der bakteriellen Erregerlast ist. Unsere Untersuchungen deckten die Existenz einer systemischen Immunabweichung“ aufgrund hoher Chemokingradienten auf. Bei der Untersuchung des Einflusses von *H.pylori* auf die Entwicklung einer *S.mansoni* Infektion in ko-infizierten Mäusen beobachteten wir im Gegensatz dazu kleinere Granulome in Verbindung mit verminderter Serumkonzentration der leberspezifi-

schen Alaninaminotransferase- (ALT) und Kollagenproduktion. Diese Beobachtung lässt eine protektive“ Rolle der *H.pylori* Ko-infektion bei *S.mansoni* assoziierten Lebererkrankungen vermuten. Zusammenfassend deuten die Daten stark auf eine unerwartete Interaktion beider in ihrer Organspezifität stark segregierten Pathogene auf immunologischer Ebene hin, welche letztendlich den Verlauf beider Krankheiten teilweise sogar günstig beeinflusst.

Contents

List of Figures

List of Tables

Acronyms

AAI	Allergic airway inflammation.
AAM	Alternatively activated macrophage.
AHR	Airway hyperresponsiveness.
ALT	Alanine aminotransferase.
APC	Antigen presenting cell.
Arg 1	Arginase 1.
BAL	Bronchoalveolar lavage.
BCG	Bacille-Calmette-Guerin.
BMDDCs	Bone marrow derived dendritic cells.
CagA	Cytotoxin associated gene A.
CFU	Colony forming units.
CTGF	Connective tissue growth factor.
CTLA4	cytotoxic T-lymphocyte antigen.
DALYs	Disability adjusted life years.
DAMPS	Damage associated molecular patters.
DC	Dendritic cell.
DOI	Degree of Infection.
ECM	Extra cellular matrix.
ELISA	Enzyme linked immunosorbent assay.
FoxP3	Forehead box P3.
gGT	g-glutamyltransferase.
GrzB	Granzyme B.

HCC	Hepatocellular carcinoma.
HE	Heamatoxylin/eosin staining.
HepB	Hepatitis B virus.
HepC	Hepatitis C virus.
HP-NAP	H.pylroi neutrophil activating protein.
HSC	Hepatic stellate cell.
HSP70	Heat shock protein 70.
IBD	Inflammatory bowel disease.
IEL	Intra epithelial lymphocyte.
Ig	Immuoglobulin.
IHC	Immunohistochemistry.
ILC	Innate lymphoid cell.
IPSE	Interleukin 4 inducing principle from S.mansoni eggs.
iTreg	Induced regulatory T cell.
LAL	Liver associated lymphocyte.
LCMV	lymphocytic choriomeningitis virus.
LPS	Lipopolysaccharide.
MALT	Mucosal associated lymphoid tissue.
MHC	Major Histocompatibility Complex.
MLN	Mesenteric lymph node.
NO	Nitric oxide.
NOD	Nucleotide binding oligomerization domain like receptors.
nTreg	Natural regulatory T cell.
OipA	Outer inflammatory protein A.
PDGF	Platelet derived growth factor.
PDL1	Programmed cell death ligand 1.
PGE2	Prostaglandin E2.
PRR	Pattern recognition receptors.
Rag	Recobinase activating gene knockout.

ROS	Reactive oxygen species.
RT	Room Temperature.
SCID	severe combined immunodeficiency.
SEA	Soluble egg antigens.
T4SS	Type-4 secretion system.
Th1	T-helper type 1.
Th17	T-helper type 17.
Th2	T-helper type 2.
TIMP	Tissue inhibitor of matrixmettaloproteinases.
TLR	Toll like receptors.
Treg	Regulatory T cell.
VacA	Vacuolating cytotoxin A.
VEGF	Vascular endothelial growth factor.
WHO	World Health Organisation.

1 Introduction

1.1 *Helicobacter pylori* (*H.pylori*)

Helicobacter pylori (*H.pylori*) is the most widespread *Helicobacter sp.* in humans and selectively colonizes the stomachs of about 4.4 billion people worldwide [1]. The discovery of this spiral, gram negative, microaerophilic bacterium and its causal relationship with severe gastric diseases led to a Nobel prize in 2005 for Robin Warren and Barry Marshall. However, they were not the first to observe that the stomach is not the sterile organ it is usually made out to be. About a century ago, W. Jaworski observed spiral shaped micro-organisms in the mucosa of gastric tissue [2]. Thereafter, several studies followed with similar reports, especially in association with ulcerative gastric tissue [3, 4, 5]. The most challenging aspect of studies with *H.pylori* was the difficulty in culturing it, which was achieved by R.Warren and B.Marshall in 1982. They were not only successful in this aspect but in order to fufil the 3rd of Koch's postulates, Marshall ingested the culture and thereafter developed gastritis, thus fulfilling all of Koch's postulates [6, 7]. The discovery of this pathogen was followed by extensive studies focused on both the pathogen as well as the host response. Inspite of this, the route of transmission has still not been explicitly demon-

strated. Since *H.pylori* has been found in feaces, saliva and vomit, it has been suggested to be transmitted by an oral-oral or feacal-oral route [8, 9, 10].

1.2 *H.pylori* prevelance and coevolution with its host

H.pylori has co-evolved with its host for more than 65000 years and is now one of the most prevalent infections in the world [11]. While the incidence varies geographically and with soci-economic status, age, diet and genetic predisposition have also been stated to play a role [12, 13]. The prevalence is highest in African countries with Nigeria at 87.7%, while Switzerland ranks lowest with 18.9% [14]. Interestingly, studies have demonstrated that co-evolution of the bacteria with its host may determine the risk for developing gastric disease. For example, an African strain of the bacteria is less deleterious to individuals with African ancestry compared to the same strain infecting Amerindian populations [15]. Furthermore *H.pylori* itself has developed several mechanisms in order to survive and thrive in one of the harshest niches of the human body- the stomach. These studies point out the important role that co-evolution has played in favour of *H.pylori*.

1.3 Interplay between *H.pylori* and the host's immune response

Primary infection with *H.pylori* in humans is always associated with a transient acute gastritis. In rare cases, particularly in children, *H.pylori* can be spontaneously eradicated by the immune response and the intitial gastritis subsides [16, 17]. However, in most humans, the bacterium colonizes the stomach and thrives for decades. Upon infection, both the innate and adative immune response come into play. The bulk of the innate response is undertaken by epithelial cells, neutrophils, macrophages, monocytes and dendritic cells [18, 19, 20]. They recognize bacterial pathogen or damage associated molecular patterns (PAMPs or DAMPS) such as flagellin and lipopolysaccharides. These are recognized via pattern recognition receptors (PRRs) like Toll-like receptors (TLRs) that are extracellular and NOD-like receptors which are intracellular. Engagement of these ligands and receptors results in a cascade of signaling pathways resulting in inflammation. To supplement this, the adaptive immune response comprising of T-cells and B-cells initiate antigen specific responses and generate immunological memory [16].

1.3.1 Innate immune responses to *H.pylori*

The first point of contact between *H.pylori* and the immune system is the gastric epithelial monolayer and intra-epithelial lymphocytes (IEL) [21, 22, 23]. When *H.pylori* infects the stomach, many signaling pathways in epithelial cells are upregulated, such as the NF-kB pathway [24, 25]. This results in induction

of pro-inflammatory cytokines such as IL-1, TNFα and IL-8, which functions as strong chemoattractants for neutrophils [26, 27]. A study of experimental *H.pylori* infection in humans demonstrated that within a fortnight, levels of IL-8 in mucosa were increased substancially upon ingestion of the bacteria [28]. Indeed, neutrophils are one of the first cells recruited to the site of infection, especially due to signals from epithelial cells as well as virulence factors such as *H.pylori* neutrophil-activating protein (HP-NAP), first identified to induce the production of reactive oxygen species (ROS) in this neutrophils [29]. This factor additionally promotes T-helper 1 (Th1) immune responses [30].

Many components of *H.pylori* induce a response from the gate-keepers of the immune system- the phagocytes. Neutrophils, macrophages, dendritic cells and monocytes initiate the first inflammatory response by releasing IL-12, IL-6 and IL-23 among other cytokines, which eventually also induces T cells to produce pro-inflammatory cytokines [31, 32, 33]. Macrophages additionally produce TNFα and induce Nitric oxide (NO) systhesis, factors which are associated with killing of the pathogen [34, 35]. While *H.pylori* has developed mechanisms to avoid phagocytic killing by macrophages (section 1.3.3), this cell type has another important function- they secrete signaling molecutes that actively recruit other immune cells, important in orchestrating an efficient immune response.

Dendritic cells (DCs) have been a much studied cell type during *H.pylori* infection as they represent a critical bridge between the innate and adaptive immune response. During *H.pylori* infection, DCs are known to be the main antigen presenting cells (APCs) that induce T cell responses. This cell type stands out due to its ability in inducing a mixed leukocyte response, especially

compared to other MHC expressing cells such as B cells and macrophages [36]. However like macrophages, these cells differ in their functions and subtypes based on anatomical location and activation status. DCs have been shown to penetrate epithelial mono-layers *in vitro* as well as have finger like projections that cross epithelial junctions *in vivo* [37]. DCs that contact *H.pylori* secretions in the stomach could migrate to the draining lymph nodes, such as the paragastric lymph nodes and prime naive T cells to induce Th1/ Th17 or even Treg responses (Figure 1.1). This maybe an active process in the beginning of the infection, however, the continuous priming of the subsequent immune response by DCs is not likely to take place in the stomach. While it is tempting to assume that dendritic cells in the stomach behaved like those in the gut, extending finger-like protrusions through epithelial cells to sample antigens, studies demonstrated that this process occurs mainly in the small bowel and this is the main priming site. Two independent studies demonstrated this by treating mice *in utero* with an IL-7Rα blocking antibody, giving rise to animals that do not have peyer's patches (PP-null) and subsequently infecting them with *H.pylori* [38, 39]. The strongest evidence was provided by Nagai et al, where the authors showed a lack of priming and *H.pylori* associated gastritis in mice that lacked peyer's patches compared to wild type mice [39]. Currently it is believed that this is one of the most critical sites of the induction and maintainance of *H.pylori* associated immune responses. The bacteria pass through to the intestine and convert to the coccoid form in the anaerobic conditions there. This coccoid form may have different surface molecules exposed that make it more susceptible to dendritic cell phagocytosis compared to the helical form in the stomach that evades engulfment. DCs in the peyer's patches

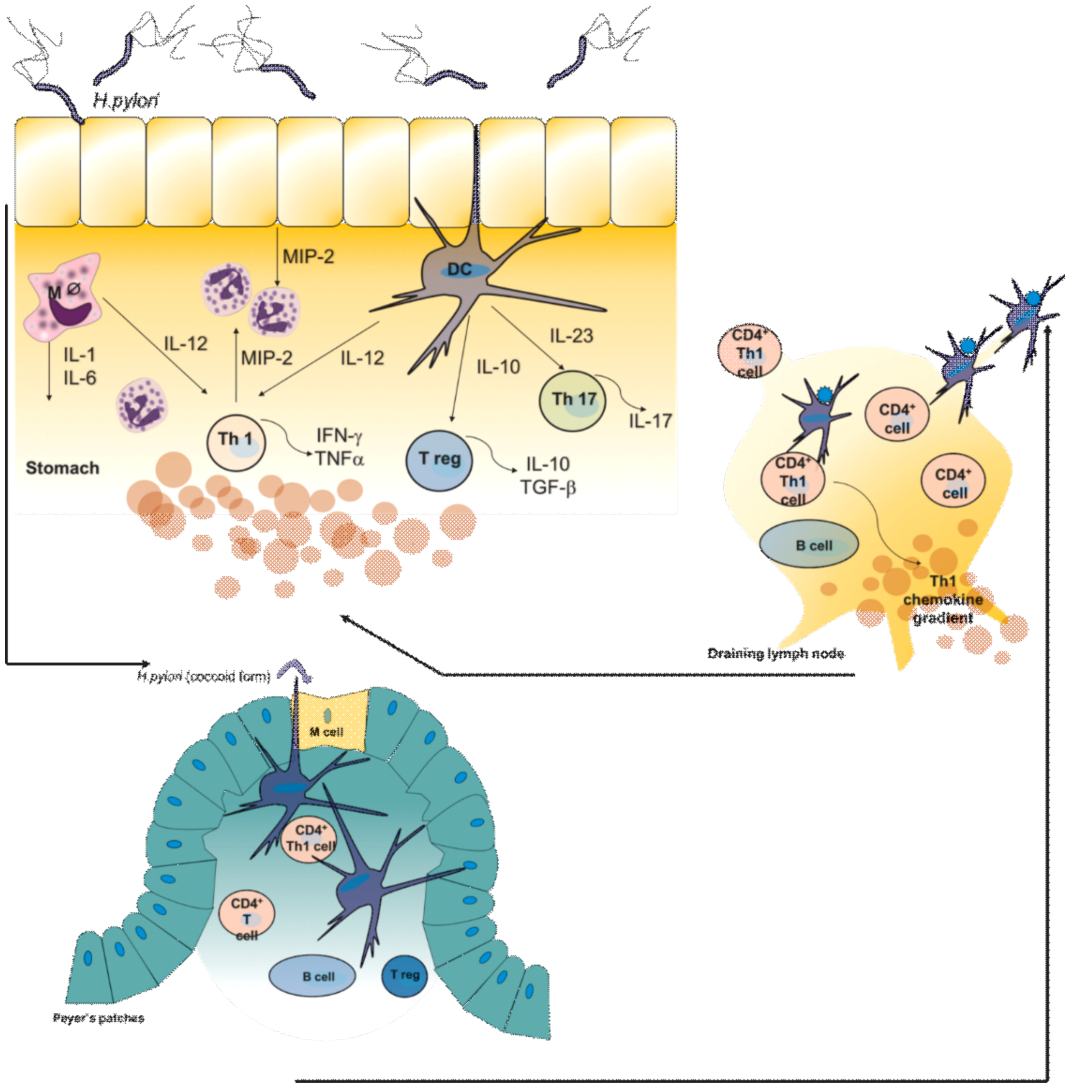


Figure 1.1: Initiation, priming and inflammatory response associated with *H.pylori* infection. Contact of *H.pylori* with gastric epithelial cells induces production of various chemokines that attract neutrophils and macrophages to the site of infection. These cells in turn produce pro-inflammatory cytokines and chemokines that attract circulating T cells. Antigens can be taken up by local dendritic cells that protrude via finger-like projections through gastric epithelial cells or more likely through dendritic cells that take up the coccoid form of the bacteria in the peyer's patches and prime T-cells. These dendritic cells / induced T cells then follow a chemokine gradient via the draining lymph nodes, to the stomach and induce inflammation, thus controlling colonization. *H.pylori* additionally induces regulatory T-cells during chronic infection.

phagocytose *H.pylori* and either migrate to lymph nodes or prime T cells in the vicinity. Finally, these activated T cells follow a Th1 cytokine and chemokine gradient back to the stomach and in turn produce pro-inflammatory cytokines (Figure 1.1).

1.3.2 Adaptive Immune responses to the bacterium

The adaptive immune response to *H.pylori* is rather complex and in some ways even contradictory. The infection is characterized by a balance of pro- (IFN-γ, TNFα, IL-17, IL-1b, IL-8) and anti-inflammatory responses (Regulatory T cells, T-regulatory type 1 cells and IL-10) [40, 41, 42, 43, 44, 45, 46]. Experimental infection in mice that lack T and B cells (Rag-/-) has shown that the colonization of the bacteria are much higher indicating that these cells play an important role in clearance of the bacteria [47, 48, 49, 50]. On the other hand the same cells also drive inflammation and gastritis, as demonstated by the lack of Rag-/- mice to develop either of those conditions [48]. Once these immune cells are transferred into infected Rag-/-, inflammation levels increase to those even higher than immunocompetent mice and colonization is effectively controlled [51, 48].

1.3.2.1 T-cell mediated responses elicited by *H.pylori*

The predominant immune response to *H.pylori* is driven by T cells. APCs prime T cells to activate and differentiate into diverse subsets. Naive CD4 cells develop into several types, especially in the case of *H.pylori* infection, into Th1 and Th17 although $CD4^+$ regulatory T cells (Treg) expressing the

transcription factor forkhead box P3 (Foxp3) also increase to significant propotions during chronic infection[52, 53, 54]. These Th1/Th17 cells are essential for suppression of bacterial expansion in gastric tissue as demonstrated by multiple knockout studies [55, 56]. Human gastric biopsies have additionally revealed the presence of high numbers of *H.pylori* specific CD4 and cytotoxic CD8 cells during infection [57]. In murine studies, both cell types are important to control colonization as a deficiency of either leads to a bloom in colonization of the stomach [58]. However, most studies have focused on CD4 T cells due to their demonstrated protective capacity on transfer during immunization studies in MHC I deficient mice. CD8 cells alone on the other hand failed to protect mice and induce control of colonization in MHC II deficient mice [59]. Additionally, the prominent role of $CD4^+$ T cells was demonstrated by transfer of splenocytes into severe combined immunodeficiency (SCID) mice infected with *H.pylori*. These animals had a reduced bacterial burden and increased gastritis only when $CD4^+$ T cells were included in the transfer [47].

H.pylori-induced Th1 responses mainly constitute of IFN-γ, TNFα and IL-17. TNFα, however, is dispensable as mice deficient in this cytokine did not demonstrate any changes in gastritis or pathology [60]. IL-17 on the other hand, produced by Th17 cells is very important in neutrophil recruitment and thus in controlling colonization [45, 61]. The cytokine acts directly on epithelial cells and promotes the production of IL-8 [62]. Surprisingly, mice deficient in this cytokine have not only lower inflammation but also decreased colonization levels compared to IL-17 proficient mice. This indicates that IL-17 not only contributes to inflammation and gastritis but may have a role in supporting *H.pylori* colonization [61, 63]. Going in line with this theory, overexpression

of IL-17 with an adenovirus system resulted in higher colonization and inflammation, whereas a neutralizing antibody treatment (αIL-17) led to a reduction in both parameters [61].

However the **most prominent cytokine** in *H.pylori* infection and also one of the most studied cytokines in this context is IFN-γ. IFN-γ expression levels are increased in stomachs of both infected humans and mice [64, 50]. The expression of this cytokine is positively correlated to inflammation and negatively to colonization [56]. IFN-γ-/- mice have been used as a surrogate marker to study lack of Th1 responses during *H.pylori* infection and have provided much clarity on the role of this cytokine. Knockout mice or those treated with an IFN-γ neutralizing antibody show a higher bacterial burden and lesser immunopathology [65, 47, 66, 50]. Additonally, adoptive transfer of IFN-γ deficient $CD4^+$ cells into immunodeficient mice led to milder gastritis [67]. However, transfer of T-bet (transcription factor for inducing IFN-γ) deficient CD4 cells still resulted in gastritis indicating that there may be other sources contributing IFN-γ such as NK cells and CD8 cells in this context [68]. The picture however is not as clear in immunization studies of IFN-$\gamma^{-/-}$ mice. Some studies showed no protection in the absence of IFN-γ while others indicate that these knockout mice are as equally protected as IFN-γ competent mice [65, 66, 56]. Nevertheless, in all cases IFN-γ correlated inversely with bacterial burden, highlighting the important role of this cytokine in controlling colonization. In addition to these, several studies analyzed the interaction and balance of Th1/Th17 responses in *H.pylori* infection. In mice deficient in both cytokines, there was milder gastritis induced compared to mice deficient in only one of the cytokines. As expected, the inflammatory response was different

since IL-17 deficient mice showed a much stronger inflammation than IFN-γ deficient mice [67]. These results collectively highlight that although both cytokines are important in the development of inflammation, IFN-γ, particulary that produced by Th1 cells clearly does so to a higher extent.

While effector cells play an import role in controlling colonization, the outcome is excessive inflammation which is chronic and sustained. In many chronic infections in humans and experimentally in mice, regulatory cells arise or are induced. In *H.pylori* infected humans, high frequencies of $CD4^+$ $CD25^{high}$ $Foxp3^+$ cells are observed, particularly in gastric tissues of patients suffering from gastric adenocarcinoma [52, 69]. $CD4^+$ Treg exist as 'natural' Tregs (nTreg) and 'induced' Tregs (iTreg), defined mainly by where they arise. nTreg arise from developing T cells in the thymus during the process of positive and negative selection whereas iTregs develop in the periphery from naive $CD4^+$ T cells. Under steady state, Treg cells control immune responses to self and foreign antigens by acting directly on effector T cells and therfore avoid autoimmunity by controlling autoreactive immune cells [70]. When exposed to antigen and in the presence of TGF-β and IL-2, naive $CD4^+$ T cells express Foxp3 and attain suppressive capacity. In fact, depletion of this population in mice led to increased gastritis and inflammation, but also to a drop in colonization [71]. Treg cells produce IL-10 and TGF-β in order to suppress inflammation. These cytokines have also been detected in the gastric mucosa of human adults [72]. Interestingly in children, the response to *H.pylori* is rather tolerogenic with high levels of Foxp3 positivite cells [73, 74]. This phenotype is associated with very low level gastritis as both Th1 and Th17 responses are diminished. IL-10 production by Treg has been clearly demonstrated to allow

persistant bacterial growth. Without IL-10, inflammatory responses are increased and colonization decreases [75, 76]. This phenotype is associated with a neutrophil infiltration and subsequent killing of the bacteria. Treg cells are also the major producers of IL-10. It is therefore accepted as a host-protective response where the presence of these cells may lead to higher colonization but it counteracts the deleterious effects of excessive, sustained inflammation.

1.3.2.2 Humoral responses generated

Humans infected with *H.pylori* are known to show strong antibody titres against multiple components of the bacteria. These include flagellin, urease and a very important component of the Type 4 secretion system (T4SS)-Cytotoxin associated gene A (CagA) [77]. However, these titres are not protective as they do not show an effect on the bacteria and have even been shown to inhibit bacterial clearance [78]. There is a positive correlation between antibody levels and protection on immunization but the antibodies by themselves do not seem adequate to induce protection since there was no change in bacterial burden in vaccinated B cell deficient mice compared to controls [78, 79, 80].

1.3.3 Immune evasion of these strategies by *H.pylori*

Inspite of such strong innate and adaptive immune responses, humans are unable to naturally clear *H.pylori*. This is due to multiple strategies employed by this bacterium that enables its survival in the gastric microenvironment (Figure 1.2). While discussion of all the pathogenicity factors and employed

techniques are out of the scope of the topic of this thesis, here, some of the most relevant pathogenicity and immune evasion strategies are highlighted.

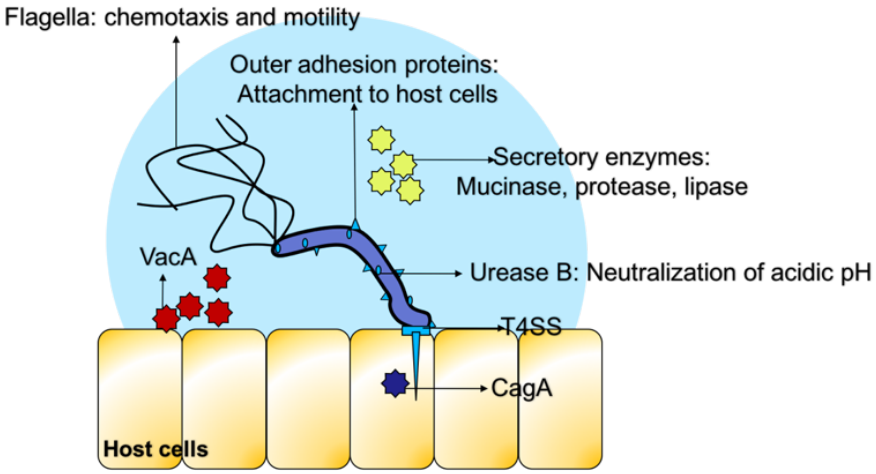


Figure 1.2: Prominent pathogenicity factors of *H.pylori*. *H.pylori* employs multiple strategies in order to arrive at and colonize the stomach mucosa, including Urease B, flagella and adhesion proteins on its surface. It further secretes VacA and injects CagA into epithelial cells through a type IV secretion system (T4SS).

One of the first obstacles that *H.pylori* encounters is the low pH of the stomach. The bacterium produces urease B that converts surrounding urea to ammonia and carbio di-oxide, thus neutralizing the acid around it [81, 82]. Urease also functions to increase the viscocity of the mucous layers, thus enabling better motility. Another factor enabling motility in this environment is *H.pylori* flagella [83, 84]. The bacteria posses about 6 polar flagella comprising of two units: FlaA and FlaB. While these flagellins are essential for reaching the host epithelium, unlike flagella from other bacteria, they do not initiate a pro-inflammatory cascade. Once *H.pylori* reaches the epithelial layer, it avoids immune cell recognition by PRRs by modulation of its surface molecules like flagellin and lipopolysaccharide (LPS) [85]. TLR5, which generally recognizes

monomers of flagellin from S. typhimurium or E.coli, does not get activated by the flagella from *H.pylori* [86]. The bacteria have sheated flagellin, which avoids the release of any pro-inflammatory mediators from these components. Apart from flagellins, which evade the host TLRs, the bacterial LPS protein consists of O-antigens which are recognized as 'self' by TLRs [85]. *H.pylori* also expresses a high abundance of Lewis antigens which are carbohydrates similar to human blood group antigens, contributing to better camouflage [87]. Indeed *H.pylori* LPS is not a strong activator of any of the usual TLRs that recongnize bacteria.

Two of the most imporatant toxins of *H.pylori* are CagA and Vacuolating cytotoxin A (VacA) [88, 89, 90]. CagA is injected into host cells via the cag-T4SS and once inside, is phosphorylated by host Abl and Src kinases. Phosphorylated CagA interacts with a viariety of host cell proteins containing SH2 domains, implicitly disrupting cell signaling pathways and causing cytoskeleton rearrangements [91]. The presence of even unphosphorylated protein is enough to disrupt mechanisms that regulate normal epithelial cell function including cell polarity, cellular tight junctions and inhibition of cell migration [92, 93, 94, 95]. On the other hand, VacA is an enzyme produced by the bacteria and intereferes with endosomal trafficking and prevents formation of a mature phagosome [96]. *H.pylori* therefore avoids phagocytic killing by delaying and reforming phagosomes in macrophages [97]. Additionally *H.pylori* produces catalase and superoxide dismutase that prevents ROS mediated killing [98]. Adaptive immune responses are also tampered with by this bacterium. We and others have demonstrated that it can affect DC maturation and activation, eventually affecting T cell responses. Interestingly, *H.pylori* can also

affect T cell function directly through VacA and g-glutamyltransferase (gGT), the latter deprives the gastric mucosa of glutamine as well as causes cell cycle arrest in T cells [99]. Additionally, *H.pylori* can skew the T cell response from Th1/Th17 to a Treg phenotype by altering dendritic cells to be tolerogenic as well as inducing IL-10 production [37, 100]. This phenomenon has been further addressed in the discussion section of this thesis.

In conclusion, *H.pylori* has developed multiple immune evasion mechanisms that actively avoid or suppress the entire plathora of immune responses including innate, T cell and B-cell directed immunity. Thus the natural response for a majority of infected individuals is mild gastritis as the immune system fights to clear the deceptive bacteria while attempting to preserve host tissue.

1.4 *H.pylori*: Gastric and extra-gastric disease

Most infected individuals do not present with clinal symptoms inspite of a life long persistence of *H.pylori* associated histological gastritis [101, 102]. However, in a significant percentage of individuals, the chronic active gastritis may lead to gastric or duodenal disease. Up to 15% of individuals develop peptic ulcers while 1-2% develop gastric adenocarcinomas [103]. Since *H.pylori* has a causal association with the development of gastric cancer, the World Health Organization (WHO) classified it as a Class I carcinogen in 1994 [104].

1.4.1 Gastric adenocarcinomas

The clinical outcome of disease depends on multiple parameters including the localization of the inflammation. An antrum predominant inflammation results

in increased acid production and consequently a higher concentration of acid deposition in the proximal part of the duodenum [105]. The consequence may be the replacement of duodenal cells with gastric epithelial cells, giving rise to gastric metaplasia, which can be further colonized by *H.pylori* [106]. The entire process may result in duodenal ulcers due to excessive inflammation. On the other hand, a corpus localized gastritis results in a loss of acid secreting cells and thus reduced acid secretion (Atrophy) [107]. These cells are replaced by mucus producing cells and fibrous tissue. This leads to cells developing an intestinal phenotype eventually leading to intestinal mataplasia and progression to dysplasia [108]. These are precursor conditions for the development of gastric adenocarcinoma. The entire progression of precancerous histological changes into development of adenocarcinoma is named the Correa's cascade.

One of the most important and widely studied virulence factors of *H.pylori* in association with gastric cancer is CagA. It is an oncoprotein strongly associated with the development of neoplasms in humans. Infact, the mere presence of CagA is associated with more severe disease [88, 109, 110, 111]. CagA disrupts host signaling and engenders genetic instability. The ratio of worldwide *cagA*-positive to the *cagA*-negative strain is around 6 to 4. However, in East Asia, almost all isolated strains are *cagA*-positive [112, 113]. The risk of developing gastric cancer is atleast one order of magnitude higher in infected individuals harbouring the *cagA*-positive strain compared to those infected with *cagA*-negative strains. This notion is also experimentally supported since infection of Mongolian gerbils with H. pylori containing a functional *cag* PAI induces a corpus-predominant atrophic gastritis, a precancerous condition [114, 115, 116, 117]. The strong oncogenic potential of CagA was

irrefutably demonstrated by the fact that transgenic mice that expressed *cagA* spontaneously, developed gastrointestinal carcinomas [118]. Even though the contribution of other H. pylori proteins such as VacA and the outer inflammatory protein A (OipA), have been implied in development of the disease, CagA plays the central role in gastric carcinogenesis.

H.pylori infection is also associated with the development of mucosal associated lymphoid tissue (MALT) lymphoma. These are B cell lymphomas arising due to chronic inflammation which can be caused by constant antigenic stimulus in the case of *H.pylori* [119]. The permanent inflammatory stimulus may lead to sustained proliferation of lymphoid cells as well as contribution of ROS by neutrophils may induce oncogenesis events, for e.g. resistance to apoptosis mediated cell death which eventually propagates the lymphoma.

1.4.2 Associated extra gastric diseases

Recently associations have been drawn between *H.pylori* infection and various extra-gastric disease like colorectal cancer, neurodegenerative disorders, cardiovascular disease, haematological malignancies, pancreatic and hepatobiliary diseases [120]. The bacterium has been isolated from the gall bladder of human patients with gallstones and *H.pylori* DNA has also been detected from liver samples of patients suffering from primary biliary cirrhosis and primary sclerosing cholangitis [121, 122]. Follow up studies with patients suffering from Hepatocellular carcinoma (HCC) showed possible associations of the disease with *H.pylori* especially dependent on its geographical location [123, 124]. Avenaud et al detected *H.pylori* in the livers of all eight patients enrolled in

a study of HCC [125]. Other studies reported presence of bacteria in the livers of at least half the HCC subjects compared to 10% found in controls [126, 127, 128]. Additionally parallels have been drawn between *H.pylori* positivity and hepatitis infections that lead to cirrhosis [129, 130, 131]. Some authors have additionally reported high *H.pylori* associated antibody titres in patients with primary cirrhosis. However, in all such cases it has been difficult to determine whether the association is due to the changes in the host occurring through carcinogenesis or true effects caused by helicobacter colonization. To our knowledge, there have been no studies, neither epidemiological nor experimental analyzying the effect on helminths that induce pathology and fibrosis in the liver (*Schistosoma mansoni*) and associations of the liver disease with *H.pylori* infection. Studies involving the helminth however have focused on the effects on the development of *H.pylori* associated gastritis as discussed in the next section.

1.5 Modulation of *H.pylori* associated gastritis and immune responses by helminth co-infection

The rates of *H.pylori* infection vary throughout the world but interestingly this does not strongly correlate with the rates of gastric cancer. Countries in Africa that have a very high prevelance of *H.pylori* infection show infact, the lowest rate of gastric cancer (Figure 1.3). Multiple theories have tried to provide an explanation for this paradox and include differences in environmental factors,

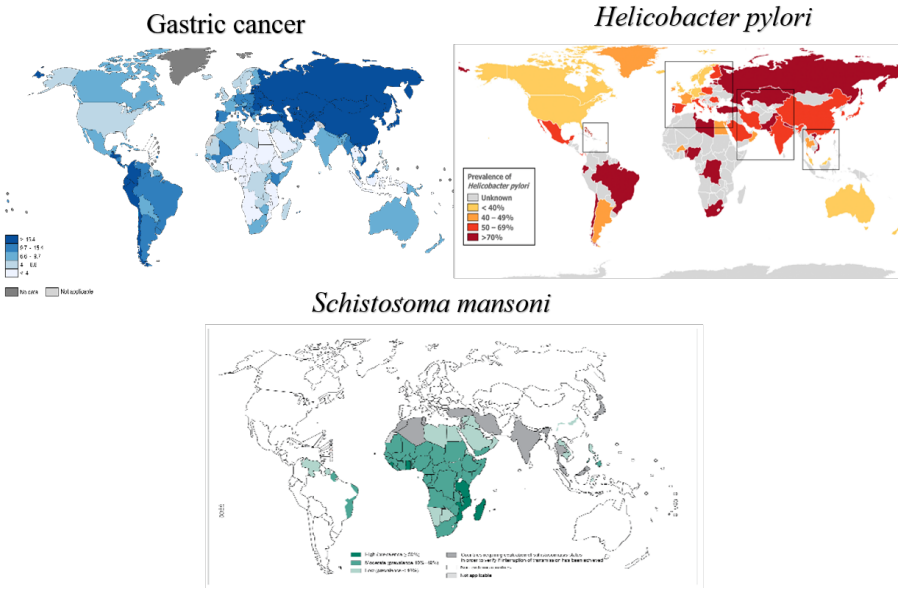


Figure 1.3: Geographical distribution of gastric cancer, *H.pylori* and *S.mansoni*. Map of gastric cancer incidence [132]. Map of *H.pylori* incidence [133]. Map of *S.mansoni* [134].

diet, genetic variance and the limited life expectancy. Yet another hypothesis suggests that co-infections with other common pathogens interfere with the potentially harmful inflammatory responses to *H.pylori* infection. This may be an important aspect to consider as most areas endemic for *H.pylori* show a high incidence of parasitic helminths which induce rather immunoregulatory processes during long term infection (Figure 1.3).

Due to the immunomodulatory abilities of helminths, it was suggested that in a Th1 dominated infection- like with *H.pylori*, a concurrent helminth infection could potentially skew the immune response towards a more protective Th2 phenotype. This would consequently reduce inflammation and decrease the risk of developing gastric cancer. Fox *et al* in 2000 presented a study using a mouse model where the Th1 mediated gastric inflammatory response

to *Helicobacter felis* (*H.felis*) was attenuated by concurrent infection with a murine enteric nematode - *Heligmosomoides polygyrus* [135]. This observation was attributed to the general polarization of the Th2 response by the parasite, ameliorating the Th1 predominant response induced by *H.felis*. Helminth co-infection was associated with milder gastritis, accompanied by reduced transcripts for Th-1 cytokines and chemokines. There was an additional impact on *H.felis*-specific IgG1 and IgG2 titres, in general, co-infected mice had lower IgG titres than *H.felis* mono infected animals. Unfortunately, the nematode is a purely rodent parasite and the helicobacter used in this study rarely ever infects humans unless upon zoonotic transfer.

Nevertheless, this experimental study encouraged epidemiological studies to investigate this phenomenon. Two epidemiological studies observed a positive correlation between co-infected patients with *H.pylori* and *S.mansoni* and protection against gastritis, with decreased nitric oxide production as well as other factors like reduced cell proliferation, apoptosis, decreased pathology and oxyradicals [136, 137]. Additionally, a study in a Chinese population demonstrated that patients co-infected with *H.pylori* and *Schistosoma japonicum* had altered bacteria specific IgG responses which further correlated to protection from gastric cancer [138].

However, much of the data in the field remains controvercial as some studies included individuals with multiple or other helminth infections, not all studies considered the status of CagA positivity of the *H.pylori* strain, which is an important factor in determining risk for development of gastric cancer. **Furthermore, surprisingly, no experimental study has studied the association between *H.pylori* and *Schistosoma mansoni*, one of the**

most common helminths endemic in countries (such as sub-saharan Africa) with the highest rate of *H.pylori* prevalence.

1.6 *Schistosoma mansoni*

Schistosoma mansoni (*S.mansoni*), commonly called the blood-fluke, is a parasitic helminth that infects humans and causes the disease known as bilhazia or schistosomiasis. While helminths include Cestodes (tapeworms), Nematodes (roundworms), Tinea (ringworms) and Trematodes (flatworms), Schistosomes are flatworms that are quite unique. They are dioecious and have distinct sexual dimorphisms between the male and female genders. The most widespread schistosomes are *S.mansoni* and *Schistosoma japonicum* which cause hepatosplenic and intestinal schistosomiasis, as well as *Schistosoma haematobium*, which leads to urogenital schistosomiasis [139].

Schistosomiasis is a very debilitating disease causing about 200,000 deaths every year along with 3.3 million DALYs or disability-adjusted life years. The latter is a measure of the sum of life years lost due to illness and disability as well as premature death. The disease is endemic in 78 countries with about 250 million people infected and 732 million people at risk for infection [140, 141]. Table 1.1 lists the different schistosome species that infect humans as well as their geographical distribution.

While hybridization between members of the *S.heamatobium* species infecting different hosts is common (e.g. a recent outbreak in locals and tourists in Corsica, France of *S.heamatobium/S.bovis*), the same between the two mem-

Species	**Geographical distribution**
Schistosoma mansoni	Africa, Middle East, Caribbean, Brazil, Venezuela, Suriname
Schistosoma japonicum	China, Indonesia, Philippines
Schistosoma malayensis	South East Asia
Schistosoma heamatobium	Africa, Middle East
Schistosoma guineensis	West Africa
Schistosoma intercalatum	Africa
Schistosoma mekongi	Cambodia and the Lao People's Democratic Republic

Table 1.1: Schistosome species infecting humans and their geographical location

bers of *S.mansoni* is rather limited to infections in wild rodents (*S.rodhaini*) [142, 143, 144].

The World Health Organization (WHO) has implemented huge efforts for the last decade e.g. by implementing mass-drug administration programs together with organisations like the Schistososomiasis Control Initiative (SCI) to eliminate the disease, however the complex life cycle and increasing chemotherapeutic resistance have made the journey a difficult one [145, 141].

1.7 Infection and the life cycle of *S.mansoni*

S.mansoni primarily utilizes humans as its definite primary host and the fresh water snail *Biomphalaria glabrata* as the intermediate host (Figure 1.3). The snails are infected by the larval schistosome stage, - the miracidium, that hatches from eggs due to photo and chemical stimuli. These primary larvae

undergo asexual reproduction in the snails and develop into the secondary larval stage, the cercariae, which are capable of infecting human skin. In the snail host, a single miracidium can produce hundreds of cercariae within a few weeks. These free swimming cercariae, stimulated by sunlight, emerge from the snails and on contact with human skin produce proteolytic enzymes that help them penetrate the intact epidermis. Once through, they lose their tails and develop into the next stage- schistosomula which resides in the skin for about twenty-four hours. Soon they migrate through the blood vessels or dermal lymphatic vessels into the lung via the pulmonary artery and reside in this organ intra-vascularly for the next week [146, 147]. The parasites then leave the lung in the direction of the blood flow and mature into male and female worms within the hepatic portal vein about 4 weeks post infection [148]. Finally, the worms pair and migrate to the mesenteric veins, where they reside while the female produces from 300 to a thousand eggs per day [144].

However, a large proportion (almost half) of the eggs do not reach the intestinal lumen and due to porto-venous shunting as well as the general portal blood flow, they are trapped in small venules in the tissue of mainly the liver. The deposition of eggs in these organs increases and the eggs degrade, resulting in inflammation and granuloma formation around the tissue-trapped eggs. This continuous immune response results in the classical pathology associated with chronic *S.mansoni* infection, namley liver fibrosis, hepatosplenomegaly and portal hypertension [149, 150, 151].

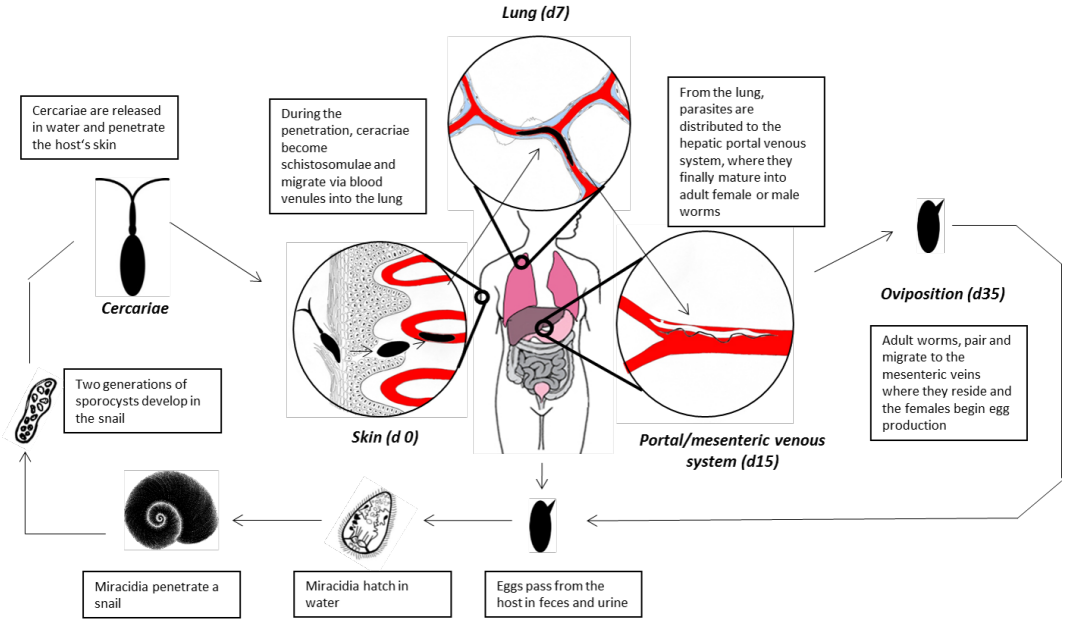


Figure 1.4: Lifecycle of *S.mansoni*. The lifecycle of *S.mansoni* begins with eggs that contain miracidia released from infected individuals with feces into fresh water. On contact with fresh water and in response to light, the free-swimming miracidium hatch out of the eggs and makes its way towards its intermediate snail host. Within the snail the parasite develops into cercariae. The free-swimming cercariae are released from the snail and actively search for their final host where penetration of the human skin is achieved by the use of proteoloytic enzymes. During penetration, the cercariae lose their tail and transform into schistosomula. The schistosomula are transported through the vasculature to the liver. There, they mature into adult worms and pair. The paired adult worms migrate to the mesenteric veins where they reside and begin to lay eggs. Released eggs penetrate the gut lumen and are released into the surrounding by stool passage again. Picture provided by Dr. Eva Loffredo Verde (PhD thesis, 2016).

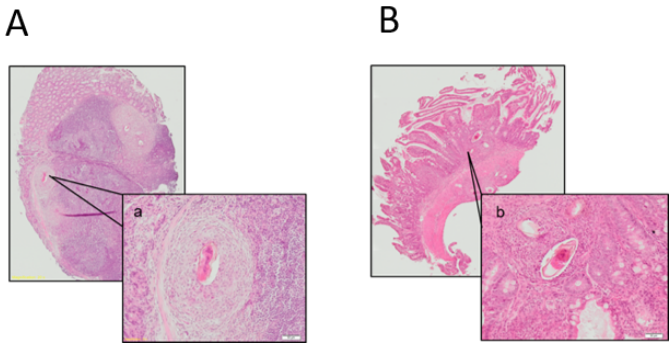


Figure 1.5: Histological sections of eggs in the intestinal tissue. Eggs passing through the peyer's patch and lodged in small intestinal tissue. H&E stained sections, scale bars represent 50 micrometres.

1.8 Immune responses induced by *S.mansoni*

The different developmental stages of the helminth give rise to distinct immune responses. The initial larval stage-cercariae can cause mild dermatitis as it penetrates human skin. A stronger acute response- Katayama's fever can take place shortly after when the schistosomula passage through the lung and develop into immature female and male worms. This is characterized by symptoms such as abdominal pain, fever, diarrhea, intestinal bleeding and development of hepatosplenomegaly [152]. However, this is a very rare occurance but can result in death if not treated immediately. Most individuals in endemic areas remain clinically asymptomatic in the primary stages of infection. The more chronic form of the disease, as mentioned before, arises from tissue trapped eggs, eventually results in severe pathology like fibrosis, anemia and undernutrition among others [153, 154, 139].

1.8.1 Immune phases: Th-1, Th-2 and Regulatory

One of the most interesting and rather unique aspects of *S.mansoni* compared to intestinal helminths is the induction of different immune phases in its host. The helminth drives an impressive switch in the immune response, which can be systemically monitored throughout the infection in a murine model [155]. There is a dynamic shift between the initial Th1 phase to a Th2 response and finally a regulatory immunosuppressive phase [156, 157, 158](Figure 1.6).

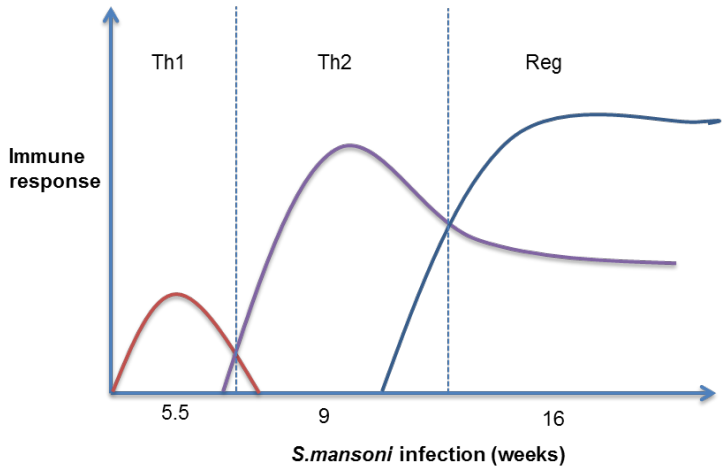


Figure 1.6: Different immune phases induced by *S.mansoni* in its host. Following cercariae penetration, a T_H1-cell response with high levels of IFN-γ production develops (Th1 phase). As the eggs are deposited, a stronger T_H2-cell response with high levels of classical T_H2 cytokines such as IL-10, IL-13 and IL-5 arise (Th2 phase) eventually ensuing in a regulatory immunosuppressive phase 16 weeks post infection (Reg).

1.8.1.1 Type 1 responses to *S.mansoni* infection

The Th1 response is an acute response to both the larval stages passing from the skin through the lungs as well as to the maturation of the worms from a juvenile to the adult stage. Since the primary stages undergo morphological changes, constantly exposing new antigens (along with old ones), and the immediate response is that of (Type 1) inflammation. DCs on contact with the shed tegument from schistosomula for example, upregulate co-stimulatory molecules like CD80 and CD40, which then drive autologous TNFα and IL-12p40 production [159]. These DCs and other innate as well as Th1 cells also induce the production of IFN-γ, the surrogate cytokine for this type of response [160]. Even though chemokines (Th1 and Th2 inducing) are one of the most important molecules in this process, majority of the studies in murine models focus on Th2 chemokines induced by the eggs at later stages. This is

primarily due to the fact that *S.mansoni* and helminths in general are known for their Th2 inducing and immunosuppressive capacities. Additionally, the main pathology of the disease is caused by the decaying eggs that are lodged in tissue.

However, epidemiological data on humans indicate that Th1 cell attracting chemokines like RANTES/CCL5 and MIP1α/CCL3 are induced during *S.mansoni* infection and may play an important role in determining severity to the disease [161, 162, 163]. A caveat here is that all *S.mansoni* infections in endemic areas do not follow a clear Th1/Th2 dichotomy, infection levels differ and reinfection is common [164]. Additionally, the likelihood of finding multiple stages of the parasite due to constant exposure to the parasite's habitat-fresh water, is very high. Although mass drug treatment programs are employed in these areas to eliminate the worms from infected individuals, they are not extremely efficient due to such common re-infection events [145, 141].

1.8.1.2 Type 2 responses induced

Eggs trapped in the liver and intestinal tissue become the focal point of inflammatory infiltrates, which eventually form granulomas; CD4 cells are the main drivers of this response but macrophages, B cells, basophils, mast cells and eosinophils also contribute in a major way [156, 165, 166]. Mice that lack CD4 T cells fail to generate a strong granulomatous response and induce much smaller granulomas that comprise mainly of neutrophils and a few macrophages [167, 168, 169]. In essence, granulomas are a host protective response, sequestering the damage-inducing eggs from surrounding tissue. In

fact, *S.mansoni* infected mice that lack T cells present with increased liver and intestinal necrosis at the onset of the Th2 phase [170].

The Th2 phase in mice is characterized by high levels of IL-13, IL-5, IL-4 and IL-10; the response is directed against the soluble egg antigen (SEA) released through degradation of the egg [171]. This results in an influx of basophils, macrophages, neutrophils and mast cells among other immune cells. Th2 chemokines like CCL22 and CCL11 (eotaxin) are induced by hepatocytes and hepatic stellate cells that attract Th2 cells expressing the corresponding receptors- CCR4 and CCR3.

The Th2 cytokine IL-4 performs a crucial role during schistosome infection [172, 173, 174, 175]. Data suggests that IL-4 supresses pro-inflammatory IFN-γ responses and limits responses to TLR agonists that may be contributed by the intestinal microflora due to diminished barrier function [172, 176, 177]. This results from the passage of eggs through the intestine, permitting an increased access of intestinal microflora or its constituents (LPS) into the venous system. Since mice develop severe intestinal pathology when IL-4 signalling is impaired, as well as that antibiotic treatment can ameliorate this severity, suggests that the microbiota may indeed have an interesting and important role in this context [177].

Th2 responses are strongly linked to the development of hepatic fibrosis, one of the main causes of morbidity in schistosomiasis [178]. While IL-4 is known to stimulate fibroblasts to produce collagen, IL-13 plays the most important role in the pathogenesis of schistosomiasis. Studies have found that IL-4 has a more important role in supressing Type 1 immune responses while IL-13 is the dominant Th2 cytokine regulating fibrosis [176]. The reduction of fibrosis in

IL-4 deficient mice is much lower than that in mice treated with a scavenging antibody to IL-13 [179, 180]. IL-13 has been shown in humans to directly correlate with the level of hepatic fibrosis during schistosomiasis [181]. It can directly stimulate hepatic stellate cells to upregulate expression of collagen I and other important fibrosis associated genes like connective tissue growth factor (CTGF). These cells are the main source of extracellular matrix (ECM) and thus assist in the progression of fibrosis [182].

1.8.1.2.1 Liver microenvironment during the Th2 phase

The liver has an extremely vital metabolic and clearance function that involves amongst others, the uptake of nutrients and filtering waste products and pathogens from the blood. Consequently, its microenvironment is set to prevent organ damage. The unique immunoregulatory function in the liver is mediated by local expression of co-inhibitory receptors and immunosuppressive mediators such as IL-10 or TGF-β during assault on the tissue, and helps to prevent inadvertent organ damage [183]. However, hepatic schistosomiasis is the most common form of the chronic manifestation of the disease. It usually is a consequence of a heavy *S.mansoni* infection burden and results from the granulomatous immune response induced by the host to the schistosomal antigens- (SEA) released by the eggs. The path of the eggs generally leads them to pass into the intestinal lumen and get excreted. However, as mentioned earlier, eggs that do not pass through successfully are carried by portal vein blood flow into small pre-sinusoidal vessels in the liver.

When the egg is released by the female worm it does not cause tissue reactions as it is still immature. In less than a week, the embryo or the miracidium start to differentiate and consequently release antigen secretions or lytic enzymes through miniscule pores in the egg shell. Since this step is initiated in a capillary venule, the vascular endothelium is the first point of contact and damage, subsequently these cells respond by proliferation. The insult to tissue results in an influx of innate immune cells like neutrophils and monocytes/-macrophages [165]. The fat storing hepatic stellate cells (HSCs) in the liver are also important responders in this scenario. They produce chemokines that attract both Th1 and Th2 cells [163]. The resulting IL-4 and IL-5 production further induces the accumulation of other immune cells like eosinophils and basophils further amplifying the Th2 induced inflammation. Simultaneously, the eggs also release factors that directly stimulate liver cells to produce osteopontin, a pro-fibrotic moiety that stimulates HSCs to become myofibroblasts [184, 185]. Myofibroblasts are one of the most potent drivers of fibrosis. To summarize, at this stage the primary type 1 pro-inflammatory response is replaced by a Th2 response which leads to the initial stages of granuloma formation (Figure 1.6).

Macrophages play a very important role in this entire process. These cells are one of the initial responders following injury (egg deposition and degradation) and drive inflammation. In fact, if macrophages are depleted during the initial phase, there is a significant decrease in the inflammatory response [186]. Although, depleting this cell type can also result in an inefficient repair and regeneration [187]. Post this early inflammatory phase, the main populations of macrophages assume wound-healing roles by producing multiple

growth factors like platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF-α) [188, 189, 190]. They additionally stimulate recruited and resident fibroblasts to differentiate into myofibroblasts that synthesize extracellular matrix (ECM) [191]. Finally, monocytes and macrophages enter into an anti-inflammatory role, they respond to IL-10 and express programmed cell death ligands 1 (PD-L1) [192]. The exact mechanisms that direct macrophages to be inflammatory, pro-fibrotic or anti-inflammatory has been a topic of intense research. It is also not clear if a single macrophage can assume all these phenotypes. Additionally, a recent study has highlighted that macrophages that are recruited or differentiated from monocytes have different roles than those resident to the tissue [193]. This was demonstrated using a LysM^{Cre} model (encoding a Cre recombinase enzyme for the lysozyme M-encoding locus (Lyz2). In *S. mansoni* infected IL-4Ra$^{flox/delta}$ LysM^{Cre} mice, recruited macrophages, in contrast to tissue resident macrophages, expressed lower levels of Lyz2 (Lyz2^{lo}) and exhibited restricted deletion of IL-4Ra through LysM^{Cre}. In response to the Th2 cytokines: IL-4 and IL-13, Lyz2^{lo} IL-4Ra^{+} macrophages expressed arginase 1 (Arg1), assumed an alternatively activated phenotype (AAM) and were responsible for downregulating fibrosis. Tissue-resident Lyz2^{high} macrophages on the other hand were identified as the subset of AAMs controlling granulomatous inflammation. AAMs expressing Arg1 are required to survive the initial inflammatory phase (Th1 as well as Th2 mediated inflammation) [194]. Expression of this factor among others like STAT6, in macrophages is important during fibrosis and converts them into an AAM phenotype [195]. The upregulation of Arg1 occurs on direct stimulation through IL-4 and IL-13:IL13Ra1 binding. AAMs also produce resistin like

molecule a (RELMα) and chitinase 3 like protein 3 (Ym1) and are known to play an important role in wound-healing and tissue repair. Arg-1 expressing AAMs compete with T cells for arginine and thereby suppress their proliferation. Along with hepatic stellate cells, AAMs contribute to the production of collagen by converting the acquired arginine to proline and polyamines, the building blocks of collagen. Collagen deposition at this stage is important to limit the extent of tissue damage caused by the developing granuloma and its immune cell infiltrate (Figure 1.7). Consequently, studies in Arg-1 deficient mice infected with *S.mansoni* demonstrated severe intestinal inflammation and portal hypertension [196].

However, since these macrophages are also potent contributors to the process of fibrosis, excessive responses from this subset can result in severe Type 2 mediated fibrosis and exaggerated pathology. Resolving macrophages that respond to IL-10, express both Arg 1 and STAT 3 are important during this phase to downregulate inflammatory and pro-fibrotic responses. They are additionally assited at this phase by Tregs and IFNg producing cells that counter balance Th2 responses (Figure 1.7).

Simultaneous to this process, tissue damage also induces a type 2 response from both innate lymphoid cells (ILC2) that act on an IL-33 signal (from hepatocytes) as well as the T cells primed by Th2-polarized dendritic cells [197, 198]. These cells subsequently release IL-13, which binds to its conventional receptor. This receptors constists of a common IL-4 receptor chain and the low affinity IL-13Ra1. The receptors have been found on B cells, mast cells, eosinophils, fibroblasts, macrophages, endothelial cells and smooth muscle cells. There has been no evidence of the receptor on either human or mouse

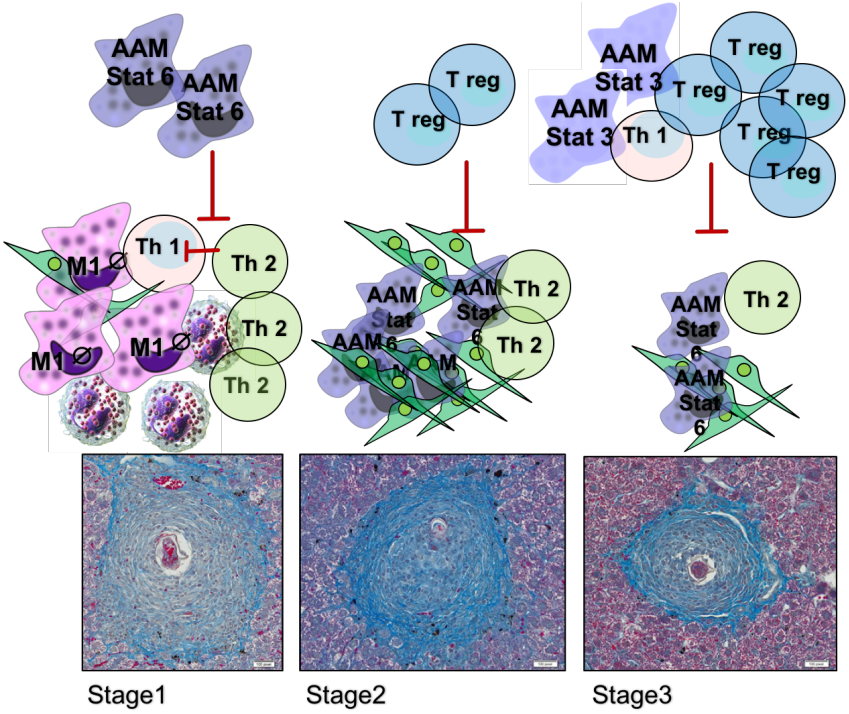


Figure 1.7: Liver microenvironment during granuloma formation. During stage 1, the pro-inflammatory response (influx of neutrophils and monocytes) as well as classical macrophages is controlled by Th2 responses induced by the eggs. However, since excessive Th2 responses are detrimental, AAMs induced by type 2 responses, expressing STAT 6, compete with T cells for arginine and dampen their proliferative capacity. Simulataneously, IL-13 induced by Th2 cells and ILC2s, act on AAMs and fibroblasts promoting collagen depositin and wound healing. At stage 2 these cells proliferate and increase the risk for fibrosis and liver damage, at this point, regulatory cells produce IL-10 as well as supress Th2 cells producing IL-13 (the trigger for precursors of collagen systhesis). Finally, at stage 3, regulatory T cells, AAMs expressing STAT 3, as well as type 1 responses regulate Type 2 responses and limit the extent of the granuloma, thus protecting the host from further tissue damage.

T cells, therefore it is an effector mechanism derived from T cells but do not act on them. Once IL-13 binds to its receptor, through Stat 6 signalling as well as direct interation with hepatic stellate cells, it initiates transcription of critical pro-fibrotic genes like matrix metalloproteinases (MMP) that induce production of collagen I and III as well as tissue inhibitors of matrix mettaloproteinases (TIMP) that slow down extracellular matrix (ECM) degradation

[182]. As collagen replaces health hepatic tissue, there is a progression to a fibrotic liver. The induced liver damage can be clinically estimated either by ultrasonography and/or by examining the levels of alanine aminotransferase (ALT) in the serum since the enzyme is preferentially present in cells of hepatic origin.

The regulation of the IL-13 pathway and thus fibrosis occurs through three main arms: Treg cells that secrete IL-10 and inhibit effector activity of Th2 cells producing IL-13, IFN-γ released by Th1 cells or innate cells which competes with the resources of Th2 cells (e.g. Arginine) as well as inhibits them, finally a decoy receptor- IL-13dRa2 which scavenges IL-13 out of the circulation (Figure 1.8) [199, 179, 200]. This decoy receptor is both found in the soluble form and on cells. It has a short cytoplasmic tail and therefore once it binds to IL-13, it does not initiate downstream signaling [179, 174]. It also has a much higher affinity for IL-13 than its low affinity conventional receptor (IL-13Ra1) [180]. The IL-13dRa2 is absolutely essential to control fibrosis and is upregulated through expression of p-selectin and via positive feedback from IL-13 itself [201]. Additionally, the role of the receptor is evident in knockout studies where IL-13dRa2 deficient mice show a very strong phenotype with marked exacerbation of fibrosis due to enhanced IL-13 activity.

In summary, Th2 responses induced by the decaying eggs limit pro-inflammatory responses while AAMs regulate excessive Th2 responses themselves. However, as AAMs perform this function on stimulation by IL-13 and thereby also produce collagen, in the long term, they induce fibrosis. At this chronic stage, type 1 responses, the levels of IL-13dRa2 and regulatory responses keep the liver damage in check by regulating the IL-13 pathway.

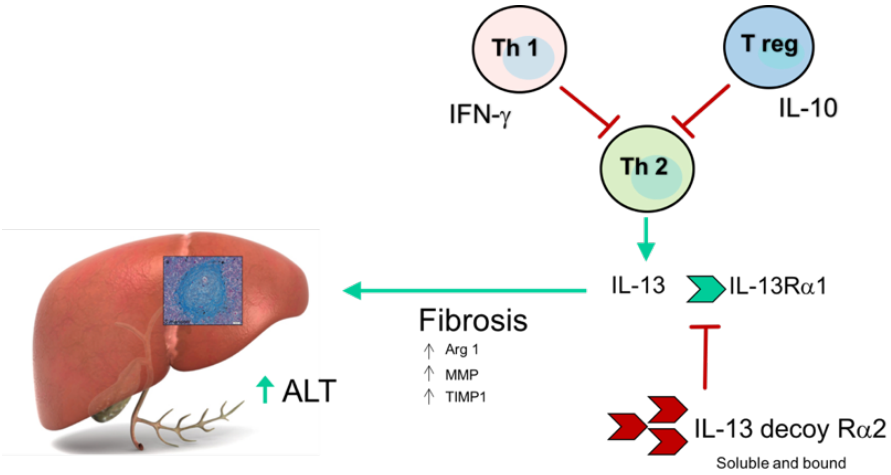


Figure 1.8: Regulation of the IL-13 pathway. IL-13 is contributed by Th2 cells and ILC2s, it binds to the IL-13Ra1 receptor and through upregulation of Stat6, induces expression of pro-fibrotic factors (Arg1, MMP, TIMP1) in both macrophages and fibroblasts. This results in production of collagen that replaces injured hepatic cells, resulting in higher levels of ALT as the enzyme is released into blood from degrading hepatocytes. The pathway is regulated by Th1 cells and regulatory cells that act on the source of IL-13 as well as the levels of soluble and bound IL-13dRa2, which competes for IL-13 with its classical receptor but does not intitiate downstream signaling.

1.8.1.3 Immune evasion and modulation strategies

S.mansoni, like *H.pylori* has also co-evolved with its human hosts and developed multiple strategies to achieve long-term persistence.Starting already at the larval stages, these parasites can produce or induce host production of eicosanoids like Prostaglandin E_2 (PGE_2) that aids in the release of IL-10 by host cells [202, 203]. Regulation through parasite derived PGD_2 can also be IL-10 independent as it acts on receptors on the dendritic cells in the skin (Langerhans cells) and inhibits their migration [204]. This in turn would delay subsequent priming of T cells in the draining lymph nodes. Adult worms can coat themselves with host antigens to avoid detection, further they release metalloaminopeptidases that have an enzymatic trypsin like activity and can

cleave bound antibodies on their surface [205]. However, the most highlighted immune evasion or rather, immune-modulation strategy of these helminths is the (egg's) ability to modulate dendritic cells in order to induce Th2 responses and the induction of Treg cells that supress inflammatory responses.

1.8.1.4 Regulatory responses

Immunoregulatory cells such as Treg and Bregs dampen overall effector functions of $CD4^+$ T cells but these functions are attributed to distinct populations of regulatory T cells and their soluble products [206]. Even though there are a variety of regulatory cell subtypes, $CD4^+CD25^+Foxp3^+$ T cells remain the most prominent of these during *S.mansoni* infection [207, 208].

Treg are absolutely essential during murine schistosomiasis since studies demonstrated their role in controlling gross immunopathology in addition to general effector T cell responses [209]. Even though these cells cannot prevent helminth infection, they serve to stand as a compromise between the parasite and the host. On one hand, they preserve host tissue from the onslaught of continuous pro-inflammatory processes and on the other they dampen effector responses to the helminth, ensuring its survival [210, 211]. Additionally, *ex vivo* isolated Treg specifically suppressed antigen-specific $CD4^+$ T cells from infected mice in an IL-10 independent manner [212]. These Treg upregulated genes such as granzyme B (GrzB), and the homing marker CD103, indicating that their phenotype changes during infection [210].

Treg secrete immunosuppressive cytokines such as IL-10 and TGF-β, the former promotes the down regulation of pro-inflammatory cytokine production

by effector cells and TGF-β leads to cell-cycle arrest and therefore decreased effector cell proliferation. Several mouse studies have highlighted the importance of IL-10 using infected mice deficient in the cytokine [213]. These mice showed an excessive T_H1- and T_H2-type mixed immune response during the chronic phase of infection, which led to severe granulomatous liver damage and increased mortality [200, 214, 215]. Additionally, Tregs that express high levels of cytotoxic T-lymphocyte antigen 4 (CTLA-4) on their surface interact with APCs inducing a down regulation of co-stimulatory molecules such as CD80/CD86 and thereby limiting the activation of other T cells by these APCs [216]. While natural Tregs mainly produce immunosuppressive IL-35 and compete with effector T cells for IL-2, induced Treg kill effector T cells and APCs by cell-to-cell contact in a granzyme- or perforin-dependent manner [216, 70].

However, suppression induced by Treg during helminth infection is not directed only toward the parasite. Although antigen specific Tregs are induced during schistosomiasis, general immunosuppression possibly leads to bystander responses that can hamper other active and necessary immune pathways especially those induced to fight a new acute infection or generate vaccine responses.

1.9 Bystander effects of *S.mansoni* associated immune responses

S.mansoni can suppress a wide range of bystander immune responses, which can range from immunopathogenic to protective in nature. Immunopathologies

such as asthma, autoimmune diseases and inflammatory diseases are reduced in prevelance in areas where helminth disease is rampant [217]. Even direct effects of helminth infections have been demonstrated on suppression of IBD symptoms leading to multiple clinical trials in this field [218, 219]. On the other hand, antitumour immunity and vaccine efficacy may be suppressed by helminth infection as well (Figure 1.9).

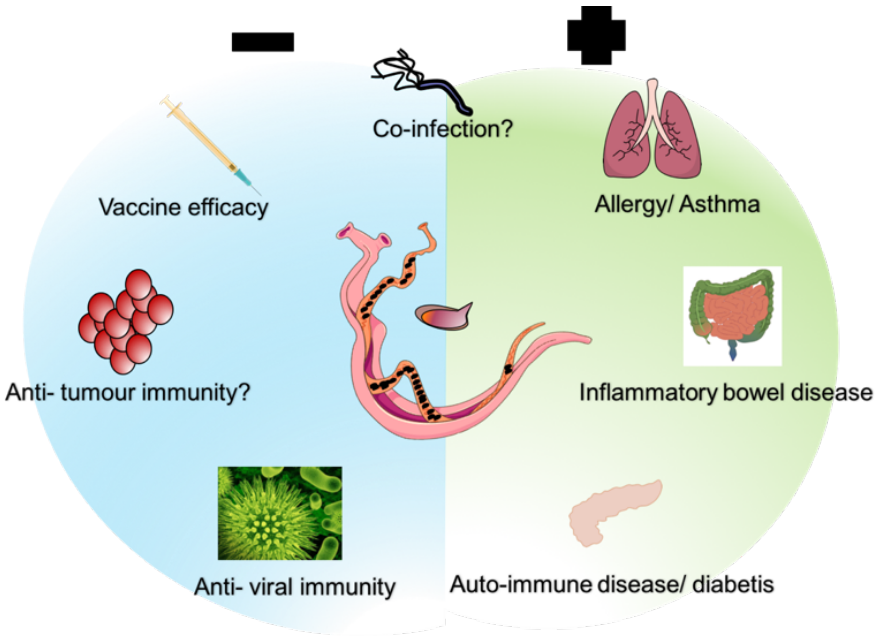


Figure 1.9: Immunoregulatory effects of helminths on bystander responses. Schistosomes can suppress a wide range of bystander immune responses, including those of both immunopathogenic and protective natures. Antitumor immunity and vaccine efficacy may be suppressed by schistosome infections. However, immunopathologies such as asthma, autoimmune diseases, and inflammatory bowel diseases are all reduced in prevalence in areas where helminth disease is endemic, and direct effects of helminth infections on the suppression of disease have been shown in clinical trials for inflammatory bowel diseases.

Here, I have highlighted the fields in which much work has been done to examine the mechanisms leading to generalized, by-stander immune regulation.

1.9.1 Allergy and vaccination

Allergic asthma is one of the most common diseases arising in the recent years, especially in industrialized countries with a prevalence of 29% [220]. However, studies have shown that individuals living in helminth endemic areas show a milder response to the classical skin-prick test to aeroallergens. Additionally, in murine models of Ovalbumin-induced allergic airway inflammation (AAI), schistosome infected mice could suppress airway hyperresponsiveness (AHR) [221, 222]. These mice presented with lower numbers of eosinophils and leukocytes in their bronchialveolar lavage (BAL) fluid. The mechanisms suggested include suppression of IgE responses and induction of high Treg frequencies as well as IL-10. Moreover, Straubinger et al have also demonstrated that this is not just limited to the infected mothers but this immunosuprresive phenotype is transferred to the uninfected offspring as well. While this phenomenon is clearly dependent on the immune phase of the helminth, the Th1 and chronic phase show a suppression of AHR [155].

As the immunosuprresion seems to be more 'systemic' and general, this positive association with prevention of asthma is unfortunately accompanied by lack of an efficient response to vaccination in populations endemic for helminths. A strong efficient immune response to a primary antigenic stimulus is the basis for long lasting immune memory. However, studies that analyzed Hepatitis B virus (HBV) vaccine efficacy in Schistosome infected individuals in Brazil and infants born to infected mothers in Egypt observed that these individuals responded less prominently to the classic HBV vaccination [223, 224]. Recently, a study also demonstrated that while individuals infected

with *S.mansoni* may develop sufficient titres to primary HBV vaccination, this does not result in memory responses[225]. In fact, the titres drop to insufficient levels within 1 year of the vaccination. Similar results have been observed in studies analyzing the efficacy of the the Bacille-Calmette-Guérin (BCG) vaccine [225]. This is especially important because these contries have the highest endimicity for the above-mentioned diseases. In conclusion, the bystander 'protection' from asthma induced by schistosomes comes at a cost of possibly 'inefficient' responses to vaccination.

An important factor to consider here is that even today large amounts of funds are invested in developing new vaccines for bacterial pathogens developing resistance to antibiotics. Especially since co-infection with helminths was not considered thoroughly when initial vaccination strategies were developed, today with increasing awareness about the immunomodulatory capacities of these parasites, this is possible. **However current strategies for developing effective prophylactic vaccination (e.g. for *H.pylori*) to our knowledge do not take this factor into account.**

1.9.2 Co-infection with other pathogens

Many countries that are endemic for schistosomiasis also naturally have a high prevelance of bacterial infections, viruses and protozoa, making co-infection a common occurance [226]. Many factors determine the outcome of this co-infection including the order and time interval between the first and the second infection, re-infection, dosage of the infectious agents, strains and pedigrees of the parasites and age of hosts at time of exposure to the infectious

pathogen. Overall, a prior infection with schistosomes, particularly a long-standing patent infection, could have an effect on a subsequent infection by a protozoan, bacterium or other helminth, a highly likely and requent event in endemic countries. In relatively few experimental models, a concomitant infection with schistosomes reduced the severity of the subsequent infection with *Fasciola hepatica* and/or *Echinostoma*. More often, however, a prior infection with *Schistosoma* increased the severity of the second infection as demonstrated with experimental *Leishmania*, *Toxoplasma gondii*, *Entamoeba histolytica*, *Staphylococcus aureus* or *Salmonella* infection [227]. In some of these infections, it was implicated that co-infection induces a more prolonged form of the disease. With respect to viral co-infections like lymphocytic choriomeningitis virus (LCMV), Hepatitis B and C, the literature is quite contradictory. In the case of LCMV, the dominant Th1 character of the viral infection supresses schistosome induced Th2 cytokine responses and thus the pathology associated with it [228].

Since the liver is a tolerogenic organ, it makes an excellent site for viral infections. One of the main complications of viral hepatitis is the chronicity that develops, especially in countries endemic for helminths infections. The immunosuppressive capacity of *S.mansoni*, especially in the liver has been discussed to be the main reason for dampening anti-viral responses. For Hepatitis C virus infections, it has been shown that that *S.mansoni* co-infected individuals have higher rates of cirrhosis and progression to hepatocellular carcinoma (HCC) [229, 230]. They additionally have higher fibrosis scores and are rather unresponsive to interferon therapy [231, 232]. Hepatitis B virus co-infections however, show a different picture. Due to the lack of suitable

animal models, the associations between *S.mansoni* and HBV have been analysed only epidemiologically. Nevertheless, the results are controversial: some studies demonstrate that co-infection with these two pathogens led to prolonged viremia and worsening of liver damage [233]. For example, in Egyptian, Brazilian and Saudi-Arabian field studies higher prevalence of HBV infection and an aggravation of hepatic disease was observed in *S.mansoni* infected patients [234, 228, 235]. On the other hand, other surveys from China, Yemen and even from Egypt again revealed no difference in HBV infection rates or development of chronicity in schistosome co-infected patients [236, 237, 238]. However since *S.mansoni* has different immune phases and strong systemic responses, it is likely that different immune phases determine the outcome of acute or chronic hepatitis B. We have indeed analysed this experimental co-infection utilizing a novel co-infection model. Our observations suggest that phase dependency is indeed an important factor to determine outcome of the disease but so is genetic predisposition (*manuscript submitted*).

However, as mentioned before, experimental studies with *H.pylori* and *S.mansoni* have not been performed.

1.10 Aims of the study

Since *H.pylori* induces a strong Th1/Th17 driven inflammatory response, helminths like *Schistosoma mansoni* (*S.mansoni*) that drive a chronic Th2/Treg response, have been suggested to skew this classical inflammatory response. However, *S.mansoni* infection gives rise to different immune phases during its distinct developmental stages within the human host. The Th1 phase, with

high levels of IFN-γ followed by the Th2 phase, where systemic IFN-γ levels drop giving rise to high levers of Th2 cytokines and finally the regulatory phase with increased Treg cells and IL-10 production. These distinct immune phases may have a different impact on the immunopathology of the co-infecting bacterium- H.pylori. On the other hand, recent evidence has suggested that *H.pylori* may be associated with a variety of extra gastric diseases as well, including liver disease. However even though schistosomiasis is endemic in 78 countries with a high prevalence of *H.pylori* infection, the very likely interaction of both diseases has not been studied in any experimental model. Therefore the aim of this study was to approach the coinfection of these two pathogens that occupy anatomically distant sites in an un-biased experimental manner. We wanted to answer the following two main questions:

1. Does *S.mansoni* infection alter the colonization and inflammation induced by H.pylori in the stomach? If so, is this dependent on the different immune phases elicited by the parasite?

2. Does H.pylori affect the immunopathology induced by *S.mansoni* in the liver? If so, what is the mechanism?

2 Material and Methods

2.1 Materials

2.1.1 Equipment

Cyan ADP Lx P8	DakoCytomation
Cytoflex^{TM} flow cytometer	Beckman Coulter
ELISA microplate reader (Sunrise^{TM})	Tecan
MoFlo^{TM} XDP	Beckman Coulter
NanoDrop® 1000 Spectrophotometer	Thermo Scientific®
Thermocycler (T3000)	Biometra
Biorad CFX	Biorad
Vi-CELL® Cell Viability Analyser	Beckman Coulter

2.1.2 Software

FlowJo v10 (Flow cytometry analysis software)	TreeStar
GraphPad Prism 5 and 7 (Biostatistics, curve fitting and scientific graphing programme)	GraphPad Software
Magellan^{TM} (Data analysis software for microplate reader)	Tecan
Nanodrop® 1000 V 3.7.0	Kisker

2.1.3 Reagents

Acid fuchsin	Morphisto
Ammonium chloride (NH4Cl)	Roth®
Aniline blue	Morphisto
Brefeldin A (5mg/mL in DMSO)	Sigma-Aldrich
Bovine serum albumin (BSA)	PAA
Bromophenol blue	Roth® , Sigma®
Chloroform	Roth®
Collagenase (from *Clostridium histolyticum*)	Sigma®
Collagenase Type IV (240 u/mg)	Worthington®
Cytofix/Cytoperm reagent	BD
Deoxynucleoside triphosphate (dNTPs)	Promega
Deoxyribonuclease I from bovine pancreas (DNAse)	Sigma®
Dithiothreitol (DTT)	Roth®
Dulbecco's PBS (Endotoxin-free)	PAA
Ethidium monoazide bromide (EMA)	Invitrogen
Eosin 1% (v/v)	Morphisto
Ethanol 70%-99.8% (v/v)	MRI Pharmacy
Ethidium bromide	Roth®
Ethylenediaminetetraacetic acid (EDTA)	Roth®
Formaldehyde solution 37% (v/v)	Sigma® , Merck
Gentamicin (10 mg/ml)	PAA
Hydrogen chloride (HCl)	Roth®
4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)	Sigma®
Light Cycler® 480 Probes Master	Roche
Haematoxylin	Morphisto

Methanol	Roth®
β-Mercaptoethanol	Roth®
Narcoren®	Merial
Paraformaldehyde (PFA)	Sigma®
Percoll^{TM}	GE Healthcare
Periodic acid	Morphisto
Perm/Wash Solution	BD
Phenol	Roth®
Phosphate buffered saline (PBS)	MRI
Phosphomolybdic acid	Morphisto
Potassium hydroxide (KOH)	Merck
Powdered PBS (Phosphate buffered saline)	Biochrom
RNAlater®	Ambion®
Roti® -Histofix 4% (v/v)	Roth®
Schiff reagent	Morphisto
Sodium chloride (0.9% v/v NaCl)	B Braun
Sulphuric acid (H_2SO_4)	Merck
3,3',5,5'-Tetramethylbenzidine (TMB) substrate	Thermo Fischer
Tris(hydroxymethyl)aminomethane (Tris)	Merck, Roth®
Trisodium citrate dihydrate	Roth®
Trypan blue solution 0.4% (v/v)	Sigma®
Tween® 20	Sigma®

2.1.4 Medium supplements

Fetal calf serum (FCS)	PAA
β-Mercaptoethanol for cell culture	Gibco®

Non-essential amino acids (100x)	PAA
Penicillin/Streptomycin (100x)	PAA
RPMI 1640 (with L-Glutamine)	PAA
Sodium pyruvate solution (100 mM)	PAA

2.1.5 Reagents for bacterial culture

Brucella broth (BB)	Oxoid
Brain heart infusion (BHI)	MP Biomedicals
Wilkins Chalgren (WC)	Invitrogen
Horse serum	Invitrogen
Dent supplement	Oxoid
Special supplement (200mg/l Bacitracin, 10mg/l Nalidixic acid, 3mg/l polymixin B)	

2.1.6 Kit systems

DNeasy stool isolation Kit	Quiagen
Foxp3 staining buffer set	Affymetrix
GoTaq® DNA polymerase (including 5x Green GoTaq® reaction buffer)	Promega
Mouse ELISA Kits (Duo Set® ; IL-13, IL-10)	R&D
Mouse ELISA Kits (Ready-Set-Go)	Affymetrix
IFN-γ,	
IL-4, IL-5	
QuantiTect Reverse Transcription Kit	Quiagen
QIAmp DNA Stool Mini Kit	Quiagen
RNeasy Mini Kit	Quiagen
Taq buffer	Invitrogen
T cell activation/expansion Kit (mouse)	Miltenyi Biotec

2.1.7 Antibodies

Table 2.1 depicts all antibodies used for this study. Commercial ELISA antibodies from the Affymetrix and R&D ELISA-Kits are not included.

α-mouseCD3 (Clone 17A2)	Affymetrix
α-mouse CD4 PE-TexasRed (Clone RM4-5)	Life Technologies
α-mouse CD4 Pe-Cy7 (Clone RM4-5)	Affymetrix
α-mouse CD4 APC (Clone RM4-5)	
α-mouse CD8 FITC (Clone 53-6.7)	Affymetrix
α-mouse CD8 APC-H7 (Clone 53-6.7)	BD
α-mouse IL-4 APC (Clone 11B11)	Biolegend
α-mouse IL-10 FITC (Clone JES5-16E3)	Affymetrix
α-mouse Foxp3 eF450 (Clone FKJ-16s)	Affymetrix
α-mouse IFN- PE (Clone XMG 1.2)	Affymetrix
α-human Granzyme B FITC (Clone GB11)	Affymetrix
α-mouse CD3e (Clone 17A2)	Affymetrix
α-mouse CD28 (Clone 37.51)	Affymetrix
α-mouse CD45.1$^+$ APC (Clone A20)	Thermo Fisher
α-mouse CD45.2 FITC (Clone 104)	Thermo Fisher

Table 2.1: Antibodies used for FACS.

2.1.8 Buffers and solutions

All buffers and solutions were prepared with Millipore Q distillated water.

2.1.8.1 Buffers and solutions for egg preparation

Vancomycin solution: 500 mg Vancomycin hydrochloride

.	10 ml	0.9% NaCl (w/v)
Collagenase solution:	500 mg	Collagenase
.	5 ml	Dulbecco‘s PBS (1x)
DNase solution:	1 g	DNase I
.	146 ml	Dulbecco‘s PBS (1x)
Egg-PBS solution:	1x	Dulbecco‘s PBS (1x)
.	0.1% (v/v)	Vancomycin solution
.	0.5% (v/v)	Gentamicin
Liver digestion solution:	25 ml	Egg-PBS solution
.	1 ml	Collagenase solution
.	3 ml	DNase solution
.	500 μl	Penicillin/Streptomycin
Percoll solution:	8 ml	PercollTM
.	32 ml	0.25M Sucrose

2.1.8.2 Buffers for erythrocyte lysis

ACT buffer:	17 mM	Tris
.	160 mM	NH_4Cl
.	pH 7.2	.

2.1.8.3 Buffers for liver-associated lymphocytes isolation

Liver digestion solution:	10 mg/liver	Collagenase Type IV
.	12,5 ml/liver	RPMI + 10% (v/v) Penicillin/Streptomycin
Percoll solution:	8 ml	Percoll^{TM}
.	32 ml	0.25 M Sucrose

2.1.8.4 Buffers and solutions for FACS

FACS buffer:	1x	PBS (pH 7.2-7.4)
.	2% (v/v)	FCS
Fc block solution:	1x	FACS buffer
.	0.1% (v/v)	α-mouse CD16/32

2.1.8.5 Buffers and solutions for ELISA

Reagent diluents:	1x	PBS (pH 7.2-7.4)
.	1% (w/v)	BSA
Washing buffer:	1x	PBS (pH 7.2-7.4)
.	0.05% (v/v)	Tween® 20
.	Stopping solution:	2 M H_2SO_4

2.1.8.6 Cell culture medium

Complete medium:	1x	RPMI 1640
.	10% (v/v)	FCS
.	1% (v/v)	Penicillin/Streptomycin
.	1% (v/v)	NEAA
.	1% (v/v)	Sodium pyruvate
.	0.1% (v/v)	β-Mercaptoethanol for cell culture

2.1.9 Primer sequences

2.1.9.1 Primer for *S.mansoni* specific qPCR

Forward tandem repeat (FP): 5'CAACCGTTCTATGAAAATCGTTGT 3'

Reverse tandem repeat (RP): 5'CCACGCTCTCGCAAATAATCT 3'

Dual labeled probe tandem repeat (labeled probe) with 5' and 3' modifications: 5'[6FAM] TCCGAAACCACTGGATTTTTATGAT[BHQ1] 3'

2.1.9.2 Primers for rt-PCR using SyBr green

Name	Forward	Reverse
IFN-γ	TCAAGTGGCATAGATGTGGAAGAA	TGGCTCTGCAGGATTTTCARG
TNF-α	CGATGGGTTGTACCTTGTC	CGGACTCCGCAAAGTCTAAG
MIP-2	AGTGAACTGCGCTGTCAATGC	AGGCAAACTTTTTGACCGCC
Foxp3	AGGAGCCGCAAGCTAAAAGC	TGCCTTCGTGCCCACTGT
CCL5	CAC CAC TCC CTG CTG CTT	ACA CTT GGC GGT TCC TTC
GAPDH	GCCTTCTCCATGGTGGTGAA	GCACAGTCAAGGCCGAGAAT
Hprt	GCCGAGGATTTGGAAAAAGT	TATAGCCCCCCTTGAGCACA

Table 2.2: Primers for rt-PCR using SyBr green.

2.2 Methods

2.2.1 Animals

2.2.1.1 Mouse strains and housing

C57BL/6 mice were obtained from Envigo (Germany) and used for all experiments. NMRI mice were acquired from Janvier (France) for the purpose of life cycle maintainance of *S.mansoni* (see section 2.2.3). Both strains were housed at the Institute of Medical Microbiology, Immunology and Hygiene (MIH), TU Munich. All mouse strains were bred and maintained under specific pathogen-free (spf) conditions in the animal house of the MIH. Experimental mice were sex- and age-matched and the experiments were performed in accordance with

local government regulations (license number for animal testing 55.2.1.54-2532-85-16).

2.2.2 *H.pylori* strain, culture and infection

H.pylori strain PMSS1 (*H.pylori* pre mouse Sydney strain 1) was used in all experiments in this thesis, both in-vitro and in-vivo. Bacteria were plated on Wilkins Chalgren (WC) blood agar plates supplemented with Dent (Oxiod) and cultured in a microaerophilic (5% O_2, 10% CO_2) humidified incubator at 37°C; split every two days. Bacteria were stored in aliquots in freezing media containing BB-Dent, 20% FCS and 20% glycerol at -80°C and freshly thawed and cultured for each infection to obtain motile and viable *H.pylori*. For infection of mice, animals were starved for 5 hours and then orally gavaged with 2 x 10^8 bacteria/0.2ml (prepared as stated above) on alternate days for a week.

2.2.3 *S.mansoni* lifecycle maintenance

NMRI mice were used as mammalian hosts to maintain the life cycle of *S.mansoni*, mice were infected every third week intraperitoneally (i.p.) with 140-200 cercariae of a Brazilian strain of *S.mansoni*, which were acquired from *Biomphalaria glabrata* snails after exposure to light and a temperature of 31°C. 8 weeks post infection, these mice were sacrificed and their intestines were digested to isolate miracidiae, which were then used to infect new snails to continue the infectious cycle. In addition, livers of infected mice were used for the preparation of eggs and SEA (section 2.2.4).

2.2.4 SEA preparation

2.2.4.1 Egg preparation from liver tissue

Fresh eggs were prepared from liver tissue, NMRI mice were sacrificed 8 weeks post *S.mansoni* infection. Livers (excluding gall bladder and bile ducts) were washed in 1.2% (v/v) NaCl solution. Subsequently livers were minced and transferred into a 50 ml falcon containing 25 ml liver digestion solution (Collagenase) along with 25ml PBS and incubated under continuous agitation at 37°C overnight. Following this, digested livers were washed twice by centrifugation at 400/5 min at 4°C and the remaining pellet was re-suspended in 25 ml PBS and filtered twice through a 250 μm sieve. The filtrate layered on a Percoll gradient and centrifuged at 800 g for 10 min at 4°C, to finally separate the eggs from liver tissue. Eggs were washed three times with 15 ml and 30 ml of 1 mM EDTA solution, respectively, and finally with 30 ml PBS. After these washing steps, the egg pellet was re-suspended in 700 μl PBS and an aliquot of 5 μl were microscopically analysed (x10 magnification) to confirm the purity and to calculate the total egg count with the following formula:

$$\text{Total egg count} = (\text{counted eggs} * 700)/5\mu\text{l}$$

Finally, the isolated eggs were frozen at -80°C or used directly for SEA preparation. Contaminations of the obtained egg suspension were analysed by standard microbiological assessments on blood – and MacConkey agar plates containing 2 μl of the egg suspension (37°C for 48 hours).

2.2.4.2 SEA preparation from liver-derived eggs

The egg suspension was transferred into a glass homogenizer and estle for at least 25 min on ice. During this procedure, the outer egg shells were destroyed and a mixture of different glycoproteins and lipids were released, this protein mixture is known as the soluble egg antigens (SEA). To separate the egg shells from the SEA, an ultracentrifugation step at 100,000 g for 1h (4°C) was performed. The supernatant, containing the SEA, was then collected and transferred to a cryotube, whereas the remaining egg shell pellet was re-suspended in 200 μl PBS. Possible contaminations were determined by plating 2 μl of both suspensions on a blood - and MacConkey agar plates (37°C for 48 hours). The protein concentration SEA and the egg shell pellet solution was measured by using the DC protein assay kit (Bio-Rad) according to the manufacturer's instructions. Both suspensions were then frozen at -80°C.

2.2.5 Experimental Helicobacter pylori and Schistosoma mansoni co-infection

To determine differential cytokine responses induced during the different immune phases of *S.mansoni* infection, C57BL/6 mice were infected with *S.mansoni* by injection of 180 cercariea s.c. Immune responses were analysed at week 4, 5, 5.5, 7.5, 8, 16 and 22 weeks post infection. These experiments were performed with Dr. Eva Loffredo Verde. Based on the peak of the Th1 response and the Th2 response, we infected mice with *H.pylori* first, waited for colonization to stabilize, followed by *S.mansoni* infection at 5.5 weeks. These animals were

Figure 2.1: Infection scheme of the co-infection model.

then analyzed at 5.5 weeks post *S.mansoni* infection for the Th1 phase, 9 weeks for the Th2 phase and 16 weeks for the regulatory phase (Figure 2.1).

2.2.5.1 Evaluation of *H.pylori* infection

The animals were sacrificed and the stomach was removed. The organ was cut along the lesser curvature and gently washed in PBS. A small piece of pre-washed stomach was cut out longitudinally with a scalpel and weighed (approx. 20mg). This piece was placed in 1ml of BHI medium and homogenized. Three dilutions (1:10, 1:100, 1:500) of this suspension were plated onto on Wilkins Chalgren (WC) blood agar plates supplemented with Dent and special supplement, cultured in a microaerophilic (5% O_2, 10% CO_2) humidified incubator at 37°C. After 6 days, the plates were taken out and the colony forming units (cfu) were counted for all plates. Based on the initial weight of the piece, the cfu was extrapolated for the stomach. The same section was taken from every stomach in order to reduce sampling error.

2.2.5.2 Evaluation of *S.mansoni* infection

The first scoring was performed qualitatively by the degree of infection (DOI) and organ damage. In essence, liver was analyzed for visible granulomas,

differences in coulour and signs of fibrosis. The infection status was graded on a scale from 0 to 3 DOI with 3 denoting the highest level of infection.

2.2.5.2.1 *S.mansoni* egg count analysis within liver Pre-weighed liver samples from each mouse was digested in 5 ml of 5% (w/v) KOH solution under continuous agitation at 37°C for 2 hours. Post incubation, the released eggs were centrifuged at 400 g for 10 min and most of the supernatant was immediately removed. 10 μl of the re-suspended remaining eggs (within 500 μl) were counted trice using a microscope and the number of eggs in the liver was calculated as indicated below:

Eggs/mg tissue = (average of counted eggs$*$50)/weight of tissue sample (mg)

2.2.6 Histological methods

H.pylori infection in the stomach is characterized by infiltrating immune cells. The preparation of the stomach was performed as indicated in section 2.2.5, one of the longitudinal sections was wrapped around a small wodden piece (in order to maintain the form) and placed in a histological cassette. Infection with *S.mansoni*, on the other hand, is characterized by chronic local inflammatory responses to tissue-trapped schistosome eggs within the liver and intestine. For the present study, liver granuloma formation and infiltration of immune cells within granulomas were analysed using histological sections.

2.2.6.1 Tissue preparation for histological staining

The stomach piece and left liver lobe from each individual mouse was fixed in Roti® -Histofix (4%), dehydrated using the Shandon Excelsior ES tissue processor and embedded in paraffin using the TB 588 paraffin embedding system. 2 μm sections were cut out using the RM 2245 automatic rotary microtome and fixed on glass slides for staining techniques

2.2.6.2 Staining techniques

For analysis of granuloma formation and immune cell infiltration within the liver, trichrome Masson‘s blue was performed whereas the stomach was analyzed using heamatoxylin/eosin (HE) staining. Additionally, Immunohistochemistry (IHC) was performed on stomach tissue samples. The trichrome Masson‘s blue stain was used to differentiate between smooth muscle and collagen in tissue. It also enables the visualization of the increase in collagen during granuloma formation, this is due to the aniline component in the staining buffer that turns collagen blue. Liver sections stained with Masson‘s blue stain were then used to analyze the size of the granulomas. The Masson‘s blue staining procedure is shown in Table 2.3.

Hemalum, is a complex formed of aluminium ions and oxidized haematoxylin, which colours the nucleus of cells blue, whereas the eosin stains eosinophilic structures within tissues in various shades of red. The HE staining procedure is shown in Table 2.4.

Immunohistochemistry was performed on stomach sections for the presence of $CD3^+$ and $CD45.1^+$+ cells. Briefly, sections were dewaxed in xylene and

Reagent	**Company**	**Incubation time**
Xylene	Engelbrecht	10 min
Ethanol (96%)	MRI	2 min
Ethanol (80%)	MRI	1 min
Ethanol (70%)	MRI	1 min
Aqua bidest	MRI	1 min
Weigert's heamatoxylin	Morphisto	2 min
Tap water	-	Constant rinsing for 5 min
Acid fuchsin	Morphisto	2 min
Phospho-molybdic acid	Morphisto	5 min
Aniline blue	Morphisto	4 min
Tap water	-	Rinsing until water becomes clear
Ethanol (96%)	MRI	2 min
Isoporopanol	MRI	1 min
Xylene	Engelbrecht	10 min
Entellan®	Merck	-

Table 2.3: Masson's blue staining protocol.

slowly rehydrated in 50-100% ethanol. Antigen retrieval was performed in 0.01M sodium citrate (pH 6) and the slides were subsequently blocked in 5% goat serum for 1hr at room temperature. A primary monoclonal CD3 antibody (Sigma) or CD45.1$^+$ antibody (Biolegend) was applied on the murine gatric tissue samples. Slides were incubated with these primary antibodies overnight at 4°C following manufacturer's instructions. HRP-conjugated secondary antibodies were then applied for 1h and the samples were developed

Reagent	Company	Incubation time
Xylene	Engelbrecht	10 min
Ethanol (96%)	MRI	2 min
Ethanol (80%)	MRI	2 min
Ethanol (70%)	MRI	2 min
Ethanol (60%)	MRI	2 min
Aqua bidest	MRI	2 min
Mayer's heamatoxylin	Morphisto	5 min
Tap water	-	Constant rinsing for 12 min
1% (v/v) Eosin	Morphisto	5 min
Tap water	-	Rinsing until water becomes clear
Ethanol (80%)	MRI	1 min
Ethanol (96%)	MRI	4 min
Xylene	Engelbrecht	10 min
Entellan®	Merck	-

Table 2.4: Heamatoxylin and eosin staining protocol.

using SignalStain DAB substrate (Cell Signaling). Sections were then counterstained with haematoxylin. Automated image acquisition was performed using the Virtual Slide Scanning System VS120 (Olympus). Five high power fields (20x magnification) were randomly selected and scored for each sample by two independent observers. The entire process was performed blinded to not introduce bias.

2.2.6.3 Microscopical analysis of stained liver sections

Masson's blue stained liver sections were analysed microscopically for granuloma formation and size, whereas HE stained sections were used to monitor immune cell infiltration. Granuloma sizes were determined by measuring the diameters of granulomas using an ocular micrometer along the longitudinal axis of the eggs at a x10 magnification. Up to 30 granulomas of each liver section was evaluated and the average size of granulomas from each liver section was calculated.

2.2.7 *S.mansoni* specific qPCR

2.2.7.1 Isolation of parasite DNA from stool

Stool samples were collected from *S.mansoni* infected mice at eight weeks post infection. The QIAmp DNA Stool Mini Kit was used according to manufacturers instructions. Briefly, the stool samples were re-suspended InhibitEX buffer, homogenized by vortexing and incubated at 95°C/20 min in a shaker to lyse the eggs and release schistosomal DNA. To purify the DNA-containing supernatant from remaining stool particles, lysed samples were centrifuged at 13000 rpm/1 min (RT). Following this, 200 μl supernatant was collected from each sample and mixed with 15 μl proteinase K and 200 μl buffer AL for digestion of proteins. Optimal working conditions for the proteinase K were achieved by incubating samples for 10 min at 70°C. Samples were then loaded onto QIAmp spin columns, followed by two washing steps (buffers: AW1 and AW2). Finally, the purified DNA was eluted from the spin column, the yield

and purity of DNA was determined using Nanodrop® 1000 (Kisker). DNA samples were then stored at –20 or -80°C for use in qPCR.

2.2.7.2 *S.mansoni* specific qPCR and running conditions

This PCR was performed with eluted DNA from the previous section in order to determine the infection status of the animals. An amplification of a 86 bp fragment of the *S.mansoni* DNA tandem repeat unit was then performed. The qPCR reaction mix was prepared as follows:

PCR-Mix	**Volume**
LightCycler® 480 Probes Master	10 μl
Forward primer (10μM)	1.0 μl
Reverse primer (10μM)	1.0 μl
Probe (6μM)	1.0 μl
Target DNA	2.0 μl
DEPC H_2O	5.0 μl

Table 2.5: qPCR reaction mix.

The primers and schistosome specific dual labeled probe tandem repeat for the PCR reaction are described in section 2.1.9.1. As a positive control, DNA isolated from the stool of a previously schistosome infected mice was utilized. The PCR was run on a LightCycler® 480 (Roche) with the following settings:

2.2.8 Serological analysis

Blood was collected from each mouse into a gel-tube with a clotting activator (Sarstedt). Tubes were centrifuged for 5min at 10000 g and the resulting

	Temperature	**Time**
Hot Start	95°C	5 min
Denaturation	95°C	10 sec
Annealing/Extension	58°C	30sec
Measure fuorescence	-	-
Cooling	45°C	15 sec

Table 2.6: PCR settings.

serum was collected and stored at -20°C. Seum was used in different dilutions for measurement of alanine aminotransferase, IL-13 and IL-13dRa2 ELISAs.

2.2.9 Collagen/ protein detection

The total collagen detection kit (Quickzyme Biosciences) was used to detect the hydroxyproline content in paraffin embedded tissue sections, obtained by hydrolyzing the samples. Briefly, 10 sections from each liver were hydrolyzed in 6M HCl at 95oC in an Eppendorf tube. The sample supernatents were diluted appropriately and then plated along with a provided standard for 20min with constant agitation. A detection reagent was added and the plate was then incubated at 60oC for 60min. Similar to this process, a protein assay kit (Quickzyme Biosciences) was used in parallel to the collagen plate. Both plates are cooled on ice and measured at 570nm. The result is calculated using values for each sample in both plates, giving the collagen/mg of protein for each sample.

2.3 Cell biological methods

2.3.1 Preparation of immune cells

2.3.1.1 Preparation of splenocytes and mesenteric lymph nodes

Mice were sacrificed using cervival dislocation or carbon dioxide inhalation for harvesting of organs. The lungs and stomach was removed first, followed by the spleen, mesenteric lymph node (MLN), Peyer's patches and finally the liver. Thereafter, in order to obtain single cell suspensions, the lymphoid organs were placed in petri dishes filled with PBS and crushed with a syringe plunger. The resulting cell suspension was transferred to a 50 ml falcon and centrifuged at 230 g/10 min (4°C). The resulting pellet was re-suspended in 5 ml ACT buffer and incubated for 5 min (RT) in order to lyse erythrocytes. Following incubation, the cell suspensions were filtered through a 70 μm cell strainer into a new tube and washed twice with PBS. After centrifugation at 230 g/10 min (4°C), the supernatant was discarded and the cell pellet was dissolved in culture medium. Finally, the cells were counted and used for cell stimulation assays and flow cytometry.

2.3.1.2 Preparation of cells from the lung

The lungs were lung into small pieces in a 5ml petri dish with a small scissor. The pieces were placed into a collagenase/DNAse digest solution and incubated at 37°C under constant shaking for 45min. The digested lungs were then pushed through a 100μm cell strainer in a 50ml tube by using a 2ml syringes piston. Everything was kept wet by using 20ml PBS/ 2% FCS with 0.01M

EDTA. The filtrate was then centrifuged at 600g for 5min. The resulting supernant was discarded and the pellet was washed twice with 20-30ml PBS/ 2% FCS, 600g for 5min. Erythrocytes lysis was then performed with 5ml of ACT for 5min. Finally, cells were washed again with 20 PBS/2% FCS, 600g for 5min and then counted for flow cytometry assays.

2.3.1.3 Preparation of cells from the stomach and Peyer's patches

The stomach was prepared as described in section 2.2.5.1. A longitudinal section from the pre-washed gastric tissue was cut up and placed in collagenase solution (1mg/ml) at 37°C for 10min under constant agitation. Following incubation, the pieces were processed using mechanical disruption between glass slides. The digested tissue was then washed and strained through a 70μm cell strainer. The cells were then washed atleast 3 times with culture medium and centrifuged to give a pellet, which was then resuspended in FACS buffer for flow cytometry analysis. Peyer's patches were prepared using a collagenase digestion for 10min first, followed by incubation in 0.01M EDTA and DTT for another 10min, the tissue was then strained through a 70μm cell strainer using a syringe plunger and then washed twice to yield a pellet. This was re-suspended as well in FACS buffer for flow cytometry analysis.

2.3.1.4 Preparation of Liver associated lymphocytes (LAL)

Liver-associated lymphocytes (LALs) were prepared post PBS-perfusion of livers. The organ was mechanically disrupted using a syringe piston, through a 100 μm cell strainer. The filtrate was then washed with RPMI1640/1%

PenStrep medium. Post centrifugation (300x g for 5 min at 4°C), the cell pellet was re-suspended in about 13 ml of liver digestion solution (Collagenase solution) and incubated for 20 min at 37°C. The digested liver solutions were then centrifuged at 300x g for 10 min at 4°C and the resulting cell pellets were re-suspended in 3 ml of 40% Percoll solution and gently layered on 3 ml of 80% Percoll solution in order to perform a gradient centrifugation at 300x g for 20 min (RT). After centrifugation, the hepatocytes were discarded, whereas the ring representing liver-associated lymphocytes was collected and washed three times in 5 ml RPMI1640. After erythrocyte lysis in 3 ml ACT buffer for 5 min, cells were centrifuged (300x g, 5 min, 4°C) to finally obtain the enriched liver-associated lymphocytes, which were then re-suspended in cell culture medium or FACS buffer for flow cytometry analysis.

2.3.1.5 Preparation of bone marrow derived dendritic cells, stimulation and co-culture

For generation of murine bone marrow–derived dendritic cells (BMDCs), bone marrow was obtained from the tibia and femur of naive donor mice. Cells were cultured for 7 d at 37°C in RPMI 1640 medium containing 10% FCS, 1% penicillin/streptomycin, 50 μM 2-ME, and 20 ng/ml GM-CSF. BMDCs were stimulated with SEA (20μg/ml), *H.pylori* PMSS1 (MOI 5) or both simultaneously. LPS (10ng) and SEA+LPS were used as a control. Following overnight stimulation, BMDCs were treated with Gentamycin (100μg/ml) for 0.5h in order to kill *H.pylori*, prior to the addition of T cells. T cells were isolated using the $CD4^+$ or $CD4^+$ $CD62L^+$ cell isolation kit (Miltenyi Biotec), accord-

ing to manufacturer's instructions. Cells were loaded in double the proportion to dendritic cells (2:1). Supernatant was collected on day 3 and 7, cells were harvested after 7d for FACS analysis.

2.3.2 Cell handling

2.3.2.1 Cell counting

Cells were counted with the Vi-CELL® Cell Viability Analyser (Beckman Coulter). Cells were diluted at a ratio of 1:10 in 200 microlitres and placed into the machine. The counter determines the cell count per ml and provides a viability percentage as it uses trypan blue as a stain.

2.3.3 Stimulation of acquired cells

2.3.3.1 Stimulation of splenocytes and MLN cells

Isolated and counted splenocytes and MLN cells (section 2.3.1.1) were added to a round-bottom 96-well plate at a concentration of $3x10^5$ cells/ml in a total volume of 200 μl complete medium per well. For *S.mansoni* specific stimulation, cells were stimulated with SEA (20μg/ml), additionally, cells were also stimulated with biotinylated αCD3 and αCD28 coated MicroBeads (Miltenyi Biotec, Germany) in a cell to bead ratio 1:1 as a positive control. Plates were then incubated for 48 hours at 37°C. Culture supernatant (185 μl) was collected and stored at –20°C for cytokine measurement by ELISA.

2.3.3.2 Enzyme-Linked ImmunoSorbent Assay (ELISA)

For the measurement of cytokine production in the culture supernatants from stimulated cells, the Ready-Set-Go® ELISAs (Affymetrix) and Duo Set® (R&D) were performed according to the manufacturer's instructions. Briefly, ELISA plates were coated overnight at 4°C or at RT with the appropriate capture antibody. Pre-blocking with either PBS/BSA or blocking buffer for an hour at room temperature, plates were washed three times with the washing buffer (1% Tween in PBS). After blocking and washing, samples and serial standard dilutions were prepared and added to the pre-coated plates. Plates were then incubated for 2 hours at room temperature in order for proteins in the samples to bind to the capture antibodies. After additional wash steps, the biotinylated detection antibody was added and incubated for 1 hour at RT. Excess detection antibodies were then washed away following the addition of streptavidin-horseradish peroxidase (HRP). Plates were then incubated for half an hour at room temperature in the dark. Following this incubation, plates were washed before addition of the substrate solution (TMB substrate). The resulting enzymatic colour reaction was stopped by adding the stopping solution (H_2SO_4). Immediately after stopping the reaction, the plates were measured at 450 nm using the Sunrise^{TM} ELISA microplate reader. Cytokine concentrations within samples were the calculated according to the standard curve on the same plate.

2.3.4 Flow Cytometry

2.3.4.1 Stimulation of immune for intracellular cytokine staining

Splenocytes and LALs were seeded in a round-bottom 96well plate at a concentration of 1x10^6 cells/ml in a total volume of 200 μl complete medium per well. Cells were either left unstimulated or stimulated with SEA (0.05mg/ml), followed by BFA in order to trap cytokines within cells. As positive controls, cells were stimulated in the presence of BFA with PMA (50 ng/ml) and Ionomycin (1 μg/ml).

2.3.4.2 Multicolour surface and intracellular cytokine staining

Cells were suspended in 50 μL FACS buffer each to which ethidium monoazide bromide (EMA, 1:1000) was added as a marker that discriminates between live and dead cells. The cells were exposed to light for 10 min on ice in order to allow the EMA staining reaction. Following this, cells were washed by adding excess FACS buffer and by centrifuging them to achieve a pellet. For surface staining, with markers such as CD3, CD45, CD4 and CD8, cell pellets were re-suspended in 50 μL/well of the respective appropriately pre-titrated dilutions of fluorophore-conjugated antibody solutions. This mixture was incubated for 30 min in the dark on ice. The cells were diluted with FACS buffer, washed by centrifugation and then re-suspended in 100 μL Cytofix/Cytoperm (BD) incubated in the dark at 4°C/20 min, to permeabilize cells for intracellular cytokine staining. After washing with 200 μl 1x Perm/Wash solution (BD), cells were re-suspended in the intracellular antibody solution such as IFNg and IL-10 diluted in 1x Perm/Wash. This was incubated for 30 min on ice in the

dark. Cells were then washed twice in 1x Perm/Wash solution. At the end, the cell pellets were resuspended in 200-300 μL FACS buffer and processed in a Cytoflex (Beckman Coulter). Samples were analyzed using FlowJo version v10 (Tree Star, Ashland, OR, USA) using the gating strategy in Figure 2.2.

2.3.4.3 Intranuclear Foxp3 staining

For intranuclear staining of Foxp3 (Forkhead box P3), cells were fixed and permeabilized with the Foxp3 staining buffer set from Affymetrix. Therefore, CD4 stained cells were centrifuged and re-suspended in 100 μl fixation/permeabilization solution (Affymetrix) before incubating them for 30 min at 4°C in the dark. Following this, cells were washed twice with 200 μl 1x Perm/Wash buffer (Affymetrix) and re-suspended in fluorophore conjugated Foxp3 antibodies, diluted in permeabilization buffer. An incubation period of 30 min at 4°C in the dark followed. After washing twice, stained cells were re-suspended in 300 μl FACS buffer and analyzed as the previous section with the Cytoflex (Beckman Coulter) and data analysis was performed according to Figure 2.2, using FlowJo v10.

2.3.5 Statistical analysis

All statistical tests were performed with PRISM® 5 and 7 (Graph- Pad Software Inc., San Diego, CA, USA). D'Agostino and Pearson omnibus normality tests were first implemented on all data sets, parametrically distributed data were analysed with unpaired t-test (2 groups) and MannWhitney U-test was used for nonparametric data. For more than two groups, 1-way ANOVA test

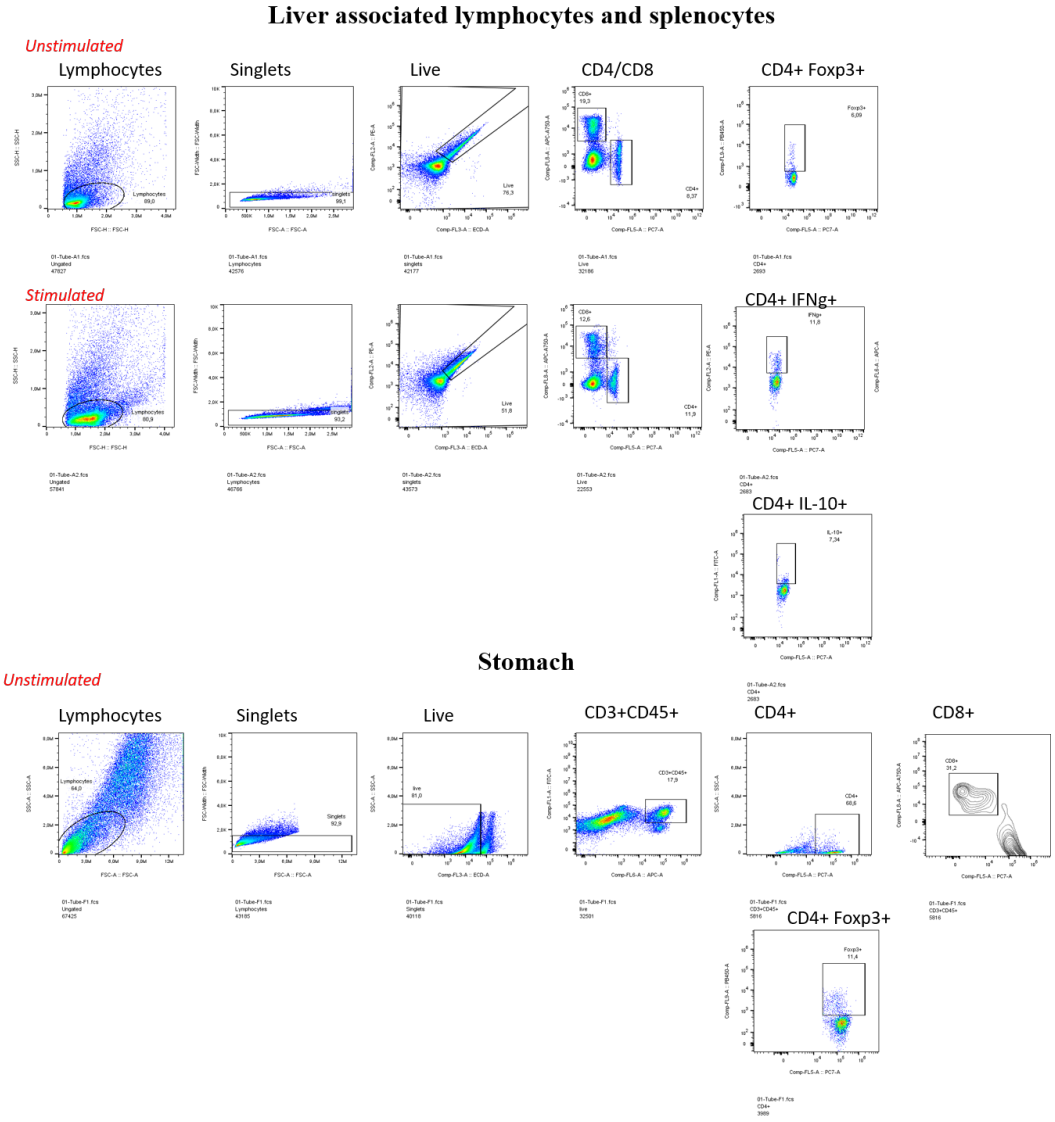


Figure 2.2: Example of the general gating strategy used to analyze cell populations via FACS.

was conducted, if the analysis revealed a significance, the data was subjected to a multiple comparsion test. If data were nonparametric, a Kruskal–Wallis test with a confidence interval of 95% was employed. Results with a P value of < 0.05 were considered as significant.

2.3.6 RNA sequencing data analysis

For the identification of differentially expressed genes we used the DESeq2 package (version 1.16.1) [239] employing a one-factor design with four levels (mono-infected stomach, co-infected stomach, mono-infected liver, co-infected liver). Genes with a fold change of two and an adjusted p-value smaller than 0.1 were considered differentially expressed.

3 Results

3.1 Kinetics and phases of *S.mansoni* infection

In order to define and determine the exact time frames within which immune responses peaked during *S.mansoni* infection in C57BL/6 mice, these immune phases were evaluated from 4 weeks post infection, up until 22 weeks of infection. These kinetic analyses were performed using splenocytes isolated from infected mice, restimulated with soluble egg antigen (SEA) derived from eggs (section 2.2.4).

As shown in Figure 3.1, IFN-γ is depicted as a surrogate cytokine of the Th1 phase in *S.mansoni* infected mice. The levels of this cytokine peaked between 5 and 6 weeks post infection. This stage involves migration of immature worms towards the portal vein. Between 7.5 and 8 weeks there was a dynamic switch in the immune response as IFN-γ levels dropped and IL-13/IL-10 levels rose. This process is induced once eggs are deposited into small venules in the liver. Decaying eggs induce strong Th2 inflammation and the resulting tissue damage simulates IL-13 production.

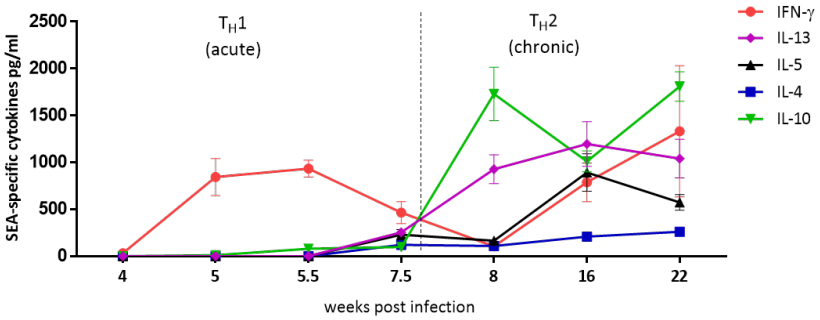


Figure 3.1: Immune responses of S. mansoni infected C57BL/6 mice. Isolated splenocytes were stimulated with SEA(20μg/ml) for 48 hours and ELISA was performed with the collected supernatent. Data is represented as mean +/- SEM. *Experiments performed with Dr. Eva Loffredo Verde.*

As the infection reached chronicity between 12-16 weeks, there was a sudden increase in IFN-γ along with IL-5 and plateaued levels of IL-10/IL-13. Even up to 22 weeks post infection, this cytokine profile remained stable. This phenomenon is quite unlike that measured in BALB/c mice, which showed almost no IFN-γ post the acute phase of the infection [155]. Interestingly, in the liver associated lymphocytes (LAL) of mice infected with *S.mansoni*, increased IFN-γ producing $CD4^+/CD8^+$ cells were only found at week 6 up till week 8, followed by a slow decline and plateau comparable to naive mice by week 22 (Figure 3.2).

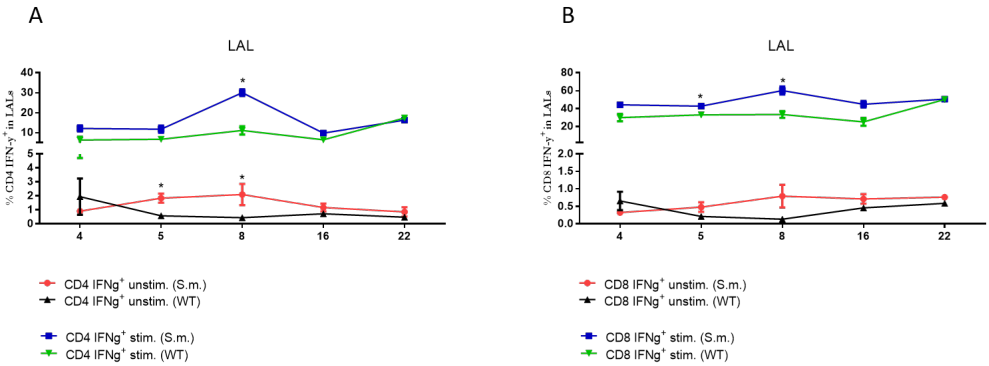


Figure 3.2: Steady state in the liver of *S.mansoni* infected mice. LAL were isolated from *S.mansoni* infected mice and were either left unstimulated or stimulated with PMA (50ng/ml) and Ionomycin (1μg/ml) for 5 hours along with Brefeldin A (BFA) (1μg/ml) for 4 hours concurrently. Percentage of lymphocytes were analysed by flow cytometry. Data is represented as mean+/- SEM.

3.2 Effect of *S.mansoni* on *H.pylori* associated parameters

To mimic the most likely infection scenario in humans where *H.pylori* infection precedes the schistosome infection due to *H.pylori* transmission within the family or from the mother to child, C57BL/6 mice were first infected with *H.pylori* (PMSS1). 5.5 weeks later, when chronic *H.pylori* infection is established, these mice were subcutaneously infected with *S.mansoni* cercariae. After another 5.5 weeks of schistosome infection (corresponding to the peak of the Th1 phase), mice were sacrificed and *H.pylori* colonization as well as gastric inflammation was analysed (Figure 3.3).

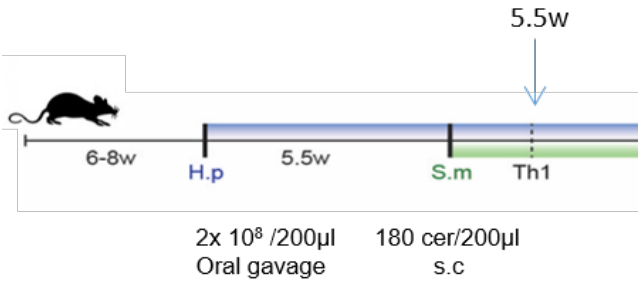


Figure 3.3: *In-vivo* infection model for the acute/Th1 phase. Adult mice were infected with *H.pylori* for 5.5 weeks followed by *S.mansoni* infection. Mice were then analysed at 5.5 weeks post *S.mansoni* infection (or 11 weeks post the first *H.pylori* infection).

3.2.1 Acute schistosomiasis induces increased *H.pylori* colonization and reduced inflammation in the stomachs of co-infected mice

Since *H.pylori* colonization is known to be controlled throughout the course of infection by Th1/Th17 responses with IFN-γ as a major mediator of bacterial clearance, it was surprising to find increased *H.pylori* (Figure 3.4A) colonization in the stomach of co-infected mice (H.p/S.m) during this Th1-prone phase of helminth infection. This was accompanied by reduced expression levels of IFN-γ TNF-α and MIP-2 (IL-8 homologue) in the gastric transcripts of co-infected mice compared to *H.pylori* mono-infected mice (Figure 3.4B). Additionally, the co-infected mice had lower inflammation scores than their *H.pylori* mono-infected counterparts (Figure 3.4C).

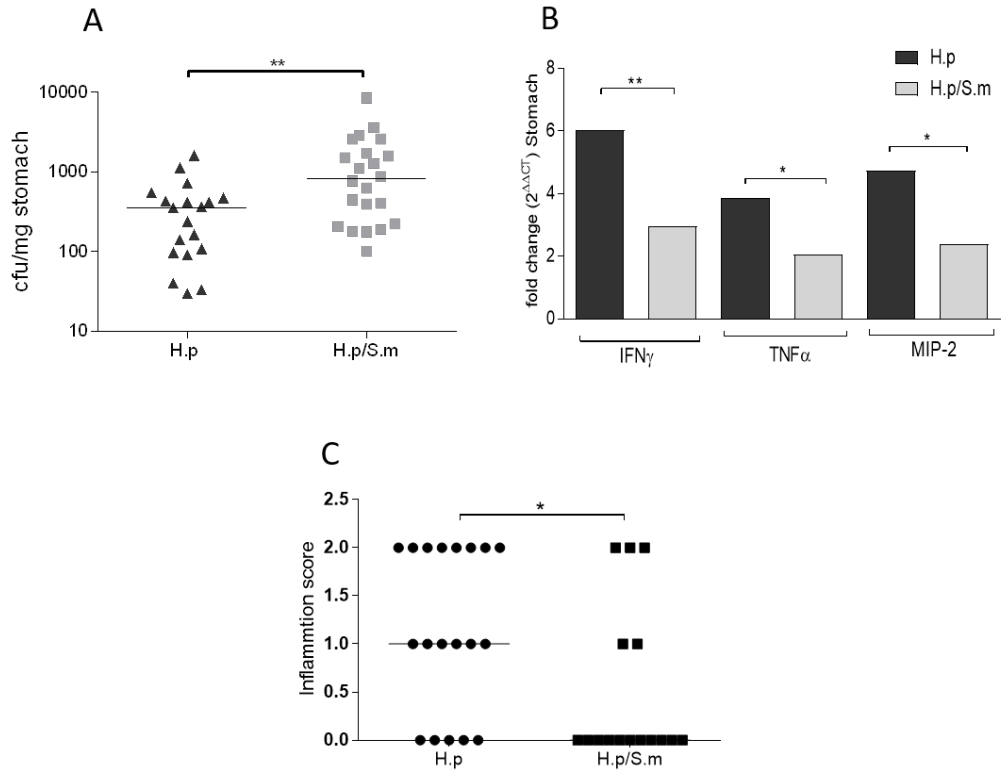


Figure 3.4: *H.pylori* colonization and inflammation parameters during the Th1 phase of *S.mansoni* infection. (A) Elevated colony forming units (CFU) from stomach isolate in concurrently infected mice at 5.5weeks post helminth infection. **(B)** mRNA fold change (over Naive mice) of IFN-γ, TNF-α and MIP-2 in the stomach of *H.pylori* mono-infected (H.p) compared to co-infected mice (H.p/S.m) during the Th-1 phase of helminth infection. **(C)** Histological inflammation score between *H.pylori* mono-infected and co-infected gastric tissue. Data is shown as group median. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data (p value: * < 0.05, ** < 0.01 and *** < 0.001).

Interestingly, these cytokines mainly stem from $CD3^+$ T cells that home to the stomach, while many chemokines induced by the innate response against *H.pylori* remained unaffected by the coinfection. RNAseq was performed on these stomach tissues, obtained from mono- and co-infected mice. Both, pro-inflammatory CC- chemokines (Figure 3.5) and CXC- chemokines (Figure 3.6)

were similar in the stomachs between *H.pylori* mono-infected and co-infected animals.

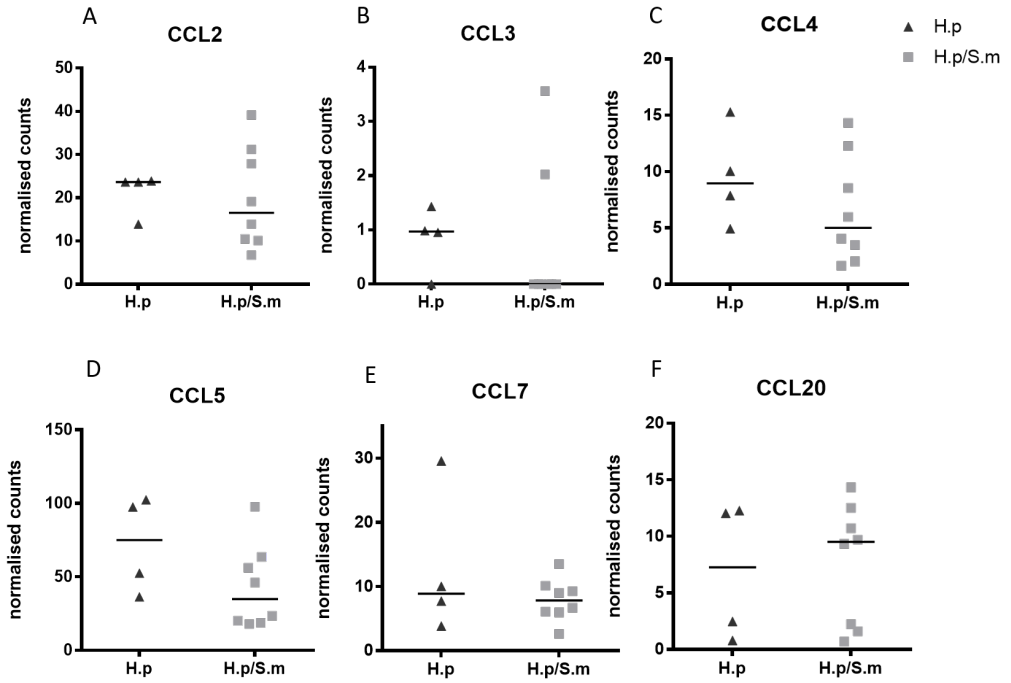


Figure 3.5: CC- chemokines upregulated in the stomach during *H.pylori* infection. Chemokine transcripts upregulated in stomachs of *H.pylori* mono- and co-infected mice 5.5 weeks post helminth infection and/or 11 weeks post *H.pylori* infection.

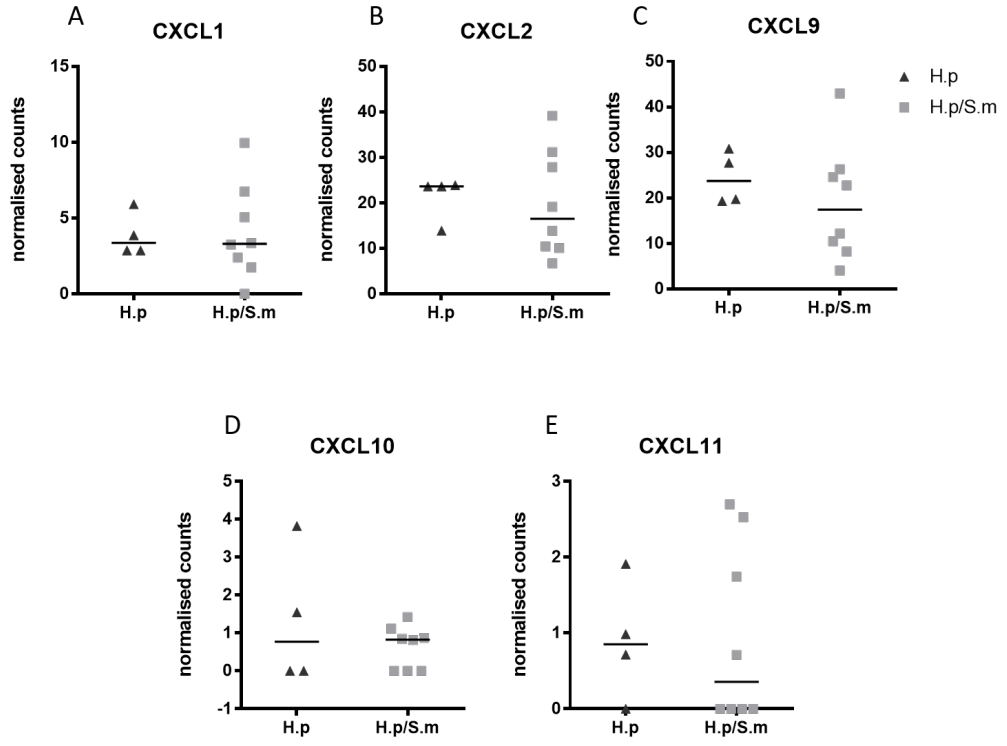


Figure 3.6: CXC- chemokines upregulated in the stomach during *H.pylori* infection. Chemokine transcripts upregulated in stomachs of *H.pylori* mono- and co-infected mice 5.5 weeks post helminth infection and/or 11 weeks post *H.pylori* infection.

Most studies analysing the immunomodulatory capacity of schistosomes have focussed on the Th2 inducing capacity of eggs that alter APC responses. However, less attention has been given to migrating stages of *S.mansoni* that may be able to modulate responses by dampening excessive Th1 responses as well. To determine the possibility of such a phenomenon already at steady state in co-infected mice, IFN-γ levels were measured from unstimulated splenocytes as well as proportions of $CD4^+/CD8^+$ IFN-γ cells after stimulation (Figures 3.7A and 3.7B). Interestingly, higher levels of IFN-γ were detected even from unstimulated splenocytes of co-infected mice compared to the mono-infected

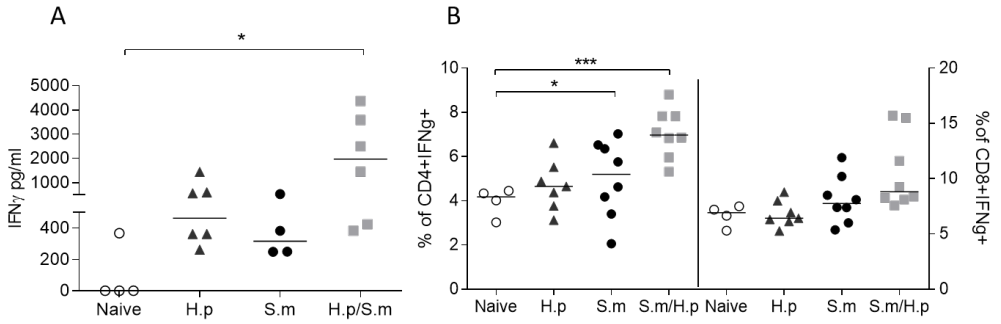


Figure 3.7: IFN-γ inducing capacity of T-cells in co-infected mice. **(A)** IFN-γ production in unstimulated splenocytes of co-infected mice after 48 hours in culture. **(B)** Frequencies of IFN-γ-producing $CD4^+$ effector cells in co-infected mice compared to *H.pylori* mono-infected mice, gated on all living lymphocytes stimulated with PMA/Ionomycin. Data is shown as group median. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data (p value: * < 0.05, ** < 0.01 and *** < 0.001).

groups (Figure 3.7A). This indicated that T cell activation occurred, but those circulating effector T cell populations could either be redirected or fail to migrate to the gastric mucosa and consequently remain in the circulation.

Therefore, next, the immune cell composition in the gastric tissue was investigated. Firstly, cells were isolated from gastric tissue and were analysed by FACS. This method enabled the analyses of the total frequencies of T cells in the stomach, the ones that were circulating, immune surveying as well as those infiltrating gastric tissue. Here, *H.pylori* mono-infected mice had the highest frequencies of $CD4^+$ cells while co-infected mice had a tendency for lower frequencies (Figure 3.8A). However, there was no difference at this point in the frequencies of $Foxp3^+$ cells (Figure 3.8B), indicating that the phenotype of the 'decreased' cells was not regulatory in nature. Since the cells that infiltrate are the ones responsible for clearance or inflammation associated with *H.pylori* infection, the gastric tissue was analysed more closely through

immunohistochemistry. Correlating with the increased *H.pylori* colonization, the infiltration of $CD3^+$ cells was significantly lower in gastric tissue from co-infected mice (Figure 3.8C). These findings indicated that schistosome-induced systemic IFN-γ e.g. as measured in the spleen, may not contribute locally to control *H.pylori* growth in the stomach. Rather, the infiltration of T-cells into gastric tissue was hindered during co-infection.

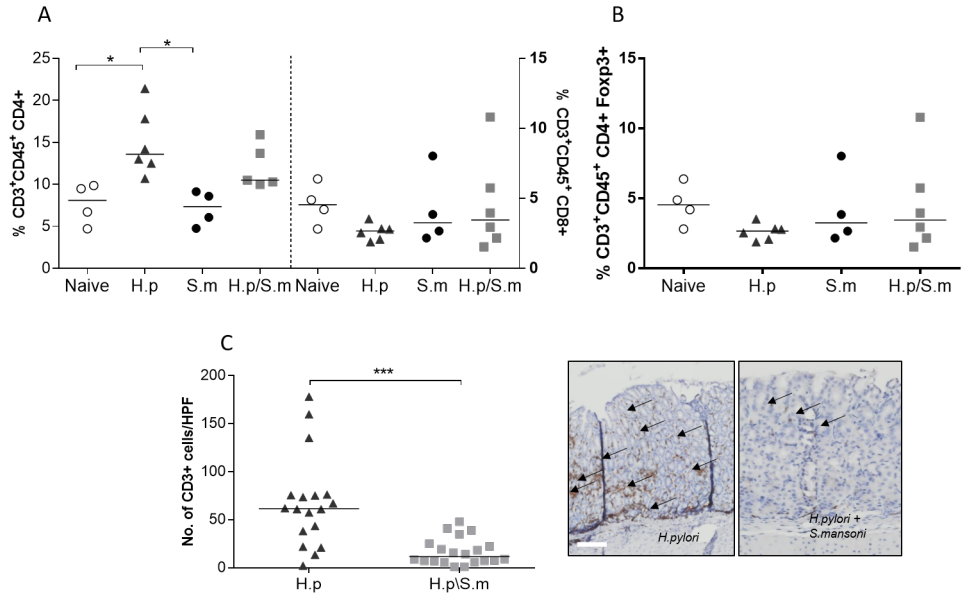


Figure 3.8: Counts of T cells in gastric tissue. **(A)** Frequency of $CD4^+$ and $CD8^+$ cells in gastric tissue via FACS analysis **(B)** Frequency of $CD4^+$ $Foxp3^+$ cells in gastric tissue via FACS **(C)** Quantification of $CD3^+$ infiltration from gastric samples during the Th1 phase of helminth infection by Immunohistochemistry (Average of 5 fields with 40x magnification). Arrows point to immune cell infiltration. HPF: High power field. Data is shown as group median. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data (p value: * < 0.05, ** < 0.01 and *** < 0.001).

A possible reason for the lack of infiltration of T lymphocytes into the stomach mucosa could stem from misdirection of these cells to a different anatomical site where another strong inflammatory response simultaneously takes place. This process would thus be independent of effective antigen-priming and specificity. In this case, migrating schistosomes could redirect homing of *H.pylori*-specific T cells from the circulation towards the liver/liver vasculature, where acute inflammatory responses take place during the peak of the Th-1 phase. Supporting this hypothesis, higher frequencies of IFN-γ producing $CD4^+$ T cells were detected in liver associated lymphocytes (LAL) from co-infected mice (Figure 3.9).

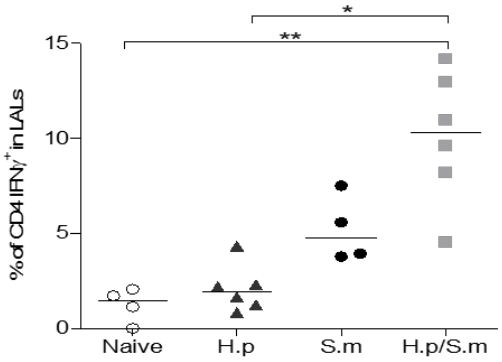


Figure 3.9: Frequency of IFN-γ producing $CD4^+$T cells in LAL. Frequencies of IFN-γ producing $CD4^+$ effector cells in co-infected mice compared to *H.pylori* mono-infected mice, gated on all living lymphocytes, within liver, stimulated with PMA/Ionomycin. Data is shown as group median. Statistical evaluation was calculated using the One-way ANOVA for multiple comparisons (p value: * $<$ 0.05, ** $<$ 0.01 and *** $<$ 0.001).

I then proceeded to determine if there was any cross-reactivity between *S.mansoni* and *H.pylori* antigens that aided in this process of immune deviation. As expected, splenocytes stimulated with *H.pylori* lysate that were

derived from *H.pylori* infected and co-infected mice responded with the production of IFN-γ. Interestingly, *S.mansoni* mono-infected (S.m) but not naive mice also responded with IFN-γ to *H.pylori* lysate (Figure 3.10) albeit not as strongly as *H.pylori* mono- or co-infected mice.

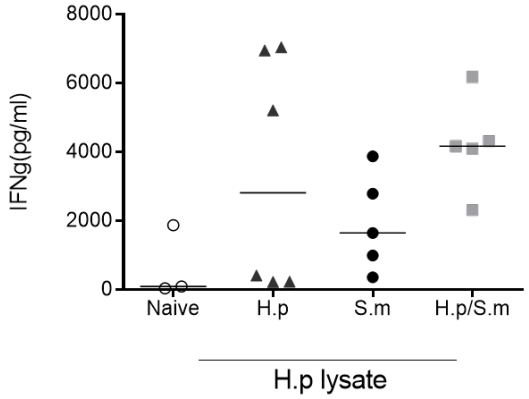


Figure 3.10: Cross reactivity between stimulated splenocytes. Splenocytes isolated from Naive, *H.pylori* infected, *S.mansoni* infected and co-infected mice, stimulated for 48 hours with *H.pylori* lysate. ELISA performed with the collected supernatant. Data is shown as group median. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data (p value: * < 0.05, ** < 0.01 and *** < 0.001).

Since *H.pylori* is a prokaryote and *S.mansoni*, a eukaryote, the likelihood of finding a high percentage similarity between proteins from these two pathogens was not high. Nevertheless, a BLAST search was performed between the entire genomes of the two pathogens, with every listed *H.pylori* protein being compared to the *S.mansoni* proteome. However, as expected, most hits were found between housekeeping enzymes such as ATPsynthases. The top ten most similar proteins/enzymes are listed below.

To further investigate if antigen experienced T cell populations were misdirected, $CD4^+$ $CD44^{hi}$ T cells from the stomach, mesenteric lymph nodes

Reference number	**Protein** [Schistosoma mansoni]	**id (%)**
$XP_0$18652499.1	putative atp synthase beta subunit	65.52%
$XP_0$18650999.1	putative heat shock protein 70 (hsp70)	59.69%
$XP_0$18652944.1	putative atp synthase alpha subunit mitochondrial	58.57%
$XP_0$18649548.1	putative DNAj domain	56.34%
$XP_0$18647947.1	peptidase Clp (S14 family)	56.32%
$XP_0$18649515.1	putative nadh-plastoquinone oxidoreductase	56.16%
$XP_0$18651000.1	putative heat shock protein 70 (hsp70)	55.56%
$XP_0$18652936.1	putative s-adenosylmethionine synthetase	53.80%
$XP_0$18654819.1	GDP-mannose 4;6-dehydratase	53.30%
$XP_0$18649548.1	putative DNAj domain	52.86%

Table 3.1: List of proteins with percentage similarity between *H.pylori* and *S.mansoni*.

(MLN) and peyer's patches of *H.pylori* infected CD45.1 congenic mice were adoptively transferred into the four different experimental groups as indicated below (Figure 3.11). CD44 was chosen as a marker that indicated antigen-experience as Th1 cells upregulate this post priming [240]. Therefore, only 50,000 $CD44^{hi}$ cells were selectively transferred from secondary lymphoid organs and the stomach. These organs are the main site of induction and activation of antigen exposed T-cells during *H.pylori* infection. After 3 days, proportions of $CD45.1^+$ cells were analysed in the stomach, liver, lung, spleen, mesenteric lymph nodes (MLN) and blood of the mice.

While the lymphoid organs (spleen and MLN) as well as blood did not show differences in numbers of transferred T cells between the groups, much more $CD45.1^+$ cells accumulated in the livers of co-infected mice when compared to *H.pylori*-mono-infected mice (Figures 3.12A and 3.12B). Interestingly, *S.mansoni* mono-infected mice also had similar levels of $CD45.1^+$ cells in their

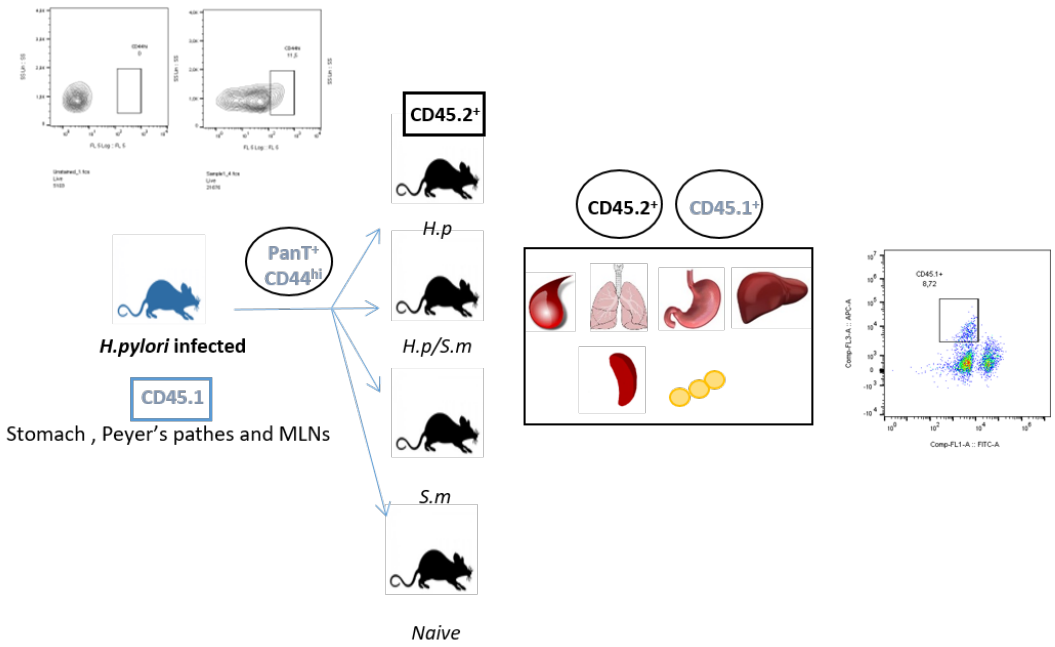


Figure 3.11: Strategy of the adoptive transfer experiment. CD45.1 mice were infected for 5.5 weeks, sacrificed and the stomach, peyer's patches and MLN was isolated. Single cell suspensions from the processed tissue was then MACS sorted for Pan T cells (CD4/CD8) followed by flow sorting of $CD44^{hi}$ antigen experienced cells. 50,000 cells were transferred into CD45.2 mice, into different experimental groups. 3 days later, all mice were sacrificed and organs were harvested for further processing and analysis.

livers, further hinting towards the role of the Th1 chemokine gradient induced by *S.mansoni* alone (Figure 3.12A). The gastric tissue of all mice revealed similar frequencies, which may be a consequence of transferring $CD45.1^+$ cells that were initially acquired amongst others from the stomach and still expressed unidentified lectin receptors that homed to the same organ.

Additionally, as mentioned before, FACS analysis of gastric tissue represents cells that are circulating, immune surveying and infiltrating therefore these cells detected in stomach tissue may not necessarily infiltrate the gastric tissue.

However, due to difficulties with certain antibodies, immunohistochemistry proved to be unspecific.

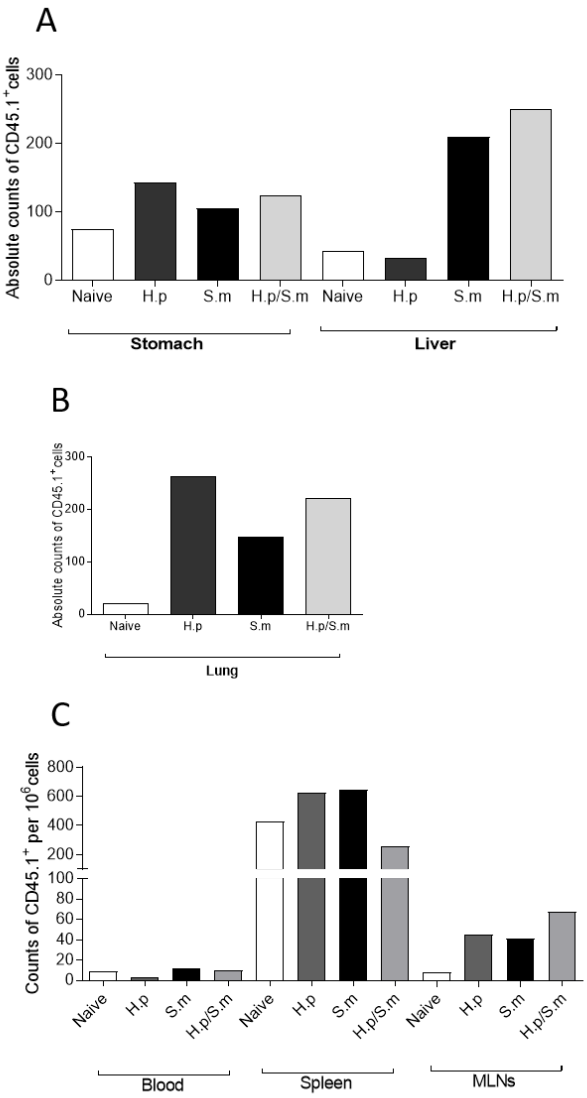


Figure 3.12: Recovery of transferred CD45.1 cells from various organs derived from CD45.2 mice. (A) Cells were isolated from the Stomach and Liver of different experimental groups and analysed for CD45.1 positivity via flow cytometry. **(B)** CD45.1 positive cells isolated from the lung. **(C)** Cells isolated from a fraction of Blood, spleen and MLNs, analysed via flow cytometry. Data is shown as group median. Statistical evaluation was calculated using the One-way ANOVA for non-parametric distributed data (p value: * < 0.05, ** < 0.01 and *** < 0.001).

In order to determine if immune deviation occurring in the above experiments were really due to similarly induced gradients by *S.mansoni* in the liver, we performed RNAseq of both the organs- stomach and liver. Both *H.pylori* infected groups showed upregulation of pro-inflammatory chemokines in the stomach as expected. However, co-infected animals showed significantly high levels of pro-inflammatory chemokines in their livers compared to *H.pylori* infected mice (Figures 3.13A-E and 3.14A-E). Interestingly, CCL20 however, was not upregulated in the livers of co-infected mice; this cytokine has been shown to attract regulatory T cells into the stomach during *H.pylori* infection (Figure 3.14F) [241].

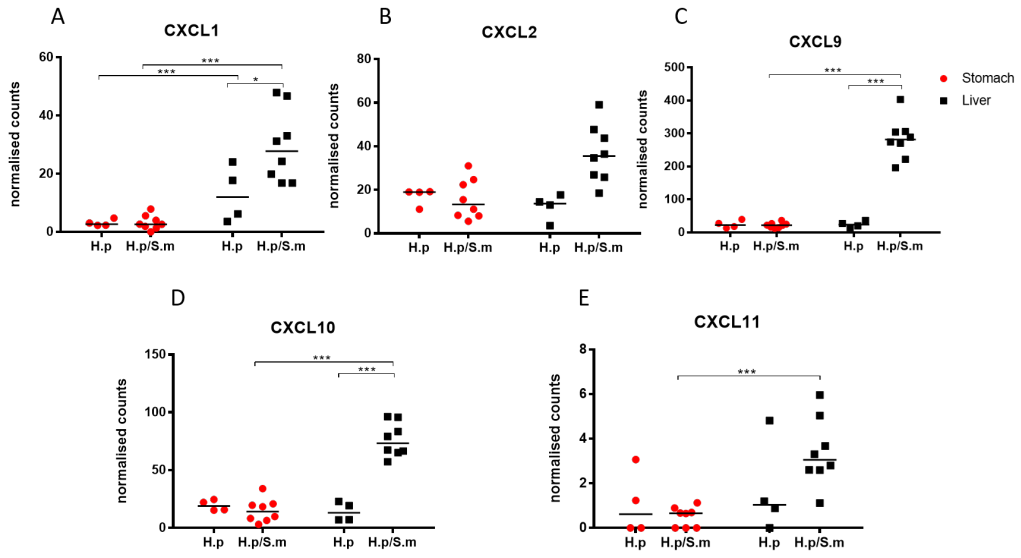


Figure 3.13: CXC- chemokines upregulated in the stomach and liver. Chemokine transcripts upregulated in stomachs of *H.pylori* mono- and co-infected mice 5.5 weeks post helminth infection and/or 11 weeks post *H.pylori* infection. Compared to transcripts upregulated in the liver during *H.pylori* mono- and co-infection. RNAseq data, normalized counts.

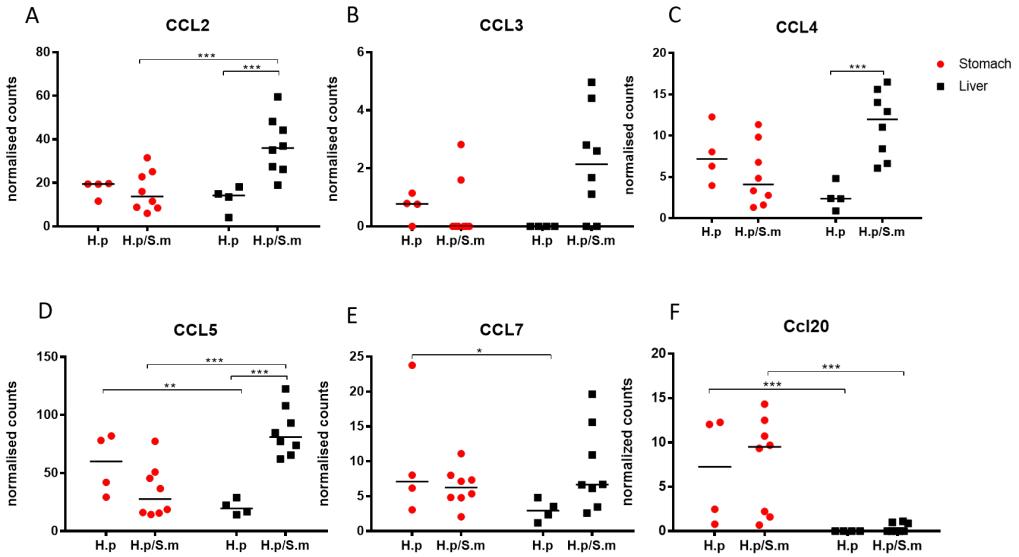


Figure 3.14: CC- chemokines upregulated in the stomach and liver. Chemokine transcripts upregulated in stomachs of *H.pylori* mono- and co-infected mice 5.5 weeks post helminth infection and/or 11 weeks post *H.pylori* infection. Compared to transcripts upregulated in the liver during *H.pylori* mono- and co-infection. RNAseq data, normalized counts.

3.2.2 *H.pylori* colonization and gastric inflammation stabilize during the Th2/chronic phase of helminth infection

As mentioned before, *S.mansoni* elicits dynamical, successive immune responses depending on its developmental stage in the host. Since we hypothesized that these individual phases will have very distinct effects on the outcome of *H.pylori* infection, we next analysed the outcome of *H.pylori*-induced immune responses during the peak of the Th2 phase (9.5 weeks) of the helminth infection. Again, mice were infected with *H.pylori* for 5.5 weeks before the infection with schistosomes, then sacrificed during the peak of the Th2 phase after 9.5 weeks of helminth infection (Figure 3.15).

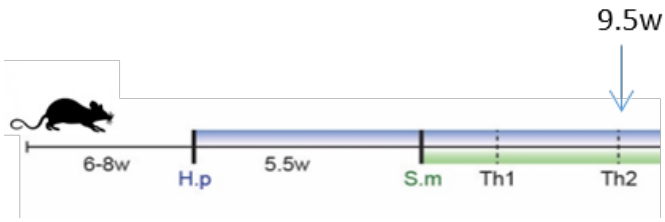


Figure 3.15: ***In-vivo*** **infection model for the chronic/Th2 phase.** Adult mice were infected with *H.pylori* and kept for 5.5 weeks followed by *S.mansoni* infection. Mice were then analysed at 9.5 weeks post concurrent *S.mansoni* infection (or 15 weeks post the first *H.pylori* infection).

Interestingly, the *H.pylori* colonization in gastric tissue of co-infected mice receded in the Th2 phase compared to the initial boost during the Th1 phase (Figure 3.16A). Additionally, the inflammatory parameters in co-infected mice matched those of their *H.pylori* mono-infected counterparts (Figure 3.16B). The co-infected mice also had similar inflammation scores as their *H.pylori* mono-infected counterparts (Figure 3.16C).

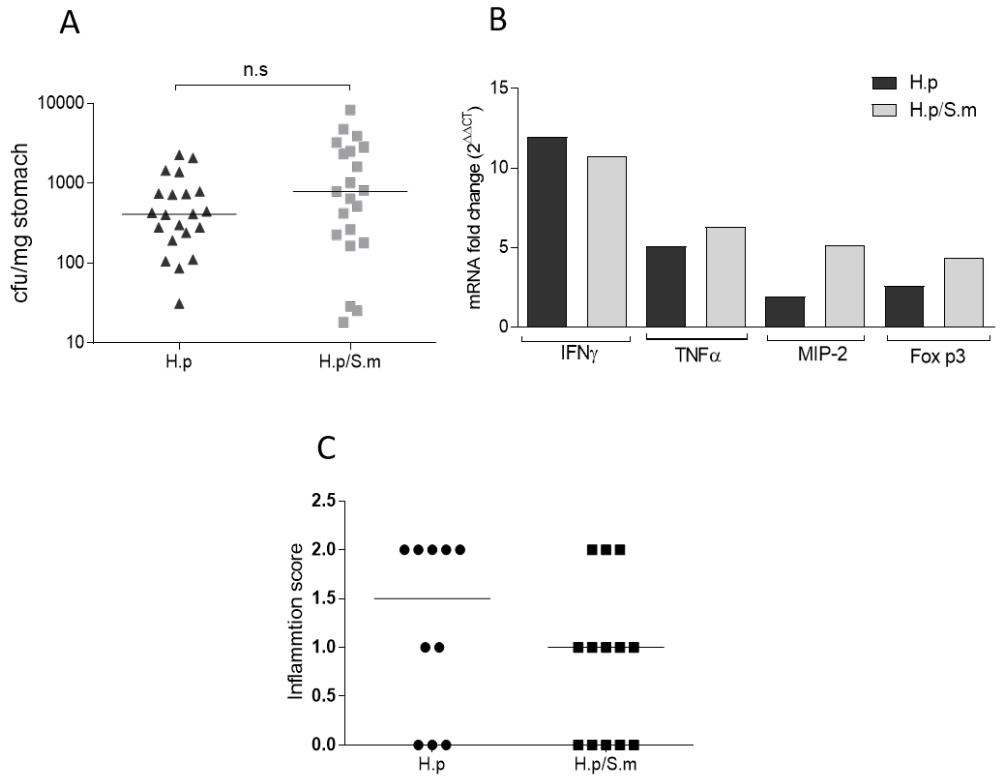


Figure 3.16: *H.pylori* colonization and inflammatory parameters in the Th2 phase. **(A)** Colony forming units (CFU) from stomach isolate in concurrently infected mice compared to *H.pylori* mono infected mice at 9.5weeks post helminth infection. **(B)** mRNA fold change over Naive mice of IFN-γ, TNF-α, MIP-2 and Foxp3 in the stomach of *H.pylori* mono-infected (H.p) compared to co-infected mice (H.p/S.m) during the Th-2 phase of helminth infection. **(C)** Histological inflammation score between *H.pylori* mono-infected and co-infected gastric tissue. Data is shown as group median. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data (p value: * < 0.05, ** < 0.01 and *** < 0.001).

All *H.pylori* infected mice displayed similar frequencies of $CD4^+/CD8^+$ cells (Figure 3.17A). However, co-infected mice at this point showed a higher frequency in $Foxp3^+$ cells (Figure 3.17B), possibly contributed by increased circulating regulatory cells due to the ongoing helminth infection. Correlating with the similar levels of *H.pylori* colonization, the infiltration of $CD3^+$ cells was similar between gastric tissue from co-infected mice and *H.pylori* mono-

infected mice (Figure 3.17C). Additionally, systemic IFN-γ levels in the spleen as well as the population of $CD4^+IFN\text{-}\gamma^+$ cells in the liver were similar between co-infected and mono infected groups (Figures 3.17D and 3.17E).

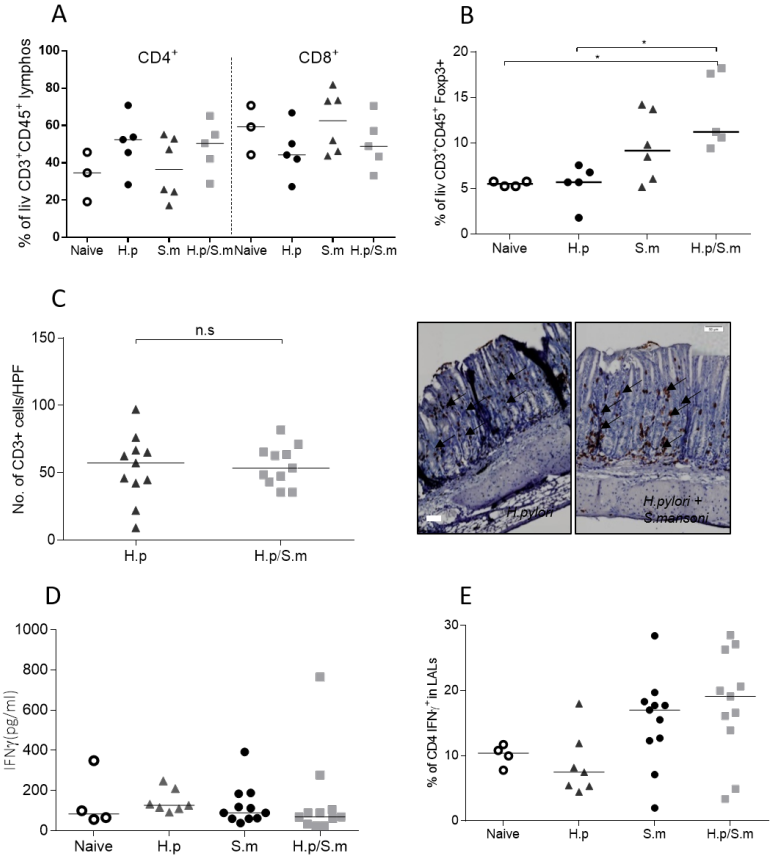


Figure 3.17: Parameters in the stomach and liver during the Th2 phase of helminth infection. (A) Frequency of $CD4^+$ and $CD8^+$ cells in gastric tissue via FACS analysis **(B)** Frequency of $CD4^+$ $Foxp3^+$ cells in gastric tissue via FACS **(C)** Quantification of $CD3^+$ infiltration from gastric samples during the Th2 phase of helminth infection by Immunohistochemistry (Average of 5 fields with 40x magnification). Arrows point to immune cell infiltration. **(D)** Levels of IFN-γ production in unstimulated splenocytes of co-infected mice after 48 hours in culture. **(E)** Frequencies of IFN-γ producing $CD4^+$ effector cells in co-infected mice compared to *S.mansoni* mono-infected mice, gated on all living lymphocytes, within liver, stimulated with PMA/Ionomycin. Data is shown as median of group. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data for figure A, One-way ANOVA was used for figures B, C (p value: * < 0.05, ** < 0.01 and *** < 0.001).

Taken together, these results indicate that the initial deviation of leukocytes due to the Th1 gradient previously induced by the helminth in the liver likely subsides and appropriate homing of the newly primed *H.pylori* experienced cells into the stomach is now possible again. This may be due to a preferential Th2 gradient induced in the liver due to decaying eggs.

3.3 Effect of H .pylori on *S.mansoni* associated parameters

Next, we investigated how *H.pylori* co-infection influenced *S.mansoni* infection parameters and the ensuing immunopathology during both, the Th1 and Th2 phases of helminth infection.

3.3.1 Systemic immune responses

In order to investigate systemic immune responses, splenocytes were isolated and stimulated with an antigen cocktail or aCD3/28 to address both antigen-specific and non-specific responses. Concerning helminth specific immune responses after stimulation with soluble egg antigens (SEA), no differences were observed between IFN-γ, IL-10 or IL-13 secretion from splenoyctes (S.m or H.p/S.m) in either the Th1 or Th2 phase (Figures 3.18A-B).

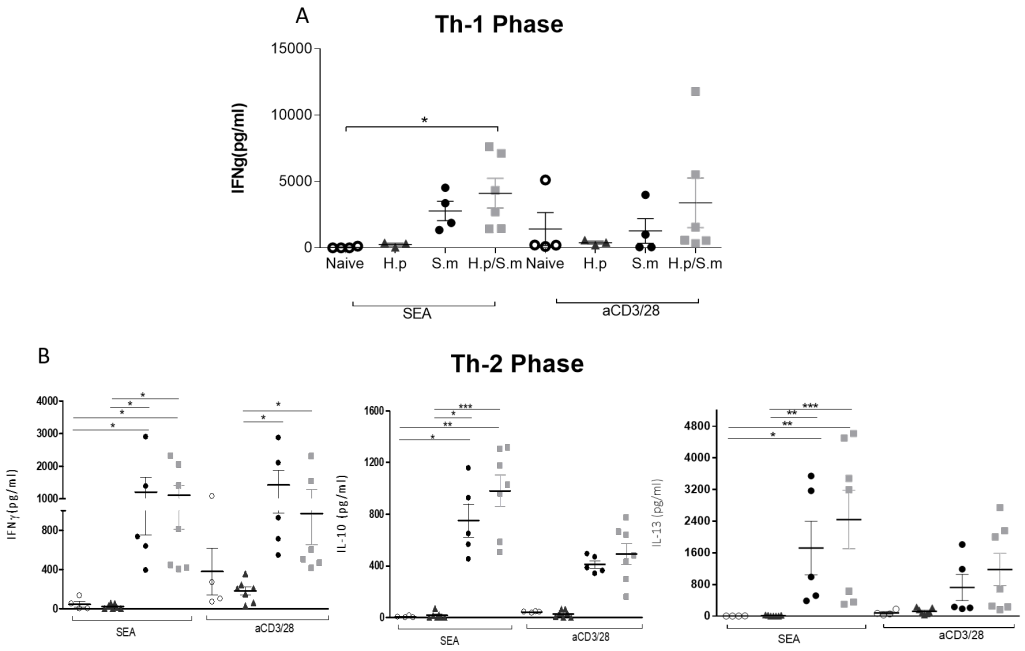


Figure 3.18: Systemic cytokine responses during the Th1 and Th2 phase. (A) IFN-γ levels from splenocytes stimulated with SEA or aCD3/28 during the Th1 phase of *S.mansoni* infection. **(B)** IFN-γ, IL-10 and IL-13 responses from splenocytes stimulated with SEA or aCD3/28 during the Th2 phase. All splenocytes were stimulated for 48hr, supernatant collected and analysed via ELISA. Data is shown as median of group. Statistical evaluation was calculated using the One-way ANOVA (p value: * < 0.05, ** < 0.01 and *** < 0.001).

T-cell responses to both PMA/Ionomycin and SEA specific responses were also investigated during the Th2 phase of the helminth infection. In the spleen, co-infected mice had lower $CD4^{+}$IFN-γ+ responses (Figure 3.19A) to unspecific stimulation compared to *S.mansoni* mono-infected mice. Co-infected mice also had lower TNF-α (Figure 3.19B) and IL-10 (Figure 3.19C) responses but similar levels of $Foxp3^{+}$ cells (Figure 3.19C) (unstimulated). However, SEA specific responses to both IFN and IL-17 remained similar between *S.mansoni* mono and co-infected mice (Figure 3.19D).

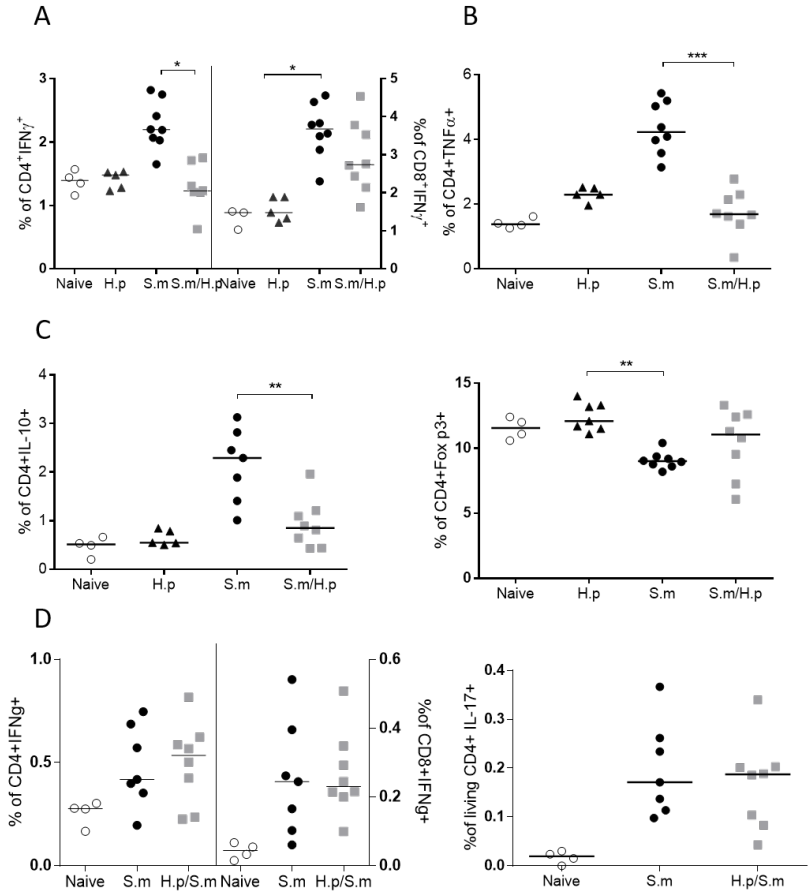


Figure 3.19: Systemic immune responses to antigen unspecific and specific stimulus during the Th2 phase of helminth infection. **(A)** Frequencies of IFN-γ-producing $CD4^+/CD8^+$ cells in co-infected mice compared to mono-infected mice, gated on all living lymphocytes stimulated with PMA/Ionomycin. **(B)** Frequencies of TNF-α, **(C)** IL-10 producing $CD4^+$ cells in co-infected mice compared to mono-infected mice, gated on all living lymphocytes stimulated with PMA/Ionomycin. $Foxp3^+$ $CD4^+$ cells as percentage of unstimulated $CD4^+$ cells. **(D)** Frequencies of IFN-γ- producing $CD4^+/CD8^+$ cells and IL-17 producing $CD4^+$ cells in co-infected mice compared to mono-infected mice, gated on all living lymphocytes stimulated with SEA (50μg/ml). Data is shown as group median. Statistical evaluation was calculated using One-way ANOVA for multiple comparisons (p value: * < 0.05, ** < 0.01 and *** < 0.001).

3.3.2 Effect on the life cycle in the host

Additionally, no differences were detected in the egg numbers or worm burden between the livers of mono and co-infected mice (Figure 3.20). Of note, liver homogenates were cultured in media suitable for *H.pylori* growth and no bacterial colonies were detected (data not shown).

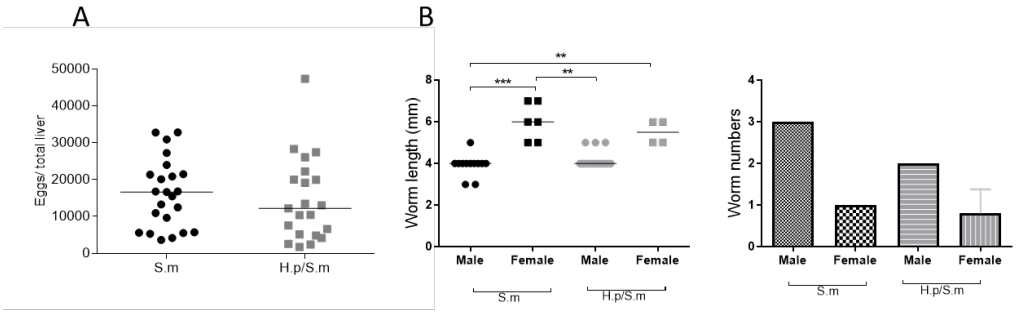


Figure 3.20: Egg burden and worm fecundity/ development during the Th2 phase of helminth infection. (A) Egg count within liver samples between *S.mansoni* mono infected (S.m) vs. co-infected (H.p/S.m). **(B)** Worm length and worm numbers isolated from the portal vein after liver perfusion of infected mice. Data is shown as median of group. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data for figure A, One-way ANOVA was used for figure B (p value: * < 0.05, ** < 0.01 and *** < 0.001).

3.3.3 Concomitant *H.pylori* reduces liver pathology elicited by *S.mansoni* infection

The most striking pathological observation however, was that the degree of infection (visual depiction of egg-induced liver damage) was significantly reduced due to co-infection (Figure 3.21A). This was accompanied by substantially smaller liver granulomas as measured in histology sections (methods section) (Figure 3.21B).

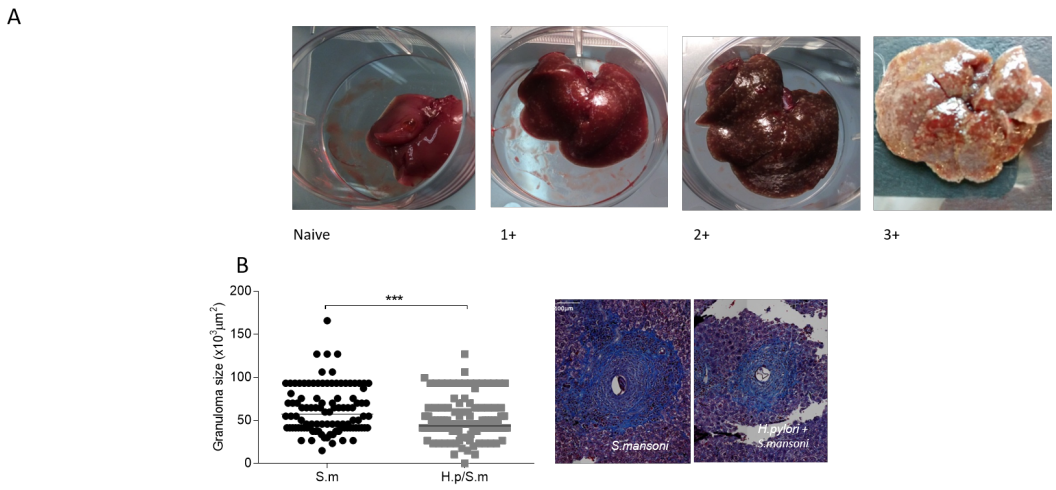


Figure 3.21: Degree of infection and granuloma formation. (A) Visual estimation of the granulomas observed in fresh liver samples display lower DOI for co-infected mice compared to *S.mansoni* mono-infected mice. **(B)** Granuloma size measured in liver sections, co-infected mice have lower granuloma size as analyzed by two independent observers. Histological evaluation of Masson stained livers. (a) An egg with a large granuloma observed frequently in *S.mansoni* infected mice. (b) A small granuloma observed in a section of liver from a co-infected mouse. Data is shown as group median. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data (p value: * < 0.05, ** < 0.01 and *** < 0.001).

Further, alanine aminotransferase (ALT) levels (a measure of liver destruction/disease) in the serum as well as collagen levels in the liver were significantly reduced in the co-infected group (Figure 3.22).

We have shown previously that granuloma size as well as the extent of Th2 immune responses and thus fibrosis is strongly controlled by Treg, which are detected mainly in the circumference of the granulomas [242]. Interestingly, while frequencies of Foxp3$^+$ cells in the compartment were hardly affected in co-infected mice, significantly more of the liver resident CD4$^+$ T cells produced IL-10 (Figures 3.23).

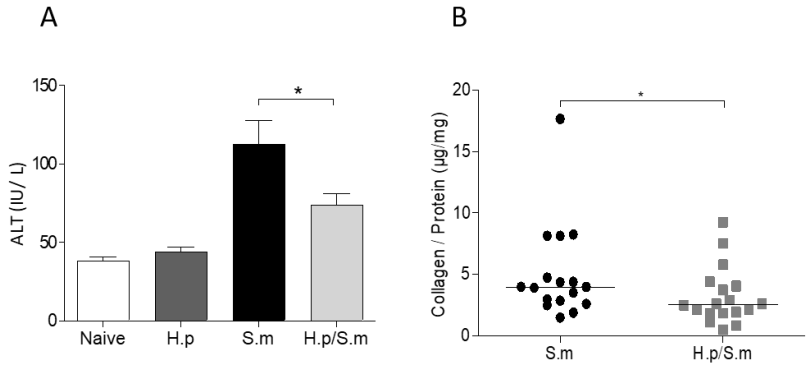


Figure 3.22: Clinical parameters of liver damage. (A) ALT levels as a measure of liver function. **(B)** Collagen/mg protein levels measured in hydrolysed liver tissue from *S.mansoni* infected and co-infected mice. Data is shown as median of the group. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data (p value: * < 0.05, ** < 0.01 and *** < 0.001). Scale bars represent 100 pixels.

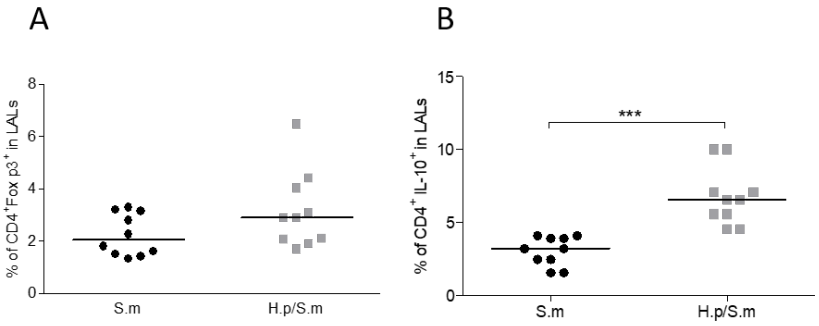


Figure 3.23: Frequencies of regulatory responses in the liver. (A) Frequencies of Foxp3$^+$ CD4$^+$ cells in co-infected mice compared to *S.mansoni* mono-infected mice. **(B)** Frequencies of IL-10 producing CD4$^+$ effector cells in co-infected mice compared to *S.mansoni* mono-infected mice, gated on all living lymphocytes, within liver, stimulated with PMA/Ionomycin. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data (p value: * < 0.05, ** < 0.01 and *** < 0.001). Scale bars represent 100 pixels.

Since both pathogens have been shown to drive dendritic cells towards a tolerogenic phenotype inducing tolerogenic, IL-10$^+$ T-cells as we have observed, we investigated the phenotype and functionality of antigen presenting cells (APCs) upon encounter of antigens from both pathogens in vitro. Upon stimulation with antigen mixtures from both pathogens together (SEA/*H.pylori*), bone marrow-derived dendritic cells (BMDDCs) from naive mice produced higher levels of IL-10 and IL-6 (Figure 3.24B,C) when compared to stimulation with individual antigens. Additionally, when these cells were co-cultured with CD4$^+$ cells from co-infected mice, we observed a significantly higher frequency of Foxp3$^+$ cells and a tendency for enhanced IL-10 production (Figure 3.24D,E), mimicking the results obtained during in vivo co-infection. This may indicate that chronic antigen exposure during these infections trigger APCs to release more IL-10 and IL-6 which are important cytokines to either prime IL-10$^+$ CD4 and/or Treg or at least lead to an increased Treg proliferation and enhance IL-10 secretion from already primed T cells.

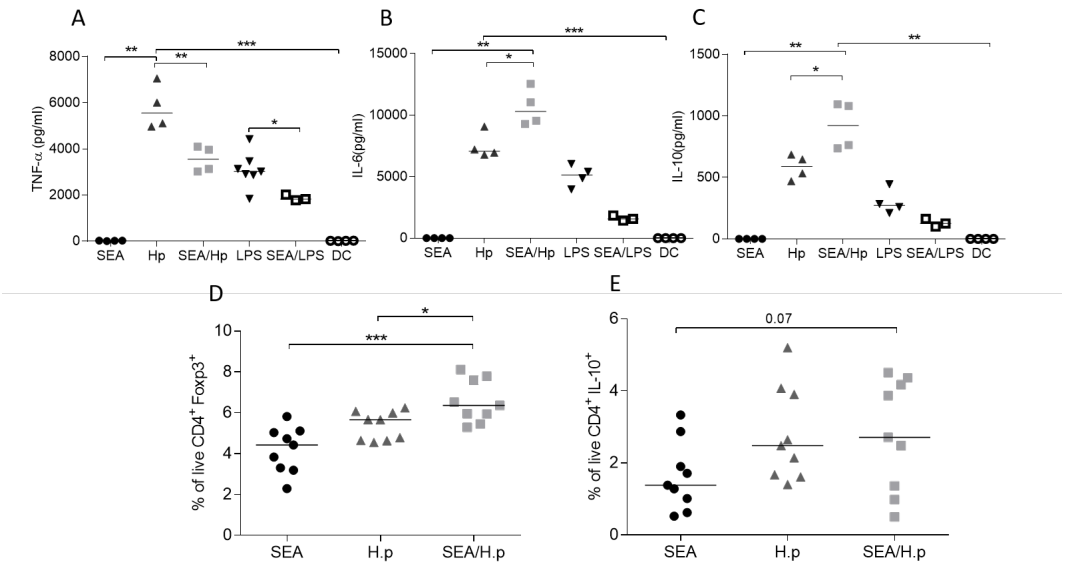


Figure 3.24: ***In-vitro*** **responses of BMDDCs and co-cultured T cells. (A-C)** BMDCs stimulated with either SEA (25μg/ml), H.p PMSS1 live bacteria (MOI 5) or both SEA (25μg/ml) and live H.p bacteria (MOI 5). Supernatents collected after 16h and analysed via ELISA (D) $CD4^+$ T cells from co-infected mice, co-cultured with stimulated BMDDCs in a 2:1 ratio and analysed via FACS. Data is depicted with the median values. Statistical evaluation was calculated using One-way ANOVA(p value: * < 0.05, ** < 0.01 and *** < 0.001).

Apart from regulatory T cells, fibrosis is known to be kept in check by various factors regulating the IL-13 pathway including the IL-13 decoy receptor 2 (IL-13dRa2) and IFN-γ. The former scavenges IL-13 and thus prevents binding of this pro-fibrotic cytokine to its own receptor and cells that produce the latter compete with the resources of profibrinogenic, IL-13+ Th2 producing cells, especially for arginine, resulting in a dampened Th2 immune response.

IL-13 levels were first undetectable in the serum of both *S.mansoni* mono and co-infected mice. Assuming this was due to binding of the IL-13dRa2 to IL-13, the antibody for the decoy receptor was added along with the serum samples in order to un-conjugate the complex. Following this, serum from

co-infected animals sacrificed during the Th1 phase showed slightly higher IL-13 already at 5.5 weeks post helminth infection (Figure 3.25A). However, the levels of IL-13 were similar between both groups at the Th2 phase of the infection (Figure 3.25B).

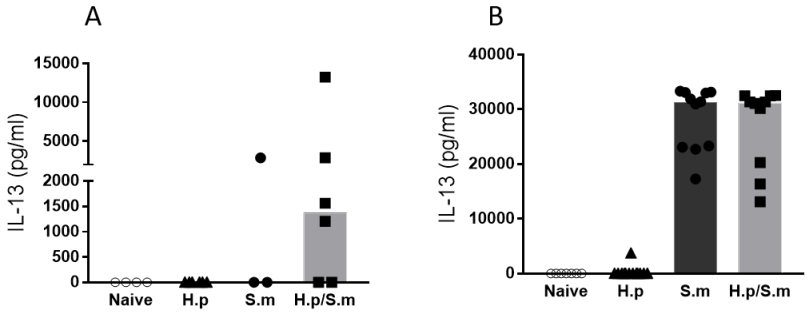


Figure 3.25: Serum IL-13 levels during the Th1 and Th2 phase of helminth infection. Serum was co-incubated with IL-13dRa2 antibody for 12hr to un-conjugate the complex. IL-13 levels were then detected via ELISA. **(A)** IL-13 at 5.5 weeks post *S.mansoni* infection. **(B)** IL-13 at 9 weeks post *S.mansoni* infection. Data is depicted with the median values. Statistical evaluation was calculated using One-way ANOVA(p value: * < 0.05, ** < 0.01 and *** < 0.001).

We also detected significantly higher transcripts of IL-13dRa2 in the livers of co-infected mice compared to monoinfected mice already in the Th-1 phase of helminth infection, and this tendency was maintained well into the Th2 phase where overall expression of the IL-13dRa2 increased in both groups (Figure 3.26A). This pattern was reflected in the soluble levels of IL-13dRa2 in the serum (Figure 3.26B). Further, higher transcripts of IFN-γ were observed in the livers of co-infected animals but interestingly, there were no differences in genes associated with alternative activation of macrophages. (Figures 3.26C,D).

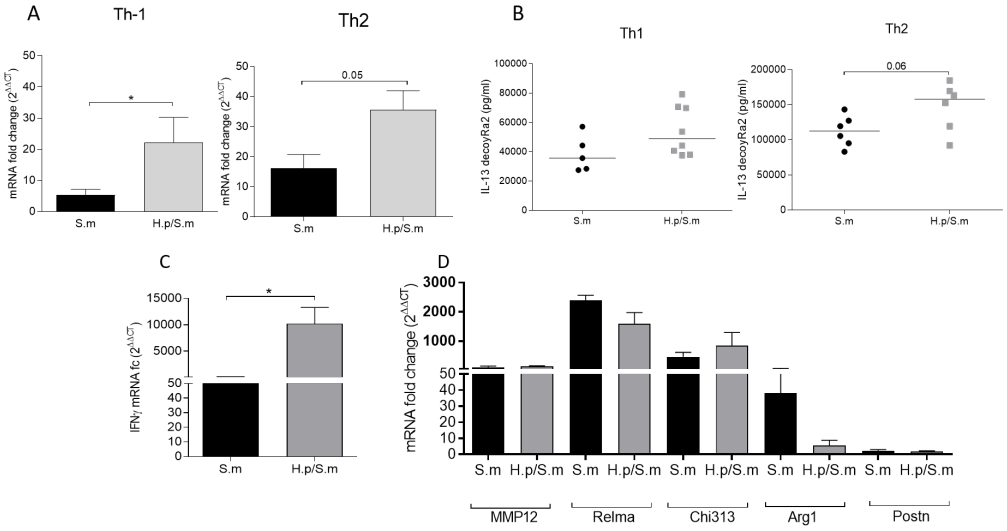


Figure 3.26: Increased regulatory environment in the liver. (A) mRNA fold change of IL-13decoy Ra2 in *S.mansoni* vs co-infected mice during the Th-1 and Th-2 phases. **(B)** Soluble levels of IL-13decoy Ra2 in serum of *S.mansoni* vs co-infected mice during the Th-1 and Th-2 phases. **(C)** mRNA fold change of IFN-γ and **(D)** AAM markers (MMP-12, Relm a, Chi313, Arg 1, Postn) in *S.mansoni* vs co-infected mice. Data is shown as median of group. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data (p value: * < 0.05, ** < 0.01 and *** < 0.001).

In order to determine possible factors contributed by *H.pylori* infection that may mediate reduction in fibrosis, the same pattern of transcripts was investigated in *H.pylori* mono-infected mice. In both, stomachs and livers of *H.pylori* mono-infected mice, higher levels of IL-13dRa2, IL-10 and IFN-γ were detected (Figures 3.27A,B). Additionally these *H.pylori* mono-infected mice had increased levels of soluble IL-13dRa2 in the serum compared to naive mice.

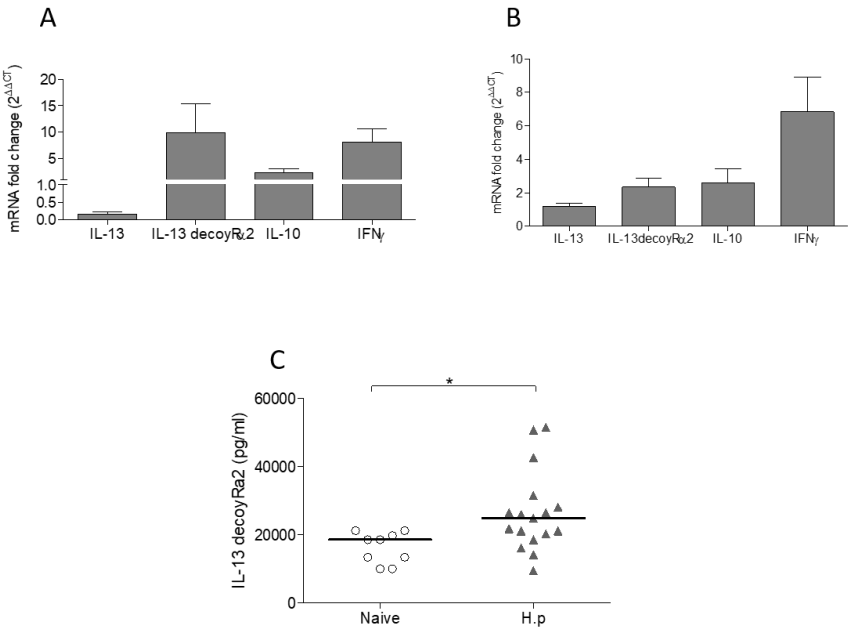


Figure 3.27: Contribution of *H.pylori* to the IL-13 pathway. (A) mRNA fold change of *H.pylori* mono-infected mice over Naive mice of IL-13, IL-13dRa2, IL-10 and IFN-γ, in the stomach and **(B)** Liver as measured by RT-PCR. **(C)** Soluble levels of IL-13dRa2 in serum of mice. Data is shown as median of group. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data (p value: * < 0.05, ** < 0.01 and *** < 0.001).

These results collectively indicate that the effect of *H.pylori* infection is not localized to the stomach only but may have potential systemic – and in this case even beneficial - effects in other organs such as the liver. Mechanistically, co-infection resulted in a positive regulation of the profibrinogenic IL-13 pathway, eventually reducing helminth-induced liver fibrosis and thus liver damage.

3.3.4 Regulatory phase of *S.mansoni* infection shows a similar but milder phenotype as the Th2 phase

Finally, we analysed the impact of *H.pylori*-induced immune responses on *S.mansoni* infection during the regulatory phase (16 weeks) of the helminth infection. Mice were infected with *H.pylori* for 5.5 weeks before the infection with schistosomes, then sacrificed during the regulatory phase after 16 weeks of helminth infection (Figure 3.28).

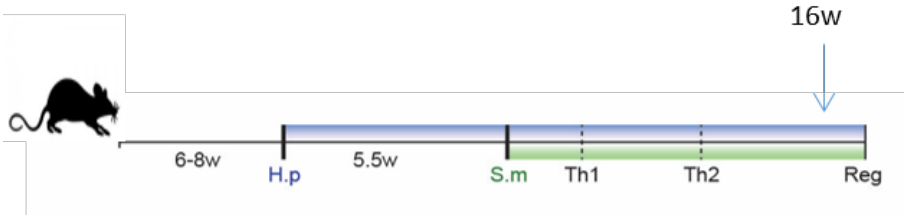


Figure 3.28: *In-vivo* infection model for the Regulatory phase. Adult mice were infected with *H.pylori* and kept for 5.5 weeks followed by *S.mansoni* infection. Mice were then analysed at 16 weeks post *S.mansoni* infection (or 21.5 weeks post the first *H.pylori* infection).

As observed during the Th2 phase, co-infection did not influence the egg burden of *S.mansoni* during the regulatory phase (Figure 3.29A). Similar to the Th2 phase, the degree of infection was significantly lower (Figure 3.29B) accompanied by smaller granuloma sizes as well (Figure 3.29C). Corresponding to this, the ALT levels were decreased (Figure 3.29D), however it was not a marked difference during the Th2 phase, as also observed with the collagen per mg protein in livers during this phase (Figure 3.29E).

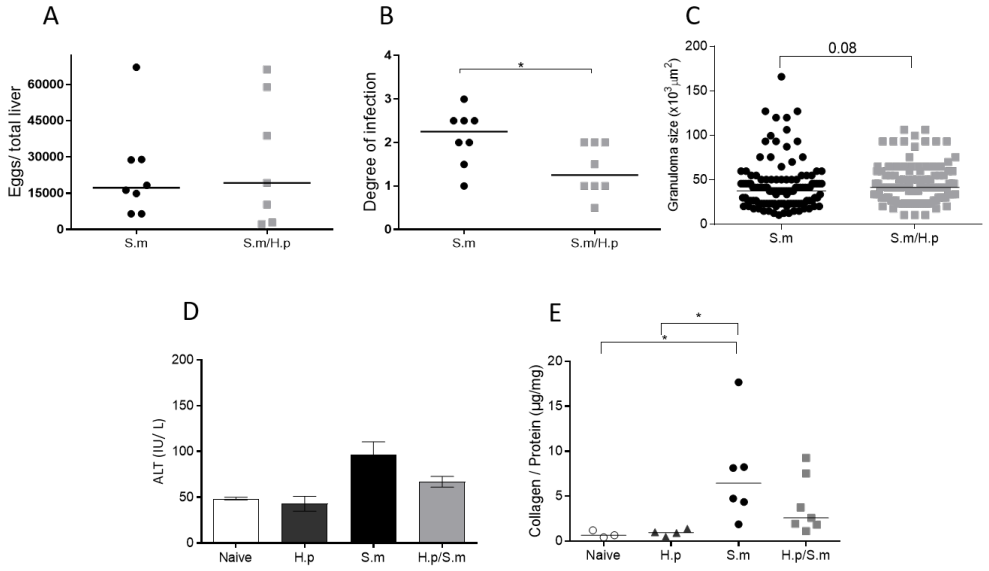


Figure 3.29: Pathology and parameters associated with the regulatory phase of *S.mansoni* infection during concomitant *H.pylori* infection. (A) Egg count within liver samples between both groups. **(B)** Visual estimation of the granulomas observed in fresh liver samples displaying DOI for co-infected mice compared to *S.mansoni* mono-infected mice. **(C)** Granuloma size measured in liver sections as analyzed by two independent observers. **(D)** ALT levels as a measure of liver function. **(E)** Collagen/mg protein levels measured in hydrolysed liver tissue from *S.mansoni* infected and co-infected mice. Data is shown as median of the group. Statistical evaluation was calculated using the Mann-Whitney Test for nonparametric distributed data (Figure A-C) and One-way ANOVA (Figures D-E) (p value: * < 0.05, ** < 0.01 and *** < 0.001).

Upon stimulation of splenocytes with SEA or aCD3/28, we did not detect differences in production of IFN-γ (Figure 3.30A) or IL-10 (Figure 3.30B) between *S.mansoni* mono and co-infected groups. However, we observed lower levels of IL-13 production in splenocytes derived from co-infected mice compared to *S.mansoni* mono-infected mice (Figure 3.30C).

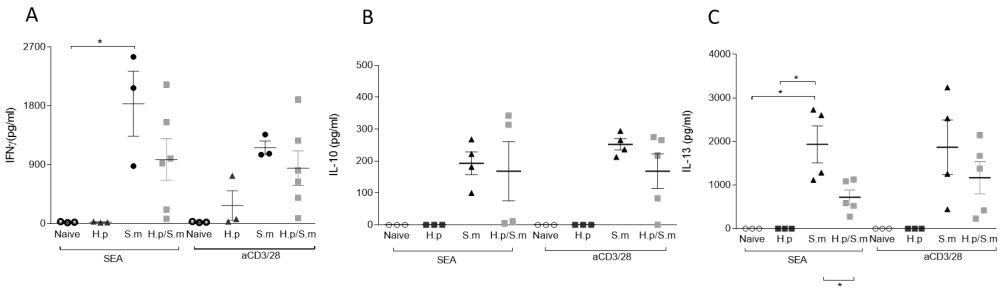


Figure 3.30: Systemic cytokine responses during the Regulatory phase. **(A)** IFN-γ levels **(B)** IL-10 and **(C)** IL-13 responses from splenocytes stimulated with SEA or aCD3/28 during the regulatory phase. All splenocytes were stimulated for 48hr, supernatant collected and analysed via ELISA. Data is shown as median of group. Statistical evaluation was calculated using the One-way ANOVA (p value: * < 0.05, ** < 0.01 and *** < 0.001).

Next, we analysed the local liver micro-environment in order to see if we could observe increased regulatory responses during the chronic phase as we saw a reduction in granuloma size. While there were no significant differences between the frequencies of $CD4^+/CD8^+$ cells, proportions of $CD4^+$ cells were lower in co-infected mice (Figure 3.31A). There was a higher tendency for more $CD4^+$ cells producing IFN-γ derived from both *S.mansoni* infected groups in the liver compared to the naive and *H.pylori* infected groups as expected (Figure 3.31B). However, in spite of overall lower frequencies of $CD4^+$ cells in co-infected LAL, the $CD4^+Foxp3^+$ cells were higher in proportion in these mice compared to the other groups (Figure 3.31C).

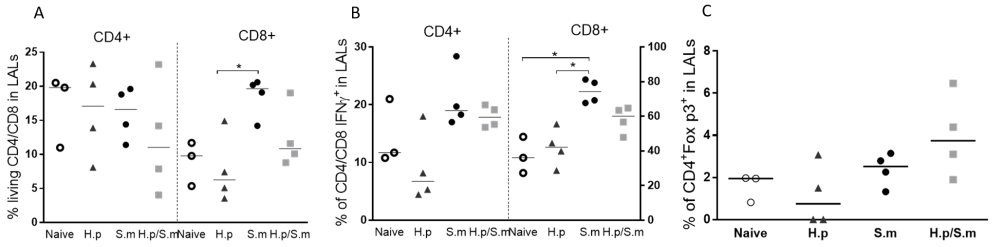


Figure 3.31: Localized liver responses. (A) Frequencies of $CD4^+/CD8^+$ cells in co-infected mice compared to mono-infected mice, gated on all living LAL, unstimulated **(B)** Frequencies of IFN-γ-producing $CD4^+/CD8^+$ cells in co-infected mice compared to mono-infected mice, gated on all living LAL stimulated with PMA/Ionomycin. **(C)** Frequencies of $Foxp3^+$ $CD4^+$ cells as percentage of unstimulated $CD4^+$ cells. Data is shown as group median. Statistical evaluation was calculated using One-way ANOVA (p value: * < 0.05, ** < 0.01 and *** < 0.001).

In summary, *S.mansoni* co-infection alters the inflammation status and therefore colonization of *H.pylori* in the stomach. *S.mansoni* stages induce a chemokine gradient in the liver, which is similar to the Th1 chemokine gradient induced during *H.pylori* infection in the stomach. This gradient in the liver is subsequently replaced by a mixed Th1/Th2 chemokine gradient during the Th2 phase of the helminth infection, which might then allow for the *H.pylori* associated T cells to home to the stomach. Consequently, during the Th2 phase of helminth infection, inflammation and colonization levels reach similarity between co-infected and *H.pylori* mono-infected mice On the other hand we observe a partially protective effect of *H.pylori* infection on *S.mansoni* induced liver pathology. While the exact mechanism is not fully clear due to the complexity of liver disease, it involves positive regulation of the IL-13 pathway, which eventually dampens the processes of fibrosis in co-infected animals.

4 Discussion

The chronic persistence of *Helicobacter pylori* in the stomach of infected individuals is strongly associated with gastric cancer. This has partially been attributed to the release of various Th1/Th17 pro-inflammatory cytokines during the ongoing immune response. As highlighted in the introduction (Section 1.5), especially in sub-Saharan African countries, the high prevalence of *H.pylori* is not associated with a high incidence of gastric cancer; this phenomenon was designated 'the African enigma'. However, these areas are endemic for schistosomiasis, implying that there may be bystander effects of the helminth infection on the development of *H.pylori* associated gastric neoplasia. Here, the classical Th1 response induced by bacteria may be counter-balanced by the strong Th2 responses induced by helminths. Some experimental and epidemiological studies further point towards a possible interaction between the opposing immune responses induced by helminths and *H.pylori*, leading to possible protection against development of gastric cancer [138, 243, 244, 245]. On the other hand, multiple studies have contradicted this hypothesis, rationalizing this 'enigma' through strain variation of *H.pylori* between these geographical areas, the genetic differences in the populations, disparities in life expectancy as well as a more stringent examination of the literature [246, 247, 248, 249]. Nevertheless,

no study could completely disprove the existence of the African enigma, which was further complicated by the fact that there were no experimental studies that analysed the interaction of *H.pylori* and *S.mansoni*. Additionally, the impact of *H.pylori* co-infection on *S.mansoni* associated liver disease is also still unknown in spite of the epidemiologically high rates of co-infection. Therefore, this project has employed a newly established co-infection model to explore how these two chronic pathogens, *S.mansoni* and *H.pylori*, impact upon each other with regard to immune response and disease outcomes despite occupying distinct anatomical niches. The results from this project have been eye opening; they have challenged some important dogmas in the field, especially about the stringency of antigen-specific immune responses and bystander effects of pathogens completely unrelated to each other.

4.1 Antigen specific kinetics of *S.mansoni* infection

The first results chapter (section 3.1) explored the kinetics of the immune response to schistosomiasis in C57BL/6 mice over a period of 22 weeks, finding clear distinctions between immune phases experienced by the host as the infection progresses, as defined by shifting cytokine responses to SEA. Interestingly, in contrast to the kinetics previously assessed in BALB/c mice, we observed increased IFN-γ levels in C57BL/6 mice even during the chronic stages of the disease [155]. This was most likely a result of the Th1-prone genetic background of C57BL/6 mice [250, 251, 252]. These commonly used inbred mouse

strains have a wide range of genetic variability in terms of gene expression and control with respect to their immune cells, which also reflects the status quo in humans [253]. Many studies have highlighted the difference between Th1-prone C57BL/6 mice and the Th2-prone BALB/c mice using various infection models, both for intra- and extracellular pathogens [254, 255, 256]. For example: C57BL/6 mice are relatively resistant to *Leishmania major* infection compared to BALB/c animals, which instead fail to control the pathogen. Similarly, knockout mutant mice on a C57BL/6 background that lack the IFN receptor or IL-12 are more susceptible to *L.major* infection and tend to mount a more Th2-polarised response, failing to control the pathogen, similar to what is observed in BALB/c animals [257, 258]. Accordingly, macrophages isolated from C57BL/6 mice also have a more type-1 pro-inflammatory response to classical M1 macrophage stimulators like LPS, compared to BALB/c mice that show a lower response [250].

Based on these results the experimental co-infection model was set up. *H.pylori* infection preceded the infection with *S.mansoni* in order to mimic the most likely situation in humans, where *H.pylori* infection occurs during the post-natal period and *S.mansoni*, post infancy [259, 260, 261, 262, 263]. Disease progression and immunological parameters of the co-infection were assessed at the peaks of each distinct phase of the helminth infection: 5.5 weeks (Th1 phase), 9 weeks (Th2 phase) and finally at 16 weeks (Reg phase).

4.2 Impact of *S.mansoni* co-infection on *H.pylori* associated inflammation and colonization

As highlighted in previous sections, *H.pylori* colonization is tightly controlled and inversely associated with inflammation. The clearance of the bacterium has been shown to be correlated with an influx of $CD4^+/CD8^+$ T cells and high levels of IFN-γ. Interestingly, bystander activation of both $CD4^+$ and $CD8^+$ T cells due to IFN-γ contributed by non-related antigen specific T cells has been demonstrated by multiple studies [264, 265, 266, 267, 268]. Therefore, as the kinetics in section 3.1 indicated a systemic surge in IFN-γ due to the acute phase of *S.mansoni*, a reduction in *H.pylori* burden was hypothesized during co-infection. However, contrary to the hypothesis, concurrent infection of *S.mansoni* during the Th1 phase resulted in lower inflammation (IFN-γ mRNA transcripts) and increased colonization of *H.pylori* in the stomach. This was reflected by a reduced infiltration of T cells into the stomach, explaining the outgrowth of *H.pylori* due to lack of colonization-control by missing antigen-specific T cells. Furthermore, higher frequencies of $CD4^+IFN\text{-}\gamma^+$ cells were observed in the liver of co-infected mice indicating a possible deviated trafficking of *H.pylori* associated T cells. An adoptive transfer of $CD44^{hi}$ *H.pylori* antigen-experienced T-cells further appropriated this result, where they were detected in the livers of *S.mansoni* infected animals and not in naive or *H.pylori* mono-infected mice.

4.2.1 Immune deviation of T cells

Therefore, ***S.mansoni infection during the Th1 phase induced deviation of H.pylori-specific T-cells towards the liver where active helminth related immune responses take place simultaneously***. The effector T cell response is critical for clearance of *H.pylori* from the stomach, two major aspects determine the success of this process: the chemokine gradient that directs trafficking of T cell sub-populations, and the effector function executed by T cells that arrive at the niche [269, 270, 271]. The expansion and effector functions of both $CD4^+$ and $CD8^+$ T cells are tightly regulated in order to prevent misdirected inflammatory responses [272, 273, 274]. $CD4^+$ T cell trafficking to particular sites of inflammation via chemokine gradients relies upon the expression of specific chemokines receptors [275]. CCR6+ T cells for example, have been shown to be recruited during gastritis induced by *H.pylori*, in human gastric biopsies. This was accompanied by increased levels of CCL20, the ligand for CCR6 [276]. In addition to migration, chemokines enhance the binding avidity of integrin adhesion receptors. Lymphocytes express not only chemokine receptors but also members of the $\beta 2$ integrin family [277]. This is required to arrest lymphocytes inside post capillary venules of non-lymphoid tissue. For example, the $\alpha 4\beta 7$ integrin enables gut tropism by acting as a ligand for MAdCAM-1; however, the signals inducing tropism for the stomach are not well-characterized [278]. However, *H.pylori* reactive T cells have been found to be $\alpha 4\beta 7$ integrin and L-selectin positive in an experimental vaccination study in *H.pylori* infected individuals. Gastric endothelial cells vessels have also been shown to express their ligands, MAdCAM-1/ ICAM-1

[279, 280, 54]. As mentioned in the introduction, effector T cells that control *H.pylori* colonization are induced in the Peyer's patches. These T cells follow a Th1 chemokine gradient back to the stomach where the antigen is most abundant and on contact, express varied effector functions like, most importantly, the production of IFN-γ, which results in reduced colonization or clearance. This cytokine, along with IL-8 and IL-1β, stimulates the expression of gastrin, a hormone that increases gastric acid secretion and this may reduce the ability of *H.pylori* to colonize the stomach [281, 282]. IFN-γ also upregulates MHC II expression on epithelial as well as antigen presenting cells, further facilitating the recognition of *H.pylori* antigens [283, 284]. Phagocytes, like macrophages respond to IFN-γ by downstream Stat1 activation and nitric oxide induction, which in turn enhances responses to microbial components such as TLR ligands [285, 286]. Additionally, signalling via Stat1 in this context, decreases expression of suppressor of cytokine signalling (SOCS) proteins that are inhibitors of signal transduction and enhances reprogramming of the effects of IL-10 [287, 288].

Therefore, these Th1 cells represent an important reservoir of a cytokine essential for controlling bacterial colonization. Their effector function depends on their ability to reach their niche, the stomach, which is facilitated to a great extent by Th1-associated chemokines. Additionally, since activated T cells have access to inflammatory sites regardless of their specificity for the antigen, the ability to guide and retain them in tissue is an important function of chemokines [289, 290, 291].

As mentioned in the introduction, *H.pylori* infection and gastritis is associated with the upregulation of pro-inflammatory chemokines like CXCL9

(MIG), CXCL10 (IP-10) MIP-1α, CCL5 (RANTES) and CCL20 [292, 276, 293]. In addition to these chemokines, our model also showed upregulation of CCL4, and CCL7 in the stomachs of *H.pylori* infected animals.

However, during co-infection, this study has shown that *S.mansoni* (Th1 phase) also elicits the production of multiple overlapping pro-inflammatory chemokines including CXCL9, CXCL10, CCL4 and CCL5 among others, such as CXCL1 and CXCL2 in the livers. Since *H.pylori*-antigen specific T-cells are assumed to express the receptors for these chemokines and rely on this gradient for arriving at the gastric tissue, the likelihood of their misdirection is high. Especially because the RNAseq analysis in this study revealed that, the livers of co-infected mice during the Th1 phase of *S.mansoni* infection express much higher levels of CXCL9 and CXCL10 compared to the stomach, two chemokines that are important to recruit the *H.pylori*-specific Th1 cells.

Many studies in the past and also currently, assume the persistent loyalty and ability of antigen-specific T cells to do their job. However, this process is dependent on multiple factors and can be deeply affected by a concurrent infection, even in an anatomically distant organ. Indeed, Ghani *et al.*. performed experiments with T-cell receptor (TCR) transgenic mice to determine the impact of antigen recognition of T cells on their capacity to migrate into the site of infection. They concluded that while there is a crucial role for antigen experienced T cells as 'pioneer cells' to initiate the cascade of inflammation, the infiltration of further effector T cells is not dependent on the antigen [294]. During this study, at 5 weeks post *H.pylori* infection, antigen-specific T cells can be assumed to have already initiated the cascade of inflammation, however newly recruited T cells are required to constantly control the colonization in

the stomach. These primed T-cells get misdirected due to similar chemokine gradients induced by the helminth and thus accumulate closer to the liver.

However, the proliferation of primed cells does depend on the presence of the antigen as demonstrated by a study using recombinant influenza virus expressing OVA. Even when Ova-specific T-cells were recruited to the site of infection without the presence of OVA, they only proliferated in the presence of the OVA epitope [295].

Another study demonstrated the unspecific recruitment of HIV-antigen-specific $CD8^+$ T cells into the central nervous system (CNS) using a model of neurotropic coronavirus-induced encephalitis. Animals were first infected with a recombinant vaccinia-HIV construct, followed by the neurotropic virus; the authors found that while cells were recruited from both infections, HIV-specific $CD8^+$ cells were recruited very early on into the CNS. However, similar to the previous study, the cells were not activated in the absence of their antigen and thus did not contribute to pathology [296]. A similar result was observed in a study analysing the effects of bystander recruitment of T cells in the context of respiratory syncytial virus (RSV) and LCMV co-infection [297].

Interestingly, Dominic *et al.*. have also demonstrated that antigen specific T cells induced by an experimental ongoing chronic infection (Mycobacterium bovis bacilli Calmette Guerin (BCG) in the liver) could be deviated to the site of an acute infection (influenza virus infection in the lung) and vice versa. The authors used monoclonal T cell populations specific for model antigens: pigeon cytochrome c (PCC) or Hen egg lysozyme (HEL). The mice were then chronically infected with a PCC-tagged BCG, localized mainly to the liver, followed by a HEL-influenza virus (HEL-flu) infection of the lung. While the

majority of T cells were found localized to where their cognate antigen was expressed, a proportion of cells were found in the second infection site. However, in this co-infection, not only were HEL-flu specific T cells present at the site of the BCG infection, they could even mediate bystander effector functions and increase the activation state of IFN-γ producing PCC-BCG specific T cells [298].

In the current study, *H.pylori* experienced T cells (during the Th1 phase of schistosome infection) failed to home to the stomach and thus to control *H.pylori* colonization, but rather accumulated within the liver, where the worms mature. This however did not have a noticeable effect on the fecundity or development of adult *S.mansoni* worm pairs and neither on egg deposition. This result is consistent with much of the literature on unspecific trafficking of antigen-specific T-cells mentioned above.

Nevertheless, I did observe cross-reactivity to antigens between the two groups, when splenocytes from co-infected, *H.pylori* mono-infected as well as from *S.mansoni* mono-infected mice responded with IFN-γ production in response to *H.pylori* lysate. As this was not observed from splenocytes isolated from naive mice, I performed a sequence similarity analysis between the proteomes of *H.pylori* and *S.mansoni* to determine if there were any overlapping immunogenic proteins. As one is a prokaryote and the other a eukaryote, the top candidates from the analysis were housekeeping enzymes such as ATP synthases. Although, interestingly the second highest similarity was observed for the heat shock protein 70 (HSP70). This protein has been implied as a potential vaccine candidate as it generates strong humoral responses against *S.mansoni* on immunization but the cellular responses to this protein have not

been analysed [299].Whether this response seen on stimulating splenocytes represents *in-vivo* cross-reactivity remains to be addressed. The *H.pylori* lysate is a sonicated preparation of cultured bacteria and this process may expose antigens like HSP70 that are not generally processed during *in-vivo* contact of bacteria and APCs.

4.2.2 *H.pylori* associated inflammation and colonization during the Th2 and Reg phase of *S.mansoni* co-infection

Once the peak of the Th2 phase of helminth infection was reached, ***H.pylori* colonization was better controlled** as $CD3^+$ T cells could home to the stomach again. Gastric inflammatory transcripts were also similar between *H.pylori* mono and co-infected groups. Additionally, frequencies between $CD4^+$ IFN-γ^+ cells were similar between the livers of *S.mansoni* mono and co-infected mice pointing to the return of a steady state *H.pylori* associated immune responses. Furthermore, the regulatory phase of the helminth infection did not greatly impact the colonization of *H.pylori* in the stomach. While there was a slight tendency for increased colonization, it remained insignificant in three independent experiments. Since studies involving co-infection of Helicobacter sp. with other helminths point to protection due to skewing towards Th2 responses, I was quite surprised to find that this did not stand true in our set-up. This was especially intriguing since *S.mansoni* is a very strong modulator of immune responses. On looking deeper into previous studies, a few aspects stood out. Firstly, in a majority of studies, *H.felis* was used. As mentioned ear-

lier, this bacterium is not a human pathogen, lacks the T4SS and CagA protein (which are extremely important for induction of inflammation during *H.pylori* infection), has a different antigenic signature and causes exaggerated gastric pathology [300, 301, 302]. Secondly, animals were infected with the helminth first and only once a proper Th2 response was established by the helminth, the mice were infected with *H.pylori*. This is a very unlikely situation in the human context. In developing countries, children are infected through familial contact within the first year(s) of life and then may later acquire a helminth infection. Lastly, other studies utilized murine nematodes instead of *S.mansoni*. *S.mansoni* is a unique helminth, one of the only parasitic helminths inducing multiple immune phases in its host. Furthermore, it resides in the blood vessels and not in the intestinal lumen like the nematodes mentioned above and can thus initiate a very different systemic immune response. Thus, the phase and dynamic of the immune response can play an important part in determining the outcome of the bystander response or co-infection [303, 155]. Nevertheless, the studies highlighted above have focussed on the development or suppression of gastric neoplasia, for which the murine wild type *H.pylori* infection model that we have used, may not be the appropriate strategy. However, instead of using pathogens that do not represent the human pathogens(*H.pylori* and *S.mansoni*), a more appropriate set-up to study this interaction could be to assist the development of neoplasia in our model, either with a transgenic or knockout mouse model that has a pre-disposition for gastric pathology. This could be supplemented with a helminth co-infection at different time points of GC development and analysed for any protective effect. This would also pave

the way for using helminth associated products for possible use in prevention of gastric neoplasia.

4.3 Impact of *H.pylori* on *S.mansoni* associated immune responses and liver pathology

4.3.1 Effects during the Th1 phase of *S.mansoni* infection

Interestingly the effects of *H.pylori* on *S.mansoni* associated parameters was only prominent during the Th2 phase. **There was no observed effect on immune responses during the Th1 phase**, neither on systemic SEA associated IFN-γ responses, nor on the number/fecundity of the worms, which were analysed at a later stage of the infection (post maturity). The inferences that can be drawn from these observations are two-fold. Firstly, systemic IFN-γ, if any, contributed by *H.pylori* infection does not seem to have any bystander effect on antigen-specific immune responses of the helminth during the Th1 phase and secondly, the *H.pylori*-experienced T cells deviated due to the Th1 chemokine gradient induced by the helminth, do not act on them. This is rather expected, as these deviated T-cells do not contact their antigen (*H.pylori*) and thus may not induce effector functions as also observed in other studies [295, 296, 297]. Furthermore, even if they induce bystander effects such as IFN-γ production from neighboring *S.mansoni*-specific cells (similar to that

observed by Dominic *et al.* in their PCC-BCG model) the cytokine has not been shown to have an effect on this stage of immature worms in any experimental model. Effects of IFN-γ during schistosomiasis have been mostly linked to implications on lung stages of the parasite, granulomas, egg distribution and liver pathology during the chronic phases [304, 305, 306, 307, 308].

Interestingly however, IL-13dRa2 mRNA transcripts were elevated in the livers of co-infected mice compared to *S.mansoni* mono-infected animals during the Th1 phase. The possible implications of this observation will be discussed in section 4.3.2.4.

4.3.2 Crosstalk between *H.pylori* and *S.mansoni* during the Th2 phase of helminth infection: Central role of the IL-13 pathway

4.3.2.1 Initiation of fibrosis and role of alternatively activated macrophages

Investigation of the liver pathology during both, the **Th2 phase and Reg phase revealed an anti-fibrotic effect of *H.pylori* induced immune responses on *S.mansoni* associated liver fibrosis**. In schistosomiasis, a plethora of factors control this slowly progressing fibrosis which consequently remains clinically balanced in most of the cases. As indicated in the introduction, hepatic stellate cells (HSC) play an important role in the initiation of fibrosis by converting to myofibroblasts when stimulated either with components from the eggs or insult to tissue. HSCs proliferate, alter matrix degradation and produce extracellular matrix. Decaying eggs at this point also induce

a simultaneous Th1/Th2 response. Excessive Type 1 responses and classical macrophages during acute granuloma formation are initially detrimental since they cause intense tissue damage due to inflammation. Th2 responses (increased production of IL-4 and IL-13) increase the frequencies of AAM that supress Th1 responses [170, 195]. While this step is essential to control inflammatory response and tissue damage, in the long run pro-fibrotic AAMs induce excessive fibrosis [172, 176, 194, 200, 309, 310, 193, 195]. Excessive deposition of collagen mediated by these cells and replacement of healthy tissue eventually results in pathogenic fibrosis. As mentioned in the introduction, these pro-fibrotic macrophages thus orchestrate the local immune responses that maintain fibrotic signals or block the initiation of pro-resolution pathways [311]. Macrophages are extremely important in controlling fibrosis during schistosomiasis and they have also been shown to be quite plastic during another co-infection model [312]. However, since there were **no observed differences in AAM associated gene transcripts in the liver** (*Arg1*, *Relma*, *Ym1*) between both co- and mono-infected mice during the Th2 or Reg phase, they were not further investigated in this thesis.

4.3.2.2 Role of Type 1 responses

IL-13 is the central effector cytokine in schistosomiasis-associated fibrosis, activating both fibroblasts and along with IL-4 : AAMs as well [179, 176, 313, 182]. The most prominent players that regulate this pathway are Type 1 responses, regulatory responses (Treg and IL-10) and the IL-13 decoy receptor [314, 315, 180, 201]. Biased Th1-prone immune responses elicited by *H.pylori*

could counterbalance Th2 responses within the liver leading to decreased granuloma size. IFN-γ treatment has also been shown to inhibit transcripts in the liver during schistosomiasis [316]. Similar to studies where mice were immunized with IL-12 before infection in order to deviate the infection towards a Th1 bias. These mice had reduced fibrosis in the chronic stages and had much lesser scarred liver tissue [317]. Recent studies have shown that even Type 1 chemokines such as CXCL9 and 10 have potent anti-fibrotic effects and can directly supress collagen production *in vitro* [161, 318, 319]. **Analysis of transcripts in livers of co-infected mice revealed increased IFN-γ, CXCL9 and CXCL10**, suggesting that there is a systemic increase in the Type 1 cytokines that can induce anti-fibrotic effects in the liver microenvironment during co-infection. This IFN-γ could also be contributed by phagocytes that have taken up *H.pylori* in the stomach or peyer's patches and are degraded in the liver, giving rise to localized IFN-γ responses in addition to systemic cytokine responses. Alternatively, another group has described the presence of *H.pylori* DNA in the livers of mice infected with the SS1 strain of the bacteria. They also detected helical bacteria within hepatocytes of these mice (16s PCR) and a mild inflammation in the livers, which may be contributed by the local liver resident macrophages (Kuppfer cells) that are activated and induce pro-inflammatory responses [320]. However, they could not culture bacteria from the liver, similar to our observations. Other groups have also described an increase in activated Kupffer cells and hydrogen peroxide levels in *H.pylori* infection. This might result in activation of HSCs (along with TGFβ), amplifying hepatic inflammation *via* release of pro-inflammatory cytokines like IFN-γ [321, 322]. Since it has been demonstrated that IFN-γ production can

differentiate naive cells into Th1 cells, the cytokine can further downregulate the pro-fibrotic activities of Th2 cells. As AAMs during chronic infection also compete with Th1 cells for arginine, increased frequencies of the latter may result in lesser collagen formation by default.

4.3.2.3 Modulation of dendritic cells and regulatory responses

Having observed smaller granulomas, lower collagen and ALT levels, our next inclination was to consider regulatory components of the IL-13 pathway that are known to control fibrosis in schistosomiasis. Here, we observed modulating effects on the induction and recruitment of regulatory T cells as well as IL-10 levels. During the Th2 phase, *S.mansoni* eggs are known to exploit the Peyer's patches to facilitate their transmission from the host [323]. Co-incidentally, *H.pylori* specific T cells are also known to be primed in the Peyer's patches via DCs,this is the primary site for maintenance of the immune response to the bacteria [38, 39]. *S.mansoni* eggs as well as *H.pylori* are expert modulators of DCs and could alter their responses, eventually affecting T cell responses.

Components within the SEA such as antioxidant peroxiredoxin, Omega 1 (T2 ribonuclease) and the glycoprotein- Interleukin 4 inducing principle from *S.mansoni* eggs (IPSE/alpha 1) drive Th2/Reg responses by exclusively conditioning dendritic cells [324, 325, 326]. Additionally, lipid fractions from SEA containing lysophosphotidylserine have been shown to prime dendritic cells in a TLR-2 dependent manner, to induce consequent Th2 and Treg development [327]. Furthermore, a lewis X containing glycan within SEA: lacto-N-fucopentaose III (LNFPIII) triggers TLR4 while schistosome dsRNA activates

TLR3 signalling in DCS polarizing them towards Th2 responses [328, 329]. Finally, SEA inhibits TLR mediated immune responses of DCs and increases their production of IL-10. Authors could also show that SEA downregulates the capacity of LPS, hyaluronic acid (HA) as well CpG, poly(I:C) as to induce IL-12 production and MHC class II (MHCII), CD80, and CD86 up-regulation in dendritic cells [330]. Studies on *H.pylori* primed dendritic cells on the other hand are not as clear. Algood *et al.*. performed experiments where BMDCs primed with *H.pylori* were shown to induce Th1 responses in para-gastric lymph node cells [331]. Human primary gastric dendritic cells were also initially shown to induce strong Th1 responses but a subsequent study demonstrated that stromal cells downregulate these inflammatory responses [332, 333]. Additionally our lab has shown that cagA impairs maturation of dendritic cells and gGT from *H.pylori* induces tolerogenic DCs [334, 335]. The mechanism of the former pathway occurs through activation of Stat3 and induction of IL-10 [335]. Chronic exposure of DCs to *H.pylori* in this context has also been shown to induce IL-10 instead of IFN [336].

Intimate contact between *S.mansoni*, *H.pylori* and DCs within the Peyer's patches could lead to altered immune responses during coinfection. Interestingly, both pathogens target TLR2 mediated signalling in DCs to induce regulatory phenotypes in these cells [242, 337, 327]. Additionally, both pathogens have also been shown to induce $CD103^{+}$DCs that are tolerogenic, they may migrate from the Peyer's patches or/and induce priming of regulatory T cells that then migrate to the liver [338, 339]. This may result in an additive effect that is contributed by each pathogen alone during co-infection.

As we have demonstrated, *in-vitro* dendritic cells are influenced by the presence of both antigens, cumulatively resulting in increased IL-10 and, upon co-culture, expansion of regulatory T-cells. These results indicate that Tregs in the vicinity of APCs that are stimulated by both antigens generate a milieu e.g. by secretion of IL-10 and IL-6 that could boost IL-10 production from primed T cells. This may be a cumulative effect where SEA downregulates Type 1 responses as seen by reduced TNFα secretion on co-incubation of the two stimulations (SEA and *H.pylori*) with DCs. This process could be similar to the ones described above with the effect of SEA on DCs stimulated with LPS. Simultaneously, both antigens enhance Stat 3 activation and increase IL-10 production from these DCs. Consequently, these 'tolerogenic' DCs could prime naive T cells to acquire a regulatory phenotype or through the secretion of IL-10, promote Treg development and proliferation.

Adding to this, *H.pylori* drives not only Th1/17 responses but also Treg responses [241, 52, 53]. T regulatory type 1 (Tr-1) cells induced by *H.pylori* infection are known to produce IL-10, which consequently also control inflammation in the stomach and could potentially contribute to the Treg pool systemically [337]. A caveat in many of these studies is that dendritic cells were generated *in-vitro* from monocytes (PBMC derived) or from bone marrow, while this is important to define mechanisms involved, *in-vivo* DCs may show a different phenotype depending on their localization [340]. Additionally, as mentioned in the introduction, live *H.pylori* can escape DC uptake while bacteria that convert into the coccoid form in the intestine and are taken up in the Peyer's patches may expose different antigens that make them vulnerable

for uptake. This may consequently induce a different cytokine repertoire from primed dendritic cells in the gut.

4.3.2.4 Importance of Th1/Th2 balance and IL13dRa2 during fibrosis

Interestingly, studies examining regulation of fibrosis have demonstrated that it is more beneficial to have both IFN-γ and IL-10 in the liver environment than each alone [341, 199, 342]. This is also reflected in our co-infection model. IL-10 has been shown to co-operate with Th1 cytokines to supress collagen deposition. In fact, IL-10 deficiency alone has little effect on fibrosis but mice deficient in both IL-10 and IFN-γ mice develop significantly stronger liver fibrosis [200].

Another mechanistically important and unexpected result was the up-regulation of IL-13dRa2 transcripts in the livers of co-infected and *H.pylori* infected mice during the Th1 phase. Especially intriguing since, to our knowledge, *H.pylori* has never been shown to elicit the production of this factor. However, IL-13 has been detected in infected patients, and as the IL-13: IL-13-decoy-interplay consists of a positive feedback loop, one can theoretically expect the induction of the decoy receptor as well [343, 344]. This is especially important because increased IL-13dRa2 levels can not only impede the process of fibrosis, but also significantly reverse many aspects of it. Currently, we believe that the early contribution of IFN-γ by *H.pylori* systemically acts to induce a protective microenvironment in the liver without really affecting antigen specific responses to *S.mansoni*. Additionally, the contribution of the decoy receptor

in the liver scavenges IL-13 induced by immune responses elicited by degrading *S.mansoni* eggs. Subsequently, the increased levels of IL-10 due to co-infection then contribute to control the extent of inflammation and fibrosis.

Interestingly in a CCL_4 dependent fibrosis model, an association has been seen between increased fibrosis and *H.pylori* positivity [345]. The authors attributed this to increased levels of TGFβ1 and consequently the effect of this pro-fibrotic cytokine on HSCs [346]. However, fibrosis during schistosomiasis has been shown to be independent of TGFβ1, which may explain the contradictory results obtained in this study [347].

Unfortunately, a reductionist approach to determine the exact mechanism in this model is extremely challenging in this co-infection setting. IFN-γ is critical for *H.pylori* control while IL-10, IL-13 and IL-13dRa2 are indispensable for the progression as well as protection from fibrosis-related morbidity and mortality in schistosomiasis. Knocking out any of the above factors influences each individual infection alone and would thus not provide us with a clearer picture of the mechanisms regulating the outcome of co-infection. However, treating the animals with the anti-helminthic drug praziquantel may isolate the direct effect of adult schistosome worms on *H.pylori* infection. Still, the effects, if any, induced by the larval stages, juvenile migrating worms or eggs would remain unaltered for a long time as the drug primarily targets adult worms and immunopathology only decreases gradually [348]. Additionally, the treatment is not guaranteed to eliminate all worms although the drug has been recently shown to reduce fecundity in worms that survive treatment [349]. Using antibiotics to clear *H.pylori* infection on the other hand would alter the microbiome of the gut and thus would not be an ideal approach

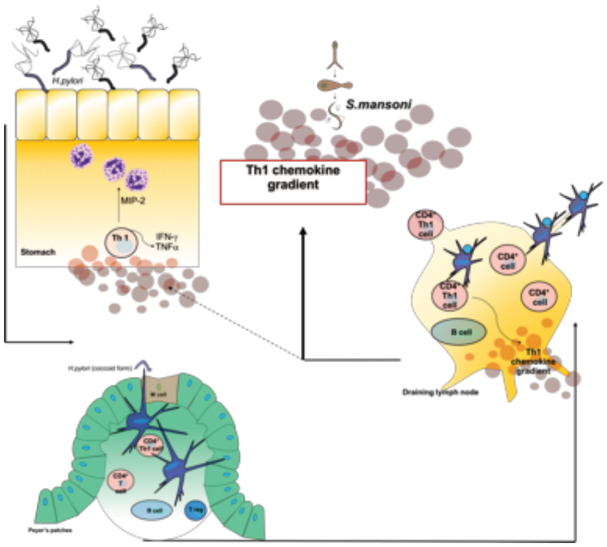
either, especially since others and we have previously demonstrated a crucial role for the commensal gut bacteria in the egress of schistosoma eggs from the intestinal tissue.

Nevertheless, this model depicts to a very large extent the situation that occurs in many countries affected by this co-infection. Most individuals have a low-level *H.pylori* induced inflammation as mimicked by our model and fewer individuals in these areas show high inflammatory biomarkers associated with gastric neoplasia [350, 351, 352, 101, 102]. The extent of fibrosis due to chronic schistosomiasis in such co-infected populations has not yet been assessed. A caveat here is that not all *S.mansoni* infections in endemic areas follow a clear Th1/Th2 dichotomy, where infection levels differ and reinfection is common. We would like to understand further how these factors could influence the dynamics of the diseases in chronic stages. Notwithstanding the above limitations, our study is the first to demonstrate a **potentially mutually beneficial relationship between these chronic pathogens**. This is critical as large amounts of funds are currently allocated for the development of prophylactic and therapeutic vaccines against *H.pylori*. This may be extremely beneficial in countries with high gastric cancer risk where *H.pylori* associated antibiotic resistance is becoming a great concern [353, 354]. However, in several countries that experience co-infections, there may be a potential side-effect. Even more so, as chronic pathogens have evolved to mutually exist and induce host-protective outcomes in these populations. Elimination of one pathogen without regard to the effects on the other can lead to increased immunopathology of the latter. Additionally, if helminths deviate antigen specific T cells during acute infection/re-infection or induce bystander

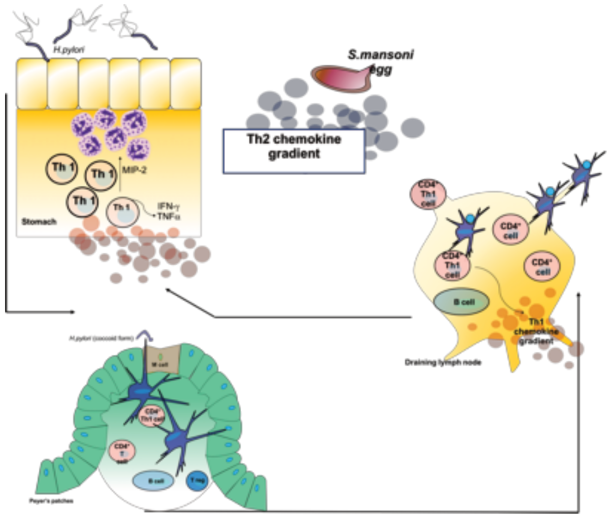
immunosuppressive responses during chronic infection, it can lead to inefficient immunization responses post vaccination. This has been made explicit by vaccination strategies employed against BCG and even Hepatitis B in helminth endemic populations. The solution could be as simple as treating the patient against the helminth before the vaccination, but without studies that delve into these questions, we cannot fully predict the outcome of such vaccination strategies. Furthermore, currently mass drug administration programs (MDA) employed by the WHO to eliminate schistosomiasis are in effect [355]. However, as mentioned before, the immunomodulatory properties of this parasite have been demonstrated in studies related to allergies, auto-immune disease, inflammatory bowel disease and even gastric neoplasia [356]. These studies have made helminths the centre stage of the 'hygiene hypothesis'. Eliminating these parasites completely from entire geographical areas may have a profound effect on the disease landscape of these countries including a surge in non-communicable diseases such as cancer.

5 Graphical Overview

Th1



Th2



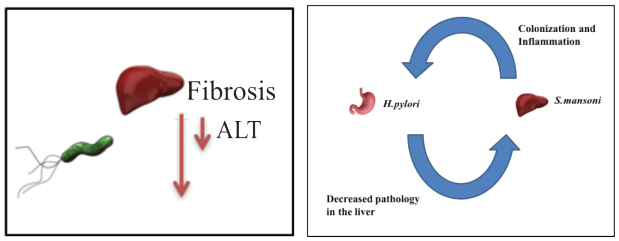


Figure 5.1: Graphical Overview.

Bibliography

[1] R. E. Ley, F. Backhed, P. Turnbaugh, C. A. Lozupone, R. D. Knight, and J. I. Gordon. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A*, 102(31):11070–5, 2005. doi:10.1073/pnas.0504978102.

[2] J. W. Konturek. Discovery by jaworski of helicobacter pylori and its pathogenetic role in peptic ulcer, gastritis and gastric cancer. *J Physiol Pharmacol*, 54 Suppl 3:23–41, 2003.

[3] J. L. Doenges. Spirochetes in gastric glands of macacus rhesus and humans without definite history of related disease. *Proceedings of the Society for Experimental Biology and Medicine*, 38(4):536–538, 1938. URL: http://journals.sagepub.com/doi/abs/10.3181/00379727-38-9924P, doi:10.3181/00379727-38-9924p.

[4] A. S. Freedberg and L. E. Barron. The presence of spirochetes in human gastric mucosa. *The American Journal of Digestive Diseases*, 7(10):443–445, 1940. URL: https://doi.org/10.1007/BF02997393, doi:10.1007/bf02997393.

[5] V. Tricottet, P. Bruneval, O. Vire, J. P. Camilleri, F. Bloch, N. Bonte, and J. Roge. Campylobacter-like organisms and surface epithelium abnormalities in active, chronic gastritis in humans: an ultrastructural study. *Ultrastruct Pathol*, 10(2):113–22, 1986.

[6] B. J. Marshall and J. R. Warren. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*, 1(8390):1311–5, 1984.

[7] J. R. Warren and B. Marshall. *Unidentified curved bacilli on gastric epithelium in active chronic gastritis*, volume 1, pages 1273–5. Lancet, England, 1983.

[8] R. P. Allaker, K. A. Young, J. M. Hardie, P. Domizio, and N. J. Meadows. Prevalence of helicobacter pylori at oral and gastrointestinal sites in children: evidence for possible oral-to-oral transmission. *J Med Microbiol*, 51(4):312–7, 2002. doi:10.1099/0022-1317-51-4-312.

[9] J. Ferguson, D. A., C. Li, N. R. Patel, W. R. Mayberry, D. S. Chi, and E. Thomas. Isolation of helicobacter pylori from saliva. *J Clin Microbiol*, 31(10):2802–4, 1993.

[10] J. Parsonnet, H. Shmuely, and T. Haggerty. Fecal and oral shedding of helicobacter pylori from healthy infected adults. *Jama*, 282(23):2240–5, 1999.

[11] Y. Moodley, B. Linz, R. P. Bond, M. Nieuwoudt, H. Soodyall, C. M. Schlebusch, S. Bernhoft, J. Hale, S. Suerbaum, L. Mugisha, S. W. van der Merwe, and M. Achtman. Age of the association between helicobacter pylori and man. *PLoS Pathog*, 8(5):e1002693, 2012. doi:10.1371/journal.ppat.1002693.

[12] M. O. Marginean, C. O. Marginean, L. E. Melit, S. Voidazan, V. Moldovan, and C. Banescu. The impact of host's genetic susceptibility on helicobacter pylori infection in children. *Medicine (Baltimore)*, 96(30):e7612, 2017. doi:10.1097/md.0000000000007612.

[13] P. M. Webb, T. Knight, S. Greaves, A. Wilson, D. G. Newell, J. Elder, and D. Forman. Relation between infection with helicobacter pylori and living conditions in childhood: evidence for person to person transmission in early life. *Bmj*, 308(6931):750–3, 1994.

[14] February 2017 2017. URL: http://www.who.int/mediacentre/factsheets/fs297/en/.

[15] N. Kodaman, A. Pazos, B. G. Schneider, M. B. Piazuelo, R. Mera, R. S. Sobota, L. A. Sicinschi, C. L. Shaffer, J. Romero-Gallo, T. de Sablet, R. H. Harder, L. E. Bravo, J. Peek, R. M., K. T. Wilson, T. L. Cover, S. M. Williams, and P. Correa. Human and helicobacter pylori coevolution shapes the risk of gastric disease. *Proc Natl Acad Sci U S A*, 111(4):1455–60, 2014. doi:10.1073/pnas.1318093111.

[16] V. Camilo, T. Sugiyama, and E. Touati. Pathogenesis of helicobacter pylori infection. *Helicobacter*, 22 Suppl 1, 2017. doi:10.1111/hel.12405.

[17] M. F. Dixon. *Pathology of Gastritis and Peptic Ulceration*, book section 38. ASM Press, Washington (DC), 2001.

[18] M. Fehlings, L. Drobbe, V. Moos, P. Renner Viveros, J. Hagen, M. Beigier-Bompadre, E. Pang, E. Belogolova, Y. Churin, T. Schneider, T. F. Meyer, T. Aebischer, and R. Ignatius. Comparative analysis of the interaction of helicobacter pylori with human dendritic cells, macrophages, and monocytes. *Infect Immun*, 80(8):2724–34, 2012. doi:10.1128/iai.00381-12.

[19] J. I. Kazi, R. Sinniah, N. A. Jaffrey, S. M. Alam, V. Zaman, S. J. Zuberi, and A. M. Kazi. Cellular and humoral immune responses in campylobacter pylori-associated chronic gastritis. *J Pathol*, 159(3):231–7, 1989. doi:10.1002/path.1711590310.

[20] T. Suzuki, K. Kato, S. Ohara, K. Noguchi, H. Sekine, H. Nagura, and T. Shimosegawa. Localization of antigen-presenting cells in helicobacter pylori-infected gastric mucosa. *Pathol Int*, 52(4):265–71, 2002.

[21] S. Backert, T. P. Schmidt, A. Harrer, and S. Wessler. Exploiting the gastric epithelial barrier: Helicobacter pylori's attack on tight and adherens junctions. *Curr Top Microbiol Immunol*, 400:195–226, 2017. doi:10.1007/978-3-319-50520-6_9.

[22] X. Fan, A. Long, P. W. Keeling, and D. Kelleher. Adhesion molecule expression on gastric intra-epithelial lymphocytes of patients with helicobacter pylori infection. *Eur J Gastroenterol Hepatol*, 7(6):541–6, 1995.

[23] D. Keilberg and K. M. Ottemann. How helicobacter pylori senses, targets and interacts with the gastric epithelium. *Environ Microbiol*, 18(3):791–806, 2016. doi:10.1111/1462-2920.13222.

[24] S. Keates, Y. S. Hitti, M. Upton, and C. P. Kelly. Helicobacter pylori infection activates nf-kappa b in gastric epithelial cells. *Gastroenterology*, 113(4):1099–109, 1997.

[25] Q. Lin, H. Xu, X. Chen, G. Tang, L. Gu, and Y. Wang. Helicobacter pylori cytotoxin-associated gene a activates tumor necrosis factor-alpha and interleukin-6 in gastric epithelial cells through p300/cbp-associated factor-mediated nuclear factor-kappab p65 acetylation. *Mol Med Rep*, 12(4):6337–45, 2015. doi:10.3892/mmr.2015.4143.

[26] F. Backhed, E. Torstensson, D. Seguin, A. Richter-Dahlfors, and B. Rokbi. Helicobacter pylori infection induces interleukin-8 receptor expression in the human gastric epithelium. *Infect Immun*, 71(6):3357–60, 2003.

[27] K. Robinson, K. Kaneko, and L. P. Andersen. Helicobacter: Inflammation, immunology and vaccines. *Helicobacter*, 22 Suppl 1, 2017. doi:10.1111/hel.12406.

[28] D. Y. Graham, A. R. Opekun, M. S. Osato, H. M. El-Zimaity, C. K. Lee, Y. Yamaoka, W. A. Qureshi, M. Cadoz, and T. P. Monath. Challenge model for helicobacter pylori infection in human volunteers. *Gut*, 53(9):1235–43, 2004. doi:10.1136/gut.2003.037499.

[29] J. Evans, D. J., D. G. Evans, T. Takemura, H. Nakano, H. C. Lampert, D. Y. Graham, D. N. Granger, and P. R. Kvietys. Characterization of a helicobacter pylori neutrophil-activating protein. *Infect Immun*, 63(6):2213–20, 1995.

[30] A. Amedei, A. Cappon, G. Codolo, A. Cabrelle, A. Polenghi, M. Benagiano, E. Tasca, A. Azzurri, M. M. D'Elios, G. Del Prete, and M. de Bernard. The neutrophil-activating protein of helicobacter pylori promotes th1 immune responses. *J Clin Invest*, 116(4):1092–101, 2006. doi:10.1172/jci27177.

[31] A. P. Gobert, J. C. Bambou, C. Werts, V. Balloy, M. Chignard, A. P. Moran, and R. L. Ferrero. Helicobacter pylori heat shock protein 60 mediates interleukin-6 production by macrophages via a toll-like receptor (tlr)-2-, tlr-4-, and myeloid differentiation factor 88-independent mechanism. *J Biol Chem*, 279(1):245–50, 2004. doi:10.1074/jbc.M307858200.

[32] W. Khamri, M. M. Walker, P. Clark, J. C. Atherton, M. R. Thursz, K. B. Bamford, R. I. Lechler, and G. Lombardi. Helicobacter pylori stimulates dendritic cells to induce interleukin-17 expression from cd4+ t lymphocytes. *Infect Immun*, 78(2):845–53, 2010. doi:10.1128/iai.00524-09.

[33] U. E. Mai, G. I. Perez-Perez, L. M. Wahl, S. M. Wahl, M. J. Blaser, and P. D. Smith. Soluble surface proteins from helicobacter pylori activate monocytes/macrophages by lipopolysaccharide-independent mechanism. *J Clin Invest*, 87(3):894–900, 1991. doi:10.1172/jci115095.

[34] L. A. Cherdantseva, O. V. Potapova, T. V. Sharkova, Y. Y. Belyaeva, and V. A. Shkurupiy. Association of helicobacter pylori and inos production by macrophages and lymphocytes in the gastric mucosa in chronic gastritis. *J Immunol Res*, 2014:762514, 2014. doi:10.1155/2014/762514.

[35] Y. Yao, G. Li, J. Wu, X. Zhang, and J. Wang. Inflammatory response of macrophages cultured with helicobacter pylori strains was regulated by mir-155. *Int J Clin Exp Pathol*, 8(5):4545–54, 2015.

[36] R. M. Steinman, B. Gutchinov, M. D. Witmer, and M. C. Nussenzweig. Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice. *J Exp Med*, 157(2):613–27, 1983.

[37] J. Y. Kao, S. Rathinavelu, K. A. Eaton, L. Bai, Y. Zavros, M. Takami, A. Pierzchala, and J. L. Merchant. Helicobacter pylori-secreted factors inhibit dendritic cell il-12 secretion: a mechanism of ineffective host defense. *Am J Physiol Gastrointest Liver Physiol*, 291(1):G73–81, 2006. doi:10.1152/ajpgi.00139.2005.

[38] K. Kiriya, N. Watanabe, A. Nishio, K. Okazaki, M. Kido, K. Saga, J. Tanaka, T. Akamatsu, S. Ohashi, M. Asada, T. Fukui, and T. Chiba. Essential role of peyer's patches in the development of helicobacter-induced gastritis. *Int Immunol*, 19(4):435–46, 2007. doi:10.1093/intimm/dxm008.

[39] C. F. Maurice, H. J. Haiser, and P. J. Turnbaugh. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell*, 152(1-2):39–50, 2013. doi:10.1016/j.cell.2012.10.052.

[40] J. Bornschein, A. Kandulski, M. Selgrad, and P. Malfertheiner. From gastric inflammation to gastric cancer. *Dig Dis*, 28(4-5):609–14, 2010. doi:10.1159/000320061.

[41] J. E. Crabtree, T. M. Shallcross, R. V. Heatley, and J. I. Wyatt. Mucosal tumour necrosis factor alpha and interleukin-6 in patients with helicobacter pylori associated gastritis. *Gut*, 32(12):1473–7, 1991.

[42] M. Galgani, I. Busiello, S. Censini, S. Zappacosta, L. Racioppi, and R. Zarrilli. Helicobacter pylori induces apoptosis of human monocytes but not monocyte-derived dendritic cells: role of the cag pathogenicity island. *Infect Immun*, 72(8):4480–5, 2004. doi:10.1128/iai.72.8.4480-4485.2004.

[43] C. Lindholm, M. Quiding-Jarbrink, H. Lonroth, A. Hamlet, and A. M. Svennerholm. Local cytokine response in helicobacter pylori-infected subjects. *Infect Immun*, 66(12):5964–71, 1998.

[44] F. Luzza, T. Parrello, G. Monteleone, L. Sebkova, M. Romano, R. Zarrilli, M. Imeneo, and F. Pallone. Up-regulation of il-17 is associated with bioactive il-8 expression in helicobacter pylori-infected human gastric mucosa. *J Immunol*, 165(9):5332–7, 2000.

[45] T. Mizuno, T. Ando, K. Nobata, T. Tsuzuki, O. Maeda, O. Watanabe, M. Minami, K. Ina, K. Kusugami, R. M. Peek, and H. Goto. Interleukin-17 levels in helicobacter pylori-infected gastric mucosa and pathologic sequelae of colonization. *World J Gastroenterol*, 11(40):6305–11, 2005.

[46] V. Ricci, M. Romano, and P. Boquet. Molecular cross-talk between helicobacter pylori and human gastric mucosa. *World J Gastroenterol*, 17(11):1383–99, 2011. doi:10.3748/wjg.v17.i11.1383.

[47] K. A. Eaton, M. Mefford, and T. Thevenot. The role of t cell subsets and cytokines in the pathogenesis of helicobacter pylori gastritis in mice. *J Immunol*, 166(12):7456–61, 2001.

[48] S. Raghavan, M. Fredriksson, A. M. Svennerholm, J. Holmgren, and E. Suri-Payer. Absence of cd4+cd25+ regulatory t cells is associated with a loss of regulation leading to increased pathology in helicobacter pylori-infected mice. *Clin Exp Immunol*, 132(3):393–400, 2003.

[49] K. A. Roth, S. B. Kapadia, S. M. Martin, and R. G. Lorenz. Cellular immune responses are essential for the development of helicobacter felis-associated gastric pathology. *J Immunol*, 163(3):1490–7, 1999.

[50] L. E. Smythies, K. B. Waites, J. R. Lindsey, P. R. Harris, P. Ghiara, and P. D. Smith. Helicobacter pylori-induced mucosal inflammation is th1 mediated and exacerbated in il-4, but not ifn-gamma, gene-deficient mice. *J Immunol*, 165(2):1022–9, 2000.

[51] K. A. Eaton and M. E. Mefford. Cure of helicobacter pylori infection and resolution of gastritis by adoptive transfer of splenocytes in mice. *Infect Immun*, 69(2):1025–31, 2001. doi:10.1128/iai.69.2.1025-1031.2001.

[52] A. Lundgren, E. Stromberg, A. Sjoling, C. Lindholm, K. Enarsson, A. Edebo, E. Johnsson, E. Suri-Payer, P. Larsson, A. Rudin, A. M. Svennerholm, and B. S. Lundin. Mucosal foxp3-expressing cd4+ cd25high regulatory t cells in helicobacter pylori-infected patients. *Infect Immun*, 73(1):523–31, 2005. doi:10.1128/iai.73.1.523-531.2005.

[53] A. Lundgren, E. Suri-Payer, K. Enarsson, A. M. Svennerholm, and B. S. Lundin. Helicobacter pylori-specific cd4+ cd25high regulatory t cells suppress memory t-cell responses to h. pylori in infected individuals. *Infect Immun*, 71(4):1755–62, 2003.

[54] M. Quiding-Jarbrink, I. Ahlstedt, C. Lindholm, E. L. Johansson, and H. Lonroth. Homing commitment of lymphocytes activated in the human gastric and intestinal mucosa. *Gut*, 49(4):519–25, 2001.

[55] I. Hitzler, E. Kohler, D. B. Engler, A. S. Yazgan, and A. Muller. The role of th cell subsets in the control of helicobacter infections and in t cell-driven gastric immunopathology. *Front Immunol*, 3:142, 2012. doi:10.3389/fimmu.2012.00142.

[56] A. Sayi, E. Kohler, I. Hitzler, I. Arnold, R. Schwendener, H. Rehrauer, and A. Muller. The cd4+ t cell-mediated ifn-gamma response to helicobacter infection is essential for clearance and determines gastric cancer risk. *J Immunol*, 182(11):7085–101, 2009. doi:10.4049/jimmunol.0803293.

[57] M. Quiding-Jarbrink, B. S. Lundin, H. Lonroth, and A. M. Svennerholm. Cd4+ and cd8+ t cell responses in helicobacter pylori-infected individuals. *Clin Exp Immunol*, 123(1):81–7, 2001.

[58] J. Pappo, D. Torrey, L. Castriotta, A. Savinainen, Z. Kabok, and A. Ibraghimov. Helicobacter pylori infection in immunized mice lacking major histocompatibility complex class i and class ii functions. *Infect Immun*, 67(1):337–41, 1999.

[59] T. H. Ermak, P. J. Giannasca, R. Nichols, G. A. Myers, J. Nedrud, R. Weltzin, C. K. Lee, H. Kleanthous, and T. P. Monath. Immunization of mice with urease vaccine affords protection against helicobacter pylori infection in the absence of antibodies and is mediated by mhc class ii-restricted responses. *J Exp Med*, 188(12):2277–88, 1998.

[60] A. M. Elliott, P. A. Mawa, E. L. Webb, M. Nampijja, N. Lyadda, J. Bukusuba, M. Kizza, P. B. Namujju, J. Nabulime, J. Ndibazza, M. Muwanga, and J. A. Whitworth. Effects of maternal and infant co-infections, and of maternal immunisation, on the infant response to bcg and tetanus immunisation. *Vaccine*, 29(2):247–55, 2010. doi:10.1016/j.vaccine.2010.10.047.

[61] Y. Shi, X. F. Liu, Y. Zhuang, J. Y. Zhang, T. Liu, Z. Yin, C. Wu, X. H. Mao, K. R. Jia, F. J. Wang, H. Guo, R. A. Flavell, Z. Zhao, K. Y. Liu, B. Xiao, Y. Guo, W. J. Zhang, W. Y. Zhou, G. Guo, and Q. M. Zou. Helicobacter pylori-induced th17 responses modulate th1 cell responses, benefit bacterial growth, and contribute to pathology in mice. *J Immunol*, 184(9):5121–9, 2010. doi:10.4049/jimmunol.0901115.

[62] L. Sebkova, A. Pellicano, G. Monteleone, B. Grazioli, G. Guarnieri, M. Imeneo, F. Pallone, and F. Luzza. Extracellular signal-regulated protein kinase mediates interleukin 17 (il-17)-induced il-8 secretion in helicobacter pylori-infected human gastric epithelial cells. *Infect Immun*, 72(9):5019–26, 2004. doi:10.1128/iai.72.9.5019-5026.2004.

[63] S. Shiomi, A. Toriie, S. Imamura, H. Konishi, S. Mitsufuji, Y. Iwakura, Y. Yamaoka, H. Ota, T. Yamamoto, J. Imanishi, and M. Kita. Il-17 is involved in helicobacter pylori-induced gastric inflammatory responses in a mouse model. *Helicobacter*, 13(6):518–24, 2008. doi:10.1111/j.1523-5378.2008.00629.x.

[64] R. Karttunen, T. Karttunen, H. P. Ekre, and T. T. MacDonald. Interferon gamma and interleukin 4 secreting cells in the gastric antrum in helicobacter pylori positive and negative gastritis. *Gut*, 36(3):341–5, 1995.

[65] A. A. Akhiani, J. Pappo, Z. Kabok, K. Schon, W. Gao, L. E. Franzen, and N. Lycke. Protection against helicobacter pylori infection following immunization is il-12-dependent and mediated by th1 cells. *J Immunol*, 169(12):6977–84, 2002.

[66] N. Sawai, M. Kita, T. Kodama, T. Tanahashi, Y. Yamaoka, Y. Tagawa, Y. Iwakura, and J. Imanishi. Role of gamma interferon in helicobacter pylori-induced gastric inflammatory responses in a mouse model. *Infect Immun*, 67(1):279–85, 1999.

[67] B. M. Gray, C. A. Fontaine, S. A. Poe, and K. A. Eaton. Complex t cell interactions contribute to helicobacter pylori gastritis in mice. *Infect Immun*, 81(3):740–52, 2013. doi:10.1128/iai.01269-12.

[68] K. A. Eaton, L. H. Benson, J. Haeger, and B. M. Gray. Role of transcription factor t-bet expression by cd4+ cells in gastritis due to helicobacter pylori in mice. *Infect Immun*, 74(8):4673–84, 2006. doi:10.1128/iai.01887-05.

[69] A. Lundgren, C. Trollmo, A. Edebo, A. M. Svennerholm, and B. S. Lundin. Helicobacter pylori-specific cd4+ t cells home to and accumulate in the human helicobacter pylori-infected gastric mucosa. *Infect Immun*, 73(9):5612–9, 2005. doi:10.1128/iai.73.9.5612-5619.2005.

[70] N. Ohkura, Y. Kitagawa, and S. Sakaguchi. Development and maintenance of regulatory t cells. *Immunity*, 38(3):414–23, 2013. doi:10.1016/j.immuni.2013.03.002.

[71] R. Rad, L. Brenner, S. Bauer, S. Schwendy, L. Layland, C. P. da Costa, W. Reindl, A. Dossumbekova, M. Friedrich, D. Saur, H. Wagner, R. M. Schmid, and C. Prinz. Cd25+/foxp3+ t cells regulate gastric inflammation and helicobacter pylori colonization in vivo. *Gastroenterology*, 131(2):525–37, 2006. doi:10.1053/j.gastro.2006.05.001.

[72] T. J. Jang. The number of foxp3-positive regulatory t cells is increased in helicobacter pylori gastritis and gastric cancer. *Pathol Res Pract*, 206(1):34–8, 2010. doi:10.1016/j.prp.2009.07.019.

[73] F. Freire de Melo, A. M. Rocha, G. A. Rocha, S. H. Pedroso, S. de Assis Batista, L. P. Fonseca de Castro, S. D. Carvalho, P. F. Bittencourt, C. A. de Oliveira, R. Correa-Oliveira, and D. M. Magalhaes Queiroz. A regulatory instead of an il-17 t response predominates in helicobacter pylori-associated gastritis in children. *Microbes Infect*, 14(4):341–7, 2012. doi:10.1016/j.micinf.2011.11.008.

[74] P. R. Harris, S. W. Wright, C. Serrano, F. Riera, I. Duarte, J. Torres, A. Pena, A. Rollan, P. Viviani, E. Guiraldes, J. M. Schmitz, R. G. Lorenz, L. Novak, L. E. Smythies, and P. D. Smith. Helicobacter pylori gastritis in children is associated with a regulatory t-cell response. *Gastroenterology*, 134(2):491–9, 2008. doi:10.1053/j.gastro.2007.11.006.

[75] W. Chen, D. Shu, and V. S. Chadwick. Helicobacter pylori infection: mechanism of colonization and functional dyspepsia reduced colonization of gastric mucosa by helicobacter pylori in mice deficient in interleukin-10. *J Gastroenterol Hepatol*, 16(4):377–83, 2001.

[76] H. F. Ismail, P. Fick, J. Zhang, R. G. Lynch, and D. J. Berg. Depletion of neutrophils in il-10(-/-) mice delays clearance of gastric helicobacter infection and decreases the th1 immune response to helicobacter. *J Immunol*, 170(7):3782–9, 2003.

[77] R. X. Tang, D. J. Luo, A. H. Sun, and J. Yan. Diversity of helicobacter pylori isolates in expression of antigens and induction of antibodies. *World J Gastroenterol*, 14(30):4816–22, 2008.

[78] A. A. Akhiani, K. Schon, L. E. Franzen, J. Pappo, and N. Lycke. Helicobacter pylori-specific antibodies impair the development of gastritis, facilitate bacterial colonization, and counteract resistance against infection. *J Immunol*, 172(8):5024–33, 2004.

[79] T. G. Blanchard, S. J. Czinn, R. W. Redline, N. Sigmund, G. Harriman, and J. G. Nedrud. Antibody-independent protective mucosal immunity to gastric helicobacter infection in mice. *Cell Immunol*, 191(1):74–80, 1999. doi:10.1006/cimm.1998.1421.

[80] N. Manojlovic, D. Babic, I. Filipovic-Ljeshovic, and D. Pilcevic. Anti helicobacter pylori igg and iga response in patients with gastric cancer and chronic gastritis. *Hepatogastroenterology*, 55(82-83):807–13, 2008.

[81] E. A. Marcus, A. P. Moshfegh, G. Sachs, and D. R. Scott. The periplasmic alpha-carbonic anhydrase activity of helicobacter pylori is essential for acid acclimation. *J Bacteriol*, 187(2):729–38, 2005. doi:10.1128/jb.187.2.729-738.2005.

[82] D. L. Weeks, S. Eskandari, D. R. Scott, and G. Sachs. A h+-gated urea channel: the link between helicobacter pylori urease and gastric colonization. *Science*, 287(5452):482–5, 2000.

[83] H. Gu. Role of flagella in the pathogenesis of helicobacter pylori. *Curr Microbiol*, 74(7):863–869, 2017. doi:10.1007/s00284-017-1256-4.

[84] K. M. Ottemann and A. C. Lowenthal. Helicobacter pylori uses motility for initial colonization and to attain robust infection. *Infect Immun*, 70(4):1984–90, 2002.

[85] T. W. Cullen, D. K. Giles, L. N. Wolf, C. Ecobichon, I. G. Boneca, and M. S. Trent. Helicobacter pylori versus the host: remodeling of the bacterial outer membrane is required for survival in the gastric mucosa. *PLoS Pathog*, 7(12):e1002454, 2011. doi:10.1371/journal.ppat.1002454.

[86] E. Andersen-Nissen, K. D. Smith, K. L. Strobe, S. L. Barrett, B. T. Cookson, S. M. Logan, and A. Aderem. Evasion of toll-like receptor 5 by flagellated bacteria. *Proc Natl Acad Sci U S A*, 102(26):9247–52, 2005. doi:10.1073/pnas.0502040102.

[87] G. Wang, Z. Ge, D. A. Rasko, and D. E. Taylor. Lewis antigens in helicobacter pylori: biosynthesis and phase variation. *Mol Microbiol*, 36(6):1187–96, 2000.

[88] M. J. Blaser, G. I. Perez-Perez, H. Kleanthous, T. L. Cover, R. M. Peek, P. H. Chyou, G. N. Stemmermann, and A. Nomura. Infection with helicobacter pylori strains possessing caga is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res*, 55(10):2111–5, 1995.

[89] S. Censini, C. Lange, Z. Xiang, J. E. Crabtree, P. Ghiara, M. Borodovsky, R. Rappuoli, and A. Covacci. cag, a pathogenicity island of helicobacter pylori, encodes type i-specific and disease-associated virulence factors. *Proc Natl Acad Sci U S A*, 93(25):14648–53, 1996.

[90] J. E. Crabtree, J. D. Taylor, J. I. Wyatt, R. V. Heatley, T. M. Shallcross, D. S. Tompkins, and B. J. Rathbone. Mucosal iga recognition of helicobacter pylori 120 kda protein, peptic ulceration, and gastric pathology. *Lancet*, 338(8763):332–5, 1991.

[91] M. Hatakeyama and H. Higashi. Helicobacter pylori caga: a new paradigm for bacterial carcinogenesis. *Cancer Sci*, 96(12):835–43, 2005. doi:10.1111/j.1349-7006.2005.00130.x.

[92] M. R. Amieva, R. Vogelmann, A. Covacci, L. S. Tompkins, W. J. Nelson, and S. Falkow. Disruption of the epithelial apical-junctional complex by helicobacter pylori caga. *Science*, 300(5624):1430–4, 2003. doi:10.1126/science.1081919.

[93] F. Bagnoli, L. Buti, L. Tompkins, A. Covacci, and M. R. Amieva. Helicobacter pylori caga induces a transition from polarized to invasive phenotypes in mdck cells. *Proc Natl Acad Sci U S A*, 102(45):16339–44, 2005. doi:10.1073/pnas.0502598102.

[94] N. Murata-Kamiya, Y. Kurashima, Y. Teishikata, Y. Yamahashi, Y. Saito, H. Higashi, H. Aburatani, T. Akiyama, J. Peek, R. M., T. Azuma, and M. Hatakeyama. Helicobacter pylori caga interacts with e-cadherin and deregulates the beta-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. *Oncogene*, 26(32):4617–26, 2007. doi:10.1038/sj.onc.1210251.

[95] M. J. Oliveira, A. M. Costa, A. C. Costa, R. M. Ferreira, P. Sampaio, J. C. Machado, R. Seruca, M. Mareel, and C. Figueiredo. Caga associates with c-met, e-cadherin, and p120-catenin in a multiproteic complex that suppresses helicobacter pylori-induced cell-invasive phenotype. *J Infect Dis*, 200(5):745–55, 2009. doi:10.1086/604727.

[96] P. Y. Zheng and N. L. Jones. Helicobacter pylori strains expressing the vacuolating cytotoxin interrupt phagosome maturation in macrophages by recruiting and retaining taco (coronin 1) protein. *Cell Microbiol*, 5(1):25–40, 2003.

[97] K. R. Jones, J. M. Whitmire, and D. S. Merrell. A tale of two toxins: Helicobacter pylori caga and vaca modulate host pathways that impact disease. *Front Microbiol*, 1:115, 2010. doi:10.3389/fmicb.2010.00115.

[98] M. Mori, H. Suzuki, M. Suzuki, A. Kai, S. Miura, and H. Ishii. Catalase and superoxide dismutase secreted from helicobacter pylori. *Helicobacter*, 2(2):100–5, 1997.

[99] R. Kabisch, R. P. Semper, S. Wustner, M. Gerhard, and R. Mejias-Luque. Helicobacter pylori gamma-glutamyltranspeptidase induces tolerogenic human dendritic cells by activation of glutamate receptors. *J Immunol*, 196(10):4246–52, 2016. doi:10.4049/jimmunol.1501062.

[100] J. Y. Kao, M. Zhang, M. J. Miller, J. C. Mills, B. Wang, M. Liu, K. A. Eaton, W. Zou, B. E. Berndt, T. S. Cole, T. Takeuchi, S. Y. Owyang, and J. Luther. Helicobacter pylori immune escape is mediated by dendritic cell-induced treg skewing and th17 suppression in mice. *Gastroenterology*, 138(3):1046–54, 2010. doi:10.1053/j.gastro.2009.11.043.

[101] K. Robinson, R. H. Argent, and J. C. Atherton. The inflammatory and immune response to helicobacter pylori infection. *Best Pract Res Clin Gastroenterol*, 21(2):237–59, 2007. doi:10.1016/j.bpg.2007.01.001.

[102] J. R. White, J. A. Winter, and K. Robinson. Differential inflammatory response to helicobacter pylori infection: etiology and clinical outcomes. *J Inflamm Res*, 8:137–47, 2015. doi:10.2147/jir.s64888.

[103] D. Basso, M. Plebani, and J. G. Kusters. Pathogenesis of helicobacter pylori infection. *Helicobacter*, 15 Suppl 1:14–20, 2010. doi:10.1111/j.1523-5378.2010.00781.x.

[104] Schistosomes, liver flukes and helicobacter pylori. iarc working group on the evaluation of carcinogenic risks to humans. lyon, 7-14 june 1994. *IARC Monogr Eval Carcinog Risks Hum*, 61:1–241, 1994.

[105] A. Hamlet and L. Olbe. The influence of helicobacter pylori infection on postprandial duodenal acid load and duodenal bulb ph in humans. *Gastroenterology*, 111(2):391–400, 1996.

[106] S. Khulusi, S. Badve, P. Patel, R. Lloyd, J. M. Marrero, C. Finlayson, M. A. Mendall, and T. C. Northfield. Pathogenesis of gastric metaplasia of the human duodenum: role of helicobacter pylori, gastric acid, and ulceration. *Gastroenterology*, 110(2):452–8, 1996.

[107] E. M. El-Omar, K. Oien, A. El-Nujumi, D. Gillen, A. Wirz, S. Dahill, C. Williams, J. E. Ardill, and K. E. McColl. Helicobacter pylori infection and chronic gastric acid hyposecretion. *Gastroenterology*, 113(1):15–24, 1997.

[108] A. Ruol, A. Parenti, G. Zaninotto, S. Merigliano, M. Costantini, M. Cagol, R. Alfieri, L. Bonavina, A. Peracchia, and E. Ancona. Intestinal metaplasia is the probable common precursor of adenocarcinoma in barrett esophagus and adenocarcinoma of the gastric cardia. *Cancer*, 88(11):2520–8, 2000.

[109] J. Q. Huang, G. F. Zheng, K. Sumanac, E. J. Irvine, and R. H. Hunt. Meta-analysis of the relationship between caga seropositivity and gastric cancer. *Gastroenterology*, 125(6):1636–44, 2003.

[110] E. J. Kuipers, G. I. Perez-Perez, S. G. Meuwissen, and M. J. Blaser. Helicobacter pylori and atrophic gastritis: importance of the caga status. *J Natl Cancer Inst*, 87(23):1777–80, 1995.

[111] J. Parsonnet, G. D. Friedman, N. Orentreich, and H. Vogelman. Risk for gastric cancer in people with caga positive or caga negative helicobacter pylori infection. *Gut*, 40(3):297–301, 1997.

[112] L. T. Nguyen, T. Uchida, K. Murakami, T. Fujioka, and M. Moriyama. Helicobacter pylori virulence and the diversity of gastric cancer in asia. *J Med Microbiol*, 57(Pt 12):1445–53, 2008. doi:10.1099/jmm.0.2008/003160-0.

[113] R. Suzuki, S. Shiota, and Y. Yamaoka. Molecular epidemiology, population genetics, and pathogenic role of helicobacter pylori. *Infect Genet Evol*, 12(2):203–13, 2012. doi:10.1016/j.meegid.2011.12.002.

[114] K. Ogura, S. Maeda, M. Nakao, T. Watanabe, M. Tada, T. Kyutoku, H. Yoshida, Y. Shiratori, and M. Omata. Virulence factors of helicobacter pylori responsible for gastric diseases in mongolian gerbil. *J Exp Med*, 192(11):1601–10, 2000.

[115] J. Peek, R. M., H. P. Wirth, S. F. Moss, M. Yang, A. M. Abdalla, K. T. Tham, T. Zhang, L. H. Tang, I. M. Modlin, and M. J. Blaser. Helicobacter pylori alters gastric epithelial cell cycle events and gastrin secretion in mongolian gerbils. *Gastroenterology*, 118(1):48–59, 2000.

[116] T. Watanabe, M. Tada, H. Nagai, S. Sasaki, and M. Nakao. Helicobacter pylori infection induces gastric cancer in mongolian gerbils. *Gastroenterology*, 115(3):642–8, 1998.

[117] H. P. Wirth, M. H. Beins, M. Yang, K. T. Tham, and M. J. Blaser. Experimental infection of mongolian gerbils with wild-type and mutant helicobacter pylori strains. *Infect Immun*, 66(10):4856–66, 1998.

[118] N. Ohnishi, H. Yuasa, S. Tanaka, H. Sawa, M. Miura, A. Matsui, H. Higashi, M. Musashi, K. Iwabuchi, M. Suzuki, G. Yamada, T. Azuma, and M. Hatakeyama. Transgenic expression of helicobacter pylori caga induces gastrointestinal and hematopoietic neoplasms in mouse. *Proc Natl Acad Sci U S A*, 105(3):1003–8, 2008. doi:10.1073/pnas.0711183105.

[119] M. Venerito, R. Vasapolli, T. Rokkas, J. C. Delchier, and P. Malfertheiner. Helicobacter pylori, gastric cancer and other gastrointestinal malignancies. *Helicobacter*, 22 Suppl 1, 2017. doi:10.1111/hel.12413.

[120] E. Goni and F. Franceschi. Helicobacter pylori and extragastric diseases. *Helicobacter*, 21 Suppl 1:45–8, 2016. doi:10.1111/hel.12340.

[121] M. Kawaguchi, T. Saito, H. Ohno, S. Midorikawa, T. Sanji, Y. Handa, S. Morita, H. Yoshida, M. Tsurui, R. Misaka, T. Hirota, M. Saito, and K. Minami. Bacteria closely resembling helicobacter pylori detected immunohistologically and genetically in resected gallbladder mucosa. *J Gastroenterol*, 31(2):294–8, 1996.

[122] J. W. Lee, D. H. Lee, J. I. Lee, S. Jeong, K. S. Kwon, H. G. Kim, Y. W. Shin, Y. S. Kim, M. S. Choi, and S. Y. Song. Identification of helicobacter pylori in gallstone, bile, and other hepatobiliary tissues of patients with cholecystitis. *Gut Liver*, 4(1):60–7, 2010. doi:10.5009/gnl.2010.4.1.60.

[123] K. Ito, M. Nakamura, G. Toda, M. Negishi, A. Torii, and T. Ohno. Potential role of helicobacter pylori in hepatocarcinogenesis. *Int J Mol Med*, 13(2):221–7, 2004.

[124] N. Leone, R. Pellicano, F. Brunello, M. A. Cutufia, M. Berrutti, S. Fagoonee, M. Rizzetto, and A. Ponzetto. Helicobacter pylori seroprevalence in patients with cirrhosis of the liver and hepatocellular carcinoma. *Cancer Detect Prev*, 27(6):494–7, 2003.

[125] P. Avenaud, A. Marais, L. Monteiro, B. Le Bail, P. Bioulac Sage, C. Balabaud, and F. Megraud. Detection of helicobacter species in the liver of patients with and without primary liver carcinoma. *Cancer*, 89(7):1431–9, 2000.

[126] H. O. Nilsson, R. Mulchandani, K. G. Tranberg, U. Stenram, and T. Wadstrom. *Helicobacter species identified in liver from patients with cholangiocarcinoma and hepatocellular carcinoma*, volume 120, pages 323–4. United States, 2001.

[127] R. Pellicano, V. Mazzaferro, W. F. Grigioni, M. A. Cutufia, S. Fagoonee, L. Silengo, M. Rizzetto, and A. Ponzetto. Helicobacter species sequences in liver samples from patients with and without hepatocellular carcinoma. *World J Gastroenterol*, 10(4):598–601, 2004.

[128] S. Y. Xuan, N. Li, X. Qiang, R. R. Zhou, Y. X. Shi, and W. J. Jiang. Helicobacter infection in hepatocellular carcinoma tissue. *World J Gastroenterol*, 12(15):2335–40, 2006.

[129] J. Pogorzelska, M. Lapinska, A. Kalinowska, T. W. Lapinski, and R. Flisiak. Helicobacter pylori infection among patients with liver cirrhosis. *Eur J Gastroenterol Hepatol*, 29(10):1161–1165, 2017. doi:10.1097/meg.0000000000000928.

[130] J. Wang, R. C. Chen, Y. X. Zheng, S. S. Zhao, N. Li, R. R. Zhou, Y. Huang, Z. B. Huang, and X. G. Fan. Helicobacter pylori infection may increase the risk of progression of chronic hepatitis b disease among the chinese population: a meta-analysis. *Int J Infect Dis*, 50:30–7, 2016. doi:10.1016/j.ijid.2016.07.014.

[131] J. Wang, W. T. Li, Y. X. Zheng, S. S. Zhao, N. Li, Y. Huang, R. R. Zhou, Z. B. Huang, and X. G. Fan. The association between helicobacter pylori infection and chronic hepatitis c: A meta-analysis and trial sequential analysis. *Gastroenterol Res Pract*, 2016:8780695, 2016. doi:10.1155/2016/8780695.

[132] URL: http://globocan.iarc.fr/old/FactSheets/cancers/stomach-new.asp.

[133] J. K. Y. Hooi, W. Y. Lai, W. K. Ng, M. M. Y. Suen, F. E. Underwood, D. Tanyingoh, P. Malfertheiner, D. Y. Graham, V. W. S. Wong, J. C. Y. Wu, F. K. L. Chan, J. J. Y. Sung, G. G. Kaplan, and S. C. Ng. Global prevalence of helicobacter pylori infection: Systematic review and meta-analysis. *Gastroenterology*, 153(2):420–429, 2017. doi:10.1053/j.gastro.2017.04.022.

[134] W. H. Organization. 2012. URL: http://www.who.int/schistosomiasis/en/.

[135] J. G. Fox, P. Beck, C. A. Dangler, M. T. Whary, T. C. Wang, H. N. Shi, and C. Nagler-Anderson. Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduces helicobacter-induced gastric atrophy. *Nat Med*, 6(5):536–42, 2000. URL: http://www.ncbi.nlm.nih.gov/pubmed/10802709, doi:10.1038/75015.

[136] M. T. Whary, N. Sundina, L. E. Bravo, P. Correa, F. Quinones, F. Caro, and J. G. Fox. Intestinal helminthiasis in colombian children promotes a th2 response to helicobacter pylori: possible implications for gastric carcinogenesis. *Cancer Epidemiol Biomarkers Prev*, 14(6):1464–9, 2005. doi:10.1158/1055-9965.epi-05-0095.

[137] S. A. Abou Holw, M. M. Anwar, R. B. Bassiouni, N. A. Hussen, and H. A. Eltaweel. Impact of coinfection with schistosoma mansoni on helicobacter pylori induced disease. *J Egypt Soc Parasitol*, 38(1):73–84, 2008.

[138] Y. Du, A. Agnew, X. P. Ye, P. A. Robinson, D. Forman, and J. E. Crabtree. Helicobacter pylori and schistosoma japonicum co-infection in a chinese population: helminth infection alters humoral responses to h. pylori and serum pepsinogen i/ii ratio. *Microbes Infect*, 8(1):52–60, 2006. doi:10.1016/j.micinf.2005.05.017.

[139] B. Gryseels. Schistosomiasis. *Infect Dis Clin North Am*, 26(2):383–97, 2012. doi:10.1016/j.idc.2012.03.004.

[140] 2010. URL: http://www.thiswormyworld.org/worms/global-burden.

[141] January 2017. URL: http://www.who.int/mediacentre/factsheets/fs115/en/.

[142] J. Boissier, S. Grech-Angelini, B. L. Webster, J. F. Allienne, T. Huyse, S. Mas-Coma, E. Toulza, H. Barre-Cardi, D. Rollinson, J. Kincaid-Smith, A. Oleaga, R. Galinier, J. Foata, A. Rognon, A. Berry, G. Mouahid, R. Henneron, H. Mone, H. Noel, and G. Mitta. Outbreak of urogenital schistosomiasis in corsica (france): an epidemiological case study. *Lancet Infect Dis*, 16(8):971–9, 2016. doi:10.1016/s1473-3099(16)00175-4.

[143] B. L. Webster, O. T. Diaw, M. M. Seye, J. P. Webster, and D. Rollinson. Introgressive hybridization of schistosoma haematobium group species in senegal: species barrier break down between ruminant and human schistosomes. *PLoS Negl Trop Dis*, 7(4):e2110, 2013. doi:10.1371/journal.pntd.0002110.

[144] J. P. Webster, G. Oliviera, D. Rollinson, and C. M. Gower. Schistosome genomes: a wealth of information. *Trends Parasitol*, 26(3):103–6, 2010. doi:10.1016/j.pt.2009.12.006.

[145] 2016. URL: http://www.who.int/neglected_diseases/news/WHO_urges_increased_access_to_praziquantel/en/.

[146] K. Fukuyama, S. Tzeng, J. McKerrow, and W. L. Epstein. The epidermal barrier to schistosoma mansoni infection. *Curr Probl Dermatol*, 11:185–93, 1983.

[147] E. Hansell, S. Braschi, K. F. Medzihradszky, M. Sajid, M. Debnath, J. Ingram, K. C. Lim, and J. H. McKerrow. Proteomic analysis of skin invasion by blood fluke larvae. *PLoS Negl Trop Dis*, 2(7):e262, 2008. doi:10.1371/journal.pntd.0000262.

[148] G. N. Gobert, M. Chai, and D. P. McManus. Biology of the schistosome lung-stage schistosomulum. *Parasitology*, 134(Pt 4):453–60, 2007. doi:10.1017/s0031182006001648.

[149] Z. A. Andrade and S. G. Andrade. Pathogenesis of schistosomal pulmonary arteritis. *Am J Trop Med Hyg*, 19(2):305–10, 1970.

[150] E. J. Pearce and A. S. MacDonald. The immunobiology of schistosomiasis. *Nat Rev Immunol*, 2(7):499–511, 2002. doi:10.1038/nri843.

[151] J. V. Weinstock and D. L. Boros. Heterogeneity of the granulomatous response in the liver, colon, ileum, and ileal peyer's patches to schistosome eggs in murine schistosomiasis mansoni. *J Immunol*, 127(5):1906–9, 1981.

[152] A. G. Ross, D. Vickers, G. R. Olds, S. M. Shah, and D. P. McManus. Katayama syndrome. *Lancet Infect Dis*, 7(3):218–24, 2007. doi:10.1016/s1473-3099(07)70053-1.

[153] M. L. Burke, M. K. Jones, G. N. Gobert, Y. S. Li, M. K. Ellis, and D. P. McManus. Immunopathogenesis of human schistosomiasis. *Parasite Immunol*, 31(4):163–76, 2009. doi:10.1111/j.1365-3024.2009.01098.x.

[154] D. J. Gray, A. G. Ross, Y. S. Li, and D. P. McManus. Diagnosis and management of schistosomiasis. *Bmj*, 342:d2651, 2011. doi:10.1136/bmj.d2651.

[155] P. J. Turnbaugh, R. E. Ley, M. A. Mahowald, V. Magrini, E. R. Mardis, and J. I. Gordon. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444(7122):1027–31, 2006. doi:10.1038/nature05414.

[156] J. M. Grzych, E. Pearce, A. Cheever, Z. A. Caulada, P. Caspar, S. Heiny, F. Lewis, and A. Sher. Egg deposition is the major stimulus for the production of th2 cytokines in murine schistosomiasis mansoni. *J Immunol*, 146(4):1322–7, 1991.

[157] E. J. Pearce, P. Caspar, J. M. Grzych, F. A. Lewis, and A. Sher. Pillars article: down-regulation of th1 cytokine production accompanies induction of th2 responses by a parasitic helminth, schistosoma mansoni. j. exp. med. 1991. 173: 159-166. *J Immunol*, 189(3):1104–11, 2012.

[158] D. Soonawala, J. W. Geerts, M. de Mos, M. Yazdanbakhsh, and L. G. Visser. The immune response to schistosome antigens in formerly infected travelers. *Am J Trop Med Hyg*, 84(1):43–7, 2011. doi:10.4269/ajtmh.2011.10-0260.

[159] F. V. Duraes, N. B. Carvalho, T. T. Melo, S. C. Oliveira, and C. T. Fonseca. Il-12 and tnf-alpha production by dendritic cells stimulated with schistosoma mansoni schistosomula tegument is tlr4- and myd88-dependent. *Immunol Lett*, 125(1):72–7, 2009. doi:10.1016/j.imlet.2009.06.004.

[160] R. A. Paveley, S. A. Aynsley, J. D. Turner, C. D. Bourke, S. J. Jenkins, P. C. Cook, L. Martinez-Pomares, and A. P. Mountford. The mannose receptor (cd206) is an important pattern recognition receptor (prr) in the detection of the infective stage of the helminth schistosoma mansoni and modulates ifngamma production. *Int J Parasitol*, 41(13-14):1335–45, 2011. doi:10.1016/j.ijpara.2011.08.005.

[161] Y. J. Liang, J. Luo, Q. Lu, Y. Zhou, H. W. Wu, D. Zheng, Y. Y. Ren, K. Y. Sun, Y. Wang, and Z. S. Zhang. Gene profile of chemokines on hepatic stellate cells of schistosome-infected mice and antifibrotic roles of cxcl9/10 on liver non-parenchymal cells. *PLoS One*, 7(8):e42490, 2012. doi:10.1371/journal.pone.0042490.

[162] M. K. Park, K. F. Hoffmann, A. W. Cheever, D. Amichay, T. A. Wynn, and J. M. Farber. Patterns of chemokine expression in models of schistosoma mansoni inflammation and infection reveal relationships between type 1 and type 2 responses and chemokines in vivo. *Infect Immun*, 69(11):6755–68, 2001. doi:10.1128/iai.69.11.6755-6768.2001.

[163] A. L. Souza, S. R. Sousa-Pereira, M. M. Teixeira, J. R. Lambertucci, and A. L. Teixeira. The role of chemokines in schistosoma mansoni infection: insights from human disease and murine models. *Mem Inst Oswaldo Cruz*, 101 Suppl 1:333–8, 2006.

[164] E. C. Mbanefo, N. T. Huy, A. A. Wadagni, C. I. Eneanya, O. Nwaorgu, and K. Hirayama. Host determinants of reinfection with schistosomes in humans: a systematic review and meta-analysis. *PLoS Negl Trop Dis*, 8(9):e3164, 2014. doi:10.1371/journal.pntd.0003164.

[165] E. J. Pearce. Priming of the immune response by schistosome eggs. *Parasite Immunol*, 27(7-8):265–70, 2005. doi:10.1111/j.1365-3024.2005.00765.x.

[166] E. J. Pearce, M. K. C, J. Sun, J. T. J, A. S. McKee, and L. Cervi. Th2 response polarization during infection with the helminth parasite schistosoma mansoni. *Immunol Rev*, 201:117–26, 2004. doi:10.1111/j.0105-2896.2004.00187.x.

[167] C. K. Hsu, S. H. Hsu, J. Whitney, R. A., and C. T. Hansen. Immunopathology of schistosomiasis in athymic mice. *Nature*, 262(5567):397–9, 1976.

[168] R. C. Mathew and D. L. Boros. Anti-l3t4 antibody treatment suppresses hepatic granuloma formation and abrogates antigen-induced interleukin-2 production in schistosoma mansoni infection. *Infect Immun*, 54(3):820–6, 1986.

[169] S. M. Phillips, J. J. DiConza, J. A. Gold, and W. A. Reid. Schistosomiasis in the congenitally athymic (nude) mouse. i. thymic dependency of eosinophilia, granuloma formation, and host morbidity. *J Immunol*, 118(2):594–9, 1977.

[170] C. Schwartz, K. Oeser, C. Prazeres da Costa, L. E. Layland, and D. Voehringer. T cell-derived il-4/il-13 protects mice against fatal schistosoma mansoni infection independently of basophils. *J Immunol*, 193(7):3590–9, 2014. doi:10.4049/jimmunol.1401155.

[171] K. Fairfax, M. Nascimento, S. C. Huang, B. Everts, and E. J. Pearce. Th2 responses in schistosomiasis. *Semin Immunopathol*, 34(6):863–71, 2012. doi:10.1007/s00281-012-0354-4.

[172] L. R. Brunet, F. D. Finkelman, A. W. Cheever, M. A. Kopf, and E. J. Pearce. Il-4 protects against tnf-alpha-mediated cachexia and death during acute schistosomiasis. *J Immunol*, 159(2):777–85, 1997.

[173] L. R. Brunet, M. A. Kopf, and E. J. Pearce. Schistosoma mansoni: Il-4 is necessary for concomitant immunity in mice. *J Parasitol*, 85(4):734–6, 1999.

[174] C. Chuah, M. K. Jones, M. L. Burke, D. P. McManus, and G. N. Gobert. Cellular and chemokine-mediated regulation in schistosome-induced hepatic pathology. *Trends Parasitol*, 30(3):141–50, 2014. doi:10.1016/j.pt.2013.12.009.

[175] H. Ndlovu and F. Brombacher. Role of il-4ralpha during acute schistosomiasis in mice. *Parasite Immunol*, 36(9):421–7, 2014. doi:10.1111/pim.12080.

[176] P. G. Fallon, E. J. Richardson, G. J. McKenzie, and A. N. McKenzie. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for il-4 and il-13: Il-13 is a profibrotic agent. *J Immunol*, 164(5):2585–91, 2000.

[177] D. R. Herbert, C. Holscher, M. Mohrs, B. Arendse, A. Schwegmann, M. Radwanska, M. Leeto, R. Kirsch, P. Hall, H. Mossmann, B. Claussen, I. Forster, and F. Brombacher. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates t helper 1 responses and immunopathology. *Immunity*, 20(5):623–35, 2004.

[178] r. Gieseck, R. L., M. S. Wilson, and T. A. Wynn. Type 2 immunity in tissue repair and fibrosis. *Nat Rev Immunol*, 2017. doi:10.1038/nri.2017.90.

[179] M. G. Chiaramonte, D. D. Donaldson, A. W. Cheever, and T. A. Wynn. An il-13 inhibitor blocks the development of hepatic fibrosis during a t-helper type 2-dominated inflammatory response. *J Clin Invest*, 104(6):777–85, 1999. doi:10.1172/jci7325.

[180] M. M. Mentink-Kane, A. W. Cheever, R. W. Thompson, D. M. Hari, N. B. Kabatereine, B. J. Vennervald, J. H. Ouma, J. K. Mwatha, F. M. Jones, D. D. Donaldson, M. J. Grusby, D. W. Dunne, and T. A. Wynn. Il-13 receptor alpha 2 down-modulates granulomatous inflammation and prolongs host survival in schistosomiasis. *Proc Natl Acad Sci U S A*, 101(2):586–90, 2004. doi:10.1073/pnas.0305064101.

[181] r. Gieseck, R. L., T. R. Ramalingam, K. M. Hart, K. M. Vannella, D. A. Cantu, W. Y. Lu, S. Ferreira-Gonzalez, S. J. Forbes, L. Vallier, and T. A. Wynn. Interleukin-13 activates distinct cellular pathways leading to ductular reaction, steatosis, and fibrosis. *Immunity*, 45(1):145–58, 2016. doi:10.1016/j.immuni.2016.06.009.

[182] T. A. Wynn. Il-13 effector functions. *Annu Rev Immunol*, 21:425–56, 2003. doi:10.1146/annurev.immunol.21.120601.141142.

[183] D. R. Herbert, T. Orekov, C. Perkins, and F. D. Finkelman. Il-10 and tgf-beta redundantly protect against severe liver injury and mortality during acute schistosomiasis. *J Immunol*, 181(10):7214–20, 2008.

[184] T. A. Pereira, W. K. Syn, F. F. Amancio, P. H. Cunha, J. F. Caporali, G. V. Trindade, E. T. Santos, M. M. Souza, Z. A. Andrade, R. P. Witek, W. E. Secor, F. E. Pereira, J. R. Lambertucci, and A. M. Diehl. Osteopontin is upregulated in human and murine acute schistosomiasis mansoni. *PLoS Negl Trop Dis*, 10(10):e0005057, 2016. doi:10.1371/journal.pntd.0005057.

[185] T. A. Pereira, W. K. Syn, M. V. Machado, P. V. Vidigal, V. Resende, I. Voieta, G. Xie, A. Otoni, M. M. Souza, E. T. Santos, I. S. Chan, G. V. Trindade, S. S. Choi, R. P. Witek, F. E. Pereira, W. E. Secor, Z. A. Andrade, J. R. Lambertucci, and A. M. Diehl. Schistosome-induced cholangiocyte proliferation and osteopontin secretion correlate with fibrosis and portal hypertension in human and murine schistosomiasis mansoni. *Clin Sci (Lond)*, 129(10):875–83, 2015. doi:10.1042/cs20150117.

[186] J. S. Duffield, S. J. Forbes, C. M. Constandinou, S. Clay, M. Partolina, S. Vuthoori, S. Wu, R. Lang, and J. P. Iredale. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J Clin Invest*, 115(1):56–65, 2005. doi:10.1172/jci22675.

[187] M. Z. Zhang, B. Yao, S. Yang, L. Jiang, S. Wang, X. Fan, H. Yin, K. Wong, T. Miyazawa, J. Chen, I. Chang, A. Singh, and R. C. Harris. Csf-1 signaling mediates recovery from acute kidney injury. *J Clin Invest*, 122(12):4519–32, 2012. doi:10.1172/jci60363.

[188] B. Berse, L. F. Brown, L. Van de Water, H. F. Dvorak, and D. R. Senger. Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. *Mol Biol Cell*, 3(2):211–20, 1992.

[189] K. Shimokado, E. W. Raines, D. K. Madtes, T. B. Barrett, E. P. Benditt, and R. Ross. A significant part of macrophage-derived growth factor consists of at least two forms of pdgf. *Cell*, 43(1):277–86, 1985.

[190] S. Willenborg, T. Lucas, G. van Loo, J. A. Knipper, T. Krieg, I. Haase, B. Brachvogel, M. Hammerschmidt, A. Nagy, N. Ferrara, M. Pasparakis, and S. A. Eming. Ccr2 recruits an inflammatory macrophage subpopulation critical for angiogenesis in tissue repair. *Blood*, 120(3):613–25, 2012. doi:10.1182/blood-2012-01-403386.

[191] P. J. Murray and T. A. Wynn. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol*, 11(11):723–37, 2011. doi:10.1038/nri3073.

[192] P. Loke and J. P. Allison. Pd-l1 and pd-l2 are differentially regulated by th1 and th2 cells. *Proc Natl Acad Sci U S A*, 100(9):5336–41, 2003. doi:10.1073/pnas.0931259100.

[193] K. M. Vannella, L. Barron, L. A. Borthwick, K. N. Kindrachuk, P. B. Narasimhan, K. M. Hart, R. W. Thompson, S. White, A. W. Cheever, T. R. Ramalingam, and T. A. Wynn. Incomplete deletion of il-4ralpha by lysm(cre) reveals distinct subsets of m2 macrophages controlling inflammation and fibrosis in chronic schistosomiasis. *PLoS Pathog*, 10(9):e1004372, 2014. doi:10.1371/journal.ppat.1004372.

[194] M. Hesse, M. Modolell, A. C. La Flamme, M. Schito, J. M. Fuentes, A. W. Cheever, E. J. Pearce, and T. A. Wynn. Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of l-arginine metabolism. *J Immunol*, 167(11):6533–44, 2001.

[195] T. A. Wynn and K. M. Vannella. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity*, 44(3):450–462, 2016. doi:10.1016/j.immuni.2016.02.015.

[196] J. T. Pesce, T. R. Ramalingam, M. M. Mentink-Kane, M. S. Wilson, K. C. El Kasmi, A. M. Smith, R. W. Thompson, A. W. Cheever, P. J. Murray, and T. A. Wynn. Arginase-1-expressing macrophages suppress th2 cytokine-driven inflammation and fibrosis. *PLoS Pathog*, 5(4):e1000371, 2009. doi:10.1371/journal.ppat.1000371.

[197] T. McHedlidze, M. Waldner, S. Zopf, J. Walker, A. L. Rankin, M. Schuchmann, D. Voehringer, A. N. McKenzie, M. F. Neurath, S. Pflanz, and S. Wirtz. Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. *Immunity*, 39(2):357–71, 2013. doi:10.1016/j.immuni.2013.07.018.

[198] G. Perona-Wright, S. J. Jenkins, and A. S. MacDonald. Dendritic cell activation and function in response to schistosoma mansoni. *Int J Parasitol*, 36(6):711–21, 2006. doi:10.1016/j.ijpara.2006.02.003.

[199] M. Booth, J. K. Mwatha, S. Joseph, F. M. Jones, H. Kadzo, E. Ireri, F. Kazibwe, J. Kemijumbi, C. Kariuki, G. Kimani, J. H. Ouma, N. B. Kabatereine, B. J. Vennervald, and D. W. Dunne. Periportal fibrosis in human schistosoma mansoni infection is associated with low il-10, low ifn-gamma, high tnf-alpha, or low rantes, depending on age and gender. *J Immunol*, 172(2):1295–303, 2004.

[200] K. F. Hoffmann, A. W. Cheever, and T. A. Wynn. Il-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. *J Immunol*, 164(12):6406–16, 2000.

[201] M. M. Mentink-Kane and T. A. Wynn. Opposing roles for il-13 and il-13 receptor alpha 2 in health and disease. *Immunol Rev*, 202:191–202, 2004. doi:10.1111/j.0105-2896.2004.00210.x.

[202] H. Harizi, M. Juzan, V. Pitard, J. F. Moreau, and N. Gualde. Cyclooxygenase-2-issued prostaglandin e(2) enhances the production of endogenous il-10, which down-regulates dendritic cell functions. *J Immunol*, 168(5):2255–63, 2002.

[203] K. Ramaswamy, P. Kumar, and Y. X. He. A role for parasite-induced pge2 in il-10-mediated host immunoregulation by skin stage schistosomula of schistosoma mansoni. *J Immunol*, 165(8):4567–74, 2000.

[204] V. Angeli, C. Faveeuw, O. Roye, J. Fontaine, E. Teissier, A. Capron, I. Wolowczuk, M. Capron, and F. Trottein. Role of the parasite-derived prostaglandin d2 in the inhibition of epidermal langerhans cell migration during schistosomiasis infection. *J Exp Med*, 193(10):1135–47, 2001.

[205] P. J. Skelly and R. Alan Wilson. Making sense of the schistosome surface. *Adv Parasitol*, 63:185–284, 2006. doi:10.1016/s0065-308x(06)63003-0.

[206] D. Jankovic, A. W. Cheever, M. C. Kullberg, T. A. Wynn, G. Yap, P. Caspar, F. A. Lewis, R. Clynes, J. V. Ravetch, and A. Sher. Cd4+ t cell-mediated granulomatous pathology in schistosomiasis is downregulated by a b cell-dependent mechanism requiring fc receptor signaling. *J Exp Med*, 187(4):619–29, 1998.

[207] J. E. Allen and R. M. Maizels. Diversity and dialogue in immunity to helminths. *Nat Rev Immunol*, 11(6):375–88, 2011. doi:10.1038/nri2992.

[208] N. Harris and W. C. Gause. To b or not to b: B cells and the th2-type immune response to helminths. *Trends Immunol*, 32(2):80–8, 2011. doi:10.1016/j.it.2010.11.005.

[209] J. J. Taylor, M. Mohrs, and E. J. Pearce. Regulatory t cell responses develop in parallel to th responses and control the magnitude and phenotype of the th effector population. *J Immunol*, 176(10):5839–47, 2006.

[210] L. E. Layland, J. Mages, C. Loddenkemper, A. Hoerauf, H. Wagner, R. Lang, and C. U. da Costa. Pronounced phenotype in activated regulatory t cells during a chronic helminth infection. *J Immunol*, 184(2):713–24, 2010. doi:10.4049/jimmunol.0901435.

[211] A. S. McKee and E. J. Pearce. Cd25+cd4+ cells contribute to th2 polarization during helminth infection by suppressing th1 response development. *J Immunol*, 173(2):1224–31, 2004.

[212] L. E. Layland, R. Rad, H. Wagner, and C. U. da Costa. Immunopathology in schistosomiasis is controlled by antigen-specific regulatory t cells primed in the presence of tlr2. *Eur J Immunol*, 37(8):2174–84, 2007. doi:10.1002/eji.200737063.

[213] S. Sakaguchi, T. Yamaguchi, T. Nomura, and M. Ono. Regulatory t cells and immune tolerance. *Cell*, 133(5):775–87, 2008. doi:10.1016/j.cell.2008.05.009.

[214] C. H. Sadler, L. I. Rutitzky, M. J. Stadecker, and R. A. Wilson. Il-10 is crucial for the transition from acute to chronic disease state during infection of mice with schistosoma mansoni. *Eur J Immunol*, 33(4):880–8, 2003. doi:10.1002/eji.200323501.

[215] T. A. Wynn, R. Morawetz, T. Scharton-Kersten, S. Hieny, r. Morse, H. C., R. Kuhn, W. Muller, A. W. Cheever, and A. Sher. Analysis of granuloma formation in double cytokine-deficient mice reveals a central role for il-10 in polarizing both t helper cell 1- and t helper cell 2-type cytokine responses in vivo. *J Immunol*, 159(10):5014–23, 1997.

[216] S. Z. Josefowicz, L. F. Lu, and A. Y. Rudensky. Regulatory t cells: mechanisms of differentiation and function. *Annu Rev Immunol*, 30:531–64, 2012. doi:10.1146/annurev.immunol.25.022106.141623.

[217] L. Janssen, G. L. Silva Santos, H. S. Muller, A. R. Vieira, T. A. de Campos, and V. de Paulo Martins. Schistosome-derived molecules as modulating actors of the immune system and promising candidates to treat autoimmune and inflammatory diseases. *J Immunol Res*, 2016:5267485, 2016. doi:10.1155/2016/5267485.

[218] E. A. Hasby, M. A. Hasby Saad, Z. Shohieb, and K. El Noby. Foxp3+ t regulatory cells and immunomodulation after schistosoma mansoni egg antigen immunization in experimental model of inflammatory bowel disease. *Cell Immunol*, 295(1):67–76, 2015. doi:10.1016/j.cellimm.2015.02.013.

[219] M. Heylen, N. E. Ruyssers, J. G. De Man, J. P. Timmermans, P. A. Pelckmans, T. G. Moreels, and B. Y. De Winter. Worm proteins of schistosoma mansoni reduce the severity of experimental chronic colitis in mice by suppressing colonic proinflammatory immune responses. *PLoS One*, 9(10):e110002, 2014. doi:10.1371/journal.pone.0110002.

[220] S. Croisant. Epidemiology of asthma: prevalence and burden of disease. *Adv Exp Med Biol*, 795:17–29, 2014. doi:10.1007/978-1-4614-8603-9_2.

[221] L. S. Cardoso, S. C. Oliveira, A. M. Goes, R. R. Oliveira, L. G. Pacifico, F. V. Marinho, C. T. Fonseca, F. C. Cardoso, E. M. Carvalho, and M. I. Araujo. Schistosoma mansoni antigens modulate the allergic response in a murine model of ovalbumin-induced airway inflammation. *Clin Exp Immunol*, 160(2):266–74, 2010. doi:10.1111/j.1365-2249.2009.04084.x.

[222] L. E. Layland, K. Straubinger, M. Ritter, E. Loffredo-Verde, H. Garn, T. Sparwasser, and C. Prazeres da Costa. Schistosoma mansoni-mediated suppression of allergic airway inflammation requires patency and foxp3+ treg cells. *PLoS Negl Trop Dis*, 7(8):e2379, 2013. doi:10.1371/journal.pntd.0002379.

[223] S. Bassily, A. Kotkat, K. C. Hyams, F. G. Youssef, N. A. El-Masry, R. Arthur, I. Z. Imam, and F. M. Brown. Immunogenicity of recombinant hepatitis b vaccine among infants of mothers with active schistosomiasis. *Am J Trop Med Hyg*, 57(2):197–9, 1997.

[224] Y. A. Ghaffar, M. Kamel, M. el Sobky, R. Bahnasy, and G. T. Strickland. *Response to hepatitis B vaccine in infants born to mothers with schistosomiasis*, volume 2, page 272. England, 1989.

[225] D. K. Riner, E. M. Ndombi, J. M. Carter, A. Omondi, N. Kittur, E. Kavere, H. K. Korir, B. Flaherty, D. Karanja, and D. G. Colley. Schistosoma mansoni infection can jeopardize the duration of protective levels of antibody responses to immunizations against hepatitis b and tetanus toxoid. *PLoS Negl Trop Dis*, 10(12):e0005180, 2016. doi:10.1371/journal.pntd.0005180.

[226] Y. Osada and T. Kanazawa. Schistosome: its benefit and harm in patients suffering from concomitant diseases. *J Biomed Biotechnol*, 2011:264173, 2011. doi:10.1155/2011/264173.

[227] A. Abruzzi and B. Fried. Coinfection of schistosoma (trematoda) with bacteria, protozoa and helminths. *Adv Parasitol*, 77:1–85, 2011. doi:10.1016/b978-0-12-391429-3.00005-8.

[228] M. J. Edwards, O. Buchatska, M. Ashton, M. Montoya, Q. D. Bickle, and P. Borrow. Reciprocal immunomodulation in a schistosome and hepatotropic virus coinfection model. *J Immunol*, 175(10):6275–85, 2005.

[229] M. Angelico, E. Renganathan, C. Gandin, M. Fathy, M. C. Profili, W. Refai, A. De Santis, A. Nagi, G. Amin, L. Capocaccia, F. Callea, M. Rapicetta, G. Badr, and G. Rocchi. Chronic liver disease in the alexandria governorate, egypt: contribution of schistosomiasis and hepatitis virus infections. *J Hepatol*, 26(2):236–43, 1997.

[230] G. T. Strickland, H. Elhefni, T. Salman, I. Waked, M. Abdel-Hamid, N. N. Mikhail, G. Esmat, and A. Fix. Role of hepatitis c infection in chronic liver disease in egypt. *Am J Trop Med Hyg*, 67(4):436–42, 2002.

[231] G. I. Gasim, A. Bella, and I. Adam. Schistosomiasis, hepatitis b and hepatitis c co-infection. *Virol J*, 12:19, 2015. doi:10.1186/s12985-015-0251-2.

[232] S. M. Kamal, J. W. Rasenack, L. Bianchi, A. Al Tawil, K. El Sayed Khalifa, T. Peter, H. Mansour, W. Ezzat, and M. Koziel. Acute hepatitis c without and with schistosomiasis: correlation with hepatitis c-specific cd4(+) t-cell and cytokine response. *Gastroenterology*, 121(3):646–56, 2001.

[233] L. Stross, J. Gunther, G. Gasteiger, T. Asen, S. Graf, M. Aichler, I. Esposito, D. H. Busch, P. Knolle, T. Sparwasser, and U. Protzer. Foxp3+ regulatory t cells protect the liver from immune damage and compromise virus control during acute experimental hepatitis b virus infection in mice. *Hepatology*, 56(3):873–83, 2012. doi:10.1002/hep.25765.

[234] H. M. Al-Freihi. Prevalence of hepatitis b surface antigenemia among patients with schistosoma mansoni. *Ann Saudi Med*, 13(2):121–5, 1993.

[235] H. A. Hammad, M. M. el Fattah, M. Moris, E. H. Madina, A. A. el Abbasy, and A. T. Soliman. Study on some hepatic functions and prevalence of hepatitis b surface antigenaemia in egyptian children with schistosomal hepatic fibrosis. *J Trop Pediatr*, 36(3):126–7, 1990.

[236] A. H. Al-Shamiri, M. A. Al-Taj, and A. S. Ahmed. Prevalence and co-infections of schistosomiasis/hepatitis b and c viruses among school children in an endemic areas in taiz, yemen. *Asian Pac J Trop Med*, 4(5):404–8, 2011. doi:10.1016/s1995-7645(11)60113-2.

[237] B. Larouze, M. C. Dazza, C. Gaudebout, M. Habib, M. Elamy, and B. Cline. Absence of relationship between schistosoma mansoni and hepatitis b virus infection in the qalyub governate, egypt. *Ann Trop Med Parasitol*, 81(4):373–5, 1987.

[238] X. P. Ye, Y. L. Fu, R. M. Anderson, and D. J. Nokes. Absence of relationship between schistosoma japonicum and hepatitis b virus infection in the dongting lake region, china. *Epidemiol Infect*, 121(1):193–5, 1998.

[239] M. I. Love, W. Huber, and S. Anders. Moderated estimation of fold change and dispersion for rna-seq data with deseq2. *Genome Biol*, 15(12):550, 2014. doi:10.1186/s13059-014-0550-8.

[240] B. J. Baaten, C. R. Li, M. F. Deiro, M. M. Lin, P. J. Linton, and L. M. Bradley. Cd44 regulates survival and memory development in th1 cells. *Immunity*, 32(1):104–15, 2010. doi:10.1016/j.immuni.2009.10.011.

[241] K. W. Cook, D. P. Letley, R. J. Ingram, E. Staples, H. Skjoldmose, J. C. Atherton, and K. Robinson. Ccl20/ccr6-mediated migration of regulatory t cells to the helicobacter pylori-infected human gastric mucosa. *Gut*, 63(10):1550–9, 2014. doi:10.1136/gutjnl-2013-306253.

[242] K. S. Warmington, L. Boring, J. H. Ruth, J. Sonstein, C. M. Hogaboam, J. L. Curtis, S. L. Kunkel, I. R. Charo, and S. W. Chensue. Effect of c-c chemokine receptor 2 (ccr2) knockout on type-2 (schistosomal antigen-elicited) pulmonary granuloma formation: analysis of cellular recruitment and cytokine responses. *Am J Pathol*, 154(5):1407–16, 1999. doi:10.1016/s0002-9440(10)65394-1.

[243] M. Nauerth, B. Weissbrich, R. Knall, T. Franz, G. Dossinger, J. Bet, P. J. Paszkiewicz, L. Pfeifer, M. Bunse, W. Uckert, R. Holtappels, D. Gillert-Marien, M. Neuenhahn, A. Krackhardt, M. J. Reddehase, S. R. Riddell, and D. H. Busch. Tcr-ligand koff rate correlates with the protective capacity of antigen-specific cd8+ t cells for adoptive transfer. *Sci Transl Med*, 5(192):192ra87, 2013. doi:10.1126/scitranslmed.3005958.

[244] M. T. Whary, S. Muthupalani, Z. Ge, Y. Feng, J. Lofgren, H. N. Shi, N. S. Taylor, P. Correa, J. Versalovic, T. C. Wang, and J. G. Fox. Helminth co-infection in helicobacter pylori infected ins-gas mice attenuates gastric premalignant lesions of epithelial dysplasia and glandular atrophy and preserves colonization resistance of the stomach to lower bowel microbiota. *Microbes Infect*, 16(4):345–55, 2014. doi:10.1016/j.micinf.2014.01.005.

[245] M. F. Elshal, I. H. Elsayed, I. M. El Kady, G. Badra, A. El-Refaei, M. El-Batanony, and O. M. Hendy. Role of concurrent s. mansoni infection in h. pylori-associated gastritis: a flow cytometric dna-analysis and oxyradicals correlations. *Clin Chim Acta*, 346(2):191–8, 2004. doi:10.1016/j.cccn.2004.03.023.

[246] A. Agha and D. Y. Graham. Evidence-based examination of the african enigma in relation to helicobacter pylori infection. *Scand J Gastroenterol*, 40(5):523–9, 2005. doi:10.1080/00365520510012280.

[247] L. E. Bravo, L. J. van Doom, J. L. Realpe, and P. Correa. Virulence-associated genotypes of helicobacter pylori: do they explain the african enigma? *Am J Gastroenterol*, 97(11):2839–42, 2002. doi:10.1111/j.1572-0241.2002.07031.x.

[248] T. E. Buffart, M. Louw, N. C. van Grieken, M. Tijssen, B. Carvalho, B. Ylstra, H. Grabsch, C. J. Mulder, C. J. van de Velde, S. W. van der Merwe, and G. A. Meijer. Gastric cancers of western european and african patients show different patterns of genomic instability. *BMC Med Genomics*, 4:7, 2011. doi:10.1186/1755-8794-4-7.

[249] D. I. Campbell, B. F. Warren, J. E. Thomas, N. Figura, J. L. Telford, and P. B. Sullivan. The african enigma: low prevalence of gastric atrophy, high prevalence of chronic inflammation in west african adults and children. *Helicobacter*, 6(4):263–7, 2001.

[250] C. D. Mills, K. Kincaid, J. M. Alt, M. J. Heilman, and A. M. Hill. M-1/m-2 macrophages and the th1/th2 paradigm. *J Immunol*, 164(12):6166–73, 2000.

[251] R. S. Sellers, C. B. Clifford, P. M. Treuting, and C. Brayton. Immunological variation between inbred laboratory mouse strains: points to consider in phenotyping genetically immunomodified mice. *Vet Pathol*, 49(1):32–43, 2012. doi:10.1177/0300985811429314.

[252] H. Watanabe, K. Numata, T. Ito, K. Takagi, and A. Matsukawa. Innate immune response in th1- and th2-dominant mouse strains. *Shock*, 22(5):460–6, 2004.

[253] S. Mostafavi, A. Ortiz-Lopez, M. A. Bogue, K. Hattori, C. Pop, D. Koller, D. Mathis, and C. Benoist. Variation and genetic control of gene expression in primary immunocytes across inbred mouse strains. *J Immunol*, 193(9):4485–96, 2014. doi:10.4049/jimmunol.1401280.

[254] K. L. Elkins, A. Cooper, S. M. Colombini, S. C. Cowley, and T. L. Kieffer. In vivo clearance of an intracellular bacterium, francisella tularensis lvs, is dependent on the p40 subunit of interleukin-12 (il-12) but not on il-12 p70. *Infect Immun*, 70(4):1936–48, 2002.

[255] S. Kumar and R. L. Tarleton. Antigen-specific th1 but not th2 cells provide protection from lethal trypanosoma cruzi infection in mice. *J Immunol*, 166(7):4596–603, 2001.

[256] Y. Yamamoto, S. Okubo, T. W. Klein, K. Onozaki, T. Saito, and H. Friedman. Binding of legionella pneumophila to macrophages increases cellular cytokine mrna. *Infect Immun*, 62(9):3947–56, 1994.

[257] F. Mattner, J. Magram, J. Ferrante, P. Launois, K. Di Padova, R. Behin, M. K. Gately, J. A. Louis, and G. Alber. Genetically resistant mice lacking interleukin-12 are susceptible to infection with leishmania major and mount a polarized th2 cell response. *Eur J Immunol*, 26(7):1553–9, 1996. doi:10.1002/eji.1830260722.

[258] K. Swihart, U. Fruth, N. Messmer, K. Hug, R. Behin, S. Huang, G. Del Giudice, M. Aguet, and J. A. Louis. Mice from a genetically resistant background lacking the interferon gamma receptor are susceptible to infection with leishmania major but mount a polarized t helper cell 1-type cd4+ t cell response. *J Exp Med*, 181(3):961–71, 1995.

[259] B. Alemayehu and Z. Tomass. Schistosoma mansoni infection prevalence and associated risk factors among schoolchildren in demba girara, damot woide district of wolaita zone, southern ethiopia. *Asian Pac J Trop Med*, 8(6):457–63, 2015. doi:10.1016/j.apjtm.2015.05.009.

[260] D. T. Cervantes, L. A. Fischbach, K. J. Goodman, C. V. Phillips, S. Chen, and C. S. Broussard. Exposure to helicobacter pylori-positive siblings and persistence of helicobacter pylori infection in early childhood. *J Pediatr Gastroenterol Nutr*, 50(5):481–5, 2010. doi:10.1097/MPG.0b013e3181bab2ee.

[261] H. Miyaji, T. Azuma, S. Ito, Y. Abe, F. Gejyo, N. Hashimoto, H. Sugimoto, H. Suto, Y. Ito, Y. Yamazaki, Y. Kohli, and M. Kuriyama. Helicobacter pylori infection occurs via close contact with infected individuals in early childhood. *J Gastroenterol Hepatol*, 15(3):257–62, 2000.

[262] D. Rothenbacher, J. Inceoglu, G. Bode, and H. Brenner. Acquisition of helicobacter pylori infection in a high-risk population occurs within the first 2 years of life. *J Pediatr*, 136(6):744–8, 2000. URL: http://www.ncbi.nlm.nih.gov/pubmed/10839870.

[263] S. A. Spencer, J. Penney, H. J. Russell, A. P. Howe, C. Linder, A. L. D. Rakotomampianina, A. M. Nandimbiniaina, S. B. Squire, J. R. Stothard, A. L. Bustinduy, and A. M. Rahetilahy. High burden of schistosoma mansoni infection in school-aged children in marolambo district, madagascar. *Parasit Vectors*, 10(1):307, 2017. doi:10.1186/s13071-017-2249-7.

[264] G. Di Genova, N. Savelyeva, A. Suchacki, S. M. Thirdborough, and F. K. Stevenson. Bystander stimulation of activated cd4+ t cells of unrelated specificity following a booster vaccination with tetanus toxoid. *Eur J Immunol*, 40(4):976–85, 2010. doi:10.1002/eji.200940017.

[265] G. Eberl, P. Brawand, and H. R. MacDonald. Selective bystander proliferation of memory cd4+ and cd8+ t cells upon nk t or t cell activation. *J Immunol*, 165(8):4305–11, 2000.

[266] S. Ehl, J. Hombach, P. Aichele, H. Hengartner, and R. M. Zinkernagel. Bystander activation of cytotoxic t cells: studies on the mechanism and evaluation of in vivo significance in a transgenic mouse model. *J Exp Med*, 185(7):1241–51, 1997.

[267] D. F. Tough, P. Borrow, and J. Sprent. Induction of bystander t cell proliferation by viruses and type i interferon in vivo. *Science*, 272(5270):1947–50, 1996.

[268] D. F. Tough, X. Zhang, and J. Sprent. An ifn-gamma-dependent pathway controls stimulation of memory phenotype cd8+ t cell turnover in vivo by il-12, il-18, and ifn-gamma. *J Immunol*, 166(10):6007–11, 2001.

[269] J. W. Griffith, C. L. Sokol, and A. D. Luster. Chemokines and chemokine receptors: positioning cells for host defense and immunity. *Annu Rev Immunol*, 32:659–702, 2014. doi:10.1146/annurev-immunol-032713-120145.

[270] M. F. Krummel, F. Bartumeus, and A. Gerard. *T cell migration, search strategies and mechanisms*, volume 16, pages 193–201. England, 2016. doi:10.1038/nri.2015.16.

[271] S. N. Mueller, T. Gebhardt, F. R. Carbone, and W. R. Heath. Memory t cell subsets, migration patterns, and tissue residence. *Annu Rev Immunol*, 31:137–61, 2013. doi:10.1146/annurev-immunol-032712-095954.

[272] S. Chikuma, M. Kanamori, S. Mise-Omata, and A. Yoshimura. Suppressors of cytokine signaling: Potential immune checkpoint molecules for cancer immunotherapy. *Cancer Sci*, 108(4):574–580, 2017. doi:10.1111/cas.13194.

[273] T. Pentcheva-Hoang, E. Corse, and J. P. Allison. Negative regulators of t-cell activation: potential targets for therapeutic intervention in cancer, autoimmune disease, and persistent infections. *Immunol Rev*, 229(1):67–87, 2009. doi:10.1111/j.1600-065X.2009.00763.x.

[274] J. Yang, S. P. Huck, R. S. McHugh, I. F. Hermans, and F. Ronchese. Perforin-dependent elimination of dendritic cells regulates the expansion of antigen-specific cd8+ t cells in vivo. *Proc Natl Acad Sci U S A*, 103(1):147–52, 2006. doi:10.1073/pnas.0509054103.

[275] A. J. Muller, O. Filipe-Santos, G. Eberl, T. Aebischer, G. F. Spath, and P. Bousso. Cd4+ t cells rely on a cytokine gradient to control intracellular pathogens beyond sites of antigen presentation. *Immunity*, 37(1):147–57, 2012. doi:10.1016/j.immuni.2012.05.015.

[276] Y. Y. Wu, H. F. Tsai, W. C. Lin, P. I. Hsu, C. T. Shun, M. S. Wu, and P. N. Hsu. Upregulation of ccl20 and recruitment of ccr6+ gastric infiltrating lymphocytes in helicobacter pylori gastritis. *Infect Immun*, 75(9):4357–63, 2007. doi:10.1128/iai.01660-06.

[277] G. Constantin, M. Majeed, C. Giagulli, L. Piccio, J. Y. Kim, E. C. Butcher, and C. Laudanna. Chemokines trigger immediate beta2 integrin affinity and mobility changes: differential regulation and roles in lymphocyte arrest under flow. *Immunity*, 13(6):759–69, 2000.

[278] C. Berlin, E. L. Berg, M. J. Briskin, D. P. Andrew, P. J. Kilshaw, B. Holzmann, I. L. Weissman, A. Hamann, and E. C. Butcher. Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin madcam-1. *Cell*, 74(1):185–95, 1993.

[279] A. Dogan, M. Du, A. Koulis, M. J. Briskin, and P. G. Isaacson. Expression of lymphocyte homing receptors and vascular addressins in low-grade gastric b-cell lymphomas of mucosa-associated lymphoid tissue. *Am J Pathol*, 151(5):1361–9, 1997.

[280] R. A. Hatz, G. Rieder, M. Stolte, E. Bayerdorffer, G. Meimarakis, F. W. Schildberg, and G. Enders. Pattern of adhesion molecule expression on vascular endothelium in helicobacter pylori-associated antral gastritis. *Gastroenterology*, 112(6):1908–19, 1997.

[281] I. Beales, S. Srinivasan, M. Blaser, J. Scheiman, J. Calam, J. Park, T. Yamada, and J. Del-Valle. Effect of helicobacter-pylori constituents and inflammatory cytokines on gastrin-release from isolated canine g-cells. In *Gastroenterology*, volume 108, pages A779–A779. WB SAUNDERS CO INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399.

[282] N. Weigert, K. Schaffer, V. Schusdziarra, M. Classen, and W. Schepp. Gastrin secretion from primary cultures of rabbit antral g cells: stimulation by inflammatory cytokines. *Gastroenterology*, 110(1):147–54, 1996.

[283] E. J. Beswick, D. A. Bland, G. Suarez, C. A. Barrera, X. Fan, and V. E. Reyes. Helicobacter pylori binds to cd74 on gastric epithelial cells and stimulates interleukin-8 production. *Infect Immun*, 73(5):2736–43, 2005. doi:10.1128/iai.73.5.2736-2743.2005.

[284] K. Schroder, P. J. Hertzog, T. Ravasi, and D. A. Hume. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol*, 75(2):163–89, 2004. doi:10.1189/jlb.0603252.

[285] S. Herbst, U. E. Schaible, and B. E. Schneider. Interferon gamma activated macrophages kill mycobacteria by nitric oxide induced apoptosis. *PLoS One*, 6(5):e19105, 2011. doi:10.1371/journal.pone.0019105.

[286] X. Hu, S. D. Chakravarty, and L. B. Ivashkiv. Regulation of interferon and toll-like receptor signaling during macrophage activation by opposing feedforward and feedback inhibition mechanisms. *Immunol Rev*, 226:41–56, 2008. doi:10.1111/j.1600-065X.2008.00707.x.

[287] C. Herrero, X. Hu, W. P. Li, S. Samuels, M. N. Sharif, S. Kotenko, and L. B. Ivashkiv. Reprogramming of il-10 activity and signaling by ifn-gamma. *J Immunol*, 171(10):5034–41, 2003.

[288] Y. B. Liang, H. Tang, Z. B. Chen, L. J. Zeng, J. G. Wu, W. Yang, Z. Y. Li, and Z. F. Ma. Downregulated socs1 expression activates the jak1/stat1 pathway and promotes polarization of macrophages into m1 type. *Mol Med Rep*, 16(5):6405–6411, 2017. doi:10.3892/mmr.2017.7384.

[289] K. H. Ely, L. S. Cauley, A. D. Roberts, J. W. Brennan, T. Cookenham, and D. L. Woodland. Nonspecific recruitment of memory cd8+ t cells to the lung airways during respiratory virus infections. *J Immunol*, 170(3):1423–9, 2003.

[290] R. Stephens, D. A. Randolph, G. Huang, M. J. Holtzman, and D. D. Chaplin. Antigen-nonspecific recruitment of th2 cells to the lung as a mechanism for viral infection-induced allergic asthma. *J Immunol*, 169(10):5458–67, 2002.

[291] D. J. Topham, M. R. Castrucci, F. S. Wingo, G. T. Belz, and P. C. Doherty. The role of antigen in the localization of naive, acutely activated, and memory cd8(+) t cells to the lung during influenza pneumonia. *J Immunol*, 167(12):6983–90, 2001.

[292] M. Eck, B. Schmausser, K. Scheller, A. Toksoy, M. Kraus, T. Menzel, H. K. Muller-Hermelink, and R. Gillitzer. Cxc chemokines gro(alpha)/il-8 and ip-10/mig in helicobacter pylori gastritis. *Clin Exp Immunol*, 122(2):192–9, 2000.

[293] Y. Yamaoka, M. Kita, T. Kodama, N. Sawai, T. Tanahashi, K. Kashima, and J. Imanishi. Chemokines in the gastric mucosa in helicobacter pylori infection. *Gut*, 42(5):609–17, 1998.

[294] S. Ghani, M. Feuerer, C. Doebis, U. Lauer, C. Loddenkemper, J. Huehn, A. Hamann, and U. Syrbe. T cells as pioneers: antigen-specific t cells condition inflamed sites for high-rate antigen-non-specific effector cell recruitment. *Immunology*, 128(1 Suppl):e870–80, 2009. doi:10.1111/j.1365-2567.2009.03096.x.

[295] T. J. Chapman, M. R. Castrucci, R. C. Padrick, L. M. Bradley, and D. J. Topham. Antigen-specific and non-specific cd4+ t cell recruitment and proliferation during influenza infection. *Virology*, 340(2):296–306, 2005. doi:10.1016/j.virol.2005.06.023.

[296] A. M. Chen, N. Khanna, S. A. Stohlman, and C. C. Bergmann. Virus-specific and bystander cd8 t cells recruited during virus-induced encephalomyelitis. *J Virol*, 79(8):4700–8, 2005. doi:10.1128/jvi.79.8.4700-4708.2005.

[297] T. Ostler, H. Pircher, and S. Ehl. "bystander" recruitment of systemic memory t cells delays the immune response to respiratory virus infection. *Eur J Immunol*, 33(7):1839–48, 2003. doi:10.1002/eji.200323460.

[298] M. Waluga, M. Kukla, M. Zorniak, A. Bacik, and R. Kotulski. From the stomach to other organs: Helicobacter pylori and the liver. *World J Hepatol*, 7(18):2136–46, 2015. doi:10.4254/wjh.v7.i18.2136.

[299] H. Y. Kanamura, K. Hancock, V. Rodrigues, and R. T. Damian. Schistosoma mansoni heat shock protein 70 elicits an early humoral immune response in s. mansoni infected baboons. *Mem Inst Oswaldo Cruz*, 97(5):711–6, 2002.

[300] B. Flahou, F. Haesebrouck, A. Smet, H. Yonezawa, T. Osaki, and S. Kamiya. Gastric and enterohepatic non-helicobacter pylori helicobacters. *Helicobacter*, 18 Suppl 1:66–72, 2013. doi:10.1111/hel.12072.

[301] T. K. Hansen, P. S. Hansen, A. Norgaard, H. Nielsen, A. Lee, and L. P. Andersen. Helicobacter felis does not stimulate human neutrophil oxidative burst in contrast to 'gastrospirillum hominis' and helicobacter pylori. *FEMS Immunol Med Microbiol*, 30(3):187–95, 2001.

[302] J. I. Keenan, R. A. Allardyce, and P. F. Bagshaw. Lack of protection following immunisation with h. pylori outer membrane vesicles highlights antigenic differences between h. felis and h. pylori. *FEMS Microbiol Lett*, 161(1):21–7, 1998.

[303] M. C. de Rezende, E. S. Araujo, J. M. Moreira, V. F. Rodrigues, J. L. Rodrigues, C. A. Pereira, and D. Negrao-Correa. Effect of different stages of schistosoma mansoni infection on the parasite burden and immune response to strongyloides venezuelensis in co-infected mice. *Parasitol Res*, 114(12):4601–16, 2015. doi:10.1007/s00436-015-4706-6.

[304] M. R. Gatlin, C. L. Black, P. N. Mwinzi, W. E. Secor, D. M. Karanja, and D. G. Colley. Association of the gene polymorphisms ifn-gamma +874, il-13 -1055 and il-4 -590 with patterns of reinfection with schistosoma mansoni. *PLoS Negl Trop Dis*, 3(2):e375, 2009. doi:10.1371/journal.pntd.0000375.

[305] A. C. La Flamme, E. A. Patton, and E. J. Pearce. Role of gamma interferon in the pathogenesis of severe schistosomiasis in interleukin-4-deficient mice. *Infect Immun*, 69(12):7445–52, 2001. doi:10.1128/iai.69.12.7445-7452.2001.

[306] A. P. Mountford, P. S. Coulson, R. M. Pemberton, L. E. Smythies, and R. A. Wilson. The generation of interferon-gamma-producing t lymphocytes in skin-draining lymph nodes, and their recruitment to the lungs, is associated with protective immunity to schistosoma mansoni. *Immunology*, 75(2):250–6, 1992.

[307] V. R. Oliveira, M. C. El-Cheikh, A. M. Aguiar, A. Balduino, B. P. M. de Fatima, L. F. Reis, and R. Borojevic. Schistosoma mansoni egg-induced hepatic granulomas in mice deficient for the interferon-gamma receptor have altered populations of macrophages, lymphocytes and connective tissue cells. *Microbes Infect*, 2(15):1817–26, 2000.

[308] H. G. Remold, A. Mednis, A. Hein, and J. P. Caulfield. Human monocyte-derived macrophages are lysed by schistosomula of schistosoma mansoni and fail to kill the parasite after activation with interferon gamma. *Am J Pathol*, 131(1):146–55, 1988.

[309] D. Jankovic, M. C. Kullberg, N. Noben-Trauth, P. Caspar, J. M. Ward, A. W. Cheever, W. E. Paul, and A. Sher. Schistosome-infected il-4 receptor knockout (ko) mice, in contrast to il-4 ko mice, fail to develop granulomatous pathology while maintaining the same lymphokine expression profile. *J Immunol*, 163(1):337–42, 1999.

[310] T. R. Ramalingam, J. T. Pesce, F. Sheikh, A. W. Cheever, M. M. Mentink-Kane, M. S. Wilson, S. Stevens, D. M. Valenzuela, A. J. Murphy, G. D. Yancopoulos, J. Urban, J. F., R. P. Donnelly, and T. A. Wynn. Unique functions of the type ii interleukin 4 receptor identified in mice lacking the interleukin 13 receptor alpha1 chain. *Nat Immunol*, 9(1):25–33, 2008. doi:10.1038/ni1544.

[311] T. A. Wynn and T. R. Ramalingam. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med*, 18(7):1028–40, 2012. doi:10.1038/nm.2807.

[312] D. Ruckerl, S. M. Campbell, S. Duncan, T. E. Sutherland, S. J. Jenkins, J. P. Hewitson, T. A. Barr, L. H. Jackson-Jones, R. M. Maizels, and J. E. Allen. Macrophage origin limits functional plasticity in helminth-bacterial co-infection. *PLoS Pathog*, 13(3):e1006233, 2017. doi:10.1371/journal.ppat.1006233.

[313] T. R. Ramalingam, R. L. Gieseck, T. H. Acciani, M. H. K, A. W. Cheever, M. M. Mentink-Kane, K. M. Vannella, and T. A. Wynn. Enhanced protection from fibrosis and inflammation in the combined absence of il-13 and ifn-gamma. *J Pathol*, 239(3):344–54, 2016. doi:10.1002/path.4733.

[314] M. G. Chiaramonte, M. Mentink-Kane, B. A. Jacobson, A. W. Cheever, M. J. Whitters, M. E. Goad, A. Wong, M. Collins, D. D. Donaldson, M. J. Grusby, and T. A. Wynn. Regulation and function of the interleukin 13 receptor alpha 2 during a t helper cell type 2-dominant immune response. *J Exp Med*, 197(6):687–701, 2003. doi:10.1084/jem.20020903.

[315] A. Erhardt, M. Biburger, T. Papadopoulos, and G. Tiegs. Il-10, regulatory t cells, and kupffer cells mediate tolerance in concanavalin a-induced liver injury in mice. *Hepatology*, 45(2):475–85, 2007. doi:10.1002/hep.21498.

[316] M. J. Czaja, F. R. Weiner, S. Takahashi, M. A. Giambrone, P. H. van der Meide, H. Schellekens, L. Biempica, and M. A. Zern. Gamma-interferon treatment inhibits collagen deposition in murine schistosomiasis. *Hepatology*, 10(5):795–800, 1989.

[317] T. A. Wynn, A. W. Cheever, D. Jankovic, R. W. Poindexter, P. Caspar, F. A. Lewis, and A. Sher. An il-12-based vaccination method for preventing fibrosis induced by schistosome infection. *Nature*, 376(6541):594–6, 1995. doi:10.1038/376594a0.

[318] H. Sahin, E. Borkham-Kamphorst, C. Kuppe, M. M. Zaldivar, C. Grouls, M. Al-samman, A. Nellen, P. Schmitz, D. Heinrichs, M. L. Berres, D. Doleschel, D. Scholten, R. Weiskirchen, M. J. Moeller, F. Kiessling, C. Trautwein, and H. E. Wasmuth. Chemokine cxcl9 attenuates liver fibrosis-associated angiogenesis in mice. *Hepatology*, 55(5):1610–9, 2012. doi:10.1002/hep.25545.

[319] H. E. Wasmuth, F. Lammert, M. M. Zaldivar, R. Weiskirchen, C. Hellerbrand, D. Scholten, M. L. Berres, H. Zimmermann, K. L. Streetz, F. Tacke, S. Hillebrandt, P. Schmitz, H. Keppeler, T. Berg, E. Dahl, N. Gassler, S. L. Friedman, and C. Trautwein. Antifibrotic effects of cxcl9 and its receptor cxcr3 in livers of mice and humans. *Gastroenterology*, 137(1):309–19, 319.e1–3, 2009. doi:10.1053/j.gastro.2009.03.053.

[320] Y. Huang, X. F. Tian, X. G. Fan, C. Y. Fu, and C. Zhu. The pathological effect of helicobacter pylori infection on liver tissues in mice. *Clin Microbiol Infect*, 15(9):843–9, 2009. doi:10.1111/j.1469-0691.2009.02719.x.

[321] E. R. Garcia-Trevijano, M. J. Iraburu, L. Fontana, J. A. Dominguez-Rosales, A. Auster, A. Covarrubias-Pinedo, and M. Rojkind. Transforming growth factor beta1 induces the expression of alpha1(i) procollagen mrna by a hydrogen peroxide-c/ebpbeta-dependent mechanism in rat hepatic stellate cells. *Hepatology*, 29(3):960–70, 1999. doi:10.1002/hep.510290346.

[322] M. D. Wheeler, H. Kono, M. Yin, M. Nakagami, T. Uesugi, G. E. Arteel, E. Gabele, I. Rusyn, S. Yamashina, M. Froh, Y. Adachi, Y. Iimuro, B. U. Bradford, O. M. Smutney, H. D. Connor, R. P. Mason, S. M. Goyert, J. M. Peters, F. J. Gonzalez, R. J. Samulski, and R. G. Thurman. The role of kupffer cell oxidant production in early ethanol-induced liver disease. *Free Radic Biol Med*, 31(12):1544–9, 2001.

[323] E. A. Sabin, M. I. Araujo, E. M. Carvalho, and E. J. Pearce. Impairment of tetanus toxoid-specific th1-like immune responses in humans infected with schistosoma mansoni. *J Infect Dis*, 173(1):269–72, 1996.

[324] B. Everts, G. Perona-Wright, H. H. Smits, C. H. Hokke, A. J. van der Ham, C. M. Fitzsimmons, M. J. Doenhoff, J. van der Bosch, K. Mohrs, H. Haas, M. Mohrs, M. Yazdanbakhsh, and G. Schramm. Omega-1, a glycoprotein secreted by schistosoma mansoni eggs, drives th2 responses. *J Exp Med*, 206(8):1673–80, 2009. doi:10.1084/jem.20082460.

[325] A. S. MacDonald, A. D. Straw, N. M. Dalton, and E. J. Pearce. Cutting edge: Th2 response induction by dendritic cells: a role for cd40. *J Immunol*, 168(2):537–40, 2002.

[326] S. Steinfelder, J. F. Andersen, J. L. Cannons, C. G. Feng, M. Joshi, D. Dwyer, P. Caspar, P. L. Schwartzberg, A. Sher, and D. Jankovic. The major component in schistosome eggs responsible for conditioning dendritic cells for th2 polarization is a t2 ribonuclease (omega-1). *J Exp Med*, 206(8):1681–90, 2009. doi:10.1084/jem.20082462.

[327] D. van der Kleij, E. Latz, J. F. Brouwers, Y. C. Kruize, M. Schmitz, E. A. Kurt-Jones, T. Espevik, E. C. de Jong, M. L. Kapsenberg, D. T. Golenbock, A. G. Tielens, and M. Yazdanbakhsh. A novel host-parasite lipid cross-talk. schistosomal lyso-phosphatidylserine activates toll-like receptor 2 and affects immune polarization. *J Biol Chem*, 277(50):48122–9, 2002. doi:10.1074/jbc.M206941200.

[328] E. Aksoy, C. S. Zouain, F. Vanhoutte, J. Fontaine, N. Pavelka, N. Thieblemont, F. Willems, P. Ricciardi-Castagnoli, M. Goldman, M. Capron, B. Ryffel, and F. Trottein. Double-stranded rnas from the helminth parasite schistosoma activate tlr3 in dendritic cells. *J Biol Chem*, 280(1):277–83, 2005. doi:10.1074/jbc.M411223200.

[329] P. G. Thomas, M. R. Carter, O. Atochina, A. A. Da'Dara, D. Piskorska, E. McGuire, and D. A. Harn. Maturation of dendritic cell 2 phenotype by a helminth glycan uses a toll-like receptor 4-dependent mechanism. *J Immunol*, 171(11):5837–41, 2003.

[330] C. M. Kane, L. Cervi, J. Sun, A. S. McKee, K. S. Masek, S. Shapira, C. A. Hunter, and E. J. Pearce. Helminth antigens modulate tlr-initiated dendritic cell activation. *J Immunol*, 173(12):7454–61, 2004.

[331] H. M. Algood, J. Gallo-Romero, K. T. Wilson, J. Peek, R. M., and T. L. Cover. Host response to helicobacter pylori infection before initiation of the adaptive immune response. *FEMS Immunol Med Microbiol*, 51(3):577–86, 2007. doi:10.1111/j.1574-695X.2007.00338.x.

[332] D. Bimczok, R. H. Clements, K. B. Waites, L. Novak, D. E. Eckhoff, P. J. Mannon, P. D. Smith, and L. E. Smythies. Human primary gastric dendritic cells induce a th1 response to h. pylori. *Mucosal Immunol*, 3(3):260–9, 2010. doi:10.1038/mi.2010.10.

[333] D. Bimczok, J. M. Grams, R. D. Stahl, K. B. Waites, L. E. Smythies, and P. D. Smith. Stromal regulation of human gastric dendritic cells restricts the th1 response to helicobacter pylori. *Gastroenterology*, 141(3):929–38, 2011. doi:10.1053/j.gastro.2011.06.006.

[334] R. Kabisch, R. P. Semper, S. Wustner, M. Gerhard, and R. Mejias-Luque. Helicobacter pylori gamma-glutamyltranspeptidase induces tolerogenic human dendritic cells by activation of glutamate receptors. *J Immunol*, 196(10):4246–52, 2016. doi:10.4049/jimmunol.1501062.

[335] R. Kaebisch, R. Mejias-Luque, C. Prinz, and M. Gerhard. Helicobacter pylori cytotoxin-associated gene a impairs human dendritic cell maturation and function through il-10-mediated activation of stat3. *J Immunol*, 192(1):316–23, 2014. doi:10.4049/jimmunol.1302476.

[336] P. Mitchell, C. Germain, P. L. Fiori, W. Khamri, G. R. Foster, S. Ghosh, R. I. Lechler, K. B. Bamford, and G. Lombardi. Chronic exposure to helicobacter pylori impairs dendritic cell function and inhibits th1 development. *Infect Immun*, 75(2):810–9, 2007. doi:10.1128/iai.00228-06.

[337] A. Sayi, E. Kohler, I. M. Toller, R. A. Flavell, W. Muller, A. Roers, and A. Muller. Tlr-2-activated b cells suppress helicobacter-induced preneoplastic gastric immunopathology by inducing t regulatory-1 cells. *J Immunol*, 186(2):878–90, 2011. doi:10.4049/jimmunol.1002269.

[338] D. B. Engler, S. Reuter, Y. van Wijck, S. Urban, A. Kyburz, J. Maxeiner, H. Martin, N. Yogev, A. Waisman, M. Gerhard, T. L. Cover, C. Taube, and A. Muller. Effective treatment of allergic airway inflammation with helicobacter pylori immunomodulators requires batf3-dependent dendritic cells and il-10. *Proc Natl Acad Sci U S A*, 111(32):11810–5, 2014. doi:10.1073/pnas.1410579111.

[339] B. Everts, R. Tussiwand, L. Dreesen, K. C. Fairfax, S. C. Huang, A. M. Smith, C. M. O'Neill, W. Y. Lam, B. T. Edelson, J. Urban, J. F., K. M. Murphy, and E. J. Pearce. Migratory cd103+ dendritic cells suppress helminth-driven type 2 immunity through constitutive expression of il-12. *J Exp Med*, 213(1):35–51, 2016. doi:10.1084/jem.20150235.

[340] N. S. Wilson and J. A. Villadangos. Lymphoid organ dendritic cells: beyond the langerhans cells paradigm. *Immunol Cell Biol*, 82(1):91–8, 2004. doi:10.1111/j.1440-1711.2004.01216.x.

[341] V. Arnaud, J. Li, Y. Wang, X. Fu, S. Mengzhi, X. Luo, X. Hou, H. Dessein, Z. Jie, Y. Xin-Ling, H. He, D. P. McManus, Y. Li, and A. Dessein. Regulatory role of interleukin-10 and interferon-gamma in severe hepatic central and peripheral fibrosis in humans infected with schistosoma japonicum. *J Infect Dis*, 198(3):418–26, 2008. doi:10.1086/588826.

[342] M. Hesse, A. W. Cheever, D. Jankovic, and T. A. Wynn. Nos-2 mediates the protective anti-inflammatory and antifibrotic effects of the th1-inducing adjuvant, il-12, in a th2 model of granulomatous disease. *Am J Pathol*, 157(3):945–55, 2000. doi:10.1016/s0002-9440(10)64607-x.

[343] E. Eskandari-Nasab, A. Sepanjnia, M. Moghadampour, M. Hadadi-Fishani, A. Rezaeifar, A. Asadi-Saghandi, B. Sadeghi-Kalani, M. D. Manshadi, F. Pourrajab, and H. Pourmasoumi. Circulating levels of interleukin (il)-12 and il-13 in helicobacter pylori-infected patients, and their associations with bacterial caga and vaca virulence factors. *Scand J Infect Dis*, 45(5):342–9, 2013. doi:10.3109/00365548.2012.737930.

[344] B. Marotti, A. Rocco, P. De Colibus, D. Compare, G. de Nucci, S. Staibano, F. Tatangelo, M. Romano, and G. Nardone. Interleukin-13 mucosal production in helicobacter pylori-related gastric diseases. *Dig Liver Dis*, 40(4):240–7, 2008. doi:10.1016/j.dld.2007.11.021.

[345] M. J. Goo, M. R. Ki, H. R. Lee, H. J. Yang, D. W. Yuan, I. H. Hong, J. K. Park, K. S. Hong, J. Y. Han, O. K. Hwang, D. H. Kim, S. H. Do, R. D. Cohn, and K. S. Jeong. Helicobacter pylori promotes hepatic fibrosis in the animal model. *Lab Invest*, 89(11):1291–303, 2009. doi:10.1038/labinvest.2009.90.

[346] M. R. Ki, M. J. Goo, J. K. Park, I. H. Hong, A. R. Ji, S. Y. Han, S. Y. You, E. M. Lee, A. Y. Kim, S. J. Park, H. J. Lee, S. Y. Kim, and K. S. Jeong. Helicobacter pylori accelerates hepatic fibrosis by sensitizing transforming growth factor-beta1-induced inflammatory signaling. *Lab Invest*, 90(10):1507–16, 2010. doi:10.1038/labinvest.2010.109.

[347] M. Kaviratne, M. Hesse, M. Leusink, A. W. Cheever, S. J. Davies, J. H. McKerrow, L. M. Wakefield, J. J. Letterio, and T. A. Wynn. Il-13 activates a mechanism of tissue fibrosis that is completely tgf-beta independent. *J Immunol*, 173(6):4020–9, 2004.

[348] D. Cioli, L. Pica-Mattoccia, A. Basso, and A. Guidi. Schistosomiasis control: praziquantel forever? *Mol Biochem Parasitol*, 195(1):23–9, 2014. doi:10.1016/j.molbiopara.2014.06.002.

[349] P. H. L. Lamberton, C. L. Faust, and J. P. Webster. Praziquantel decreases fecundity in schistosoma mansoni adult worms that survive treatment: evidence from a laboratory life-history trade-offs selection study. *Infect Dis Poverty*, 6(1):110, 2017. doi:10.1186/s40249-017-0324-0.

[350] J. C. Atherton and M. J. Blaser. Coadaptation of helicobacter pylori and humans: ancient history, modern implications. *J Clin Invest*, 119(9):2475–87, 2009. doi:10.1172/jci38605.

[351] V. Conteduca, D. Sansonno, G. Lauletta, S. Russi, G. Ingravallo, and F. Dammacco. H. pylori infection and gastric cancer: state of the art (review). *Int J Oncol*, 42(1):5–18, 2013. doi:10.3892/ijo.2012.1701.

[352] P. Michetti. Experimental helicobacter pylori infection in humans: a multifaceted challenge. *Gut*, 53(9):1220–1, 2004. doi:10.1136/gut.2004.042135.

[353] C. Alba, A. Blanco, and T. Alarcon. Antibiotic resistance in helicobacter pylori. *Curr Opin Infect Dis*, 30(5):489–497, 2017. doi:10.1097/qco.0000000000000396.

[354] I. Thung, H. Aramin, V. Vavinskaya, S. Gupta, J. Y. Park, S. E. Crowe, and M. A. Valasek. Review article: the global emergence of helicobacter pylori antibiotic resistance. *Aliment Pharmacol Ther*, 43(4):514–33, 2016. doi:10.1111/apt.13497.

[355] 2016. URL: http://www.who.int/neglected_diseases/news/WHO_urges_increased_access_to_praziquantel/en/.

[356] F. Mutapi, R. Maizels, A. Fenwick, and M. Woolhouse. Human schistosomiasis in the post mass drug administration era. *Lancet Infect Dis*, 17(2):e42–e48, 2017. doi:10.1016/s1473-3099(16)30475-3.

A Publications (Published, submitted and in-prep)

Klar K, Perchermeier S, **Bhattacharjee S.**, Harb H, Adler T, Istvanffy R, Loffredo-Verde E, R A Oostendorp R, Renz H, Prazeres da Costa C., Chronic schistosomiasis during pregnancy epigenetically reprograms T cell differentiation in offspring of infected mothers. Eur J Immunol. 2017 May;47(5):841-847. doi:10.1002/eji.201646836. Epub 2017 Apr 11.

Bhattacharjee, S., Kalbfuss, N. and Prazeres da Costa, C., Parasites, microbiota and metabolic disease. Parasite Immunol. 2017 May;39(5). doi: 10.1111/pim.12390. Epub 2016 Dec 14.

Loffredo-Verde E, **Bhattacharjee S.**, Malo.A, Reisinger.F, Ringelhan.M, Heikenwaelder.M, Protzer.U, Prazeres da Costa C., , Helminth immunomodulation enhances rather than suppresses anti-viral immunity in a dynamic pattern and determines the outcome of acute or chronic Hepatitis B virus infection. Submitted November 2017

Bhattacharjee S., Loffredo-Verde E, Mejias-Luque R, Gerhard M, Prazeres da Costa C, Concomitant infection of *S.mansoni* and *H.pylori* promotes promiscuity of antigen experienced cells and primes the liver for a lower fibrotic response. Submitted November 2017.

Other projects with manuscripts in prep:
Bhattacharjee S. et al. Overcoming inefficient immunization responses in *S.mansoni* infected mothers and their offspring.
Bhattacharjee S. et al. Changes in the microbiome and metabolome of *S.mansoni* infected mice during chronic infection.

B Declaration

I, Sonakshi Bhattacharjee, hereby declare that I independently prepared the present thesis, using only the references and resources stated. This work has not been submitted to any examination board, yet. Parts of this work will be published in two scientific journals.

Munich, November 2017

C Acknowledgements

Firstly, I would like to thank **Prof. Dr.med Markus Gerhard** and **Prof. Dr.med Clarissa da Costa**. This was a challenging project from the beginning but they have always believed in me, encouraged my ideas, pushed me to achieve more than I believed was possible and made time for me no matter what. I would also like to extend my gratitude towards **Prof. Dr.med Dirk Haller**, for his critical feedback and candid comments, that led us to modify some initial ideas and figures. The next person I want to thank is not only a colleague and friend but has grown to be family. **Dr. Eva Loffredo Verde** has been there for me from the first day I entered the lab, her encouragement, personality and outlook to life changed my perception of it. I would not be the same person if I had not met this wonderful woman. **Dr. Raquel Mejias-Luque** is another person whom I am deeply indebted to, her abilities as a scientist are unapologetically clear but her kindness, patience, encouragement and love for what she does makes her an exceptional teacher, scientist and most importantly, one of my closest friends. I cannot thank **Marija Ram** enough for her help, not just with this project but with the countless ideas and projects that we have followed up on together. She has stayed with me through experiments that lasted longer than her responsibility as a technician

and never stepped back from an opportunity to learn something new. I would like to also thank **Sabine Paul**, **Ursula Henn** and **Stefanie Fretzer** for their technical help and taking care of the schistosome life cycle, making it much easier to plan and perform our experiments. **Dr. Raphaela Semper** for teaching me the basics of working with mice in the field of immunology and all the troubleshooting we did together. **Matthew Lacorcia** for endless scientific discussions and great company in the lab. Finally, I would like to thank **Maximilian Miller**, his support, scientific contributions, attitude to life and love are unparalled. I could not have asked for a better partner or friend. In the end, I owe everything to **my parents**, living continents away since I began university was not easy. They have always, always stood by me, pushed me to achieve the best and supported every decision I have made.