Max von Pettenkofer-Institut
Institut der Ludwig-Maximilians-Universität München
Vorstand: Prof. Dr. Sebastian Suerbaum / Prof. Dr. Oliver T. Keppler


# *Innate human cell activation by bacterial lipopolysaccharide core heptose metabolites and Helicobacter pylori*

Dissertation
zum Erwerb des Doktorgrades der Naturwissenschaften
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von
**Larissa Faaß**
aus
**Aalen**

Jahr
**2023**

Mit Genehmigung der Medizinischen Fakultät

der Universität München

| | |
|---|---|
| **Betreuerin:** | Prof. Dr. Christine Josenhans |
| **Zweitgutachterin:** | PD Dr. Hanna-Mari Baldauf |
| **Dekan:** | Prof. Dr. med. Thomas Gudermann |
| **Tag der mündlichen Prüfung:** | 06. November 2023 |

# 1 AFFIDAVIT

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN | Promotionsbüro Medizinische Fakultät | MMRS

**Eidesstattliche Versicherung**

Faaß, Larissa

Name, Vorname

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Titel:

"Innate human cell activation by bacterial lipopolysaccharide core heptose metabolites and *Helicobacter pylori*"

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

München, 29.12.2023 — Ort, Datum

Larissa Faaß — Unterschrift Doktorandin bzw. Doktorand

**Table of contents**

## 2 SUMMARY

Lipopolysaccharide (LPS) inner core heptose metabolites, including ADP-heptose, play an essential role in the activation of cell-autonomous innate immune responses in eukaryotic cells via the ALPK1-TIFA-NF-κB signaling pathway, as demonstrated for several pathogenic bacteria. The important role of LPS heptose metabolites in the pro-inflammatory activation of gastric epithelial cells during an infection with the pathogenic bacterium *Helicobacter pylori* (*H. pylori*) was demonstrated before, in studies by our group and other scientists. The human pathogen *H. pylori* colonizes more than half of the world's population, causing chronically active gastritis and ulcers, and long-term infection can lead to gastric cancer. Disease progression and severity depend on virulence factors of the pathogen, such as the Cag type 4 secretion system (T4SS) that enables active transport of proteins and metabolites into host cells.

The impact of heptose metabolites and pure ADP-heptose on human myeloid cells was not yet investigated. Therefore, in my project, we aimed to gain a better understanding of the activation and modulation potential of bacterial heptose metabolites on human macrophages and neutrophils. For this purpose, we used pure heptose metabolites and, as a bacterial model, *H. pylori*, which appears to transport heptose metabolites into the human host cell, most probably via its T4SS. Main questions of my thesis were how bacterial heptose metabolites affect the pro-inflammatory activation and maturation, alone and in the bacterial context, of human macrophages and neutrophils. In addition, we wanted to characterize the effect of heptose metabolites on macrophage functions including phagocytosis and antigen presentation.

The main findings of my project were that human macrophages and neutrophils respond with high sensitivity to pure heptose metabolites in cell culture and primary cell models (manuscript I and III). In addition, we discovered that activation of these cells with pure ADP-heptose was dependent on active cellular uptake. Our results are based on pro-inflammatory cytokine production and transcriptional upregulation of pro-inflammatory genes (IL-8) and the transcription factor NF-κB after exposure to ADP-heptose. When comparing different cell types, we found that transcriptional changes after co-incubation with pure ADP-heptose were very similar in human gastric epithelial cells, monocyte/macrophage-like cells, and neutrophil-like cells (manuscript III). Using knock-down experiments, I demonstrated that ADP-heptose signaling in monocyte/macrophage-like cells is dependent on TIFA (manuscript I). Moreover, we discovered that stimulation with pure ADP-heptose polarized monocytes into macrophages of predominantly pro-inflammatory type M1 and pre-mature neutrophil-like cells rather into the pro-inflammatory type N1 (manuscript I and III). Furthermore, I found that activation of human

macrophages and neutrophils by live *H. pylori* is strongly influenced by the presence of LPS heptose metabolites and the functionality of its T4SS. In addition, we showed the ability of macrophages to phagocytose does not appear to be affected by heptose metabolites, but ADP-heptose is the decisive factor to reduce antigen presentation by macrophages upon heptose- or bacteria-exposure, due to upregulation of miRNA146b (manuscript I and II).

In summary, my work contributed significantly to our new findings that specific heptose metabolites or bacteria producing heptose metabolites elicit a strong activity and modulation of cell-autonomous innate immune responses and cellular functions of human macrophages and neutrophils.

# 3 ZUSAMMENFASSUNG

Die Heptose-Metaboliten des inneren Kerns von Lipopolysacchariden (LPS), einschließlich ADP-Heptose, spielen eine wesentliche Rolle bei der Aktivierung von zellautonomen angeborenen Immunreaktionen in eukaryotischen Zellen über den ALPK1-TIFA-NF-κB Signalweg, wie schon für mehrere pathogene Bakterien gezeigt wurde. Die wichtige Rolle von LPS-Heptose-Metaboliten bei der pro-inflammatorischen Aktivierung von Magenepithelzellen während einer Infektion mit dem pathogenen Bakterium *Helicobacter pylori* (*H. pylori*) wurde bereits, in Forschungsarbeiten unserer Arbeitsgruppe und von anderen Wissenschaftler*innen, nachgewiesen. Das humanpathogene Bakterium *H. pylori* besiedelt mehr als die Hälfte der Weltbevölkerung und verursacht eine chronisch aktive Gastritis und Magengeschwüre, und eine langfristige Infektion kann zu Magenkrebs führen. Krankheitsverlauf und Schweregrad hängen von Virulenzfaktoren des Erregers ab, wie z. B. dem Cag Typ-4-Sekretionssystem (T4SS), das den aktiven Transport von Proteinen und Metaboliten in Wirtszellen ermöglicht.

Die Auswirkungen von Heptose-Metaboliten und reiner ADP-Heptose auf menschliche myeloide Zellen wurden bisher nicht untersucht. Deshalb haben wir in meinem Projekt versucht, das Aktivierungs- und Modulationspotenzial von bakteriellen Heptose-Metaboliten auf menschliche Makrophagen und Neutrophile besser zu verstehen. Zu diesem Zweck verwendeten wir reine Heptose-Metaboliten und, als bakterielles Modell, *H. pylori*, welcher Heptose-Metaboliten, höchstwahrscheinlich über sein T4SS, in menschliche Wirtszellen transportieren kann. Die Hauptfragen meines Projekts waren, wie bakterielle Heptose-Metaboliten die pro-inflammatorische Aktivierung und Reifung von menschlichen Makrophagen und Neutrophilen, allein und im bakteriellen Kontext, beeinflussen. Darüber hinaus wollten wir die Wirkung von Heptose-Metaboliten auf verschiedene Funktionen von Makrophagen, wie Phagozytose und Antigenpräsentation, charakterisieren.

Die wichtigsten Ergebnisse meines Projekts waren, dass menschliche Makrophagen und Neutrophile, in Zellkultur-Zellen und Primärzellen, mit hoher Empfindlichkeit auf reine Heptose-Metaboliten reagieren (Manuskript I und III). Darüber hinaus entdeckten wir, dass die Aktivierung dieser Zellen mit reiner ADP-Heptose von einer aktiven zellulären Aufnahme abhängig ist. Unsere Ergebnisse beruhen auf der Produktion pro-inflammatorischer Zytokine und der transkriptionellen Hochregulierung pro-inflammatorischer Gene (IL-8) und des Transkriptionsfaktors NF-κB nach Koinkubation mit ADP-Heptose. Beim Vergleich verschiedener Zelltypen stellten wir fest, dass die transkriptionellen Veränderungen nach Ko-Inkubation mit reiner ADP-Heptose in menschlichen Magenepithelzellen, Monozyten-

/Makrophagenartigen Zellen und Neutrophilen sehr ähnlich waren (Manuskript III). Mit Hilfe von knock-down Experimenten konnte ich zeigen, dass der ADP-Heptose aktivierte Signaltransduktionsweg in Monozyten- und Makrophagen-artigen Zellen von TIFA abhängig ist (Manuskript I). Darüber hinaus entdeckten wir, dass die Stimulation mit reiner ADP-Heptose Monozyten in Makrophagen des überwiegend pro-inflammatorischen Typs M1 und unreife Neutrophilen-ähnliche Zellen eher in den pro-inflammatorischen Typ N1 polarisiert (Manuskript I und III). Ebenfalls fand ich heraus, dass die Aktivierung menschlicher Makrophagen und Neutrophilen durch lebende *H. pylori* Bakterien stark von LPS Heptose-Metaboliten abhängt und von der Funktionalität des T4SS beeinflusst wird. Darüber hinaus haben wir gezeigt, dass die Fähigkeit der Makrophagen zur Phagozytose nicht durch Heptose-Metaboliten beeinträchtigt zu werden scheint, das ADP-Heptose jedoch der entscheidende Faktor für die Verringerung der Antigenpräsentation durch Makrophagen ist, wenn sie Heptose oder Bakterien ausgesetzt sind, was auf die Hochregulierung von miRNA146b zurückzuführen ist (Manuskript I und II).

Zusammenfassend lässt sich sagen, dass meine Arbeit wesentlich zu unseren neuen Erkenntnissen beigetragen hat, dass spezifische Heptose-Metaboliten oder Bakterien, die Heptose-Metaboliten produzieren, eine starke Aktivität und Modulation der zellautonomen angeborenen Immunreaktionen und Zellfunktionen menschlicher Makrophagen und Neutrophile hervorrufen.

## 4 LIST OF ABBREVIATIONS

3′-UTR = 3′ untranslated region

ADP-hep = ADP-glycero-β-D-manno-heptose-1-phosphate

AlpA = Adherence-associated lipoprotein A

AlpB = Adherence-associated lipoprotein B

ALPK1 = *Alpha* protein kinase 1

AP-1 = Activator protein 1

BabA = Blood group antigen binding adhesin

CagA = Cytotoxin-associated gene A

*cag*PAI = *Cag* pathogenicity island

CD4 = Cluster of differentiation 4

CD40L = Cluster of differentiation 40 ligand

CD8 = Cluster of differentiation 8

CEACAM = Carcinoembryonic antigen-related cell adhesion molecule

CIAP1 = Cellular inhibitor of apoptosis 1

CLR = C-type lectin receptor

CTL = C-type lectin

DC = Dendritic cell

DC-SIGN = Dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin

DNA = Deoxyribonucleic acid

DSBs = Double strand breaks

*Et al.* = *et alia*

GC = Gastric cancer

GEC = Gastric epithelial cell

GGT = Gamma-glutamyl transpeptidase

GSC = Gastric stem cell

HBP = D-glycero-β-D-manno-heptose-1,7-BP

hCEACAM = human carcinoembryonic antigen-related cell adhesion molecule

HMP-1 = D-glycero-β-D-manno-heptose-1-phosphate

HMP-7 = D-glycero-α,β-D-manno-heptose-7-phosphate

HopQ = *Helicobacter pylori* outer protein Q

*H. pylori* = *Helicobacter pylori*

HSC = Hematopoietic stem cell

IFN = Interferon

IgA = Immunglobulin A

IgG = Immunglobulin G

IκB-α = Nuclear factor *kappa*-B cell inhibitor *alpha*

IKK = I *kappa* B kinase

IKKα = I *kappa* B kinase *alpha*

IKKβ = I *kappa* B kinase *beta*

IKKγ = I *kappa* B kinase *gamma*

IL-1β = Interleukin 1 *beta*

IL-8 = Interleukin 8

IL-6 = Interleukin 6

IL-10 = Interleukin 10

IL-18 = Interleukin 18

IRF3 = Interferon regulatory factor 3

IRF7 = Interferon regulatory factor 7

Kb = Kilo base

Lgr4 = Leucine rich repeat containing G protein-coupled receptor 4

LPS = Lipopolysaccharide

LTβR = Lymphotoxin-*beta* receptor

MALT lymphoma = Mucosa-associated lymphoid tissue lymphoma

MAMP = Microbe associated molecular pattern

MINCLE = Macrophage-inducible C-type lectin

MiRNA = Micro ribonucleic acid

NF-κB = Nuclear factor *kappa*-B

NF-κB2 = Nuclear factor *kappa*-B 2

NIK = NF-κB-inducing kinase

NLR = Nod-like receptor

NLRP3 = Nod-like receptor family pyrin domain containing 3

NOD1 = Nucleotide-binding oligomerization domain-containing 1

NOS = Nitric oxide synthase

Nt = Nucleotide

OLFM4 = Olfactomedin 4

PRR = Pattern recognition receptor

RIG-I = Retinoic acid inducible gene I

RLR = Retinoic acid-inducible gene-I-like receptor

RNA = Ribonucleic acid

Rspo3 = R-spondin 3

S-7-P = Sedoheptulose-7-phosphate

SabA = Sialic acid binding adhesin

SiRNA = Silencing ribonucleic acid

SS1 = *H. pylori* sydney strain 1

T4SS = Cag type 4 secretion system

Th1-cell = T helper type 1 cell

Th17-cell = T helper type 17 cell

TIFA = Tumor necrosis factor receptor associated factor-interacting protein with a forkhead - associated domain

TLR = Toll-like receptor

TLR2 = Toll-like receptor 2

TLR4 = Toll-like receptor 4

TLR5 = Toll-like receptor 5

TLR9 = Toll-like receptor 9

TNF-$\alpha$ = Tumor necrosis factor *alpha*

TNFAIP3 = Tumor necrosis factor *alpha*-induced protein 3

TRAF2 = Tumor necrosis factor receptor associated factor 2

TRAF6 = Tumor necrosis factor receptor associated factor 6

Treg-cell = T regulatory cell

Trim30a = Tripartite motif-containing 30 a

VacA = Vacuolating cytotoxin A

# 5 INTRODUCTION

## 5.1 *Helicobacter pylori:* History, microbiological characterization, prevalence and therapy

The pathogenic bacterium *Helicobacter pylori (H. pylori)* was discovered by Barry Marshall and Robin Warren in 1982 (Warren and Marshall, 1983). It is a Gram-negative, spiral-shaped, flagellated, mainly extracellular, microaerophilic bacterium that colonizes the gastric mucosa of more than 50% of the world's population (Suerbaum *et al*., 2002). Transmission of the pathogen usually occurs in childhood via oral-to-oral and fecal-to-oral routes (Schwarz *et al*., 2008;Goh *et al*., 2011;Didelot *et al*., 2013;Krebes *et al*., 2014). The prevalence of an *H. pylori* infection within populations is related to socioeconomic status, community type and habitational crowding conditions, and *H. pylori* is known to have co-evolved with humans (Falush *et al.*, 2003;Linz *et al.*, 2007;Moodley *et al.*, 2012;Salama *et al.*, 2013;Hooi *et al.*, 2017). Infected individuals develop chronic gastritis, which is usually asymptomatic, and some develop gastric ulcers, MALT lymphomas and gastric cancer (GC) over time (Suerbaum and Michetti, 2002;Amieva and El-Omar, 2008). For this reason, the World Health Organization defined *H. pylori* as group 1 carcinogen in 1994 (IARC, 1994). *H. pylori* is the greatest risk factor for the development of GC, causing up to ~75% of all GCs (Asghar and Parsonnet, 2001). According to the Global Cancer Statistics of 2020 (Sung *et al.*, 2021), GC is the fifth most common cancer and the fourth leading cause of cancer-related death worldwide. In 2020 alone, 768,793 people died from GC (Sung *et al.*, 2021). Disease progression and severity are influenced by the host genetic predisposition, genotype of the bacterium, and environmental parameters (Suerbaum and Josenhans, 2007). The pathogen exhibits high genetic diversity, globally and within individual hosts (Achtman *et al.*, 1999;Suerbaum and Josenhans, 2007;Olbermann *et al.*, 2010;Ailloud *et al.*, 2019). *H. pylori* has evolved pro-inflammatory and immune-evasive mechanisms that support its long-term survival and persistence in the human stomach unless treated (Mejias-Luque and Gerhard, 2017). An antibiotic combination therapy with either proton-pump inhibitors or bismuth salts are the mainstay of treatment for confirmed infections and currently recommended (Malfertheiner *et al.*, 2017). The increasing antibiotic resistance of *H. pylori* has led to its inclusion in a high-priority list of pathogens for which research and development of new effective drugs is needed (Tacconelli *et al.*, 2018). To date, there is no efficient vaccination against *H. pylori* available (Sutton and Boag, 2019).

## 5.2 The human gastric niche

The gastric epithelium consists of a monolayer of columnar gastric epithelial cells (GECs) covered with mucus and forming invaginations called gastric pits within the underlying lamina propria. Each pit gives rise to a gastric gland, which consist of a variety of secretory cells,

including chief cells, enteroendocrine cells, parietal cells and mucous neck cells that produce the overlaying mucus and gastric juice (Alzahrani *et al.*, 2014;Kayisoglu *et al.*, 2021a). Gastric stem cells (GSCs) are located deep inside the gastric gland and are essential for continuous dynamic gastric cell renewal (Xiao and Zhou, 2020). An essential function of this GEC layer is to maintain a protective barrier that separates the luminal contents containing pathogenic microorganisms from the underlying tissue compartments (Wroblewski LE, Peek RM Jr., 2011). During infection, GECs provide a first line of defense against invading pathogens (Alzahrani *et al.*, 2014).

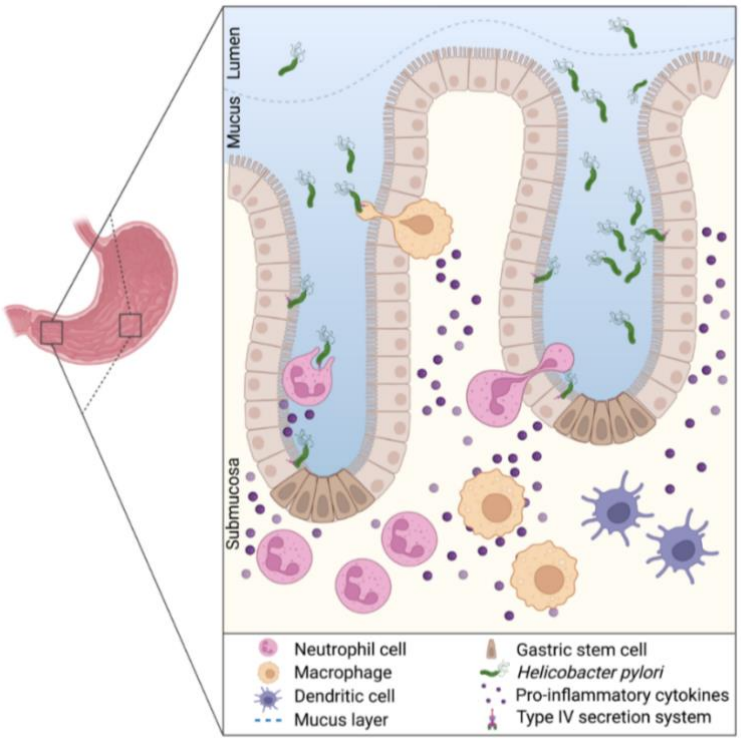


**FIG 1: A schematic overview of the stomach habitat of *H. pylori*.** *H. pylori* persists in the lower layers of the stomach mucus and in the gastric crypts, where it proliferates and forms micro-colonies. In particular in the gastric crypts, *H. pylori* can get into contact with GSCs and myeloid cell types with phagocytic and antigen-presenting properties that are recruited through the lamina propria, as indicated. Its Cag type 4 secretion system (T4SS) promotes interaction with epithelial and non-epithelial cells and secretes various proteins and metabolites, which can influence cell responses. The artwork of this figure was created using BioRender and is also contained in our recent review Faass *et al.* 2023 (Faass *et al.*, 2023).

### 5.3 *H. pylori* survival and persistence in the human gastric niche

After ingestion, *H. pylori* is able to survive the acidic pH of the stomach with the help of its enzyme urease. Using urea channels, *H. pylori* transports urea to its cytoplasm, where urease hydrolyzes urea to ammonia (Krulwich *et al.*, 2011). This buffers the periplasmic pH and the

immediate environment of the pathogen and enables it to maintain a stable cytoplasmic pH (Weeks *et al.*, 2000;Krulwich *et al.*, 2011). In addition, the bacterium is able to move within the gastric mucus layer based on chemotaxis, using its flagella (Josenhans and Suerbaum, 2002). It can reside in the lower layers of the mucus or deep within the gastric crypts, where the pH is more neutral and GSCs are harbored (Schreiber *et al.*, 2004;Sigal *et al.*, 2015;Sigal *et al.*, 2017). When *H. pylori* arrives deep down at the gastric niche, it can adhere to GECs (Hessey *et al.*, 1990;Thomsen *et al.*, 1990;Kim *et al.*, 2004). There, the bacterium can form micro-colonies and creates a microenvironment beneficial for bacterial proliferation (see Figure1) (Tan *et al.*, 2009;Fung *et al.*, 2019).

### 5.4 *H. pylori cag* pathogenicity island and T4SS

*H. pylori* possesses the *cag* pathogenicity island (*cag*PAI), which is one of its major virulence factors. The *cag*PAI is about 40 kb long and contains approximately 28 genes (Fischer *et al.*, 2001). The genomic island genes encode the structural components of the *H. pylori* transport machine, the T4SS, and the effector protein cytotoxin-associated gene A (CagA). The secretion system spans the inner and outer membrane of the bacterium and is capable of transporting molecules into host cells (Kutter *et al.*, 2008;Frick-Cheng *et al.*, 2016;Chung *et al.*, 2019). It is able to transport CagA (Segal *et al.*, 1999;Backert *et al.*, 2000;Odenbreit *et al.*, 2000;Odenbreit *et al.*, 2001;Hatakeyama, 2008), bacterial DNA (Varga et al., 2016a), peptidoglycan molecules (Viala *et al.*, 2004), and, most probably, lipopolysaccharide (LPS) heptose metabolites (Gall *et al.*, 2017;Stein *et al.*, 2017;Zimmermann *et al.*, 2017). *H. pylori* strains can also be classified according to the presence or absence of the genomic island. Many strains contain a *cag*PAI and account for approximately 70% of all global isolates (Olbermann *et al.*, 2010). Strains carrying a functional *cag*PAI cause a stronger inflammation and ulceration in the stomach (Wiedemann *et al.*, 2009;Phuc *et al.*, 2021). *H. pylori* was shown to express *cag*PAI genes in the infected human stomach (Boonjakuakul *et al.*, 2005;Aviles-Jimenez *et al.*, 2012).

### 5.5 Human GECs and their immunological features

In addition to their important barrier function within the gastric niche, GECs perform an initial immune response after recognizing gastric pathogens (Alzahrani *et al.*, 2014). Although GECs are not classical immune cells, they possess many pattern recognition receptors (PRRs) that enable the detection of microbe-associated molecular patterns (MAMPs). After immune-activation, GECs are able so secrete pro-inflammatory cytokines to recruit innate immune cells (Kayisoglu *et al.*, 2021a). Furthermore, GECs produce antimicrobial peptides, including defensins, to fight the infection (O'Neil *et al.*, 2000).

### 5.6 The interaction of *H. pylori* with GECs and consequences

The interaction of *H. pylori* with GECs was well studied in the past using human GEC lines (Stein *et al.*, 2017), human gastric biopsies (Hazell *et al.*, 1986), primary cell organoids grown from isolated human donor gastric gland material (Bartfeld *et al.*, 2015) and animal models (Amalia *et al.*, 2023). Adhesion of *H. pylori* to GECs occurs through a variety of its outer membrane proteins. For example, the adhesin BabA binds difucosylated ABO/Lewis blood antigens expressed by GECs, and SabA binds to sialylated Lewis antigens on GECs *(*Ilver *et al.*, 1998;Mahdavi *et al.*, 2002). AlpA/B bind to host laminin (Senkovich *et al.*, 2011), and HopQ attaches to host cell CEACAMs (Javaheri *et al.*, 2016;Königer *et al.*, 2016). In addition, the outer membrane protein CagL of the T4SS interacts with the $\alpha5\beta1$ integrin on the host cell (Kwok *et al.*, 2007). Cell adhesion and interaction with *H. pylori,* as well as secretion and translocation of *H. pylori* molecules can activate GECs. *H. pylori* is able to activate the extracellular PRR TLR2 (Mandell *et al*., 2004), the intracellular PRRs TLR9 via T4SS-translocated DNA (Varga et al., 2016a), and NOD1 via translocated peptidoglycan *(Viala et al.*, 2004). Although known for other bacteria, other PRRs, including TLR4 and TLR5, are not classically activated by *H. pylori.* The pathogen modifies its MAMPs LPS lipidA and O-chain and its flagellins in an immune-evasive manner (Aspinall *et al.*, 1996;Moran *et al.*, 1997;Monteiro *et al.*, 1998;Lee *et al.*, 2003;Gewirtz *et al.*, 2004;Mandell *et al.*, 2004;Li *et al.*, 2017). Recently, LPS heptose metabolites of Gram-negative bacteria, including *H. pylori*, were discovered as new MAMPs (Gaudet *et al.*, 2015;Milivojevic *et al.*, 2017;Zhou *et al.*, 2018). *H. pylori* LPS heptose metabolites activate the intracellular PRR *alpha* protein kinase 1 (ALPK1) and thus pro-inflammatory signaling in GECs (Gall e*t al.*, 2017;Stein *et al.*, 2017;Zhou *et al.*, 2018;Pfannkuch *et al.*, 2019). Downstream pro-inflammatory signaling cascades lead to the upregulation of pro-inflammatory genes and production of cytokines (Alzahrani *et al.*, 2014). In addition to the induction of immune responses, *H. pylori* infection elicits DNA double strand breaks (DSBs) in GECs, depending on a functional T4SS, thus causing genetic instability in host cells (Koeppel *et al.*, 2015).

### 5.7 The human innate immune system

The innate immune system is the body's first line of defense against invading pathogens and encompasses cells and proteins that respond with non-specific immune reactions (Janeway and Medzhitov, 2002). Phagocytes such as macrophages and neutrophils, dendritic cells (DCs), mast cells, basophils and eosinophils are part of the innate immune system (Marshall *et al.*, 2018). Their role during infection is to directly eliminate the pathogens and transmit the danger signal to cells of the adaptive immune system (Marshall *et al.*, 2018). In addition to cells, the complement system is an important part of the innate immune system. The proteins are able to identify and opsonize pathogens in order to support the uptake of pathogens by phagocytic cells or to kill them directly (Medzhitov, 2007;Marshall *et al.*, 2018).

### 5.8 Innate immune system cell activation and modulation by *H. pylori*

The interaction of *H. pylori* with GECs has been well studied in the past. However, little is known about the mechanism of interaction and activation between cells of the myeloid lineage, for instance the phagocytic and antigen-presenting cells, and *H. pylori*. Upon activation by *H. pylori*, GECs recruit myeloid cells to the side of infection via cytokine secretion, such as IL-8 (see Figure1) (Noach *et al.*, 1994). During infection and after disruption of the cell integrity of the gastric epithelial barrier, phagocytes, including neutrophils, DCs, monocytes, and macrophages, are found in the gastric mucosal tissue (Noach *et al.*, 1994;Fehlings *et al.*, 2012). Activation of the innate immune system is aimed at combating the pathogen, directly or with the help of the adaptive response. However, *H. pylori* has evolved multiple strategies to evade and dampen immune recognition and response, resulting in lifelong persistence in the human stomach (Mejias-Luque and Gerhard, 2017). During *H. pylori* infection, infiltration of innate immune cells increases the severity of the inflammation and the degree of mucosal damage (Fu *et al.*, 2016;Mejias-Luque and Gerhard, 2017).

While it is well known how *H. pylori* interacts with and activates GECs, the molecular mechanisms of its primary surface interaction with lymphoid and phagocytic cells is less well understood. Adhesion of *H. pylori* with myeloid cells may occur via primary interactions similar to that known for GECs, since Unemo *et al.* showed that adhesion of *H. pylori* to neutrophils via SabA is essential for phagocyte activation (Unemo *et al.*, 2005). Similar to GECs, phagocytes recognize *H. pylori* via a variety of PRRs, including TLRs, NLRs, and CLRs, which contribute to pro-inflammatory or tolerogenic effects (Mejias-Luque and Gerhard, 2017;Faass *et al.*, 2023). In addition to interacting with hosts via MAMPs and PRRs, the bacterium interacts with phagocytic cells via its T4SS (Sanchez-Zauco *et al.*, 2014). There may be other innate immune players and strategies for myeloid cell activation and pathogen elimination during infection that were not yet explored.

### 5.9 Macrophages

Macrophages are long-lived phagocytes that mostly differentiate from circulating blood monocytes, which were produced by the bone marrow (Grainger *et al.*, 2017). During an infection, monocytes leave the blood circulation, migrate into tissues and differentiate into macrophages (Grainger *et al.*, 2017). In the gastric niche, monocyte-derived macrophages can be found in the lamina propria right beneath the gastric epithelial monolayer where they are able to capture and eliminate bacteria that breach the epithelial barrier (Grainger *et al.*, 2017). In the mouse model, it was shown that macrophages in the lamina propria are able to sample pathogens using trans-epithelial dendrites without disrupting the epithelial integrity *(Niess et al.*, 2005). Upon activation by a danger signal, the macrophages can polarize into the pro-inflammatory M1 phagocytes or the inhibitory and immune-modulating M2 subtype (Italiani and

Boraschi, 2014). The main characteristics of M1 macrophages are pathogen phagocytosis, antigen presentation to T-cells, NOS production and recruitment of further immune cells to the side of infection by cytokine secretion (Orecchioni *et al.*, 2019).

### 5.10 Macrophages and *H. pylori*

*H. pylori* activates many different immunological pathways in macrophages and leads to pro-inflammatory polarization and signaling. Upon interaction, *H. pylori* induces inflammatory cytokine production in macrophages via TLR2 signaling and independent of TLR4 (Mandell *et al.*, 2004). Besides TLRs, NLRs are also involved in the activation of macrophages, as Li *et al.* showed that *H. pylori* enhances the production of the pro-inflammatory cytokines IL-1β and IL-18 via the NLRP3 inflammasome in human macrophage-like cells (Kim *et al.*, 2013;Li *et al.*, 2015). Furthermore, *H. pylori* uses its LPS Lewis blood antigens to interact with MINCLE to escape clearance by macrophages by the induction of anti-inflammatory cytokine production (Chmiela *et al.*, 1997;Matsumoto *et al.*, 1999;Devi *et al.*, 2015). In mouse macrophages, Koch *et al.* showed that macrophages are able to recognize the pathogens' T4SS independently of TLRs (Koch *et al.*, 2016). Overall, *H. pylori* activation of macrophages and pro-inflammatory cytokine production involves the NF-κB pathway (Maeda *et al.*, 2001). During infection with *H. pylori*, presence of M1 and M2 polarized macrophages (predominantly M1) were reported in human gastric biopsies from infected individuals and mouse models (Quiding-Järbrink *et al.*, 2010;Fehlings *et al.*, 2012). After phagocytosis, macrophages present *H. pylori* antigens to T-cells of the adaptive immune system and induce their pro-inflammatory differentiation within the *H. pylori*-infected mouse mucosa (Zhuang *et al.*, 2011). However, macrophages infected with *H. pylori* downregulate (Pagliari *et al.*, 2017) or upregulate (Codolo *et al.*, 2019) inflammation-related microRNAs (miRNAs), which reduces their pro-inflammatory potential as they decrease the exposure of HLA-II molecules on the plasma membrane, compromising the bacterial antigen presentation toward T-cells (Pagliari *et al.*, 2017;Codolo *et al.*, 2019).

### 5.11 Neutrophils

Neutrophils are short-lived polymorphonuclear leukocytes and arise from hematopoietic stem cells (HSC) in the bone marrow (Nathan, 2006). Under homeostatic conditions, mature neutrophils leave the bone marrow, enter the blood stream and can migrate to various tissues. The main antimicrobial functions of neutrophils are phagocytosis, degranulation, neutrophil extracellular trap formation and oxidative burst to eliminate pathogens (Mayer-Scholl *et al.*, 2004;Mantovani *et al.*, 2011;Boeltz *et al.*, 2019;Burn *et al.*, 2021). During an acute infection, neutrophils are the first cell type recruited to the site of infection. Neutrophils can polarize into a pro-inflammatory subtype, N1, and an immune-suppressive subtype, N2 (Fridlender *et al.*, 2009;Silvestre-Roig *et al.*, 2019). Neutrophils sense the pathogens and their MAMPs via their PRRs and communicate the danger signal to cells of the innate and adaptive immune system,

including macrophages, DCs, and B- and T-cells (Mantovani *et al.*, 2011;Polak and Bohle, 2022).

### 5.12 Neutrophils and *H. pylori*

Various signaling pathways were identified that are activated after interaction of *H. pylori* with neutrophils, inducting immune responses and pro-immunogenic polarization. *H. pylori* induces secretion of pro-inflammatory cytokines IL-8, IL-1β and TNF-α early after interaction, and the anti-inflammatory cytokine IL-10 at later time points via TLR2 and TLR4 (Alvarez-Arellano *et al.*, 2007). In addition, *H. pylori* and *H. pylori* DNA trigger TLR9-dependent IL-8 production in human neutrophils (Alvarez-Arellano *et al.*, 2014). *H. pylori*-induced secretion of IL-8 depends on the *cag*PAI in primary neutrophils, whereas *cag*PAI-negative strains elicit production of the anti-inflammatory cytokine IL-10 (Sanchez-Zauco *et al.*, 2014). Furthermore, Perez-Figueroa *et al.* showed that *H. pylori* infection enhances activation of the NLRP3 inflammasomes and secretion of IL-1β in human neutrophils, independent of a functional T4SS, TLR2, and TLR4, using inhibitory antibodies (Perez-Figueroa *et al.*, 2016). In contrast, in mouse primary cells and partially in human neutrophil-like cell culture cells, Jang *et al*. demonstrated the important role of the *H. pylori* NLRP3 inflammasome for IL-1β cytokine production dependent on the T4SS, flagellin motility and TLR2, using knock-out cells (Jang *et al.*, 2020). For the *H. pylori* infection, it was shown that the degree of gastric mucosal damage correlates with the amounts of locally infiltrated neutrophils, as a high number of neutrophils leads to more severe gastritis and a more rapid clearance of the bacteria (Warren and Marshall, 1983) (Davies *et al.*, 1994;Fiocca *et al.*, 1994).

### 5.13 Dendritic cells

DCs are potent antigen-presenting cells that are critical for the induction of downstream adaptive immune responses and are derived from HSCs in the bone marrow. Among other body sites, DC can be found in the gastrointestinal tract where their main goals are phagocytosis and antigen presentation to the adaptive immune system (Worbs *et al.*, 2017). During infection, DCs are essential for T-cell activation and differentiation (Hilligan and Ronchese, 2020).

### 5.14 Dendritic cells and *H. pylori*

Within the gastric niche, DCs come in contact with *H. pylori* either in the gut lumen, where mucosal DCs insert dendrites through the tight junctions of the gut epithelial monolayer, or after disruption of the epithelial barrier (Rescigno *et al.*, 2001;Necchi *et al.*, 2009;Kao *et al.*, 2010). *H. pylori* has evolved a variety of strategies to manipulate DC cytokine production toward a rather tolerogenic response, and therefore induces an adaptive immune response that may contribute to bacterial persistence (Mejias-Luque and Gerhard, 2017). DCs recognize *H. pylori* via several PRRs, which elicit either pro-inflammatory or anti-inflammatory responses.

In mouse DCs, *H. pylori* activates pro-inflammatory IL-6 cytokine signaling via TLR2, TLR9 and, to a minor extent, via TLR4 (Rad *et al.*, 2009). In addition to the pro-inflammatory cytokine production, *H. pylori* induces anti-inflammatory IL-10 cytokine secretion in mouse DCs dependent on TLR2 (Rad *et al.*, 2009). Furthermore, *H. pylori* RNA activates the RLR RIG-I and induces IFN secretion (Rad *et al.*, 2009). In murine bone marrow-derived DCs, IL-1β secretion was shown to be depended on the presence of the *cag*PAI and involved TLR2, NOD2 and NLRP3 receptors (Kim *et al.*, 2013). Moreover, *H. pylori* LPS containing Lewis blood antigens for immune evasion activates the CTL DC-SIGN on DCs, leading to a subsequent expression of the anti-inflammatory cytokine IL-10 (Bergman *et al.*, 2004;Gringhuis *et al.*, 2009;Miszczyk *et al.*, 2012).

### 5.15 The adaptive immune system

The adaptive immune system is activated and recruited to the site of infection by cells and responses of the innate immune system. In conjunction with the innate responses, it matures and is enabled to recognize specific antigens, and it initiates a pathogen-specific immune response upon activation to eliminate specific pathogens or infected cells. After pathogen removal, the adaptive immune system stores an immune memory to enable rapid immune response in the event of re-infection (Medzhitov, 2007). T- and B-cells are the central players in the adaptive immune response and emerge during hematopoiesis from pluripotent HSCs in the bone marrow (Marshall *et al.*, 2018). Lymphocyte differentiation occurs in a tightly regulated process that gives rise to a variety of progenitor cells. B-cells develop in the bone marrow, while T-cells migrate to the thymus for further development (Reyes and Peniche, 2019). Both cell types fully mature in secondary lymphoid organs including the spleen and lymph nodes or locally in tissues (Reyes and Peniche, 2019). Activation of T-cells occurs in these secondary lymphoid organs through the presentation of foreign antigens by antigen-presenting cells via direct cell-to-cell interaction and the secretion of certain cytokines. In contrast, B-cells are able to recognize antigens themselves and do not require activation by antigen-presenting cells (Reyes and Peniche, 2019). The diverse functions of T-cells during infection include to directly kill infected host cells, activate other immune cells, produce cytokines, and regulate the immune response. The main function of B-cells during an infection are to produce antibodies against the antigens and to participate in the activation of T-cells via antigen presentation, co-stimulation, and antibody as well as cytokine production. T-cells can be classified into CD4- or CD8-positive cells. CD8-positive cells are also known as cytotoxic T-cells and perform mainly cytotoxic activities including killing of infected cells. CD4-positive T-cells can differentiate into a variety of different cell subsets, including Th1-, Th17- and Treg-cells, depending on the activation, having individual roles during inflammation (Reyes and Peniche, 2019). Th1-cells are responsible for identification and eradication of intracellular pathogens, inducing cell-mediated immunity. In contrast, Th17-cells are important in immune

responses against extracellular pathogens (Saravia *et al.*, 2019). Regulatory T-cells, Treg-cells, are known to limit and suppress immune response in order to maintain immune homeostasis and self-tolerance (Saravia *et al.*, 2019).

### 5.16 Role of the adaptive immune system during *H. pylori* infection

During *H. pylori* infection, CD4- and CD8-positive cells are found in the gastric niche, with CD4-positive cells forming the majority (Bamford *et al.*, 1998;Nurgalieva *et al.*, 2005). T-cells in the infected stomach were demonstrated to resemble the Th1 T-cell type upon activation via HLA-II and cytokines (D'Elios *et al.*, 1997;Bamford *et al.*, 1998;Sayi *et al.*, 2009). In addition to Th1-cells, Treg- and Th17-cells were also found in the infected gastric mucosa of humans and mice (Lundgren *et al.*, 2005;Shi *et al.*, 2010;Hitzler *et al.*, 2012). The balance of Treg- and Th17-cells is of great importance for an efficient and homeostatic immune response. However, *H. pylori* can shift this balance, as mostly shown in mouse models, towards a majority of Treg-cells, preventing an effective *H. pylori* specific Th17 immune response (Gebert *et al.*, 2003;Gerhard *et al.*, 2005;Kao *et al.*, 2010;Omenetti and Pizarro, 2015). To underline this, Arnold *et al.* showed in infected mice that depletion of Treg-cells facilitated clearance of *H. pylori* (Arnold *et al.*, 2011). Moreover, Treg-cells accumulate in the *H. pylori*-infected human gastric mucosa and effectively suppress *H. pylori*-specific memory T-cell responses (Lundgren *et al.*, 2003). The exact role of B-cells during *H. pylori* infection is not fully understood, although it is known that *H. pylori*-carriers produce *H. pylori*-specific IgA and IgG antibodies (Futagami *et al.*, 1998;Nurgalieva *et al.*, 2005). Very insightful reviews addressing the role of the adaptive immune response during the *H. pylori* infection was recently published (Reyes and Peniche, 2019;Zhang *et al.*, 2020).

### 5.17 Nuclear factor *kappa*-B signaling

The nuclear factor *kappa*-B (NF-κB) family are key transcription factors regulating the transcription of various immunological and pro-inflammatory genes (Sen and Baltimore, 1986) and an important regulator for cell survival (Luo *et al.*, 2005). In addition, NF-κB stimulation was shown to promote tumor growth as being the link between inflammation and carcinogenesis (Karin *et al.*, 2002;Karin and Greten, 2005). NF-κB is found in the cytoplasm of cells but is masked until activated by NF-κB inhibitors. Pro-inflammatory signaling can be activated via the canonical, the classical, and the non-canonical, the alternative, NF-κB pathways (Feige *et al.*, 2018). Canonical NF-κB signaling is involved in almost all aspects of the immune response, whereas the non-canonical pathway appears to be a supplementary signaling axis that cooperates with the canonical pathway (Sun, 2017). The canonical NF-κB pathway is rapidly activated by stimuli like pro-inflammatory cytokines and MAMPs and their interaction with, for example, cytokine receptors, PRRs, TNF receptors, T- and B-cell receptors (Newton and Dixit, 2012;Taniguchi and Karin, 2018). Upon host cell activation via PRRs, for

example, the NF-κB inhibitor IκB-α is phosphorylated by the IKK complex (IKKβ, IKKα, IKKγ), whereby RelA/p50 is activated and translocated into the nucleus (Taniguchi and Karin, 2018). Activation of the non-canonical pathway happens slower, due to *de novo* NIK synthesis, and is induced by TNF superfamily members, including TNF and CD40L, and subsets of TNF receptor superfamily members, such as the LTβR. NIK initiates the phosphorylation of IKKα, which induces processing of the p100/RelB complex to p52/RelB and its translocation into the nucleus (Sun, 2017;Taniguchi and Karin, 2018). Translocation of NF-κB transcription factors into the cell nucleus results in upregulation of cytokine transcripts and secretion of pro-inflammatory cytokines such as IL-8 and IL-6 (Taniguchi and Karin, 2018). In addition to cytokines, NF-κB *de novo* transcribes its negative feedback regulator A20 (*tnfaip3*). Furthermore, NF-κB transcribes the *nlrp3* gene and is involved in the regulation of the NLRP3 inflammasome (Bauernfeind *et al.*, 2009;Shembade and Harhaj, 2012;Guo *et al.*, 2015).

### 5.18 *H. pylori* induced NF-κB signaling

*H. pylori* is capable of activating canonical and non-canonical NF-κB signaling along various routes. *H. pylori* is able to activate the canonical NF-κB pathway and the secretion of pro-inflammatory cytokines, including IL-8, in host cells dependent on its Cag T4SS (Hirata *et al.*, 2006a;Gorrell *et al.*, 2013). It was long assumed that the transport of the virulence factor CagA via the *H. pylori* T4SS activates the canonical NF-κB pathway after translocation into the host cytoplasm (Ying and Ferrero, 2019). However, researchers showed conflicting results and it turned out that NF-κB activation in host cells is clearly dependent on the T4SS but rather independent of CagA (Schweitzer *et al.*, 2010;Sokolova *et al.*, 2013). A role of CagA in NF-κB activation does not have to be completely excluded in long-term infection experiments (Suzuki *et al.*, 2009;Schweitzer *et al.*, 2010;Gall *et al.*, 2017;Ying and Ferrero, 2019). *H. pylori*, in addition to CagA delivery, can translocate peptidoglycan into host cells via its T4SS and induces NF-κB activation via NOD1 receptor signaling (Viala *et al.*, 2004;Gall *et al.*, 2017), especially at later time points after exposure. However, conflicting results were published for this signaling pathway, too, as some research groups questioned the role of NOD1 for the induction of NF-κB-transcribed pro-inflammatory cytokine secretion (Hirata *et al.*, 2006b;Gall *et al.*, 2017;Ying and Ferrero, 2019). In summary, inhibition, knock-down and knock-out of NOD1 appeared to reduce rather than inhibit NF-κB driven IL-8 secretion (Ying and Ferrero, 2019), suggesting that additional agents and signaling pathways may be involved in the activation of this pro-inflammatory pathway. Furthermore, T4SS transported *H. pylori* DNA activates NF-κB signaling through TLR9 (Varga *et al.*, 2016b). Recently, a new pro-inflammatory pathway contributing to the *H. pylori*-induced activation of NF-κB was discovered in epithelial cells that is based on the intra cytoplasmic proteins ALPK1 and TNF receptor-activating factor (TRAF)-interacting protein with forkhead-associated (FHA) domain TIFA (Gall

*et al.*, 2017;Stein *et al.*, 2017;Zimmermann *et al.*, 2017). The pathway is activated by *H. pylori* LPS heptose metabolites and is dependent on a functional T4SS (Gall *et al.*, 2017;Stein *et al.*, 2017;Zimmermann *et al.*, 2017;Zhou *et al.*, 2018). In addition, *H. pylori* triggers non-canonical activation of the NF-κB pathway via LTβR in a T4SS dependent manner (Ohmae *et al.*, 2005;Mejias-Luque *et al.*, 2017;Feige *et al.*, 2018).

### 5.19 LPS heptose metabolites and their role during infection

The MAMP LPS can be found in the outer membrane of most Gram-negative bacteria, such as *H. pylori* (Garcia-Weber and Arrieumerlou, 2020). LPS is generally composed of three main regions: the lipidA part that is anchored into the outer membrane of Gram-negative bacteria and is the main MAMP of LPS, usually detected via TLR4 (Park and Lee, 2013). The O-antigen chain, which reaches out into the aqueous surroundings of the bacteria and the core part forms the linker between lipidA and outer chains. The core part is divided into an inner and outer core part and consists mostly of hexose residues, such as heptoses and keto-deoxy-octonate sugars (Stein *et al.*, 2017;Garcia-Weber and Arrieumerlou, 2020). These oligosaccharides are not known to be immunogenic MAMPs. LPS inner core heptose units are synthesized by different enzymes and during the synthesis, various intermediate heptose metabolites arise and are well conserved across many species, although the synthesis pathway varies slightly between bacteria (Garcia-Weber and Arrieumerlou, 2020). However, not all Gram-negative bacteria possess a heptose synthesis pathway, for instance *Legionella* replace the heptose residues by other sugars (Knirel *et al.*, 1996;Garcia-Weber and Arrieumerlou, 2020) and some Gram-positive bacteria can synthesize heptose metabolites, too (Tang *et al.*, 2018;Garcia-Weber and Arrieumerlou, 2020).

Recently, these LPS heptose metabolites were discovered to act as MAMPs and activate pro-inflammatory responses in host cells (Gaudet *et al.*, 2015;Milivojevic *et al.*, 2017;Garcia-Weber *et al.*, 2018;Zhou *et al.*, 2018). The intermediate heptose metabolites D-glycero-β-D-manno-heptose-1,7-BP (HBP) and ADP-glycero-β-D-manno-heptose-1-phosphate (ADP-heptose), produced by different bacteria, were identified to contribute significantly to the pro-inflammatory activation and modulation of human epithelial cells (Gaudet *et al.*, 2015;Stein *et al.*, 2017;Zimmermann *et al.*, 2017;Pfannkuch *et al.*, 2019).

### 5.20 *H. pylori* LPS biosynthesis, heptose metabolites and signaling

*H. pylori* heptose units are synthesized in a five-step pathway (see Figure 2) that generates different intermediate heptose metabolites. The precursor metabolite D-sedoheptulose-7-phosphate (S-7-P) is converted to D-glycero-α,β-D-manno-heptose-7-phosphate (HMP-7) by the isomerase GmhA. Phosphorylation of HMP-7 by the bifunctional HldE kinase forms HBP. The subsequent dephosphorylation of HBP at position 7 by the phosphatase GmhB leads to D-glycero-β-D-manno-heptose-1-phosphate (HMP-1) (Pfannkuch *et al.*, 2019;Garcia-Weber

and Arrieumerlou, 2020). Transfer of an adenylyl moiety by the ADP transferase HldE forms ADP-heptose which, in a final step, gets transformed to ADP-L-glycero-β-D-manno-heptose by the epimerase HldD and integrated into the LPS core by heptose transferase (Pfannkuch *et al*., 2019;Garcia-Weber and Arrieumerlou, 2020).

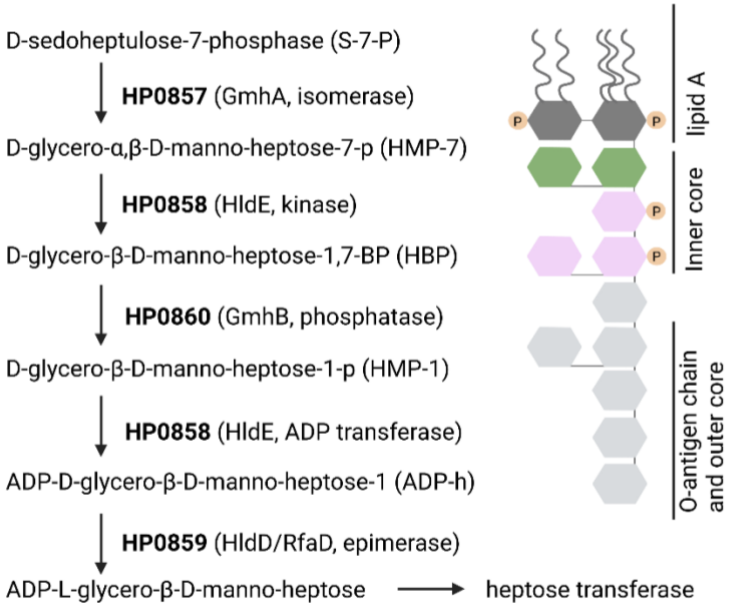


**FIG 2: A schematic overview of the five-step heptose synthesis pathway of *H. pylori* inner core heptose units**. *H. pylori* LPS generally consist of three main regions: the lipidA part that is anchored into the outer membrane (dark grey), the O-antigen chain (bright grey) and the inner core part, which forms the linker between lipidA and outer core and consists mostly of heptose units (pink) and keto-deoxy-octonate sugars (green). Synthesis of the inner core heptose units is a five-step pathway. Using the enzymes GmhA, HldE, GmhB, HldD, *H. pylori* synthesizes ADP-L-glycero-β-manno-heptose from D-sedoheptulose-7-phosphase. During this process, intermediate heptose metabolites arise, including HBP and ADP-heptose. The artwork of this figure is modified according to (Stein *et al.*, 2017) and was created using BioRender.

*H. pylori* appears to primarily use its T4SS to mediate direct cell transfer of heptoses (Gall *et al.*, 2017;Stein *et al.*, 2017;Zimmermann *et al.*, 2017), whereas free heptoses are only marginally released into the environment by live *H. pylori*. For *H. pylori*, the intermediate heptose metabolites HBP and ADP-heptose are known to induce pro-inflammatory signaling in GECs (Gall *et al.*, 2017;Stein *et al.*, 2017;Zimmermann *et al.*, 2017). After translocation into the host cell cytoplasm, the heptose intermediate metabolites bind and activate the PRR ALPK1 in the host cells. This subsequently phosphorylates TIFA in its threonine residue T9 and leads to self-oligomerization of TIFA in the cytosol (Milivojevic *et al.*, 2017;Zimmermann

*et al.*, 2017;Zhou *et al.*, 2018). Several members of the TRAF family, including TRAF6 and TRAF2 (Zimmermann *et al.*, 2017), are recruited to the polymerized TIFA proteins and form the multiprotein complex TIFAsome (Milivojevic *et al.*, 2017), which induces the NF-κB pathway (Gaudet *et al.*, 2015;Stein *et al.*, 2017;Garcia-Weber *et al.*, 2018;Zhou *et al.*, 2018;Pfannkuch *et al.*, 2019). The *H. pylori* LPS metabolite HBP cannot enter the cell without active transport whereas ADP-heptose is cell-permeable (Pfannkuch *et al.*, 2019). In addition, ADP-heptose binds to ALPK-1 with a higher affinity than HBP (Zhou *et al.*, 2018), and ADP-heptose was reported to be about 10-times higher concentrated within the pathogen than HBP (Pfannkuch *et al.*, 2019).

### 5.21 MicroRNAs and their role during infection and malignancies

MiRNAs are short, non-coding single-stranded RNAs (19–24 nt) that inhibit the transduction or induce the degradation of target genes upon the interaction with their 3′-UTR (Vella *et al.*, 2004). MiRNAs are found in various organisms, including mice and humans, and play important roles in, for example, cell apoptosis, proliferation, migration, and differentiation by post-transcriptionally regulating genes (Bartel, 2004;2009). More than thousand miRNAs were identified, and each miRNA may regulate the expression of up to thousands of target genes (Bartel, 2009). It is estimated that 30%-60% of human genes are regulated by miRNA (Lewis et al., 2003;Bartel, 2004). MiRNAs were shown to be involved in modulating the innate and adaptive immune responses (Chen *et al.*, 2004). In addition, miRNAs were shown to affect the expression of tumor suppressor genes and oncogenes, potentially contributing to the development and progression of malignancies (Saito *et al.*, 2012).

### 5.22 MiRNAs and *H. pylori*

It was reported that the expression of several miRNAs is altered by *H. pylori* in GECs and immune cells (Matsushima *et al.*, 2011;Noto and Peek, 2011). *H. pylori* infection was shown to manipulate the expression of specific miRNAs involved in pro-inflammatory responses and GC (Noto and Peek, 2011;Libanio *et al.*, 2015;Zou *et al.*, 2019). For example, *H. pylori* is able to affect the ability of macrophages to present antigens to the adaptive immune system by inhibiting the expression of the immune receptor CD300E through downregulation of miR-4270 (Pagliari *et al.*, 2017). In addition, the pathogen was reported to induce upregulation of the miRNAs Let-7f-5p, Let-7i-5p, miR-146b-5p, and miR-185-5p, which inhibit *H. pylori* antigen presentation due to inhibition of HLA-II gene expression (Codolo *et al.*, 2019).

# 6 AIMS OF THIS THESIS

The gastric pathogen *H. pylori* colonizes the stomach of more than half of the world's population. Although the colonization elicits innate and adaptive immune responses and causes gastric inflammation, *H. pylori* can persist in the host up to a lifetime. Elimination of the pathogen *in vivo* by the host's immune system is hampered, as it developed many different immune evasive strategies. Long-term infection with *H. pylori* increases the risk of gastric pathologies and development of GC, and antibiotic therapy is becoming increasingly difficult due to rising antibiotic resistance. Thus, it is of great importance to better understand the pathogen's strategies of immune activation, evasion and persistence in order to develop new therapeutic options.

*H. pylori* activates pro-inflammatory signaling in host cells via various MAMPs, which signal through different immune pathways. The important role of LPS heptose metabolites to activate human cells during infection with the pathogen has recently been well studied in GECs. During *H. pylori* infection, GECs recruit cells of the innate immune system to the site of infection. When I started this project, there was no knowledge available about the effects of LPS heptose metabolites on cells of the myeloid lineage during gastric infection with *H. pylori*. To expand our knowledge on the role of LPS heptose metabolites during infection, in this project, I aimed to gain a better understanding of the activation and modulation potential of *H. pylori* LPS heptose metabolites for macrophages and neutrophil cells.

My main research questions were how bacterial LPS heptose metabolites influence the pro-inflammatory activation, alone and in the bacterial context. In addition, we wanted to analyze how they affect maturation and polarization of human macrophages and neutrophils during infection with *H. pylori*. Furthermore, we wanted to get more information about the influence of ADP-heptose on the ability of macrophages to present antigens to the adaptive immune system.

During the first part of the project, I wanted to focus on the question whether and how heptose metabolites and the *cag*PAI contribute to the activation of monocytes/macrophages by *H. pylori*.

In the second part of the project, we chose to examine, within a collaboration with colleagues from Padua, Italy, whether ADP-heptose and *H. pylori* produced heptose metabolites suppress the antigen-presentation of macrophages.

For the third part, I aimed to understand whether the bacterial metabolite ADP-heptose and other heptose metabolites of *H. pylori* are able to activate human neutrophils and neutrophil-like cells.

# 7 MANUSCRIPTS

## 7.1 Manuscript I

# "Contribution of Heptose Metabolites and the *cag* Pathogenicity Island to the Activation of Monocytes/Macrophages by *Helicobacter pylori*"

**Faass L, Stein SC, Hauke M, Gapp M, Albanese M, Josenhans C**.

Front Immunol. 2021 May 19;12:632154. doi: 10.3389/fimmu.2021.632154. PMID: 34093525; PMCID: PMC8174060.

https://www.frontiersin.org/articles/10.3389/fimmu.2021.632154/full

First author:

Conceptual contributions/experimental design; Investigation: Performing and developing experiments, evaluation of results, formal analysis and statistics, establishing and improving methodology; Manuscript - writing of original draft methods part, figures and figure design, references, contributions to final review and editing.

First authorship is shared with Stein SC, who has laid the foundation of this research project with her work for the Stein *et al.*, 2017 paper (Stein *et al.*, 2017), which shows the important role of heptose metabolites for pro-inflammatory signaling in GECs upon infection with *H. pylori*. In addition, she started with the initiation of the macrophage-focused project with the Josenhans laboratory when still located at Hannover Medical School and performed initial experiments. When the Josenhans laboratory moved to Munich, I took over the project, established new experimental methods and performed and analyzed numerous experiments. My contributions were essential to compile the manuscript.

## 7.2 Manuscript II

# "ADP-heptose enables *Helicobacter pylori* to exploit macrophages as a survival niche by suppressing antigen-presenting HLA-II expression"

**Coletta S, Battaggia G, Della Bella C, Furlani M, Hauke M, Faass L, D'Elios MM, Josenhans C, de Bernard M**. FEBS Lett. 2021 Aug;595(16):2160-2168. doi: 10.1002/1873-3468.14156. Epub 2021 Jul 14. PMID: 34216493.

https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.14156

Coauthor:

Contributions to experimental design; Investigation: preparation, contribution and pretesting of central materials and resources; Manuscript - review and final editing. My contributions were essential to compile the manuscript.

# 8 DISCUSSION

The important role of bacteria-produced LPS heptose metabolites on epithelial cells has been well studied in the past (Gaudet *et al.*, 2015;Milivojevic *et al.*, 2017;Zhou *et al.*, 2018;Garcia-Weber and Arrieumerlou, 2020). This also includes its major activating function during the GEC infection by *H. pylori* (Gall *et al.*, 2017;Stein *et al.*, 2017;Zimmermann *et al.*, 2017;Pfannkuch *et al.*, 2019), mostly dependent on the presence of the *cag*PAI and a functional T4SS. Signaling of heptose metabolites in GECs involves ALPK1, TIFA, members of the TRAF family and the NF-κB pathway (Gaudet *et al.*, 2015;Gall *et al.*, 2017;Milivojevic *et al.*, 2017;Stein *et al.*, 2017;Zimmermann *et al.*, 2017;Zhou *et al.*, 2018;Pfannkuch *et al.*, 2019). Until today, little is known about the activation and modulation potential of heptose metabolites for myeloid lineage cells, which are recruited to the site of bacterial infection (Noach *et al.*, 1994).

In this project, we aimed to gain a better understanding of the activation and modulation potential of the bacterial metabolite ADP-heptose and other *H. pylori* heptose metabolites for macrophages and neutrophils. We addressed our research questions by exposing macrophages and neutrophils of different maturation stages to pure heptose metabolites, live bacteria, and their enzymatically treated bacterial lysates. Details of the methods and conditions were outlined in manuscripts I to III.

## 8.1 Which conclusive results were obtained and how has the field moved forward?

### 8.1.1 Heptose-mediated activation is a fundamental principle of innate human cell activation in various cell types

In this project, we were able to contribute to understanding the potential of bacterial heptose metabolites on the activation and modulation of human monocyte/macrophage-like cells and primary macrophages as well as human neutrophil-like cells and primary neutrophils. In addition, we gained insights into the role of *H. pylori* heptose metabolites during active infection of these phagocytes with the live pathogen.

#### 8.1.1.1 The role of *H. pylori* lipopolysaccharide heptose metabolites during activation of macrophages

We demonstrated that human monocyte/macrophage-like cells and primary macrophages responded strongly to pure heptose metabolites, as the NF-κB pathway was activated and the cells produced high amounts of pro-inflammatory cytokines (manuscript I and II). In addition, we confirmed the important role of heptose metabolites during infection with live *H. pylori* strains, as heptose mutant strains showed a significant reduction in their pro-inflammatory potential (manuscript I). We also demonstrated that the functionality of the T4SS affects the

activation potential of the pathogen, suggesting that transport of heptose metabolites by bacteria into host cells is predominantly mediated by the T4SS (manuscript I). Transcriptome analysis of macrophage-like cells stimulated with pure ADP-heptose, *H. pylori* wild-type, and the *H. pylori* heptose metabolite mutant strains confirmed the activation potential of pure ADP-heptose (probably involving active uptake by the cells) and the important role of heptose metabolites in live *H. pylori* for pro-inflammatory signaling in monocyte/macrophage-like cells (manuscript I). Using silencing RNA, we discovered the important role of TIFA for pro-inflammatory signaling in monocyte/macrophage-like cells (manuscript I). Activation of monocyte/macrophage-like cells by pure ADP-heptose induced polarization into a rather pro-inflammatory phenotype, called M1, which we analyzed via their gene expression profile (manuscript I). In our study, we were not able to detect NLRP3 inflammasome activation by *H. pylori* and pure ADP-heptose without a second signal (supplementary unpublished results SU1, in appendix). In primary human macrophages, we identified ADP-heptose and other heptose metabolites to be essential and sufficient to induce pro-inflammatory responses (manuscript I) and upregulation of the host cell miRNA miR146b during *H. pylori* infection, leading to a reduction in antigen presentation via HLA-II and compromising the antigen presentation to T-cells (manuscript II).

#### 8.1.1.2 The role of *H. pylori* LPS heptose metabolites during activation of neutrophils

For neutrophil-like cells and neutrophils, we also found that pure ADP-heptose is able to activate pro-inflammatory signaling after co-incubation without transfection (manuscript III). Upon infection with live *H. pylori*, we again demonstrated the important role of heptose metabolites (manuscript III). Moreover, the intensity of the pro-inflammatory response was dependent on a functional T4SS, suggesting that the T4SS also plays an important role for the bacterial transport of heptose metabolites into neutrophils (manuscript III). In untreated neutrophil-like cells, we detected regulation of maturation genes after the co-incubation with pure ADP-heptose, highlighting its intrinsic differentiating potential (manuscript III). Transcriptome analysis of neutrophil-like cells stimulated with ADP-heptose revealed broad upregulation of innate immune-relevant and other genes (manuscript III). Comparison of the transcriptomic changes resulting from co-incubation with pure ADP-heptose in neutrophil-like cells with the genetic profile of ADP-heptose-stimulated gastric epithelial-like and monocyte/macrophage-like cells revealed extensive similarities and also differences between the transcriptomic changes of the different cell types (manuscript III). Based on the strong pro-inflammatory response we can postulate polarization of neutrophil-like cells into a rather N1-like phenotype by pure ADP-heptose (manuscript III).

### 8.1.2 Findings of other research groups that advanced the field of *H. pylori and* heptose metabolites

In parallel with my thesis, other researchers contributed to the study of *H. pylori* heptose metabolites and their inflammatory potential and further advanced the field.

#### 8.1.2.1 Role of heptose metabolites during infection and cancer progression

Parallel to my thesis work, other research groups have discovered that bacterial heptose metabolites are not only instrumental for inducing pro-inflammatory reactions, but also have other impactful functions.

A major advance in answering the long-standing question of which factor of *H. pylori* contributes in a major way to causing DNA DSBs in human cells as a prerequisite to cancerogenesis was made by Bauer, Nascakova and Mihai *et al.* in 2020 (Bauer *et al.*, 2020). They showed in cell culture and organoid model experiments that DNA damage induced by *H. pylori cag*PAI-positive strains in GECs is dependent on ALPK1-TIFA-NF-κB signaling. Further experiments revealed that *H. pylori* LPS heptose metabolites, including ADP-heptose, are able to induce DNA damage and replication stress in GECs, co-transcriptionally in S-phase, depending on the presence of R-loops (Bauer *et al.*, 2020).

*H. pylori* forms colonies deep in the gastric glands where stem and progenitor cells reside, triggering stem cell expansion, gastritis, and precursor lesions for GC development (Sigal *et al.*, 2015;Fischer and Sigal, 2019). The pathogen increases the expression of R-spondin 3 (Rspo3) in the stromal cells beneath the glands, leading to gland expansion and consequent gland hyperplasia, as a possible precursor of malignant transformation (Sigal *et al.*, 2017). Using mouse and organoid models, Wizenty *et al.* discovered that *H. pylori* and ADP-heptose induce Rspo3 signaling via its receptor Lgr4 to activate cell proliferation and NF-κB signaling in GSCs. This new data reveals how the gastric gland cells elicit a pro-inflammatory response to the pathogen and its heptose metabolites and how they structurally adapt to the infection (Wizenty *et al.*, 2022).

#### 8.1.2.2 Signaling induced by heptose metabolites in epithelial cells

It was known that *H. pylori* is able to induce both the canonical and the non-canonical NF-κB pathway in GECs (Ohmae *et al.*, 2005;Hirata *et al.*, 2006a;Gorrell *et al.*, 2013), but the role of TIFA as part of both signaling cascades was not clear until, Maubach *et al.* shed more light on the field in 2021 (Maubach et al., 2021). On the basis of previous findings by our lab and others (Gall *et al.*, 2017;Stein *et al.*, 2017;Zimmermann *et al.*, 2017;Pfannkuch *et al.*, 2019) they first demonstrated that induction of the canonical and non-canonical NF-κB pathway by *H. pylori* depends on ADP-heptose and the metabolite's interaction with ALPK1 and TIFA. In addition, they identified the TRAF family members TRAF6 and TRAF2 as binding partners of TIFA for

the formation of the TIFAsome upon *H. pylori* infection. Subsequently, they showed that the interaction of TIFA with TRAF6 results in canonical NF-κB activation involving TAK1, whereas the interaction of TIFA and TRAF2 results in the degradation of cIAP1 and accumulation of NIK, leading to activation of the non-canonical NF-κB pathway (Maubach *et al.*, 2021).

Snelling *et al*. further expanded the knowledge on the NF-κB signaling cascade induced by heptose metabolites. They showed that pure ADP-heptose is able to stimulate the formation of ubiquitin chains on target proteins that are required for both, canonical and non-canonical NF-κB signaling. In addition, they further identified and characterized NF-κB pathway interaction partners. Furthermore, they showed for the first time that ALPK1 phosphorylates TIFA directly at Thr177 in addition to Thr9 (Huang *et al.*, 2012;Gaudet *et al.*, 2015;Zhou *et al.*, 2018) as a second phosphorylation site (Snelling *et al.*, 2022).

#### 8.1.2.3 Interaction of *H. pylori* with myeloid cells

A new paper by Behrens *et al.* further analyzed the interaction of *H. pylori* with cells of the myeloid lineage (Behrens *et al.*, 2020). It was already known that *H. pylori* uses its adhesin HopQ to interact with CEACAMs on host GECs to translocate CagA (Belogolova *et al.*, 2013;Javaheri *et al.*, 2016;Königer *et al.*, 2016). To expand the knowledge of this interaction for immune cells, they analyzed human and mouse primary neutrophils, macrophages and DCs, as well as mouse neutrophils, macrophages and DCs expressing human CEACAMs and their interaction with *H. pylori* HopQ. Human and CEACAM-humanized mouse neutrophils allowed an increased HopQ-dependent cell interaction that strongly enhanced CagA translocation, significantly increased pro-inflammatory actions upon *H. pylori* contact and allowed prolonged survival. In contrast, human or murine bone marrow-derived macrophages and DCs showed low CEACAM expression and low bacterial binding and only small amounts of translocated CagA, which was independent of the HopQ-CEACAM interaction (Behrens *et al.*, 2020). In conclusion, *in vitro* interaction data have shown that the interaction of *H. pylori* HopQ and CEACAMs expressed by myeloid cells has a major impact on the subsequent induction of pro-inflammatory responses, especially for neutrophils, and thus could have an impact on further ligands of *H. pylori* and their signaling.

In Figure 3, a summary of current knowledge about the activation of host cells mediated by various T4SS translocated factors of *H. pylori* is visualized (see Figure 3).

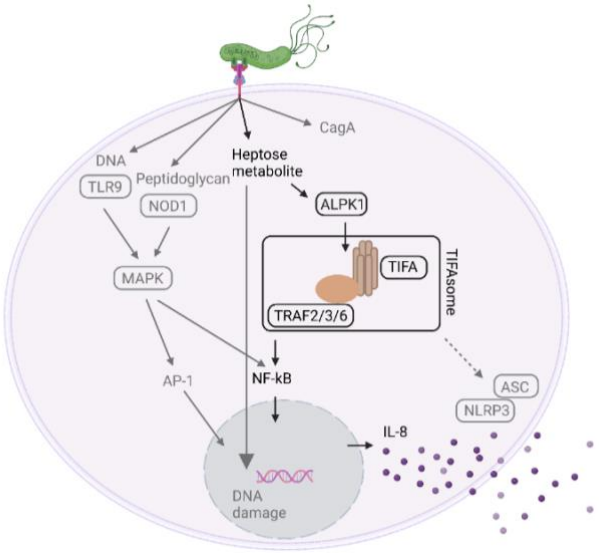


**FIG 3**: A schematic overview of the signaling cascades activated by *H. pylori* virulence factors transported via the T4SS in an exemplary human host cell is visualized. The translocated small molecules activate intracellular receptors such as ALPK1, TLR9 and NOD1. Activation of the PRRs leads to translocation of pro-inflammatory transcription factors, such as NF-κB, into the nucleus and transcription of pro-inflammatory genes, including the cytokine gene *IL-8*. In addition to immune responses, heptose metabolites induce DNA DSBs. The artwork of this figure was created using BioRender.

### 8.2 Is there a difference in the activation potential of *H. pylori* ADP-heptose between different cell types?

One important role of GECs is to protect the host from pathogens and other damaging agents by forming a physical barrier in the gastric niche that separates the gastric lumen from underlying tissue. Upon contact with pathogens, they initiate an initial inflammatory response and recruit cells of the innate immune system. Both neutrophils and macrophages are recruited to the site of infection, probe the pathogens, and subsequently aim to eradicate them via phagocytosis (Medzhitov, 2007). In a next step, they present the antigen to cells of the adaptive immune system to enhance the pro-inflammatory response (Medzhitov, 2007;Vono *et al.*, 2017). All three different cell types have their individual, important role during an infection with *H. pylori*. Their individual or common response to heptose metabolites, as we know so far, will be discussed in the next chapter.

Cell culture and primary cell GECs, macrophages, and neutrophils respond to ADP-heptose with activation as they show upregulation of mainly pro-inflammatory signaling cascades, gene transcription, and secretion of corresponding cytokines, e.g. IL-8 (manuscript I, II, III). In addition to pure ADP-heptose, all three different cell types show a significant decrease in their pro-inflammatory activation after co-incubation with *H. pylori* deficient in heptose metabolite production in comparison to the wild-type strain. Comparing the quantities of IL-8 secretion or

NF-κB activation between cell types to evaluate the cell-type specific activation potential is rather complex, since they rely on many factors, starting with bacterial adherence. Our results are based on cell culture and primary cell infection-models and are strongly influenced by the individual experimental designs, including incubation times, the cells' prior maturation status, and, for primary cells, donor specificity. However, comparing the extent of IL-8 transcript upregulation after addition of ADP-heptose with other transcriptional changes within each individual cell type highlights the major impact on pro-inflammatory signaling for all of the tested cell types. Moreover, the same amount of pure ADP-heptose induced comparably high pro-inflammatory IL-8 cytokine secretion in all three different cell types (manuscript I to III)(Stein *et al.*, 2017). Even though the GECs are non-phagocytic cells, and macrophages and neutrophils are phagocytes, we were able to show in manuscript I and III that the pro-inflammatory activation potential of *H. pylori* heptose metabolites in all cell types strongly depend on a functional T4SS, which transports heptose metabolites into cells.

Heptose metabolites signal through the ALPK1-TIFA-NF-κB pathway in epithelial cells (Gaudet *et al.*, 2015;Gall *et al.*, 2017;Milivojevic *et al.*, 2017;Stein *et al.*, 2017;Zhou *et al.*, 2018;Pfannkuch *et al.*, 2019;Garcia-Weber and Arrieumerlou, 2020). In manuscript I, II, and III, we showed that ADP-heptose induces NF-κB signaling in all three different cell types, as we were able to measure high IL-8 cytokine secretion. In manuscript II, we used a NF-κB pathway inhibitor to confirm that IL-8 transcript expression in primary macrophages was dependent on NF-κB upon stimulation with ADP-heptose. Inhibitor-treated cells showed a significant reduction in IL-8 transcripts after stimulation with ADP-heptose compared with the positive control (manuscript II). We also proved the presence of ALPK1 and TIFA at the transcriptional level for AGS, Thp-1 and HL-60 cells and on the protein level for HL-60 cells (manuscript III). Furthermore, we demonstrated in manuscript I that ADP-heptose-induced NF-κB activation was significantly reduced when TIFA was knocked-down in human monocyte/macrophage-like cells (manuscript I).

Maubach *et al.* have recently published that ADP-heptose is able to activate the canonical and non-canonical NF-κB pathways in GECs (Maubach *et al.*, 2021). Since the pro-inflammatory cytokine IL-8 was highly upregulated in our experiments using ADP-heptose stimulated GECs, macrophages and neutrophils, we expect a high activation of the canonical NF-κB pathway (manuscript III). Furthermore, as the transcriptome analysis results of AGS, Thp-1 and HL-60 cells showed an increase of RelB transcripts, activation of the non-canonical NF-κB pathway can be expected in all tested cell types, too, especially at longer incubation times (manuscript III).

GECs are known to undergo phenotypic changes after infection with *cag*PAI-positive *H. pylori* strains by epithelial to mesenchymal transition and resemble the hummingbird phenotype, dependent on CagA translocation (Azuma *et al.*, 2004). However, morphological changes of GECs are not known to be dependent on LPS heptose metabolites (Stein *et al.*, 2017) and do not occur after stimulation with ADP-heptose alone. In manuscript I and III, we have shown that both macrophages and neutrophils polarize into a rather pro-inflammatory (M1/N1) phenotype in response to *H. pylori* and LPS heptose metabolites.

In summary, it appears that *H. pylori* heptose metabolites activate human GECs, monocytes and neutrophils in a very similar manner and lead to similar initial responses, including pro-inflammatory cytokine expression and secretion. In particular, in manuscript III, where we compared the highest differentially expressed genes after stimulation with ADP-heptose between all three different cell types, it became clear that the pro-inflammatory response elicited by the heptose metabolite is very similar. Ratios of IL-8 transcripts for heptose-activated vs. mock, as one measure of pro-inflammatory activation, were higher for Thp-1 and AGS (< 40-fold), while the absolute RPKM counts for IL-8 were much higher in HL-60 neutrophils than in the other two cell types (manuscript III). For primary cells, human neutrophils seemed to be somewhat less responsive to ADP-heptose than macrophages, a phenotype that can be influenced by many cellular factors including cellular heptose uptake activity (manuscript I and III). The overlapping upregulated genes in all three cell types included important key genes of the NF-κB pathway such as *il-8*, *nf-κb2* and *relb*. Although the initial activation of the cells seems to be similar, it is important to consider that all three cell types have different roles during infection, while we mainly analyzed the effect of heptose metabolites on their function to secrete pro-inflammatory cytokines. We have only begun to analyze other functions of myeloid cells like phagocytosis and presentation of antigens to the adaptive immune system. These questions and their investigation should definitively be pursued in the future.

### 8.3 Suitability of study models for *H. pylori* innate immunity research - which models fit best to address our particular research questions?

To study the interaction between the pathogenic bacterium *H. pylori* and cells in the human stomach niche, several experimental models were developed to mimic the situation of an active gastric infection. The question of which of these models are most suitable to study the role of heptose metabolites during *H. pylori* infection will be addressed in this chapter.

*In vitro* cell culture models based on cancer cell lines or genetically transformed cells are a versatile tool to study the direct interaction of *H. pylori* with specific cell types and resulting cellular events. Cell lines are easily accessible, as they are readily available from various cell collections and relatively painless to maintain, as they are immortalized (Pompaiah and

Bartfeld, 2017). In addition, cell line cells are often well characterized. However, cell lines differ from *in vivo* cells as they are clones and can exhibit genetic alterations (Pompaiah and Bartfeld, 2017). Furthermore, cancer cell lines are sometimes infected with bacteria or viruses, which affects their cellular and phenotypic characteristics (Pompaiah and Bartfeld, 2017). A wide range of cell lines is available for the study of *H. pylori* infection in the gastric niche. In our studies, we used the well characterized and widely used cell lines AGS, Thp-1 and HL-60 cells to analyze the role of ADP-heptose during *H. pylori* infection (manuscript I, II, III)(Tsuchiya *et al.*, 1980;Collins, 1987). During our studies for manuscript III, some limitations of cell culture cells became apparent. In HL-60 cells, ligands known to stimulate primary neutrophils did not activate the cell culture neutrophils. Transcriptome analysis of the neutrophil-like cells showed a lack of PRR receptor gene expression in HL-60 cells necessary for pro-inflammatory signaling (manuscript III). In addition, literature search on HL-60 cells then revealed known chromosome aberrations in the culture cells and prior knowledge of loss of TLR2/4 functionality (Rincon *et al.*, 2018).

Primary human cells are a good *in vitro* alternative to cell culture models. They can be isolated from host tissues of interest or blood, present the physiological state of the cell of interest *in vivo*, and retain their *in vivo* functions (Pompaiah and Bartfeld, 2017). To study gastric infections, gastric epithelial and myeloid primary cell models can be generated using isolated cells from human gastric biopsies or donor blood for subsequent co-incubation with *H. pylori* (Richter-Dahlfors *et al.*, 1998;Quiding-Järbrink *et al.*, 2010). A disadvantage of primary cell models is that they cannot be maintained and expanded over a longer period of time (Pompaiah and Bartfeld, 2017). In addition, the behavior of the cells is frequently donor-specific, also depending on the donor's past and current infection history. Furthermore, blood myeloid cells might not necessarily resemble the immune cells that can be found in the human stomach during infection. During this project, we isolated monocytes and neutrophils from human donor blood, differentiated the monocytes into macrophages, and analyzed the pro-inflammatory activation upon stimulation with ADP-heptose (manuscript I, II, III). Both cell types responded with pro-inflammatory signaling to the heptose metabolite, but blood neutrophils nevertheless responded less avidly than primary blood macrophages (manuscript I, II, III). The results we obtained from primary cells partially recapitulated experiments with cell culture cells, validating both our study models. As expected, we observed donor specific variations in between independent donors of primary cells (manuscript I, II, III).

Another model that has been widely advocated during the last years for infection research are organoids derived from primary pluripotent cells (Pompaiah and Bartfeld, 2017). Organoids are tiny, three-dimensional and self-assembled tissues composed of different cell types that can model functionalities of their organ of origin (Rossi *et al.*, 2018;Hofer and Lutolf, 2021). Organoids grown from human GSCs or gastric tumor cells provide a model that resembles an

organized epithelial structure comprising different cell types of the stomach (Bartfeld *et al.*, 2015). A unique feature of gastric organoids is the ability to grow cell types in this structure that could not previously be cultured, for example, mucus gland cells, and that multiple cell types can be co-incubated in parallel with *H. pylori*. Co-incubation of gastric organoids with strains of *H. pylori* has already led to interesting new findings in the field of *H. pylori* and *H. pylori* heptose metabolites (Bartfeld *et al.*, 2015;Bauer *et al.*, 2020;Aguilar *et al.*, 2022). Pompaiah and Bartfeld provide a very good overview of the development of gastric organoids and their usefulness to study *H. pylori* infection in their review (Pompaiah and Bartfeld, 2017). In order to study the role of immune cells during *H. pylori* infection of the gastric niche, a gastric organoid immune cell co-culture model was developed (Holokai *et al.*, 2019).

Immunohistochemical analysis of human or mouse tissue samples is a way to gain insight into the *status quo* of gastric inflammation *in situ.* To study *H. pylori*, samples are often obtained from gastric biopsies of healthy or diseased donors and allow visualization and analysis of the *H. pylori*-infected stomach. This study model revealed the localization of *H. pylori* close to the GECs, deep in the human gastric niche and allowed the analysis of involved myeloid cells during infection (Quiding-Järbrink *et al.*, 2010;Fehlings *et al.*, 2012). Immunohistochemical analysis in the context of heptose metabolites was not done yet. The availability of tissue samples from humans is limited, very locally restricted and only a snapshot of already dead cells (Faass *et al.*, 2023).

Animal models are essential for *in vivo* infection research with *H. pylori* because they provide some organismal insight into some aspects of the systemic and local consequences of a *H. pylori* infection, which can - to some extent - recapitulate disease processes in humans. However, there is an ongoing debate about which animal models can be used for which specific question and how they resemble the human-adapted infection. It must be kept in mind that animals differ from humans in their genetic profiles, cellular functions and in the structure of their organs. Some animals were successfully infected with *H. pylori*, including mice, Mongolian gerbils and nonhuman primates (Amalia *et al.*, 2023). However, animal models have drawbacks, such as the fact that most human *H. pylori* strains cannot colonize the stomach of animal models, making it difficult to extrapolate these results to the human situation and compare colonization and inflammation in a strain-dependent manner.

*H. pylori* is a pathogen exclusively colonizing the human host, not mice. However, Lee *et al.* identified and adapted the *H. pylori* Sydney strain 1 (SS1), which can colonize the stomach of mice long-term, but elicits a relatively low inflammatory response (Lee *et al.*, 1997). Most wild-type mouse models do not develop strong inflammation or GC, but only mild inflammations after *H. pylori* infection and are therefore not suitable for studying *H. pylori*-induced pathologies (Ferrero *et al.*, 1998;van Doorn *et al.*, 1999). Therefore, mutant mice were developed that can

be induced to develop GC (Wang *et al.*, 1993;Lofgren *et al.*, 2011). Amalia *et al.* published a nice summary of mouse models for *H. pylori* inflammation and cancer development (Amalia *et al.*, 2023). Interestingly, unlike humans, *H. pylori* infections in mice show no differences in pathology between *cag*PAI-positive and -negative strains (Philpott *et al.*, 2002). Passage of *H. pylori* through mice frequently causes loss of T4SS function due to rearrangements in CagY (Philpott *et al.*, 2002;Barrozo *et al.*, 2013;Draper *et al.*, 2017). As *H. pylori* mutant strains deficient for heptose biosynthesis showed strongly impaired fitness (Stein *et al.*, 2017), the prospect of comparative experiments with wild type strains in any animal model might be rather difficult. However, Zhou *et al.* demonstrated that C57BL/6 mice responded to ADP-heptose with ALPK1-NF-κB signaling, pro-inflammatory cytokine secretion, and neutrophil recruitment in an air pouch model (Zhou *et al.*, 2018).

In contrast to mice, Mongolian gerbils exhibit a significant level of gastric inflammation and can develop cancer when colonized with certain strains of *H. pylori* alone, and results are similar to human stomach carcinogenesis (Hirayama *et al.*, 1996;Watanabe *et al.*, 1998). An overview about *Mongolian g*erbil models for *H. pylori* inflammation and cancer development was recently published by Amalia *et al.* (Amalia *et al.*, 2023). Presence or absence of the *cag*PAI plays an important role during *H. pylori* infection of Mongolian gerbils, as gerbils colonized with *cag*PAI mutant strains develop significantly less severe gastritis (Wirth *et al.*, 1998;Ogura *et al.*, 2000;Rieder *et al.*, 2005;Wiedemann *et al.*, 2009).

In conclusion, there are several suitable models available to study the role of heptose metabolites during *H. pylori* infection, each having its individual advantages and disadvantages. Which model should be chosen depends on the exact research question to be answered by the experiment. In order to address our research question, the cell culture and primary cell model already gave very good insights about the direct activation potential of heptose metabolites alone or in the context of live bacteria and further experimental designs will for sure answer more questions.

### 8.4 How does *H. pylori* benefit from the consequences of LPS heptose metabolite release in the gastric niche?

The big question that remained after the discovery of the APLK1 pathway, activated by LPS heptose metabolites, is how *H. pylori* benefits from the activation of this signaling cascade. How does translocation of heptose metabolites into host cells shape the immune response, possibly in favor for the pathogen, which is adapted to persist in the gastric niche? What is the role of heptose metabolites in *H. pylori* triggered GC development? In the next chapter, these questions will be addressed.

### 8.4.1 Overview of the microbiota and *H. pylori* in the human gastric niche

To answer the above questions, I will first give a general, clinical insight into a stomach infected with *H. pylori*. For a long time, the human stomach was considered as an inhospitable environment for bacteria because of its acidic pH. However, it could be demonstrated that the stomach is not a completely sterile organ and particularly under diseased conditions is estimated to harbor different bacterial species (Bik *et al.*, 2006), albeit in low numbers (Yang *et al.*, 2013). Genetic analysis of the gastric mucosa of healthy adult subjects revealed different dominant genera, mostly Gram-negative ones (Bik *et al.*, 2006;Li *et al.*, 2009;Engstrand and Lindberg, 2013). An early study by Bik *et al.* showed that presence of *H. pylori* did not affect the composition of the microbiotal community (Bik *et al.*, 2006). However, when present, *H. pylori* usually is the most abundant bacterium in the human gastric niche (Bik *et al.*, 2006). *H. pylori* colonization is not evenly distributed in the gastric mucosa and is concentrated in the antrum and cardia of the human stomach, and patterns of infection vary between individuals (Ailloud *et al.*, 2019;Fung *et al.*, 2019). In addition to close to the gastric epithelium, *H. pylori* was found deep in the gastric glands and Sigal *et al*. demonstrated that *H. pylori* directly interacts with human gastric progenitor and stem cells (Sigal *et al.*, 2015). Furthermore, Aguilar *et al.* showed that *H. pylori* preferentially attaches to differentiated cells in the pit region of gastric units directed by chemotaxis towards host cell-released urea (Aguilar *et al.*, 2022). During *H. pylori* infection of the human stomach, the amount of the pro-inflammatory cytokine IL-8 is significantly increased, whereas in non-infected human stomach tissue samples no IL-8 was detected (Lindholm *et al.*, 1998). This result indicates that IL-8 is upregulated only during *H. pylori* infection, and that the threshold above which IL-8 can be interpreted as marker for pro-inflammatory response may be rather low. Under the conditions of pro-inflammatory immune activation, modulation and IL-8 induction, *H. pylori* stays persistently in the human stomach.

### 8.4.2 Which role do *H. pylori* heptose metabolites play for establishing chronic inflammation and persistent infection - immune evasion or activation?

During the long co-existence between *H. pylori* and the human host, the bacterium has evolved many mechanisms to activate and evade the innate and adaptive immune response, which is considered one important basis for its persistent infection. In addition, *H. pylori* is able to manipulate functions of host cells, including cytokine secretion, the ability to phagocytose and kill pathogens, and antigen presentation to the adaptive immune system in order to favor its persistence (Faass *et al.*, 2023). Since the pathogen is able to prevent its own eradication by the host immune system, chronic inflammation results from *H. pylori* infection (Mejias-Luque and Gerhard, 2017). How inflammation promotes *H. pylori* persistence is not fully understood. Aihara *et al.* showed in a mouse model that the pathogen prefers to colonize gastric lesions over healthy epithelium (Aihara *et al.*, 2014). One reason for this preference could be the

increased pH that results from the gastric lesions (Nishio *et al.*, 2007;Aihara *et al.*, 2014). In addition, gastric lesions could provide metabolites or other nutritious molecules from damaged tissue that favor the pathogen (Papini *et al.*, 1998). In the following, an overview about the mechanism of immune evasion of *H. pylori* is provided, followed by a discussion about the role of heptose metabolites for persistence strategies.

As highlighted in the introduction of this thesis, *H. pylori* activates pro-inflammatory signaling in host cells via different MAMPs. In addition, *H. pylori* has evolved several strategies to evade or modulate immune responses in host cell. The pathogen has modified its LPS components lipidA, by phosphorylation and hypo-acylation (Muotiala *et al.*, 1992;Moran *et al.*, 1997;Cullen *et al.*, 2011), and O-chain, by mimicking host Lewis antigens (Bergman *et al.*, 2004;Li *et al.*, 2017), and its flagellin (Lee *et al.*, 2003;Gewirtz *et al.*, 2004), resulting in weak classical TLR4 and TLR5 immune response in host cells. In mouse dendritic cells, *H. pylori* activates pro-inflammatory TLR2 signaling, but also upregulates the expression of high levels of the anti-inflammatory cytokine IL-10 via MYD88 (Rad *et al.*, 2009). Sun *et al.* supported these findings by showing that TLR2 -/- mice were less colonized by *H. pylori* than wild-type mice and elicited stronger pro-inflammatory immune response with increased neutrophilic infiltration (Sun *et al.*, 2013). Similar observations were made by Otani *et al.* as they found a strong upregulation in TLR9 in the gastric mucosa of *H. pylori* infected mice and revealed a significant increase in gastric inflammation in TLR9 KO mice infected with *H. pylori* (Otani *et al.*, 2012). Varga *et al*. confirmed these results and emphasized the dependence on the CagT4SS for the anti-inflammatory effects (Varga *et al.*, 2016a). Furthermore, interaction of *H. pylori* Lewis antigens with the host CTR DC-SIGN increases the production of anti-inflammatory IL-10 and reduces Th1-cell development (Bergman *et al.*, 2004). In addition, *H. pylori* actively dissociates the signaling complex downstream of DC-SIGN to suppress pro-inflammatory signaling (Gringhuis *et al.*, 2009). Another CTR, MINCLE, was shown to interact with host Lewis antigens and to elicit an anti-inflammatory response upon *H. pylori* infection (Chmiela *et al.*, 1997;Matsumoto *et al.*, 1999). SiRNA-mediated knock-down of MINCLE in human macrophages resulted in upregulation of pro-inflammatory cytokines and consequent downregulation of anti-inflammatory cytokines (Devi *et al.*, 2015). Furthermore, just recently Dooyema *et al.* showed that *H. pylori* suppresses STING and RIG-I signaling in host cells by downregulating IRF3 and inducing the STING suppressor Trim30a (Dooyema *et al.*, 2022). The PRRs NLR and ALPK1 are activated by the pathogen, and to date there appears to be no evidence that *H. pylori* evades NLR and ALPK1 signaling, including NOD1, or NLRP3.

Bacterial phagocytosis is a central host defense mechanism for the elimination of invading bacteria and is performed by phagocytes. *H. pylori* is able to evade uptake by these cells, thereby manipulates the subsequent pro-inflammatory response. The evasion depends on the

*cag*PAI encoded T4SS (Ramarao and Meyer, 2001). In addition to strategies to evade phagocytosis, *H. pylori* has evolved strategies to survive phagocytosis. One survival strategy of *cag*PAI-positive *H. pylori* is based on delayed host cell entry, actin polymerization and phagosome formation in macrophages, followed by the fusion of phagosomes to megasomes, allowing *H. pylori* to resist intracellular killing (Allen *et al.*, 2000). Other apparent strategies to avoid bacterial killing within phagosomes include interruption or arrest of phagosome maturation (Zheng and Jones, 2003;Borlace *et al.*, 2011;Du *et al.*, 2016).

Another important role of activated myeloid cells during infection is to activate the adaptive immune system to elicit immune responses against the pathogen. However, *H. pylori* was shown to induce tolerogenic DCs, which subsequently lead to the differentiation of native T-cells into Treg-cells, instead of effector Th17- and Th1-cells (Oertli *et al.*, 2013). In addition, *H. pylori* is able to directly inhibit and manipulate T-cells via different virulence factors. VacA was shown to interact with and invade T-cells during infection, thereby impairing T-cell receptor signaling and preventing T-cell activation, immune response, and proliferation (Gebert *et al.*, 2003;Sundrud *et al.*, 2004;Sewald *et al.*, 2008). In addition, the pathogen uses GGT to arrest the host cell cycle, inhibit proliferation of infected T-cells, (Gerhard *et al.*, 2005;Schmees *et al.*, 2007) and impede effector T-cell response (Wustner *et al.*, 2015).

Summarizing current knowledge, *H. pylori* induces pro-inflammatory activation in GECs and cells of the innate and adaptive immune system, but attempts to specifically impair and manipulate them. Insightful reviews focusing on immune evasion strategies of *H. pylori* were published by Mejías-Luque and Gerhard in 2017 (Mejias-Luque and Gerhard, 2017), Salama *et al.* in 2013 (Salama *et al.*, 2013) and Zhang *et al.* 2020 (Zhang *et al.*, 2020). What we know today about the role of LPS heptose metabolites in *H. pylori*-mediated immune activation and evasion is also ambiguous. LPS heptose metabolites activate the PRR APLK1 in the host cell cytoplasm, signal along the NF-κB pathway and induce cytokine production in a T4SS dependent manner (manuscript I)(Stein *et al.*, 2002;Gall *et al.*, 2017;Zimmermann *et al.*, 2017;Pfannkuch *et al.*, 2019). The cytokines that we measured in this project after co-incubation of cells with heptose metabolites include pro-inflammatory cytokines such as IL-8, but no anti-inflammatory cytokines such as IL-10, at least for the short incubation times we analyzed in GECs, macrophages and neutrophils (manuscript I and III). This might indicate that ADP-heptose does not sufficiently induce tolerogenic feedback signaling in contrast to TLR activation. On the other hand, A20 as a feedback mechanism was induced (Maubach *et al.*, 2021;Lim *et al.*, 2022)(manuscript I and III). The balance between pro-inflammatory and homeostatic signaling in this context should be studied further. Even though *H. pylori* is known to manipulate phagocytosis (Allen *et al.*, 2000;Ramarao and Meyer, 2001;Zheng and Jones, 2003;Borlace *et al.*, 2011;Du *et al.*, 2016), in our experiments, ADP-heptose did not influence

the ability of cell culture macrophages to phagocytose coated beads (manuscript I). However, in manuscript II, we were able to demonstrate that heptose metabolites manipulate antigen presentation of primary macrophages as they downregulate HLA-II expression via miRNA upregulation. Therefore, *H. pylori* appears to use its heptose metabolites to inhibit recognition by the adaptive immune system. To be able to conclude about whether and how *H. pylori* uses ADP-heptose to activate, manipulate or evade immune response in the context of the bacterium to favor its persistence, more research is necessary.

### 8.4.3 How could bacteria benefit from ADP-heptose and the ALPK1-TIFA-NF-κB pathway in comparison to cell activation by other MAMPS and their signaling cascades?

Human host cells are able to sense bacteria via different PRRs, including TLRs, NLRs, CLRs, and ALPK1 to trigger immunological responses (Smith, 2014;Munoz-Wolf and Lavelle, 2016;Garcia-Weber and Arrieumerlou, 2020). Each PRR is activated by its respective MAMP, on or in the host cell, signals along its individual signaling pathway and leads to the activation of certain transcription factors (Li and Wu, 2021). LPS heptose metabolites were recently identified as new potent MAMP that activates the newly defined PRR, ALPK1, and signals along TIFA and the NF-κB pathway (Gaudet *et al.*, 2015;Gall *et al.*, 2017;Milivojevic *et al.*, 2017;Stein *et al.*, 2017;Zimmermann *et al.*, 2017;Zhou *et al.*, 2018;Pfannkuch *et al.*, 2019). To get a better idea of how bacteria might benefit from these heptose metabolites during infection, current knowledge about its ALPK1 PRR with other well-known PRRs, which are activated by ligands of bacteria, including *H. pylori*, will be compared. Due to conceptual similarities of PRRs, the focus will be on comparing TLRs, NLRs and ALPK1.

The PRR TLR2 (TLR) is located on the cell surface of the host cells, whereas the PRRs TLR9 (TLR), NOD1 (NLR), and ALPK1 are located in endosomes or the cytoplasm. TLR2 and TLR9 are known to be membrane bound receptors. In contrast, NOD1 and ALPK1 are cytosolic receptors. Extracellular TLR2 is activated by MAMPs located on the membrane of the extracellular bacterium, for instance *H. pylori* (Mandell *et al.*, 2004). To activate the intracellular receptors TLR9, via DNA (Varga *et al.*, 2016b), NOD1, via peptidoglycan (Viala *et al.*, 2004), and ALPK1, via heptose metabolites (Gall *et al.*, 2017;Stein *et al.*, 2017;Zimmermann *et al.*, 2017), *H. pylori* actively transports ligands into the host cell via its T4SS. A major difference between fixed surface ligands and transported ligands is that the latter can be constantly and variably produced during infection. In addition, their production can be modulated or, in case of bacteria that can actively transport metabolites, their transport might be inhibited by mechanisms like off-switching of their bacterial secretion systems, as known for *H. pylori* (Barrozo *et al.*, 2013;Fischer *et al.*, 2020). Therefore, the pathogens might be able to actively regulate the intensity of immune response activated by heptose metabolites in host cells in

their favor. Our group has obtained some evidence for this in another thesis (Hauke *et al.*, 2023).

The PRRs TLR2, TLR9, NOD1, and ALPK1 induce NF-κB signaling in pathogens such as *H. pylori* (Mandell *et al.*, 2004;Munoz-Wolf and Lavelle, 2016;Stein *et al.*, 2017;Li and Wu, 2021). In addition, TLRs and NLRs activate further transcription factors, including AP-1 and IRF3/7 (Allison *et al.*, 2009;Müller *et al.*, 2011). Until today, ALPK1-TIFA is known to only activate the pro-inflammatory NF-κB pathway. As ALPK1 and TLRs share TRAF6 as signaling hub involved in their individual NF-κB pathway activation, and NOD1 and ALPK1 both involve TRAF3, it is likely that the pro-inflammatory pathways overlap and interfere. Furthermore, ADP-heptose is able to induce canonical and non-canonical NF-κB signaling, whereas TLRs only activate the canonical NF-κB pathway (Maubach *et al.*, 2021).

It is known for TLR ligands that after initial activation of the NF-κB pathway, negative feedback leads to inhibition of pro-inflammatory responses and tolerance induction (Medvedev *et al.*, 2000;Kubo-Murai *et al.*, 2008;Faber *et al.*, 2016). Negative regulation was also observed for NLRs through NF-κB transcribed OLFM4, which inhibits NOD-mediated NF-κB activation upon *H. pylori* infection (Liu *et al.*, 2010). During this project, I could measure an increased expression of the NF-κB negative regulator A20 (Lim *et al.*, 2022) after stimulation of host cells with ADP-heptose (manuscript III). However, the impact of A20 for the long-term ADP-heptose stimulation of host cells is not yet clear. Since A20 inhibits NF-κB signaling downstream of TRAF6 (Skaug *et al.*, 2011), which takes part in both TLR and ALPK1 signaling, a negative regulation in between or of multiple pathways in parallel might be possible.

As a preliminary conclusion, the question of how the ALPK1 pathway differs from other PRRs induced inflammatory signaling pathways and how bacteria might benefit from its individual activation cannot be fully answered yet. On one hand, there is still much information lacking for the newly defined ALPK1 pathway and about the interaction with other PRRs pathways activated upon infection. On the other hand, there is some overlap between the ALPK1 and other pathways, wherefore it appears likely that the different pathways substantially overlap and interfere with each other rather than each pathway having its own individual signals and immunological outcome. Furthermore, one important consideration is that the activation strategies might differ in between bacteria species and depend on the infected body niche.

### 8.4.4 *H. pylori* ADP-heptose during chronic inflammation and cancer development

The development of GC begins with the transition of normal mucosa to chronic superficial gastritis caused by chronic *H. pylori* infection (Correa and Houghton, 2007). Anyone infected with *H. pylori* develops gastritis, which usually persists for many years (Suerbaum and Michetti, 2002;Amieva and El-Omar, 2008). However, only a small percentage of those infected develop

GC (Asghar and Parsonnet, 2001). Over time, gastritis may progress to atrophic gastritis, then to intestinal metaplasia, and finally to dysplasia and adenocarcinoma (Correa, 1992;Correa and Houghton, 2007). Antibiotic therapy during atrophic gastritis induced by *H. pylori* can significantly reduce the risk of GC. However, antibiotic eradication must occur before the patient shows pre-malignant lesions (Wong *et al.*, 2004), as treatment of patients with intestinal metaplasia and dysplasia did not reduce the risk of GC (Chen *et al.*, 2016). In the following, I discuss the role of LPS heptose metabolites during *H. pylori*-induced GC development.

Many different virulence factors of *H. pylori* enable its long-term persistence and promote the development of gastric malignancies through pro-inflammatory cell activation, perturbation of cell junctions and polarity, induction of cell proliferation and death, and DNA damage (Amieva *et al.*, 2003;Cover *et al.*, 2003;Saadat *et al.*, 2007;Bronte-Tinkew *et al.*, 2009;Lee *et al.*, 2010;Koeppel *et al.*, 2015;Gonciarz *et al.*, 2019). Heptose metabolites induce pro-inflammatory cytokine secretion in host cells, including the neutrophil recruiting chemokine IL-8 (manuscript I, II, III)(Stein *et al.*, 2017;Pfannkuch *et al.*, 2019). IL-8 is an important factor in cancer progression and patient clinical prognosis, as several research groups have found a significant correlation between high expression levels of IL-8 in the gastric mucosa and the risk and severity of GC (Crabtree *et al.*, 1994;Lee *et al.*, 2004;Macri *et al.*, 2006;Yamada *et al.*, 2013). This is partly due to the fact that continuous and increasing neutrophil infiltration is associated with gastric carcinogenesis (Fu *et al.*, 2016). Since we discovered that heptose metabolites activated macrophages and neutrophils produce high amounts IL-8 during active *H. pylori* infection, the high numbers of infiltrating immune cells and the increased cancer risk can be strongly attributed to heptose metabolites (manuscript I, II, III). In addition to IL-8, NF-κB is an important player in the development of GC (Taniguchi and Karin, 2018). Both the canonical and non-canonical NF-κB pathways were linked to the development of gastric carcinogenesis (Greten *et al.*, 2004;Pikarsky *et al.*, 2004;Merga *et al.*, 2016), and both NF-κB pathways are induced by heptose metabolites (Maubach et al., 2021)(manuscript I and III). Furthermore, our studies showed that cell culture monocyte/macrophages-like and neutrophil-like cells are polarized into a rather pro-inflammatory and pro-cancerogenic M1 and N1 subtype, upon stimulation with heptose metabolites, leading to increased pro-inflammatory and pro-cancerogenic cytokines (manuscript I, III)(Li *et al.*, 2022). MiRNAs are known for their ability to act as oncogenes (Zabaleta, 2012). As we discovered the potential of heptose metabolites to regulate miRNAs (manuscript II), miRNAs regulation could be another way of heptose metabolites to increase the risk of GC. Futhermore, stem cells play an important role during *H. pylori* induced GC, and the interaction between the pathogen and GSCs was proposed to facilitate carcinogenesis during chronic infection (Sigal *et al.*, 2015;Amieva and Peek, 2016;Wroblewski and Peek, 2016). Especially as it is known that gland-colonizing bacteria, not the bacteria colonizing the surface, induce the precursor lesions for GC (Howitt *et al.*,

2011;Sigal *et al.*, 2015;Fischer and Sigal, 2019;Wizenty *et al.*, 2022). Most importantly, it was discovered that heptose metabolites directly trigger DSB in GECs via ALPK1-TIFA-NF-κB signaling (Bauer *et al.*, 2020), which highly increases the risk for cancer development due to genomic instability. Summarizing the above-discussed findings, *H. pylori* LPS heptose metabolites appear to have great cancerogenic potential.

### 8.4.5 Why does *H. pylori* appear to use the active transport of heptose metabolites through its T4SS and what are the effects of active transport versus release?

To answer the above questions, I will give a general overview about release and transport options known for bacteria that possess heptose metabolites and shortly discuss the possible strategy of the assumed active transport of heptose metabolites performed by *H. pylori*. Today, several pathogenic bacteria are known to elicit a pro-inflammatory response via the ALPK1-TIFA pathway in host cells upon heptose metabolite release (Garcia-Weber and Arrieumerlou, 2020). The mechanisms by which the bacterial heptose metabolites enter the host cell vary among species and occur by active or passive transport either extracellularly or intracellularly. *Yersinia pseudotuberculosis*, for example, transports heptose metabolites via a type 3 secretion system (Zhou *et al.*, 2018). In contrast, *Neisseria meningitidis* does not have a specific secretion system, and heptose metabolites are suggested to be locally secreted by bacteria adhering at the cell surface or released during cell growth or upon bacterial lysis (Gaudet *et al.*, 2015). In addition, the release of heptose metabolites during cell growth and lysis within the host cell is possible (Gaudet *et al.*, 2017;Garcia-Weber *et al.*, 2018;Bauer *et al.*, 2020;Garcia-Weber and Arrieumerlou, 2020). For the mostly extracellular *H. pylori*, host cell activation via ALPK1-TIFA strongly depends on a functional T4SS, wherefore active transport of heptose metabolites is expected (manuscript I and III)(Gall *et al.*, 2017;Stein *et al.*, 2017;Pfannkuch *et al.*, 2019). One hypothesis for the secretion system-dependent release of heptose metabolites is that it allows *H. pylori* to activate specific cells and, through on and off-switching the T4SS (Barrozo *et al.*, 2013;Fischer *et al.*, 2020), only when wanted. However, for GECs it was shown that not only infected cells are able to form TIFAsomes but also non-infected bystander cells through cell-cell communication (Milivojevic *et al.*, 2017).This information limits the hypothesis of specific cell activation but still, only a small, defined region would be activated by active transport of heptose metabolites. In contrast, secretion of heptose metabolites independent of transport systems would activate more cells at once, leading to a more efficient and stronger immune response. Given the rather immune evasive colonization strategy of *H. pylori*, a precise and controlled activation of host cells via heptose metabolites appears logical.

# 9 OUTLOOK AND OPEN QUESTIONS

In this thesis, we discovered the important role of LPS heptose metabolites for the activation of human myeloid cells by *H. pylori*. Nevertheless, there are still many unanswered questions about the effects of heptose metabolites on various host cell functions and the overall strategies that *H. pylori* pursues using these small molecules. In the outlook chapter, I want to present my ideas for further experimental designs that might help answer our persisting research questions and further open new questions, which will certainly advance the field.

## 9.1 Further study models to analyze the role of heptose metabolites

Within the discussion section of this thesis, I provided an overview about commonly used study models for *H. pylori* infections. In the following, I want to propose study models that might be helpful to further answer our specific research questions.

We demonstrated that the early NF-κB activation and induction of IL-8 secretion by live *H. pylori* in human GECs, macrophages and neutrophils is strongly dependent on heptose metabolites (manuscript I and III). Moreover, we measured *H. pylori* strain-specific levels of IL-8 secretion, despite similar MOIs. Therefore, we ask ourselves the question, whether strains of *H. pylori*, context- or strain-dependently, can locally produce and transport different amounts of heptoses. Furthermore, we find it interesting to know how the severity of inflammation induced by *H. pylori* correlates with the amount of heptose transported *in vivo* in the human stomach. To address these questions, an immunohistochemistry study of human biopsies using anti-heptose metabolite antibodies would be of great interest. Using the antibodies, tissue samples from infected patients or from infected or heptose stimulated animal models could be analyzed for heptose quantity and, in addition, correlated with disease severity or strain specificity. A similar method was already established for the quantification of CagA (Kuo *et al.*, 2013). To address our research questions of how *H. pylori* heptose metabolites affect activation, maturation and functions of myeloid cells in the gastric niche and to gain further insight into the systemic interplay of GECs and myeloid cells, a gastric organoid myeloid cell co-culture model (Holokai *et al.*, 2019) would certainly be very helpful. The study design would include generation of human gastric organoids co-incubated with donor blood macrophages, neutrophils and DCs and stimulated with *H. pylori* strains or pure heptose metabolites. In addition, and to build on findings from Zhou *et al.* (Zhou *et al.*, 2018), a wild-type and myeloid cell depletion mouse model to study the effect of ADP-heptose targeted to the gastric niche would be of great help to study the activation potential of the heptose metabolites in the gastric niche. For sure, one must consider the different microbiota in mice, compared to the human stomach. In conclusion, the best strategy to further analyze our research questions will be a combination of different study models.

### 9.2 Further investigation of the effect of heptose metabolites on innate immune cells

In my project, we investigated the effects of LPS heptose and human macrophages and neutrophils (manuscript I, II, III). In addition to further exploring the impact of heptose metabolites on macrophages, I propose that studying additional effects of heptose metabolites on neutrophils, in addition to cytokine production and maturation, would be of great interest. Furthermore, I am sure that investigating the effect of heptose metabolites on DCs and other innate and adaptive immune cells would add to the picture of LPS heptose metabolites and their role during *H. pylori* infection.

### 9.3 Further insights on the uptake and transport of different heptose metabolites

Until today, the *H. pylori* heptose metabolites HBP and ADP-heptose were identified to induce pro-inflammatory signaling via ALPK1-TIFA-NF-κB in host cells (Stein *et al.*, 2017;Pfannkuch *et al.*, 2019). There are still many unanswered questions about the activation potential, the transport and the signaling cascade of LPS heptose metabolites. Below, I propose research questions and ideas that might be helpful to better understand how the pathogen benefits from the individual heptose metabolites.

First, it would be of great interest to understand how and to identify which *H. pylori* heptose metabolites are transported into host cells. The ability to track the translocation of heptose metabolites into host cells, identify and quantify the individual metabolites in host cells and to determine transport timings would rapidly advance the field. ADP-heptose can activate ALPK1 directly after translocation (Zhou *et al.*, 2018), whereas HBP must be converted into ADP-heptose-7-phosphate in the host cell cytoplasm to activate the PRR (Zhou *et al.*, 2018). Does *H. pylori* somehow benefit from other heptose metabolites versus ADP-heptose? Furthermore, it is of great importance to investigate the pro-inflammatory potential of further heptose metabolites besides HBP and ADP-heptose. Although Zhou *et al.* showed that only ADP-heptose and converted HBP are able to activate ALPK1 (Zhou *et al.*, 2018), other metabolites, could also reach the hosts cytoplasm and subsequently activate the ALPK1-TIFA pathway (Adekoya *et al.*, 2018). ADP-heptose induces inflammatory responses along the ALPK1-TIFA axis independent of active bacterial transport (Pfannkuch *et al.*, 2019)(manuscript I, II, III). What receptor or transport channel allows the metabolite to cross the host cell membrane? Why is HBP unable to enter the host cell independently of active transport (Zhou *et al.*, 2018)?

### 9.4 The homeostatic/non-infected human stomach and the role of ADP-heptose from commensal Gram-negative bacteria activating the ALPK1-TIFA pathway

In the last outlook chapter, I will introduce another, in my opinion very important, broader additional role for the PRR ALPK1 and heptose metabolites from Gram-negative commensals and point to questions whose answers are very important for understanding the role of heptose metabolites in the infected and uninfected human stomach and intestinal tract.

The PRR ALPK1 was shown to be a key driver for maintaining intestinal homeostasis, which is the interplay of GECs, immune cells and the local microbiota (Ryzhakov *et al.*, 2018;Garcia-Weber and Arrieumerlou, 2020). ALPK1 was discovered to be a negative regulator of intestinal inflammation in mice, as in an *alpk1*-/- mouse model, ALPK1 was found to promote intestinal homeostasis by regulating the balance of T-cells upon infection (Ryzhakov *et al.*, 2018). In addition, the commensal Gram-negative bacterium *Akkermansia muciniphila* was reported to use heptose to induce ALPK1-TIFA-TRAF6-NF-κB signaling in intestinal epithelial cells (Martin-Gallausiaux *et al.*, 2022). Activation of the pathway by the commensal bacterium induces expression of genes involved in maintenance of intestinal barrier function and positively promotes intestinal homeostasis (Martin-Gallausiaux *et al.*, 2022). Although the commensal also induced pro-inflammatory IL-8 secretion via ALPK1-TIFA *in vitro*, the authors do not expect the amount to be enough to induce detrimental intestinal inflammation *in vivo*, especially as the commensal is known for its rather beneficial effects in the intestinal niche (Ottman *et al.*, 2017;Bian *et al.*, 2019;Martin-Gallausiaux *et al.*, 2022).

Some bacterial species were identified that might be resident in the human stomach, in low numbers and mostly under diseased conditions, most of which are Gram-negative (Bik *et al.*, 2006;Li *et al.*, 2009;Engstrand and Lindberg, 2013). Since the LPS heptose synthesis pathway is well conserved among Gram-negative bacteria, ADP-heptose from human commensal bacteria can be occasionally expected in the human stomach (Garcia-Weber and Arrieumerlou, 2020). The above findings showing a dual role for ALPK1 were obtained for the intestinal niche. It is therefore of great importance to gain data on the impact of ALPK1 for gut homeostasis. In particular, it will be interesting to know whether the effects of heptose metabolites differ between commensal and pathogenic bacteria in the gut or in between different gastrointestinal niches to better understand the role of heptose metabolites during *H. pylori* infection. Since the expression level of the PRR ALPK1 is comparable in the human gut and intestine, a similar role of ALPK1 might be expected in both niches (Kayisoglu *et al.*, 2021a;Kayisoglu *et al.*, 2021b). In addition, mutations in *alpk1* lead to severe inflammatory diseases independent of the gastrointestinal niche (Sangiorgi *et al.*, 2019;Hecker *et al.*, 2022;Kozycki *et al.*, 2022), supporting the important immune regulatory role of ALPK1 in different body regions (Garcia-Weber and Arrieumerlou, 2020;Hecker *et al.*, 2022). Further understanding of the systemic role of the ALPK1 pathway could, in my opinion, also help elucidating how *H. pylori* benefits from activating this specific pathway during infection of the human stomach.

## 10 REFERENCES


Achtman, M., Azuma, T., Berg, D.E., Ito, Y., Morelli, G., Pan, Z.J., Suerbaum, S., Thompson, S.A., Van Der Ende, A., and Van Doorn, L.J. (1999). Recombination and clonal groupings within *Helicobacter pylori* from different geographical regions. *Mol Microbiol* 32**,** 459-470.

Adekoya, I.A., Guo, C.X., Gray-Owen, S.D., Cox, A.D., and Sauvageau, J. (2018). d-Glycero-beta-d-Manno-Heptose 1-Phosphate and d-Glycero-beta-d-Manno-Heptose 1,7-Biphosphate Are Both Innate Immune Agonists. *J Immunol* 201**,** 2385-2391.

Aguilar, C., Pauzuolis, M., Pompaiah, M., Vafadarnejad, E., Arampatzi, P., Fischer, M., Narres, D., Neyazi, M., Kayisoglu, O., Sell, T., Bluthgen, N., Morkel, M., Wiegering, A., Germer, C.T., Kircher, S., Rosenwald, A., Saliba, A.E., and Bartfeld, S. (2022). *Helicobacter pylori* shows tropism to gastric differentiated pit cells dependent on urea chemotaxis. *Nat Commun* 13**,** 5878.

Aihara, E., Closson, C., Matthis, A.L., Schumacher, M.A., Engevik, A.C., Zavros, Y., Ottemann, K.M., and Montrose, M.H. (2014). Motility and chemotaxis mediate the preferential colonization of gastric injury sites by *Helicobacter pylori*. *PLoS Pathog* 10**,** e1004275.

Ailloud, F., Didelot, X., Woltemate, S., Pfaffinger, G., Overmann, J., Bader, R.C., Schulz, C., Malfertheiner, P., and Suerbaum, S. (2019). Within-host evolution of *Helicobacter pylori* shaped by niche-specific adaptation, intragastric migrations and selective sweeps. *Nat Commun* 10**,** 2273.

Allen, L.A., Schlesinger, L.S., and Kang, B. (2000). Virulent strains of *Helicobacter pylori* demonstrate delayed phagocytosis and stimulate homotypic phagosome fusion in macrophages. *J Exp Med* 191**,** 115-128.

Allison, C.C., Kufer, T.A., Kremmer, E., Kaparakis, M., and Ferrero, R.L. (2009). *Helicobacter pylori* induces MAPK phosphorylation and AP-1 activation via a NOD1-dependent mechanism. *J Immunol* 183**,** 8099-8109.

Alvarez-Arellano, L., Camorlinga-Ponce, M., Maldonado-Bernal, C., and Torres, J. (2007). Activation of human neutrophils with *Helicobacter pylori* and the role of Toll-like receptors 2 and 4 in the response. *FEMS Immunol Med Microbiol* 51**,** 473-479.

Alvarez-Arellano, L., Cortes-Reynosa, P., Sanchez-Zauco, N., Salazar, E., Torres, J., and Maldonado-Bernal, C. (2014). TLR9 and NF-kappaB are partially involved in activation of human neutrophils by *Helicobacter pylori* and its purified DNA. *PLoS One* 9**,** e101342.

Alzahrani, S., Lina, T.T., Gonzalez, J., Pinchuk, I.V., Beswick, E.J., and Reyes, V.E. (2014). Effect of *Helicobacter pylori* on gastric epithelial cells. *World J Gastroenterol* 20**,** 12767-12780.

Amalia, R., Panenggak, N.S.R., Doohan, D., Rezkitha, Y.a.A., Waskito, L.A., Syam, A.F., Lubis, M., Yamaoka, Y., and Miftahussurur, M. (2023). A comprehensive evaluation of an animal model for *Helicobacter pylori*-associated stomach cancer: Fact and controversy. *Helicobacter* 28**,** e12943.

Amieva, M., and Peek, R.M., Jr. (2016). Pathobiology of *Helicobacter pylori*-Induced Gastric Cancer. *Gastroenterology* 150**,** 64-78.

Amieva, M.R., and El-Omar, E.M. (2008). Host-bacterial interactions in *Helicobacter pylori* infection. *Gastroenterology* 134**,** 306-323.

Amieva, M.R., Vogelmann, R., Covacci, A., Tompkins, L.S., Nelson, W.J., and Falkow, S. (2003). Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA. *Science* 300**,** 1430-1434.

Arnold, I.C., Lee, J.Y., Amieva, M.R., Roers, A., Flavell, R.A., Sparwasser, T., and Müller, A. (2011). Tolerance rather than immunity protects from *Helicobacter pylori*-induced gastric preneoplasia. *Gastroenterology* 140**,** 199-209.

Asghar, R.J., and Parsonnet, J. (2001). *Helicobacter pylori* and risk for gastric adenocarcinoma. *Semin Gastrointest Dis* 12**,** 203-208.

Aspinall, G.O., Monteiro, M.A., Pang, H., Walsh, E.J., and Moran, A.P. (1996). Lipopolysaccharide of the *Helicobacter pylori* type strain NCTC 11637 (ATCC 43504): structure of the O antigen chain and core oligosaccharide regions. *Biochemistry* 35**,** 2489-2497.

Aviles-Jimenez, F., Reyes-Leon, A., Nieto-Patlan, E., Hansen, L.M., Burgueno, J., Ramos, I.P., Camorlinga-Ponce, M., Bermudez, H., Blancas, J.M., Cabrera, L., Ribas-Aparicio, R.M., Solnick, J.V., and Torres-Lopez, J. (2012). In vivo expression of *Helicobacter pylori* virulence genes in patients with gastritis, ulcer, and gastric cancer. *Infect Immun* 80**,** 594-601.

Azuma, T., Yamazaki, S., Yamakawa, A., Ohtani, M., Muramatsu, A., Suto, H., Ito, Y., Dojo, M., Yamazaki, Y., Kuriyama, M., Keida, Y., Higashi, H., and Hatakeyama, M. (2004). Association between diversity in the Src homology 2 domain--containing tyrosine phosphatase binding site of *Helicobacter pylori* CagA protein and gastric atrophy and cancer. *J Infect Dis* 189**,** 820-827.

Backert, S., Ziska, E., Brinkmann, V., Zimny-Arndt, U., Fauconnier, A., Jungblut, P.R., Naumann, M., and Meyer, T.F. (2000). Translocation of the *Helicobacter pylori* CagA protein in gastric epithelial cells by a type IV secretion apparatus. *Cell Microbiol* 2**,** 155-164.

Bamford, K.B., Fan, X., Crowe, S.E., Leary, J.F., Gourley, W.K., Luthra, G.K., Brooks, E.G., Graham, D.Y., Reyes, V.E., and Ernst, P.B. (1998). Lymphocytes in the human gastric mucosa during *Helicobacter pylori* have a T helper cell 1 phenotype. *Gastroenterology* 114**,** 482-492.

Barrozo, R.M., Cooke, C.L., Hansen, L.M., Lam, A.M., Gaddy, J.A., Johnson, E.M., Cariaga, T.A., Suarez, G., Peek, R.M., Jr., Cover, T.L., and Solnick, J.V. (2013). Functional plasticity in the type IV secretion system of *Helicobacter pylori*. *PLoS Pathog* 9**,** e1003189.

Bartel, D.P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116**,** 281-297.

Bartel, D.P. (2009). MicroRNAs: target recognition and regulatory functions. *Cell* 136**,** 215-233.

Bartfeld, S., Bayram, T., Van De Wetering, M., Huch, M., Begthel, H., Kujala, P., Vries, R., Peters, P.J., and Clevers, H. (2015). In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology* 148**,** 126-136 e126.

Bauer, M., Nascakova, Z., Mihai, A.I., Cheng, P.F., Levesque, M.P., Lampart, S., Hurwitz, R., Pfannkuch, L., Dobrovolna, J., Jacobs, M., Bartfeld, S., Dohlman, A., Shen, X., Gall, A.A., Salama, N.R., Topfer, A., Weber, A., Meyer, T.F., Janscak, P., and Müller, A. (2020). The ALPK1/TIFA/NF-kappaB axis links a bacterial carcinogen to R-loop-induced replication stress. *Nat Commun* 11**,** 5117.

Bauernfeind, F.G., Horvath, G., Stutz, A., Alnemri, E.S., Macdonald, K., Speert, D., Fernandes-Alnemri, T., Wu, J., Monks, B.G., Fitzgerald, K.A., Hornung, V., and Latz, E. (2009). Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J Immunol* 183**,** 787-791.

Behrens, I.K., Busch, B., Ishikawa-Ankerhold, H., Palamides, P., Shively, J.E., Stanners, C., Chan, C., Leung, N., Gray-Owen, S., and Haas, R. (2020). The HopQ-CEACAM Interaction Controls CagA Translocation, Phosphorylation, and Phagocytosis of *Helicobacter pylori* in Neutrophils. *mBio* 11.

Belogolova, E., Bauer, B., Pompaiah, M., Asakura, H., Brinkman, V., Ertl, C., Bartfeld, S., Nechitaylo, T.Y., Haas, R., Machuy, N., Salama, N., Churin, Y., and Meyer, T.F. (2013). *Helicobacter pylori* outer membrane protein HopQ identified as a novel T4SS-associated virulence factor. *Cell Microbiol* 15**,** 1896-1912.

Bergman, M.P., Engering, A., Smits, H.H., Van Vliet, S.J., Van Bodegraven, A.A., Wirth, H.P., Kapsenberg, M.L., Vandenbroucke-Grauls, C.M., Van Kooyk, Y., and Appelmelk, B.J. (2004). *Helicobacter pylori* modulates the T helper cell 1/T helper cell 2 balance through phase-variable interaction between lipopolysaccharide and DC-SIGN. *J Exp Med* 200**,** 979-990.

Bian, X., Wu, W., Yang, L., Lv, L., Wang, Q., Li, Y., Ye, J., Fang, D., Wu, J., Jiang, X., Shi, D., and Li, L. (2019). Administration of *Akkermansia muciniphila* Ameliorates Dextran Sulfate Sodium-Induced Ulcerative Colitis in Mice. *Front Microbiol* 10**,** 2259.

Bik, E.M., Eckburg, P.B., Gill, S.R., Nelson, K.E., Purdom, E.A., Francois, F., Perez-Perez, G., Blaser, M.J., and Relman, D.A. (2006). Molecular analysis of the bacterial microbiota in the human stomach. *Proc Natl Acad Sci U S A* 103**,** 732-737.

Boeltz, S., Amini, P., Anders, H.J., Andrade, F., Bilyy, R., Chatfield, S., Cichon, I., Clancy, D.M., Desai, J., Dumych, T., Dwivedi, N., Gordon, R.A., Hahn, J., Hidalgo, A., Hoffmann, M.H., Kaplan, M.J., Knight, J.S., Kolaczkowska, E., Kubes, P., Leppkes, M., Manfredi, A.A., Martin, S.J., Maueroder, C., Maugeri, N., Mitroulis, I., Munoz, L.E., Nakazawa, D., Neeli, I., Nizet, V., Pieterse, E., Radic, M.Z., Reinwald, C., Ritis, K., Rovere-Querini, P., Santocki, M., Schauer, C., Schett, G., Shlomchik, M.J., Simon, H.U., Skendros, P., Stojkov, D., Vandenabeele, P., Berghe, T.V., Van Der Vlag, J., Vitkov, L., Von Kockritz-Blickwede, M., Yousefi, S., Zarbock, A., and Herrmann, M. (2019). To NET or not to NET:current opinions and state of the science regarding the formation of neutrophil extracellular traps. *Cell Death Differ* 26**,** 395-408.

Boonjakuakul, J.K., Canfield, D.R., and Solnick, J.V. (2005). Comparison of *Helicobacter pylori* virulence gene expression in vitro and in the Rhesus macaque. *Infect Immun* 73**,** 4895-4904.

Borlace, G.N., Jones, H.F., Keep, S.J., Butler, R.N., and Brooks, D.A. (2011). *Helicobacter pylori* phagosome maturation in primary human macrophages. *Gut Pathog* 3**,** 3.

Bronte-Tinkew, D.M., Terebiznik, M., Franco, A., Ang, M., Ahn, D., Mimuro, H., Sasakawa, C., Ropeleski, M.J., Peek, R.M., Jr., and Jones, N.L. (2009). *Helicobacter pylori* cytotoxin-associated gene A activates the signal transducer and activator of transcription 3 pathway in vitro and in vivo. *Cancer Res* 69**,** 632-639.

Burn, G.L., Foti, A., Marsman, G., Patel, D.F., and Zychlinsky, A. (2021). The Neutrophil. *Immunity* 54**,** 1377-1391.

Chen, C.Z., Li, L., Lodish, H.F., and Bartel, D.P. (2004). MicroRNAs modulate hematopoietic lineage differentiation. *Science* 303**,** 83-86.

Chen, H.N., Wang, Z., Li, X., and Zhou, Z.G. (2016). *Helicobacter pylori* eradication cannot reduce the risk of gastric cancer in patients with intestinal metaplasia and dysplasia: evidence from a meta-analysis. *Gastric Cancer* 19**,** 166-175.

Chmiela, M., Czkwianianc, E., Wadstrom, T., and Rudnicka, W. (1997). Role of *Helicobacter pylori* surface structures in bacterial interaction with macrophages. *Gut* 40**,** 20-24.

Chung, J.M., Sheedlo, M.J., Campbell, A.M., Sawhney, N., Frick-Cheng, A.E., Lacy, D.B., Cover, T.L., and Ohi, M.D. (2019). Structure of the *Helicobacter pylori* Cag Type IV secretion system. *Elife* 8.

Codolo, G., Toffoletto, M., Chemello, F., Coletta, S., Soler Teixidor, G., Battaggia, G., Munari, G., Fassan, M., Cagnin, S., and De Bernard, M. (2019). *Helicobacter pylori* Dampens HLA-II Expression on Macrophages via the Up-Regulation of miRNAs Targeting CIITA. *Front Immunol* 10**,** 2923.

Collins, S.J. (1987). The HL-60 promyelocytic leukemia cell line: proliferation, differentiation, and cellular oncogene expression. *Blood* 70**,** 1233-1244.

Correa, P. (1992). Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 52**,** 6735-6740.

Correa, P., and Houghton, J. (2007). Carcinogenesis of *Helicobacter pylori*. *Gastroenterology* 133**,** 659-672.

Cover, T.L., Krishna, U.S., Israel, D.A., and Peek, R.M., Jr. (2003). Induction of gastric epithelial cell apoptosis by *Helicobacter pylori* vacuolating cytotoxin. *Cancer Res* 63**,** 951-957.

Crabtree, J.E., Wyatt, J.I., Trejdosiewicz, L.K., Peichl, P., Nichols, P.H., Ramsay, N., Primrose, J.N., and Lindley, I.J. (1994). Interleukin-8 expression in *Helicobacter pylori* infected, normal, and neoplastic gastroduodenal mucosa. *J Clin Pathol* 47**,** 61-66.

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

D'elios, M.M., Manghetti, M., De Carli, M., Costa, F., Baldari, C.T., Burroni, D., Telford, J.L., Romagnani, S., and Del Prete, G. (1997). T helper 1 effector cells specific for *Helicobacter pylori* in the gastric antrum of patients with peptic ulcer disease. *J Immunol* 158**,** 962-967.

Davies, G.R., Banatvala, N., Collins, C.E., Sheaff, M.T., Abdi, Y., Clements, L., and Rampton, D.S. (1994). Relationship between infective load of *Helicobacter pylori* and reactive oxygen metabolite production in antral mucosa. *Scand. J. Gastroenterol* 29**,** 419-424.

Devi, S., Rajakumara, E., and Ahmed, N. (2015). Induction of Mincle by *Helicobacter pylori* and consequent anti-inflammatory signaling denote a bacterial survival strategy. *Sci Rep* 5**,** 15049.

Didelot, X., Nell, S., Yang, I., Woltemate, S., Van Der Merwe, S., and Suerbaum, S. (2013). Genomic evolution and transmission of *Helicobacter pylori* in two South African families. *Proc Natl Acad Sci U S A* 110**,** 13880-13885.

Dooyema, S.D.R., Noto, J.M., Wroblewski, L.E., Piazuelo, M.B., Krishna, U., Suarez, G., Romero-Gallo, J., Delgado, A.G., and Peek, R.M. (2022). *Helicobacter pylori* actively suppresses innate immune nucleic acid receptors. *Gut Microbes* 14**,** 2105102.

Draper, J.L., Hansen, L.M., Bernick, D.L., Abedrabbo, S., Underwood, J.G., Kong, N., Huang, B.C., Weis, A.M., Weimer, B.C., Van Vliet, A.H., Pourmand, N., Solnick, J.V., Karplus, K., and Ottemann, K.M. (2017). Fallacy of the Unique Genome: Sequence Diversity within Single *Helicobacter pylori* Strains. *mBio* 8.

Du, S.Y., Wang, H.J., Cheng, H.H., Chen, S.D., Wang, L.H., and Wang, W.C. (2016). Cholesterol glucosylation by *Helicobacter pylori* delays internalization and arrests phagosome maturation in macrophages. *J Microbiol Immunol Infect* 49**,** 636-645.

Engstrand, L., and Lindberg, M. (2013). *Helicobacter pylori* and the gastric microbiota. *Best Pract Res Clin Gastroenterol* 27**,** 39-45.

Faass, L., Hauke, M., Stein, S.C., and Josenhans, C. (2023). Innate immune activation and modulatory factors of *Helicobacter pylori* towards phagocytic and nonphagocytic cells. *Curr Opin Immunol* 82**,** 102301.

Faber, E., Gripp, E., Maurischat, S., Kaspers, B., Tedin, K., Menz, S., Zuraw, A., Kershaw, O., Yang, I., Rautenschlein, S., and Josenhans, C. (2016). Novel Immunomodulatory Flagellin-Like Protein FlaC in *Campylobacter jejuni* and Other *Campylobacterales*. *mSphere* 1.

Falush, D., Wirth, T., Linz, B., Pritchard, J.K., Stephens, M., Kidd, M., Blaser, M.J., Graham, D.Y., Vacher, S., Perez-Perez, G.I., Yamaoka, Y., Megraud, F., Otto, K., Reichard, U., Katzowitsch, E., Wang, X., Achtman, M., and Suerbaum, S. (2003). Traces of human migrations in *Helicobacter pylori* populations. *Science* 299**,** 1582-1585.

Fehlings, M., Drobbe, L., Moos, V., Renner Viveros, P., Hagen, J., Beigier-Bompadre, M., Pang, E., Belogolova, E., Churin, Y., Schneider, T., Meyer, T.F., Aebischer, T., and Ignatius, R. (2012). Comparative analysis of the interaction of *Helicobacter pylori* with human dendritic cells, macrophages, and monocytes. *Infect Immun* 80**,** 2724-2734.

Feige, M.H., Vieth, M., Sokolova, O., Tager, C., and Naumann, M. (2018). *Helicobacter pylori* induces direct activation of the lymphotoxin beta receptor and non-canonical nuclear factor-kappa B signaling. *Biochim Biophys Acta Mol Cell Res* 1865**,** 545-550.

Ferrero, R.L., Thiberge, J.M., Huerre, M., and Labigne, A. (1998). Immune responses of specific-pathogen-free mice to chronic *Helicobacter pylori* (strain SS1) infection. *Infect Immun* 66**,** 1349-1355.

Fiocca, R., Luinetti, O., Villani, L., Chiaravalli, A.M., Capella, C., and Solcia, E. (1994). Epithelial cytotoxicity, immune responses, and inflammatory components of *Helicobacter pylori* gastritis. *Scand. J. Gastroenterol* 29 Suppl. 205**,** 11-21.

Fischer, A.S., and Sigal, M. (2019). The Role of Wnt and R-spondin in the Stomach During Health and Disease. *Biomedicines* 7.

Fischer, W., Puls, J., Buhrdorf, R., Gebert, B., Odenbreit, S., and Haas, R. (2001). Systematic mutagenesis of the *Helicobacter pylori* cag pathogenicity island: essential genes for

CagA translocation in host cells and induction of interleukin-8. *Mol Microbiol* 42**,** 1337-1348.

Fischer, W., Tegtmeyer, N., Stingl, K., and Backert, S. (2020). Four Chromosomal Type IV Secretion Systems in *Helicobacter pylori*: Composition, Structure and Function. *Front Microbiol* 11**,** 1592.

Frick-Cheng, A.E., Pyburn, T.M., Voss, B.J., Mcdonald, W.H., Ohi, M.D., and Cover, T.L. (2016). Molecular and Structural Analysis of the *Helicobacter pylori* cag Type IV Secretion System Core Complex. *mBio* 7**,** e02001-02015.

Fridlender, Z.G., Sun, J., Kim, S., Kapoor, V., Cheng, G., Ling, L., Worthen, G.S., and Albelda, S.M. (2009). Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell* 16**,** 183-194.

Fu, H., Ma, Y., Yang, M., Zhang, C., Huang, H., Xia, Y., Lu, L., Jin, W., and Cui, D. (2016). Persisting and Increasing Neutrophil Infiltration Associates with Gastric Carcinogenesis and E-cadherin Downregulation. *Sci Rep* 6**,** 29762.

Fung, C., Tan, S., Nakajima, M., Skoog, E.C., Camarillo-Guerrero, L.F., Klein, J.A., Lawley, T.D., Solnick, J.V., Fukami, T., and Amieva, M.R. (2019). High-resolution mapping reveals that microniches in the gastric glands control *Helicobacter pylori* colonization of the stomach. *PLoS Biol* 17**,** e3000231.

Futagami, S., Takahashi, H., Norose, Y., and Kobayashi, M. (1998). Systemic and local immune responses against *Helicobacter pylori* urease in patients with chronic gastritis: distinct IgA and IgG productive sites. *Gut* 43**,** 168-175.

Gall, A., Gaudet, R.G., Gray-Owen, S.D., and Salama, N.R. (2017). TIFA Signaling in Gastric Epithelial Cells Initiates the cag Type 4 Secretion System-Dependent Innate Immune Response to *Helicobacter pylori* Infection. *MBio* 8.

Garcia-Weber, D., and Arrieumerlou, C. (2020). ADP-heptose: a bacterial PAMP detected by the host sensor ALPK1. *Cell Mol Life Sci*.

Garcia-Weber, D., Dangeard, A.S., Cornil, J., Thai, L., Rytter, H., Zamyatina, A., Mulard, L.A., and Arrieumerlou, C. (2018). ADP-heptose is a newly identified pathogen-associated molecular pattern of *Shigella flexneri*. *EMBO Rep* 19.

Gaudet, R.G., Guo, C.X., Molinaro, R., Kottwitz, H., Rohde, J.R., Dangeard, A.S., Arrieumerlou, C., Girardin, S.E., and Gray-Owen, S.D. (2017). Innate Recognition of Intracellular Bacterial Growth Is Driven by the TIFA-Dependent Cytosolic Surveillance Pathway. *Cell Rep* 19**,** 1418-1430.

Gaudet, R.G., Sintsova, A., Buckwalter, C.M., Leung, N., Cochrane, A., Li, J., Cox, A.D., Moffat, J., and Gray-Owen, S.D. (2015). INNATE IMMUNITY. Cytosolic detection of the bacterial metabolite HBP activates TIFA-dependent innate immunity. *Science* 348**,** 1251-1255.

Gebert, B., Fischer, W., Weiss, E., Hoffmann, R., and Haas, R. (2003). *Helicobacter pylori* vacuolating cytotoxin inhibits T lymphocyte activation. *Science* 301**,** 1099-1102.

Gerhard, M., Schmees, C., Voland, P., Endres, N., Sander, M., Reindl, W., Rad, R., Oelsner, M., Decker, T., Mempel, M., Hengst, L., and Prinz, C. (2005). A secreted low-molecular-weight protein from *Helicobacter pylori* induces cell-cycle arrest of T cells. *Gastroenterology* 128**,** 1327-1339.

Gewirtz, A.T., Yu, Y., Krishna, U.S., Israel, D.A., Lyons, S.L., and Peek, R.M., Jr. (2004). *Helicobacter pylori* flagellin evades toll-like receptor 5-mediated innate immunity. *J Infect Dis* 189**,** 1914-1920.

Goh, K.L., Chan, W.K., Shiota, S., and Yamaoka, Y. (2011). Epidemiology of *Helicobacter pylori* infection and public health implications. *Helicobacter* 16 Suppl 1**,** 1-9.

Gonciarz, W., Krupa, A., Hinc, K., Obuchowski, M., Moran, A.P., Gajewski, A., and Chmiela, M. (2019). The effect of *Helicobacter pylori* infection and different *H. pylori* components on the proliferation and apoptosis of gastric epithelial cells and fibroblasts. *PLoS One* 14**,** e0220636.

Gorrell, R.J., Guan, J., Xin, Y., Tafreshi, M.A., Hutton, M.L., Mcguckin, M.A., Ferrero, R.L., and Kwok, T. (2013). A novel NOD1- and CagA-independent pathway of interleukin-8 induction mediated by the *Helicobacter pylori* type IV secretion system. *Cell Microbiol* 15**,** 554-570.

Grainger, J.R., Konkel, J.E., Zangerle-Murray, T., and Shaw, T.N. (2017). Macrophages in gastrointestinal homeostasis and inflammation. *Pflugers Arch* 469**,** 527-539.

Greten, F.R., Eckmann, L., Greten, T.F., Park, J.M., Li, Z.W., Egan, L.J., Kagnoff, M.F., and Karin, M. (2004). IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 118**,** 285-296.

Gringhuis, S.I., Den Dunnen, J., Litjens, M., Van Der Vlist, M., and Geijtenbeek, T.B. (2009). Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to Mycobacterium tuberculosis, HIV-1 and *Helicobacter pylori*. *Nat Immunol* 10**,** 1081-1088.

Guo, H., Callaway, J.B., and Ting, J.P. (2015). Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med* 21**,** 677-687.

Hatakeyama, M. (2008). SagA of CagA in *Helicobacter pylori* pathogenesis. *Curr Opin Microbiol* 11**,** 30-37.

Hauke, M., Metz, F., Rapp, J., Faass, L., Bats, S., Radziej, S., Link, H., Eisenreich, W., and Josenhans, C. (2023). *Helicobacter pylori* modulates heptose metabolite biosynthesis and heptose-dependent innate immune host cell activation by multiple mechanisms. *Microbiology Spectrum*, 10.1128/spectrum.03132-22.

Hazell, S.L., Lee, A., Brady, L., and Hennessy, W. (1986). *Campylobacter pyloridis* and gastritis: association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium. *J Infect Dis* 153**,** 658-663.

Hecker, J., Letizia, M., Loescher, B.S., Siegmund, B., and Weidinger, C. (2022). Early Onset of TNFalpha-Driven Arthritis, Auto-inflammation, and Progressive Loss of Vision in a Patient with ALPK1 Mutation. *J Clin Immunol* 42**,** 880-884.

Hessey, S.J., Spencer, J., Wyatt, J.I., Sobala, G., Rathbone, B.J., Axon, A.T., and Dixon, M.F. (1990). Bacterial adhesion and disease activity in *Helicobacter* associated chronic gastritis. *Gut* 31**,** 134-138.

Hilligan, K.L., and Ronchese, F. (2020). Antigen presentation by dendritic cells and their instruction of CD4+ T helper cell responses. *Cell Mol Immunol* 17**,** 587-599.

Hirata, Y., Maeda, S., Ohmae, T., Shibata, W., Yanai, A., Ogura, K., Yoshida, H., Kawabe, T., and Omata, M. (2006a). *Helicobacter pylori* induces IkappaB kinase alpha nuclear translocation and chemokine production in gastric epithelial cells. *Infect Immun* 74**,** 1452-1461.

Hirata, Y., Ohmae, T., Shibata, W., Maeda, S., Ogura, K., Yoshida, H., Kawabe, T., and Omata, M. (2006b). MyD88 and TNF receptor-associated factor 6 are critical signal transducers in *Helicobacter pylori*-infected human epithelial cells. *J Immunol* 176**,** 3796-3803.

Hirayama, F., Takagi, S., Kusuhara, H., Iwao, E., Yokoyama, Y., and Ikeda, Y. (1996). Induction of gastric ulcer and intestinal metaplasia in mongolian gerbils infected with *Helicobacter pylori*. *J Gastroenterol* 31**,** 755-757.

Hitzler, I., Kohler, E., Engler, D.B., Yazgan, A.S., and Müller, A. (2012). The role of Th cell subsets in the control of Helicobacter infections and in T cell-driven gastric immunopathology. *Front Immunol* 3**,** 142.

Hofer, M., and Lutolf, M.P. (2021). Engineering organoids. *Nat Rev Mater* 6**,** 402-420.

Holokai, L., Chakrabarti, J., Broda, T., Chang, J., Hawkins, J.A., Sundaram, N., Wroblewski, L.E., Peek, R.M., Jr., Wang, J., Helmrath, M., Wells, J.M., and Zavros, Y. (2019). Increased Programmed Death-Ligand 1 is an Early Epithelial Cell Response to *Helicobacter pylori* Infection. *PLoS Pathog* 15**,** e1007468.

Hooi, J.K.Y., Lai, W.Y., Ng, W.K., Suen, M.M.Y., Underwood, F.E., Tanyingoh, D., Malfertheiner, P., Graham, D.Y., Wong, V.W.S., Wu, J.C.Y., Chan, F.K.L., Sung, J.J.Y., Kaplan, G.G., and Ng, S.C. (2017). Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. *Gastroenterology* 153**,** 420-429.

Howitt, M.R., Lee, J.Y., Lertsethtakarn, P., Vogelmann, R., Joubert, L.M., Ottemann, K.M., and Amieva, M.R. (2011). ChePep controls *Helicobacter pylori* Infection of the gastric glands and chemotaxis in the Epsilonproteobacteria. *mBio* 2.

Huang, C.C., Weng, J.H., Wei, T.Y., Wu, P.Y., Hsu, P.H., Chen, Y.H., Wang, S.C., Qin, D., Hung, C.C., Chen, S.T., Wang, A.H., Shyy, J.Y., and Tsai, M.D. (2012). Intermolecular

binding between TIFA-FHA and TIFA-pT mediates tumor necrosis factor alpha stimulation and NF-kappaB activation. *Mol Cell Biol* 32**,** 2664-2673.

Iarc (1994). Schistosomes, liver flukes and *Helicobacter pylori*. *IARC Monogr Eval Carcinog Risks Hum* 61**,** 1-241.

Ilver, D., Arnqvist, A., Ogren, J., Frick, I.M., Kersulyte, D., Incecik, E.T., Berg, D.E., Covacci, A., Engstrand, L., and Boren, T. (1998). *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science* 279**,** 373-377.

Italiani, P., and Boraschi, D. (2014). From Monocytes to M1/M2 Macrophages: Phenotypical vs. Functional Differentiation. *Front Immunol* 5**,** 514.

Janeway, C.A., Jr., and Medzhitov, R. (2002). Innate immune recognition. *Annu Rev Immunol* 20**,** 197-216.

Jang, A.R., Kang, M.J., Shin, J.I., Kwon, S.W., Park, J.Y., Ahn, J.H., Lee, T.S., Kim, D.Y., Choi, B.G., Seo, M.W., Yang, S.J., Shin, M.K., and Park, J.H. (2020). Unveiling the Crucial Role of Type IV Secretion System and Motility of *Helicobacter pylori* in IL-1beta Production via NLRP3 Inflammasome Activation in Neutrophils. *Front Immunol* 11**,** 1121.

Javaheri, A., Kruse, T., Moonens, K., Mejias-Luque, R., Debraekeleer, A., Asche, C.I., Tegtmeyer, N., Kalali, B., Bach, N.C., Sieber, S.A., Hill, D.J., Koniger, V., Hauck, C.R., Moskalenko, R., Haas, R., Busch, D.H., Klaile, E., Slevogt, H., Schmidt, A., Backert, S., Remaut, H., Singer, B.B., and Gerhard, M. (2016). *Helicobacter pylori* adhesin HopQ engages in a virulence-enhancing interaction with human CEACAMs. *Nat Microbiol* 2**,** 16189.

Josenhans, C., and Suerbaum, S. (2002). The role of motility as a virulence factor in bacteria. *Int J Med Microbiol* 291**,** 605-614.

Kao, J.Y., Zhang, M., Miller, M.J., Mills, J.C., Wang, B., Liu, M., Eaton, K.A., Zou, W., Berndt, B.E., Cole, T.S., Takeuchi, T., Owyang, S.Y., and Luther, J. (2010). *Helicobacter pylor*i immune escape is mediated by dendritic cell-induced Treg skewing and Th17 suppression in mice. *Gastroenterology* 138**,** 1046-1054.

Karin, M., Cao, Y., Greten, F.R., and Li, Z.W. (2002). NF-kappaB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer* 2**,** 301-310.

Karin, M., and Greten, F.R. (2005). NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* 5**,** 749-759.

Kayisoglu, O., Schlegel, N., and Bartfeld, S. (2021a). Gastrointestinal epithelial innate immunity-regionalization and organoids as new model. *J Mol Med (Berl)* 99**,** 517-530.

Kayisoglu, O., Weiss, F., Niklas, C., Pierotti, I., Pompaiah, M., Wallaschek, N., Germer, C.T., Wiegering, A., and Bartfeld, S. (2021b). Location-specific cell identity rather than exposure to GI microbiota defines many innate immune signalling cascades in the gut epithelium. *Gut* 70**,** 687-697.

Kim, D.J., Park, J.H., Franchi, L., Backert, S., and Nunez, G. (2013). The Cag pathogenicity island and interaction between TLR2/NOD2 and NLRP3 regulate IL-1beta production in *Helicobacter pylori* infected dendritic cells. *Eur J Immunol* 43**,** 2650-2658.

Kim, N., Marcus, E.A., Wen, Y., Weeks, D.L., Scott, D.R., Jung, H.C., Song, I.S., and Sachs, G. (2004). Genes of *Helicobacter pylori* regulated by attachment to AGS cells. *Infect Immun* 72**,** 2358-2368.

Knirel, Y.A., Moll, H., and Zahringer, U. (1996). Structural study of a highly O-acetylated core of Legionella pneumophila serogroup 1 lipopolysaccharide. *Carbohydr Res* 293**,** 223-234.

Koch, M., Mollenkopf, H.J., and Meyer, T.F. (2016). Macrophages recognize the *Helicobacter pylori* type IV secretion system in the absence of toll-like receptor signalling. *Cell Microbiol* 18**,** 137-147.

Koeppel, M., Garcia-Alcalde, F., Glowinski, F., Schlaermann, P., and Meyer, T.F. (2015). *Helicobacter pylori* Infection Causes Characteristic DNA Damage Patterns in Human Cells. *Cell Rep* 11**,** 1703-1713.

Königer, V., Holsten, L., Harrison, U., Busch, B., Loell, E., Zhao, Q., Bonsor, D.A., Roth, A., Kengmo-Tchoupa, A., Smith, S.I., Mueller, S., Sundberg, E.J., Zimmermann, W.,

Fischer, W., Hauck, C.R., and Haas, R. (2016). *Helicobacter pylori* exploits human CEACAMs via HopQ for adherence and translocation of CagA. *Nat Microbiol* 2**,** 16188.

Kozycki, C.T., Kodati, S., Huryn, L., Wang, H., Warner, B.M., Jani, P., Hammoud, D., Abu-Asab, M.S., Jittayasothorn, Y., Mattapallil, M.J., Tsai, W.L., Ullah, E., Zhou, P., Tian, X., Soldatos, A., Moutsopoulos, N., Kao-Hsieh, M., Heller, T., Cowen, E.W., Lee, C.R., Toro, C., Kalsi, S., Khavandgar, Z., Baer, A., Beach, M., Long Priel, D., Nehrebecky, M., Rosenzweig, S., Romeo, T., Deuitch, N., Brenchley, L., Pelayo, E., Zein, W., Sen, N., Yang, A.H., Farley, G., Sweetser, D.A., Briere, L., Yang, J., De Oliveira Poswar, F., Schwartz, I.V.D., Silva Alves, T., Dusser, P., Kone-Paut, I., Touitou, I., Titah, S.M., Van Hagen, P.M., Van Wijck, R.T.A., Van Der Spek, P.J., Yano, H., Benneche, A., Apalset, E.M., Jansson, R.W., Caspi, R.R., Kuhns, D.B., Gadina, M., Takada, H., Ida, H., Nishikomori, R., Verrecchia, E., Sangiorgi, E., Manna, R., Brooks, B.P., Sobrin, L., Hufnagel, R.B., Beck, D., Shao, F., Ombrello, A.K., Aksentijevich, I., Kastner, D.L., and Undiagnosed Diseases, N. (2022). Gain-of-function mutations in ALPK1 cause an NF-kappaB-mediated autoinflammatory disease: functional assessment, clinical phenotyping and disease course of patients with ROSAH syndrome. *Ann Rheum Dis* 81**,** 1453-1464.

Krebes, J., Didelot, X., Kennemann, L., and Suerbaum, S. (2014). Bidirectional genomic exchange between *Helicobacter pylori* strains from a family in Coventry, United Kingdom. *Int J Med Microbiol* 304**,** 1135-1146.

Krulwich, T.A., Sachs, G., and Padan, E. (2011). Molecular aspects of bacterial pH sensing and homeostasis. *Nat Rev Microbiol* 9**,** 330-343.

Kubo-Murai, M., Hazeki, K., Nigorikawa, K., Omoto, T., Inoue, N., and Hazeki, O. (2008). IRAK-4-dependent degradation of IRAK-1 is a negative feedback signal for TLR-mediated NF-kappaB activation. *J Biochem* 143**,** 295-302.

Kuo, S.H., Chen, L.T., Lin, C.W., Wu, M.S., Hsu, P.N., Tsai, H.J., Chu, C.Y., Tzeng, Y.S., Wang, H.P., Yeh, K.H., and Cheng, A.L. (2013). Detection of the *Helicobacter pylori* CagA protein in gastric mucosa-associated lymphoid tissue lymphoma cells: clinical and biological significance. *Blood Cancer J* 3**,** e125.

Kutter, S., Buhrdorf, R., Haas, J., Schneider-Brachert, W., Haas, R., and Fischer, W. (2008). Protein subassemblies of the *Helicobacter pylori* Cag type IV secretion system revealed by localization and interaction studies. *J Bacteriol* 190**,** 2161-2171.

Kwok, T., Zabler, D., Urman, S., Rohde, M., Hartig, R., Wessler, S., Misselwitz, R., Berger, J., Sewald, N., Konig, W., and Backert, S. (2007). *Helicobacter exploits* integrin for type IV secretion and kinase activation. *Nature* 449**,** 862-866.

Lee, A., O'rourke, J., De Ungria, M.C., Robertson, B., Daskalopoulos, G., and Dixon, M.F. (1997). A standardized mouse model of *Helicobacter pylori* infection: introducing the Sydney strain. *Gastroenterology* 112**,** 1386-1397.

Lee, I.O., Kim, J.H., Choi, Y.J., Pillinger, M.H., Kim, S.Y., Blaser, M.J., and Lee, Y.C. (2010). *Helicobacter pylori* CagA phosphorylation status determines the gp130-activated SHP2/ERK and JAK/STAT signal transduction pathways in gastric epithelial cells. *J Biol Chem* 285**,** 16042-16050.

Lee, K.H., Bae, S.H., Lee, J.L., Hyun, M.S., Kim, S.H., Song, S.K., and Kim, H.S. (2004). Relationship between urokinase-type plasminogen receptor, interleukin-8 gene expression and clinicopathological features in gastric cancer. *Oncology* 66**,** 210-217.

Lee, S.K., Stack, A., Katzowitsch, E., Aizawa, S.I., Suerbaum, S., and Josenhans, C. (2003). *Helicobacter pylori* flagellins have very low intrinsic activity to stimulate human gastric epithelial cells via TLR5. *Microbes Infect* 5**,** 1345-1356.

Lewis, B.P., Shih, I.H., Jones-Rhoades, M.W., Bartel, D.P., and Burge, C.B. (2003). Prediction of mammalian microRNA targets. *Cell* 115**,** 787-798.

Li, D., and Wu, M. (2021). Pattern recognition receptors in health and diseases. *Signal Transduct Target Ther* 6**,** 291.

Li, H., Yang, T., Liao, T., Debowski, A.W., Nilsson, H.O., Fulurija, A., Haslam, S.M., Mulloy, B., Dell, A., Stubbs, K.A., Marshall, B.J., and Benghezal, M. (2017). The redefinition of *Helicobacter pylori* lipopolysaccharide O-antigen and core-oligosaccharide domains. *PLoS Pathog* 13**,** e1006280.

Li, X., Liu, S., Luo, J., Liu, A., Tang, S., Liu, S., Yu, M., and Zhang, Y. (2015). *Helicobacter pylori* induces IL-1beta and IL-18 production in human monocytic cell line through activation of NLRP3 inflammasome via ROS signaling pathway. *Pathog Dis* 73.

Li, X.X., Wong, G.L., To, K.F., Wong, V.W., Lai, L.H., Chow, D.K., Lau, J.Y., Sung, J.J., and Ding, C. (2009). Bacterial microbiota profiling in gastritis without *Helicobacter pylori* infection or non-steroidal anti-inflammatory drug use. *PLoS One* 4**,** e7985.

Li, Z., Zhang, W., Bai, J., Li, J., and Li, H. (2022). Emerging Role of *Helicobacter pylori* in the Immune Evasion Mechanism of Gastric Cancer: An Insight Into Tumor Microenvironment-Pathogen Interaction. *Front Oncol* 12**,** 862462.

Libanio, D., Dinis-Ribeiro, M., and Pimentel-Nunes, P. (2015). *Helicobacter pylori* and microRNAs: Relation with innate immunity and progression of preneoplastic conditions. *World J Clin Oncol* 6**,** 111-132.

Lim, M.C.C., Maubach, G., Birkl-Toeglhofer, A.M., Haybaeck, J., Vieth, M., and Naumann, M. (2022). A20 undermines alternative NF-kappaB activity and expression of anti-apoptotic genes in *Helicobacter pylori* infection. *Cell Mol Life Sci* 79**,** 102.

Lindholm, C., Quiding-Järbrink, M., Lonroth, H., Hamlet, A., and Svennerholm, A.M. (1998). Local cytokine response in *Helicobacter pylori*-infected subjects. *Infect Immun* 66**,** 5964-5971.

Linz, B., Balloux, F., Moodley, Y., Manica, A., Liu, H., Roumagnac, P., Falush, D., Stamer, C., Prugnolle, F., Van Der Merwe, S.W., Yamaoka, Y., Graham, D.Y., Perez-Trallero, E., Wadstrom, T., Suerbaum, S., and Achtman, M. (2007). An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature* 445**,** 915-918.

Liu, W., Yan, M., Liu, Y., Wang, R., Li, C., Deng, C., Singh, A., Coleman, W.G., Jr., and Rodgers, G.P. (2010). Olfactomedin 4 down-regulates innate immunity against *Helicobacter pylori* infection. *Proc Natl Acad Sci U S A* 107**,** 11056-11061.

Lofgren, J.L., Whary, M.T., Ge, Z., Muthupalani, S., Taylor, N.S., Mobley, M., Potter, A., Varro, A., Eibach, D., Suerbaum, S., Wang, T.C., and Fox, J.G. (2011). Lack of commensal flora in *Helicobacter pylori*-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. *Gastroenterology* 140**,** 210-220.

Lundgren, A., Stromberg, E., Sjoling, A., Lindholm, C., Enarsson, K., Edebo, A., Johnsson, E., Suri-Payer, E., Larsson, P., Rudin, A., Svennerholm, A.M., and Lundin, B.S. (2005). Mucosal FOXP3-expressing CD4+ CD25high regulatory T cells in *Helicobacter pylori*-infected patients. *Infect Immun* 73**,** 523-531.

Lundgren, A., Suri-Payer, E., Enarsson, K., Svennerholm, A.M., and Lundin, B.S. (2003). *Helicobacter pylori*-specific CD4+ CD25high regulatory T cells suppress memory T-cell responses to *H. pylori* in infected individuals. *Infect Immun* 71**,** 1755-1762.

Luo, J.L., Kamata, H., and Karin, M. (2005). IKK/NF-kappaB signaling: balancing life and death--a new approach to cancer therapy. *J Clin Invest* 115**,** 2625-2632.

Macri, A., Versaci, A., Loddo, S., Scuderi, G., Travagliante, M., Trimarchi, G., Teti, D., and Famulari, C. (2006). Serum levels of interleukin 1beta, interleukin 8 and tumour necrosis factor alpha as markers of gastric cancer. *Biomarkers* 11**,** 184-193.

Maeda, S., Akanuma, M., Mitsuno, Y., Hirata, Y., Ogura, K., Yoshida, H., Shiratori, Y., and Omata, M. (2001). Distinct mechanism of *Helicobacter pylori*-mediated NF-kappa B activation between gastric cancer cells and monocytic cells. *J Biol Chem* 276**,** 44856-44864.

Mahdavi, J., Sonden, B., Hurtig, M., Olfat, F.O., Forsberg, L., Roche, N., Angstrom, J., Larsson, T., Teneberg, S., Karlsson, K.A., Altraja, S., Wadstrom, T., Kersulyte, D., Berg, D.E., Dubois, A., Petersson, C., Magnusson, K.E., Norberg, T., Lindh, F., Lundskog, B.B., Arnqvist, A., Hammarstrom, L., and Boren, T. (2002). *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. *Science* 297**,** 573-578.

Malfertheiner, P., Megraud, F., O'morain, C.A., Gisbert, J.P., Kuipers, E.J., Axon, A.T., Bazzoli, F., Gasbarrini, A., Atherton, J., Graham, D.Y., Hunt, R., Moayyedi, P., Rokkas, T., Rugge, M., Selgrad, M., Suerbaum, S., Sugano, K., El-Omar, E.M., European, H., Microbiota Study, G., and Consensus, P. (2017). Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut* 66**,** 6-30.

Mandell, L., Moran, A.P., Cocchiarella, A., Houghton, J., Taylor, N., Fox, J.G., Wang, T.C., and Kurt-Jones, E.A. (2004). Intact gram-negative *Helicobacter pylori*, *Helicobacter felis*, and *Helicobacter hepaticus* bacteria activate innate immunity via toll-like receptor 2 but not toll-like receptor 4. *Infect Immun* 72**,** 6446-6454.

Mantovani, A., Cassatella, M.A., Costantini, C., and Jaillon, S. (2011). Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol* 11**,** 519-531.

Marshall, J.S., Warrington, R., Watson, W., and Kim, H.L. (2018). An introduction to immunology and immunopathology. *Allergy Asthma Clin Immunol* 14**,** 49.

Martin-Gallausiaux, C., Garcia-Weber, D., Lashermes, A., Larraufie, P., Marinelli, L., Teixeira, V., Rolland, A., Beguet-Crespel, F., Brochard, V., Quatremare, T., Jamet, A., Dore, J., Gray-Owen, S.D., Blottiere, H.M., Arrieumerlou, C., and Lapaque, N. (2022). *Akkermansia muciniphila* upregulates genes involved in maintaining the intestinal barrier function via ADP-heptose-dependent activation of the ALPK1/TIFA pathway. *Gut Microbes* 14**,** 2110639.

Matsumoto, M., Tanaka, T., Kaisho, T., Sanjo, H., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., and Akira, S. (1999). A novel LPS-inducible C-type lectin is a transcriptional target of NF-IL6 in macrophages. *J Immunol* 163**,** 5039-5048.

Matsushima, K., Isomoto, H., Inoue, N., Nakayama, T., Hayashi, T., Nakayama, M., Nakao, K., Hirayama, T., and Kohno, S. (2011). MicroRNA signatures in *Helicobacter pylori*-infected gastric mucosa. *Int J Cancer* 128**,** 361-370.

Maubach, G., Lim, M.C.C., Sokolova, O., Backert, S., Meyer, T.F., and Naumann, M. (2021). TIFA has dual functions in *Helicobacter pylori*-induced classical and alternative NF-kappaB pathways. *EMBO Rep* 22**,** e52878.

Mayer-Scholl, A., Averhoff, P., and Zychlinsky, A. (2004). How do neutrophils and pathogens interact? *Curr Opin Microbiol* 7**,** 62-66.

Medvedev, A.E., Kopydlowski, K.M., and Vogel, S.N. (2000). Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression. *J Immunol* 164**,** 5564-5574.

Medzhitov, R. (2007). Recognition of microorganisms and activation of the immune response. *Nature* 449**,** 819-826.

Mejias-Luque, R., and Gerhard, M. (2017). Immune Evasion Strategies and Persistence of *Helicobacter pylori*. *Curr Top Microbiol Immunol* 400**,** 53-71.

Mejias-Luque, R., Zoller, J., Anderl, F., Loew-Gil, E., Vieth, M., Adler, T., Engler, D.B., Urban, S., Browning, J.L., Müller, A., Gerhard, M., and Heikenwalder, M. (2017). Lymphotoxin beta receptor signalling executes *Helicobacter pylori*-driven gastric inflammation in a T4SS-dependent manner. *Gut* 66**,** 1369-1381.

Merga, Y.J., O'hara, A., Burkitt, M.D., Duckworth, C.A., Probert, C.S., Campbell, B.J., and Pritchard, D.M. (2016). Importance of the alternative NF-kappaB activation pathway in inflammation-associated gastrointestinal carcinogenesis. *Am J Physiol Gastrointest Liver Physiol* 310**,** G1081-1090.

Milivojevic, M., Dangeard, A.S., Kasper, C.A., Tschon, T., Emmenlauer, M., Pique, C., Schnupf, P., Guignot, J., and Arrieumerlou, C. (2017). ALPK1 controls TIFA/TRAF6-dependent innate immunity against heptose-1,7-bisphosphate of gram-negative bacteria. *PLoS Pathog* 13**,** e1006224.

Miszczyk, E., Rudnicka, K., Moran, A.P., Fol, M., Kowalewicz-Kulbat, M., Druszczynska, M., Matusiak, A., Walencka, M., Rudnicka, W., and Chmiela, M. (2012). Interaction of *Helicobacter pylori* with C-type lectin dendritic cell-specific ICAM grabbing nonintegrin. *J Biomed Biotechnol* 2012**,** 206463.

Monteiro, M.A., Chan, K.H., Rasko, D.A., Taylor, D.E., Zheng, P.Y., Appelmelk, B.J., Wirth, H.P., Yang, M., Blaser, M.J., Hynes, S.O., Moran, A.P., and Perry, M.B. (1998). Simultaneous expression of type 1 and type 2 Lewis blood group antigens by *Helicobacter pylori* lipopolysaccharides. Molecular mimicry between *H. pylori* lipopolysaccharides and human gastric epithelial cell surface glycoforms. *J Biol Chem* 273**,** 11533-11543.

Moodley, Y., Linz, B., Bond, R.P., Nieuwoudt, M., Soodyall, H., Schlebusch, C.M., Bernhoft, S., Hale, J., Suerbaum, S., Mugisha, L., Van Der Merwe, S.W., and Achtman, M. (2012). Age of the association between *Helicobacter pylori* and man. *PLoS Pathog* 8**,** e1002693.

Moran, A.P., Lindner, B., and Walsh, E.J. (1997). Structural characterization of the lipid A component of *Helicobacter pylori* rough- and smooth-form lipopolysaccharides. *J Bacteriol* 179**,** 6453-6463.

Müller, A., Oertli, M., and Arnold, I.C. (2011). *H. pylori* exploits and manipulates innate and adaptive immune cell signaling pathways to establish persistent infection. *Cell Commun Signal* 9**,** 25.

Munoz-Wolf, N., and Lavelle, E.C. (2016). Innate Immune Receptors. *Methods Mol Biol* 1417**,** 1-43.

Muotiala, A., Helander, I.M., Pyhala, L., Kosunen, T.U., and Moran, A.P. (1992). Low biological activity of *Helicobacter pylori* lipopolysaccharide. *Infect Immun* 60**,** 1714-1716.

Nathan, C. (2006). Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* 6**,** 173-182.

Necchi, V., Manca, R., Ricci, V., and Solcia, E. (2009). Evidence for transepithelial dendritic cells in human *H. pylori* active gastritis. *Helicobacter* 14**,** 208-222.

Newton, K., and Dixit, V.M. (2012). Signaling in innate immunity and inflammation. *Cold Spring Harb Perspect Biol* 4.

Niess, J.H., Brand, S., Gu, X., Landsman, L., Jung, S., Mccormick, B.A., Vyas, J.M., Boes, M., Ploegh, H.L., Fox, J.G., Littman, D.R., and Reinecker, H.C. (2005). CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 307**,** 254-258.

Nishio, H., Terashima, S., Nakashima, M., Aihara, E., and Takeuchi, K. (2007). Involvement of prostaglandin E receptor EP3 subtype and prostacyclin IP receptor in decreased acid response in damaged stomach. *J Physiol Pharmacol* 58**,** 407-421.

Noach, L.A., Bosma, N.B., Jansen, J., Hoek, F.J., Van Deventer, S.J., and Tytgat, G.N. (1994). Mucosal tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8 production in patients with *Helicobacter pylori* infection. *Scand J Gastroenterol* 29**,** 425-429.

Noto, J.M., and Peek, R.M. (2011). The role of microRNAs in *Helicobacter pylori* pathogenesis and gastric carcinogenesis. *Front Cell Infect Microbiol* 1**,** 21.

Nurgalieva, Z.Z., Conner, M.E., Opekun, A.R., Zheng, C.Q., Elliott, S.N., Ernst, P.B., Osato, M., Estes, M.K., and Graham, D.Y. (2005). B-cell and T-cell immune responses to experimental *Helicobacter pylori* infection in humans. *Infect Immun* 73**,** 2999-3006.

O'Neil, D.A., Cole, S.P., Martin-Porter, E., Housley, M.P., Liu, L., Ganz, T., and Kagnoff, M.F. (2000). Regulation of human beta-defensins by gastric epithelial cells in response to infection with *Helicobacter pylori* or stimulation with interleukin-1. *Infect Immun* 68**,** 5412-5415.

Odenbreit, S., Gebert, B., Puls, J., Fischer, W., and Haas, R. (2001). Interaction of *Helicobacter pylori* with professional phagocytes: role of the cag pathogenicity island and translocation, phosphorylation and processing of CagA. *Cell Microbiol* 3**,** 21-31.

Odenbreit, S., Puls, J., Sedlmaier, B., Gerland, E., Fischer, W., and Haas, R. (2000). Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. *Science* 287**,** 1497-1500.

Oertli, M., Noben, M., Engler, D.B., Semper, R.P., Reuter, S., Maxeiner, J., Gerhard, M., Taube, C., and Müller, A. (2013). *Helicobacter pylori* gamma-glutamyl transpeptidase and vacuolating cytotoxin promote gastric persistence and immune tolerance. *Proc Natl Acad Sci U S A* 110**,** 3047-3052.

Ogura, K., Maeda, S., Nakao, M., Watanabe, T., Tada, M., Kyutoku, T., Yoshida, H., Shiratori, Y., and Omata, M. (2000). Virulence factors of *Helicobacter pylori* responsible for gastric diseases in Mongolian gerbil. *J Exp Med* 192**,** 1601-1610.

Ohmae, T., Hirata, Y., Maeda, S., Shibata, W., Yanai, A., Ogura, K., Yoshida, H., Kawabe, T., and Omata, M. (2005). *Helicobacter pylori* activates NF-kappaB via the alternative pathway in B lymphocytes. *J Immunol* 175**,** 7162-7169.

Olbermann, P., Josenhans, C., Moodley, Y., Uhr, M., Stamer, C., Vauterin, M., Suerbaum, S., Achtman, M., and Linz, B. (2010). A global overview of the genetic and functional diversity in the *Helicobacter pylori* cag pathogenicity island. *PLoS Genet* 6**,** e1001069.

Omenetti, S., and Pizarro, T.T. (2015). The Treg/Th17 Axis: A Dynamic Balance Regulated by the Gut Microbiome. *Front Immunol* 6**,** 639.

Orecchioni, M., Ghosheh, Y., Pramod, A.B., and Ley, K. (2019). Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. *Front Immunol* 10**,** 1084.

Otani, K., Tanigawa, T., Watanabe, T., Nadatani, Y., Sogawa, M., Yamagami, H., Shiba, M., Watanabe, K., Tominaga, K., Fujiwara, Y., and Arakawa, T. (2012). Toll-like receptor 9 signaling has anti-inflammatory effects on the early phase of *Helicobacter pylori*-induced gastritis. *Biochem Biophys Res Commun* 426**,** 342-349.

Ottman, N., Reunanen, J., Meijerink, M., Pietila, T.E., Kainulainen, V., Klievink, J., Huuskonen, L., Aalvink, S., Skurnik, M., Boeren, S., Satokari, R., Mercenier, A., Palva, A., Smidt, H., De Vos, W.M., and Belzer, C. (2017). Pili-like proteins of *Akkermansia muciniphila* modulate host immune responses and gut barrier function. *PLoS One* 12**,** e0173004.

Pagliari, M., Munari, F., Toffoletto, M., Lonardi, S., Chemello, F., Codolo, G., Millino, C., Della Bella, C., Pacchioni, B., Vermi, W., Fassan, M., De Bernard, M., and Cagnin, S. (2017). *Helicobacter pylori* Affects the Antigen Presentation Activity of Macrophages Modulating the Expression of the Immune Receptor CD300E through miR-4270. *Front Immunol* 8**,** 1288.

Papini, E., Satin, B., Norais, N., De Bernard, M., Telford, J.L., Rappuoli, R., and Montecucco, C. (1998). Selective increase of the permeability of polarized epithelial cell monolayers by *Helicobacter pylori* vacuolating toxin. *J Clin Invest* 102**,** 813-820.

Park, B.S., and Lee, J.O. (2013). Recognition of lipopolysaccharide pattern by TLR4 complexes. *Exp Mol Med* 45**,** e66.

Perez-Figueroa, E., Torres, J., Sanchez-Zauco, N., Contreras-Ramos, A., Alvarez-Arellano, L., and Maldonado-Bernal, C. (2016). Activation of NLRP3 inflammasome in human neutrophils by *Helicobacter pylori* infection. *Innate Immun* 22**,** 103-112.

Pfannkuch, L., Hurwitz, R., Traulsen, J., Sigulla, J., Poeschke, M., Matzner, L., Kosma, P., Schmid, M., and Meyer, T.F. (2019). ADP heptose, a novel pathogen-associated molecular pattern identified in *Helicobacter pylori*. *FASEB J* 33**,** 9087-9099.

Philpott, D.J., Belaid, D., Troubadour, P., Thiberge, J.M., Tankovic, J., Labigne, A., and Ferrero, R.L. (2002). Reduced activation of inflammatory responses in host cells by mouse-adapted *Helicobacter pylory* isolates. *Cell Microbiol* 4**,** 285-296.

Phuc, B.H., Tuan, V.P., Dung, H.D.Q., Binh, T.T., Tung, P.H., Tri, T.D., Thuan, N.P.M., Van Khien, V., Trang, T.T.H., Akada, J., Matsumoto, T., and Yamaoka, Y. (2021). *Helicobacter pylori* type 4 secretion systems as gastroduodenal disease markers. *Sci Rep* 11**,** 4584.

Pikarsky, E., Porat, R.M., Stein, I., Abramovitch, R., Amit, S., Kasem, S., Gutkovich-Pyest, E., Urieli-Shoval, S., Galun, E., and Ben-Neriah, Y. (2004). NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature* 431**,** 461-466.

Polak, D., and Bohle, B. (2022). Neutrophils-typical atypical antigen presenting cells? *Immunol Lett* 247**,** 52-58.

Pompaiah, M., and Bartfeld, S. (2017). Gastric Organoids: An Emerging Model System to Study *Helicobacter pylori* Pathogenesis. *Curr Top Microbiol Immunol* 400**,** 149-168.

Quiding-Järbrink, M., Raghavan, S., and Sundquist, M. (2010). Enhanced M1 macrophage polarization in human *Helicobacter pylori*-associated atrophic gastritis and in vaccinated mice. *PLoS One* 5**,** e15018.

Rad, R., Ballhorn, W., Voland, P., Eisenacher, K., Mages, J., Rad, L., Ferstl, R., Lang, R., Wagner, H., Schmid, R.M., Bauer, S., Prinz, C., Kirschning, C.J., and Krug, A. (2009). Extracellular and intracellular pattern recognition receptors cooperate in the recognition of *Helicobacter pylori*. *Gastroenterology* 136**,** 2247-2257.

Ramarao, N., and Meyer, T.F. (2001). *Helicobacter pylori* resists phagocytosis by macrophages: quantitative assessment by confocal microscopy and fluorescence-activated cell sorting. *Infect Immun* 69**,** 2604-2611.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J.P., and Ricciardi-Castagnoli, P. (2001). Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2**,** 361-367.

Reyes, V.E., and Peniche, A.G. (2019). *Helicobacter pylori* Deregulates T and B Cell Signaling to Trigger Immune Evasion. *Curr Top Microbiol Immunol* 421**,** 229-265.

Richter-Dahlfors, A., Heczko, U., Meloche, R.M., Finlay, B.B., and Buchan, A.M. (1998). *Helicobacter pylori*-infected human antral primary cell cultures: effect on gastrin cell function. *Am J Physiol* 275**,** G393-401.

Rieder, G., Merchant, J.L., and Haas, R. (2005). *Helicobacter pylori* cag-type IV secretion system facilitates corpus colonization to induce precancerous conditions in Mongolian gerbils. *Gastroenterology* 128**,** 1229-1242.

Rincon, E., Rocha-Gregg, B.L., and Collins, S.R. (2018). A map of gene expression in neutrophil-like cell lines. *BMC Genomics* 19**,** 573.

Rossi, G., Manfrin, A., and Lutolf, M.P. (2018). Progress and potential in organoid research. *Nat Rev Genet* 19**,** 671-687.

Ryzhakov, G., West, N.R., Franchini, F., Clare, S., Ilott, N.E., Sansom, S.N., Bullers, S.J., Pearson, C., Costain, A., Vaughan-Jackson, A., Goettel, J.A., Ermann, J., Horwitz, B.H., Buti, L., Lu, X., Mukhopadhyay, S., Snapper, S.B., and Powrie, F. (2018). Alpha kinase 1 controls intestinal inflammation by suppressing the IL-12/Th1 axis. *Nat Commun* 9**,** 3797.

Saadat, I., Higashi, H., Obuse, C., Umeda, M., Murata-Kamiya, N., Saito, Y., Lu, H., Ohnishi, N., Azuma, T., Suzuki, A., Ohno, S., and Hatakeyama, M. (2007). *Helicobacter pylori* CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. *Nature* 447**,** 330-333.

Saito, Y., Suzuki, H., Tsugawa, H., Imaeda, H., Matsuzaki, J., Hirata, K., Hosoe, N., Nakamura, M., Mukai, M., Saito, H., and Hibi, T. (2012). Overexpression of miR-142-5p and miR-155 in gastric mucosa-associated lymphoid tissue (MALT) lymphoma resistant to *Helicobacter pylori* eradication. *PLoS One* 7**,** e47396.

Salama, N.R., Hartung, M.L., and Müller, A. (2013). Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. *Nat Rev Microbiol* 11**,** 385-399.

Sanchez-Zauco, N.A., Torres, J., Perez-Figueroa, G.E., Alvarez-Arellano, L., Camorlinga-Ponce, M., Gomez, A., Giono-Cerezo, S., and Maldonado-Bernal, C. (2014). Impact of cagPAI and T4SS on the inflammatory response of human neutrophils to *Helicobacter pylori* infection. *PLoS One* 8**,** e64623.

Sangiorgi, E., Azzara, A., Molinario, C., Pietrobono, R., Rigante, D., Verrecchia, E., Sicignano, L.L., Genuardi, M., Gurrieri, F., and Manna, R. (2019). Rare missense variants in the ALPK1 gene may predispose to periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA) syndrome. *Eur J Hum Genet* 27**,** 1361-1368.

Saravia, J., Chapman, N.M., and Chi, H. (2019). Helper T cell differentiation. *Cell Mol Immunol* 16**,** 634-643.

Sayi, A., Kohler, E., Hitzler, I., Arnold, I., Schwendener, R., Rehrauer, H., and Müller, A. (2009). The CD4+ T cell-mediated IFN-gamma response to *Helicobacter* infection is essential for clearance and determines gastric cancer risk. *J Immunol* 182**,** 7085-7101.

Schmees, C., Prinz, C., Treptau, T., Rad, R., Hengst, L., Voland, P., Bauer, S., Brenner, L., Schmid, R.M., and Gerhard, M. (2007). Inhibition of T-cell proliferation by *Helicobacter pylori* gamma-glutamyl transpeptidase. *Gastroenterology* 132**,** 1820-1833.

Schreiber, S., Konradt, M., Groll, C., Scheid, P., Hanauer, G., Werling, H.O., Josenhans, C., and Suerbaum, S. (2004). The spatial orientation of *Helicobacter pylori* in the gastric mucus. *Proc Natl Acad Sci U S A* 101**,** 5024-5029.

Schwarz, S., Morelli, G., Kusecek, B., Manica, A., Balloux, F., Owen, R.J., Graham, D.Y., Van Der Merwe, S., Achtman, M., and Suerbaum, S. (2008). Horizontal versus familial transmission of *Helicobacter pylori*. *PLoS Pathog* 4**,** e1000180.

Schweitzer, K., Sokolova, O., Bozko, P.M., and Naumann, M. (2010). *Helicobacter pylori* induces NF-kappaB independent of CagA. *EMBO Rep* 11**,** 10-11; author reply 11-12.

Segal, E.D., Cha, J., Lo, J., Falkow, S., and Tompkins, L.S. (1999). Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. *Proc Natl Acad Sci U S A* 96**,** 14559-14564.

Sen, R., and Baltimore, D. (1986). Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. *Cell* 47**,** 921-928.

Senkovich, O.A., Yin, J., Ekshyyan, V., Conant, C., Traylor, J., Adegboyega, P., Mcgee, D.J., Rhoads, R.E., Slepenkov, S., and Testerman, T.L. (2011). *Helicobacter pylori* AlpA and AlpB bind host laminin and influence gastric inflammation in gerbils. *Infect Immun* 79**,** 3106-3116.

Sewald, X., Gebert-Vogl, B., Prassl, S., Barwig, I., Weiss, E., Fabbri, M., Osicka, R., Schiemann, M., Busch, D.H., Semmrich, M., Holzmann, B., Sebo, P., and Haas, R. (2008). Integrin subunit CD18 Is the T-lymphocyte receptor for the *Helicobacter pylori* vacuolating cytotoxin. *Cell Host Microbe* 3**,** 20-29.

Shembade, N., and Harhaj, E.W. (2012). Regulation of NF-kappaB signaling by the A20 deubiquitinase. *Cell Mol Immunol* 9**,** 123-130.

Shi, Y., Liu, X.F., Zhuang, Y., Zhang, J.Y., Liu, T., Yin, Z., Wu, C., Mao, X.H., Jia, K.R., Wang, F.J., Guo, H., Flavell, R.A., Zhao, Z., Liu, K.Y., Xiao, B., Guo, Y., Zhang, W.J., Zhou, W.Y., Guo, G., and Zou, Q.M. (2010). *Helicobacter pylori*-induced Th17 responses modulate Th1 cell responses, benefit bacterial growth, and contribute to pathology in mice. *J Immunol* 184**,** 5121-5129.

Sigal, M., Logan, C.Y., Kapalczynska, M., Mollenkopf, H.J., Berger, H., Wiedenmann, B., Nusse, R., Amieva, M.R., and Meyer, T.F. (2017). Stromal R-spondin orchestrates gastric epithelial stem cells and gland homeostasis. *Nature* 548**,** 451-455.

Sigal, M., Rothenberg, M.E., Logan, C.Y., Lee, J.Y., Honaker, R.W., Cooper, R.L., Passarelli, B., Camorlinga, M., Bouley, D.M., Alvarez, G., Nusse, R., Torres, J., and Amieva, M.R. (2015). *Helicobacter pylori* Activates and Expands Lgr5(+) Stem Cells Through Direct Colonization of the Gastric Glands. *Gastroenterology* 148**,** 1392-1404 e1321.

Silvestre-Roig, C., Fridlender, Z.G., Glogauer, M., and Scapini, P. (2019). Neutrophil Diversity in Health and Disease. *Trends Immunol* 40**,** 565-583.

Skaug, B., Chen, J., Du, F., He, J., Ma, A., and Chen, Z.J. (2011). Direct, noncatalytic mechanism of IKK inhibition by A20. *Mol Cell* 44**,** 559-571.

Smith, S.M. (2014). Role of Toll-like receptors in *Helicobacter pylori* infection and immunity. *World J Gastrointest Pathophysiol* 5**,** 133-146.

Snelling, T., Shpiro, N., Gourlay, R., Lamoliatte, F., and Cohen, P. (2022). Co-ordinated control of the ADP-heptose/ALPK1 signalling network by the E3 ligases TRAF6, TRAF2/c-IAP1 and LUBAC. *Biochem J* 479**,** 2195-2216.

Sokolova, O., Borgmann, M., Rieke, C., Schweitzer, K., Rothkotter, H.J., and Naumann, M. (2013). *Helicobacter pylori* induces type 4 secretion system-dependent, but CagA-independent activation of IkappaBs and NF-kappaB/RelA at early time points. *Int J Med Microbiol* 303**,** 548-552.

Stein, M., Bagnoli, F., Halenbeck, R., Rappuoli, R., Fantl, W.J., and Covacci, A. (2002). c-Src/Lyn kinases activate *Helicobacter pylori* CagA through tyrosine phosphorylation of the EPIYA motifs. *Mol Microbiol* 43**,** 971-980.

Stein, S.C., Faber, E., Bats, S.H., Murillo, T., Speidel, Y., Coombs, N., and Josenhans, C. (2017). *Helicobacter pylori* modulates host cell responses by CagT4SS-dependent translocation of an intermediate metabolite of LPS inner core heptose biosynthesis. *PLoS Pathog* 13**,** e1006514.

Suerbaum, S., and Josenhans, C. (2007). *Helicobacter pylori* evolution and phenotypic diversification in a changing host. *Nat Rev Microbiol* 5**,** 441-452.

Suerbaum, S., and Michetti, P. (2002). *Helicobacter pylori* infection. *N Engl J Med* 347**,** 1175-1186.

Sun, S.C. (2017). The non-canonical NF-kappaB pathway in immunity and inflammation. *Nat Rev Immunol* 17**,** 545-558.

Sun, X., Zhang, M., El-Zataari, M., Owyang, S.Y., Eaton, K.A., Liu, M., Chang, Y.M., Zou, W., and Kao, J.Y. (2013). TLR2 mediates *Helicobacter pylori*-induced tolerogenic immune response in mice. *PLoS One* 8**,** e74595.

Sundrud, M.S., Torres, V.J., Unutmaz, D., and Cover, T.L. (2004). Inhibition of primary human T cell proliferation by *Helicobacter pylori* vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. *Proc Natl Acad Sci U S A* 101**,** 7727-7732.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 71**,** 209-249.

Sutton, P., and Boag, J.M. (2019). Status of vaccine research and development for *Helicobacter pylori*. *Vaccine* 37**,** 7295-7299.

Suzuki, M., Mimuro, H., Kiga, K., Fukumatsu, M., Ishijima, N., Morikawa, H., Nagai, S., Koyasu, S., Gilman, R.H., Kersulyte, D., Berg, D.E., and Sasakawa, C. (2009). *Helicobacter pylori* CagA phosphorylation-independent function in epithelial proliferation and inflammation. *Cell Host Microbe* 5**,** 23-34.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E.M., Houchens, C.R., Grayson, M.L., Hansen, P., Singh, N., Theuretzbacher, U., Magrini, N., and Group, W.H.O.P.P.L.W. (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 18**,** 318-327.

Tan, S., Tompkins, L.S., and Amieva, M.R. (2009). *Helicobacter pylori* usurps cell polarity to turn the cell surface into a replicative niche. *PLoS Pathog* 5**,** e1000407.

Tang, W., Guo, Z., Cao, Z., Wang, M., Li, P., Meng, X., Zhao, X., Xie, Z., Wang, W., Zhou, A., Lou, C., and Chen, Y. (2018). d-Sedoheptulose-7-phosphate is a common precursor for the heptoses of septacidin and hygromycin B. *Proc Natl Acad Sci U S A* 115**,** 2818-2823.

Taniguchi, K., and Karin, M. (2018). NF-kappaB, inflammation, immunity and cancer: coming of age. *Nat Rev Immunol* 18**,** 309-324.

Thomsen, L.L., Gavin, J.B., and Tasman-Jones, C. (1990). Relation of *Helicobacter pylori* to the human gastric mucosa in chronic gastritis of the antrum. *Gut* 31**,** 1230-1236.

Tsuchiya, S., Yamabe, M., Yamaguchi, Y., Kobayashi, Y., Konno, T., and Tada, K. (1980). Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int J Cancer* 26**,** 171-176.

Unemo, M., Aspholm-Hurtig, M., Ilver, D., Bergstrom, J., Boren, T., Danielsson, D., and Teneberg, S. (2005). The sialic acid binding SabA adhesin of *Helicobacter pylori* is essential for nonopsonic activation of human neutrophils. *J Biol Chem* 280**,** 15390-15397.

Van Doorn, N.E., Namavar, F., Sparrius, M., Stoof, J., Van Rees, E.P., Van Doorn, L.J., and Vandenbroucke-Grauls, C.M. (1999). *Helicobacter pylori*-associated gastritis in mice is host and strain specific. *Infect Immun* 67**,** 3040-3046.

Varga, M.G., Piazuelo, M.B., Romero-Gallo, J., Delgado, A.G., Suarez, G., Whitaker, M.E., Krishna, U.S., Patel, R.V., Skaar, E.P., Wilson, K.T., Algood, H.M., and Peek, R.M., Jr. (2016a). TLR9 activation suppresses inflammation in response to *Helicobacter pylori* infection. *Am J Physiol Gastrointest Liver Physiol* 311**,** G852-G858.

Varga, M.G., Shaffer, C.L., Sierra, J.C., Suarez, G., Piazuelo, M.B., Whitaker, M.E., Romero-Gallo, J., Krishna, U.S., Delgado, A., Gomez, M.A., Good, J.A., Almqvist, F., Skaar, E.P., Correa, P., Wilson, K.T., Hadjifrangiskou, M., and Peek, R.M. (2016b). Pathogenic *Helicobacter pylori* strains translocate DNA and activate TLR9 via the cancer-associated cag type IV secretion system. *Oncogene* 35**,** 6262-6269.

Vella, M.C., Choi, E.Y., Lin, S.Y., Reinert, K., and Slack, F.J. (2004). The C. elegans microRNA let-7 binds to imperfect let-7 complementary sites from the lin-41 3'UTR. *Genes Dev* 18**,** 132-137.

Viala, J., Chaput, C., Boneca, I.G., Cardona, A., Girardin, S.E., Moran, A.P., Athman, R., Memet, S., Huerre, M.R., Coyle, A.J., Distefano, P.S., Sansonetti, P.J., Labigne, A., Bertin, J., Philpott, D.J., and Ferrero, R.L. (2004). Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. *Nat Immunol* 5**,** 1166-1174.

Vono, M., Lin, A., Norrby-Teglund, A., Koup, R.A., Liang, F., and Lore, K. (2017). Neutrophils acquire the capacity for antigen presentation to memory CD4(+) T cells in vitro and ex vivo. *Blood* 129**,** 1991-2001.

Wang, T.C., Bonner-Weir, S., Oates, P.S., Chulak, M., Simon, B., Merlino, G.T., Schmidt, E.V., and Brand, S.J. (1993). Pancreatic gastrin stimulates islet differentiation of transforming growth factor alpha-induced ductular precursor cells. *J Clin Invest* 92**,** 1349-1356.

Warren, J.R., and Marshall, B. (1983). Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1**,** 1273-1275.

Watanabe, T., Tada, M., Nagai, H., Sasaki, S., and Nakao, M. (1998). *Helicobacter pylori* infection induces gastric cancer in mongolian gerbils. *Gastroenterology* 115**,** 642-648.

Weeks, D.L., Eskandari, S., Scott, D.R., and Sachs, G. (2000). A H+-gated urea channel: the link between *Helicobacter pylori* urease and gastric colonization. *Science* 287**,** 482-485.

Wiedemann, T., Loell, E., Mueller, S., Stoeckelhuber, M., Stolte, M., Haas, R., and Rieder, G. (2009). *Helicobacter pylori* cag-Pathogenicity island-dependent early immunological response triggers later precancerous gastric changes in Mongolian gerbils. *PLoS One* 4**,** e4754.

Wirth, H.P., Beins, M.H., Yang, M., Tham, K.T., and Blaser, M.J. (1998). Experimental infection of Mongolian gerbils with wild-type and mutant *Helicobacter pylori* strains. *Infect Immun* 66**,** 4856-4866.

Wizenty, J., Mullerke, S., Kolesnichenko, M., Heuberger, J., Lin, M., Fischer, A.S., Mollenkopf, H.J., Berger, H., Tacke, F., and Sigal, M. (2022). Gastric stem cells promote inflammation and gland remodeling in response to *Helicobacter pylori* via Rspo3-Lgr4 axis. *EMBO J* 41**,** e109996.

Wong, B.C., Lam, S.K., Wong, W.M., Chen, J.S., Zheng, T.T., Feng, R.E., Lai, K.C., Hu, W.H., Yuen, S.T., Leung, S.Y., Fong, D.Y., Ho, J., Ching, C.K., Chen, J.S., and China Gastric Cancer Study, G. (2004). *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 291**,** 187-194.

Worbs, T., Hammerschmidt, S.I., and Forster, R. (2017). Dendritic cell migration in health and disease. *Nat Rev Immunol* 17**,** 30-48.

Wroblewski, L.E., and Peek, R.M., Jr. (2016). *Helicobacter pylori*: Pathogenic enablers - toxic relationships in the stomach. *Nat Rev Gastroenterol Hepatol* 13**,** 317-318.

Wustner, S., Mejias-Luque, R., Koch, M.F., Rath, E., Vieth, M., Sieber, S.A., Haller, D., and Gerhard, M. (2015). *Helicobacter pylori* gamma-glutamyltranspeptidase impairs T-lymphocyte function by compromising metabolic adaption through inhibition of cMyc and IRF4 expression. *Cell Microbiol* 17**,** 51-61.

Xiao, S., and Zhou, L. (2020). Gastric Stem Cells: Physiological and Pathological Perspectives. *Front Cell Dev Biol* 8**,** 571536.

Yamada, S., Kato, S., Matsuhisa, T., Makonkawkeyoon, L., Yoshida, M., Chakrabandhu, T., Lertprasertsuk, N., Suttharat, P., Chakrabandhu, B., Nishiumi, S., Chongraksut, W., and Azuma, T. (2013). Predominant mucosal IL-8 mRNA expression in non-cagA Thais is risk for gastric cancer. *World J Gastroenterol* 19**,** 2941-2949.

Yang, I., Nell, S., and Suerbaum, S. (2013). Survival in hostile territory: the microbiota of the stomach. *FEMS Microbiol Rev* 37**,** 736-761.

Ying, L., and Ferrero, R.L. (2019). Role of NOD1 and ALPK1/TIFA Signalling in Innate Immunity Against *Helicobacter pylori* Infection. *Curr Top Microbiol Immunol* 421**,** 159-177.

Zabaleta, J. (2012). MicroRNA: A Bridge from *H. pylori* Infection to Gastritis and Gastric Cancer Development. *Front Genet* 3**,** 294.

Zhang, X., Arnold, I.C., and Müller, A. (2020). Mechanisms of persistence, innate immune activation and immunomodulation by the gastric pathogen *Helicobacter pylori*. *Curr Opin Microbiol* 54**,** 1-10.

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

Zhou, P., She, Y., Dong, N., Li, P., He, H., Borio, A., Wu, Q., Lu, S., Ding, X., Cao, Y., Xu, Y., Gao, W., Dong, M., Ding, J., Wang, D.C., Zamyatina, A., and Shao, F. (2018). Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. *Nature* 561**,** 122-126.

Zhuang, Y., Shi, Y., Liu, X.F., Zhang, J.Y., Liu, T., Fan, X., Luo, J., Wu, C., Yu, S., Chen, L., Luo, P., Guo, G., Liu, Z., Tang, B., Mao, X.H., Guo, Y., and Zou, Q.M. (2011). *Helicobacter pylori*-infected macrophages induce Th17 cell differentiation. *Immunobiology* 216**,** 200-207.

Zimmermann, S., Pfannkuch, L., Al-Zeer, M.A., Bartfeld, S., Koch, M., Liu, J., Rechner, C., Soerensen, M., Sokolova, O., Zamyatina, A., Kosma, P., Maurer, A.P., Glowinski, F., Pleissner, K.P., Schmid, M., Brinkmann, V., Karlas, A., Naumann, M., Rother, M., Machuy, N., and Meyer, T.F. (2017). ALPK1- and TIFA-Dependent Innate Immune Response Triggered by the *Helicobacter pylori* Type IV Secretion System. *Cell Rep* 20**,** 2384-2395.

Zou, D., Xu, L., Li, H., Ma, Y., Gong, Y., Guo, T., Jing, Z., Xu, X., and Zhang, Y. (2019). Role of abnormal microRNA expression in *Helicobacter pylori* associated gastric cancer. *Crit Rev Microbiol* 45**,** 239-251.

## 11 ACKNOWLEDGEMENTS

I want to thank my supervisor Prof. Dr. Christine Josenhans for the great opportunity to work on this exciting research project to expand my knowledge in the field of medical microbiology and immunology. I am very grateful for her scientific support and advices and the many helpful discussions during the practical and theoretical parts of my project. Furthermore, I want to thank her for the opportunity to attend national and international conferences, where I had the chance to present my scientific work, meet and listen to inspiring scientist and their research in my field.

In addition, I am very grateful for the inspiring discussions and the constructive feedback I received from my co-supervisors Dr. Tobias Geiger and Prof. Dr. Bernd Lepenies during our TAC meetings.

It was my great pleasure to be part of the graduate program “Infection Research on Human Pathogens @MvPI”. I am very thankful for the manifold support offered by the program in the form of workshops and retreats and especially for the great cohesion amongst us doctorate students.

I am very grateful to the Examination Committee for examining my thesis.

Furthermore, I want to thank all current members and alumni of the Josenhans and Suerbaum labs for their technical support and the enriching scientific discussions. Special thanks goes to Martina, Annemarie and Bettina – I am very grateful for all the great laughter we had together.

Moreover, I want to gratefully thank all co-authors and cooperation partners without whom this project, including the published work, would not have been possible.

A big shout out goes to my dear friends who have sweetened the last few years with pony farm trips, lake garda escapes, regular halloween sleepovers, yoga & brunch rituals and numerous bar and party nights, spezi-walks, hour-long phone calls and hikes filled with love and laughter.

Ein riesiges Dankeschön auch an euch, liebster Faaß-Clan! 1000 Dank, dass auf euch, Pia, Rolf, Frank und Erik, immer Verlass ist und ihr mir, egal wann und wo, immer den Rücken stärkt und mich bei allen Vorhaben unterstützt und ermutigt.

A big thank you also to you, Jann, for being my biggest fan and most supportive ally.

# 12 LIST OF OWN PUBLICATIONS, PRESENTATIONS AND AWARDS

## 12.1 Own publications

**Faass L, Hauke M, Stein SC, Josenhans C**. Innate activation of human neutrophils and neutrophil-like cells by the proinflammatory bacterial metabolite ADP-heptose and *Helicobacter pylori*. In revision in the “International Journal of Medical Microbiology”.

**Hauke M, Metz F, Rapp J, Faass L, Bats S, Radziej S, Link H, Eisenreich W, Josenhans C**. *Helicobacter pylori* modulates heptose metabolite biosynthesis and heptose-dependent innate immune host cell activation by multiple mechanisms. In print in the Journal “Microbiology Spectrum”.

**Krone L, Faass L, Hauke M, Josenhans C, Geiger T**. Chitinase A, a tightly regulated virulence factor of *Salmonella enterica* serovar Typhimurium, is actively secreted by a Type 10 Secretion System. PLoS Pathog. 2023 Apr 5;19(4):e1011306.doi: 10.1371/journal.ppat.1011306. Epub ahead of print. PMID: 37018381.

**Faass L, Hauke M, Stein SC, Josenhans C**. Innate immune activation and modulatory factors of *Helicobacter pylori* towards phagocytic and nonphagocytic cells. Curr Opin Immunol. 2023 Mar 16;82:102301. doi: 10.1016/j.coi.2023.102301. Epub ahead of print. PMID: 36933362.

**Ngo QV, Faass L, Sähr A, Hildebrand D, Eigenbrod T, Heeg K, Nurjadi D**. Inflammatory Response Against *Staphylococcus aureus via* Intracellular Sensing of Nucleic Acids in Keratinocytes. Front Immunol. 2022 Feb 24;13:828626. doi: 10.3389/fimmu.2022.828626. PMID: 35281009; PMCID: PMC8907419.

**Coletta S, Battaggia G, Della Bella C, Furlani M, Hauke M, Faass L, D'Elios MM, Josenhans C, de Bernard M**. ADP-heptose enables *Helicobacter pylori* to exploit macrophages as a survival niche by suppressing antigen-presenting HLA-II expression. FEBS Lett. 2021 Aug;595(16):2160-2168. doi: 10.1002/1873-3468.14156. Epub 2021 Jul 14. PMID: 34216493.

**Faass L, Stein SC, Hauke M, Gapp M, Albanese M, Josenhans C**. Contribution of Heptose Metabolites and the *cag* Pathogenicity Island to the Activation of Monocytes/Macrophages by *Helicobacter pylori*. Front Immunol. 2021 May 19;12:632154. doi: 10.3389/fimmu.2021.632154. PMID: 34093525; PMCID: PMC8174060.

### 12.2 Oral and poster presentations

**Faass L, Hauke M, Stein SC, Marina de Bernard, Albanese M, Keppler O. T, Hornung V, Josenhans C**. "Innate human cell activation by bacterial LPS core heptose metabolites and *H. pylori*" presented at 14th International Workshop on Pathogenesis and Host Response in *Helicobacter* Infections" from June 29 - July 2, 2022 in Helsingor, Denmark (Talk).

**Faass L, Hauke M, Stein SC, Gapp M, Albanese M, Josenhans C**. "Contribution of heptose Metabolites and the *cag* pathogenicity island to the activation of monocytes/macrophages by *Helicobacter pylori*" presented at EMBO workshop "The inflammasomes: the next frontier" from September 21 - 24, 2021 in Martinsried, Germany (Poster).

**Faass L, Hauke M ,Stein SC , Gapp M, Albanese M, Josenhans C**. "Contribution of heptose Metabolites and the *cag* pathogenicity island to the activation of monocytes/macrophages by *Helicobacter pylori*" presented at "Keystone Symposia eSymposia meeting Innate Immunity: Mechanisms and Modulation | EK39" from April 12 - 15, 2021, Virtual Meeting (Poster).

**Faass L, , Nurjadi D, Sähr A, Heeg K. "**The role of indoleamine 2,3-dihydrogenase (IDO) in skin colonization by *Staphylococcus aureus*" presented at the 20th Symposium "Infektion und Immunabwehr" by DGfI from March 09 – 11, 2016 at Burg Rothenfels, Germany (Talk).

### 12.3 Awards and achievements

**Doc@MvPI International Travel Award 2022** by the graduate program "Infection Research on Human Pathogens @MvPI" to support my attendance at the 14th International Workshop on Pathogenesis and Host Response in *Helicobacte*r Infections" from June 29 - July 2, 2022 in Helsingor, Denmark where I presented the talk "Innate human cell activation by bacterial LPS core heptose metabolites and *H. pylori*".

# 13 APPENDIX I

## 13.1 Supplementary unpublished results (to manuscript I)

**“Influence of the NLRP3 inflammasome on the concentration-dependent pro-inflammatory activation of Thp-1 cells with pure ADP-heptose, and lack of potential of the metabolite to contribute to the activation of the NLRP3 inflammasome”**

## Supplementary unpublished results (to manuscript I)

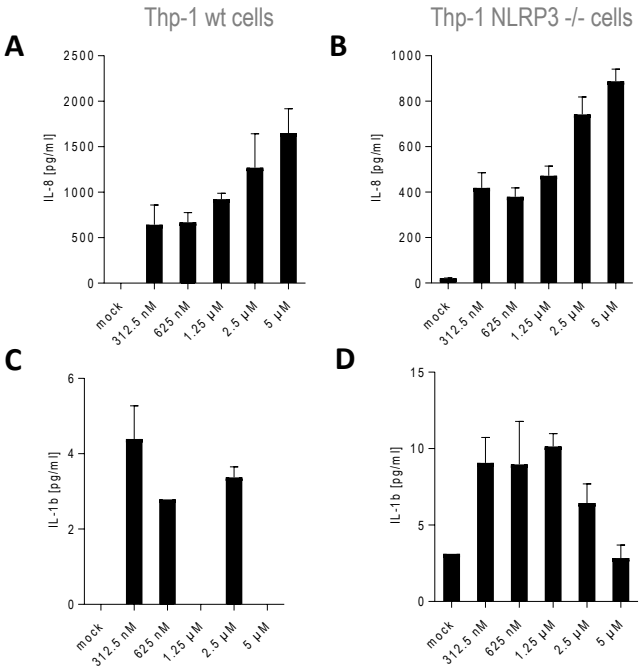


**FIG SU1**: **Influence of the NLRP3 inflammasome on the concentration-dependent pro-inflammatory activation of Thp-1 cells with pure ADP-heptose, and lack of potential of the metabolite to contribute to the activation of the NLRP3 inflammasome.**
**A)** Amount of IL-8 cytokines released by Thp-1 wild type cells incubated with different concentrations of ADP-heptose, ranging from 5 µM to 312.5 nM for 16 h. **B)** Amount of IL-8 cytokines released by Thp-1 NLRP3 CRISPR-Cas9 knock-out cells (kind gift from V. Hornung, LMU Munich), incubated with different concentrations of ADP-heptose, ranging from 5 µM to 312.5 nM for 16 h. **C)** Amount of IL-1β cytokines released by Thp-1 wild type cells incubated with different concentrations of ADP-heptose ranging from 5 µM to 312.5 nM for 16 h. The lack of measuring a detectable quantity of IL-1β in one concentration of ADP-heptose in panel C is due to low production below detection limit of this particular experiment. **D)** Amount of IL-1β cytokines released by Thp-1 NLRP3 knock-out cells incubated with different concentrations of ADP-heptose ranging from 5 µM to 312.5 nM for 16 h. IL-8 and IL-1β secretion into the supernatants were quantitated with ELISA after co-incubation of Thp-1 wild type and mutant cells in 24-well plates.

Within manuscript I, we examined the role of the NLRP3 inflammasome in the pro-inflammatory activation of Thp-1 cells by live *H. pylori* strains and found that the inflammasome did not seem to play a significant role for the bacteria-triggered or ADP-heptose-triggered IL-8 or IL-1β release in this cell type (tested by siRNA knock-down of NLRP3).
Here, I tested true NLRP3 knock-out cells to verify those findings in a clearer model. Conclusions: Although the level of IL-8 expression was again very high after co-incubation of Thp-1 cells with live bacteria (see above), in both wild type and NLRP3 knock-out cells, we did not measure significant upregulations or NLRP3-dependent changes of IL-1β. Thus, NLRP3 is not involved in heptose-mediated IL-8 release, and ADP-heptose alone does not seem to be able to induce NLRP3 activation at all. The presented supplementary unpublished results indicate that pure ADP-heptose does indeed induce a high, pro-inflammatory IL-8 response in NLRP3 k/o cells, making this effect totally independent of the NLRP3 inflammasome in Thp-1 cells. Moreover, in our experimental setup, it appears that the pure metabolite is not able to elicit the assembly and signaling of the NLRP3 inflammasome, in contrast to live *H. pylori* bacteria, demonstrated by very low IL-1β cytokine release.

# 14 APPENDIX II

## 14.1 Manuscript III

## "Innate activation of human neutrophils and neutrophil-like cells by the proinflammatory bacterial metabolite ADP-heptose and *Helicobacter pylori*"

**Faass L, Hauke M, Stein SC, Josenhans C**. Int J Med Microbiol. 2023 Jul;313(4):151585. doi: 10.1016/j.ijmm.2023.151585. Epub 2023 Jun 28. PMID: 37399704.

https://www.sciencedirect.com/science/article/pii/S1438422123000139?via%3Dihub

First author:

Conceptual contributions/experimental design; Investigation: performing and developing experiments, evaluation of results, formal analysis and statistics, establishing and improving methodology; Manuscript - writing: contribution to writing original draft; all figures and figure design, continuous editing of manuscript text, figures, references, contributions to final editing. My contributions were essential to compile the manuscript.