Trafficking of Human Dendritic Cells and B cells in *Helicobacter pylori*-induced Gastritis

Malin Hansson

UNIVERSITY OF GOTHENBURG

Department of Microbiology and Immunology,
Institute of Biomedicine at Sahlgrenska Academy,
The University of Gothenburg,
Sweden, 2009

ISBN 978-91-628-7934-1
http://hdl.handle.net/2077/21196

Printed by Geson Hylte Tryck, Göteborg, Sweden 2009

Denna avhandling tillägnas

Mamma och Pappa

Fritz och Axel som fyller mitt hjärta

ABSTRACT

Infection with the bacterium *Helicobacter pylori* is widespread throughout the world, and is associated with development of gastric and duodenal ulcer disease as well as gastric adenocarcinoma and mucosa associated lymphoid tissue lymphoma. The infection generally leads to a large infiltration of immune cells, among them dendrite cells (DC) and IgA-secreting cells. Even though there is a strong innate and adaptive immune response, the bacteria are not eliminated from the stomach and the infection usually remains throughout life.

The inductive site for the adaptive immune responses to *H. pylori* has not yet been identified and very little is known about the role of DC in the immune defense of the human stomach. The migration of DC from sites of antigen capture in peripheral tissues to the secondary lymphoid organs and the simultaneous maturation are crucial for initiation and amplification of primary immune responses. In this thesis we hypothesized that gastric DC fails to migrate to the lymph node and instead remains in the tissue and contribute to the chronic inflammation.

Tissue-specific lymphocyte homing to the intestinal mucosa tissue is dependent on interactions between specific adhesion molecules. These are, however, not changed during *H. pylori* infection. Instead, we hypothesized that mucosal chemokines contribute to recruitment of B cells to the *H. pylori* infected gastric mucosa. Therefore, the overall aims of this thesis were to evaluate how *H. pylori* infection affect the recruitment, functions and migration of DC and to investigate the role of chemokines for B cell homing to the gastric mucosa.

We have shown that DC stimulated with live *H. pylori in vitro* up-regulate the expression of the chemokine receptor CCR7, important for migration to the secondary lymphoid tissue, and that *H. pylori* stimulated DC migrate toward the CCR7 ligand CCL19. *H. pylori* stimulated DC were also capable of presenting antigen to T cells and secreted Th1 inducing cytokines. Using human gastric tissue we could also show that there is an accumulation of mature DC associated with lymphoid follicles and $CD4^+$ T cells, in the infected gastric mucosa, and also increased levels of CCL19.

Further, we have shown that production of the mucosal chemokine CCL28 is increased in *H. pylori* infections and that there is a correlation between CCL28 and IgA concentration in the gastric tissue of *H. pylori* infected individuals. Moreover, gastric IgA-secreting cells from *H. pylori* infected, but not uninfrcted, tissue had a robust migration toward CCL28.

Based on our results in this thesis we suggest that mature DC are retained in the gastric mucosa due to *H. pylori* infection, and that they contribute to sustaining the chronic inflammation. We have also shown that the expression of CCL28 is increased in human *H. pylori*-induced gastritis and that CCL28 may contribute to effector B-cell recruitment to the gastric mucosa in *H. pylori*-induced gastritis.

Keywords: *Helicobacter pylori*, migration, homing, dendritic cells, B cells, gastric mucosa.

SWEDISH SUMMARY, SVENSK SAMMANFATTNING

Helicobakter pylori är känd som magsårsbakterien och koloniserar den humana magslemhinnan. Infektionen är vanlig i hela världen och kan ge upphov till magsår eller cancer i magsäcken. Infektionen leder till en ökning av vita blodkroppar i magslemhinnan, till dessa hör neutrofiler, makrofager, dendritceller (DC), T och B celler. Vid infektionen sker även en stor ackumulering av celler som producerar antikroppar i magslemhinnan, en typ som kallas IgA. Trots det starka immunsvaret elimineras inte bakterierna, utan infektionen varar livet ut. Immuncellerna är spridda runt omkring i slemhinnan men i *H. pylori* infekterad vävnad bildas också organiserade små ansamlingar av vita blodkroppar, s.k. lymfoida folliklar, vilket man inte ser i oinfekterad magslemhinna.

Det är ännu inte känt var i kroppen de vita blodkropparna som finns i magslemhinnan har aktiverats, det finns inte heller så mycket kunskap om DC roll i immunförsvaret i magen. I normal fallet vandrar DC från vävnaden, t ex. magslemhinnan, till närliggande lymfkörtlar, där de kan aktivera vita blodkroppar genom att visa upp bakteriekomponenter på sin yta. Syftena med den här avhandlingen har varit att dels utvärdera hur *H. pylori* kan reglera funktionen och migrationen hos DC, dels undersöka hur en typ av vita blodkroppar, B celler, rekryteras till magslemhinnan.

Genom försök har vi visat att DC som stimulerats med *H. pylori* ökar uttrycket av kemokin receptorn CCR7. Denna receptor är viktig för DC vandring till lymfkörtlarna, där aktivering av vita blodkroppar celler sker. Kemokiner är små proteiner som”visar vägen” för vita blodkroppar, genom att dessa vandrar mot ökande koncentration av kemokinen. I våra försök visade vi också att DC kan vandra mot kemokinen CCL19 som kan signalera genom CCR7. I dessa in *vitro* försök fann vi inga fel i migrationen hos de *H. pylori* stimulerade DC som skulle kunna förklara den *H. pylori*-inducerade kroniska inflammationen i magslemhinnan. Möjligheten att andra celler som inte finns med i cellkultur försöken påverkar migrationen av DC kan dock inte uteslutas, och vi undersökte därför DC i magen från både *H. pylori* infekterade och oinfekterade individer. Dessa resultat visade att mogna DC, vilka annars hittas i lymfkörtlarna, finns associerade med de lymfoida folliklarna i den infekterade magslemhinnan, och även att det är en hög nivå av CCL19 i magslemhinnan från *H. pylori* infekterade individer. Det kan alltså vara så att mogna DC stannar kvar i magslemhinnan och på så sätt hjälper till upprätthålla den kroniska inflammationen.
Vidare har vi för första gången visat att uttrycket av den slemhinne- associerade kemokinen, CCL28 ökar i den humana magslemhinnan vid *H. pylori*-inducerad inflammation. Vi kunde också visa att CCL28 rekryterar IgA producerande vita blodkroppar från de *H. pylori* infekterade individerna.

Resultaten i denna avhandling tyder på att mogna DC kan stanna kvar i vävnaden hos *H. pylori* infekterade individer och därigenom hjälper till att upprätthålla den kroniska inflammationen. Vi har även visat att uttrycket av CCL28 ökar i magslemhinnan hos de *H. pylori* infekterade individerna och att CCL28 kan rekrytera antikropps-producerande celler till magslemhinnan.

ORIGINAL PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals (I-III):

I. **Hansson M., Lundgren A., Elgbratt K., Quiding-Järbrink M., Svennerholm A-M., Johansson E-L.**
Dendritic cells express CCR7 and migrate in response to CCL19 (MIP-3β) after exposure to *Helicobacter pylori*.
Microbes and Infection. 8 (2006) 841-850

II. **Hansson M., Sundquist M., Hering S., Hermansson M., Quiding-Järbrink M.**
Retention of mature dendritic cells in the gastric mucosa of patients with *Helicobacter pylori*-induced gastritis.
In manuscript.

III. **Hansson M., Hermansson M., Svensson H., Elfvin A., Hansson L-E., Johnsson E., Sjöling Å., Quiding-Järbrink M.**
CCL28 is increased in human *Helicobacter pylori*-induced gastritis and mediates recruitment of gastric immunoglobulin A-secreting cells.
Infection and Immunity, July 2008, p 3304-3311

Reprints were made with permission from the publisher

TABLE OF CONTENTS

Abstract 5

Swedish summary, Svensk sammanfattning 6

Original paper 7

Abbreviations 10

Introduction 11

Trafficking of leukocytes **11**
Recirculation of leukocyte subsets 11
Transendothelial migration 12
Tissue specific homing of lymphocytes 13
Migratory pathways of leukocyte subpopulations 15

Dendritic cells **16**
Human dendritic cell subsets 16
Monocyte derived DC 18
Lymphoid-tissue resident DC 19
DC-SIGN$^+$ immature DC 19
CD303$^+$ plasmacytoid DC 20
Maturation of DC 20
DC as antigen presenting cells 21

B cells **23**
Development of B cells 23
B cell activation 24
B cell homing to mucosal tissues 25

Helicobacter pylori **26**
H. pylori virulence factors 27
Immune responses in *H. pylori* infection 29
DC in the gastric mucosa 31

Aims of the thesis **33**

Materials and methods **34**

Results and discussion **45**
The influence of *H. pylori* on DC in the gastric mucosa 45
Recruitment of IgA antibody secreting cells by increased expression of the chemokine CCL28 in human *H. pylori*-induced gastritis 53

Concluding remarks **60**

Acknowledgements **64**

References **66**

Paper I-III

ABBREVIATIONS

AS	asymptomatic
APC	antigen presenting cell
ASC	antibody secreting cell
BabA	blood group binding adhesin A
cagPAI	cyotoxin associated gene pathogenicity island
CLA	cutaneous lymphocyte-associated antigen
CFU	colony forming unit
DC	dendritic cell
DC-LAMP	dendritic cell lysosome-associated membrane glycoprotein
DC-SIGN	dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin
DU	duodenal ulcer
FCM	flow cytometry
GC	gastric adenocarcinoma
GlyCAM-1	glycosylatio-dependent cell adhesion molecule-1
HEV	high endothelial venule
ICAM-1	intracellular cell adhesion molecule 1
iDC	immature dendritic cell
IFN-γ	interferon gamma
IHC	immunhistochemistry
Le	Lewis antigen
LN	lymph node
LPL	lamina propria lymphocytes
LPS	lipopolysaccharide
MadCAM-1	mucosal addressin cell adhesion molecule-1
MALT	mucosa associated lymphoid issue
mDC	mature dendritic cell
MHC	major histocompatibility complex
MP	membrane protein
PBMC	peripheral blood mononuclear cells
PNAd	peripheral lymph node addressin
PP	Peyer's patches
SabA	sialic acid binding adhesin A
TEM	transendothelial migration
TGF-β	transforming growth factor β
Th	T helper
TLR	Toll like receptor
TNF-α	tumour necrosis factor α
T4SS	type IV secretion system
VacA	vacuolating cytotoxin A
VCAM-1	vascular cell adhesion molecule

INTRODUCTION

We are all the time exposed to different microbes. Both the skin and the mucosal surfaces are covered by different types of microbes, both pathogenic and non-pathogenic. The internal surfaces of the human body are covered by epithelial cells. The mucosal surfaces serve many functions such as respiration, absorption, excretion and reproduction but also as a barrier against pathogens and toxins. When the mucosal or skin barrier is broken, the immune system has to decide if a response is necessary or not. It is also important that the mounted response is able to fight that particular microbe.

Dendritic cells (DC) serve like a bridge between the innate immune system, including epithelial cells, monocytes, macrophages, neutrophils and the adaptive immune system which is composed of lymphocytes. The gut mucosa contains DC that are specialized to sense external antigen and to activate the adapted immune system. Pathogen invasion leads to activation of innate immune cells such as neutrophils and macrophages that secrete pro-inflammatory cytokines, which in turn induce maturation of DC. However, in the gut there is a continuous uptake of nutrients and fluids through the epithelial layer and the DC has to decide whether to induce tolerance or active immunity.

The ability to re-circulate, migrate or home to specific tissues or organs is important for the immune cells of both innate and adapted immunity. Selectins, integrins, cytokines and chemokines are important for the trafficking between the blood, tissue and lymphoid tissue. Both in steady state and upon microbial infection, different cytokines and chemokines and the corresponding receptors regulate the different immune cells. This is very important for the outcome of the immune response.

Trafficking of leukocytes

Recirculation of leukocyte subsets

The immune system works like a single organ but its parts are located in many regions of the human body. Due to this, recirculation of leukocytes is very important. Recirculation means that cells leave the blood, migrate through the tissues and return to the blood via efferent lymphatic vessel. Leukocyte migration has an essential role in the function of the immune

system by for example enabling antigen presenting cells (APC) to encounter T cells in lymphoid organs where activation of naïve lymphocytes occurs. Stem cells in the bone marrow differentiate into myeloid and lymphoid precursor cells. Upon infection new leukocytes from the bone marrow are recruited. Myeloid precursors give rise to monocyte and granulocyte precursors where the terminal differentiation stage of this myeloid precursor leads to granulocytes (mainly neutrophils) and monocytes which exit from the bone marrow into the blood where they circulate. After recruitment to peripheral tissues, monocytes further differentiate into DC or macrophages (MΦ).
Lymphocyte precursors give rise to B and T lymphocytes that mature in the bone marrow and thymus, respectively. They enter the circulation as naïve mature lymphocytes, ready to be activated. Circulation of naïve lymphocytes from blood into lymph nodes and back to the blood via the lymphatic is mediated by interaction between selectins, chemokine receptors and integrins on the lymphocyte and corresponding ligands expressed by endothelial cells. After naïve lymphocytes have been activated they become either effector or memory cells.

Transendothelial migration

Transendothelial migration (TEM) is very important for tissue recruitment of leucocytes both during steady state as well as during inflammation. Leukocytes are recruited locally to the site of inflammation, via post-capillary venules or in secondary lymphoid tissues in a series of adhesive steps that allow them to attach to the vessel wall, roll along the endothelial border, traverse the endothelium and the subendothelial basement membrane, and migrate through the endothelial tissue [1, 2]. The recruitment of leukocytes from the blood to the lymph or tissue is a multistep process involving selectins, chemokine receptors and integrins and their respective ligands. Selectins are cell surface proteins that react and bind weakly to carbohydrate ligands present on glycoproteins on endothelial cells. This initiates the adhesion and transmigration of the rolling leukcocytes. Chemokines induce a firm arrest via integrin conformational change *in vitro* [3] and *in vivo* [4]. This results in firm adhesion of the leukocyte to the endothelium. Finally, migration between the endothelial cells (diapedesis) requires interaction with endothelial cell molecules, such as the junctional adhesion molecule (JAM), and cadherins [5, 6], but also proteolytic degradation of junctional complexes and the basal membrane (Fig.1).

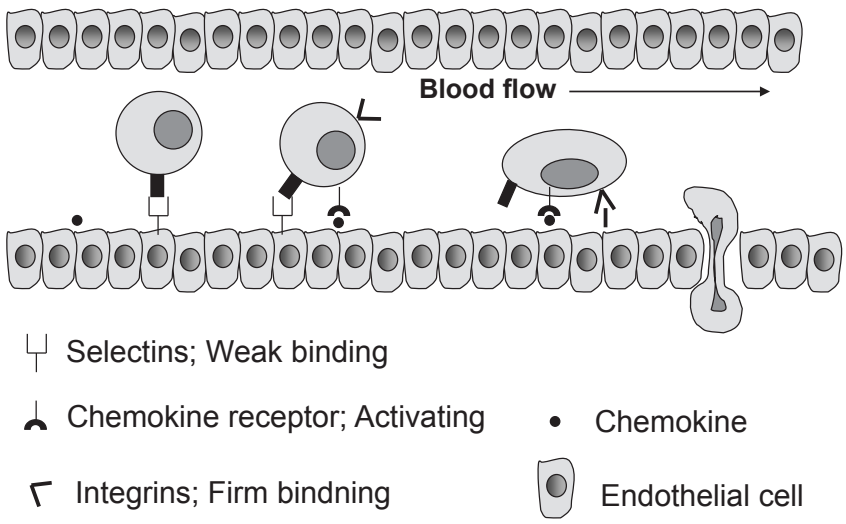

Figure 1. Transendothelial migration (TEM).

Tissue specific homing of lymphocytes

During steady state, immune cells belonging to both the innate and adapted immune systems circulate in the blood and lymph. Naïve lymphocytes circulate from the blood into lymph nodes and back to the blood via lymphatic vessel.

Migration of naïve T and B cells from the blood into the lymph nodes is mediated by interaction between lymphocyte L-selectin on the lymphocyte and peripheral node addressin (PNAd) expressed on CD34 or GlyCAM on endothelial cells in high endothelial venules (HEV) [7]. HEV are specialized post-capillary venous swellings that enable naïve lymphocytes to move in and out of the lymph nodes from the circulatory system. In contrast to the endothelial cells from other vessels, the endothelial cells of HEVs have a distinct appearance of cuboidal morphology. In Peyer´s patches the interaction between lymphocytes and the endothelium can be mediated between L-selectin and PNAd expression on mucosal addressin cell adhesion molecule-1 (MAdCAM-1) [8, 9]. The firm adhesion of lymphocytes to the endothelial cells is also mediated by MAdCAM-1and the integrin $\alpha 4\beta 7$ after signaling from chemokine receptors [8]. Based on their functional properties, chemokines can be divided into homeostatic and inflammatory chemokines. Homeostatic chemokines mediate

recruitment of naïve and memory lymphocytes to lymphoid and effector tissues and also to the correct microenvironment within the tissue. Inflammatory chemokines are up-regulated during infection due to inflammatory stimuli. This results in recruitment of cells from both the innate immune system, such as neutrophils, macrophages and DC, and from the adapted immune system, in the form of effector lymphocytes into the effector site [10, 11]. The recruitment of naïve T and B cells to the lymph nodes or other lymphoid tissue is mediated by the chemokines CCL19, produced by HEV, and CCL21 (SLC), produced in lymphoid tissue. CCL19 and CCL21 bind to the chemokine receptor CCR7, which is expressed by naïve lymphocytes and mature DC. In addition, naïve B cells express the chemokine receptor CXCR4 which binds to the chemokine CXCL12 (SDF-1). Once the naïve B cells have entered the lymph node the chemokine CXCL13 (BCA-1) and its corresponding receptor CXCR5 mediate the migration into the follicle. Naïve T and B cells recirculate through the blood and secondary lymphoid tissue until they encounter their specific antigen presented by DC in the lymph node, or until they die. When naïve lymphocytes have been activated in the lymph node by DC they down regulate the expression of L-selectin and CCR7 and are able to leave the lymph node and migrate to the inflamed tissue.

Effector and memory lymphocytes as well as cells in the innate immune system, such as neutrophils, macrophages and DC are recruited from the circulation into peripheral tissues. In principal, the mechanism is the same as when lymphocytes circulate from blood to secondary lymphoid tissue. Distinct subsets of effector and memory lymphocytes migrate preferentially through non-lymphoid tissues with a tissue specific, pattern of recirculation [12]. Memory cells are imprinted so that they return to the type of tissue where they first encountered the antigen. This has been best described for the integrated mucosal immune system. Activated lymphocytes from one mucosal surface can recirculate and selectively home to other mucosal surfaces, this observation formed the basis for the concept of an integrated mucosal immune system [13]. However, studies have shown a compartmentalization of the mucosal immune system in that antibody responses are stronger in the mucosa at which the antigen is delivered than at more distal sites [14, 15]. Tissue-specific lymphocyte homing to gastrointestinal mucosal tissue is dependent on the expression of the mucosal homing receptor integrin $\alpha4\beta7$ [16-18]. $\alpha4\beta7$ interacts with the MAdCAM-1, which is expressed on post-capillary endothelial cells in Peyer`s patches and the gastrointestinal mucosa [19, 20]. Chemokines also play an essential role during tissue-specific homing to different organs [21, 22]. The mucosa-associated epithelial chemokine CCL28 is a

common mucosal chemokine which is constitutively expressed by epithelial cells in most mucosal tissues [23, 24]. The second mucosal chemokine, CCL25 on the other hand, is mainly expressed by epithelial cells in the small intestine [25-27]. Although produced by epithelial cells, these chemokines are enriched by endothelial cells and presented to migrating lymphocytes on their apical side [22, 28]. A recent report has shown that antibody-secreting B cells activated by intestinal, but not systemic, immunization respond strongly to CCL28 and CCL25 [29]. It has also been demonstrated that DC from different tissues have the capacity to imprint T cells with tissue-specific homing receptors and that DC subsets in Peyer's patches have the capacity to induce expression of $\alpha 4\beta 7$ and CCR9 on $CD8^+$ T cells [30]. These migration pathways, which correlate with different functional properties of lymphocyte subsets, increase the efficiency of the immune system [12].

Migratory pathways of leukocyte subpopulations

Monocyte migration into tissues is regulated by at least two mechanisms. One of these is the production of chemotactic factors by inflamed tissue such as interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1) and members of the GRO family of chemoattractants [31, 32]. The other mechanism involves the activation of vascular endothelial cells by cytokines such as IL-1, TNF-α, or IFN-γ or by bacterial antigens such as the endotoxin lipopolysaccharide (LPS), leading to the expression of several proteins on the endothelial surface that facilitate adhesion and migration of monocytes [33, 34]. Monocytes can differentiate into myeloid DC when recruited to the tissue upon inflammation. Trafficking of myeloid DC differs from that of naïve lymphocyte [17]. Neither immature nor mature myeloid DC enter the lymph node directly from the circulation through HEV; instead, they enter the peripheral tissues [35, 36]. Antigen-loaded mature DC then migrate into the draining lymph node via afferent lymphatic vessels [37]. In contrast, plasmacytoid DC (see below) precursors appear to directly migrate to secondary lymphoid tissues across activated HEVs in a CXCL9 and E-selectin dependent manner [38].

Dendritic cells

Dendritic cells (DC) are a group of bone-marrow-derived leukocytes that are specialized on the uptake, transport, processing and presentation of antigens [39, 40]. In 1973, Steinman and Cohn discovered these cells in mouse spleen and named them DC. A few years later they showed that DC were 100-fold better at activating T cells compared to other antigen-presenting cells such as macrophages and B cells [41-43]. DC constitute an important link between the innate and adaptive immune system since they are the only antigen presenting cells capable of activating naïve T cells. Immature DC (iDC) act as immunological sensors that recognize microbial components or signals from the innate immune system when it is exposed to microbes. DC maturation (see below) occurs when DC encounter inflammation or tissue damage. They will then capture antigen in the infected tissue followed by migration to the secondary lymphoid tissue and subsequent up-regulation of their antigen presenting capacity. Migration of DC from the site of antigen capture to the secondary lymphoid organ is crucial for both the initiation and amplification of primary immune responses [37]. As mentioned above, migration is dependent on chemokine receptors and the corresponding ligands as well as secreted cytokines. In steady state, DC reside in both peripheral tissues and lymphoid organs and they also circulate in the blood. In this thesis we have been looking at monocyte derived DC, DC-SIGN$^+$ immature DC (iDC), DC-LAMP$^+$ mature DC (mDC) and CD303$^+$ plasmacytoid DC in tissue from both *H. pylori* infected and uninfected individuals.

Human dendritic cell subsets

DC are a heterogeneous group of cells where different subtypes differ in location, migratory pathway, and detailed immunological function. There are relatively few studies on mature human DC freshly isolated from tissue compared to studies on DC from mouse. Most of the insights into human DC subsets and their developmental origins have come from studies of development in culture from iDC or pDC.

Different DC subtypes arise from separate developmental pathways but their development and function are modulated by exogenous factors. Therefore, it is important to study the dynamics of the DC in response to microbial invasion There are two main pathways of DC ontogeny from hematopoietic progenitor cells. One pathway generates myeloid DC, while another generates plasmacytoid DC (pDC) (Fig. 2).

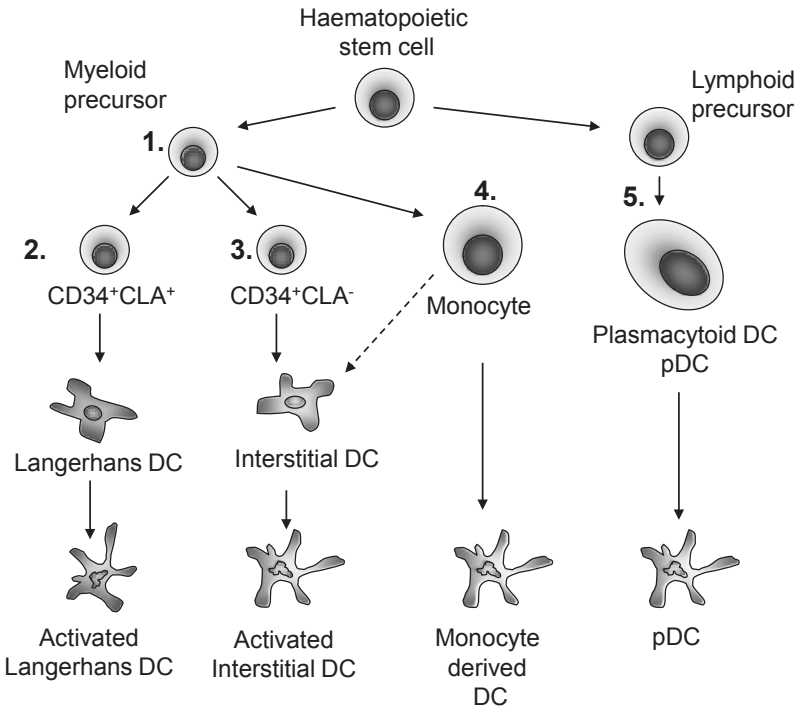

Figure 2. Pathways of dendritic cells (DC) development.

1. **Myeloid CD34+ precursor** cells, isolated from bone marrow or umbilical-cord blood. When these cells are in culture supplemented with GM-CSF and TNF-α, they give rise to two separate pathways of DC development: $CD34^+CLA^+$ and $CD34^+CLA^-$. Culture together with TGF-β gives rise to $CD34^+CLA^+$ cells and subsequently Langerhans DC.
2. **Langerhans DC** arise from the $CD34^+CLA^+$ pathway. This is a separate DC subtype which exhibits Birbeck granules and express CD1a, langerin and E-cadherin.
3. **Interstitial DC** also arise from the CD34+ precursor but the intermediates along this pathway lack cutaneous lymphocyte-associated antigen (CLA, a skin homing receptor) and CD1a, but express CD14 and resemble blood monocytes. In skin, two distinct types of myeloid DC are found in two distinct layers. Langerhans DC reside in the epidermis, while interstitial/dermal dendritic cells DC are present in the dermis [44].

4. **Blood monocytes.** These are the most common precursor cells for generating human DC in culture. In the presence of macrophage colony-stimulating factor (M-CSF) they generate macrophages but in the presence of GM-CSF and IL-4, iDC are produced. Maturation is induced by pro-inflammatory cytokines or microbial products. In paper I we have used monocyte derived DC during the *in vitro* studies. However, the *in vivo* significance of this route of DC generation is not clear.
5. **Plasmacytoid DC (pDC)** express lymphoid markers and lack myeloid surface markers. They are found in blood and many lymphoid tissues. In this thesis pDC were assessed by staining for CD303 (BDCA-2). After stimulation pDC gain a more dendrite phenotype.

Myeloid DC exist in at least three compartments, peripheral-tissue-resident DC, secondary lymphoid organ-resident DC and circulating blood myeloid DC. In mice, all DC express the CD11c integrin in varying amounts and MHC II molecules. They are further distinguished by their differential expression of CD8α, CD4, CD11b, Langerin and PDCA-1 as well as a growing list of other markers [45]. However, human DC lack the expression of CD8 on the surface which excludes comparison with mouse $CD8^{+}$DC.

Monocyte derived DC

Blood monocytes are circulating cells that may consist of several subpopulations of cells that differ in size and function.Two subsets of monocytes have been identified in humans, mice and rats [46-48]. One population corresponds to the main monocyte population present in humans, i.e. the classical $CD14^{hi}CD16^{low}CCR2^{hi}$ sub-population that constitutes 90-95% of total monocytes. These behave in a similar way to the murine $CX_3CR1^{low}CCR2^{hi}Gr\text{-}1^{hi}$ monocytes, which only constitute 50% and 10-20% of total monocytes, in mice and rats respectively. It has been reported that murine $CX_3CR1^{low}CCR2^{hi}Gr\text{-}1^{hi}$ monocytes are so called inflammatory monocytes, since they are recruited to inflammatory tissue [49]. The other sub-population of monocytes identified in humans, the $CD14^{low}CD16^{hi}CCR2^{low}$ monocytes, resembles the $Gr\text{-}1^{low}$ murine monocytes in that they are smaller in size and less granular. The murine $CX_3CR1^{hi}CCR2^{low}Gr\text{-}1^{low}$ have been proposed to be a resident cell population while human $CD14^{lo}$ $CD16^{+}CCR2^{-}$ monocytes are called proinflammatory monocytes.

However, it has also been described that $Gr\text{-}1^{low}$ monocytes are recruited during infection [50] and that they migrate to the lungs, brain, and gut independently of inflammation [46]. This

indicates that specific recruitment strategies of inflammatory and resident monocytes may exist. When monocytes have been recruited to the tissue, they differentiate either into macrophages or myeloid DC. These myeloid DC migrate to the draining lymph node in response to an inflammatory signal and following antigen capture. In addition, monocytes have also been shown to enter inflammatory lymph nodes through HEV [51, 52]. However, if monocytes migrate from the blood to the tissue and from the tissue to the lymph node without stimuli is not clear.

Treatment of blood $CD14^+$ monocytes with interleukin 4 (IL-4) and granulocyte-macrophage colony stimulating factor (GM-CSF) is used for generating human iDC in culture [53]. In the presence of macrophage colony-stimulating factor (M-CSF), blood monocytes will instead generate macrophages ($CD68^+$). The iDC derived from $CD14^+$ monocytes are then stimulated by cytokines or bacterial components to become mDC.

Lymphoid-tissue resident DC

Lymphoid tissue–resident DC are the most studied DC populations in mice, but little information is available on their human counterparts. Lymphoid-tissue resident DC are resident in lymphoid organs and do not migrate, instead they collect and present antigen in the lymphoid organ itself. In the thymus and spleen most of DC are lymphoid tissue-resident DC. However, even in the lymph nodes around half the DC in steady state seems to be lymphoid-tissue resident DC rather than migrants from the lymphatic vessels [54]. The migrating DC have a mature phenotype when arriving into lymph nodes, with a shut down of antigen uptake. By contrast, the lymphoid tissue-resident DC have an immature phenotype ready to capture and process antigen [54].

$DC\text{-}SIGN^+$ immature DC ($DC\text{-}SIGN^+$ iDC)

DC sub-populations can be identified by their expression of cell surface markers. DC-SIGN is a specific DC marker that is highly expressed by myeloid iDC. It is a C-type lectin that has a high affinity for the ICAM3 molecule and binds various microorganisms by recognizing high-mannose-containing glycoproteins on their envelopes. However, small subsets of macrophages have also been shown to express DC-SIGN [55].

DC-SIGN acts both as a pattern recognition receptor and as an adhesion molecule. As an adhesion molecule, DC-SIGN is able to mediate rolling and adhesion over endothelial cells under shear flow. ICAM-2 is abundantly expressed by vascular and lymph endothelium [56] and the interaction between DC-SIGN on DC and ICAM-2 on endothelial cells is strictly

glycan-specific [57]. The DC-SIGN-ICAM-2 interaction also regulates chemokine-induced transmigration of DCs across both resting and activated endothelium [58].
Further, it has been reported that ICAM-3 expressed by resting T cells is important in the initial contact with DC. It has been described that DC-SIGN and not the common ICAM-3 receptors LFA-1 and αDβ2 binds ICAM-3 with high affinity.
H. pylori as well as other pathogens bind to DC-SIGN via pathogen-associated molecular patterns (PAMPs) consisting of high mannose and / or Lewis (Le) blood group antigens [59]. Several of these pathogens, such as human immunodeficiency virus (HIV-1), target DC-SIGN to modulate DC functions and escape immune activation [60, 61]. A further finding is that *H. pylori* phase variation influences the production of Th1 and Th2 inducing cytokines by the DC, thereby contributing to shaping the resulting T cell response [59].

CD303$^+$plasmacytoid DC

CD303 is a calcium dependent type II lectin also known as Blood-Dendritic-Cell-Antigen-2 (BDCA-2), and is specifically expressed by human pDC [62]. They are found in blood and many lymphoid tissues, entering lymph nodes by L-selectin dependent interactions. pDC are a subtype of circulating dendritic cells which express intracellular MHC-II. They express the surface markers CD123, CD303 and CD304, but not CD11c or CD14, and their main function is to produce large amounts of type I interferon upon detection of viral and bacterial nucleic acids [63, 64]. In steady state they resemble plasma cells but upon viral stimulation they produce type 1 interferon [64] and also convert from plasmacytoid morphology to a more dendritic shaped cell and acquire some DC antigen-processing and antigen-presentation properties [64] .

Maturation of DC

As mentioned above, migration of DC from the site of antigen capture to the secondary lymphoid organs and the simultaneous maturation is crucial for both initiation and amplification of primary immune responses [37]. The maturation processes include changes in morphology and the acquisition of cellular motility, the loss of endocytic / phagocytic receptors and secretion of chemokines according to the type of immune cells that need to be attracted.
DC capture and process antigen as immature DC but upon exposure to inflammatory cytokines and bacterial components such as tumour necrosis factor (TNF)-α, interleukin (IL)-1, and LPS they transform into mature DC that are capable of delivering co-stimulatory

signals and activate naïve T cells. Molecules important for activation and costimulation of T cells, such as major histocompatibility complex class I and II (MHC- I and -II, CD40, CD80 and CD86, are also induced. Maturation also correlates with down-regulation of inflammatory chemokine receptors, involved in tissue-specific homing, such as CCR1, CCR2, CCR5 and CXCR-1 and up-regulation of the chemokines receptor CCR7 and CXCR4 [65, 66]. DC-LAMP is a type I transmembrane glycoprotein that is absent on iDC but is rapidly upregulated upon maturation of DC [67]. It is expressed in the endosomal/lysosomal compartment and may be involved in MHC-II processing and promotes Th1-type responses.

Pathogen recognition through pattern recognition receptors (PRRs) activates DC to increase their expression of the chemokine receptor CCR7. It has been shown in CCR7 knock out mice that the interaction of CCR7 and its ligand CCL19 is important for the generation of primary immune responses [68]. The interaction with antigen also leads to the secretion of cytokines. Maturation of DC and the acquisition of antigen presenting capacity are also associated with expression of DC-LAMP [67]. However, a mature phenotype does not necessarily correlate with a functional immunogenic stage of the DC, but can in fact be related to DC that induce tolerance [69].

DC as antigen presenting cells

DC are the only antigen presenting cells (APC) that can activate naïve T cells. DC are among the first cells to recognize an incoming pathogen through a set of PRR. DC present endogenous antigens on MHC-I for CD8 T cells. Exogenous antigens are presented on MHC-II for CD4 T cells. Peptides from endogenous antigens are generated by proteolytic degradation by proteasome or other enzymes in the cytosol. Thereafter the peptides are transported to endoplasmic reticulum (ER) via TAP and loaded on MHC-I. Exogenous antigens on the other hand are captured by surface receptors and internalize into intracellular membrane-bound vesicles, called endosomes. Endosomes contain proteolytic enzymes that degrade the protein into peptides. MHC-II is synthesized in the ER and transported to the endosomes. Peptide loading onto MHC-II occurs in endosomal compartments after cleavage of the stabilizing invariant chain and HL-DM mediated exchange of the invariant chain peptide, CLIP, which is present in the peptide binding groove. DC are also able to recycle antigen back to the surface in an intact form where it can be presented to antigen-specific B cells. They also have the ability to ingest virus or tumor cells and present these antigens on

MHC-I molecules and as APC inducing the primary response of $CD8^+$ T cells. This process whereby DC present antigens from other cells to T cells is called cross-presentation.
Naïve T cells need two signals from the APC for specificity, activation and proliferation. The first signal is when DC presents the peptide-MHC complexes to naïve T cells and the second signal is provided by co-stimulatory molecules, CD80 and CD86 (collectively called B7), that bind to CD28 on the T cell. Activated T cells express CD40L on their surface, which binds to CD40 on APC, and deliver signals that further enhance the expression of B7 on the APC. In addition, mDC determine the character of the ensuing immune response by secreting cytokines that drive the development of T cells into T helper cell type 1 (Th1), type 2 (Th2), Th17 or T regulatory effector cells [69, 70]. DC are a major source of IL-12, whereas IL-4 is mainly produced by activated T cells. IL-12 induces differentiation of naïve CD4+ T cells to Th1, which mainly produce IFN-γ, TNF-α and IL-2, and promote cell-mediated immunity [71]. In contrast, IL-4 dives Th2 polarization, resulting in T cells that mainly produce IL-4, IL-5, IL-10 and IL-13 and promote humoral immunity.

B cells

The main functions of B cells are to produce and secrete antibodies and to develop into memory B cells. Every day the human body makes over a million different types of B cells that circulate between the blood and lymph. After B cells encounter their specific antigen and are activated by signals from $CD4^+$ T helper cells, they differentiate into either plasma cells or memory B cells. In response to T cell activation, isotype switch to IgG, IgA and IgE occurs. The most abundant isotype produced in the human body is IgA which is produced at a level of around 3 g/day. As much as 80 % of all IgA-antibody-secreting cells reside in the gut mucosa [72, 73]. Dimeric IgA, is produced by plasma cells in the lamina propria. It is secreted as a dimer that is held together by the coordinately produced J chain. IgA are then transported through the epithelial cell by binding to the poly-Ig receptor at the base of an epithelial cell and secreted into the gut lumen [74]. In the lumen the newly created secretory IgA (SIgA) adhere to bacteria or bacterial toxins and thus inhibit bacterial adhesion or penetration through the mucosal barrier in the stomach [74]. Therefore, migration and homing of B cells and ASC to the gut is critical for protection against intestinal pathogens [75, 76].

Development of B cells

B cells develop from haematopoitic stem cells in the bone marrow and fetal liver [77]. Bone marrow-derived haematopoitic stem cells give rise to B2 B cells while fetal liver-derived haematopoitic stem cells give rise to B1 B cells. The majority of B cells are B2 B cells and these can further be divided into marginal zone B2 B cells and follicular B2 B cells. Follicular B cells are also called mature or recirulating B cells, and they express IgM and IgD on the surface and migrate between lymphoid organs. Development in the bone marrow occurs through several stages where each stage corresponds to a change in the antibody loci. Antibodies are made up of two light (L) and two heavy (H) chains. The H chain loci contain three regions, V, D and J, while the L loci contain two regions, V and J. Each B cell has a unique B cell receptor (BCR) on the surface, specific for one specific antigen, creating a great diversity of the BCR repertoire. During VDJ recombination, V_H, D_H, and J_H sequences are randomly combined to produce a unique variable domain in the immunoglobulin of each B cell. The process is the same for the L chain locus except that there are only two regions. Before B cells are released into the blood and lymph they are selected on the basis not to react with self antigen, if they do they will undergo apoptosis.

B cell activation

As mentioned before, naïve mature B cells migrate between the blood and the secondary lymphoid tissue until they meet their specific antigen. B cells recognize their specific free antigen in its naïve form using their BCR. T cells, on the other hand, only recognize the antigen in a processed form, bound to MHC I or MHC II on an antigen presenting cell. However, when BCR have bound the specific antigen, it is internalized into endosomal vesicles and subsequently presented on MHC II for $CD4^+$ T cells. Activated $CD4^+$ T cells express the surface molecule called CD40 ligand (CD40L). Ligation of CD40L with the CD40 receptor, expressed on B cells, leads to proliferation and differentiation of the specific B cell clone which results in generation of plasma cells that secrete antibodies, and memory cells [78].

Interaction between B and T cells is mediated at the border of the B cell follicle and the signals result in formation of a germinal center (GC). The secondary follicle up-regulates the chemokine ligand CXCL13, which attracts CXCR5 positive B cells to migrate into the follicle. The GC reaction is responsible for the generation of high affinity plasma cells (ABS) and memory B cells, and the GC can be divided into a light and a dark region [79]. Follicular DC seem to provide B cells with signals for rapid proliferation as well as survival signals throughout the germinal centre reaction. In the dark zone, the Ig gene of the B cells hypermutates before the cells migrate to the light zone. In the light zone, B cells carrying high affinity antibodies are selected and the cells also undergo class switch recombination to form IgA, IgE or IgG isotypes.

B cells will further differentiate into either long-lived plasma cells secreting high-affinity antibodies against their specific antigen or circulating memory B lymphocytes expressing high-affinity antibodies ready to efficiently respond if re-infection occurs [80]. T cells mediate the differentiation of the B cells by secreting IL-4, which drives differentiation of B cells to memory B cells, or IL-10, which results in differentiation into plasma cells.

Antibodies, also known as immunoglobulins (Ig), are gamma globulin proteins that identify and neutralize foreign objects such as bacteria and virus. Antibodies produced in the lymph nodes, spleen or bone marrow enter the blood and circulate to the site were the antigen is located. High affinity antibodies eliminate pathogens by several different mechanisms dependent on the antibody isotype. The different isotypes activate and recruit components of the immune system. Secreted antibodies perform different effector functions such as neutralizing antigens, activating complement and promoting leukocyte destruction of

microbes. The primary functions of SIgA at mucosal sites are thought to be to prevent pathogens from adhering to the host cells as well as to neutralize the toxins [81]. Dimeric IgA is produced by plasma cells in the lamina propria and secreted into the lumen. *H. pylori* infection is associated with a large accumulation of IgA secreting cells in the gastric mucosa [82].

DC are not only essential for T cell activation; they also influence B cell responses [83]. Thus, DC are also able to present unprocessed antigen to B cells *in vivo*. Since antigen-exposed DC also induce humoral immunity, DC must also retain antigen in its native state for the engagement of BCR on B cells [84]. It has been shown that DC can regulate the antibody isotype switch to IgA by production of BAFF and APRIL in a T cell independent class switch recombination [85, 86].

B cells homing to mucosal tissues

Homing of lymphoid cells is dependent on tissue-specific adhesion molecules together with tissue specific production of chemokines. As mentioned before, the extravasation from blood into tissue is a multistep process involving rolling along the vessel wall, loose interaction between selectins and their ligands, and chemokines that induce firm adhesion by activating surface integrins. During inflammation, endothelial cells may increase or decrease expression of existing homing receptors or up-regulate new ones.

Tissue-specific homing of B cells to gastrointestinal mucosa is dependent on the expression of the mucosal homing receptor integrin $\alpha4\beta7$ [17] which interacts with MAdCAM-1, expressed by endothelial cells in Peyer's patches and the gastrointestinal mucosa [19, 20]. Chemokines also help to direct transmigration into the surrounding tissues. The site where the pathogen enters the body upon infection determines the microenvironment where naïve B cells are activated. This in turn influences the homing commitment of the effector B cells (ASC). It has been shown that DC in Peyer's patches and DC from the lamina propria of the small intestine in mice can imprint $\alpha4\beta7$, CCR9 and gut-homing capacity on ASC [87, 88]. CCL25 (TECK) is a chemokine which is mainly expressed by epithelial cells in the small intestine and its receptor is CCR9. [25-27]. The chemokine CCL28 (MEC) is another chemokine that guides cells to the tissue. CCL28 is expressed in most mucosal tissues and its receptor CCR10 is expressed on IgA^+ plasmablasts from all mucosal sites that have been examined [23, 24, 89].

Helicobacter pylori

The discovery of the bacterium *Helicobacter pylori* has changed the notion that the human stomach was sterile and containing no bacteria. The human stomach produces extensive amounts of acid and the conventional thinking around 1982/83 was that no bacterium could live in this environment. However, in 1983 Dr Barry J. Marshall and Dr J. Robin Warren from Australia were able to culture the gram negative, microaerophilic, spiral shaped bacterium that lives in the human gastric mucosa from human gastric biopsies [90]. Infection with *H. pylori* is widespread in humans and approximately half of the world's population is estimated to be or to have been infected [91, 92]. *H. pylori* is habitat specific and only colonize the human gastric mucosa and the gastric-like metaplasia in the duodenum. The infection is usually acquired in childhood and is often life-long in the absence of antibiotic therapy. The transmission route is thought to be oral-oral or fecal-oral even if it has not been completely elucidated [93]. However, a recent study indicated that the predominant route of *H. pylori* transmission is likely to be other than waterborne [94]. Although all infected individuals develop gastritis, most individuals remain more or less asymptomatic (approximately 85%). However, about 10-15% of all infected individuals develop gastric or duodenal ulcer disease and 1% develop gastric adenocarcinoma or mucosa associated lymphoid tissue lymphoma (MALT lymphoma) (Fig. 3). The risk of development of these disorders in the presence of *H. pylori* infection depends on a variety of bacterial, host and environmental factors that are related to the pattern and severity of gastritis (Fig. 3). *H. pylori* infection in the human stomach results in active chronic gastritis in almost all infected individuals. Furthermore, in individuals with low acid secretion and pangastritis, atrophic gastritis associated with intestinal metaplasia may occur and these individuals have an increased risk of developing gastric adenocarcinoma (Fig.3) [95]. The *H. pylori* bacterium is one of the most genetically variable organisms and it was early realized that almost every infected individual has a unique strain of the bacteria [96]. The current treatment against *H. pylori* is a combination of two different antibiotics together with a proton-pump inhibitor [97].

The infection leads to a large infiltration of immune cells such as neutrophils, macrophages, DC, and T and B cells into the gastric mucosa [98-100]. These cells are found scattered in the lamina propria but they also form organized lymphoid follicles, which are not present in the uninfected mucosa [101]. In particular, *H. pylori* infection gives rise to a large accumulation of IgA-secreting cells in the gastric mucosa, many of which are specific for *H. pylori*

virulence factors [82]. Earlier studies have shown that α4β7-MAdCAM-1 interactions do not seem to explain the increased B cell migration to the *H. pylori* infected gastric mucosa. Even though the *H. pylori* infection induces an inflammatory response, the immune response fails to eradicate the bacteria and the infection becomes chronic. Still very little is known about the role of DC in the immune defence of the human stomach.

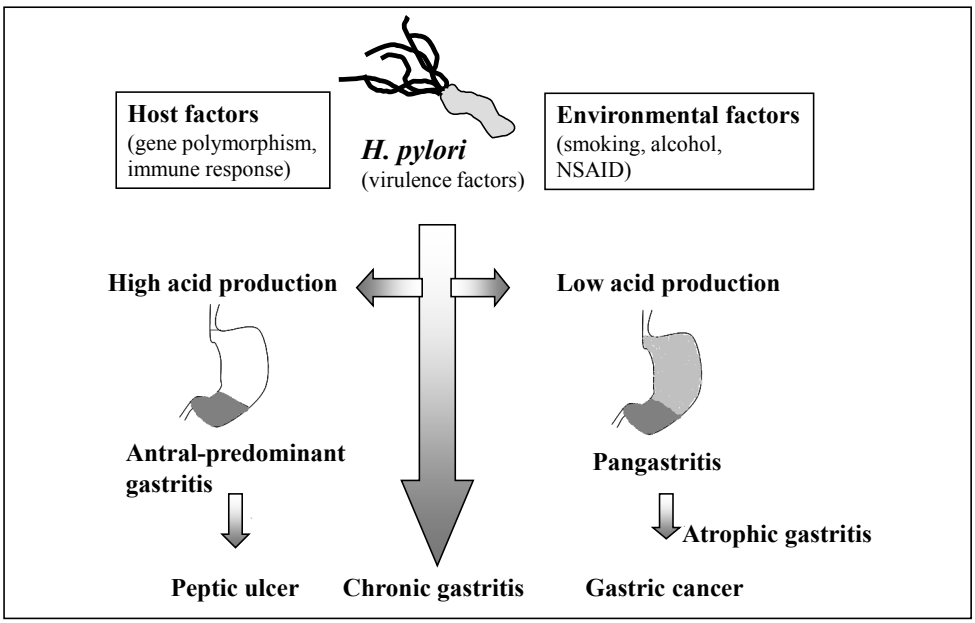

Figure 3. Factors that contribute to gastric pathology and disease outcome in *H. pylori* infection.

***H. pylori* virulence factors**

H. pylori have several virulence factors such as an ability to swim through the mucus, enzymes that protect the bacteria from the acidic environment, proteins that are involved in the attachment to epithelial cells, a secretion system that can inject proteins into host cells and proteins on the surface that attract leukocytes. These factors permit *H. pylori* to colonize and survive in the hostile environment (Fig. 4).

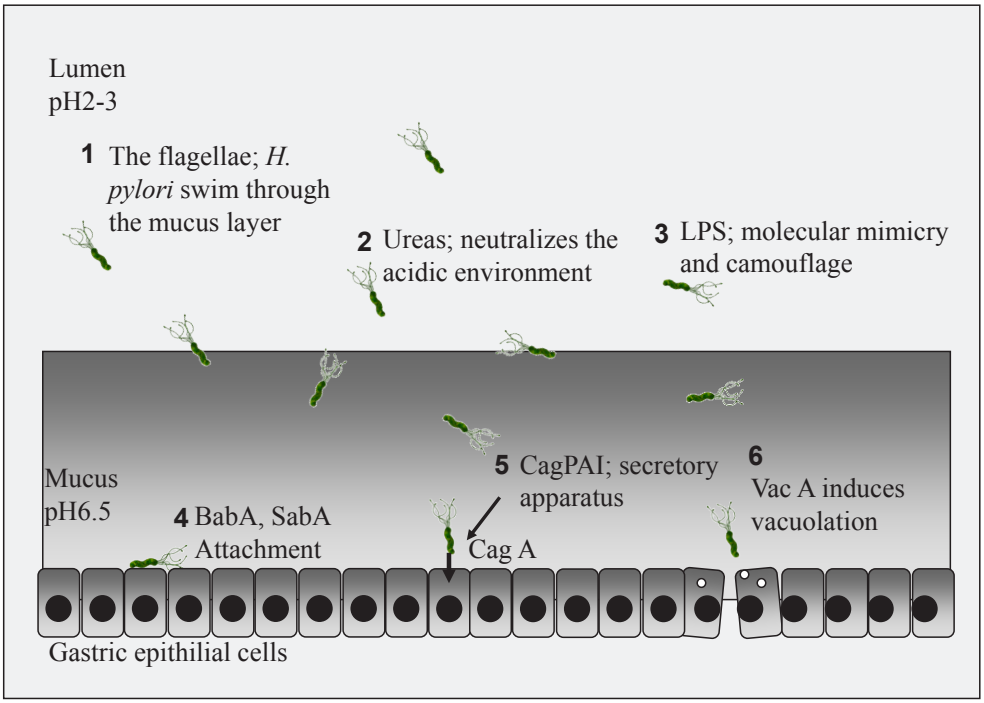

Figure 4. *H. pylori* virulence factors is involved during colonization in the gastric mucosa.

1. Flagellae: *H. pylori* can move through the mucus layer due to its helicoidal shape and because they have 4-8 polar flagellae that function as a propeller. *H. pylori* senses the pH gradient within the mucus layer and moves away from the acidic contents of the lumen towards the more neutral pH environment of the epithelial cell surface.

2. Urease: Urease is an enzyme that converts gastric urea to ammonia and carbon dioxide and in this way buffers the intracellular pH in *H. pylori*, although the human stomach is highly acidic. Urease protects the bacteria from the acidic environment and enables colonization of the gastric mucosa.

3. LPS: LPS is a major component in the membrane of gram-negative bacteria. It consists of three domains, lipid A, a core oligosaccharide, and an O-specific polysaccharide chain. Lipid A anchors the molecule in the outer membrane and is responsible for the endotoxic activity. However, *H. pylori* LPS is known to have a much lower biological activity compared to *E. coli* or *Salmonella* LPS [102]. The core oligosaccharide attaches directly to lipid A and

additional sugars are attached to the core oligosaccharide, forming the o-antigens that comprise the outermost domain of the LPS molecule. O-antigens (the outer carbohydrates) are the most variable portion of the LPS molecule, imparting the antigenic specificity. In contrast, lipid A is the most conserved part. Furthermore, LPS from *H. pylori* strains contains Lewis (Le) blood group antigens and it has been suggested that the bacteria can escape immune responses by mimicking the host [103].

4. Adhesins: Gastritis induced by *H. pylori* is triggered by *H. pylori* attaching to epithelial cells [104]. The best characterized adhesin is blood group antigen binding adhesin (BabA) that binds to Lewis b receptors [105] and the sialic acid binding adhesin (SabA) that binds to sialyl-Le x [106] in the gastric mucosa, but other adhesins are also believed to exist. Once attached to the gastric epithelial cells *H. pylori* inject effectors molecules into the gastric epithelial cells or the lamina propria.

5. Vacuolating cytotoxin A (VacA): The VacA protein has the ability to induce vacuoles in the mucosal epithelial cells and to cause epithelial erosion [107]. It has also be found that VacA can induce apoptosis in epithelial cells [108]. The gene consists of one s-region (s1a, s1b or s2) and one m-region (m1 or m2). The s-region is important for the toxic activity of the protein while the m-region is involved of the binding of the protein to epithelial cells. The s1/m1 genotype is most virulent while strains expressing s2 fails to release the toxin.

6. The cytotoxin associated gene A (Cag A) and pathogenicity island (PAI): The *cag* PAI encodes a type IV secretion system (T4SS) which is activated and induces insertion of the Cag A protein into the host cell upon binding of the bacteria to the gastric epithelial cells. Not all strains have this T4SS. However, strains with an intact *cag* PAI (type I strains) have been shown to be more virulent than strains that lack *cag* PAI (type II strains). Type I strains are also associated with activation of the NF-κβ complex and increased production of cytokines, especially IL-8, by epithelial cells, that recruit neutrophils to the infected tissue [109].

Immune responses in *H. pylori* infection

Infection with *H. pylori* results in a strong immune response against the bacterial strain. However, the strong immune response seldom results in clearance of the infection and the activities of the host immune response are more associated with the pathology rather than with direct bacterial activity. The nature of the cells that first interact with the bacteria is

important for the different cytokines, chemokines and interleukins that they released when activated. *H. pylori*-induced active gastritis is triggered by *H. pylori* attaching to epithelial cells [104] and once this has occurred they inject effectors molecules into the gastric epithelial cells or the lamina propria. Following contact with *H. pylori*, the gastric epithelium produces IL-8, a strong chemotactic and activating chemokine for neutrophils [109]. Increased infiltration of neutrophils to the gastric mucosa results in a higher concentration of reactive oxygen, which, together with bacterial toxins will damage the epithelial layer in the mucosa. *H. pylori* infection also induces up-regulation of CCL20 (MIP-3α) gene expression in gastric epithelial cells during colonization resulting in an influx of monocytes into the lamina propria of the gastric mucosa [110]. Activation of macrophages also results in release of several pro-inflammatory cytokines such as IL-12, IFN-γ, TNF-α and IL-1β, that will recruit effector cells to the infected tissue. DC present antigen to naïve T lymphocytes and this results in two different subtypes of T cells, Th1 and Th2 cells. Somewhat simplified, Th1 cells secrete IL-2, IFN-γ and TNF-α and are most active against intracellular pathogens. Th2 cells on the other hand secrete IL-4, IL-5, IL-13 and IL-10 and are required for stimulation of B cell and antibody production necessary for extracellular pathogens. Recent studies have suggested that the type of T cell responses may by responsible for the host's inability to clear the infection. Furthermore, the presence of regulatory T cells ($CD4^{+}CD25^{high}$) down-regulate the memory T cell response to *H. pylori* in the blood of asymptomatic *H. pylori* infected individuals [111]. The number of $CD4^{+}CD25^{high}$ cells was also increased in infected antral and duodenal mucosa compared to uninfected mucosa [112]. It has also been reported that DC generated in the continued presence of bacteria in the gut may have an altered phenotype and that this leads to exhausted DC [113].

Activation of DC by microbes is mediated by pattern recognition receptors including the Toll-like receptors (TLR) and it has been shown that intracellular TLR (2 and 4) signalling by bacteria is necessary for an optimal DC response [114]. DC have been found to interact with *H. pylori* glycoconjugates *in vitro* through DC-SIGN/CD209 [115], and to undergo maturation and activation after incubation with *H. pylori* products such as LPS, urease, or outer membrane proteins (OMPs) [116-118].

H. pylori appear not to be invasive and rarely infiltrate the gastric mucosa. However, reports have shown the presence of whole bacteria or the non-culturable bacteria in coccoidal form in the gastric antrum [119-125]. This indicates that *H. pylori* are able to cross the epithelial membrane. Necchi et al. have also shown that at sites of dense *H. pylori* infection and active

inflammatory response, DC appear to be present inside the gastric epithelium, thereby gaining direct access to luminal *H. pylori.* Other cells belonging to the innate immune system are also recruited to the *H. pylori* infected epithelium, and the same authors have shown that these cells are able to phagocytose bacteria and /or to accumulate VacA, OMP and urease in their phagolysomes or cytoplasm. This may play a role in the outcome of innate and the adaptive immune responses [126].

During *H. pylori* infection lymphoid follicles are formed and it is not clear if these can support local antigen presentation and B cell differentiation into antibody-secreting cells. During B and T cell interaction a germinal centre is formed at the border of the follicle and activated B cells migrating into the B cells zone of the follicle.

Our group has shown that gastric IgA responses were not dependent on local antigen uptake and processing but were caused by an increased recruitment of circulating plasma cell precursors [127]. However, when comparing *H. pylori* infected and uninfected tissue there was no difference in MAdCAM-1 expression which indicates that something else recruits lymphocytes to the gastric mucosa during *H. pylori* infection [128].

DC in the gastric mucosa

As mentioned before, chemokines are essential for the recruitment of cells to the tissue. Resident and immigrating DC are important for the immune response and dependent on the cytokines and chemokines present in a given tissue. It has been shown that there is an increased production of CCL20 by the gastric epithelium of patients with *H. pylori*-associated gastritis [129]. CCL20 is a known chemo-attractant for both iDC and memory T cells through interaction with CCR6 [130-132]. Immunohistochemical analysis has shown that CCL20 protein expression is localized exclusively in the mucosal epithelium [133], and that there was an increased expression of CCR6 on $CD3^+$ T cells infiltrating the gastric mucosa. Thus, up-regulation of CCL20 may maintain the recruitment of immature myeloid DC and memory T

Very little is know about the role of DC in the mucosa immune system in the stomach. We and others have shown that monocyte derived DC are activated and secrete cytokines when they are cultured in the presence of *H. pylori* [116-118, 134, 135]. More recent studies have also shown that MyD88-dependent Toll-like receptor (TLR) signaling is a crucial pathway by which DC sense *H. pylori* and become activated [136, 137].

Further, DC with a mature phenotype have been identified in gastric mucosa in humans and mice with autoimmune gastritis [138] and also within proliferating T cell clusters in colonic

tissue affected by Crohn's disease [139]. The intestinal inflammation seen in Crohn's disease may give indications as to what is happening in the gastric mucosa. For example, an increased expression of the lymphoid chemokines CCL19 and CCL21 was found in Crohn's disease versus normal colonic tissue, which indicates that there is a microenvironment in colonic tissue normally found in lymph nodes [139]. Plasmacytoid DC on the other hand were randomly distributed within the lamina propria and submucosa in colonic tissue and not associated with a T cell cluster [139]. However, DC have also been characterized in the subepithelial dome in ileal Peyer's patches from individuals with Crohn's disease [140]. These results show an increased number of DC and an unusual phenotype of mDC expressing CD83 but not the chemokine receptor CCR7 [140] in the subepithelial dome. This is of interest since it has been suggested that Peyer's patches in the small intestine play a critical roll in *H. pylori*-induced gastritis [141, 142]. *H. pylori* antigens can easily access the intestinal lumen where they can induce mucosal immune responses in the well organized Peyer's patches. It has been shown that the development of *Helicobacter*-induced gastritis and the production of anti-*Helicobacter* antibodies were severely impaired in *Helicobacter*- infected mice that lack Peyer's patches [141, 142]. CD83+ mature DC have also been localized in the vicinities of injured bile ducts in liver from patients with primary biliary cirrhosis [143]. Further, Katou et al have analysed the nature of T cell-mDC (DC-LAMP+) interactions in chronically inflamed skin and lymph nodes. These results show a difference between the function of mDC in lymph nodes compared to chronically inflamed skin. The majority of DC-LAMP$^+$ mDCs in the T-cell area of lymph nodes expressed the chemokine CCL19 and was surrounded by CCR7$^+$ naïve lymphocytes. However, the majority of DC-LAMP$^+$mDC in inflamed skin were totally negative for CCL19 and were surrounded by CCR7$^-$ memory-type T cells [144]. The interaction seen between T-cells and DC-LAMP$^+$ DC in chronically inflamed skin may give indications that there are differences between different sub-populations of mDC in the chronically inflamed gastric mucosa and lymph nodes. Inflammation induced by *H. pylori* infection is characterized by high levels of IFN-γ, also known as a Th1 response. Thus, although *H. pylori* bacteria are generally considered as non-invasive, gastric DC may come into contact with whole bacteria as well as free antigens that penetrate the epithelial layer. It has also been suggested that gut DC may sample the antigen in the lumen by opening tight junctions between epithelial cells [126]. Studies have shown that in some areas of small intestine, LP DC extends their dendrites through the epithelial layer into the lumen by forming tight junctions with adjacent epithelial cells [145].

AIMS OF THE THESIS

The main objective of this thesis was to investigate migration of DC and homing of B cells in *H. pylori* associated gastritis. The specific aims were:

- To determine if *H. pylori* is able to induce maturation and migration of human DC.

- To analyse gastric DC subpopulations *in vivo* and elucidate if DC are retained in the *H. pylori* infected gastric mucosa.

- To investigate the role of chemokines in and B cell homing to the human *H. pylori* infected gastric mucosa.

MATERIALS AND METHODS

Volunteers, patients, and collection of specimens

This study was approved by the regional research ethics committee of west Sweden, and informed consent was obtained from all participants. Participating donors were recruited among blood donors at Sahlgrenska University Hospital after serologic analysis and *H. pylori* infection was subsequently confirmed or excluded by culture on Scirrow plates [146] and pathology reports. One biopsy from each volunteer was fixed in formalin, paraffin embedded, and examined by an experienced histopathologist for the grade of gastritis and the presence of *Helicobacter*-like organisms (HLO) using the updated Sydney system [146].

Paper II

For immunohistochemical staining gastric antrum biopsies were collected from eight *H. pylori* infected (males, aged between 34-66 years) and ten uninfected volunteers (5 males and 5 females, aged between 29-70 years) by endoscopy.

For RNA preparation gastric antrum biopsies were collected from five *H. pylori* infected (2 males and 3 females, aged between 36-68 years) and eight uninfected volunteers (4 males and 4 females, aged between 27-54 years) by endoscopy. Biopsies from the same volunteers as for RNA preparation were also collected for protein extraction together with biopsies collected from three different *H. pylori* infected and one *H. pylori* uninfected volunteers.

Paper III

Eight *H. pylori*-infected volunteers (two females and six males, aged 26 to 60 years) and eight uninfected volunteers (three females and five males, aged 24 to 34 years) were recruited from blood donors at Sahlgrenska University Hospital by serological screening. Ten antrum biopsy samples were collected from each volunteer by endoscopy. Three biopsy samples were immediately embedded in OCT compound and frozen in liquid nitrogen for immunofluorescence analysis, two biopsy samples were immediately frozen in liquid nitrogen for RNA purification, and four biopsy samples were collected on ice for protein extraction. Gastric tissues from 14 individuals (7 females and 7 males, aged 30 to 80 years) undergoing gastric resection due to gastric adenocarcinoma (n = 7), severe gastric dysplasia (n = 1), duodenal adenocarcinoma (n = 1), bile duct carcinoma (n = 1), endocrine gastrointestinal stromal tumor (GIST; n = 1), pancreatic carcinoma (n = 2), or chronic pancreatitis (n = 1) were used to isolate gastric lymphocytes for migration experiments. Directly after

gastrectomy, a strip of gastric tissue encompassing antrum and corpus mucosa was collected. In patients with gastric cancer, tissue was collected at least 5 cm distant from the tumor. *H. pylori* status was subsequently determined by serology, as previously described [148], and 9 out of these 14 individuals were found to be *H. pylori* positive. The five uninfected individuals suffered from pancreatic (n = 2), bile duct, or gastric carcinoma or endocrine GIST. Three different enzyme-linked immunosorbent assays (ELISAs) (in-house serology for IgG and IgA antibodies and the EIA-G III ELISA from Orion Diagnostics) were used to determine *H. pylori* status, since previous studies have shown that it is not always possible to culture *H. pylori* from gastric cancer patients, even though the presence of antibodies indicates infection [149].

A second group of 19 patients (8 females and 11 males, aged 30 to 81 years) undergoing gastric resection due to gastric adenocarcinoma (n = 11), endocrine GIST (n = 1), bile duct carcinoma (n = 2), pancreatic carcinoma (n = 3), or benign gastric ulcer (n = 2) was subsequently used to isolate gastric lymphocytes for flow cytometry analyses. Ten out of these 19 individuals were found to be *H. pylori* positive, and out of the 10 positives, 9 had gastric, 2 pancreatic, and 1 bile duct tumors. None of the patients received any medication related to their cancer disease before surgery.

Bacteria and antigen preparation

H. pylori strains are highly diverse on the genetic level [150]. Due to this, different strains of *H. pylori* were selected based on their expression of the Cag PAI, Vac A, and BabA antigens for stimulation of iDC. Four strains with different characteristics were isolated in our laboratory from Swedish volunteers and one strain and the corresponding mutant were kindly provided by Dr S. Backert (University College Dublin, School of Biomolecular and Biomedical Sciences, Dublin, Ireland) [151] (Table 1). After isolation and initial culture, strains were stored in a freeze-drying medium containing 20% glycerol at -70°C. *H. pylori* bacteria were cultured on Colombia-iso agar plates for 3 days, prior to stimulation of iDC, under microaerobic conditions at 37°C. The bacteria were resuspended in PBS and adjusted to a final OD_{600} = 1, corresponding to 1.1×10^9 colony forming units (CFU) for Hel 312, 1.8×10^9 CFU for Hel 333, 3.0×10^9 CFU for Hel 340, 1.5×10^8 CFU for Hel 305, 5.2×10^8 CFU for P1 CagPAI, and 3.2×10^8 CFU for P1wt.

The *H. pylori* suspensions were then further diluted in cell culture medium and the DC were stimulated with *H. pylori* at a concentration corresponding to 10^6 CFU/ml (ratio of DC to

CFU; 1:1) or 10^8 CFU/ml (ratio of DC to CFU; 1:100) for all strains except for Hel 305 where a concentration of 10^7 CFU/ml (ratio of DC to CFU; 1:10) was used since the bacteria of this strain aggregate at higher concentrations. For comparison, DC were also stimulated with the non-pathogenic *E. coli* strain K12, which had been cultured on Luria-Bertani (LB) plates after incubation over night at 37°C. The bacteria were harvested and diluted to OD_{600}= 0.8 which corresponds to 10^9 CFU/ml, and DC were stimulated with *E. coli* bacteria at a concentration corresponding to 10^4 CFU/ml (DC:CFU; 100:1) or 10^6 CFU/ml (DC:CFU; 1:1).
DC were also stimulated with a membrane protein preparation (MP) of the *H. pylori* strain Hel 305 (5 µg/ml). The MP was prepared by sonication and differential centrifugation of the bacteria, as previously described [152]. Gel electrophoresis of the MP showed that it contained more than 20 different proteins, among which urease, neutrophil activating protein, *H. pylori* adhesin A (HpaA) and flagellin were identified by Western blotting using monoclonal antibodies specific for the different antigens [153, 154]. The MP preparation contained <50% LPS w/w, as determined by the Limulus test. LPS was purified from strain Hel 305, using hot-phenol-water extraction, as previously described (38), and DC were stimulated with 2.5 µg LPS/ml.

Table. 1 Characteristics of the *H. pylori* strains used.

Strain	Origin	Virulernce factors		
		CagPAI[1]	VacA[2]	BabA[3]
Hel 312	AS[4]	+	s1/m1	-
Hel 340	AS[4]	+	s1/m2	+
Hel 305	DU[5]	+	s1/m1	+
Hel 333	DU[5]	-	S2/m2	-
P1	NUD[6]	+	s1/m2	-
P1Δ *cag*PAI	NUD[6]	-	s1/m2	-

[1]CagPAI = Cytotoxin associated gene pathogenicity island
[2]VacA = Vacuolating toxin A, s=signal sequence,
m = middle region
[3]BabA = Blood group binding adhesin A
[4]AS = Asymptomatic individual
[5]DU = Duodenal ulcer patient
[6]NUD = Non-ulcer-dyspepsia

Isolation of PBMC, $CD14^+$, $CD4^+$ and $CD8^+$ cells from blood

Peripheral blood mononuclear cells (PBMC) were obtained from 50 ml of leukocyte-enriched buffy coat or from 70 ml of heparinized venous blood by density gradient centrifugation on Ficoll-Paque. For stimulation of T cells by mature DC a second blood sample of 70 ml was collected one week later from each volunteer. Monocytes ($CD14^+$, MACS), $CD4^+$ and $CD8^+$ T cells (Dynabeads) were isolated by positive selection using magnetic microbeads as described by the manufacturer.

Isolation of gastric lamina propria mononuclear cells (LPMNC)

Mucosal LPMC were isolated from gastric tissue from *H. pylori* infected and uninfected patients undergoing gastrectomy due to gastric adenocarcinoma, or chronic pancreatitis. The cells were isolated on the same day as the gastrectomy and used for chemotaxis and flow cytometric analysis on the following day.

Tumour-free tissue (at least 5 cm from the tumour) was collected and cut into small pieces after removal of the muscle and fat layers. The epithelium was removed by incubation in Hanks balanced salt solution (HBSS) containing EDTA and dithiothritol (DTT). Thereafter, the remaining tissue was incubated for 2 h at 37°C in collagenase and DNase to release LPMNC, which were then run through a nylon mesh to remove remaining tissue fragments. Cells used for migration assays or flow cytometric analyses were resuspended in Iscove´s medium with 5 % fetal calf serum, 50µg/ml gentamicin, and 3 µg/ml of L-glutamine at 10^6cells/ml and kept over night at 37°C in a humidified atmosphere containing 5% CO_2 (migration assay) or 4°C (flow cytometric analysis).

Generation of DC

Treatment of monocytes with interleukin 4 (IL-4) and granulocyte-macrophage colony stimulating factor (GM-CSF) leads to differentiation of monocytes to immature dendritic cells (iDCs). $CD14^+$ monocytes isolated from PBMC were cultured at 10^6 cells/ml in Iscove´s medium, supplemented with 800 U/ml of GM-CSF, 500 U/ml of IL-4, 3 µg/ml of L-glutamine, 50 µg/ml of gentamicin and 5% human AB+ serum for 7 days. Every second day, fresh medium was added to the cells. After five days of culture, different strains of *H. pylori* or *E. coli* (K12) bacteria, MP preparation or LPS from *H. pylori* (Hel 305), or 100 U/ml of human recombinant TNF-α was added to the iDC to induce maturation. iDC incubated with medium alone were used as control cells. After 24h and 48h of stimulation, supernatants were

collected. Triplicate samples were pooled and stored at -70°C until analysis of the cytokine content was performed.

DC and B cell chemotaxis assays

Chemotaxis describes cell migration either toward or away from a particular chemical signal. In our studies we analyzed, by chemotaxis assays, the migration of cells toward a chemoattractrant on the opposite side of a membrane. Migration of DC and B cells are regulated by chemokine receptors and their corresponding ligands. Migration of DC towards the ligand CCL19 or B cell migration toward the ligands CCL25, CCL28 and the positive control CXCL12 (SDF-1α) was evaluated using chemotaxis assays performed in 24-well Transwell cell culture chambers containing a 5.0 µm-pore size membrane to separate the upper and lower chambers (Fig. 5).

The upper chamber was loaded with 100 µl DC cell suspension containing 4-6 $\times$ 10^5 cells/ml after stimulation with different strains of *H. pylori* or 1-2.5 $\times$ 10^5 cells/ml (P1Δ*cag*PAI or P1 wt). The lower compartment contained 600 µl RPMI-1640 medium and 0.5 % fetal calf serum with or without CCL19 (1 µg/ml) (Paper I). After incubation at 37°C for 90 minutes, cells that had migrated into the lower compartment were collected and counted under light microscopy. The experiments were performed in duplicates and the average frequencies of DC that migrated towards CCL19 (specific migration) were compared to the average frequencies of cells that spontaneously migrated towards medium (Paper I).

In paper II, gastric LPMNC from *H. pylori*-infected and uninfected gastric cancer patients were resuspended at 5 $\times$ 10^6 cells/ml in RPMI-1640 medium with 0.5 % fetal calf serum and 50 µg/ml gentamicin. The upper chamber was loaded with 100 µl of cell suspension and the lower compartment contained 600 µl of medium, with or without CCL28, CCL25, or CXCL12. The experiments were performed in duplicates, and the chemokine concentration had been titrated in pilot experiments. After incubation at 37°C for 2.5 h, cells that had migrated into the lower compartment were collected in complete Iscove´s medium.

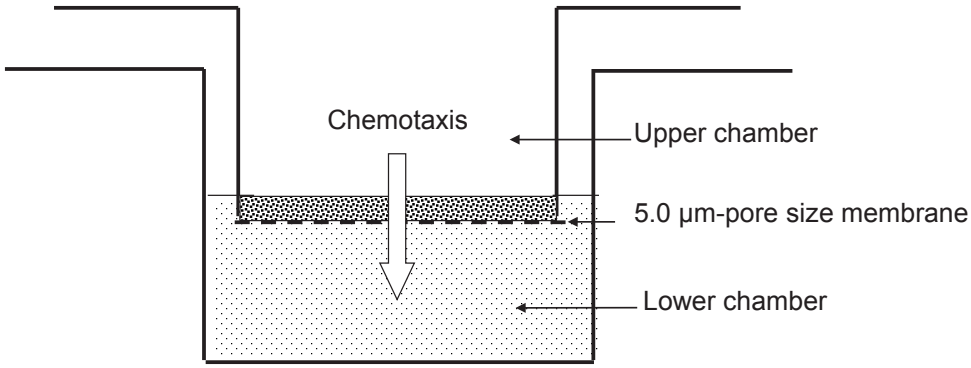

Figure 5. Schematic picture of the Transwell system.

Flow cytometric analysis of DC and lymphocytes

H. pylori induced maturation of DC was analysed by flow cytometric analysis. The DC were incubated with fluorescently labelled antibodies 48 hours after stimulation with different *H. pylori* strains, MP or LPS from Hel 305, for 30 min on ice. In order to investigate the maturation status, and their potential for T cell activation, we analysed their expression of CD40, HLA-DR, CD80, CD86, and the chemokine receptor CCR7.

DC were labelled with antibodies against CCR7 followed by goat-anti-mouse-IgM-biotin as a secondary antibody and streptavidin-PE. FITC-labelled anti-HLA-DR and anti-CD40, and CD80 and CD86 followed by rat-anti-mouse-IgG1-PE as a secondary antibody. Propidium iodide was used to exclude dead cells. The DC were analysed on a FACS-Calibur using CellQuest and Flow Jo software.

Half of the migrating LPMNC from the Transwell experiments were used to enumerate memory B cells by flow cytometry analysis using true-count beads as previously described [155]. Memory B cells were defined as small resting lymphocytes that had undergone isotype switching to IgA or IgG. In addition, most IgA^+ and IgG^+ cells coexpress CD27 [155], confirming their memory cell status. The expression of IgA and IgG on the cell surface was determined by FITC labeled rabbit anti-IgA and -IgG antibodies and was combined with PE labeled anti-CD19, peridinin chlorophyll protein (perCP)-labeled anti-CD3, and allophycocyanin (APC)-labeled anti-CD69 antibodies. Appropriate isotype control antibodies were used to determine unspecific staining. Cells were analyzed on a FACSCalibur using CellQuest and FlowJo software.

In addition, chemokine receptor expression by IgA^+ and IgG^+ memory B cells and plasmablasts (identified as large $CD3^-$ $CD38^{high}$ lymphocytes expressing surface immunoglobulins) isolated from the stomach mucosa of *H. pylori*-infected and uninfected individuals was examined. Chemokine receptor expression was visualized by using a biotinylated mouse monoclonal antibody to CCR9 (clone 3C3, kindly provided by D. Picarella, Millennium Inc., Cambridge, MA) followed by APC-conjugated streptavidin, APC-labeled anti-CCR10, and anti-CXCR4 antibodies.

RNase protection assay

RNase protection assay has the capacity to quantify several mRNA species in a single sample of total RNA. Total RNA was extracted from DC following 48 hours stimulation using the RNA isolation kit RNeasy (Qiagen). The multi-probe template set hCR6 (containing DNA templates for CXCR-1, CXCR-2, CXCR-3, CXCR4, CXCR5/BLR-1, CCR7/BLR-2, V28/CXCR1, L32 and GAPDH) and the RNase protection assay was performed according to the standard protocol. Briefly, the DNA templates were used to synthesize the [α ^{35}S] UTP-labeled probes in the presence of a GACU pool using a T7 RNA polymerase. Hybridization with 2-3 µg target RNA was performed overnight followed by digestion with RNase A and T1. The samples were treated with proteinase K and then extracted with tris-saturated phenol, and chloroform:isoamyl alcohol and precipitated in the presence of ammonium acetate. The samples were loaded on an acrylamide-urea sequencing gel next to labelled DNA molecular weight markers and to the labelled probes, and run at 40 W with 0.5×TBE (Tris/Borate/EDTA) buffer. The gel was absorbed onto filter paper, dried under vacuum and exposed on a Kodak Biomax MR film for one week.

Detection of CCL25, CCL28, CCL19 and CCL21 mRNA in gastric tissue

Expression of CCL25 and CCL28 mRNA in gastric tissues was assessed by reverse transcriptase PCR (paper III). Total RNA was purified by use of a total RNA extraction kit for mammalian RNA. Residual genomic DNA was removed by DNase treatment with a DNase I amplification-grade kit. The concentration and integrity of the RNA was assessed by use of NanoDrop and by gel electrophoresis. cDNA was synthesized using 600 ng total RNA and oligo(dT) primers with the Omniscript RT-PCR kit (Qiagen, Hilden, Germany) in a total volume of 20 µl, as described by the manufacturer. The cDNA was stored at −20°C. Primers for CCL25 were designed by Primer3 software (forward primer, CCATCGTGGCCTTGGCTGTCTGTG; reverse primer,

GCCGTATGTTTCGTGTTTCCCCTG). CCL28 and hypoxanthine phosphoribosyltransferase (HPRT) primers were used as previously described [23, 156]. Reverse transcriptase -PCR was performed by multiplex PCR using either the CCL25 or CCL28 primers in combination with the HPRT primers according to standard procedures for 35 cycles. The expression of CCL25 and CCL28 was expressed as the ratio of the optical density for the chemokine band relative to the HPRT band.

Detection of CCL19 and CCL21 mRNA in gastric tissue was determined by real time-PCR (Paper III). Total RNA was purified by the RNeasy minikit (Qiagen, Hilden, Germany). cDNA was synthesised from 500 ng total RNA and oligo-dT primers using the Omniscript RT-PCR Kit (Qiagen). Real time-PCR was performed with CCL19, CCL21, and HPRT primers using standard procedures for 40 cycles (Applied Biosystems, Foster City, CA). The relative levels of CCL19 and CCL21 mRNA was calculated using the ΔΔCT method, with HPRT as an internal standard and a sample from a non-infected volunteer with a low and stable CT value as calibrator.

Protein extraction from gastric tissue

Four antral biopsy samples from each subject were incubated overnight at 4^{o}C in 600 µl phosphate-buffered saline containing 2% saponin, 100 mg/ml soybean trypsin inhibitor, 350 mg/ml phenylmethylsulfonyl fluoride, and 0.1% bovine serum albumin. Each suspension was centrifuged at 13,000 × *g* for 5 min, and the supernatants were collected and frozen at −70^{o}C until used for chemokine analyses.

Detection of chemokines and antibodies in tissue extracts by ELISA

The concentration of CCL19, CCL25 and CCL28 was determined by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer´s instructions. The detection limit for CCL25 was 30 pg/ml and that for CCL28 was 10 pg/ml. Before CCL19 protein analysis, the samples were passed through desalting columns to remove detergents from the extraction procedure. Total IgA concentration in the tissue extracts was determined in ELISA as previously described [157]. Purified human IgA was used to construct a standard curve, and the tissue extracts were diluted 100-fold before analysis. Chemokine and antibody concentrations were related to the total protein concentration in the respective samples, which was determined by a protein assay kit.

Immunohistochemical staining (paper III)

Biopsies from both *H. pylori* infected and uninfected volunteers were collected and immediately embedded in OCT compound, snap frozen and stored at -70 °C for immunohistochemical analysis. The number of iDC, mDC and plasmacytoid DC (pDC) was determined by different surface markers. Cryo-cut tissue sections (8µm thick) were fixed for 10 minutes in ice-cold acetone and endogenous peroxidase was blocked with glucose-oxidase for 20 minutes in the dark. Thereafter, the slides were incubated for 30 minutes at room temperature with primary mouse monoclonal antibodies to dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) which defines iDC, dendritic cell lysosome-associated membrane glycoprotein (DC-LAMP) which defines mDC, or CD303 which defines pDC. This was performed in PBS containing human serum (5 %) and rabbit serum (5 %) and was followed by addition of a HRP-conjugated rabbit antibody against mouse immunoglobulins. Mouse IgG1 was used as an isotype control for DC-LAMP and CD303 and mouse IgG_{2B} was used as an isotype control for for DC-SIGN.

Immunofluorescence detection

Cryocut tissue sections (8 µm) from biopsy samples from each volunteer were fixed in ice-cold acetone. The expression of CCL28 was detected using immunofluorescent staining (paper II). Endogenous peroxidase was blocked with glucose oxidase followed by blocking of biotin in the tissue. Thereafter, the slides were incubated with mouse IgG1 anti-CCL28 or with mouse IgG1 as the negative control. Primary antibodies were used at optimal dilutions in phosphate-buffered saline with 0.05% Tween at room temperature for 1 h. The samples were then incubated with goat anti-mouse IgG1 conjugated to AlexaFluor 594 followed by detection using tyramide amplification. Finally, slides were mounted using a DAPI (4′,6′-diamidino-2-phenylindole)-containing mounting medium.

The potential interaction between mDC and $CD4^+$ T cells was assessed by immunofluorescence staining of frozen sections from both *H. pylori* infected and uninfected volunteers. The slides were blocked with 5% of human serum and stained with anti-DC-LAMP and AlexaFluor 594-conjugated rabbit antibody against mouse immunoglobulins. Thereafter, the slides were blocked with 5% mouse serum and then stained with mouse anti-human CD4 conjugated to AlexaFluor 488 (BD, diluted 1:5). Finally, slides were mounted using a DAPI-containing mounting medium.

To detect potential expression of DC-LAMP and CD68 on the same cells, antibodies against CD68 were first pre-incubated with the secondary antibody, goat anti-mouse IgG conjugated to AlexaFluor 488. Thereafter, the remaining binding sites were blocked by adding 5% mouse serum. The slides were blocked with 5 % human serum and double stained with DC-LAMP as described above together with the pre-incubated CD68 antibody for 1 hour.
To further examine the identity of the DC-LAMP$^+$ mDC, CD11c was examined in serial sections following DC- LAMP staining. The slides were blocked with of human serum (5%) and then incubated with a mouse monoclonal antibody against human CD11c and then goat anti-mouse IgG conjugated to AlexaFluor 488 for 1 hour. Finally, slides were mounted using a DAPI-containing mounting medium.

Activation of T cells

For T-cell proliferation analysis, a new blood sample was drawn from each volunteer one week after the isolation of CD14$^+$ cells and CD4$^+$ and CD8$^+$ T cells (5×10^4/well) were isolated by positive selection. Thereafter they were co-cultured with autologous DC (10^4/well), that had been stimulated with *H. pylori* (Hel 305 and Hel 333) or *E. coli* (KI) (DC:CFU; 1:1) or with MP (Hel 305) for 48h in round-bottomed 96-well plates (paper I). After 24h or 48h of coculture, 100 µl of the medium was removed for subsequent cytokine analysis and replaced with 100µl fresh medium. After five days of cultivation, the proliferation of the cells was measured by a standard thymidine incorporation assay.

Detection of cytokines by Cytometric Bead Array (CBA)

CBA is a flow cytometry application that makes it possible to quantify different proteins simultaneously. Antibodies are coated with beads which have unique fluorescence intensities, respectively, so that beads can be mixed and run simultaneously in a single tube. This method reduces the amount of sample required and also the time required to investigate different proteins. Supernatants were taken from the stimulated DC in the T-cell proliferation assay. The concentration of IL-12 p70, IL-10, TNF-α, IL-6, IL-1β, and IL-8 in culture supernatants collected at 24 and 48h after simulation with *H. pylori* (Hel 305 and Hel 333), *E. coli* or MP was measured by CBA analysis.

Analysis of IgA and IgG ASC migration by Enzyme-linked immunosorbent spot (ELISPOT) assay

The ELISPOT assay is a method able to enumerate B cells secreting antigen-specific antibodies [158]. IgA- and IgG-secreting cells that migrated toward CCL28, CCL25, or CXCL12 were detected in two-color ELISPOT assays as previously described [159] [160]. Briefly, wells were coated with goat antibodies against the $F(ab)_2$ fragment of human IgG . Cell suspension (50 µl) was added to each well and the experiments were performed in duplicate. Cells were incubated overnight at 37°C in a humidified atmosphere containing 5% CO_2. During this incubation period, the immobilized antibody, in the immediate vicinity of the secreting cells, binds secreted IgA or IgG antibodies. The assay was developed by the addition of horseradish peroxidase-conjugated goat antibodies against human IgA and alkaline phosphatase-conjugated goat antibodies against human IgG for 4 h followed by addition of chromogen substrates. The frequency of IgA and IgG ASC, represented by spots, was determined under low magnification with a stereomicroscope. Untreated LPMNC were assayed in parallel as a positive control, and incubation of these cells for 2.5 h with the respective chemokines did not influence ASC frequencies or the amounts of IgA secreted into the medium.

Statistical analysis

The two-tailed Mann-Whitney test was used to evaluate differences in proliferation, migration, cytokine secretion, expression of the chemokine receptors in DC and comparisons between *H. pylori*-infected and uninfected subjects. P values of <0.05 were considered statistically significant. Correlation was evaluated using the two-tailed Pearson test (paper II).

RESULTS AND DISCUSSION

The influence of *Helicobacter pylori* on DC in the gastric mucosa
(Papers I and II)

H. pylori infection results in chronic gastritis characterized by infiltration of neutrophils, macrophages, DC and lymphocytes into the infected stomach mucosa [154, 161, 162]. However, the role of DC in *H. pylori*-induced chronic inflammation has been poorly defined. Therefore we investigated DC populations in the human gastric mucosa of *H. pylori* infected and uninfected individuals and how *H. pylori* influence monocyte derived DC *in vitro* (papers I and II). Since the immune response does not eradicate the infection, we hypothesized that DC fail to migrate to the lymph nodes and instead remain localized in the tissue, where they contribute to the local inflammation by producing inflammatory mediators and activating local T cells.

DC accumulation in *H. pylori* infected tissue (paper II)

To identify iDC in the gastric mucosa, biopsies from both uninfected and *H. pylori* infected individuals were stained with DC-SIGN, a specific marker that is highly expressed by iDC [55, 163]. DC-SIGN$^+$ iDC were found scattered in the lamina propria both in uninfected and *H. pylori* infected individuals. However, DC-SIGN$^+$ iDC were found in significantly higher frequencies in *H. pylori* infected individuals compared to in uninfected. DC have previously been observed to be recruited following infection with *H. pylori* in mice [110, 164]. The findings in paper II show that this may also be the case in humans. There seems to exist two groups of *H. pylori* infected individuals with regard to iDC frequencies; one with relatively few DC-SIGN$^+$ iDC at the same levels as in the uninfected volunteers (25%), and one group with substantially higher numbers of DC-SIGN$^+$ iDC (75%). This partition could not be explained by age, sex or symptoms of the individuals. The staining with DC-SIGN also showed that the large majority of iDC were scattered throughout the lamina propria while only a few iDC were found close to or in a lymphoid follicle. Some iDC were positioned just underneath the epithelium. Previous studies have shown that iDC express inflammatory chemokine receptors such as CCR1, CCR2, CCR5 and CXCR1, and that they respond to their respective chemokine, which are produced at inflammatory sites [65]. However, the chemokine CCL20 is a known chemoattractant for iDC and memory T cells [130, 131, 165] and it has recently been shown that there is an increased production of CCL20 by the gastric

epithelium of patients with *H. pylori*-associated gastritis [129]. High production of CCL20 by epithelial cells in *H. pylori* infection may thus explain the higher frequencies of DC-SIGN$^+$ iDC in the gastric mucosa. Furthermore, CCR6-dependent regulation of DC is responsible for a localized T cell dependent defense in Peyer's patches against entero-invasive pathogens [166]. CCL20 production is controlled directly by the transcriptional factor NF-κβ, which is in turn up-regulated by TNF-α. We have also shown that DC stimulated with *H. pylori* secretes TNF-α (paper I), suggesting that *H. pylori* infection may result in recruitment of iDC expressing CCR6 into the tissue, through epithelial production of CCL20.
Since the mucosa-associated epithelial chemokine CCL28 is a common mucosal chemokine which is constitutively expressed by epithelial cells in most mucosal sites [23, 24], we investigated if *H. pylori* stimulated monocyte derived DC were able to migrate towards CCL28 in a chemotaxis assay. However, the result showed that *H. pylori* stimulated monocyte derived DC did not migrate towards CCL28 (unpublished data).
We also stained plasmacytoid DC (pDC) for CD303 to see if they were recruited upon *H. pylori* infection. However, they were found in very low numbers in both uninfected and *H. pylori* infected individuals. Furthermore, there was no difference between the two groups of volunteers with regard to CD303 expression.

***H. pylori* induce expression of the chemokine receptor CCR7 on DC (paper I)**
In this study we compared DC responses induced by pathogenic *H. pylori* bacteria with a non-pathogenic *Escherichia coli* strain to evaluate if there was any difference between pathogenic and non-pathogenic bacteria.
Earlier studies have shown that CCR7 and CXCR4 mRNA is expressed at very low levels by iDC, but are strongly up-regulated following maturation [65]. We could also show that iDC expressed CCR7 and CXCR4 mRNA at very low levels. To analyse expression of CCR7 and CXCR4 mRNA we stimulated monocyte derived iDC *in vitro* with different strains of live *H. pylori* bacteria or *H. pylori* MP. For comparison, iDC were also stimulated with non-pathogenic *E. coli* bacteria (strain K12). Stimulation of iDC with *H. pylori* strains Hel 312 (asymptomatic individual, CagPAI$^+$, VacA$^+$BabA$^-$), Hel 330 (asymptomatic individual, CagPAI$^+$, VacA$^+$ and BabA$^+$), and Hel 305 (duodenal ulcer, CagPAI$^+$, VacA$^+$ and BabA$^+$), as well as *E. coli* (DC:CFU; 1:1), resulted in a significant up-regulation of both CCR7 and CXCR4 mRNA. However, stimulation with *H. pylori* strain Hel 333 (duodenal ulcer patient, VacA+ CagPAI$^-$, and BabA$^-$) did not induce an up-regulation of either receptor unless 100-

fold more bacteria was used, and even then the increase in CXCR4 and CCR7 mRNA was much lower compared to the levels induced by the other *H. pylori* strains and *E. coli*. Stimulation of DC with Hel 305 MP (5µg/ml) also induced increased CCR7 and CXCR4 mRNA expression but to a lower extent as compared to stimulation with whole bacteria. LPS was not responsible for this expression, since stimulation of DC with *H. pylori* LPS at the same concentration as that present in the membrane preparation did not induce CCR7 and CXCR4 mRNA.

We went on to analyse the *H. pylori*-induced expression of the CCR7 protein on the DC surface since this chemokine receptor is essential for the migration of mature DC to the lymph node. In agreement with the results of the mRNA expression analysis, stimulation with both *H. pylori* and *E. coli* resulted in a significant up-regulation of the expression of CCR7 on the DC surface. However, *E. coli* induced higher levels of CCR7 protein expression than *H. pylori*, using the same concentration of live bacteria for stimulation (DC:CFU; 1:1). To achieve a comparable level of surface expression of CCR7 protein, a 100-fold higher concentration of *H. pylori* bacteria than *E. coli* was required for all strains. However, we only analysed the surface expression of CCR7, and therefore the possibility remains that intracellular CCR7 protein expression might explain the discrepancy between mRNA and protein data after *H. pylori* stimulation. Finally, stimulation with MP, but not LPS, induced a low, but consistent up-regulation of CCR7 surface expression in all experiments. In conclusion, our data demonstrate that live *H. pylori* bacteria can induce expression of CCR7 receptors on human DC. Thus, *H. pylori* is likely to induce DC migration to the lymph nodes.

***H. pylori* induces specific migration of monocyte derived DC to CCL19**

Since our results showed that *H. pylori* induce the chemokine receptor CCR7 important for migration to lymph nodes we evaluated the capacity of *H. pylori* to induce DC migration towards the lymph node expressed ligand CCL19 by a chemotaxis.

Stimulation of DC with either *H. pylori* or *E. coli* (DC:CFU; 1:1) induced a significant specific migration towards CCL19, although the migration efficiency varied between different strains of *H. pylori*. (Due to practical reasons *H. pylori* strain Hel 340 was not included in the migration assay.) After stimulation with Hel 312 and Hel 305, 42 % and 33 %, of the DC migrated in response to CCL19, respectively. This was comparable to stimulation with *E. coli*, which induced 44 % of the DC to migrate towards CCL19. However, the specific migration of DC after stimulation with Hel 333 was low, only 8%. To achieve a somewhat higher specific migration of DC stimulated with Hel 333 (12%) a 100-fold higher

concentration of *H. pylori* Hel 333 bacteria compared to *E. coli* was required. MP-stimulation also induced specific DC migration in all experiments (22% migrated cells), while unstimulated DC did not migrate towards either CCL19 or medium.

Hel 333 was the only strain investigated that lacked the CagPAI. To investigate if the presence of CagPAI in *H. pylori* influenced the migration of *H. pylori* stimulated DC, we evaluated the capacity of an isogenic *H. pylori* CagPAI deficient mutant (P1Δ*cag*PAI) and the corresponding wild type strain (P1wt) to induce DC migration in an *in vitro* chemotaxis assay. We also included Hel 333 and *E. coli* bacteria for comparison in these new experiments. Both the P1Δ*cag*PAI and the wt strain induced a comparable migration of DC towards CCL19 compared to Hel 333 that induced less migration (Fig. 6). Thus, we have no evidence to suggest that CagPAI is essential for induction of DC migration. However, presence of CagPAI seems to influence cytokine responses (see below).

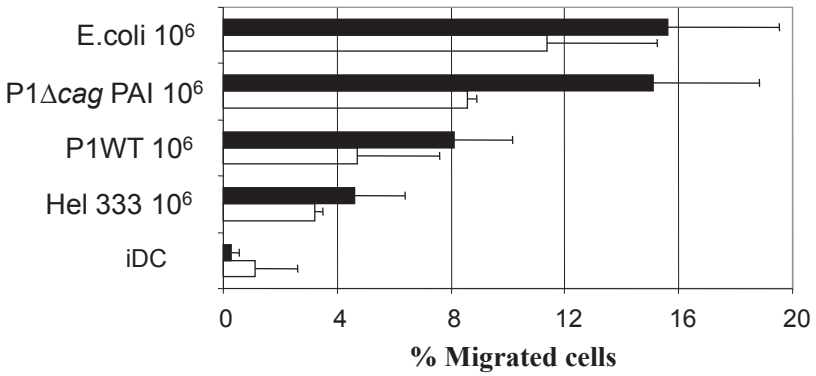

Figure 6. Migration of DC towards CCL19 analysed by an *in vitro* chemotaxis assay. The upper chamber was loaded with 1-2.5 × 10^5 *H. pylori*-stimulated or unstimulated DC (iDC). The lower chamber contained medium (open bars) or medium supplemented with CCL19 (filled bars).

***H. pylori* induce cytokine production by DC**

Although our results suggest that DC are likely to up-regulate CCR7 and migrate to lymph nodes after exposure to *H. pylori*, DC-derived cytokines and chemokines secreted immediately after exposure may influence local inflammation in the mucosa. Monocyte

derived DC were prepared from both *H. pylori* infected and uninfected individuals and supernatants were collected from DC cultures at 24 and 48 h after stimulation. We found that *H. pylori*-stimulated DC secretes TNF-α, IL-6 and IL-8 within 24 h of stimulation. These cytokines are likely to activate other immune cells and epithelial cells and IL-8 is known to recruit neutrophils to the mucosa. Stimulation with *H. pylori* (Hel 305) also induced secretion of relatively high levels of IL-12, but less IL-10. These findings are in agreement with another study, where *H. pylori* was shown to induce DC production of IL-12, but not IL-10 [117, 118, 167]. Increased expression of interferon (IFN)-γ and IL-12 in biopsies has also been observed in *H. pylori* infected compared to uninfected volunteers [134, 135, 168]. DC production of IL-12 in response to *H. pylori* infection may contribute directly to the local inflammation as well as to Th1 polarization of naïve T cells in lymph nodes. In contrast, the non-pathogenic *E. coli* (K12) induces higher levels of IL-10, which is associated with immunosuppression [169, 170].

Mature DC are detected in association with lymphoid follicles in *H. pylori* infected gastric mucosa

We have seen *in vitro* that *H. pylori* stimulated DC up-regulate CCR7 and migrate towards the ligand CCL19. However, these studies were performed *in vitro*. To investigaste whether mDC are actually retained in the *H. pylori* infected tissue we stained biopsies from both uninfected and *H. pylori* infected individuals with DC-LAMP, which is absent on iDC but upregulated upon maturatin of DC [67]. In contrast to the iDC, DC-LAMP$^+$ mDC were mainly found in close association with lymphoid follicles in biopsies from *H. pylori* infected individuals, while they were scarce in the tissue from healthy individuals.

DC-LAMP$^+$ mDC were found in both uninfected and *H. pylori* infected gastric mucosa, but the frequency of DC-LAMP$^+$ mDC was significantly increased in the gastric mucosa of *H. pylori* infected individuals as compared to uninfected volunteers. Lymphoid follicles were not found in the uninfected volunteers but were present in the sections from six out of the nine *H. pylori* infected individuals. In five of these individuals, DC-LAMP$^+$ mDC were found close to and inside the follicles and only a few were scattered throughout the lamina propria. Furthermore, DC-LAMP$^+$ mDC and CD4$^+$ T cells were found in close association, an observation that might indicate that mDC are able to present antigen to and activate T cells. To exclude the possibility that DC-LAMP was expressed by mucosal macrophages, double staining with antibodies to DC-LAMP and CD68 (expressed on monocytes and macrophages) was performed. However, DC-LAMP and CD68 were not co-expressed by the same cells, and

the markers were usually found in different areas of the lamina propria. To further investigate the identity of the DC-LAMP$^+$ mDC, their expression of CD11c was examined in serial sections following DC- LAMP staining. CD11c is expressed by many human hematopoetic cells, including subpopulations of human DC. CD11c cells were mainly found in the lymphoid follicles, but also scattered in the lamina propria and in association with the epithelium unlike DC-LAMP$^+$ mDC, which were gathered close to or just inside the lymphoid follicle. This indicates that DC-LAMP$^+$ mDC constitute a CD11c$^-$ subpopulation. Preliminary data from flow cytometry analyses also show that DC-LAMP$^+$ mDC in the gastric mucosa express MHC-II, further suggesting their potential to act as antigen presenting cells. These data reinforce our previous conclusions that DC stimulated with *H. pylori* upregulate MHC-II. A similar association was previously suggested by Ladányi et al. [171], showing that DC-LAMP$^+$ DC expressing maturation markers were predominantly found in lymphocyte aggregates in the cancer stroma, sometimes forming clusters with T cells. The latter study also showed a significant association between the density of DCs and activated T lymphocytes. Earlier studies have shown that presence of lymphoid follicles in *H. pylori* infection correlate strongly with the degree and severity of gastritis [172]. Therefore, it is hard to judge whether the presence of lymphoid follicles *per se* or presence of a more intense inflammation is the cause of the mDC accumulation.

***H. pylori* induce maturation of DC and activation of T cells**

As mentioned above, *H. pylori* have the capacity to induce the chemokine receptor CCR7, necessary for migration to the lymph node, on DC. However, DC maturation usually also leads to up-regulation of co-stimulatory molecules, such as CD40, HLA-DR, CD80, and CD86 necessary for T cell activation. In order to evaluate the maturation stage of the stimulated DC and their potential for T-cell activation, we analysed their expression of these molecules. Analysis showed that the majority, >90%, of the iDC expressed CD40 and HLA-DR. However, the expression of HLA-DR increased further, as revealed by a shift in the median fluorescence intensity, in response to stimulation with *H. pylori* live bacteria (DC:CFU; 1:1), but not in response to MP. The CD40 expression was relatively unaffected by bacterial stimulation. However, the positive control TNF-α induced increased expression of CD40. Unstimulated iDC expressed low levels of CD80 and CD86, but stimulation with *H. pylori* or *E. coli* bacteria resulted in increased expression of both of these markers. Stimulation of iDC with *H. pylori* MP result in increased expression of CD80 and CD86, although to a lesser extent than stimulation with whole live bacteria. Taken together, we

found that DC stimulated with live *H. pylori* bacteria expressed elevated levels of MHC II molecules as well as the co-stimulatory molecules CD80 and CD86. To verify that the mature, CCR7 expressing DC are able to induce a T-cell response, we treated iDC with *H. pylori* (Hel 305 and Hel 333), *E. coli* bacteria (DC:CFU; 1:1), or MP for 48 h and then used these DC as antigen presenting cells for autologous T-cell activation. *H. pylori* (Hel 305) and *E. coli* stimulated DC induced $CD4^+$ T-cell proliferation, as did the MP. In contrast, stimulation of DC with *H. pylori* (Hel 333) required 100-fold more bacteria to induce a significant $CD4^+$ T-cell response. There was no difference in the T-cell response to any of the *H. pylori* strains between *H. pylori* infected and uninfected individuals [111]. Since we isolated total $CD4^+$ T cells, both naïve and memory T cells were present during activation by DC induced *by H. pylori*. In a similar study by Lundgren at al, the $CD4^+$ T cells were also separated into memory and naïve cells and their response to *H. pylori* was monitored [111]. Results from the latter study showed that *H. pylori* infection does not increase the memory T cell response to *H. pylori* antigens above the baseline found in uninfected individuals. This was shown to be linked to existence of *H. pylori*-specific regulatory T cells, and depletion of regulatory T cells lead to increased proliferation in infected individuals [111]. The existence of *H. pylori*-reactive memory cells in uninfected individuals may be explained by priming by other related bacteria *in vivo*. However, we could show that *H. pylori*-induced DC are able to activate both memory and naïve $CD4^+$ T cells, while $CD8^+$ T-cells did not proliferate in response to any of the stimulations.

CCR7-CCL19 interaction is a possible mechanisms for accumulation of mDC in the *H. pylori* infected tissue

Having established the presence of mDC in human *H. pylori* associated gastritis, we sought to determine the mechanisms of mDC retention. Since our previous *in vitro* experiments had demonstrated an increased expression of CCR7 on *H. pylori* stimulated DC, we determined the presence of the CCR7 ligands CCL19 and CCL20 in the gastric mucosa.

The relative expression of CCL19 mRNA but not CCL20 mRNA was significantly higher in *H. pylori* infected individuals compared to in uninfected volunteers. Furthermore, there were significantly higher concentrations of CCL19 protein in biopsies from *H. pylori* infected than uninfected individuals. These results indicate that a subpopulation of maturing DC remain in the gastric mucosa due to CCL19-CCR7 interaction, instead of migrating to draining gastric lymph nodes. Still, the inductive site for the adaptive immune response to *H. pylori* has not yet been clearly identified, but it was recently shown in murine studies that DC in the Peyer´s

patches are able to prime Th1 T cell responses after exposure to coccoid *H. pylori* [141, 142], suggesting that the Peyer´s patches of the intestine may be an inductive site during murine *H. pylori* infection.

Thus, these mature DC that remain in the gastric mucosa may contribute to the local inflammation by producing inflammatory mediators. This can also be one reason for Th1 like immune responses in human gastritis [173], since *H. pylori* activate DC to drive Th1 polarization [118, 167, 174].

Recruitment of IgA antibody secreting cells by increased expression of the chemokine CCL28 in human *H. pylori*-induced gastritis

(Paper III)

H. pylori infection gives rise to a large accumulation of IgA secreting cells in the gastric mucosa. Since α4β7-MAdCAM-1interactions do not seem to explain the increased B-cell migration to the *H. pylori*-infected gastric mucosa, we hypothesized that it might instead be mediated by altered chemokine production. The chemokines CCL28 and CCL25 have been shown to be essential for lymphocyte migration to gastrointestinal tissues [26-28, 89, 175], and we therefore evaluated their contribution to B-cell migration into *H. pylori*-infected gastric tissues. Biopsies from gastric antrum mucosa were collected from both asymptomatic *H. pylori*-infected and uninfected individuals.

Increased chemokine CCL28 content in *H. pylori*-infected gastric tissue

As a first experiment, we analysed the concentration of CCL25 and CCL28 in gastric biopsies. Protein analyses of the chemokines CCL25 and CCL28 showed that gastric tissue from *H. pylori*-infected individuals contained significantly more ($P < 0.001$) CCL28 than gastric tissues from *H. pylori* uninfected individuals. On average, *H. pylori*-infected tissues contained 2.8 times more CCL28 than did uninfected tissues (33 ± 21 pg/mg protein compared to 12 ± 3 pg/mg). Since the *H. pylori*-infected group contained some individuals that were older than the uninfected volunteers, we also investigated if there was a correlation between CCL28 concentration and age in the *H. pylori*-infected group. This was, however, not the case ($r = 0.457$, $P > 0.05$). To validate the results for protein expression, we used RT-PCR to examine mRNA in gastric tissue from the same individuals and compared the result to those for the housekeeping gene HPRT. These results showed a significantly higher CCL28 mRNA expression level ($p<0.001$) in *H. pylori*-infected gastric tissue compared to uninfected tissue. However, there was a baseline production of CCL28 in the stomach of *H. pylori* uninfected individuals, confirming previous studies of CCL28 mRNA expression in human tissue [23, 24]. Therefore, these studies bring forward the concept that in parallel to being constitutively expressed at most mucosal surfaces, CCL28 production can also be induced by mucosal infections.

The CCL25 protein concentrations were more variable than CCL28, but there was no difference between *H. pylori* infected and uninfected individuals as a whole. However, almost

all subjects had detectable levels of CCL25 in the gastric mucosa, although these levels were generally much lower than what we have previously detected in small intestinal tissue extract (C. Lindholm and M. Quiding-Järbrink, unpublished observations). There was also no difference between infected and uninfected tissue with regard to CCL25 mRNA expression. Therefore, changes in CCL25 expression do not seem to mediate lymphocyte influx during *H. pylori*-induced gastritis. Since HLO and chronic inflammation scores did not vary much between the volunteers (all but one had scores of 2 for both parameters), we could not evaluate if chemokine levels correlate to *H. pylori* density or the infiltration of lymphocytes.

The chemokine CCL28 is localized in the epithelium of the neck region and the surface epithelium of *H. pylori*-infected individuals

Our results suggest that *H. pylori* infection can induce CCL28 production in the gastric tissue. Therefore, we analysed the localization of CCL28 within the gastric mucosa by immunofluorescence staining on frozen tissue from the same individuals.

These analyses revealed CCL28 reactivity exclusively in the epithelia in all individuals except two uninfected volunteers for whom no CCL28 staining could be detected. The staining was, however, always more intense in the *H. pylori*-infected individuals. The CCL28 staining was cytoplasmatic and found mainly in the deep zone of the antral glands in the uninfected volunteers. In the *H. pylori*-infected individuals, on the other hand, CCL28 was also detected in the epithelium of the neck region and the surface epithelium. However, as mentioned above, there was a low baseline CCL28 expression for most of the *H. pylori* uninfected individuals also during these analyses.

In order to access the ability of *H. pylori* to induce CCL28 production in epithelial cells, we stimulated different gastric epithelial cell lines (AGS and KatoIII) and freshly isolated epithelial cells with live *H. pylori* bacteria, TNF-α or IFN-γ and analysed the production and secretion of CCL28. However, we could not detect either intracellular CCL28 or CCL28 secreted by the epithelial cells. Furthermore, stimulation of whole gastric biopsies from uninfected individuals with live *H. pylori* did not induce CCL28 secretion either (unpublished results). The hypothesis of inducible CCL28 expression is, however, supported by a study by Ogawa et al. [176] showing increased CCL28 levels in inflamed colonic tissue from patients suffering from ulcerative colitis. In the case of *H. pylori* infection, it is not possible at this stage to determine if the increased CCL28 expression is a direct effect of *H. pylori* on the epithelial cells or if pro-inflammatory mediators in the tissue influence CCL28 expression.

Since the study by Ogawa et al. [176] demonstrated that both pro-inflammatory cytokines and bacteria can induce CCL28 in colonic cell lines, a combination of the two seems most likely.

Higher levels of gastric IgA content in *H. pylori*-infected gastric tissue

To investigate whether there is a correlation between CCL28 and IgA in the *H. pylori*-infected gastric mucosa we determined the concentration of total IgA in the gastric tissue extract previously used for chemokine detection. As previously reported [157], *H. pylori*-infected subjects had higher levels of gastric IgA than did the uninfected. On average, tissue extracts from *H. pylori*-infected subjects contained 19.3 ± 13.0 μg IgA per mg protein (mean ± standard deviation), and extracts from uninfected subjects contained 2.8 ± 0.7 μg/mg. Furthermore, when the IgA concentrations in the gastric extracts from infected individuals were plotted against CCL28 concentrations, there was a significant positive correlation (r = 0.897, $P < 0.01$). Therefore, the results show that CCL28 production can be induced by mucosal infections and thereby probably enabling the recruitment of additional IgA-secreting cells from the circulation.

Chemokine CCL28 induces migration of gastric antibody secreting cells (ASC) from *H. pylori* infected individuals

Once we had established that CCL28 production was increased in *H. pylori*-associated gastritis, we asked whether gastric B cells could respond to CCL28. We therefore examined if gastric cells from *H. pylori*-infected and uninfected individuals would respond to CCL25 or CCL28. Lamina propria mononuclear cells (LPMNC) isolated from tissue collected at gastrectomy surgery were allowed to migrate in the Transwell system toward CCL25, CCL28, or the positive control, CXCL12 (SDF-1α), known to recruit IgG ASC [177]. The frequency of ASC among the migrating cells was analyzed by ELISPOT assays. Gastric IgA-secreting cells from all *H. pylori*-infected subjects displayed a robust migration toward CCL28, and this is in fact the first demonstration that human mucosal IgA-secreting cells chemotax to CCL28, a feature that was previously shown only for murine cells [89].

This response was not seen among cells from uninfected individuals ($P < 0.01$ comparing infected and uninfected individuals). In contrast, there was no IgG ASC response in any of the patient groups to CCL28 and this is consistent with our earlier observations that the frequency of gastric IgG-secreting cells remain unchanged during *H. pylori* infection [82]. Likewise, there was no response to CCL25 in any of the patient groups. Both IgA- and IgG-secreting gastric cells from *H. pylori*-infected, but not uninfected, individuals responded to the positive

control, CXCL12, but only the IgG ASC response was significantly different ($P < 0.05$) between infected and uninfected patients.

The frequencies of IgA and IgG ASC are represented by spots in the ELISPOT assay. These spots were bigger and more intense for the IgA-secreting cells that had migrated towards CCL28 than in uninfected cells. Therefore we analysed whether CCL28 is able to induce ASC to secrete larger amounts of IgA antibodies per cell, using ALS. However, there was no difference in IgA-secretion between cells incubated together with CCL28 or without (Fig.7), and we could not explain the finding of larger spots following migration towards CCL28.

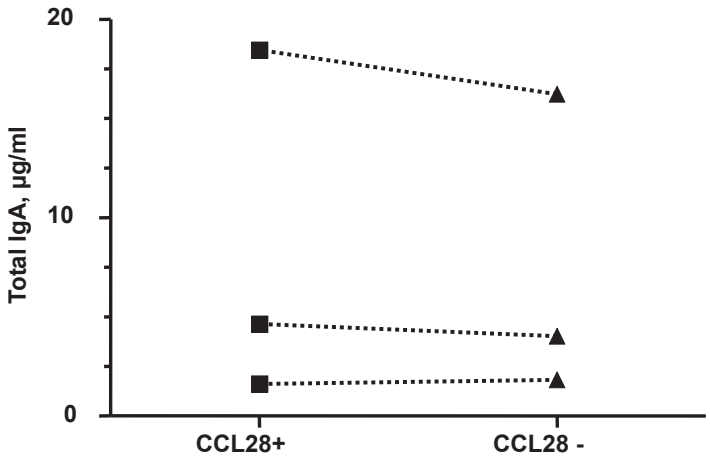

Figure 7. CCL28 induced IgA response.

Chemokine CCL28 induces migration of gastric IgA$^+$ and IgG$^+$ memory B cells from *H. pylori* infected individuals.

In parallel with the analysis of CCL28-induced ASC migration, we analyzed the migration of IgA$^+$ and IgG$^+$ memory B cells from the same individuals by flow cytometry.

IgA$^+$ memory B cells from *H. pylori*-infected individuals responded significantly ($P < 0.05$) to CCL28 compared to uninfected subjects but not to the same extent as ASC. While the median response of IgA ASC migration to CCL28 was more than 10-fold in relation to

spontaneous migration, the IgA^+ memory cells only exhibited a 2-fold-increased response towards CCL28. In contrast, IgG^+ memory B cells did not respond to CCL28.
Similar to what was seen for the ASC response, CCL25 did not induce any migration of memory B cells, except for in two individuals. These results are in agreement with our previous result that circulating memory B cells do not respond to any large extent to either CCL25 or CCL28 [155]. On the other hand, the positive control, CXCL12, induced a robust migration of both IgA^+ and IgG^+ memory B cells isolated from infected individuals. Gastric memory B cells from uninfected subjects, however, did not respond to CXCL12 ($P < 0.05$ and $P < 0.01$ compared to infected individuals). In relation to CCL28-induced migration of gastric ASC, it is interesting that circulating IgA ASC induced by intestinal immunization respond to CCL28 in chemotaxis assays [29]. This is not the case for the general pool of circulating IgA ASC [155], suggesting that most cells recruited to the gastric mucosa may be recently activated B cells originating from intestinal inductive sites. This would explain the accumulation of vaccine-specific ASC in the gastric mucosa following oral immunization and would also suggest that the use of CCL28-inducing adjuvant formulations might promote mucosal antibody responses to vaccination.

Chemokine receptor expression on gastric B-cell subsets

We have seen that *H. pylori* infection is able to induce increased concentrations of the CCR10 ligand CCL28 in the gastric tissue while the CCR9 ligand CCL25 remains unchanged. ASC from the *H. pylori* infected tissue also had a robust migration to CCL28 and the CXCR4 ligand CXCL12. Thus, we also examined the expression of chemokine receptors CCR9, CCR10, and CXCR4 by IgA^+ and IgG^+ memory B cells and plasmablasts isolated from the stomach mucosa of *H. pylori*-infected and uninfected individuals.

Small, resting naïve, and memory gastric B cells:

Within the population of small, resting naïve, and memory gastric lymphocytes, 46% ± 10% were IgA^+ and 6% ± 4% were IgG^+ in the infected individuals, and 43% ± 28% were IgA^+ and 12% ± 10% were IgG^+ in uninfected subjects. More than half of the IgA^+ and IgG^+ memory B cells expressed CCR9, regardless of whether they were isolated from *H. pylori*-infected or uninfected tissue (Table 1). Furthermore, the majority of memory cells expressed CCR10, and there were no large differences between IgA^+ and IgG^+ cells or between infected and uninfected individuals (Table1). The expression of CXCR4 was generally high on the memory B cells, with a somewhat higher expression for cells from *H. pylori*-infected patients.

Large CD3$^-$ CD38^{high} cells expressing IgA or IgG on the surface:

Chemokine receptor expression was also analyzed on plasmablasts, defined as large CD3$^-$ CD38^{high} cells expressing IgA or IgG on the surface. A majority of cells expressed IgA in both *H. pylori*-infected and uninfected individuals (68% ± 10% in infected and 62% ± 9% in uninfected), whereas 7% ± 7% expressed IgG in the infected individuals and 6% ± 6% of cells did so in the uninfected individuals. A majority of IgA$^+$ plasmablasts expressed CCR9, and there was also a very high expression of CCR10 on IgA$^+$ plasmablasts, with no difference between cells from infected and uninfected individuals (Table 1). CXCR4 expression was lower and more variable on plasmablasts than on memory cells, but no difference was seen between *H. pylori*-infected and uninfected volunteers. The expression of chemokine receptors on IgG$^+$ plasmablasts was hard to evaluate, since the frequency of cells was often too low to allow for proper analysis.

Table 2. CCR9, CCR10, and CXCR4 expression in gastric B-cell subsets isolated from gastric mucosa of *H. pylori-infected* and uninfected individuals.

Mucosa	**% of indicated cell type (mean ± SD) expressing the indicated chemokine receptors[a]**								
	IgA$^+$ memory B cells			**IgG$^+$ memory B cells**			**IgA$^+$ plasmablasts**		
	CCR9$^+$	**CCR10$^+$**	**CXCR4$^+$**	**CCR9$^+$**	**CCR10$^+$**	**CXCR4$^+$**	**CCR9$^+$**	**CCR10$^+$**	**CXCR4$^+$**
H. pylori$^+$	64 ± 15	70 ± 13	91 ± 9	72 ± 25	72 ± 9	90 ± 8	67 ± 21	99 ± 1	58 ± 37
H. pylori$^-$	66 ± 19	63 ± 23	78 ± 32	61 ± 21	74 ± 19	78 ± 32	63 ± 21	94 ± 9	44 ± 37

[a] n = 3 to 7 in the infected group; n = 3 to 5 in the uninfected group.

A study by Kunkel et al. [178] has shown that the vast majority of gastric plasma cells express the CCL28 receptor CCR10. However, the *H. pylori* status of patients in that study was not determined. In this study, we compared the chemokine receptor expression on B cells isolated from *H. pylori*-infected and uninfected individuals, and we could show a large and uniform expression of CCR10 on both IgA$^+$ and IgG$^+$ gastric plasmablasts, regardless of *H. pylori*

status. This finding is apparently contradictory to the distinct differences in migration between IgA ASC from infected and uninfected individuals and between IgA and IgG ASC. Clearly, additional factors that have not yet been identified contribute critically to CCL28-induced migration of the gastric IgA-secreting cells. A similar phenomenon has previously been described for CXCR4-expressing plasma cells in the bone marrow, hematopoietic stem cells, and B cells [179-181]. In this study they show that responsiveness to SDF-1, a ligand to CXCR4, was markedly decreased for immature and mature B cells despite relatively high levels of CXCR4 expression. These findings raise the possibility that CXCR4 function is differentially controlled and may be relevant to the compartmentalization of B-cell precursors in the bone marrow [180].

However, our studies are the first to show increased CCL28 production during mucosal infection in humans and may provide an explanation for the large influx of IgA-secreting cells into the gastric mucosa in *H. pylori*-infected individuals.

CONCLUDING REMARKS

H. pylori infection is characterized by formation of organized lymphoid follicles that are not present in the uninfected gastric mucosa. The role of antibodies for protection against *H. pylori* is less clear than the role of $CD4^+$ T cells. It has been shown that the $CD4^+$ T cell response of the Th1 type is associated with protection against *H. pylori*. However, the inductive site for the adaptive immune response has not yet been identified. Our findings are discussed below and refer to numbers in figure 8.

During our *in vitro* experiments we have shown that stimulation of monocyte derived DC with *H. pylori* membrane preparation results in maturation of DC and migration towards CCL19, but to a lesser extent than stimulation with whole bacteria. Even though *H. pylori* are generally thought to be extracellular bacteria there is evidence that whole bacteria or coccoidal forms as well as bacterial components could be present in the gastric antrum tissue during infection [123, 125]. This indicates that *H. pylori* may be able to cross the epithelial membrane. Thus, gastric DC may come into contact with whole bacteria as well as free antigens that penetrate the epithelial layer (**1**). It has also been suggested that mucosal DC may sample antigen in the lumen by opening tight junctions between epithelial cells [145] (**2**).

We have also shown that *H. pylori* are able to induce accumulation of DC-$SIGN^+$ iDC in the *H. pylori* infected human gastric mucosa (**3**). DC-SIGN has been shown to be involved in emigration from blood into tissues (**4**). There is also an increased production of the chemokine CCL20 by the gastric epithelium in *H. pylori*-induced gastritis [129]. Since we have shown that DC stimulated by *H. pylori* secrete TNF-α, which indirectly regulates the production of CCL20 by up-regulation of NF-κβ, we suggest that together with DC-SIGN, this may explain the higher frequencies of DC-SIGN+ iDC in the *H. pylori* infected gastric mucosa. However, since we have not investigated the recruitment mechanisms of iDC we can just suggest that DC-SIGN are involved in the emigration from blood into tissue. We also found that DC-$SIGN^+$ iDC were scattered throughout the lamina propria where they are well situated to recognize, capture and process antigen (**3**). *H. pylori* and other pathogens have been shown to bind to DC-SIGN via PAMPs, and several of these pathogens have also been shown to modulate DC functions and escape immune activation [60, 61].
Based on the results in this thesis we also know that there is a subpopulation of mDC that accumulates in *H. pylori* infected human gastric mucosa, in close association with $CD4^+$ T

cells in the lymphoid follicles (**5**). Since DC-LAMP is a marker for myeloid mature DC we speculate that these cells are derived from myeloid $CD14^+$ monocytes or DC-$SIGN^+$ iDC but of course we can not really know until further experiments are performed. Our *in vitro* experiments showed an increased expression of CCR7 on *H. pylori* stimulated monocyte derived mDC and also that these mDC were able to migrate towards the CCR7 ligand CCL19 (**6**). Thus, *H. pylori* is likely to induce DC migration to the secondary lymphoid follicle. However, a subpopulation of mDC is retained in the gastric mucosa (**5**). This result correlates with other studies on DC in inflamed tissue. For Crohn's disease it has been shown that an increased number of mature myeloid DC form clusters with naïve and memory T cells in the bowel wall. The mDC as well as the naïve and memory T cells express CCR7 and there was also increased expression of the CCR7 ligand CCL19 [139]. However, an accumulation of mDC that do not express CCR7 has been reported for Crohn's disease [140]. The different results may be due to examination of different locations in the intestine.

Further we sought to determine the mechanisms of mDC retention and we could demonstrate increased levels of both CCL19 mRNA and protein in *H. pylori* infected gastric mucosa (**7**). Therefore, it is reasonable to propose that a subpopulation of maturing DC remain in the gastric mucosa due to a CCR7-CCL19 interaction, instead of migrating to draining gastric lymph nodes. However, to be really sure the expression of CCR7 on DC-$LAMP^+$ mDC has to be determined. In our *in vitro* experiments we could show that DC stimulated by *H. pylori* up-regulate the costimulatory molecules important during activation of T cells, and that they induce T cell proliferation (**8**). We also demonstrated that $CD4^+$ T cells were in close association with DC-$LAMP^+$ mDC in the lymphoid follicles in the *H. pylori* infected human gastric mucosa. These findings suggest that some of the mDC remaining in the gastric mucosa may contribute to the activation of local T cells and maybe also to the activation of memory IgA B cells (**9**).

The role of antibodies, particularly mucosal IgA, for protection against *H. pylori* is less clear than the role of T cells. However, it has been shown that patients with IgA deficiency have an increased risk of developing gastrointestinal carcinomas [182, 183]. Previous studies in our group have also shown that patients suffering from gastric adenocarcinoma have a decreased local IgA production in the stomach compared to asymptomatic *H. pylori* infected individuals [184]. These studies show that a protective role for antibodies against *H. pylori* infection and *H. pylori*-associated diseases cannot be ruled out.

Based on these considerations, we investigated the recruitment of B cells to the *H. pylori* infected tissue. We could show that CCL28 expression is increased in human *H. pylori*-induced gastritis (**10**) and that CCL28 efficiently recruits gastric IgA-ASC from *H. pylori*-infected individuals (**11**). This study is the first to show an increase in CCL28 production during mucosal infection in humans and to provide an explanation for the large influx of IgA-secreting cells into the gastric mucosa in *H. pylori*-infected individuals. In line with this findings, circulating IgA-ASC induced by intestinal immunization also respond to CCL28 in chemotaxis assays [29], which is not the case for the general pool of circulating IgA-ASC [155]. We could also show that IgA memory B cells from *H. pylori* infected individuals respond significantly more to CCL28 compared to those from uninfected individuals, but not to the same extent as IgA-ASC. This indicates that IgA memory B cells can be recruited to the inflamed gastric tissue and that they may be activated in the lymphoid follicles (**12**). However, this has to be investigated before we can draw any conclusions.

Moreover, DC-derived cytokines and chemokines that are secreted immediately after exposure to antigen may influence local inflammation in the mucosa. We have found that monocyte derived DC stimulated by *H. pylori* secrete IL-12, IL-10, TNF-α, IL-6 and IL-8 within 24h of stimulation (**13**). These cytokines and chemokines activate other immune cells and epithelial cells, and IL-8 is known to recruit neutrophils to the mucosa (**14**). The fact that *H. pylori* induce relatively high levels of IL-12, but less IL-10 in DC, may contribute directly to the local inflammation as well as to Th1 polarization of naïve T cells in the lymphoid follicles.

In conclusion, this thesis may contribute to a better understanding of how DC and B cells are recruited into the *H. pylori* infected tissue. The results presented in this thesis suggest that some mature DC may contribute to the activation of local T cells in the *H. pylori*-induced chronic inflammation. Further we have shown that *H. pylori* infection increase the level of the chemokine CCL28 and that CCL28 efficiently recruits gastric IgA ASC but also leads to a lower level of IgA memory B cells. These results will probably be important when designing a future vaccine against *H. pylori* infection.

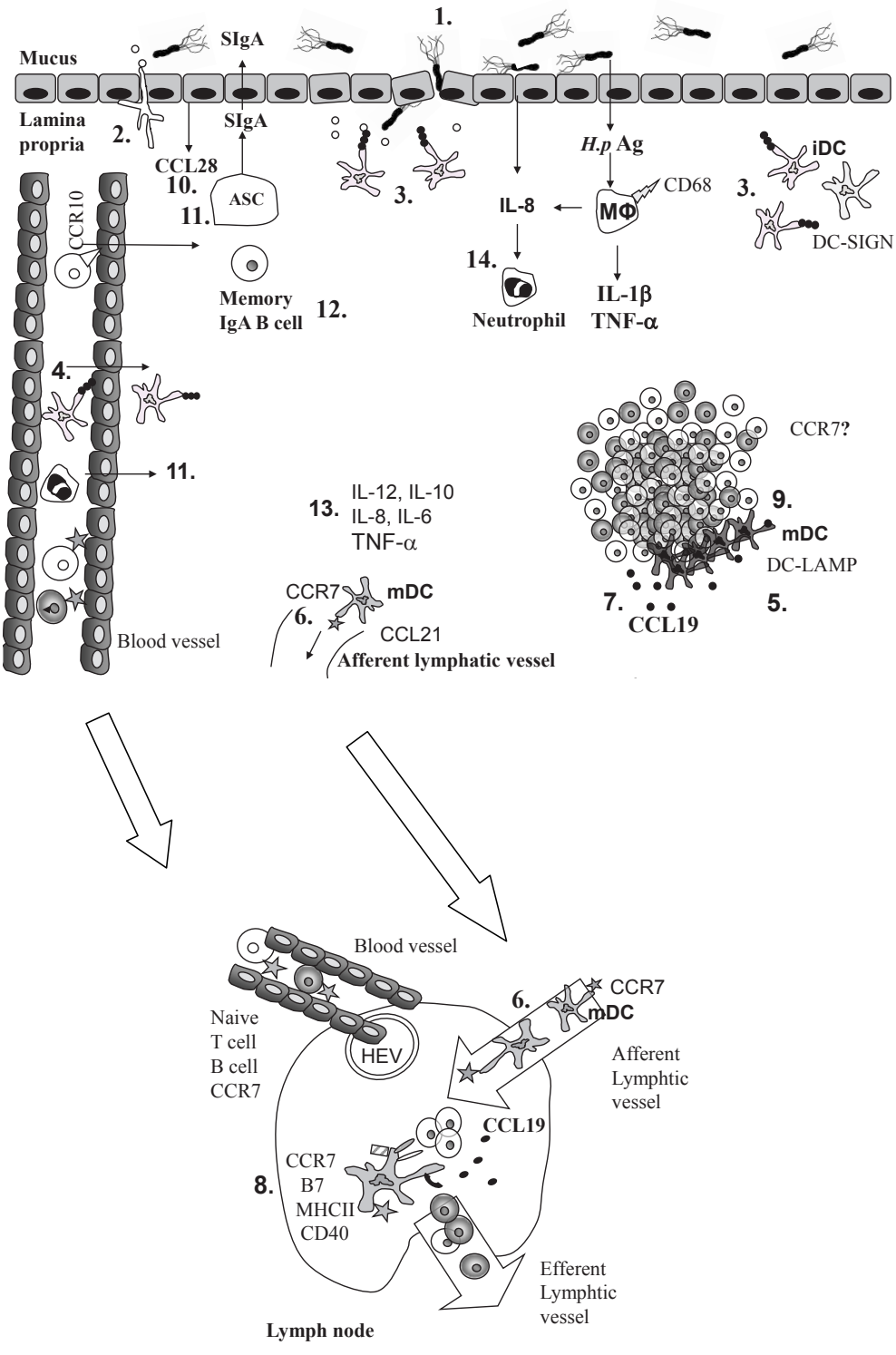
Mucus
Lamina propria
SIgA
SIgA
1.
2.
CCL28
10.
11.
ASC
3.
H.p Ag
CD68
MΦ
IL-8
3.
iDC
DC-SIGN
14.
Neutrophil
IL-1β
TNF-α
CCR10
Memory IgA B cell
12.
4.
11.
13.
IL-12, IL-10
IL-8, IL-6
TNF-α
CCR7?
9.
mDC
DC-LAMP
7.
CCL19
5.
CCR7
mDC
6.
CCL21
Afferent lymphatic vessel
Blood vessel
Blood vessel
Naive
T cell
B cell
CCR7
HEV
6.
CCR7
mDC
Afferent
Lymphtic
vessel
CCL19
8.
CCR7
B7
MHCII
CD40
Efferent
Lymphtic
vessel
Lymph node

Figure 8.

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank everyone who has contributed in various ways to complete this thesis. I would especially like to thank:

Marianne Quiding-Järbrink för att du gav mig plats i din grupp. Du har bidragit till den fina stämningen i gruppen. Din djupa kunskap tillsammans med en avspänd attityd och härliga skratt gör det roligt och intressant att jobba tillsammans med dig.

Ann-Mari Svennerholm min bihandledare. Du är en fantastisk person som har förmågan att leda och entusiasmera. Jag glömmer inte hur du stöttade mig vid min första muntliga presentation på en konferens.

Jan Holmgren som antog mig som doktorand på institutionen.

Gunilla Bogren och Magdalena Granung för hjälp att rekrytera försökspersoner och hjälp vid gastroskopi . **Michael Hermansson och Anders Elfvin** för hjälp med insamling av biopsier.

Ingela Jonson för hjälp med RNA och den ”stora” acrylamide-urea sequencing gelen. Det var alltid trevligt att komma ner till dig.

Anna Lundgren för allt du lärde mig och visade mig då Eva-Liz slutade. Du är en fantastisk person Anna med mycket värme, skratt och kamratskap. Förresten skratt ”have you ever seen so many bulls”……

Matilda Nicklasson det har varit intressant och roligt att prata med dig. Jag är så tacksam för att du hjälpte mig med att korrektur läsa avhandlingen.

Malin Sundquist tack så jätte mycket för korrektur läsning av avhandlingen.

Anna Holmberg för hjälp med bilden till manuset.

Joanna Kaim det är alltid roligt att prata med dig och tack för hjälp med insamling av biopsier.

Jenni Adamsson för hjälp med insamling av biopsier och trevliga samtal.

Camilla Johansson och **Kerstin Andersson** för all hjälp med att ta blod från mina försökspersoner.

Åsa Sjöling för hjälp med RNA. Har någon sagt att ditt skratt är smittsamt?

Gudrun Wiklund du är alltid trevlig och har ända sedan jag började frågat hur det går. Du hjälpte mig även i början då jag gjorde mitt examensarbete.

Ulf Yrlid för samtal om DC.

Karin Enarsson och Johanna Asklin ni har verkligen varit två jättebra vänner. Jag tycker jag hade tur som fick göra huvuddelen av min doktorand tid tillsammans med er. Ni har hjälpt

och stöttat mig i mycket och så var ni väldigt roliga att vara med. För tänk så hemskt det kan vara att behöva stanna i Italien ett extra dygn.
Bert Kindlund och Anders Janzon det var trevligt att dela rum med er och prata om all möjlig vetenskap och annat.
Vår grupp, Hanna Stenstad, Helena Svensson, Veronica Olofsson, Malin Sundquist och Patrik Sundström men även dom gamla Karin Enarsson och Phil Bergin. Ni är mycket trevliga personer med mycket kunskap. Det är lätt att fråga er om hjälp och det betyder mycket.
Susanne Uhlan och **Harald Estling** utan er hade inte mycket fungerat. Ni är så hjälpsamma.
Jag vill även tacka alla övriga på Med mikrobiologi och immunologi för att ni gjort det så trevligt.
Tack även **Anna Rudin** för att du tog en snowboard lektion av mig och sedan presenterade mig för **Eva-Liz Baunge** min första handledare som tyvärr slutade men jag är glad att du antog mig, och satte mig in i dendritcellernas värld.

Ulrika Einald, vilken tur jag har! Du flyttar hem och får en son, Lewin, i samma ålder som Fritz. Vår vänskap betyder mycket.
Malin Pettersson min vän sedan länge. Det betyder så mycket att veta att du alltid finns.
Anncatrin Nilsson tack för att du alltid lyssnar och bryr dig. Du är verkligen en god vän.
Helena Svensson för en rolig studietid tillsammans, nu har jag dock fått ”labba” själv men säger lite tyst ja ändå.
Mamma det är så tråkigt att du inte fick uppleva denna och alla de andra dagarna hemma med Fritz och Axel. Jag vet och känner att du är med mig och tack för att du alltid stöttat och trott på mig. **Pappa** som hela tiden ställer upp, vad det än är. ”Vill du ha kaffe” är något som hjälpt mig genom alla dessa år. Det är sådan tur att vi har dig och så nära. Du betyder allt för Fritz och Axel. Jag är så glad att jag haft er två till mamma och pappa. Jag älskar er.
Killarna Karlbäck, vad vore livet utan er, lugnt, enkelt, tyst och väldigt tråkigt.
Fritz och **Axel** mina underbara pojkar! Ni har saknat mamma mycket nu i höst men snart är jag klar och då är det jul. Ni har varit så rediga när jag bara ”måste” jobba. Jag älskar er så mycket.
Per jag är så tacksam för att du är du. Ingen skulle klara det bättre med allt som hänt oss. Du betyder jätte mycket för mig och snart kan vi starta livet igen. Jag älskar dig.

This study was supported by grants from Swedish Science Council, Swedish Cancer Foundation, Sahlgrenska Hospital Funds for clinical research (LUA/Alf), The Sahlgrenska Academy, Professor Nanna Svartz Foundation, Clas Groshinsky's Foundation. Wilhelm and Martina Lundgren's Foundation.

REFERENCES

1. **Muller, W., et al., *Origin and migration of the Alpine Iceman.* Science, 2003. 302(5646): p. 862-6.**
2. **Ley, K., et al., *Getting to the site of inflammation: the leukocyte adhesion cascade updated.* Nat Rev Immunol, 2007. 7(9): p. 678-89.**
3. **Campbell, J.J., et al., *Chemokines and the arrest of lymphocytes rolling under flow conditions.* Science, 1998. 279(5349): p. 381-4.**
4. **Campbell, J.J., et al., *The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells.* Nature, 1999. 400(6746): p. 776-80.**
5. **Kim, M., C.V. Carman, and T.A. Springer, *Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins.* Science, 2003. 301(5640): p. 1720-5.**
6. **Ayalon, O., et al., *Spatial and temporal relationships between cadherins and PECAM-1 in cell-cell junctions of human endothelial cells.* J Cell Biol, 1994. 126(1): p. 247-58.**
7. **Michie, S.A., et al., *The human peripheral lymph node vascular addressin. An inducible endothelial antigen involved in lymphocyte homing.* Am J Pathol, 1993. 143(6): p. 1688-98.**
8. **Berg, E.L., et al., *L-selectin-mediated lymphocyte rolling on MAdCAM-1.* Nature, 1993. 366(6456): p. 695-8.**
9. **Rosen, S.D. and C.R. Bertozzi, *The selectins and their ligands.* Curr Opin Cell Biol, 1994. 6(5): p. 663-73.**
10. **Zlotnik, A. and O. Yoshie, *Chemokines: a new classification system and their role in immunity.* Immunity, 2000. 12(2): p. 121-7.**
11. **Olson, T.S. and K. Ley, *Chemokines and chemokine receptors in leukocyte trafficking.* Am J Physiol Regul Integr Comp Physiol, 2002. 283(1): p. R7-28.**
12. **Mackay, C.R., *Homing of naive, memory and effector lymphocytes.* Curr Opin Immunol, 1993. 5(3): p. 423-7.**
13. **McDermott, M.R. and J. Bienenstock, *Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory, and genital tissues.* J Immunol, 1979. 122(5): p. 1892-8.**
14. **Quiding, M., et al., *Induction of specific antibody responses in the human nasopharyngeal mucosa.* Adv Exp Med Biol, 1995. 371B: p. 1445-50.**
15. **Eriksson, K., et al., *Anatomic segmentation of the intestinal immune response in nonhuman primates: differential distribution of B cells after oral and rectal immunizations to sites defined by their source of vascularization.* Infect Immun, 1999. 67(11): p. 6210-2.**
16. **Wagner, N., et al., *Critical role for beta7 integrins in formation of the gut-associated lymphoid tissue.* Nature, 1996. 382(6589): p. 366-70.**
17. **Butcher, E.C. and L.J. Picker, *Lymphocyte homing and homeostasis.* Science, 1996. 272(5258): p. 60-6.**
18. **Farstad, I.N., et al., *Human intestinal B-cell blasts and plasma cells express the mucosal homing receptor integrin alpha 4 beta 7.* Scand J Immunol, 1995. 42(6): p. 662-72.**
19. **Berlin, C., et al., *Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1.* Cell, 1993. 74(1): p. 185-95.**

20. **Briskin, M., et al., *Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue.* Am J Pathol, 1997. 151(1): p. 97-110.**
21. **Cyster, J.G., *Homing of antibody secreting cells.* Immunol Rev, 2003. 194: p. 48-60.**
22. **Kunkel, E.J., D.J. Campbell, and E.C. Butcher, *Chemokines in lymphocyte trafficking and intestinal immunity.* Microcirculation, 2003. 10(3-4): p. 313-23.**
23. **Pan, J., et al., *A novel chemokine ligand for CCR10 and CCR3 expressed by epithelial cells in mucosal tissues.* J Immunol, 2000. 165(6): p. 2943-9.**
24. **Wang, W., et al., *Identification of a novel chemokine (CCL28), which binds CCR10 (GPR2).* J Biol Chem, 2000. 275(29): p. 22313-23.**
25. **Kunkel, E.J., et al., *Lymphocyte CC chemokine receptor 9 and epithelial thymus-expressed chemokine (TECK) expression distinguish the small intestinal immune compartment: Epithelial expression of tissue-specific chemokines as an organizing principle in regional immunity.* J Exp Med, 2000. 192(5): p. 761-8.**
26. **Papadakis, K.A., et al., *The role of thymus-expressed chemokine and its receptor CCR9 on lymphocytes in the regional specialization of the mucosal immune system.* J Immunol, 2000. 165(9): p. 5069-76.**
27. **Zabel, B.A., et al., *Human G protein-coupled receptor GPR-9-6/CC chemokine receptor 9 is selectively expressed on intestinal homing T lymphocytes, mucosal lymphocytes, and thymocytes and is required for thymus-expressed chemokine-mediated chemotaxis.* J Exp Med, 1999. 190(9): p. 1241-56.**
28. **Hieshima, K., et al., *CC chemokine ligands 25 and 28 play essential roles in intestinal extravasation of IgA antibody-secreting cells.* J Immunol, 2004. 173(6): p. 3668-75.**
29. **Sundstrom, P., et al., *Human IgA-secreting cells induced by intestinal, but not systemic, immunization respond to CCL25 (TECK) and CCL28 (MEC).* Eur J Immunol, 2008. 38(12): p. 3327-38.**
30. **Mora, J.R., et al., *Reciprocal and dynamic control of CD8 T cell homing by dendritic cells from skin- and gut-associated lymphoid tissues.* J Exp Med, 2005. 201(2): p. 303-16.**
31. **Schwartz, D., et al., *Role of the GRO family of chemokines in monocyte adhesion to MM-LDL-stimulated endothelium.* J Clin Invest, 1994. 94(5): p. 1968-73.**
32. **Yla-Herttuala, S., et al., *Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions.* Proc Natl Acad Sci U S A, 1991. 88(12): p. 5252-6.**
33. **Bevilacqua, M.P. and R.M. Nelson, *Endothelial-leukocyte adhesion molecules in inflammation and metastasis.* Thromb Haemost, 1993. 70(1): p. 152-4.**
34. **Springer, T.A., *Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm.* Cell, 1994. 76(2): p. 301-14.**
35. **Steinman, R.M. and M. Pope, *Exploiting dendritic cells to improve vaccine efficacy.* J Clin Invest, 2002. 109(12): p. 1519-26.**
36. **Kupiec-Weglinski, J.W., J.M. Austyn, and P.J. Morris, *Migration patterns of dendritic cells in the mouse. Traffic from the blood, and T cell-dependent and -independent entry to lymphoid tissues.* J Exp Med, 1988. 167(2): p. 632-45.**
37. **Banchereau, J., et al., *Immunobiology of dendritic cells.* Annu Rev Immunol, 2000. 18: p. 767-811.**
38. **Yoneyama, H., et al., *Evidence for recruitment of plasmacytoid dendritic cell precursors to inflamed lymph nodes through high endothelial venules.* Int Immunol, 2004. 16(7): p. 915-28.**

39. **Steinman, R.M., *The dendritic cell system and its role in immunogenicity.* Annu Rev Immunol, 1991. 9: p. 271-96.**
40. **Hart, D.N., *Dendritic cells: unique leukocyte populations which control the primary immune response.* Blood, 1997. 90(9): p. 3245-87.**
41. **Steinman, R.M. and Z.A. Cohn, *Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution.* J Exp Med, 1973. 137(5): p. 1142-62.**
42. **Steinman, R.M. and Z.A. Cohn, *Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro.* J Exp Med, 1974. 139(2): p. 380-97.**
43. **Steinman, R.M. and M.D. Witmer, *Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice.* Proc Natl Acad Sci U S A, 1978. 75(10): p. 5132-6.**
44. **Valladeau, J. and S. Saeland, *Cutaneous dendritic cells.* Semin Immunol, 2005. 17(4): p. 273-83.**
45. **Shortman, K. and S.H. Naik, *Steady-state and inflammatory dendritic-cell development.* Nat Rev Immunol, 2007. 7(1): p. 19-30.**
46. **Geissmann, F., S. Jung, and D.R. Littman, *Blood monocytes consist of two principal subsets with distinct migratory properties.* Immunity, 2003. 19(1): p. 71-82.**
47. **Gordon, S. and P.R. Taylor, *Monocyte and macrophage heterogeneity.* Nat Rev Immunol, 2005. 5(12): p. 953-64.**
48. **Yrlid, U., C.D. Jenkins, and G.G. MacPherson, *Relationships between distinct blood monocyte subsets and migrating intestinal lymph dendritic cells in vivo under steady-state conditions.* J Immunol, 2006. 176(7): p. 4155-62.**
49. **Ancuta, P., et al., *Fractalkine preferentially mediates arrest and migration of CD16+ monocytes.* J Exp Med, 2003. 197(12): p. 1701-7.**
50. **Auffray, C., et al., *Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior.* Science, 2007. 317(5838): p. 666-70.**
51. **Palframan, R.T., et al., *Inflammatory chemokine transport and presentation in HEV: a remote control mechanism for monocyte recruitment to lymph nodes in inflamed tissues.* J Exp Med, 2001. 194(9): p. 1361-73.**
52. **Janatpour, M.J., et al., *Tumor necrosis factor-dependent segmental control of MIG expression by high endothelial venules in inflamed lymph nodes regulates monocyte recruitment.* J Exp Med, 2001. 194(9): p. 1375-84.**
53. **Sallusto, F. and A. Lanzavecchia, *Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha.* J Exp Med, 1994. 179(4): p. 1109-18.**
54. **Wilson, N.S., et al., *Most lymphoid organ dendritic cell types are phenotypically and functionally immature.* Blood, 2003. 102(6): p. 2187-94.**
55. **Soilleux, E.J., et al., *Constitutive and induced expression of DC-SIGN on dendritic cell and macrophage subpopulations in situ and in vitro.* J Leukoc Biol, 2002. 71(3): p. 445-57.**
56. **Nortamo, P., et al., *The expression of human intercellular adhesion molecule-2 is refractory to inflammatory cytokines.* Eur J Immunol, 1991. 21(10): p. 2629-32.**
57. **Garcia-Vallejo, J.J., et al., *DC-SIGN mediates adhesion and rolling of dendritic cells on primary human umbilical vein endothelial cells through LewisY antigen expressed on ICAM-2.* Mol Immunol, 2008. 45(8): p. 2359-69.**

58. **Geijtenbeek, T.B., et al., *DC-SIGN-ICAM-2 interaction mediates dendritic cell trafficking.* Nat Immunol, 2000. 1(4): p. 353-7.**
59. **Bergman, M.P., et al., *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, 2004. 200(8): p. 979-90.**
60. **van Kooyk, Y., B. Appelmelk, and T.B. Geijtenbeek, *A fatal attraction: Mycobacterium tuberculosis and HIV-1 target DC-SIGN to escape immune surveillance.* Trends Mol Med, 2003. 9(4): p. 153-9.**
61. **Geijtenbeek, T.B., et al., *DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells.* Cell, 2000. 100(5): p. 587-97.**
62. **Rock, J., et al., *CD303 (BDCA-2) signals in plasmacytoid dendritic cells via a BCR-like signalosome involving Syk, Slp65 and PLCgamma2.* Eur J Immunol, 2007. 37(12): p. 3564-75.**
63. **Ito, T., Y.H. Wang, and Y.J. Liu, *Plasmacytoid dendritic cell precursors/type I interferon-producing cells sense viral infection by Toll-like receptor (TLR) 7 and TLR9.* Springer Semin Immunopathol, 2005. 26(3): p. 221-9.**
64. **Liu, Y.J., *IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors.* Annu Rev Immunol, 2005. 23: p. 275-306.**
65. **Sallusto, F., et al., *Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation.* Eur J Immunol, 1998. 28(9): p. 2760-9.**
66. **Sozzani, S., et al., *Differential regulation of chemokine receptors during dendritic cell maturation: a model for their trafficking properties.* J Immunol, 1998. 161(3): p. 1083-6.**
67. **de Saint-Vis, B., et al., *A novel lysosome-associated membrane glycoprotein, DC-LAMP, induced upon DC maturation, is transiently expressed in MHC class II compartment.* Immunity, 1998. 9(3): p. 325-36.**
68. **Forster, R., et al., *CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs.* Cell, 1999. 99(1): p. 23-33.**
69. **Reis e Sousa, C., *Dendritic cells in a mature age.* Nat Rev Immunol, 2006. 6(6): p. 476-83.**
70. **Mazzoni, A. and D.M. Segal, *Controlling the Toll road to dendritic cell polarization.* J Leukoc Biol, 2004. 75(5): p. 721-30.**
71. **Spellberg, B. and J.E. Edwards, Jr., *Type 1/Type 2 immunity in infectious diseases.* Clin Infect Dis, 2001. 32(1): p. 76-102.**
72. **Suzuki, K., et al., *Intestinal IgA synthesis: a primitive form of adaptive immunity that regulates microbial communities in the gut.* Semin Immunol, 2007. 19(2): p. 127-35.**
73. **Macpherson, A.J. and E. Slack, *The functional interactions of commensal bacteria with intestinal secretory IgA.* Curr Opin Gastroenterol, 2007. 23(6): p. 673-8.**
74. **Macpherson, A.J., et al., *The immune geography of IgA induction and function.* Mucosal Immunol, 2008. 1(1): p. 11-22.**
75. **Youngman, K.R., et al., *Correlation of tissue distribution, developmental phenotype, and intestinal homing receptor expression of antigen-specific B cells during the murine anti-rotavirus immune response.* J Immunol, 2002. 168(5): p. 2173-81.**
76. **Williams, M.B., et al., *The memory B cell subset responsible for the secretory IgA response and protective humoral immunity to rotavirus expresses the intestinal homing receptor, alpha4beta7.* J Immunol, 1998. 161(8): p. 4227-35.**

77. **LeBien, T.W. and T.F. Tedder, *B lymphocytes: how they develop and function.* Blood, 2008. 112(5): p. 1570-80.**
78. **Garside, P., et al., *Visualization of specific B and T lymphocyte interactions in the lymph node.* Science, 1998. 281(5373): p. 96-9.**
79. **Manser, T., *Textbook germinal centers?* J Immunol, 2004. 172(6): p. 3369-75.**
80. **Slifka, M.K., et al., *Humoral immunity due to long-lived plasma cells.* Immunity, 1998. 8(3): p. 363-72.**
81. **Lycke, N., L. Eriksen, and J. Holmgren, *Protection against cholera toxin after oral immunization is thymus-dependent and associated with intestinal production of neutralizing IgA antitoxin.* Scand J Immunol, 1987. 25(4): p. 413-9.**
82. **Mattsson, A., et al., *Antibody-secreting cells in the stomachs of symptomatic and asymptomatic Helicobacter pylori-infected subjects.* Infect Immun, 1998. 66(6): p. 2705-12.**
83. **Craxton, A., et al., *Macrophage- and dendritic cell--dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF.* Blood, 2003. 101(11): p. 4464-71.**
84. **Bergtold, A., et al., *Cell surface recycling of internalized antigen permits dendritic cell priming of B cells.* Immunity, 2005. 23(5): p. 503-14.**
85. **Castigli, E., et al., *TACI and BAFF-R mediate isotype switching in B cells.* J Exp Med, 2005. 201(1): p. 35-9.**
86. **Litinskiy, M.B., et al., *DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL.* Nat Immunol, 2002. 3(9): p. 822-9.**
87. **Uematsu, S., et al., *Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5.* Nat Immunol, 2008. 9(7): p. 769-76.**
88. **Mora, J.R., et al., *Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells.* Science, 2006. 314(5802): p. 1157-60.**
89. **Lazarus, N.H., et al., *A common mucosal chemokine (mucosae-associated epithelial chemokine/CCL28) selectively attracts IgA plasmablasts.* J Immunol, 2003. 170(7): p. 3799-805.**
90. **Marshall, B.J. and J.R. Warren, *Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration.* Lancet, 1984. 1(8390): p. 1311-5.**
91. **Kuipers, E.J., J.C. Thijs, and H.P. Festen, *The prevalence of Helicobacter pylori in peptic ulcer disease.* Aliment Pharmacol Ther, 1995. 9 Suppl 2: p. 59-69.**
92. **Rothenbacher, D. and H. Brenner, *Burden of Helicobacter pylori and H. pylori-related diseases in developed countries: recent developments and future implications.* Microbes Infect, 2003. 5(8): p. 693-703.**
93. **Mitchell, H. and F. Megraud, *Epidemiology and diagnosis of Helicobacter pylori infection.* Helicobacter, 2002. 7 Suppl 1: p. 8-16.**
94. **Janzon, A., et al., *Failure to detect Helicobacter pylori DNA in drinking and environmental water in Dhaka, Bangladesh, using highly sensitive real-time PCR assays.* Appl Environ Microbiol, 2009. 75(10): p. 3039-44.**
95. **Correa, P., *Is gastric cancer preventable?* Gut, 2004. 53(9): p. 1217-9.**
96. **Akopyanz, N., et al., *DNA diversity among clinical isolates of Helicobacter pylori detected by PCR-based RAPD fingerprinting.* Nucleic Acids Res, 1992. 20(19): p. 5137-42.**
97. **Bazzoli, F., P. Pozzato, and T. Rokkas, *Helicobacter pylori: the challenge in therapy.* Helicobacter, 2002. 7 Suppl 1: p. 43-9.**
98. **Figueiredo, C., J.C. Machado, and Y. Yamaoka, *Pathogenesis of Helicobacter pylori Infection.* Helicobacter, 2005. 10 Suppl 1: p. 14-20.**

99. **Bodger, K. and J.E. Crabtree,** ***Helicobacter pylori and gastric inflammation.*** **Br Med Bull, 1998. 54(1): p. 139-50.**
100. **Del Giudice, G. and P. Michetti,** ***Inflammation, immunity and vaccines for Helicobacter pylori.*** **Helicobacter, 2004. 9 Suppl 1: p. 23-8.**
101. **Genta, R.M., H.W. Hamner, and D.Y. Graham,** ***Gastric lymphoid follicles in Helicobacter pylori infection: frequency, distribution, and response to triple therapy.*** **Hum Pathol, 1993. 24(6): p. 577-83.**
102. **Birkholz, S., et al.,** ***Immunological activity of lipopolysaccharide of Helicobacter pylori on human peripheral mononuclear blood cells in comparison to lipopolysaccharides of other intestinal bacteria.*** **FEMS Immunol Med Microbiol, 1993. 6(4): p. 317-24.**
103. **Moran, A.P., M.M. Prendergast, and B.J. Appelmelk,** ***Molecular mimicry of host structures by bacterial lipopolysaccharides and its contribution to disease.*** **FEMS Immunol Med Microbiol, 1996. 16(2): p. 105-15.**
104. **Marshall, B.,** ***Helicobacter pylori: 20 years on.*** **Clin Med, 2002. 2(2): p. 147-52.**
105. **Boren, T., et al.,** ***Attachment of Helicobacter pylori to human gastric epithelium mediated by blood group antigens.*** **Science, 1993. 262(5141): p. 1892-5.**
106. **Mahdavi, J., et al.,** ***Helicobacter pylori SabA adhesin in persistent infection and chronic inflammation.*** **Science, 2002. 297(5581): p. 573-8.**
107. **Reyrat, J.M., et al.,** ***Towards deciphering the Helicobacter pylori cytotoxin.*** **Mol Microbiol, 1999. 34(2): p. 197-204.**
108. **Boquet, P., et al.,** ***Gastric cell apoptosis and H. pylori: has the main function of VacA finally been identified?*** **Trends Microbiol, 2003. 11(9): p. 410-3.**
109. **Crabtree, J.E., et al.,** ***Helicobacter pylori induced interleukin-8 expression in gastric epithelial cells is associated with CagA positive phenotype.*** **J Clin Pathol, 1995. 48(1): p. 41-5.**
110. **Nishi, T., et al.,** ***Involvement of myeloid dendritic cells in the development of gastric secondary lymphoid follicles in Helicobacter pylori-infected neonatally thymectomized BALB/c mice.*** **Infect Immun, 2003. 71(4): p. 2153-62.**
111. **Lundgren, A., et al.,** ***Helicobacter pylori-specific CD4+ CD25high regulatory T cells suppress memory T-cell responses to H. pylori in infected individuals.*** **Infect Immun, 2003. 71(4): p. 1755-62.**
112. **Lundgren, A., et al.,** ***Mucosal FOXP3-expressing CD4+ CD25high regulatory T cells in Helicobacter pylori-infected patients.*** **Infect Immun, 2005. 73(1): p. 523-31.**
113. **Mitchell, P.,** ***Chronic exposure to Helicobacter pylori impairs dendritic cell function.*** **Infection and immunity, 2007. 75(2): p. 810.**
114. **Uronen-Hansson, H., et al.,** ***Toll-like receptor 2 (TLR2) and TLR4 are present inside human dendritic cells, associated with microtubules and the Golgi apparatus but are not detectable on the cell surface: integrity of microtubules is required for interleukin-12 production in response to internalized bacteria.*** **Immunology, 2004. 111(2): p. 173-8.**
115. **Appelmelk, B.J., et al.,** ***Cutting edge: carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells.*** **J Immunol, 2003. 170(4): p. 1635-9.**
116. **Kranzer, K., et al.,** ***Impact of Helicobacter pylori virulence factors and compounds on activation and maturation of human dendritic cells.*** **Infect Immun, 2005. 73(7): p. 4180-9.**
117. **Guiney, D.G., P. Hasegawa, and S.P. Cole,** ***Helicobacter pylori preferentially induces interleukin 12 (IL-12) rather than IL-6 or IL-10 in human dendritic cells.*** **Infect Immun, 2003. 71(7): p. 4163-6.**

118. **Hafsi, N., et al., *Human dendritic cells respond to Helicobacter pylori, promoting NK cell and Th1-effector responses in vitro.* J Immunol, 2004. 173(2): p. 1249-57.**
119. **Andersen, L.P. and S. Holck, *Possible evidence of invasiveness of Helicobacter (Campylobacter) pylori.* Eur J Clin Microbiol Infect Dis, 1990. 9(2): p. 135-8.**
120. **Mai, U.E., et al., *Surface proteins from Helicobacter pylori exhibit chemotactic activity for human leukocytes and are present in gastric mucosa.* J Exp Med, 1992. 175(2): p. 517-25.**
121. **Morozov, I.A., *[Possibility of Helicobacter pylori invasion into the lamina propria of the gastric mucosa].* Arkh Patol, 1994. 56(3): p. 19-22.**
122. **Ko, G.H., et al., *Invasiveness of Helicobacter pylori into human gastric mucosa.* Helicobacter, 1999. 4(2): p. 77-81.**
123. **Necchi, V., et al., *Intracellular, intercellular, and stromal invasion of gastric mucosa, preneoplastic lesions, and cancer by Helicobacter pylori.* Gastroenterology, 2007. 132(3): p. 1009-23.**
124. **Ito, T., et al., *Helicobacter pylori invades the gastric mucosa and translocates to the gastric lymph nodes.* Lab Invest, 2008. 88(6): p. 664-81.**
125. **Dubois, A. and T. Boren, *Helicobacter pylori is invasive and it may be a facultative intracellular organism.* Cell Microbiol, 2007. 9(5): p. 1108-16.**
126. **Necchi, V., et al., *Evidence for transepithelial dendritic cells in human H. pylori active gastritis.* Helicobacter, 2009. 14(3): p. 208-22.**
127. **Quiding-Jarbrink, M., et al., *Human gastric B cell responses can be induced by intestinal immunisation.* Gut, 2001. 49(4): p. 512-8.**
128. **Quiding-Jarbrink, M., et al., *Homing commitment of lymphocytes activated in the human gastric and intestinal mucosa.* Gut, 2001. 49(4): p. 519-25.**
129. **Yoshida, A., et al., *Enhanced expression of CCL20 in human Helicobacter pylori-associated gastritis.* Clin Immunol, 2009. 130(3): p. 290-7.**
130. **Greaves, D.R., et al., *CCR6, a CC chemokine receptor that interacts with macrophage inflammatory protein 3alpha and is highly expressed in human dendritic cells.* J Exp Med, 1997. 186(6): p. 837-44.**
131. **Baba, M., et al., *Identification of CCR6, the specific receptor for a novel lymphocyte-directed CC chemokine LARC.* J Biol Chem, 1997. 272(23): p. 14893-8.**
132. **Kaser, A., et al., *Increased expression of CCL20 in human inflammatory bowel disease.* J Clin Immunol, 2004. 24(1): p. 74-85.**
133. **Wu, Y.Y., et al., *Upregulation of CCL20 and recruitment of CCR6+ gastric infiltrating lymphocytes in Helicobacter pylori gastritis.* Infect Immun, 2007. 75(9): p. 4357-63.**
134. **Bauditz, J., et al., *Production of IL-12 in gastritis relates to infection with Helicobacter pylori.* Clin Exp Immunol, 1999. 117(2): p. 316-23.**
135. **D'Elios, M.M., et al., *T helper 1 effector cells specific for Helicobacter pylori in the gastric antrum of patients with peptic ulcer disease.* J Immunol, 1997. 158(2): p. 962-7.**
136. **Rad, R., et al., *Extracellular and intracellular pattern recognition receptors cooperate in the recognition of Helicobacter pylori.* Gastroenterology, 2009. 136(7): p. 2247-57.**
137. **Rad, R., et al., *Toll-like receptor-dependent activation of antigen-presenting cells affects adaptive immunity to Helicobacter pylori.* Gastroenterology, 2007. 133(1): p. 150-163 e3.**
138. **Ninomiya, T., et al., *Localization and characterization of antigen-presenting dendritic cells in the gastric mucosa of murine and human autoimmune gastritis.* Eur J Clin Invest, 2000. 30(4): p. 350-8.**

139. Middel, P., et al., *Increased number of mature dendritic cells in Crohn's disease: evidence for a chemokine mediated retention mechanism.* Gut, 2006. 55(2): p. 220-7.
140. Salim, S.Y., et al., *CD83+CCR7- dendritic cells accumulate in the subepithelial dome and internalize translocated Escherichia coli HB101 in the Peyer's patches of ileal Crohn's disease.* Am J Pathol, 2009. 174(1): p. 82-90.
141. Kiriya, K., et al., *Essential role of Peyer's patches in the development of Helicobacter-induced gastritis.* Int Immunol, 2007. 19(4): p. 435-46.
142. Nagai, S., et al., *Role of Peyer's patches in the induction of Helicobacter pylori-induced gastritis.* Proc Natl Acad Sci U S A, 2007. 104(21): p. 8971-6.
143. Tanimoto, K., et al., *Immunohistochemical localization of antigen presenting cells in liver from patients with primary biliary cirrhosis; highly restricted distribution of CD83-positive activated dendritic cells.* Pathol Res Pract, 1999. 195(3): p. 157-62.
144. Katou, F., et al., *Differential expression of CCL19 by DC-Lamp+ mature dendritic cells in human lymph node versus chronically inflamed skin.* J Pathol, 2003. 199(1): p. 98-106.
145. Rescigno, M., et al., *Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria.* Nat Immunol, 2001. 2(4): p. 361-7.
146. Hamlet, A., et al., *Duodenal Helicobacter pylori infection differs in cagA genotype between asymptomatic subjects and patients with duodenal ulcers.* Gastroenterology, 1999. 116(2): p. 259-68.
147. Dixon, M.F., et al., *Histological classification of gastritis and Helicobacter pylori infection: an agreement at last? The International Workshop on the Histopathology of Gastritis.* Helicobacter, 1997. 2 Suppl 1: p. S17-24.
148. Enarsson, K., et al., *Differential mechanisms for T lymphocyte recruitment in normal and neoplastic human gastric mucosa.* Clin Immunol, 2006. 118(1): p. 24-34.
149. Enroth, H., et al., *Does the method of Helicobacter pylori detection influence the association with gastric cancer risk?* Scand J Gastroenterol, 2002. 37(8): p. 884-90.
150. Hook-Nikanne, J., G.I. Perez-Perez, and M.J. Blaser, *Antigenic characterization of Helicobacter pylori strains from different parts of the world.* Clin Diagn Lab Immunol, 1997. 4(5): p. 592-7.
151. Selbach, M., et al., *Functional analysis of the Helicobacter pylori cag pathogenicity island reveals both VirD4-CagA-dependent and VirD4-CagA-independent mechanisms*, in *Infect Immun*. 2002. p. 665-71.
152. Bolin, I., H. Lonroth, and A.M. Svennerholm, *Identification of Helicobacter pylori by immunological dot blot method based on reaction of a species-specific monoclonal antibody with a surface-exposed protein.* J Clin Microbiol, 1995. 33(2): p. 381-4.
153. Lindholm, C., J. Osek, and A.M. Svennerholm, *Quantification of conserved antigens in Helicobacter pylori during different culture conditions.* Infect Immun, 1997. 65(12): p. 5376-80.
154. Thoreson, A.C., et al., *Differences in surface-exposed antigen expression between Helicobacter pylori strains isolated from duodenal ulcer patients and from asymptomatic subjects.* J Clin Microbiol, 2000. 38(9): p. 3436-41.
155. Johansson, C., et al., *Differential expression of chemokine receptors on human IgA+ and IgG+ B cells.* Clin Exp Immunol, 2005. 141(2): p. 279-87.
156. Pernas-Alonso, R., et al., *Multiplex semi-quantitative reverse transcriptase-polymerase chain reaction of low abundance neuronal mRNAs.* Brain Res Brain Res Protoc, 1999. 4(3): p. 395-406.

157. **Bergquist, C., et al., *Development of a new method for the determination of immune responses in the human stomach.* J Immunol Methods, 2000. 234(1-2): p. 51-9.**
158. **Czerkinsky, C.C., et al., *A solid-phase enzyme-linked immunospot (ELISPOT) assay for enumeration of specific antibody-secreting cells.* J Immunol Methods, 1983. 65(1-2): p. 109-21.**
159. **Czerkinsky, C., et al., *A novel two colour ELISPOT assay. I. Simultaneous detection of distinct types of antibody-secreting cells.* J Immunol Methods, 1988. 115(1): p. 31-7.**
160. **Mattsson, A., et al., *Induction of B cell responses in the stomach of Helicobacter pylori- infected subjects after oral cholera vaccination.* J Clin Invest, 1998. 102(1): p. 51-6.**
161. **Ernst, P.B. and B.D. Gold, *The disease spectrum of Helicobacter pylori: the immunopathogenesis of gastroduodenal ulcer and gastric cancer.* Annu Rev Microbiol, 2000. 54: p. 615-40.**
162. **Sarsfield, P., et al., *A study of accessory cells in the acquired lymphoid tissue of helicobacter gastritis.* J Pathol, 1996. 180(1): p. 18-25.**
163. **Geijtenbeek, T.B., et al., *Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses.* Cell, 2000. 100(5): p. 575-85.**
164. **Kao, J.Y., et al., *Helicobacter pylori-secreted factors inhibit dendritic cell IL-12 secretion: a mechanism of ineffective host defense.* Am J Physiol Gastrointest Liver Physiol, 2006. 291(1): p. G73-81.**
165. **Liao, F., et al., *CC-chemokine receptor 6 is expressed on diverse memory subsets of T cells and determines responsiveness to macrophage inflammatory protein 3 alpha.* J Immunol, 1999. 162(1): p. 186-94.**
166. **Salazar-Gonzalez, R.M., et al., *CCR6-mediated dendritic cell activation of pathogen-specific T cells in Peyer's patches.* Immunity, 2006. 24(5): p. 623-32.**
167. **Kranzer, K., et al., *Induction of maturation and cytokine release of human dendritic cells by Helicobacter pylori.* Infect Immun, 2004. 72(8): p. 4416-23.**
168. **Hida, N., et al., *Increased expression of IL-10 and IL-12 (p40) mRNA in Helicobacter pylori infected gastric mucosa: relation to bacterial cag status and peptic ulceration.* J Clin Pathol, 1999. 52(9): p. 658-64.**
169. **D'Andrea, A., et al., *Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells.* J Exp Med, 1993. 178(3): p. 1041-8.**
170. **Jotwani, R., et al., *Mature dendritic cells infiltrate the T cell-rich region of oral mucosa in chronic periodontitis: in situ, in vivo, and in vitro studies.* J Immunol, 2001. 167(8): p. 4693-700.**
171. **Ladanyi, A., et al., *Density of DC-LAMP(+) mature dendritic cells in combination with activated T lymphocytes infiltrating primary cutaneous melanoma is a strong independent prognostic factor.* Cancer Immunol Immunother, 2007. 56(9): p. 1459-69.**
172. **Zaitoun, A.M., *The prevalence of lymphoid follicles in Helicobacter pylori associated gastritis in patients with ulcers and non-ulcer dyspepsia.* J Clin Pathol, 1995. 48(4): p. 325-9.**
173. **Bamford, K.B., et al., *Lymphocytes in the human gastric mucosa during Helicobacter pylori have a T helper cell 1 phenotype.* Gastroenterology, 1998. 114(3): p. 482-92.**

174. **Hansson, M., et al., *Dendritic cells express CCR7 and migrate in response to CCL19 (MIP-3beta) after exposure to Helicobacter pylori.* Microbes Infect, 2006. 8(3): p. 841-50.**
175. **Bowman, E.P., et al., *The intestinal chemokine thymus-expressed chemokine (CCL25) attracts IgA antibody-secreting cells.* J Exp Med, 2002. 195(2): p. 269-75.**
176. **Ogawa, H., et al., *Regulated production of the chemokine CCL28 in human colon epithelium.* Am J Physiol Gastrointest Liver Physiol, 2004. 287(5): p. G1062-9.**
177. **Odendahl, M., et al., *Generation of migratory antigen-specific plasma blasts and mobilization of resident plasma cells in a secondary immune response.* Blood, 2005. 105(4): p. 1614-21.**
178. **Kunkel, E.J., et al., *CCR10 expression is a common feature of circulating and mucosal epithelial tissue IgA Ab-secreting cells.* J Clin Invest, 2003. 111(7): p. 1001-10.**
179. **Hauser, A.E., et al., *Chemotactic responsiveness toward ligands for CXCR3 and CXCR4 is regulated on plasma blasts during the time course of a memory immune response.* J Immunol, 2002. 169(3): p. 1277-82.**
180. **Honczarenko, M., et al., *SDF-1 responsiveness does not correlate with CXCR4 expression levels of developing human bone marrow B cells.* Blood, 1999. 94(9): p. 2990-8.**
181. **Shen, H., et al., *CXCR-4 desensitization is associated with tissue localization of hemopoietic progenitor cells.* J Immunol, 2001. 166(8): p. 5027-33.**
182. **Filipovich, A.H., et al., *Lymphoproliferative disorders and other tumors complicating immunodeficiencies.* Immunodeficiency, 1994. 5(2): p. 91-112.**
183. **Cunningham-Rundles, C., et al., *Selective IgA deficiency and neoplasia.* Vox Sang, 1980. 38(2): p. 61-7.**
184. **Quiding-Jarbrink, M., et al., *Decreased IgA antibody production in the stomach of gastric adenocarcinoma patients.* Clin Immunol, 2009. 131(3): p. 463-71.**