

Review

Role of Regulatory T-cells in *H. pylori*-induced Gastritis and Gastric Cancer

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Abstract. *The current model of gastric carcinogenesis comprises the interaction of multiple risk factors. Besides Helicobacter pylori (H. pylori) infection as the major risk factor for gastric carcinogenesis, environmental factors (e.g. high saline- or nitrosamine-containing food) and genetic susceptibility contribute to the development of gastric cancer (GC). It has been established that the topographical pattern of gastritis and its immune response are the main causes for the persistence of bacteria and the final clinical outcome. Regulatory immune cells, mostly regulatory FOXP3⁺CD4⁺CD25^{high} T-cells (Treg cells), have been identified as the major regulatory component of the adaptive immune response and involved in H. pylori-related inflammation and bacterial persistence. The functional activity of these cells is either mediated by direct cell-cell contact or by the secretion of the immune-modulating cytokines TGF- β 1 and IL-10. Based on the differentiation process, Treg cells comprise various lineages that differ in the expression of cell surface marker and pattern of secreted cytokines. Numerous studies have demonstrated important functions of Treg cells for controlling acute and chronic inflammatory processes. This paper reviews the role of Treg for gastric carcinogenesis and precursor lesions related to H. pylori.*

Epidemiological Changes of Gastric Cancer

Although the incidence of gastric cancer (GC) has been rapidly declining in most Western countries (1), the disease remains a challenge and burden worldwide, individually and socio-economically. Due to the predicted growth of the world

population and the increased life expectancy in most countries, the absolute number of GC cases is likely to stabilise or even to increase in the future. Recent estimates calculated roughly 900,000 new GC cases each year, with an annual associated death toll of 700,000 (1). Regional incidence rates are reported to vary by a factor of 10, with the highest rates in East Asia, Eastern Europe, and parts of Central and Southern America, and the lowest rates in Southern Asia, North and East Africa, Australia and North America (2). The decrease of GC incidence has been steady and well documented for decades (2-4), and is most likely a result of significant reduction of various risk factors that include changes in food preservation (cooling and freezing instead of salting, smoking and fermentation) and a decreasing prevalence of *Helicobacter pylori* by birth cohort (2).

Regarding the clinical management of GC, the 5-year survival rate has not significantly improved and is still below 30% in most countries, except Japan. The decreasing mortality rates for GC are explained by a lower incidence and are not affected significantly by therapeutic improvements. As exemplarily illustrated for Germany, the standardised mortality rate has declined by more than 80% in the last six decades. In West Germany, 24,600 and 8,200 individuals died from GC in 1952 and 2007 respectively. Actual numbers after reunification of Germany in 1990 demonstrate that the incidence of this disease has continuously decreased, with an average annual rate of 3% in the last 16 years (Table I). Similar changes in the incidence rates have been reported for most other European countries, with a reduction of up to 5.4% per year between 1994 and 2005 (4).

Gastric Cancer – A Multifactorial Disease

Gastric cancer develops sporadically in about 97% of all cases, while the remaining cases are attributed to germline mutations, in most cases mutations of the E-cadherin (*CDH-1*) gene (5, 6). Currently, several pathogenetic models exist for the development of sporadic GC. The first one, dating back about 25 years, describes the process of gastric carcinogenesis as a

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Table I. Annual change of gastric cancer mortality in Germany.

| Year | Number of patients who died from GC | Change relative to previous year (%) |
|---------|-------------------------------------|--------------------------------------|
| 1991 | 17240 | |
| 1992 | 16938 | -1.8% |
| 1993 | 16242 | -4.1% |
| 1994 | 15929 | -2.0% |
| 1995 | 15389 | -3.4% |
| 1996 | 15244 | -1.0% |
| 1997 | 14217 | -6.7% |
| 1998 | 13821 | -2.8% |
| 1999 | 13145 | -4.9% |
| 2000 | 13132 | -0.1% |
| 2001 | 12451 | -5.2% |
| 2002 | 12388 | -0.5% |
| 2003 | 11844 | -4.4% |
| 2004 | 11473 | -3.1% |
| 2005 | 11300 | -1.5% |
| 2006 | 10623 | -6.0% |
| 2007 | 10487 | -1.3% |
| Average | | -3.05±2.0% |

The table illustrates the reduction of annual mortality rates from gastric cancer in Germany. Due to limited prognosis and overall survival (5-year survival <25 %), the decline of GC-related deaths mirrors the decreased incidence of the disease. Data were taken from the website of the German Centre of Cancer Research ("Deutsches Krebsforschungszentrum"), Heidelberg, Germany (http://www.dkfz.de/de/krebsatlas/organe/151_tab.html).

stepwise progression through histopathologically defined stages: (I) chronic gastritis, (II) atrophy and intestinal metaplasia, and (III) intraepithelial neoplasia (formerly known as "dysplasia"). This model relates to the majority of intestinal type GC according to the Lauren classification (7-9). Alternatively, predominantly in the diffuse type of GC, the development of malignant disease occurs without prior histologically recognisable alterations (8) (Figure 1).

In recent years, the role of mesenchymal- or bone marrow-derived stem cells for the malignant transformation has been studied intensively, mostly in animal models (10, 11). It has been demonstrated that this cell population can serve as a "seeding point" for gastric carcinogenesis, but the relevance with respect to human disease remains still unclear (11).

The strong association of GC with *H. pylori* infection led to the classification of this bacterium as "definite carcinogen" (class I) by the World Health Organization in 1994 (12). This classification was reconfirmed in 2009 after intensive re-evaluation of data published between 1994 and 2008 (13). As discussed for other tumours, chronic inflammation, triggered by *H. pylori* infection, is considered a risk factor for malignant transformation (14). Furthermore, several other risk factors, such the high intake of salty and nitrosamine-containing food, as well as smoking, contribute to gastric carcinogenesis (15).

H. pylori infection is associated with an up to 6-fold elevated risk of developing GC compared to non-infected individuals (16, 17). Although, 85% of *H. pylori*-infected individuals remain asymptomatic throughout their life, and only one out of seven eventually develops severe disease such as duodenal ulcer (10-12%), gastric ulcer (3-5%), adenocarcinoma (1-2%) or mucosa-associated lymphoid tissue lymphoma (0.1%), all infected individuals present gastric inflammation (gastritis) histologically (17, 18). From clinical observations and multiple studies it has been concluded that in particular, duodenal ulcer (DU) disease and GC are mutually exclusive diseases because of different physiological sequences (17). The major differences in the pathophysiology of the stomach of *H. pylori*-infected individuals are antrum-predominant gastritis that predisposes them to develop DU (with higher acid secretion) (19) and corpus-predominant gastritis that transits into atrophic changes that eventually can lead to GC (Figure 1).

Bacterial Virulence Factors

Bacterial virulence factors play an important role for the topology and the degree of inflammatory alterations. The most important virulence factor of *H. pylori* is the presence of the cag pathogenicity island (cagPAI). CagPAI encodes for a type 4 secretory system that is responsible for the transfer of CagA into the host cell, where it affects various pathways such as the intracellular signal transduction and cytoskeletal rearrangements (20, 21). In general, cagPAI-positive *H. pylori* strains are associated with more severe gastritis and bear an increased risk for the development of subsequent diseases such as ulcer and GC compared to strains lacking cagPAI (17, 21). Other important virulence factors encoded in the bacterial chromosome are *vacA*, *babA* and *sabA*. The *H. pylori* vacuolating toxin (VacA) is secreted and leads to epithelial vacuolisation, and was reported to mediate immunosuppressive effects, while the *H. pylori* outer-membrane proteins BabA and SabA represent key molecules for the adherence of *H. pylori* to epithelial cells (22, 23).

Besides bacterial virulence factors, genetic susceptibility plays a role in gastric carcinogenesis. To date, more than 1,500 papers have been published regarding genetic susceptibility and gastric carcinogenesis. In 2001, El-Omar *et al.* identified the association of 3 haplotypes of the *IL1* gene with an increased risk for GC with a hazard ratio of up to 5.3 for developing noncardia cancer (24). Since then, genetic polymorphisms linked to GC have been demonstrated for cytokines (*e.g.* TNF- α , IL-10, IL-8, IFN- γ), pattern recognition factors (TLR-4, NOD-1, NOD-2), proteases (MMP9), xenobiotic metabolism enzymes, cell cycle regulators, HLA molecules and DNA repair enzymes (25-27). There is no doubt that these genetic factors contribute to gastric carcinogenesis, but so far no "genetic risk profile" has

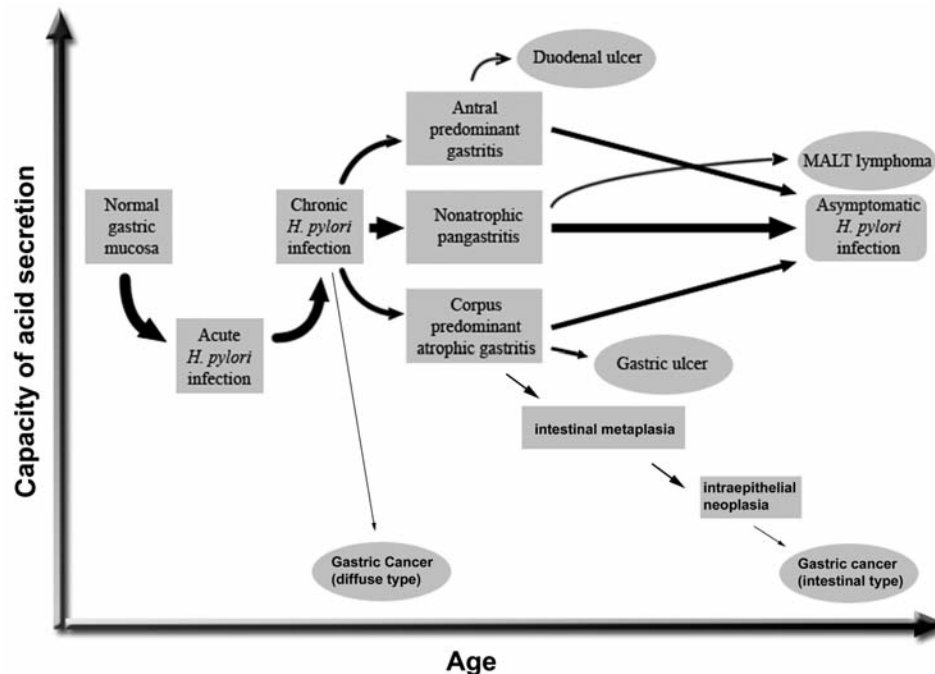


Figure 1. Natural history of *H. pylori*-mediated gastroduodenal diseases. *H. pylori* infection is usually acquired in early childhood and leads to chronic gastritis in virtually all patients. Ten to 15% of *H. pylori*-infections will eventually develop into severe pathologies depending on the presence of bacterial and host factors. Patients with higher acid output have a predisposition for antrum-predominant gastritis that is associated with duodenal ulcer. In contrast, those with lower acid secretion are more likely to have corpus-predominant gastritis that is associated with histomorphological alterations such as atrophy, intestinal metaplasia and intraepithelial neoplasia. These lesions have been a matter of debate as to whether they represent “pre-malignant” or “indicator” lesions in gastric tumourigenesis. Alternatively, GC of the diffuse type can arise without these histomorphological changes. Another rare complication, which is independent of the pattern of colonisation or gastritis, is the formation of mucosa-associated lymphoid tissue (MALT) that can give rise to malignant lymphoma. This figure was adapted from Suerbaum & Michetti (17).

been shown to be of clinical practicability for *H. pylori* infection (18). While the functional relevance of these polymorphisms has been mostly demonstrated on cellular level, their pathogenetic role for gastric carcinogenesis is understood poorly and discussed controversially (28-31).

Regulatory T-cells - Heterogeneous Subpopulations of Immune-suppressive T-cells

Recent publications have reported a complex histological and immune pattern of *H. pylori*-induced gastritis (32-34). In particular, regulatory T-cells (Treg), mostly CD4⁺CD25^{high} Treg, are reported to be important regulators of the immune response to *H. pylori* and involved in the pathogenesis of *H. pylori*-related diseases such as peptic ulcer disease and even gastric malignancy (35).

Naturally occurring regulatory T-cells. By suppressing the activation and proliferation of antigen-specific T effector cells, regulatory T-cells (Treg cells) are key regulators of the immune system in maintaining immunological tolerance (36-38). Treg cells comprise different subsets, the naturally

occurring FOXP3-expressing CD4⁺CD25^{high} Treg cells, peripherally induced FOXP3-expressing Treg cells, as well as historically described Tr1 cells secreting interleukin 10 (IL-10) and Th3 cells characterized by TGF-β1 secretion, formerly known as Th3 cells (39) (Figure 2). The most distinct and comprehensively investigated sub-population of Treg cells is the subset of naturally occurring thymus-derived CD4⁺CD25^{high} Treg cells. They represent 5 to 10% of CD4-positive T-cells and are characterised by a higher expression of CD25 compared to other CD4⁺ T-cells and the expression of the nuclear transcription factor FOXP3. FOXP3 encodes a forkhead family transcription factor and acts as a master switch gene for Treg cell development and function that is involved directly in generating CD4⁺CD25^{high} Treg cells. A loss of function mutation in mice leads to a fatal hyperproliferative autoimmune disease (40). In humans, the mutation of the *FOXP3* gene causes the fatal X-linked immune dysregulation, polyendocrinopathy, enteropathy and the X-linked syndrome (IPEX) (41). This fatal monogenetic disorder is accompanied by high incidences of other autoimmune diseases, including type 1 diabetes, inflammatory bowel disease and severe allergy (42). In

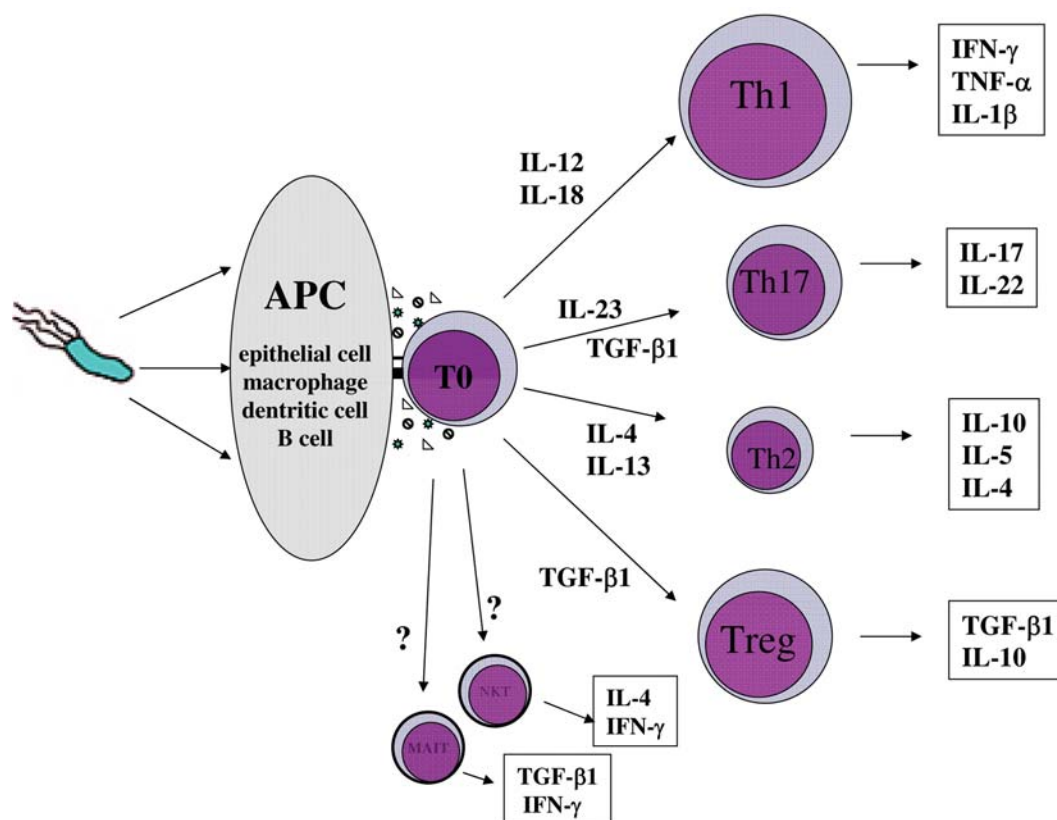


Figure 2. Regulatory T-cell subpopulation related to *H. pylori* infection.

contrast to other cell surface proteins, such as CD25, CTLA-4 and GITR that are also found on activated T-cells, FOXP3 is specifically expressed by CD4⁺CD25^{high} Treg cells and is therefore considered to be a molecular marker for this cell lineage (38).

Induced regulatory T-cells. Among the FOXP3-expressing CD4⁺CD25^{high} T-cells, the peripherally induced FOXP3-expressing CD4⁺CD25^{high} Treg and the inducible type 1 IL-10-secreting regulatory T-cells (Tr1) should be distinguished (44). For the differentiation of the latter subset, TGF- β 1 plays an important regulatory role by the induction of FOXP3 gene expression in naive CD4⁺CD25⁻FOXP3⁻ T-cells at the site of inflammation (44). It has been demonstrated that the peripheral induction of Treg cells is strongly related to gut-associated lymphoid tissue (GALT) and mucosal dendritic cells (DCs) (45).

The immunoregulatory function of Treg has been reported to be both cell-cell contact-mediated and related to the secretion of the cytokines TGF- β 1 and IL-10 (Tr1) (46). The pathophysiological role of Treg cells has been established for several inflammatory disorders such as Crohn's disease, ulcerative colitis and rheumatoid arthritis (46, 47). In the

following, we review the role of Treg cells in *H. pylori* infection, gastritis and *H. pylori*-associated gastric carcinogenesis.

Immunological determinants in *H. pylori*-induced gastritis and GC. *H. pylori* induces various mechanisms of the innate immune system that lead to predominant Th1-polarized mucosal immune response. Immune recognition of *H. pylori* is mediated by Toll-like receptors (TLR) and cytoplasmic NOD-like receptors (NLR) (48). However, *H. pylori*-derived LPS and flagellin are rather weak ligands for their corresponding receptors, TLR-4 and TLR-5 (49, 50). Furthermore, many *H. pylori* strains express O-antigens related to the Lewis blood antigen of host's cells leading to "molecular mimicry" and evasion of immune recognition (51). Therefore, the activation of intracellular signaling pathways and the immune response seems to be lower than those against other intestinal luminal bacteria (52). In addition to the low immune-stimulatory properties of key antigens, *H. pylori* expresses various proteins that interfere directly with the host's immune system. As best investigated, the VacA inhibits the transcriptional activation of T-cells and prevents subsequent antigen-induced proliferation (53), as

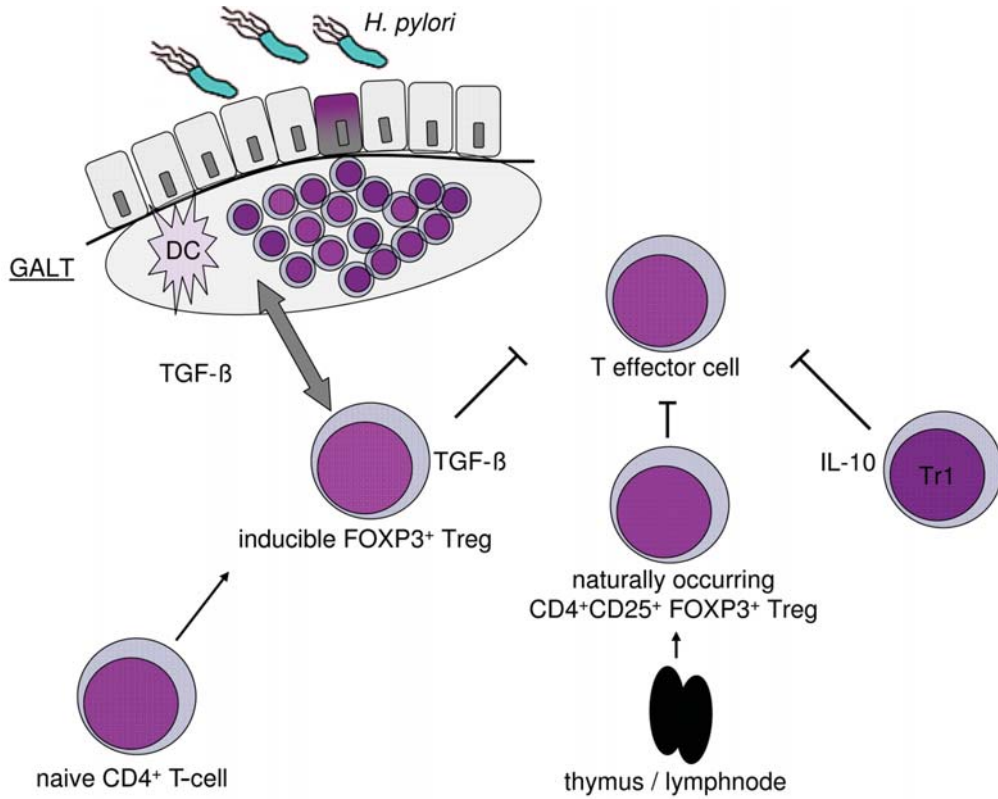


Figure 3. Immunological determinants of *H. pylori*-associated inflammation. *H. pylori* is recognised by the innate immune system that leads to the differentiation of different T-cell lineages in the adaptive immune response. *H. pylori*-induced gastritis is characterised by a predominant Th1/Th17 response that is regulated by Treg cells. The various T-cell lineages secrete different cytokines that modulate further the immune response that is a critical factor for the development of severe diseases such as ulcer or GC (see also Figure 1).

Regulatory T-cells in the equilibrium between H. pylori and human immune system

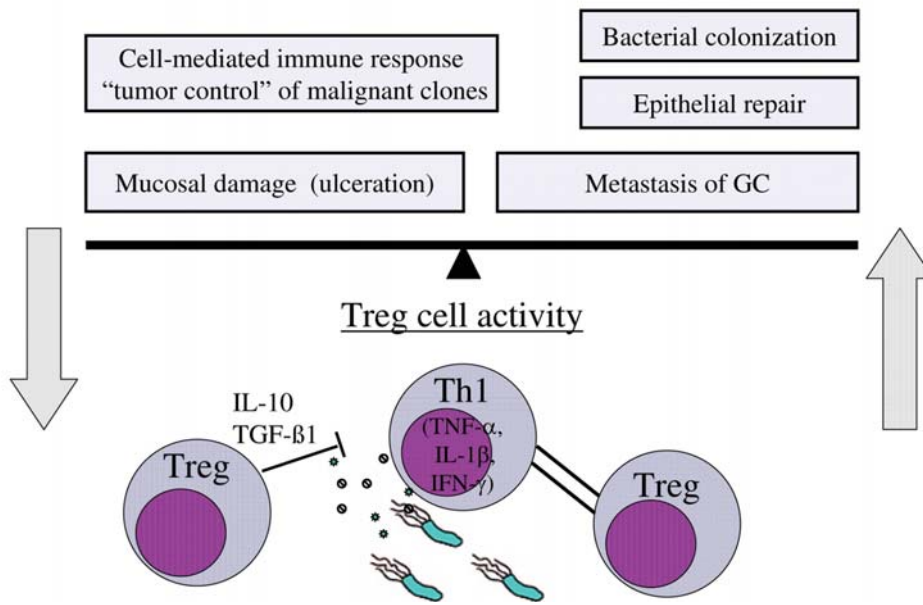


Figure 4. *H. pylori* bacterial–host immunological equilibrium, Treg cell activity balancing inflammation and bacterial persistence.

well as arginase and γ -glutamyl transferase that target T-cells and macrophages (54, 55). Very recently, it was shown that *H. pylori* incorporates cholesterol-containing membrane fractions with human antigens from gastric epithelial cells as a novel "hiding strategy" from immune recognition (56). Together, these factors and mechanisms are thought to play an important role in regulating the activity of gastritis, and providing a molecular basis for the lifetime persistence of *H. pylori* infection in humans as an almost perfect *H. pylori*-host equilibrium.

Although 80-85% of all these infected by *H. pylori* do not present with symptomatic clinical symptoms, it is notable that chronic gastritis is always present in all (17, 18, 57). The degree of gastritis is defined by the number of infiltrating immune cells in the gastric mucosa (*i.e.* polymorphonuclear granulocytes=activity of gastritis; macrophages, mast cells B and T lymphocytes=chronicity of gastritis) that correlate with mucosal damage (57). It has been well established that this inflammatory infiltration is induced by chemokines that are secreted by epithelial cells or immune cells after *H. pylori* antigen stimulation. RANTES, MIP- α and IL-8 are key players that lead to mucosal chemoattraction and infiltration of granulocytes and other immune cells. The recognition and presentation of *H. pylori*-derived antigens by gastric epithelial cells, DCs and macrophages leads to a complex cytokine milieu, promoting the differentiation of various T-cell lineages (Figure 3), the Th1 cells (characterized by IL-12, 18, TNF- α , IL-1 β), Th2 cells (IL-4, IL-5), Th17 cells (IL17A, F, IL-23) and Treg cells (TGF- β 1, IL-10) (32-34, 58-60). The functional activity of these lineages contributes to the pathological nature of the underlying gastritis. The two rather novel subsets (Th17 and Treg cells) have separated from the classical Th1 (Th17) and Th2 (Treg cells) lineages. Numerous studies have been published on Th1-dominated cytokine milieu (TNF- α , IL-1 β , IFN- γ) in *H. pylori*-induced gastritis (59-62). Whether these alterations can be assigned to the novel Th17 cells, or whether the strong pro-inflammatory Th17 cells are the real cause of rheumatoid arthritis and inflammatory bowel disease is actively discussed (63, 64) and this needs still to be proven for *H. pylori*-induced gastritis.

In addition to these four conventional T-cell lineages, characterised by the expression of T-cell receptor (TCR), there are two unconventional T-cell subsets that express invariant antigen-specific TCRs. These are the Cd1d-restricted natural killer T (NKT) cells (65) and the mucosal-associated invariant T (MAIT) cells (66), (Figure 2). Both unconventional T-cell lineages are abundantly present in the gastric mucosa; NKT cells can make up between 4-10% of T-cells in the lamina propria (67, 68). A few studies have analysed these cells *in vitro* and *ex vivo* in relation to *H. pylori* infection, and revealed differences among individuals infected by this bacterium, but their

pathophysiological importance for the *H. pylori*-induced pathologies has not been demonstrated (68). Keeping in mind that NKT cells have been demonstrated to play an important role in tumour surveillance (69) and counteract the suppressive action of antitumour immunity of Treg cells (70), the interaction of both cell lineages could potentially affect gastric tumourgenesis (68). MAIT cells are abundant in the intestinal mucosa, and their development depends on the presence of luminal bacteria (68). This cell population has not yet been investigated in response to *H. pylori* infection (68).

Role of Regulatory T-cells in *H. pylori*-induced Pathologies

Pathophysiology of Treg cell activity for H. pylori infection and gastritis. As outlined above, Treg cells comprise different regulatory T-cells that differ in the expression of cell surface markers and functional activity (cytokines, contact-dependent). The first evidence for their pathophysiological role in stomach-related pathologies came from Sakaguchi *et al.* who demonstrated that CD25-expressing T-cells mediate self-tolerance to autoantigens in autoimmune gastritis (71, 72). Later studies provided evidence that Treg cells suppress autoimmune gastritis by two mechanisms: (i) by inhibiting the differentiation of autoreactive H⁺/K⁺-ATPase-specific effector cells (73) and (ii) by suppressing the expansion of these cells after antigen contact (74). Notably, naturally occurring Treg cells are capable of suppressing Th1-derived effector cells, but lack corresponding activity towards antigen-specific Th17 cells (75). The activity of these pro-inflammatory Th17 cells are controlled by inducible Treg cells only, as shown by transfer experiments (75-77). However, it should be mentioned and kept in mind that these excellent functional studies have been performed mostly in mice models, and therefore may not reflect the complete pathophysiology of autoimmune gastritis in humans.

A similar situation is present in the field of Treg cells and *H. pylori*-induced pathologies. Several human-based studies demonstrated that the degree of active *H. pylori*-induced inflammation correlates negatively with the numbers of Treg cells in gastric mucosa or periphery (77-81). Interestingly, a similar activity of peripheral Treg cells was reported towards *H. pylori*-stimulated AGS cells that showed impaired IL-8 secretion than those cultivated with Treg cells (78). Lundgren *et al.* isolated peripheral CD4⁺CD25^{high} T-cells from *H. pylori*-infected individuals and showed their immune-suppressive activity towards the CD4 cells primed by *H. pylori*-presenting DCs (78).

Consistently in all studies, elevated numbers of Treg cells were identified in the gastric mucosa of *H. pylori*-infected patients (78, 80-82). Treg cells were found to be associated with increasing bacterial colonisation (80), chronic

inflammatory changes (80-82) and the expression of immuno-suppressive cytokines (80, 83). Eradication therapy of the infection led to a significant reduction of Treg cells and corresponding cytokine levels in gastroduodenal mucosa (82). Analysing the chronic inflammation at the cardia with respect to the pathophysiological condition (*H. pylori*-infection or gastroesophageal reflux (GERD)-associated inflammation), it has been demonstrated that there are elevated numbers of Treg cells only in association with *H. pylori* infection, but not with inflammatory changes related to GERD (84).

Bearing in mind that *H. pylori* infection is usually transmitted within a family in early childhood, it is an interesting observation that children revealed a strong correlation among increased numbers of mucosal Treg cells, lower inflammatory scores and elevated cytokine levels (IL-10, TGF- β 1) characteristic of the functional importance of Treg cell activity in early stages of the acute infection (85). Although limited knowledge is available concerning the interaction between Treg response and *H. pylori* infection from acute childhood infection, Treg cell activity appears to be a plausible explanation for a more moderate acute phase of infection leading to chronic changes and the persistence of the bacteria in a inflammatory equilibrium between *H. pylori* and the immune system of the host (86, 87).

In order to overcome the limitation of human studies addressing the function of Treg cells, comprehensive descriptive analysis of Treg cells and their gene expression patterns have been performed (78-87). Although associations do not prove causality, a consistent correlation between Treg markers (CD4⁺CD25^{high} and/or FOXP3) and the cytokine levels of IL-10 or TGF- β 1 in the gastric mucosa strongly supports the functional relevance of Treg cells for gastric pathologies. In a functional assay, Robinson *et al.* isolated mucosal T-cells from biopsies and stimulated those with *H. pylori* antigens (83). Compared to uninfected donors, the stimulated cells presented a predominant CD4⁺IL-10⁺ response that was characterized by a 36-fold increase in IL-10 production. Furthermore, the authors showed that patients suffering from peptic ulcer disease were characterized by decreased Treg cell activity and dramatically reduced amounts of IL-10, indicating that a low IL-10 expression by Treg cells favours acute inflammatory changes and immune-mediated cell damage leading to peptic ulceration. In agreement with Strömberg *et al.* (79), it has been demonstrated that IL-10 is capable of down-regulating the inflammatory response of epithelial cells (AGS) by interfering with the NF κ B signal pathway, which subsequently leads to reduced IL-8 secretion after *H. pylori*-stimulation (83).

The hypothesis of an association between *H. pylori* infection/gastritis and Treg cells has been confirmed by several animal studies, mostly mouse models (88-91). Besides descriptive analyses, these models allow functional

analyses by gene targeting, cell transfer and *in vivo* application of mediators. Suppressive activities of mouse-derived Treg cells on antigen-stimulated cells have been shown in various C57BL/6 mice colonies (88, 89), and have been found to depend strongly on the presence of IL-10 (90, 91). Depletion of murine Treg cells by monoclonal anti-CD25 antibodies results in stronger inflammation, elevated proinflammatory cytokine expression and reduced bacterial colonisation (89).

Furthermore, the humoral immune response is affected by increased titers of anti-*H. pylori* IgG levels and altered isotype distribution (89, 92). Murine Treg cells mediate their function by inducing anergy in *H. pylori*-stimulated effector cells (93), and CD73 (ecto-5'-nucleotidase) seems to be a critical protein involved in the functional activity of Treg cells (94).

Recently, various human studies aimed at the protective vaccination against *H. pylori* infection have been performed with inconclusive results (33, 95). T-cell-mediated mechanisms, in particular IL-17 and IFN- γ producing cells, seem to play the predominant role in immunity against *H. pylori* (96-98). Regulatory T-cells are capable of suppressing these T-cell functions (100). Therefore, a modulatory role of Treg cells in any vaccination strategy can be assumed, and should be investigated further.

Treg cell activity in H. pylori-associated gastric adenocarcinoma. Similarly to *H. pylori* infection, several studies demonstrated elevated numbers of Treg cells in patients with gastric adenocarcinoma (99-101). Analysing 711 *H. pylori*-infected patients including patients suffering from GC, Wang *et al.* showed also an increased number of Treg cells in the peripheral mononuclear cell population besides the local mucosal changes (100). Notably, the ratio of Th1/Th2-derived cytokines was found to decline starting from asymptomatic gastritis *via* gastric atrophy, intestinal metaplasia and intraepithelial neoplasia towards gastric adenocarcinoma. The steady decline was associated with a concomitant increase of the Treg cell compartment in peripheral blood and the presence of CagA⁺ *H. pylori* strains favouring a Treg cell-mediated chronic inflammation and the persistence of CagA⁺ strains (100). In agreement with Wang *et al.* (100) and colleagues, Jang (81) recently reported about elevated numbers of mucosal Tregs in *H. pylori*-associated gastritis, where they correlated positively with the grade of chronic gastritis. Mucosal Treg cells were found to increase further in patients with dysplastic changes and with the highest density for gastric adenocarcinoma (81). Supporting data demonstrated elevated levels of peripheral blood FOXP3-expressing CD4⁺CD25^{high}CD117^{low} Treg cells in patients with GC. The authors also described increased numbers of FOXP3⁺CD4⁺CD25^{high}CD117^{low} Treg in the tumour tissue and the mucosal microenvironment as well as in the adjacent lymph nodes and the ascitic fluid of advanced

tumour stages. The authors found a positive correlation with the tumour-nodes-metastasis (TNM) stage and particularly high numbers in advanced tumour stages. Further evidence for the functional activity was demonstrated by Treg cell-mediated antiproliferative effect on T effector cells (101). A retrospective immunohistochemical study found increased numbers of Treg cells associated with vascular, lymphatic and perineural invasion of gastric tumour cells. Higher numbers of Treg cells were correlated with advanced tumour stage and correlated negatively to the overall survival in a study population of 110 patients, leading the authors to propose FOXP3⁺ Treg cells as an additional marker for identifying high-risk GC patients that need further therapy after R0 resection (102). The total number of Treg cells and the pattern of mucosal infiltration has been characterized immunohistochemically with respect to TNM tumour stage and survival, presenting a more diffuse pattern of Treg cell infiltration correlating with a poor prognosis and survival (103).

Taken together, data from clinical studies and animal models uniformly support a role of Treg in *H. pylori*-induced pathologies, even at the early stages of gastric tumorigenesis, the chronic gastritis. Immunosuppressive mechanisms and involved molecules mediating Treg cell activity have been characterized in detail, mostly in mouse studies. The clinical relevance of these findings to humans needs to be studied further in order to transfer this knowledge from the bench to the bedside and for the development of new therapeutic options.

Conclusion

Recent data from animal models as well as human studies imply an important role of regulatory T-cells for *H. pylori* infection and complications. A predominant Th1/Th17-immune response against *H. pylori* infection indicates a rather mild chronic inflammation that allows the lifelong persistence of bacteria. The gradual elevation and high numbers of infiltrating Treg cells in the course of *H. pylori*-related pathologies (atrophy, intraepithelial neoplasia and GC) imply their pathophysiological relevance to the regulation of gastritis and antitumor surveillance. Once gastric tumorigenesis is initiated and malignant clones have appeared, the functional activity of Treg cells impairs adequate immunological response to tumour cells, and favours their dissemination and metastasis (Figure 4). Whether antigenic specificity of these Treg cells is directed against tumour antigens and/or *H. pylori*-derived antigens is an open question and needs to be investigated further. Similar to other immune-modulated diseases, the modulation of Treg activity in relation to *H. pylori* or GC may be a potential target for new therapeutic agents, but future work is needed to address these issues successfully for the clinical setting.

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