REVIEW

The effect of *Helicobacter pylori* on asthma and allergy

Amedeo Amedei[1]
Gaia Codolo[2]
Gianfranco Del Prete[1]
Marina de Bernard[2]
Mario M D'Elios[1]

[1]Policlinico AOU Careggi, Department Internal Medicine, University of Florence, Italy; [2]Venetian Institute of Molecular Medicine, University of Padua, Italy

Abstract: Current evidence indicates an inverse association between *Helicobacter pylori* and asthma and allergy. *H. pylori* is a Gram-negative bacterium which represents the major cause of peptic ulcer and gastric cancer, and preferentially elicits a T helper (Th)-1 response. Many *H. pylori* factors, such as the neutrophil-activating factor of *H. pylori* (HP-NAP), are able to drive Th-1 polarization and to display a powerful inhibition of allergic Th-2 response. This article proposes an overview of the actual knowledge about the effects of *H. pylori* on asthma and allergy. Special attention has been drawn to HP-NAP as a potential novel strategy for the prevention and treatment of asthma and atopy.

Keywords: *Helicobacter pylori* neutrophil-activating factor, protein, Th-1/Th-2, Treg, asthma

Introduction

The prevalence of airway allergic disease such as asthma has over the years increased in developed countries. The causes of this increase remain largely unknown. Proposed associations include changes in smoking habits,[1] exposure to food-borne and orofecal infections,[2,3] types of dwellings,[4] ownership of furry animals,[5] number of siblings, family income/education level,[6] and the presence of particulates in diesel exhaust.[7] The inverse association between family size and manifestations of allergy has been consistently found,[8–11] and there is also a much-published potential link between allergy and childhood infection, especially with *Helicobacter pylori*.[12–14]

Until the late 1980s, interest in the role of infections in allergic diseases focused principally upon the process of primary allergic sensitization. The literature of the time contained several observations which argued for a role for infections, including the ability of bacterial-derived immunostimulants such as pertussigen to selectively improve priming for immunoglobulin (Ig)E antibody production,[15] and the potential of lipopolysaccharide to bypass tolerance to mucosally applied allergens. Also, other studies reported that respiratory viral infections such as influenza could subvert the generation of protective "inhalation tolerance" to aeroallergens.[16] More recently, signals such as enterotoxins from skin-dwelling bacteria have been invoked as important contributors to the pathogenesis of atopic dermatitis.[17] However, it was also clear from other observations that microbial exposure per se could not be considered in generic terms as "pro-atopic". For example, other microbial-derived agents exemplified by the components of Freund's adjuvant displayed atopy-antagonistic activity,[18] and stimuli derived from normal gut flora were demonstrated to be necessary to facilitate the expression of oral tolerance to fed

Correspondence: Mario M D'Elios
Policlinico AOU Careggi, Department of Internal Medicine, University of Florence, Italy
Email delios@unifi.it

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allergens,[19] and also inhalation tolerance to aeroallergens.[20] These observations suggested that microbial-derived stimuli had potential to modulate the etiology and pathogenesis of atopic diseases in dichotomous ways, their ultimate effects perhaps being context-dependent.

In this review we will focus our attention on the unambiguous effects of *H. pylori* on asthma and atopy.

The role of *Helicobacter pylori* in asthma and allergy

H. pylori, a Gram-negative bacillus that colonizes the human stomach, is the main cause of peptic ulceration, gastric lymphoma, and gastric adenocarcinoma, the second leading cause of death from cancer worldwide. The World Health Organization classifies *H. pylori* as a human carcinogen for distal gastric cancer, and eradicating the bacterium in high risk populations reduces incidence of gastric cancer.[21] *H. pylori* also may contribute to other conditions, including iron and vitamin B12 deficiencies, idiopathic thrombocytopenic purpura, and growth retardation in children. *H. pylori* colonization occurs in childhood and persists throughout life, causing disease mainly in adults.[22,23]

In 1989, Strachan proposed the "hygiene hypothesis", stating that the exposure to infectious agents and living in an unhygienic environment might "educate" the immune system and thus protect against the development of allergic diseases.[24] The idea originated from epidemiological observations suggesting a general hypothesis that infections in early childhood acquired from older siblings might confer protection against the development of atopic diseases such as atopic eczema, allergic rhinoconjunctivitis, and asthma. Subsequent research into the association between childhood infections and atopic sensitization or atopic disease have offered conflicting results. Indeed, our understanding of the timing, the mechanism, and the specific infections that might carry antiallergenic potential are by no means satisfactory.[25,26] The T helper (Th)-1/Th-2 paradigm of adaptive immune responses provided the initial immunological backbone for the hygiene hypothesis.[27–29] On the basis of the cytokine production patterns, T cell responses may be divided into counter-regulatory Th-1 and Th-2 subtypes. Th-2 responder phenotype is associated with atopic sensitization and atopic disease. Indeed, inflammation of the Th-2 type appears to be active in the initial stage of the pathogenesis of atopic eczema,[30,31] allergic rhinoconjunctivitis,[32,33] and asthma.[34,35]

In detail, the histopathological characteristics of bronchial asthma, even a mild one, are represented by inflammatory infiltrates consisting of T lymphocytes and accumulation of activated eosinophils, epithelial shedding, and basal membrane thickening. Immunological and molecular studies of bronchial biopsies and bronchoalveolar lavage samples obtained in baseline disease or taken after natural or "experimentally" induced asthma exacerbations have shown that a complex and fascinating inflammatory mechanism sustains the pathogenesis of bronchial asthma, including the participation of different types of Th cells and peculiar cytokine and chemokine networks.[36] In allergic asthmatic patients, allergen exposure induces a predominant activation of Th-2 lymphocytes in the airways, able to over-express several Th-2 cytokines, such as interleukin (IL)-4 and IL-5.[34,37] Moreover, the degree of IL-5 expression at the bronchial level is associated with the disease severity both in atopic and in nonatopic asthma.[38] IL-5 and granulocyte macrophage colony-stimulating factor (GM-CSF) can be considered the most important cytokines for eosinophil accumulation in asthmatic inflammation. Th-2 cytokines in bronchial asthma are produced not only by CD4$^+$ but also by CD8$^+$ T cells, which contribute to the genesis of asthma and to the clinical expression of the disease.[39] In *H. pylori* infection, a predominant activation of Th-1 cells, with the production of interferon (IFN)-γ, IL-12, IL-18, IL-23, and tumor necrosis factor (TNF)-α, occurs in vivo in the stomach of humans and in animal models, and the inhibition of the allergic Th-2 inflammation by Th-1 responses can explain the inverse relationship between *H. pylori* and asthma.[40]

Mechanism of action of *Helicobacter pylori*

H. pylori colonizes the human stomach in childhood and persists for decades.[23] This implies near perfect adaptation to the niche and an ability to evade the human immune response. Its spiral shape and flagella allow it to corkscrew through the gastric mucus gel, and numerous adhesins enable selective adherence to the epithelium. *H. pylori* has multiple mechanisms for protection against gastric acid;[41] notably, 15% of its protein content comprises preformed cytoplasmic urease that, when the external pH is less than 6.5, neutralizes the periplasm, allowing maintenance of the cytoplasmic membrane potential.[42]

Like many human commensal bacteria, *H. pylori* has evolved specific mechanisms to avoid stimulating the immune response. For example, innate immune recognition by several Toll-like receptors (TLRs) is attenuated for *H. pylori*.[43,44] Despite this, colonization is associated with inflammatory and mucosa infiltration of polymorphonuclear leukocytes, macrophages, and Th-1 lymphocytes, with active production

of IL-12 and IFN-γ.[45] Such an immune response is expected to play a role in the pathogenesis of *H. pylori*-associated diseases in humans.[45,46] Accordingly, a Th-1-directed immune response, induced by *H. pylori* infection, increases gastric inflammation and atrophy, whereas Th-2 redirection reduces them.[47,48] Different pathways are responsible for the predominant *H. pylori*-induced mucosal Th-1 response.[45,46] Stimulation of human neutrophils, monocytes, and dendritic cells with *H. pylori* neutrophil-activating protein (HP-NAP) strongly upregulates both IL-12 and IL-23 production, via TLR2 activation. In the gastric mucosa of *H. pylori*-infected patients, a considerable proportion of Th cells is specific for different *H. pylori* antigens, including HP-NAP, CagA, urease, VacA, and heat shock proteins, and HP-NAP drives the production of high levels of IFN-γ and TNF-α by gastric Th cells, thus promoting a polarized Th-1 response (Figure 1).[45–49]

Protective properties of *Helicobacter pylori* on asthma and allergies

Asthma, a chronic inflammatory disease of the airways is a multifaceted disorder characterized by airway hyper-responsiveness to a multiplicity of specific and nonspecific stimuli, and mucus hypersecretion by goblet cells.

The severity and incidence of asthma have increased drastically in the developed nations over recent decades. Although the underlying reason is still unknown, clinical, epidemiological, and experimental evidence indicate that infectious diseases can influence the development of allergic disorders.[24] Accordingly, an inverse correlation has been demonstrated between the onset of allergic disorders and the incidence of infections. This may be the result of an inhibition of allergic Th-2 inflammation exerted by Th-1 responses; the latter are elicited by infectious agents and are able to induce the production of IFN-γ, IL-12, IL-18, and IL-23.[50] This view is supported by studies showing that development of asthma can be prevented in animals by administering live or killed bacteria or their components, which induce Th-1 responses.[51] Also, we demonstrated that *H. pylori* inhibited Th-2 responses in asthmatic patients.[49] Interestingly, on the basis of large epidemiological studies, recently, a consistent negative association between *H. pylori* infection and the presence of allergic disorders, such as asthma and rhinitis, has been proposed.[52] Table 1 summarizes some recent studies in which the relationships of *H. pylori* with asthma, atopy, allergic rhinitis, and/or eczema were examined.[2,8,53–60] In general, the cross-sectional studies, involving a variety of populations and somewhat differing definitions of atopy

and asthma, show significant inverse relationships of these conditions with *H. pylori*. The published case-control studies, in general much smaller in scale, do not show any significant direct or inverse relationships.

Although it is an undoubtedly interesting theory, no convincing molecular mechanism has been proposed to support it. Our studies, carried out with *H. pylori* may help in understanding this complex issue. We have shown that addition of HP-NAP (a dodecamer formed by four-helix bundled subunits with a hollow central part) to allergen-induced T-cell lines derived from allergic asthmatic patients led to a drastic increase in IFN-γ-producing T cells and to a decrease in IL-4-secreting cells, thus resulting in a redirection of the immune response from a Th-2 to a Th-1 phenotype.[49] Furthermore, in the gastric mucosa of *H. pylori*-infected patients a remarkable proportion of Th cells showed significant proliferation to different *H. pylori* antigens, including HP-NAP; upon HP-NAP stimulation, Ag-specific gastric Th cells produced large amounts of IFN-γ and TNF-α, and displayed a powerful cytotoxic activity, thus showing a polarizing Th-1 effector phenotype.

Likewise, HP-NAP stimulation of neutrophils, monocytes, and dendritic cells resulted in a remarkable upregulation of cytokines, including IL-12 and IL-23, contributing to the induction of an IL-12- and IL-23-enriched milieu, which has the potential to drive the differentiation of antigen-stimulated T cells towards a polarized Th-1 phenotype (Figure 1).[56,61]

An issue to be considered in studies showing negative associations between *H. pylori* and various atopic and allergic diseases is that *H. pylori* positivity is linked with more crowded living conditions and poor hygiene in infancy. Since these factors also are associated with other childhood infections, *H. pylori* status may simply be a marker for these. However, the negative association with childhood asthma is stronger for *cagA*+ *H. pylori* strains.[56]

Another hypothetical explanation for the inverse association between *H. pylori* and asthma is that the high levels of regulatory T cells (Tregs) associated with *H. pylori* infection may contribute to prevention of allergic diseases, and *H. pylori*-free humans are thus more susceptible to these diseases (Figure 2). In support of this, *H. pylori*-positive people have higher levels of gastric Tregs than those without the organism,[62,63] and more importantly also, circulating Tregs are increased in number.[64] In addition, in mice experimentally infected with *H. pylori*, systemic Tregs are increased, and these suppress other immune responses, one effect of which is to facilitate *H. pylori* colonization.[65] The excess Tregs may have immunosuppressive activity in humans as well: among *H. pylori*-positive persons, those
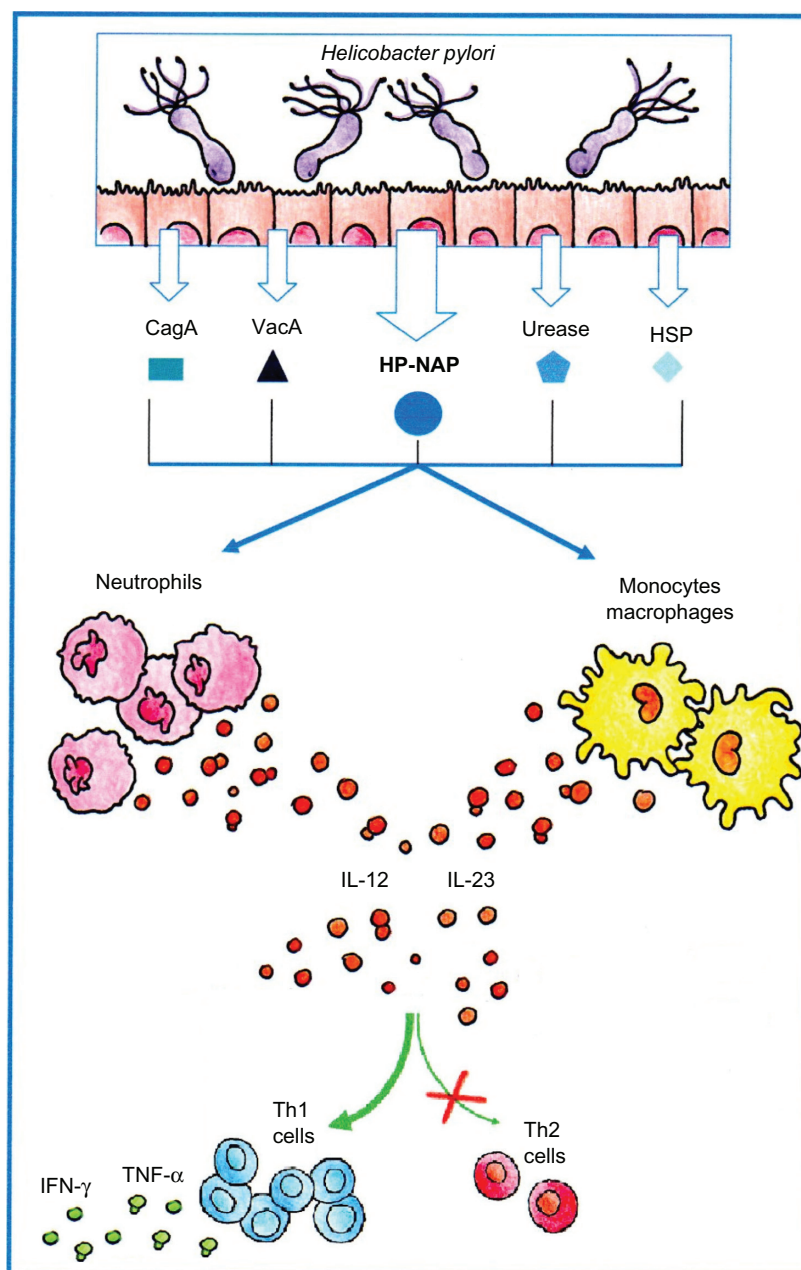
Figure 1 Schematic representation of *Helicobacter pylori*-driven inhibition of allergic Th-2 inflammation via activation of Th-1 responses. Following *H. pylori* infection, HP-NAP and other *H. pylori* factors induce the production of IL-12 and IL-23 that both promote the preferential development of Th-1 cells and repress the Th-2 allergic response.
Abbreviations: HP-NAP, *H. pylori* neutrophil-activating protein; Th, T helper; IL, interleukin; IFN, interferon; TFN, tumor necrosis factor.

with fewer Tregs are more likely to have peptic ulcers[62] and so presumably have more intense gastritis. Finally, in *cagA+ H. pylori* colonization, mucosal Tregs may be more numerous, and mucosal levels of the immunomodulatory cytokine IL-10 may be higher than in *cagA−* colonization.[66] If the same phenomenon applies to circulating Tregs, it could potentially explain the stronger, negative association with childhood asthma of *cagA+* strains.[56] Taken together, these studies imply a theoretical plausible link between *H. pylori*, Tregs, and reduction in risk of allergic diseases. However,

interventional studies in relevant animal models and in humans are needed to verify the hypothesis.

Effect on immune system and lung function

So far we have considered what happens in the stomach and at the systemic level, following *H. pylori* colonization; but a very pertinent question about the link between asthma and *H. pylori* is whether *H. pylori* may have some effects in the lung region. To answer this question we created a mouse

Table 1 Major studies showing a negative association between *Helicobacter pylori* and asthma/atopic diseases

Location/type of study	Study population	*H. pylori* detection	Clinical and laboratory findings of the atopic patients studied	Reference
Japan/C-C	46 patients with asthma/48 HC	IgG ELISA IgG CagA	Current asthma diagnosed by ATS guidelines.	53
Scotland/C-C	97 patients/208 C	IgG ELISA	Skin and specific IgE tests. Atopy: weal $\geq$ 3 mm, or any IgE > 0.35 IU/mL. Self-reported adult-onset wheeze and asthma.	8
Italy/C-C	240 atopic patients/ 240 nonatopic C	IgG ELISA	Total IgE. Atopy: logRU > 1.2. Nonatopic < logRU < 0.	2
Hong Kong/C-C	90 patients with asthma/97 C	IgG ELISA	Current asthma diagnosed by ATS guidelines.	54
UK/C-S	3244 patients	13C-urea breath test	Asthma (treated with inhalers), allergic rhinitis (treated with antihistamines), and eczema (treated with topical corticosteroids).	55
USA/C-S	7663 atopic adults	IgG ELISA IgG CagA	Self-reported asthma and hay fever (current and lifetime). Skin sensitization tests.	56
Finland, Russian/C-S	1177 patients	IgG Elisa	Skin prick testing with a panel of 11 common airborne allergens. Atopy: any wheal diameter $\geq$ 3 mm.	57
Germany/C-S	321 with blood samples from 930 randomly selected from 3112 inhabitants	IgG ELISA IgG CagA	Specific IgE against a panel of aeroallergens. Atopy: any IgE > 0.70 kU/L.	58
Denmark/C-S	1011 patients	IgG ELISA	Self-reported allergic rhinitis. Specific IgE to 6 allergens. Atopy: any IgE > 0.35 kU/L.	59
Iceland, Sweden, Estonia/C-S	1249 patients	IgG ELISA	Detection of specific atopy: any IgE > 0.35 kU/L.	60

Abbreviations: ATS, American Thoracic Society; C-C, case-control; C-S, cross-sectional; C, controls; HC, healthy controls; Ig, immunoglobulin; ELISA, enzyme-linked-immunosorbent serologic assay test.

model of allergic asthma,[67] and demonstrated that in vivo administration of HP-NAP prevents the typical eosinophil accumulation in the lung, as well as the increase of serum IgE. These results suggest the possibility that HP-NAP might be a part of the molecular mechanism underlying the negative association between *H. pylori* infection and allergy, corroborating the epidemiological observations with a plausible scientific explanation. To address whether HP-NAP, on the basis of its immune-modulating activity, could be beneficial for the prevention and treatment of bronchial asthma, it was administered via the intraperitoneal or the intranasal route using a mouse model of allergic asthma induced by inhaled ovalbumin (OVA). Groups of nine C57BL/6j, wild type or *tlr2*$^{-/-}$, mice were treated with OVA alone, or with OVA plus HP-NAP intraperitoneally or mucosally administered. In both systemic and mucosal protocols, mice were treated with OVA according to a standardized procedure consisting of a first phase of sensitization with OVA intraperitoneally and

a second phase of induction of the allergic response with aerosolized OVA on day 8, followed by repeated aerosol challenge with the allergen on days 15–18. Control animals were injected with phosphate-buffered saline (PBS) alone and then exposed to aerosolized PBS. In the systemic protocol, mice were treated with intraperitoneal HP-NAP on day 1, whereas in the mucosal protocol mice received intranasal HP-NAP on days 7 and 8.[67] After priming and a repeated aerosol challenge with OVA, Th-2 responses were induced in the mouse lung. Accordingly, following OVA treatment, eosinophils were recruited and activated in bronchial airways, and serum IgE levels increased. Both systemic and mucosal administration of HP-NAP strongly inhibited the development of airway eosinophilia and bronchial inflammation. Likewise, HP-NAP treatment strongly affected the lung cytokine release, reducing the production of IL-4, IL-5, and GM-CSF. Systemic HP-NAP also significantly resulted in both the reduction of total serum IgE and an increase of IL-12 plasma levels.
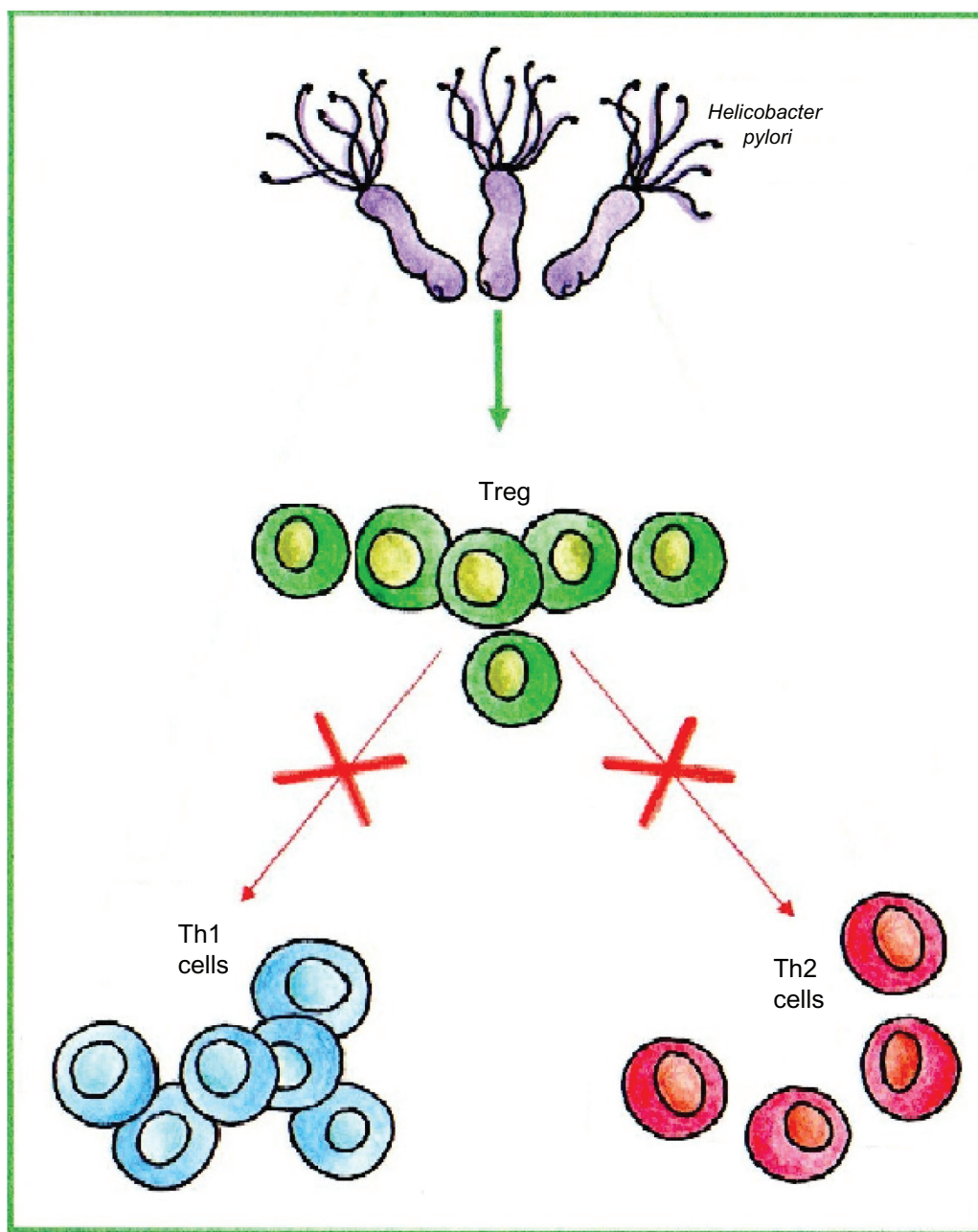
Figure 2 Inhibition of Th-2 response might hypothetically occur by *Helicobacter pylori*-induced activation of regulatory T cells.
Abbreviations: Th, T helper; Treg, regulatory T cell.

However, no suppression of lung eosinophilia and bronchial Th-2 cytokines was observed in TLR2 knock-out mice following treatment with the TLR2 ligand HP-NAP.[67] The results obtained in our studies suggest that HP-NAP might be the key element responsible for the decrement of allergy frequency in *H. pylori*-infected patients.

Conclusion

H. pylori and humans have coevolved for at least 50,000 years and probably for much longer. As such, *H. pylori*

colonization has been essentially universal, and the usual pattern of inflammation has likely been pan-gastric. *H. pylori* is the main cause of peptic ulceration, gastric lymphoma, and gastric adenocarcinoma.

The loss of this ancient, dominant, and persistent member of the normal biota of humans would be predicted to have consequences, and now there is much information about the beneficial and deleterious aspects of this change on the health and disease of the gastrointestinal tract. However, increasing evidence is pointing to extra-intestinal manifestations of the

disappearance of *H. pylori*, including asthma. An inverse association of *H. pylori* and childhood asthma, allergic rhinitis, and atopy is becoming increasingly obvious.

This phenomenon might be explained by the inhibition of the allergic Th-2 inflammation by the Tregs that could be present during *H. pylori* infection and could suppress the atopy-associated Th-2 response.

However, a realistic hypothesis, based on clinical and experimental evidence in humans and animal models,[45,49,67] is that the allergic Th-2 response is redirected by the Th-1 response elicited by *H. pylori*, that is able to induce the production of IFN-γ, IL-12, IL-18, and IL-23. Several studies were devoted to the definition of new immune modulating factors able to inhibit Th-2 responses, and different compounds have been proposed for the treatment and prevention of asthma and atopic diseases, including several TLR ligands mimicking the effects of microbial components such as dsRNA, CpG-oligodeoxynucleotides, and imidazoquinolines.[68–70] In detail, it has been shown that HP-NAP, by acting on both neutrophils and monocytes following the engagement of TLR2, significantly contributes to create an IL-12- and IL-23-enriched milieu, and as such it represents a key bacterial factor able to drive the differentiation of antigen-stimulated T cells toward a polarized Th-1 phenotype. HP-NAP has the potential to redirect the in vitro allergen-specific T-cell response from a predominant Th-2 to a Th-1 response. Also, HP-NAP administration in vivo resulted in inhibition of the typical Th-2-mediated bronchial inflammation of allergic bronchial asthma. Thus, altogether, these results support the view that the increased prevalence and severity of asthma and allergy in Western countries may be related, at least in part, to the decline of *H. pylori* infection, which is able to induce a long-lasting Th-1 background, and suggest also that *H. pylori* compounds such as HP-NAP could be important candidates for novel strategies of the prevention and treatment of asthma and allergic diseases.

Acknowledgments

Disclosure

Mario M D'Elios, Amedeo Amedei, Gianfranco del Prete, and Marina de Bernard are applicants of EU Patent 05425666.4 for HP-NAP as a potential therapeutic agent in asthma, allergic and infectious diseases, and cancer. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

1. Dijkstra L, Houthuijs D, Brunekreef B, et al. Respiratory health effects of the indoor environment in a population of Dutch children. *Am Rev Respir Dis*. 1990;142(5):1172–1178.
2. Matricardi PM, Rosmini F, Riondino S, et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ*. 2000;320(7232):412–417.
3. Matricardi PM, Rosmini F, Panetta V, et al. Hay fever and asthma in relation to markers of infection in the United States. *J Allergy Clin Immunol*. 2002;110(3):381–387.
4. Wickman M, Nordvall SL, Pershagen G, et al. House dust mite sensitization in children and residential characteristics in a temperate region. *J Allergy Clin Immunol*. 1991;88(1):89–95.
5. Strachan DP, Carey IM. Home environment and severe asthma in adolescence: a population based case-control study. *BMJ*. 1995;311(7012):1053–1056.
6. Litonjua AA, Carey VJ, Weiss ST, et al. Race, socioeconomic factors, and area of residence are associated with asthma prevalence. *Pediatr Pulmonol*. 1999;28(6):394–401.
7. Takafuji S, Suzuki S, Koizumi K, et al. Diesel-exhaust particulates inoculated by the intranasal route have an adjuvant activity for IgE production in mice. *J Allergy Clin Immunol*. 1987;79(4):639–645.
8. Bodner C, Anderson WJ, Reid TS, et al. Childhood exposure to infection and risk of adult onset wheeze and atopy. *Thorax*. 2000;55(5):383–387.
9. Peat JK, van den Berg RH, Green WF, et al. Changing prevalence of asthma in Australian children. *BMJ*. 1994;308(6944):1591–1596.
10. von Mutius E, Martinez FD, Fritzsch C, et al. Skin test reactivity and number of siblings. *BMJ*. 1994;308(6930):692–695.
11. Wickens K, Crane J, Pearce N, et al. The magnitude of the effect of smaller family sizes on the increase in the prevalence of asthma and hay fever in the United Kingdom and New Zealand. *J Allergy Clin Immunol*. 1999;104(3 Pt 1):554–558.
12. Kosunen TU, Hook-Nikanne J, Salomaa A, et al. Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to *Helicobacter pylori* infections. *Clin Exp Allergy*. 2002;32(3):373–378.
13. Strachan DP. Family size, infection and atopy: the first decade of the 'hygiene hypothesis'. *Thorax*. 2000;55 Suppl 1:S2–S10.
14. Sheikh A, Strachan DP. The hygiene theory: fact or fiction? *Curr Opin Otolaryngol Head Neck Surg*. 2004;12(3):232–236.
15. Munoz JJ, Peacock MG. Action of pertussigen (pertussis toxin) on serum IgE and Fce receptors on lymphocytes. *Cell Immunol*. 1990;127(2):327–336.
16. Holt PG, Vines J, Bilyk N. Effect of influenza virus infection on allergic sensitization to inhaled antigen in mice. *Int Arch Allergy Appl Immunol*. 1988;86(1):121–123.
17. Sicherer SH, Leung DYM. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects. *J Allergy Clin Immunol*. 2005;116(1):153–163.
18. Ishizaka K. Regulation of IgE synthesis. *Annu Rev Immunol*. 1984;2:159–182.
19. Sudo N, Sawamura S-A, Tanaka K, et al. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol*. 1997;159(4):1739–1745.
20. Holt PG, Sedgwick JD. Suppression of IgE responses following antigen inhalation: a natural homeostatic mechanism which limits sensitization to aeroallergens. *Immunol Today*. 1987;8:14–15.

21. Schistosomes, liver flukes and *Helicobacter pylori*. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 1994 Jun 7–14. IARC *Monogr Eval Carcinog Risks Hum*. 1994;61:1–241.

22. Blaser MJ, Atherton JC. Helicobacter pylori persistence: biology and disease. *J Clin Invest*. 2004;113(3):321–333.

23. Banatvala N, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and *Helicobacter pylori*. *J Infect Dis*. 1993;168(1):219–221.

24. Strachan DP. Hay fever, hygiene, and household size. *BMJ*. 1989;299(6710):1259–1260.

25. Kemp A, Björkstén B. Immune deviation and the hygiene hypothesis: a review of the epidemiological evidence. *Pediatr Allergy Immunol*. 2003;14(2):74–80.

26. Kilpi T, Kero J, Jokinen J, et al. Common respiratory infections early in life may reduce the risk of atopic dermatitis. *Clin Infect Dis*. 2002;34(5):620–626.

27. Mosmann TR, Cherwinski H, Bond MW, et al. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol*. 1986;136(7):2348–2357.

28. Del Prete GF, de Carli M, Mastromauro C, et al. Purified protein derivative of Mycobacterium tuberculosis and excretorysecretory antigen(s) of Toxocara canis expand in vitro human T cells with stable and opposite (type 1 T helper or type 2 T helper profile of cytokine production. *J Clin Invest*. 1991;88(1):346–350.

29. Romagnani S. Th1 and Th2 in human diseases. *Clin Immunol Immunopathol*. 1996;80(3 Pt 1):225–235.

30. Reinhold U, Kukel S, Goeden B, et al. Functional characterization of skin-infiltrating lymphocytes in atopic dermatitis. *Clin Exp Immunol*. 1991;86(3):444–448.

31. Leung DY, Bieber T. Atopic dermatitis. *Lancet*. 2003;361(9352):151–160.

32. Del Prete GF, de Carli M, D'Elios MM, et al. Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. *Eur J Immunol*. 1993;23(7):1445–1449.

33. Maggi E, Biswas P, Del Prete G, et al. Accumulation of Th-2-like helper T cells in the conjunctiva of patients with vernal conjunctivitis. *J Immunol*. 1991;146(4):1169–1174.

34. Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med*. 1992;326(5):298–304.

35. Robinson D, Hamid Q, Bentley A, et al. Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol*. 1993;92(2):313–324.

36. Chatila TA, Li N, Garcia-Lloret M, et al. T-cell effector pathways in allergic diseases: transcriptional mechanisms and therapeutic targets. *J Allergy Clin Immunol*. 2008;121(4):812–823.

37. Romagnani S, Parronchi P, D'Elios MM, et al. An update on human Th1 and Th2 cells. *Int Arch Allergy Immunol*. 1997;113(1–3):153–156.

38. Kon OM, Kay AB. T cells and chronic asthma. *Int Arch Allergy Immunol*. 1999;118(2–4):133–135.

39. Betts RJ, Kemeny DM. CD8 T cells in asthma: friend or foe? *Pharmacol Ther*. 2009;121(2):123–131.

40. Reibman J, Marmor M, Filner J, et al. Asthma is inversely associated with *Helicobacter pylori* status in an urban population. *PLoS One*. 2008;3(12):e4060.

41. Sachs G, Weeks DL, Melchers K, Scott DR. The gastric biology of *Helicobacter pylori*. *Annu Rev Physiol*. 2003;65:349–369.

42. Weeks DL, Eskandari S, Scott DR, Sachs G. A H+-gated urea channel: the link between *Helicobacter pylori* urease and gastric colonization. *Science*. 2000;287(5452):482–485.

43. Bäckhed F, Rokbi B, Torstensson E, et al. Gastric mucosal recognition of *Helicobacter pylori* is independent of Toll like receptor 4. *J Infect Dis*. 2003;187(5):829–836.

44. Lee SK, Stack A, Katzowitsch E, Aizawa SI, Suerbaum S, Josenhans C. *Helicobacter pylori* flagellins have very low intrinsic activity to stimulate human gastric epithelial cells via TLR5. *Microbes Infect*. 2003;5(15):1345–1356.

45. D'Elios MM, Manghetti M, de Carli M, et al. Th1 effector cells specific for *Helicobacter pylori* in the gastric antrum of patients with peptic ulcer disease. *J Immunol*. 1997;158(2):962–967.

46. D'Elios MM, Amedei A, Benagiano M, et al. *Helicobacter pylori*, T cells and cytokines: the "dangerous liaisons". *FEMS Immunol Med Mic*. 2005;44(2):113–119.

47. Fox JG, Beck P, Dangler CA, et al. Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduces helicobacter-induced gastric atrophy. *Nat Med*. 2000;6(5):536–542.

48. D'Elios MM, Manghetti M, de Carli M, et al. Different cytokine profile and antigen-specificity repertoire in *Helicobacter pylori*-specific T cell clones from the antrum of chronic gastritis patients with or without peptic ulcer. *Eur J Immunol*. 1997;27(7):1751–1755.

49. Amedei A, Cappon A, Codolo G, et al. The neutrophil-activating protein of *Helicobacter pylori* promotes Th1 immune responses. *J Clin Invest*. 2006;116(4):1092–1101.

50. Herz U, Lacy P, Renz H, Erb K. The influence of infections on the development and severity of allergic disorders. *Curr Opin Immunol*. 2000;12(6):632–640.

51. Wohlleben G, Erb KJ. Immune stimulatory strategies for the prevention and treatment of asthma. *Curr Pharm Design*. 2006;12(25):3281–3292.

52. Blaser MJ, Chen Y, Reibman J. Does *Helicobacter pylori* protect against asthma and allergy? *Gut*. 2008;57(5):561–567.

53. Jun ZJ, Lei Y, Shimizu Y, et al. *Helicobacter pylori* seroprevalence in patients with mild asthma. *Tohoku J Exp Med*. 2005;207(4):287–291.

54. Tseng KW, Lam WK, Chan KN, et al. *Helicobacter pylori* seroprevalence in asthma. *Respir Med*. 2000;94(8):756–759.

55. McCune A, Lane A, Murray L, et al. Reduced risk of atopic disorders in adults with *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol*. 2003;15(6):637–640.

56. Chen Y, Blaser MJ. Inverse associations of *Helicobacter pylori* with asthma and allergy. *Arch Intern Med*. 2007;167(8):821–827.

57. von Hertzen LC, Laatikainen T, Makela MJ, et al. Infectious burden as a determinant of atopy – a comparison between adults in Finnish and Russian Karelia. *Int Arch Allergy Immunol*. 2006;140(2):89–95.

58. Radon K, Windstetter D, Eckart J, et al. Farming exposure in childhood, exposure to markers of infections and the development of atopy in rural subjects. *Clin Exp Allergy*. 2004;34(8):1178–1183.

59. Linneberg A, Ostergaard C, Tvede M, et al. IgG antibodies against microorganisms and atopic disease in Danish adults: the Copenhagen Allergy Study. *J Allergy Clin Immunol*. 2003;111(4):847–853.

60. Janson C, Asbjornsdottir H, Birgisdottir A, et al. The effect of infectious burden on the prevalence of atopy and respiratory allergies in Iceland, Estonia, and Sweden. *J Allergy Clin Immunol*. 2007;120(3):673–679.

61. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nature Rev Immunol*. 2003;3(2):133–146.

62. Robinson K, Kenefeck R, Pidgeon EL, et al. *Helicobacter pylori* induced peptic ulcer disease is associated with inadequate regulatory T cell responses. *Gut*. 2008;57(10):1375–1385.

63. Lundgren A, Strömberg E, Sjöling A, et al. Mucosal FOXP3-expressing CD4+ CD25 high regulatory T cells in *Helicobacter pylori*-infected patients. *Infect Immun*. 2005;73(1):523–531.

64. Wang SK, Zhu HF, He BS, et al. CagA+ H pylori infection is associated with polarization of T helper cell immune responses in gastric carcinogenesis. *World J Gastroenterol*. 2007;13(21):2923–2931.

65. Rad R, Brenner L, Bauer S. CD25+/Foxp3+ T cells regulate gastric inflammation and *Helicobacter pylori* colonization in vivo. *Gastroenterology*. 2006;131(2):525–537.

66. Harris PR, Wright SW, Serrano C, et al. *Helicobacter pylori* gastritis in children is associated with a regulatory T-cell response. *Gastroenterology*. 2008;134(2):491–499.

67. Codolo G, Mazzi P, Amedei A, et al. The neutrophil-activating protein of *Helicobacter pylori* down-modulates Th2 inflammation in ovalbumin-induced allergic asthma. *Cell Microbiol*. 2008;10(11): 2355–2363.

68. Ball HA, van Scott MR, Robinson CB. Sense and antisense: therapeutic potential of oligonucleotides and interference RNA in asthma and allergic disorders. *Clin Rev Allergy Immunol*. 2004;27(3):207–217.

69. Vollmer J, Krieg AM. Immunotherapeutic applications of CpG oligodeoxynucleotide TLR9 agonists. *Adv Drug Deliv Rev*. 2009; 61(3):195–204.

70. Moisan J, Camateros P, Thuraisingam T, et al. TLR7 ligand prevents allergen-induced airway hyperresponsiveness and eosinophilia in allergic asthma by a MYD88-dependent and MK2-independent pathway. *Am J Physiol Lung Cell Mol Physiol*. 2006;290(5):987–995.