Review

**Helicobacter pylori** and asthma pathogenesis, role of HP-NAP?

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**Helicobacter pylori** is a Gram-negative bacterium which chronically infects the stomach of > 50% of the human population and is the major cause of gastro-duodenal pathologies ranging from peptic ulcers to gastric cancer. There is a strong inflammatory component to these diseases and *H. pylori* gastric colonization is typically accompanied by mucosal infiltration of neutrophils, macrophages and Th1 lymphocytes, with a production of IL-12 and IFN-γ. However, an inverse association between the *H. pylori* infection and asthma has been also recently reported. Bronchial asthma is characterized by Th2 inflammation, which is inhibited by IL-12 and IFN-γ. A number of studies demonstrated that in allergic asthmatic patients, the Th2 responses can be redirected toward Th1 by *H. pylori*, specifically through the activities of its protein HP-NAP. Moreover, administration of HP-NAP limits the accumulation of eosinophils in the lung and prevents an increase of serum IgE in a mouse model of allergic asthma. These results could provide a possible biological function for HP-NAP and might be a part of the molecular mechanism underlying the inverse association between *H. pylori* infection and asthma.

**Key words:** Cytokines, **Helicobacter pylori**, asthma, HP-NAP.

INTRODUCTION

**Helicobacter pylori** (*H. pylori*), a Gram-negative bacillus is a frequent colonizer of the human stomach. This organism is also the causative agent of peptic ulceration, gastric lymphoma, and gastric adenocarcinoma (Ernst and Gold, 2000; Piazuelo et al., 2010; Blaser and Atherton, 2004). The World Health Organization (WHO) classifies *H. pylori* as a human carcinogen for gastric cancer, and eradicating the bacterium in high risk populations reduces incidence of gastric cancer (WHO, 2006; D'Elios and Andersen, 2009). It is estimated that approximately half of the world's population is infected with or are carriers of *H. pylori* (Go, 2002). *H. pylori* colonization occurs in childhood and can persist lifelong, causing disease mainly in adults (Banatvala et al., 1993; Brown, 2000). Colonization with *H. pylori* represents an interesting and unique paradigm in infectious diseases for its broad-range of consequences beyond peptic ulcers or gastric cancer. A strong association between *H. pylori* infections and iron deficiency anemia, and idiopathic thrombocytopenic purpura has been made and the contribution of the bacteria to the disease was confirmed by demonstration of clinical benefit of antibiotic therapy (Dubois and Kearney, 2005; Duques et al., 2010; Franchini and Veneri and 2004). However, the most fascinating aspect of the co-evolution of *H. pylori* with human hosts is the result of the interaction of these organisms with the immune system, causing certain diseases while protecting against others (Cover and Blaser, 2009).

Observations in the stomach in human and animal models indicate that *H. pylori* infections activate predominantly T-helper type 1 (Th1) cells, with the production of interferon-gamma (IFN-γ), interleukin-12 (IL-12), IL-18, IL-23 and tumor necrosis factor-alpha (TNF-α), (Tomita et al., 2001; Vivas et al., 2008). Complex and fascinating
mechanisms are responsible for the mucosal Th1 polarization (Del Giudice et al., 2001; D’Ellios et al., 2005). Furthermore, H. pylori infection is accompanied by a large infiltration of the mucosa by neutrophils, which are believed to contribute substantially to H. pylori–induced gastritis (Bayerdorfer et al., 1992). In the gastric mucosa of H. pylori–infected patients, a considerable proportion of Th cells show significant proliferation in response to various H. pylori antigens, including the cytotoxin–associated protein CagA, urease, vacuolating toxin VacA and heat shock proteins (D’Ellios et al., 1997). Several studies have provided evidence that a small oligomeric protein termed H. pylori neutrophil-activating protein (HP-NAP) plays an important role in activating the Th1 response while promoting neutrophil adhesion to endothelial cells via the production of reactive oxygen intermediates (ROI) (Marchetti et al., 1995; Evans et al., 1995). Furthermore, the stimulation of neutrophils with HP-NAP results in prompt and remarkable upregulation of IL-12 and IL-23 mRNA expression and protein secretion, via Toll-like receptor 2 (TLR2) activation. HP-NAP drives the production of high levels of IFN-γ and TNF-α by antigen specific gastric Th cells and induces a powerful cytotoxic activity, thus promoting a polarized Th1 response (Amedei et al., 2006). Re-direction of Th cells towards Th1 subtype is at the expense of the Th2 response, typically associated with asthma. There is a substantial body of evidence that H. pylori childhood infections are protective against asthma and HP-NAP is directly involved in the modulation of this immune disorder (deBernard and D’Ellios, 2010).

Asthma, defined by the WHO as a ‘chronic inflammatory disease of the airways,’ is a complex disorder which characterized by airway hyperresponsiveness to a variety of specific and nonspecific stimuli, and mucus hypersecretion by goblet cells (WHO, 2003). The bronchial asthma is characterized by basal membrane thickening, epithelial shedding, inflammatory infiltrates consisting of T lymphocytes and accumulation of activated eosinophils.

Asthma is driven by bronchial persistence of activated memory T cells, previously sensitized against allergenic, occupational, or viral antigens. In asthmatic patients, the exposure to allergen can induce a predominant activation of a cluster of differentiation-4+ (CD4+) Th2 lymphocytes in the airways, able to overexpress several Th2 cytokines, including IL-4 and IL-5 (Robinson et al., 1992; Del Prete et al., 1993). Moreover, the degree of IL-5 expression at the bronchial level is associated with disease severity both in atopic and in nonatopic asthma (Kon and Kay, 1999). IL-5 and granulocyte macrophage colony-stimulating factor (GM-CSF) can be considered the most important cytokines for eosinophil accumulation in asthmatic inflammation. Th2 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 (Betts and Kemeny, 2009).

The present review will focuses on the role of HP-NAP on the immunopathological process of bronchial asthma and H. pylori infection. Moreover, the potential use of HP-NAP as a therapeutic agent for prevention and treatment of asthma will also be considered.

### HP-NAP structure and function

HP-NAP is a 17-kDa protein folded into a 4-helix bundle monomer and is most frequently isolated as a dodecamer. Formation of a stable dimeric precursor of the oligomer requires iron binding and each dodecamer is capable of binding a maximum of 500 atoms of iron (Tonello et al., 1999). Although resembling bacterial ferritin in sequence and iron-binding activity, it is unclear, whether HP-NAP is involved in H. pylori iron metabolism and it is expressed independently of the iron content of the media (Dundon et al., 2001). Based on the absence of any extracellular targeting sequences, it is predicted to be localized in the bacterial cytosol and therefore HP-NAP very likely released upon autolysis. HP-NAP can bind to the external surface of the bacterial outer membrane (Namavar et al., 1998). In such a location, HP-NAP can mediate the binding of H. pylori to the mammalian cell surface and mucins via specific interactions with carbohydrates (Teneberg et al., 1997, Navamar et al., 1998). Moreover, soluble forms of HP-NAP can induce degranulation of peritoneal mast cells to release β-hexosaminidase and IL-6. HP-NAP can also translocate across polarized epithelial cells and induce IL-6 production by mast cells on the basolateral side (Montemurro et al., 2002).

HP-NAP is one of several virulence factors which produced by the bacterium H. pylori but has the most diverse physiological acities (Yoshida et al., 1993). When purified from aqueous extracts of H. pylori, HP-NAP was shown to have the following activities: 1) induce adhesion of neutrophil to endothelial cells in vitro as well as in vivo, 2) increase the adhesion of neutrophils to endothelial cells, 3) induce migration and activation of human neutrophils and monocytes (Satin et al., 2000), 4) is a potent stimulant of mast cells, 5) binds to the stomach mucus layer and 6) is capable of inducing reactive oxygen intermediates (ROI) production (Evans et al., 1995). The production of free radicals by neutrophils is an important component of the innate immune system and is an effective antimicrobial agent against H. pylori. However, bacterially-induced oxygen radicals could very well contribute to mucosal damage and gastritis as well.

### HP-NAP and the inverse association between H. pylori and asthma

There has been considerable epidemiological evidence accumulated over the last decades suggesting that
infectious diseases can influence the severity and incidence of asthma in the developed nations (Strachan, 1989). An inverse correlation has been demonstrated between the onset of asthma and the incidence of infections (Herz et al., 2000). This can be explained by the inhibition of allergic Th2 inflammation by Th1 responses elicited by immunostimulants produced by infectious agents, which are able to induce the production of IFN-γ, IL-12, IL-18 and IL-23 (Herz et al., 2000; Wohlleben and Erb, 2001). This view is supported by studies showing that development of asthma can be prevented in animals by administration of alive or killed bacteria or their components, which induce Th1 responses (Wohlleben and Erb, 2006) as illustrated for *H. pylori* in Figure 1.

Recently, on the basis of large epidemiological studies,
a consistent negative association between *H. pylori* infection and asthma and allergy has been described particularly when examined in children (Chen and Blaser, 2007; Chen and Blaser, 2008; Blaser et al., 2008). Further studies have shown that addition of HP-NAP to allergen-induced T-cell lines derived from allergic asthmatic patients led to a drastic increase in IFN-γ-producing T cells and a decrease in IL-4-secreting cells, thus resulting in a redirection of the immune response from a Th2 to a Th1 phenotype (Amedei et al., 2006). These results suggest that HP-NAP could be the key factor responsible for the reduction of allergy frequency in *H. pylori*-infected patients. Considering that there is also evidence for an inverse relationship between the presence of *H. pylori* and esophageal disease (Soneneberg et al., 2010) it is tempting to speculate that HP-NAP could play a protective role in this disease by a similar immunoologically-based mechanism.

**Exploitation of HP-NAP as a therapeutic for the treatment of asthma**

Using a mouse model of ovalbumin (OVA)-induced asthma, Codolo et al (2008) investigated whether HP-NAP might provide a novel therapeutic approach for redirecting Th2 to Th1 responses. Mice were first primed intraperitoneally (IP) with OVA, and then Th2 responses induced in their lungs by repeated OVA aerosol challenges. To determine the effect of HP-NAP on asthma development, mice were injected IP with either phosphate buffered saline (PBS) or HP-NAP, simultaneously with the initial OVA sensitization. Eighteen days following initial priming and subsequent challenge with OVA, differential white blood cell counts were measured in lung washing (bronchoalveolar lavages (BALs)) collected from mice initially treated with OVA alone or OVA+PBS or OVA+HP-NAP. As expected, administration of OVA resulted in the recruitment and activation of eosinophils in the bronchial airways of the mice and a subsequent increase in serum IgE levels. However mice co-administered HP-NAP and OVA had significantly reduced numbers of BAL and airway eosinophils (*P* < 0.01), and OVA-induced airway eosinophilia was prevented.

In contrast, the numbers of macrophages, neutrophils and lymphocytes in systemic HP-NAP treated mice were similar to those of OVA treated mice. Furthermore, mucosal administration of HP-NAP also suppressed the development of OVA-induced asthma. Finally they compared the potential immunomodulating activity of (TLR2) and HP-NAP by measuring the Th2 cytokines, IL-4, IL-5 and (GM-CSF), in the BAL of wild-type and TLR2 mice. While a significant reduction in IL-4, IL-5 and GM-CSF was observed in BAL from wild-type mice following systemic and mucosal treatment with HP-NAP, no such reduction were observed in TLR2 mice, suggesting that TLR2 expression is required for the beneficial effect of HP-NAP. These finding suggest that both systemic and mucosal administration of HP-NAP may be effective in preventing allergic asthma (Codolo et al., 2008; Sutton and Mitchell, 2010).

**CONCLUSION**

Asthma consists of airway inflammation, bronchial hyper-responsiveness and airway obstruction. The pathological features include infiltration of the airways by activated lymphocytes, specially Th2 cells and eosinophils. The epidemiological studies and experimental data provide evidence suggesting that infectious diseases, such as *H. pylori* infection, can affect the development of allergic disorders. This can be explained by the inhibition of the allergic Th2 inflammation by Th1 responses elicited by *H. pylori*, able to induce the production of IFN-γ, IL-12, IL-18 and IL-23. HP-NAP contributes toward inducing an IL-12- and IL-23-, and its a key bacterial factor able to drive the differentiation of antigen-stimulated T cells toward a polarized Th1 phenotype. HP-NAP is able to redirect the allergen-specific T-cell response from a Th2 to a Th1 response. Moreover, HP-NAP administration *in vivo* can result in inhibition of Th2-mediated bronchial inflammation of allergic bronchial asthma. These results support the view that the increased prevalence and severity of asthma in certain countries may be related to the decline in *H. pylori* infections capable of inducing a long lasting Th1 response, and suggest that HP-NAP may represent an important factor for novel strategies for the prevention and treatment of asthma and allergic diseases. Full appreciation of the role of HP-NAP in inflammation and its dual role in disease and health is important in light of a possibility that this highly immunogenic protein may be included in the next generation vaccine against *H. pylori* (Malfertheiner et al., 2008). Inclusion of HP-NAP in a vaccine raises the risk that vaccination against peptic disease and gastric cancer may carry an undesirable side effect of a further increase in the incidence of asthma or any other disease that show negative correlation with *H. pylori* infections. Therapeutic intervention using HP-NAP or a suitable mimicks of this immunostimulatory molecule could overcome this limitation and provide a novel strategy for the treating complex immunological disorders.

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