

Hp(2-20) peptide of *Helicobacter pylori* and the innate immune receptors: specific role(s) of the formyl peptide receptors

Il peptide Hp(2-20) dell'*Helicobacter pylori* e i recettori dell'immunità innata: ruolo specifico dei recettori per i peptidi formilati

Francesca W. Rossi¹, Nella Prevede¹, Nunzia Montuori², Pia Ragno³, Carmine Selleri⁴, Gianni Marone¹, Amato de Paulis¹

¹Department of Clinical Immunology and Allergy and Center for Basic and Clinical Immunology Research, University of Naples Federico II and Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy;

²Department of Cellular and Molecular Biology and Pathology, University of Naples Federico II, Italy;

³Department of Chemistry and Biology, University of Salerno, Italy;

⁴Hematology Branch, Department of Medicine, University of Salerno, Italy

INTRODUCTION

H*elicobacter pylori* (*H. pylori*), first isolated by Warren and Marshall in 1983 [1], is a Gram-negative microaerophilic, spiral bacterium specialized in colonization of the human stomach [2].

The bacterium, transmitted from person to person, has infected over half the global population; the infection, being prevalent among more than 90% of adults in developing countries, is the most common bacterial infection worldwide [3]. Typically acquired during childhood, the infection can persist in the gastric environment throughout the lifespan of the host, if untreated [4].

A well-choreographed equilibrium between the pathogen and the host, which is in turn dependent on strain-specific bacterial factors, host genotypic traits and permissive environmental factors, permits microbial persistence and health of the host, but confers a risk for serious diseases.

Indeed, infection with *H. pylori* is a co-factor in the development of three important upper gastrointestinal diseases: duodenal or gastric ulcers, gastric cancer, and gastric mucosa-asso-

ciated lymphoid-tissue (MALT) lymphoma. The risk that infected patients develop some of these diseases varies widely among populations. The great majority of patients with *H. pylori* infection will not present, throughout their life, any clinically significant complication [5]. Increasingly evidence indicates that, during its long coexistence with humans, *H. pylori* has developed complex strategies to limit the degree and extent of gastric mucosal damage and inflammation, as well as immune effectors activity. The innate immune response in the gastrointestinal tract consists of many components, including innate immune receptors [6], thus rendering its interaction with *H. pylori* particularly complex.

Several *H. pylori* constituents that are required for colonization or virulence are now well known; identification of bacterial and host mediators that augment disease risk has profound implications for both researchers and clinicians since such findings will not only provide a mechanistic insight into inflammatory carcinogenesis but may also serve to identify high-risk populations of *H. pylori*-infected individuals, who can then be targeted for therapeutic intervention.

■ ROLE OF FORMYL PEPTIDE RECEPTORS IN MEDIATING INFECTION AND DISEASE

Formyl peptide receptors (FPRs) are a small group of seven-transmembrane domain, G protein-coupled receptors, expressed mainly by mammalian phagocytic leukocytes and involved in host defense and inflammation. The three human FPRs (FPR1, FPR2/ALX, and FPR3) share significant sequence homology and are encoded by clustered genes. Taken together, these receptors are able to bind an extraordinarily numerous and structurally different group of agonistic ligands, including *N*-formyl and non-formyl peptides of different composition, that mediate phagocytes chemotactic activity in addition to different phagocytes functions [7] (Figure 1).

Several natural *n*-formyl peptides have been studied, including the prototype *N*-formyl-methionyl-leucyl-phenylalanine (fMet-Leu-Phe, or fMLF). These peptides, purified from bacterial supernatants, are biologically relevant ligands for formyl peptide receptors [7]. FPR1 and its homologue FPR2 are expressed on neutrophils, whereas monocytes and basophils express all the three receptors: FPR1, FPR2, and FPR3 [8]. Recent studies indicate that the *in vitro* activation of FPR family members induces leukocyte chemotactic migration and bactericidal activity via superoxide anion generation in neutrophils and monocytes [7].

Since bacteria are the natural source of these peptides, and FPRs are expressed in abundance by cells involved in the host defense system,

such as neutrophils, a view emerged that the FPRs family has evolved as chemoattractant receptors that support the organism in counteracting bacterial infections, in particular by facilitating phagocytes migration at the site of bacterial and viral invasion [9, 10]. Moreover, FPRs also participate in crucial pathophysiologic processes, including intestinal epithelial cell restitution [11].

■ ROLE OF HP(2-20) PEPTIDE AS A VIRULENCE FACTOR

Several peptides produced by *H. pylori* appear to be involved in inflammation associated with the infection. Among the other proteins associated with *H. pylori* virulence, which are associated with gastric ulcers and cancer (VacA, HP-NAP, CagA, etc.) [12, 13], a cecropin-like *H. pylori* peptide Hp(2-20) has been recently identified and found to be an important factor as a monocyte chemoattractant [14]. Cecropins are small peptides composed of two amphipathic helices joined by a hinge. *H. pylori* synthesizes the cecropin-like amino-terminal peptide Hp(2-20) derived from the ribosomal protein, L1 [8]. This peptide can be defined an antimicrobial peptide (AMP). AMPs are innate immune components ubiquitous in plants and animals, variously active against a wide range of pathogens, such as gram-positive and gram-negative bacteria, fungi and protozoa [15]. In particular Hp(2-20) possesses several important functional characteristics: it is bactericidal, it ac-

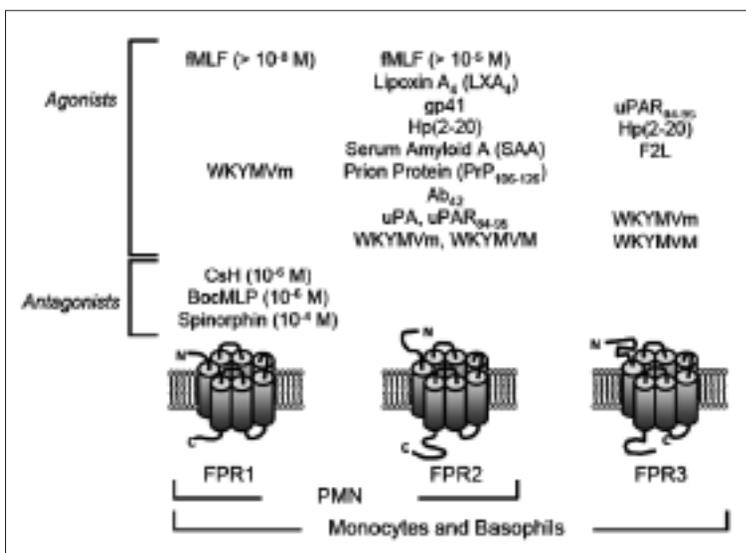


Figure 1 - The three *n*-formyl peptide receptors, the innate immune cells on which receptors are expressed and the relative agonists and antagonists acting on the specific receptors.

tivates phagocyte NADPH oxidase to produce reactive oxygen species, and it induces neutrophil, basophil, and monocyte migration through interactions with n-formyl peptide receptors [8, 14]. AMPs therefore have been proposed as some of the most likely substitutes for common antibiotics, to confront an increasingly serious threat to human health caused by antibiotic-resistant bacterial infections [15].

Based on Betten's *et al.* work [14] we decided to further extend these observations by evaluating the presence of basophils in gastric mucosa of *H. pylori*-infected patients, and the *in vitro* and *in vivo* effects of *H. pylori*-derived Hp(2-20) on basophils and gastric epithelial cells, thus clarifying the striking role of the interaction between this peptide and FPRs, in modulating the pathogenesis of *H. pylori* infection.

■ ROLE OF FC ϵ RI⁺ CELLS IN HELICOBACTER PYLORI INFECTION

Mast cells and basophils are the main effector cells in IgE-mediated allergic responses, but they also play important roles in innate immune responses against bacteria by releasing pro-inflammatory mediators and cytokines [16-18]. In particular, these cells synthesize several pro-inflammatory (histamine, PAF, cysteinyl leukotrienes, etc.), immunomodulating (cytokines and chemokines) and angiogenic mediators (vascular endothelial growth factors, etc.) [19, 20].

Even though mast cells are present in the gastric mucosa in normal conditions and in *H. pylori*-infected patients [17, 20], their role in Hp in-

fection in man is still largely obscure. On the other hand, it has been demonstrated in animals that several *H. pylori* products (VacA and HP-NAP) are chemotactic for mast cells and are able to induce the release of proinflammatory mediators and cytokines [20, 22].

In vitro studies have demonstrated that several *H. pylori*-derived peptides induce and/or potentiate mediator release from Fc RI⁺ cells. For instance, *H. pylori* extracts potentiate histamine release from rat mast cells [23]. In addition, the neutrophil-activating protein of *H. pylori* induces the release of preformed mediators and IL-6 from peritoneal mast cells [21]. Finally, VacA activates mast cells so leading to the migration and production of proinflammatory cytokines. Moreover, oral treatment of mice with VacA causes acute inflammation of gastric mucosa and mast cell accumulation [22]. Thus, several *H. pylori* proteins activate rodent mast cells, presumably through different immunologic mechanisms. Human basophils express receptors for fMLF that induce their chemotaxis and the release of proinflammatory mediators [8].

Granulocyte and mononuclear cell infiltration is characteristic of bacterial infections. By contrast, to our knowledge basophils have never been detected at sites of bacterial infections. The interactions between basophils and microbial agents are exceedingly complex, reflecting long periods of co-evolution [8].

It has been shown that peripheral blood basophils may be increased in *H. pylori*-positive patients [24]. Starting from these data, we have demonstrated that basophils can contribute to *H. pylori*-induced mucosal inflammation and that a bacterial infection is associated with ba-

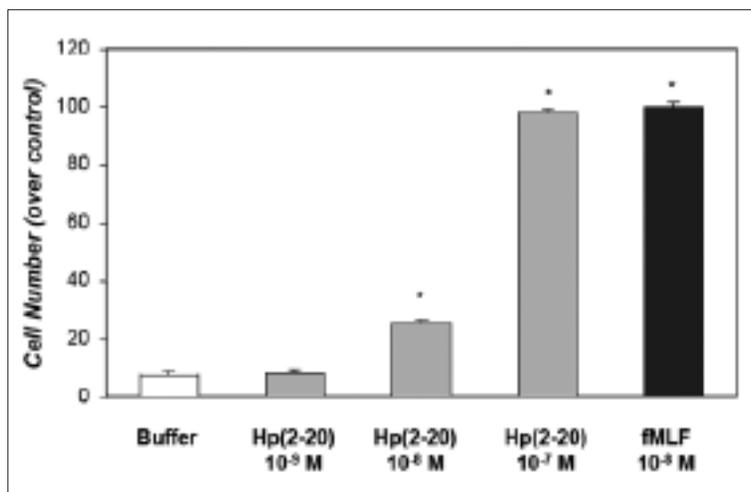


Figure 2 - Effects of Hp(2-20), at different concentrations, and fMLF (10⁻⁸ M) on human basophil chemotaxis. Basophils obtained from peripheral blood of donors negative for *H. pylori*, HIV-1 and HIV-2 Abs were allowed to migrate in the presence of the indicated concentrations of peptides, for 1 h at 37°C in a humidified (5%CO₂) incubator. Values are the mean ± SEM of six experiments with different basophil preparations. **p*<0.01 vs buffer.

sophil infiltration [8]. Although we found that Hp(2-20) is chemotactic for human basophils, through interaction with FPR2 and FPR3, this peptide was not found to induce the release of mediators from basophils purified from either *H. pylori*-positive or -negative individuals [8]. However, several stimuli can locally activate infiltrating basophils. For example, a mechanism by which basophils may be activated in *H. pylori* gastritis could involve IL-8, which is secreted from inflamed mucosa and is able to induce histamine release from basophils. In addition, it cannot be excluded that basophils from *H. pylori*-positive individuals can be activated through an IgE-mediated mechanism. Our study suggests that the Hp(2-20) peptide contributes to the recruitment of basophils to the inflammatory component of *H. pylori*-infected gastric tissue (Figure 2). Betten *et al.* [14] demonstrated that Hp(2-20) activates human monocytes to induce lymphocyte dysfunction and apoptosis. Taken together, these observations suggest that Hp(2-20) may contribute through various mechanisms to the dysregulation of the immune system in *H. pylori* infection. The basophil infiltration of gastric mucosa in *H. pylori*-infected individuals was unexpected and unprecedented. The role(s) of basophils and their mediators in the inflammatory response to *H. pylori* remains to be determined. It is possible that platelet-activating factor, histamine, cysteinyl leukotriene C₄, and other proinflammatory mediators released by basophils contribute to *H. pylori*-associated gastric damage. In addition, we cannot exclude that T_H2-like cytokines (IL-4 and IL-13), synthesized by basophils, modulate T_H1-mediated mucosal inflammation [8].

■ INTERACTION BETWEEN GASTRIC EPITHELIAL CELLS, HP(2-20) AND FPRS

The activation of the innate immune response represents the first line of defence for the eradication of the pathogens. One of the first step of the innate immune response to be engaged by *H. pylori* in the stomach is the gastric epithelial cell [6, 25].

H. pylori-derived peptide RpL1 (aa 2-20 (Hp(2-20))) in addition to its antimicrobial action exerts several immunomodulatory effects in eukaryotic cells by interacting with *N*-formyl peptide receptors (FPRs). Extending the findings of Babbini *et al.* [11], who found that activa-

tion of FPR is associated with increased migration of intestinal epithelial cells *in vitro*, a process that is relevant to the recovery of gastrointestinal mucosal integrity after induced injury, we have recently investigated the role of Hp(2-20) in wound healing, by using gastric epithelial cells.

Gastric mucosal defence can be defined in terms of the ability of the gastric mucosa to resist injury, as well as its capacity to respond appropriately to injury so that tissue damage is limited and the survival of the organism is not compromised. The ability of the gastric mucosa to repair itself after damage involves three different processes. First, viable surface mucous cells and mucous neck cells migrate from areas adjacent to the injured surface to reestablish epithelial continuity. Second, lost cells are replaced by cell division at the proliferative zone in the neck of gastric glands. Finally, granulation tissue, consisting of fibroblasts, macrophages, and proliferating microvessels formed through the process of neoangiogenesis, contributes to the complete anatomic and functional recovery of the mucosa. Several factors, including epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and prostaglandins play a role in the healing of injured gastric mucosa. However, the precise mechanisms underlying recovery of damaged gastric mucosal architecture are not completely understood. It is likely that a variety of physical and chemical signals sensed by cell surface receptors can initiate intracellular signalling pathways that co-ordinate the cellular changes observed during mucosal healing.

Most investigations focused on *H. pylori*-epithelial interactions have utilized human gastric epithelial cells. AGS human gastric epithelial cells (ATCC CRL-1739) are one of the most frequently used models for *in vitro* *H. pylori* studies and were derived from a patient with gastric adenocarcinoma. Several groups have demonstrated that the interaction between AGS cells and *H. pylori* is a useful *in vitro* model to study specific aspects of pathogenesis *in vivo*, such as the production of innate immune cytokines. MKN28 cells (JCRB0253) represent another commonly used human gastric epithelial cell model and were derived from a patient with a moderately differentiated tubular gastric adenocarcinoma [6].

In our study, using AGS and MKN epithelial cells, we have demonstrate that Hp(2-20) plays a role in regulating the process of gastric mu-

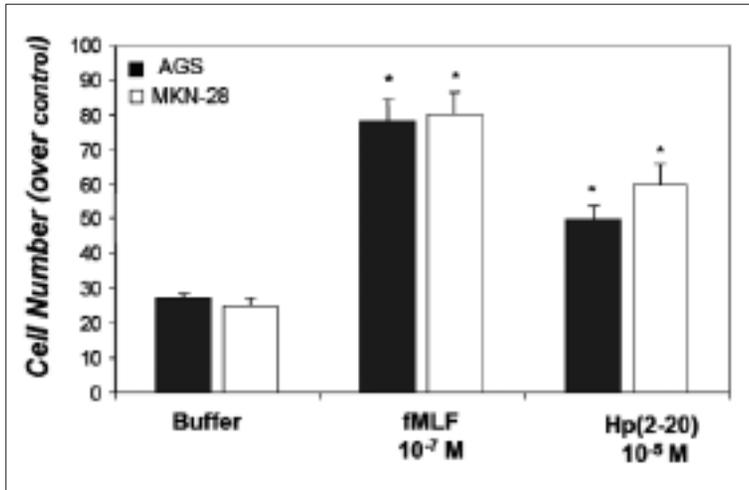


Figure 3 - Effects of fMLF (10^{-7} M), and Hp(2-20) (10^{-5} M) on gastric epithelial cell chemotaxis. AGS cells (■) and MKN-28 (□) were allowed to migrate in the presence of the indicated concentrations of fMLF and Hp(2-20), for 5 h at 37°C in a humidified (5% CO₂) incubator. Values are the means ± SEM of six experiments. * $p < 0.01$ vs buffer.

cosal healing by facilitating cell migration, proliferation, and neoangiogenesis. Gastric epithelial cells expressed FPR1, FPR2, and FPR3 both at mRNA and protein levels. In particular, we provided evidence that Hp(2-20), stimulates gastric epithelial cell migration (Figure 3) and proliferation and up-regulates VEGF-A expression at both transcriptional and translational levels through the interaction with FPR2 and FPR3 receptors. Consistently with these findings, treatment of gastric epithelial cells with Hp(2-20) activates the ERK, Akt, and STAT3 signaling pathways. Finally, using a specific in vivo model of indomethacin induced gastritis in rat stomach, we demonstrated that Hp(2-20) orally administered, was able to promote gastric mucosal healing [9].

These results suggest that Hp(2-20) can positively affect the remodeling phase of gastric mucosal healing. In fact, re-vascularization of damaged tissue through neoangiogenesis is a necessary part of wound healing, and deranged angiogenesis leads to abnormal healing of ulcers [9]. Our findings suggest that submicromolar concentrations of Hp(2-20) play a role in the healing of gastric mucosa. The biologic effect of Hp(2-20) is probably potentiated by other FPRs ligands produced by *H. pylori*.

H. pylori-induced gastroduodenal disease depends on the inflammatory response of the host and on the production of specific virulence factors that damage gastric epithelial cells and disrupt the gastric mucosal barrier, such as urease, responsible for ammonia generation, and the vacuolating cytotoxin VacA. Cytokines contribute to mucosal damage, either directly or indirectly, by mediating inflammatory response to

H. pylori. The gastric mucosal levels of the proinflammatory cytokines interleukin 1 β (IL-1 β), IL-6, IL-8 and tumour necrosis factor (TNF- α) are increased in *H. pylori*-infected subjects [26].

CONCLUSIONS

The human stomach is an optimal niche for *H. pylori* to survive, proliferate, and potentially pass to other hosts. Evidence accumulated over recent years suggests that *H. pylori* may positively or negatively regulate gastric acid secretion as well as the degree of gastric inflammation in order to best adapt gastric conditions to its own requirements [27].

The interactions between innate immune cells and microbial agents are exceedingly complex, reflecting long periods of co-evolution. This extraordinary ability to thrive in the face of a robust and vigorous local and systemic immune response is due to elaborate evolutionary adaptations of *H. pylori* that allow the bacteria to not only escape detection by pattern recognition receptors on innate immune cells, but also to evade adaptive immunity.

Several crucial bacterial factors, implicated in the progression of the infection and associated with pathogenicity, have been subsequently studied; in particular the role of Hp(2-20) seems very interestingly, and the observations provided by our group support the hypothesis that this peptide could be biologically relevant not only in the recruitment of innate immune cells, such as basophils, but also in gastric mucosal healing [8,9]. Moreover the results obtained in our studies provide further evidence of the

multifaceted relationship between *H. pylori* and innate immune cells. Our experiments clarified some of the pathogenetic aspects of the *H. pylori* infection, opening possible scenarios where a bacterial product (i.e. Hp(2-20)) may prove useful in the therapy and/or prevention of exogenous injury to the human stomach.

Key words: *Helicobacter pylori*, formyl peptide receptors, innate immune receptors

Conflict of interest disclosure

The authors declare that the article has not been sponsored, that no financial support has been given and finally that there is no conflict of interest.

SUMMARY

Helicobacter pylori (*H. pylori*) is a microaerophilic, Gram-negative bacterium that affects more than half of the world's population. *H. pylori* has co-evolved with humans to be transmitted from person to person and to persistently colonize the stomach. A well-choreographed equilibrium between bacterial effectors and host responses permits microbial persistence and health of the host but confers risk of serious diseases. During its long coexistence with humans, *H. pylori* has evolved complex strategies to limit the degree and extent of gastric mucosal damage and inflammation as well as immune effector activity. In this complex strategy an important role is played by

the interaction of *H. pylori* with a specific class of innate immune receptors, named n-formyl peptide receptor family (FPRs). In the last years several virulence factors have been studied in an effort to correlate bacterial phenotype with specific gastric manifestations and to clarify the pathogenetic mechanisms. Several peptides produced by *H. pylori* appear to be involved in inflammation associated with the infection. A particular interest has been focused on the Hp(2-20) peptide derived from the bacteria.

Thus, aim of the article is to comment on some advances in the elucidation of specific interactions between the Hp(2-20) peptide and FPRs.

RIASSUNTO

L'*Helicobacter pylori* (*H. pylori*) è un batterio microaerofilo, Gram-negativo che colpisce più della metà della popolazione mondiale. L'*H. pylori* è co-evoluto con l'uomo per essere trasmesso da individuo ad individuo colonizzando in maniera persistente lo stomaco. Un delicato equilibrio tra i prodotti batterici e le risposte di difesa dell'ospite consente la persistenza del microbo all'interno dello stomaco senza causare malattia nell'uomo, ma conferendo un elevato rischio di sviluppare malattie gastriche gravi.

Durante la sua lunga convivenza con l'uomo, l'*H. pylori* ha sviluppato complesse strategie atte a limitare il danno della mucosa gastrica e l'infiammazione, così come le risposte effettrici immunitarie. In questo comples-

so sistema un ruolo cruciale è svolto dall'interazione dell'*H. pylori* con una specifica classe di recettori dell'immunità innata, denominati recettori per peptidi formilati (FPRs). Negli ultimi anni diversi fattori di virulenza sono stati studiati nel tentativo di correlare il fenotipo batterico con specifiche manifestazioni gastriche e per chiarire i meccanismi patogenetici che sottendono il danno. Diversi peptidi prodotti dall'*H. pylori* sembrano essere coinvolti in infiammazione associata con l'infezione. Particolare interesse è stato focalizzato sul peptide Hp(2-20) derivato dall'*H. pylori*.

Pertanto, scopo di quest'articolo è di commentare alcuni progressi interazioni specifiche tra il peptide Hp(2-20) e gli FPRs.

REFERENCES

[1] Marshall B.J., Warren J.R. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 16, 1311-1315, 1984.
[2] Blaser M.J., Parsonnet J. Parasitism by the "slow" bacterium *Helicobacter pylori* leads to altered gastric homeostasis and neoplasia. *J. Clin. Invest.* 94, 4-8, 1994.

[3] Goh K.L., Chan W.K., Shiota S., Yamaoka Y. Epidemiology of *Helicobacter pylori* infection and public health implications. *Helicobacter* 16, 1-9, 2011.
[4] Ricci V., Romano M., Boquet P. Molecular cross-talk between *Helicobacter pylori* and human gastric mucosa. *World J. Gastroent.* 17, 1383-1399, 2011.
[5] Mccoll K.E. Clinical practice. *Helicobacter pylori* infection. *N. Eng. J. Med.* 362, 1597-1604, 2010.
[6] Peek R.M. Jr., Fiske C., Wilson K.T. Role of innate

immunity in *Helicobacter pylori*-induced gastric malignancy. *Physiol. Rev.* 90, 831-858, 2010.

[7] Ye R.D., Boulay F., Wang J.M., et al. International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. *Pharmacol. Rev.* 61, 119-161, 2009.

[8] De Paulis A., Prevete N., Fiorentino I., et al. Basophils infiltrate human gastric mucosa at sites of *Helicobacter pylori* infection, and exhibit chemotaxis in response to H. pylori-derived peptide Hp(2-20). *J. Immun.* 172, 7734-7743, 2004.

[9] De Paulis A., Prevete N., Rossi F.W., et al. *Helicobacter pylori* Hp(2-20) promotes migration and proliferation of gastric epithelial cells by interacting with formyl peptide receptors in vitro and accelerates gastric mucosal healing in vivo. *J. Immun.* 183, 3761-3769, 2009.

[10] De Paulis A., De Palma R., Di Gioia L., et al. Tat protein is an HIV-1-encoded beta-chemokine homolog that promotes migration and up-regulates CCR3 expression on human Fc epsilon RI+ cells. *J. Immun.* 165, 7171-7179, 2000.

[11] Babbin B.A., Jesaitis A.J., Ivanov A.I., et al. Formyl peptide receptor-1 activation enhances intestinal epithelial cell restitution through phosphatidylinositol 3-kinase-dependent activation of Rac1 and Cdc42. *J. Immun.* 179, 8112-8121, 2007.

[12] Blaser M.J., Berg D.E. *Helicobacter pylori* genetic diversity and risk of human disease. *J. Clin. Invest.* 107, 767-773, 2001.

[13] Romero-Adrian T.B., Leal-Montiel J., Monsalve-Castillo F., et al. *Helicobacter pylori*: bacterial factors and the role of cytokines in the immune response. *Current Microbiology*, 60, 143-155, 2010.

[14] Betten A., Bylund J., Christophe T., Boulay F., Romero A., Hellstrand K., Dahlgren C. A proinflammatory peptide from *Helicobacter pylori* activates monocytes to induce lymphocyte dysfunction and apoptosis. *J. Clin. Invest.* 108, 1221-1228, 2001.

[15] Liu L., Fang Y., Huang Q., Wu J. A rigidity-enhanced antimicrobial activity: a case for linear cationic alpha-helical peptide HP(2-20) and its four analogues. *PLoS One*, 6, e16441, 2011.

[16] Malaviya R., Ikeda T., Ross E., Abraham S.N. Mast cell modulation of neutrophil influx and bacte-

rial clearance at sites of infection through TNF-alpha. *Nature*. 381, 77-80, 1996.

[17] Wedemeyer J., Tsai M., Galli S.J. Roles of mast cells and basophils in innate and acquired immunity. *Curr. Opin. Immun.* 12, 624-631, 2000.

[18] Marone G., Triggiani M., Genovese A., De Paulis A. Role of human mast cells and basophils in bronchial asthma. *Adv. Immun.* 88, 97-160, 2005.

[19] Boesiger J., Tsai M., Maurer M., Yamaguchi M., et al. Mast cells can secrete vascular permeability factor/vascular endothelial cell growth factor and exhibit enhanced release after immunoglobulin E-dependent upregulation of Fc epsilon receptor 1 expression. *J. Exp. Med.* 188, 1135-1145, 1998.

[20] Detoraki A., Staiano R.I., Granata F., et al. Vascular endothelial growth factors synthesized by human lung mast cells exert angiogenic effects. *J. Allergy Clin. Immun.* 123, 1142-1149, 2009.

[21] Montemurro P., Nishioka H., Dundon W.G., et al. The neutrophil-activating protein (HP-NAP) of *Helicobacter pylori* is a potent stimulant of mast cells. *Eur. J. Immun.* 32, 671-676, 2002.

[22] Supajatura V., Ushio H., Wada A., et al. Cutting edge: VacA, a vacuolating cytotoxin of *Helicobacter pylori*, directly activates mast cells for migration and production of proinflammatory cytokines. *J. Immun.* 168, 2603-2607, 2002.

[23] Masini E., Bechi P., Dei R., Di Bello M.G., Sacchi T.B. *Helicobacter pylori* potentiates histamine release from rat serosal mast cells induced by bile acids. *Dig. Dis. Sci.* 39, 1493-500, 1994.

[24] Karttunen T.J., Niemela S., Kerola T. Blood leukocyte differential in *Helicobacter pylori* infection. *Dig. Dis. Sci.* 41, 1332-1336, 1996.

[25] Molnar B., Galamb O., Sipos F., Leiszter K., Tullassay Z. Molecular pathogenesis of *Helicobacter pylori* infection: the role of bacterial virulence factors. *Dig. Dis. Sci.* 28, 604-608, 2010.

[26] Zarrilli R., Ricci V., Romano M. Molecular response of gastric epithelial cells to *Helicobacter pylori*-induced cell damage. *Cell. Microbiol.* 1, 93-99, 1999.

[27] Blaser M.J., Atherton J.C. *Helicobacter pylori* persistence: biology and disease. *J. Clin. Invest.* 113, 321-333, 2004.