MICROBIAL PATHOGENICITY

Modulatory action of *Helicobacter pylori* on histamine release from mast cells and basophils *in vitro*

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Summary. *Helicobacter pylori* is important in the aetiology of peptic ulceration. Despite inducing an inflammatory response in the mucosa, the organism persists, suggesting that it has efficient protective mechanisms. Some bacterial and viral products modulate histamine secretion from inflammatory cells. Therefore, this study examined the modulatory effects of *H. pylori* preparations on histamine release from rat peritoneal mast cells and human basophils. Eleven clinical isolates of *H. pylori* were prepared in different ways: as whole washed bacteria, washed sonicated bacteria, and formalin-killed bacteria, and as outer-membrane and lipopolysaccharide (LPS) extracts. Histamine release from mast cells or basophils was not elicited by any of these bacterial preparations alone. However, when mixed with various secretory stimulants, the bacterial preparations caused inhibition of histamine release from rat mast cells (calcium ionophore A23187, compound 48/80, concanavalin A, anti-rat IgE) and human basophils (A23187, *N*-formyl Met-Leu-Phe). The degree of inhibition ranged from 48 % to 97 %. These results indicate that *H. pylori* exerts an inhibitory effect on cells of the immune system that contributes to its persistence within the gastric mucosa.

Introduction

Helicobacter pylori (*H. pylori*) is recognised as a significant cause of chronic antral gastritis and is important in the aetiology of duodenal ulceration.^{1–3} There is also evidence to support a role for *H. pylori* as a risk factor for gastric carcinoma.^{4–6} The pathogenic mechanisms of *H. pylori* are incompletely understood. Several of the histological features associated with *H. pylori* infection (e.g., neutrophil infiltration and increased numbers of mononuclear cells—chiefly lymphocytes and plasma cells) are similar to those observed in human allergic reactions.^{7,8} The importance of mast cells and basophils in immediate hypersensitivity reactions (e.g., hay fever, atopic asthma) has long been recognised⁹ and recent evidence suggests that they may be involved in other disease states involving chronic inflammatory processes.¹⁰ Mast cells and basophils may be activated either by immunological mechanisms (*via* antigen cross-linking of specific IgE bound to the plasma membrane of mast cells and basophils) or *via* non-immunological pathways, whereby the agent directly activates the

cells.¹¹ *Haemophilus influenzae*, *Salmonella typhimurium* and several other bacteria, as well as bacterial and viral products, have been found to induce or modulate histamine release from mast cells or basophils.^{12,13}

Duodenal ulcer patients, both children¹⁴ and adults,¹⁵ with *H. pylori* infection have a lower concentration of histamine within their oxyntic mucosa than *H. pylori*-negative patients. This suggests that the mucosal mast cells in the *H. pylori*-positive patients are either actively secreting histamine, or that histamine synthesis is reduced. Furthermore, basophils from 84 % of chronic gastritis patients with *H. pylori* infection were found to have bound *H. pylori*-specific IgE, and to release histamine when challenged with surface antigens of *H. pylori*. Histamine liberation, by an IgE-mediated mechanism, was similarly achieved when normal basophils were passively sensitised with serum from IgE-positive patients.¹⁶

In-vitro studies have shown that washed cells, formalin-killed cells and crude cell-wall preparations of *H. pylori* were unable to induce histamine release from rat serosal, peritoneal and pleural mast cells. However, similar preparations from several *H. pylori* strains were found to potentiate mast cell histamine release induced by compound 48/80, calcium

ionophore A23178 and cholic acid.^{17,18} Enhancement was maximal with the crude cell-wall preparations and differences in the degree of histamine release were obtained with different strains of *H. pylori*.

Thus, *H. pylori* may be involved in mast cell and basophil activation and subsequent mediator release. In patients with specific IgE, an antibody-allergen reaction may occur leading to mediator release and the attendant inflammatory response. However, in patients lacking specific IgE, *H. pylori* products may potentiate the release of mediators from mast cells or basophils which have been primed by other agents. Despite these studies, the precise role of *H. pylori* in histamine release remains unclear.

The aim of the present study was to investigate further the direct and modulatory actions of *H. pylori* preparations on rat peritoneal mast cells and human basophils. The effect of *H. pylori* on mediator release via both IgE-dependent and IgE-independent non-immunological mechanisms was examined.

Materials and methods

Isolation, characterisation and culture of *H. pylori*

Antral biopsy specimens from patients were inoculated directly on to Columbia Agar Base (Oxoid) containing defibrinated horse blood 7% v/v and on to agar with antibiotic supplement (vancomycin 10 mg/L, trimethoprim lactate 5 mg/L, cefsulodin 5 mg/L, amphotericin B 5 mg/L and nalidixic acid 20 mg/L). Plates were incubated at 37°C for up to 5 days in a micro-aerophilic atmosphere of O₂ 5%, H₂ 1%, CO₂ 7% and N₂ 87% (variable atmosphere incubator; Don Whitley Scientific, Shipley, W. Yorks). Isolates were identified as *H. pylori* if they were gram-negative with a typical morphology, hydrolysed urea rapidly and were catalase- and oxidase-positive.¹⁹ The identification was confirmed by gene amplification by the polymerase chain reaction.²⁰ Isolates were stored at -70°C in peptone 1% w/v solution containing glycerol 25% v/v.

For control experiments, a recently isolated non-capsulate strain of *Hae. influenzae* was grown aerobically for 24 h at 37°C on Columbia chocolate agar, which was made by adding defibrinated horse blood 7% v/v to Columbia agar base and heating at 75°C for 30 min.

Preparation of bacteria for histamine release experiments

For whole washed *H. pylori*, bacteria were harvested after 2-3 days, washed three times in 0.01 M phosphate-buffered saline, pH 7.4 (PBS), and resuspended in Tyrode's buffer.²¹ Sonicated bacteria were prepared by sonication on ice, three times for 2 min at an amplitude of 18 µm (MSE Soniprep 150). These *H. pylori* preparations were used at a final protein concentration of 0.15 mg/ml, which was chosen as appropriate from the previously published work of Bechi *et al.*¹⁷

Formalin-killed *H. pylori* were obtained by incubation of whole bacteria in formalin 2% v/v in PBS for 1 h at 37°C. The bacteria were subsequently washed four times in PBS and resuspended in Tyrode's buffer to a final concentration of 10 mg wet weight/ml as used in previous studies by Stahl Skov *et al.*²²

Bacterial outer membranes were extracted by suspension of whole washed *H. pylori* in *N*-lauroyl sarcosinate 3% v/v for 1 h with rotation.²³ The extraction mixture was then centrifuged (12000 *g*, 1 h, 4°C) and the pellet was resuspended in Tyrode's buffer to a final concentration of 10 µg of protein/ml.²⁴ Lipopolysaccharide (LPS) was extracted by the proteinase K method of Clementsen *et al.*²⁵ and Brown *et al.*²⁶ Briefly, whole washed *H. pylori* cells were suspended in Tris buffer (1.5 ml, 0.025 M, pH 7.4) to an absorbance of 0.6 at 525 nm. They were then incubated (1 h, 37°C) with an insoluble proteinase K-agarose compound (25 µg; Sigma P-9290). The enzyme-agarose complex was removed by centrifugation (200 *g*, 5 min, 4°C) and any possible remaining enzyme activity in the supernate was inactivated by heating (30 min, 70°C). The supernate preparation was then diluted 1 in 4 in Tyrode's buffer for use in the histamine release experiments, as recommended by Professor S. Norn (personal communication).

Hae. influenzae was harvested at 24 h, washed three times in PBS and resuspended in Tyrode's buffer. The bacteria were used in the histamine release assay at a final protein concentration of 0.15 mg/ml, as above.

Isolation of human basophils

Blood (30 ml) was drawn by venepuncture, anticoagulated with heparin, and mixed with 5 ml of dextran 70 (Lomodex 70) and 150 mg of glucose. After sedimentation for 90 min, the supernate was collected. The cells were obtained by centrifugation (200 *g*, 7 min, 20°C), and washed twice in Tyrode's buffer. The basophils were pre-incubated at 37°C for 10 min before challenge.

Isolation of rat peritoneal mast cells

Mixed peritoneal cells were isolated from female Hooded Lister rats by direct lavage with heparinised Tyrode's buffer as described previously.²¹ The mast cell suspensions were pre-warmed for 5 min at 37°C before challenge.

Histamine release assay

Mast cells or basophils were incubated, for 10 and 45 min, respectively, with the various bacterial preparations alone, or in the presence of several known immunological or non-immunological stimulants (as described in the *Results*). All samples were incubated, in duplicate, at 37°C within a metabolic shaker. The reactions were stopped by placing the tubes in an ice-water bath and the addition of ice-cold Tyrode's buffer. The cells were recovered by centrifugation, and

the histamine concentration was assayed in both the cellular and the supernatant (released histamine) fractions. Histamine was measured by an automated fluorimetric assay based on the method of Shore *et al.*^{27, 28}

Histamine release was expressed as a percentage of the total histamine (cells and supernates) and corrected for the spontaneous release (i.e., that occurring in the absence of a stimulant). In several experiments, the effects of *H. pylori* preparations on histamine release were expressed as percentage inhibition. This was calculated from the formula:

Inhibition (%) =

$$100 - \left[\frac{\text{histamine release by secretagogue in the presence of } H. \text{ pylori}}{\text{histamine release by secretagogue alone}} \times 100 \right]$$

Data are given as means and standard error of the mean (SEM) for the number (*n*) of experiments performed, unless otherwise stated.

Results

In the initial experiments, whole washed bacteria of 11 different strains of *H. pylori* were tested. None of the strains elicited a significant histamine release from rat peritoneal mast cells when tested alone and, therefore, two *H. pylori* strains (HP1 and HP2) were selected for the further experiments.

Table. Effect of different concentrations of whole washed *H. pylori* (strain HP1) on histamine release from rat peritoneal mast cells induced by calcium ionophore A23187 (0.25 μ M)

Protein concentration (mg/ml)	Mean (SEM) percentage histamine release	
	<i>H. pylori</i> alone	<i>H. pylori</i> plus A23187
0	0	29.6 (5.9)
0.005	1.5 (1.1)	29.5 (5.1)
0.01	-0.2 (0.2)	26.7 (5.3)
0.025	0.8 (0.3)	13.3 (4.6)
0.05	0.6 (0.6)	6.7 (1.8)
0.1	2.5 (1.4)	8.6 (3.5)

*All data are given as means (SEM) for 4–5 experiments. Negative values occur when the histamine release in the presence of bacteria is less than that obtained for the spontaneous release.

No significant histamine release (< 3%) was induced when *H. pylori* preparations HP1 and HP2 (whole washed or sonicated cells) were incubated alone with rat peritoneal mast cells (data not shown). However, these preparations inhibited histamine release induced by either calcium ionophore A23187 or compound 48/80 (fig. 1). There was a concentration-dependent inhibition of histamine release (table). In all experiments with whole washed bacteria, preparations were standardised to 0.15 mg of protein/ml. This value was chosen in light of the data of Bechi *et al.*¹⁷ who studied the dose response effects of similar bacterial preparations. A further nine randomly selected clinical isolates of *H. pylori* (HP3–HP11) inhibited histamine

Fig. 1. Inhibitory effects of whole washed (HP1 and HP2) and sonicated (HP1S and HP2S) preparations of two strains of *H. pylori* on histamine release from rat peritoneal mast cells induced by calcium ionophore A23187 (0.25 μ M, □) or compound 48/80 (0.04 μ g/ml, ■). Values shown are means (and SEM) of four experiments, each performed in duplicate. The histamine release values in the absence of *H. pylori* preparations were 61.0 SEM 2.9% (calcium ionophore A23187) and 20.7 SEM 2.5% (compound 48/80).

Fig. 2. Comparison of the inhibitory effects of different clinical isolates of whole washed *H. pylori* (HP3–HP11) on histamine release from rat peritoneal mast cells induced by calcium ionophore A23187 (0.25 μ M). The control histamine release values in the absence of *H. pylori* preparations were 37.6% and 81.1% for the experiments involving strains HP3–HP7 and HP8–HP11, respectively.

Fig. 3. Inhibitory effects of formalin-killed preparations (□) or LPS extracts (■) of *H. pylori* (HP1) on histamine release from rat peritoneal mast cells induced by compound 48/80 (0.1 μ g/ml), concanavalin A (1 μ g/ml) or anti-rat IgE (1 in 5000 dilution). Values shown are means (and SEM) for three experiments, each performed in duplicate. The histamine release values in the absence of *H. pylori* preparations were 44.6 SEM 2.5% (compound 48/80), 37.3 SEM 10.1% (concanavalin A) and 40.4 SEM 15.2% (anti-rat IgE).

release induced by calcium ionophore A23187. The inter-strain variation in this inhibition of histamine release by *H. pylori* is illustrated in fig. 2.

The effects of formalin-killed bacteria, outer-

membrane preparations and LPS extracts of *H. pylori* (HP1) were examined. Minimal histamine release was induced (< 10%) when these preparations were incubated alone with rat peritoneal mast cells (data

Fig. 4. Effect of different concentrations of outer membrane preparations of *H. pylori* on histamine release from rat peritoneal mast cells induced by the calcium ionophore A23187 (0.25 µM, —■—) or compound 48/80 (0.4 µg/ml, ...○...).

Fig. 5. Inhibitory effects of formalin-killed preparations (□) or LPS extracts (■) of *H. pylori* (HP1) on histamine release from human basophils induced by calcium ionophore A23187 (0.25 µM) or *N*-formyl Met-Leu-Phe (0.01 µM). Values shown are means (and SEM) for 5–6 experiments, each performed in duplicate. The histamine release values in the absence of *H. pylori* preparations were 32.1 SEM 3.1% (calcium ionophore A23187) and 15.2 SEM 1.3% (*N*-formyl Met-Leu-Phe).

not shown). Histamine release induced by compound 48/80, concanavalin A, or anti-rat IgE was inhibited by formalin-killed preparations and LPS extracts of *H. pylori* (fig. 3). *H. pylori* outer-membrane preparations caused a dose-dependent inhibition of histamine release induced by compound 48/80 and A23187 (fig. 4).

With human basophils, no significant histamine release (< 3%) was induced when formalin-killed preparations or LPS extracts of *H. pylori* (HP1) were incubated alone with these cells (data not shown). These preparations inhibited histamine release from human basophils induced by calcium ionophore A23187 and *N*-formyl Met-Leu-Phe (fig. 5). *H. pylori* outer-membrane preparations (10 ng of protein/ml) caused a slight but significant inhibition (17%) of A23187-induced histamine release but higher concentrations were not tested.

No significant histamine release was obtained when whole washed *Hae. influenzae* were incubated alone with rat peritoneal mast cells. The histamine release induced by calcium ionophore A23187 (0.25 μ M) was approximately doubled (from 13% to 23%) by co-incubation with *Hae. influenzae* (0.15 mg of protein/ml).

Discussion

Histamine plays an essential role in the regulation of gastric acid secretion.²⁹ Factors that stimulate the production or liberation of histamine may contribute to the pathogenesis of duodenal ulcer disease, which is characterised by gastric hypersecretion. *H. pylori* is known to be an important pathogen in duodenal ulcer disease;³⁰ however, the influence of *H. pylori* on mast cell and basophil histamine release remains unclear.

Previous findings^{17,18} that histamine liberation does not occur when whole washed, whole formalin-killed or crude cell-envelope extracts of *H. pylori* are incubated *in vitro* with rat peritoneal mast cells were confirmed. This work was extended and it was found that, in addition, histamine release was not induced when rat peritoneal mast cells were exposed to LPS or outer-membrane extracts of *H. pylori*. Furthermore, in similar experiments with human basophils, histamine release was not stimulated by incubation with LPS or whole formalin-killed *H. pylori* preparations. However, in contrast to previous reports,^{17,18} each of the five *H. pylori* preparations studied was shown to inhibit the release of histamine from rat peritoneal mast cells simultaneously stimulated by calcium ionophore A23187 or compound 48/80. Inhibition was consistently obtained with whole washed bacteria of each of 11 different clinical isolates of *H. pylori*. The Italian workers found that co-incubation of purified rat peritoneal mast cells with several strains of *H. pylori* (whole washed, whole formalin-killed or crude cell-envelope preparations) and calcium ionophore A23187 or compound 48/80, caused a marked potentiation of histamine release.^{17,18} In the present

study, inhibition of histamine release from rat peritoneal mast cells was also seen with other stimulants (anti-rat IgE, concanavalin A) and with an additional cell type, the human basophil (calcium ionophore A23187, *N*-formyl Met-Leu-Phe). Where possible, the concentration of bacterial preparations used was one that produced potentiation in previous studies.^{17,18}

This inconsistency in the modulatory effects of *H. pylori* preparations on histamine release may reflect variations between the strains of *H. pylori* isolated in two different countries (Italy^{17,18} and Northern Ireland). It is known that there is considerable genetic variation between different isolates of *H. pylori*.^{31,32} The modulatory effect of *H. pylori* was found previously to differ between strains and, in some strains, no significant potentiation of histamine release was obtained.¹⁷ However, in the present study inhibition of histamine liberation was obtained with each of 11 different clinical isolates of *H. pylori* (48–97% inhibition). Although, in the current study, unpurified target cell populations were used, it is unlikely that the different effect was due to problems associated with either the cells or test systems examined, as potentiation of histamine liberation from rat peritoneal mast cells was achieved after co-incubation with calcium ionophore A23187 and *Hae. influenzae*. This is in agreement with a previous study in which calcium ionophore A23187 enhanced histamine release from human basophils after stimulation with endotoxin from *Hae. influenzae*.¹³

The differences observed in the action of *H. pylori* preparations may also be due to the isolation procedures for the rat peritoneal mast cells. In the present study the cells were not purified, as marked functional alterations can occur after purification by density gradient methods.³³ In contrast, in the previously reported work, cell preparations purified by density gradient centrifugation and elutriation were used.^{17,18}

Variation in the modulatory potential of *H. pylori* may reflect differences employed in the *in vitro* growth conditions of the bacterium. *H. pylori* was harvested from solid culture media in the present study, whereas previous investigations were performed with broth cultures.^{17,18} *H. pylori* strains cultured on a solid medium synthesise Paf-acether, whereas the same isolates cultured in brucella broth do not.³⁴ Therefore, it would be of interest to compare directly the ability of *H. pylori* isolates grown under different conditions to modulate mediator release.

Investigations of the interactions of *H. pylori* with the host cellular immune system have shown an increase in the expression of class II transplantation antigens and in the proportion of γ/δ T cells in *H. pylori*-infected compared to non-infected mucosa.^{35,36} Stimulation of mononuclear cells,³⁷ cytokine production (IL-2, IL-4, IL-6, TNF α) and soluble CD8 and IL-2 receptor molecule production have been demonstrated *in vitro*.^{38,39}

In contrast, *H. pylori* has been found to exert a

down-regulating or inhibitory effect on cells involved in the mucosal defences. This includes a reduction in the in-vitro proliferative response of monocytes to stimuli, without altering cell viability.⁴⁰ T lymphocyte proliferation is also inhibited and this is associated with a reduction of IL-2 receptor expression.⁴¹ This, together with the current findings that *H. pylori* inhibits histamine release from mast cells and basophils, may support the theory that the persistence of *H. pylori* infection is related to modulation of the host immune system by this bacterium. Differences in the ability of various *H. pylori* strains to influence the host immune response may influence the outcome of infection. Furthermore, the environmental conditions within the host tissues and the immune status of

the host may influence the expression of virulence determinants by the infecting bacterium. Hence the outcome of infection (chronic gastritis or duodenal ulceration) may reflect a complex series of interactions between the host and the infecting bacterium.

In the present study, *H. pylori* has been shown to inhibit histamine release from rat peritoneal mast cells and human basophils. Further studies are necessary to determine the role of this bacterium in the modulation of histamine release from human mucosal mast cells.

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