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MUCOSAL CHEMOKINES IN *HELICOBACTER PYLORI* INFECTION

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Chemokines are a family of low-molecular-weight proinflammatory cytokines that have leukocyte chemotactic and activating properties. Chemokine protein and mRNA are increased in the gastric mucosa of *Helicobacter pylori* infection and they are considered to regulate migration of leukocyte populations. The increase of C-X-C chemokines (e.g. IL-8, GRO-α) which effect primarily neutrophils is significantly associated with gastric polymorphonuclear cell activity suggesting that these chemokines play a primary role in active gastritis induced by *H. pylori* infection. *In vitro* enhanced epithelial chemokine responses are induced by *cagA* positive strains which have been clinically associated with more severe clinical outcome. Infection with *cagA*-positive *H. pylori* strains associates *in vivo* specifically with a C-X-C profile and enhanced polymorphonuclear infiltration in the gastric mucosa. Whilst infection with *H. pylori*, especially *cag* positive strains, is associated with more severe disease, genetic variability in host chemokine responses may also contribute to disease outcome.

K e y w o r d s: *Helicobacter pylori, chemokine, gastritis, cagA.*

INTRODUCTION

Since the discovery of *Helicobacter pylori,* many studies have implicated infection with this bacterium in the pathogenesis of chronic gastritis. Histologically, the host response to *H. pylori* infection is characterised by infiltration of plasma cells, lymphocytes, neutrophils and monocytes into the gastric mucosa. Several studies have shown *H. pylori* infection is associated with increased gastric mRNA expression of cytokines and chemokines (1—3). Increased gastric cytokine and chemokine protein concentrations have also been documented (4—7). Chemokines are now considered to play a significant role in the nature of the inflammatory host response to *H. pylori* infection.

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Chemokines are a recently described family of low-molecular-weight proinflammatory cytokines that have leukocyte chemotactic and activating properties (8—10). Chemokines have a relatively high degree of specificity, interleukin (IL)-8 and monocyte chemoattractant protein (MCP)-1 being specific for neutrophils and monocytes respectively. Chemokines are considered to be important in the recruitment and activation of specific leukocytes in a number of diseases (10). Recent interest has been focused on the relevance and importance of members of the chemokine family in many pathological conditions. IL-8, which is the best studied chemokine to date, and other chemokines have been implicated in several gastrointestinal bacterial infections (11, 12), including *H. pylori* infection in the gastric mucosa (1—4).

The chemokine family

Chemokines exhibit a high degree of structural similarities and have two pairs of conserved cysteine residues (8—10). The chemokine superfamily has been divided into two major subgroups: the C-X-C sub-family which has an intervening non-conserved amino acid residue between the first two cysteines and the C-C chemokines which lack an intervening amino acid at this position. These two sub-families differ in their biological properties and chromosomal locations. Members of C-X-C chemokines (e.g. IL-8, GRO-α) located on chromosome 4 have specific chemotactic activity for neutrophils but not monocytes. On the other hand, members of C-C chemokines (e.g. RANTES, MCP-1) located on chromosome 17 attract monocytes and lymphocytes but not neutrophils. Recent studies have identified a new chemokine, lymphotactin, which lacks the first and third cysteine residues suggesting that the superfamily may have an additional branch (13). This peptide, located on chromosome 1, is categorised as a member of the C chemokine branch. Lymphotactin is chemoattractant for T cells but not for monocytes or neutrophils (13).

C-X-C chemokines can be divided into two groups which may illuminate chemokine function. The majority of C-X-C chemokines contain the N-terminal sequence Glu-Leu-Arg (ELR) (14, 15). This ELR motif is common to chemokines which attract neutrophils and have the capacity to bind the C-X-C receptor activating neutrophils, but is absent in three C-X-C chemokines which do not seem to bind the C-X-C receptor and are devoid of such activities (15). Thus, the chemokine superfamily has four different structural and functional sub-families, E-L-R-C-X-C sub-family, C-X-C without E-L-R sub-family, C-C sub-family and C sub-family (14). (*Table 1*).

Table 1. The chemokine family

C-X-C chemokines
E-L-R
Interleukin-8 (IL-8)
Epithelial neutrophil-activating protein-78 (ENA-78)
Melanoma growth-stimulating factor (MGSA)/growth-related oncogene α, β and γ (GRO-α, β and γ)
Stromal cell-derived factors-1α, β (SDF-1α/β)
Platelet basic protein (PBP)
β-thromboglobulin (β-TG)
Neutrophil activating protein-II (NAP-2)
Connective tissue-activating protein-III (CTAP-II)
non E-L-R
Monokine induced by interferon-γ (mig)
Interferon-γ-induced protein (IP-10)
Platelet factor-4 (PF-4)
C-C chemokines
Regulated on activation normal T-cell-expressed and-secreted (RANTES)
Macrophage inflammatory protein-1α, β (MIP-1α, β)
Monocyte chemotactic protein-1, 2 and 3 (MCP-1, 2 and 3)
T-cell activation gene 3 (TCA-3/I309)
Eotaxin
C-chemokine
Lymphotactin

The ability of chemokines to attract various leukocyte populations is one of their most extensively studied functions. C-X-C chemokiness attract and most of them also activate neutrophils (10). Macrophage inflammatory protein (MIP)-1α is the only C-C chemokine with some stimulatory effect on neutrophils but it induces no functional chemotactic effect (16). Some C-X-C chemokines are also chemotactic to basophils and eosinophils but C-C chemokines are much more active. RANTES is known to be the most effective basophil and eosinophil chemoattractant (17, 18). Monocytes are also highly responsive to C-C chemokines. Many C-C chemokines such as RANTES, MCP-1 and MIP-1α attract monocytes whereas IL-8 is not chemotactic to monocytes (10). Human T lymphocytes also respond to the C-C chemokines RANTES, MIP-1α and MIP-1β (19, 20). Recently, some C-X-C chemokines IL-8 and IP-10 have been described as chemotactic for T cells (21, 22). A specific role in migration of intestinal intraepithelial lymphocytes has been reported (22). IL-8 and GRO-α are chemotactic for $CD8^+CD45RO^+$ intraepithelial lymphocytes (22) which have a promiscuous IL-8 receptor (23). In general, however, the C-X-C chemokines seem to affect primarily neutrophils, the C-C chemokines have functional action on basophils,

eosinophils, monocytes and lymphocytes, and the C chemokine acts on lymphocytes. These selective chemoattractant activities of chemokines play a major role in regulating leukocyte populations migrating into tissues.

Chemokines in gastroduodenal mucosa in H. pylori infection

The prevalence and role of chemokines in many gastrointestinal inflammatory conditions has been the subject of recent interest (1—4, 11, 12, 24, 25). However few studies have investigated expression of chemokines, other than IL-8, in the human gastrointestinal mucosa and gastric epithelial cells lines. Early studies showed high concentration of IL-8 in culture supernatants of *H. pylori* positive antral biopsy specimens and an association between mucosal IL-8 secretion and polymorphonuclear activity (4, 26). This association was also described and confirmed by investigation of IL-8 protein concentrations in gastric biopsy homogenates (3) and IL-8 mRNA expression in gastric biopsies (2).

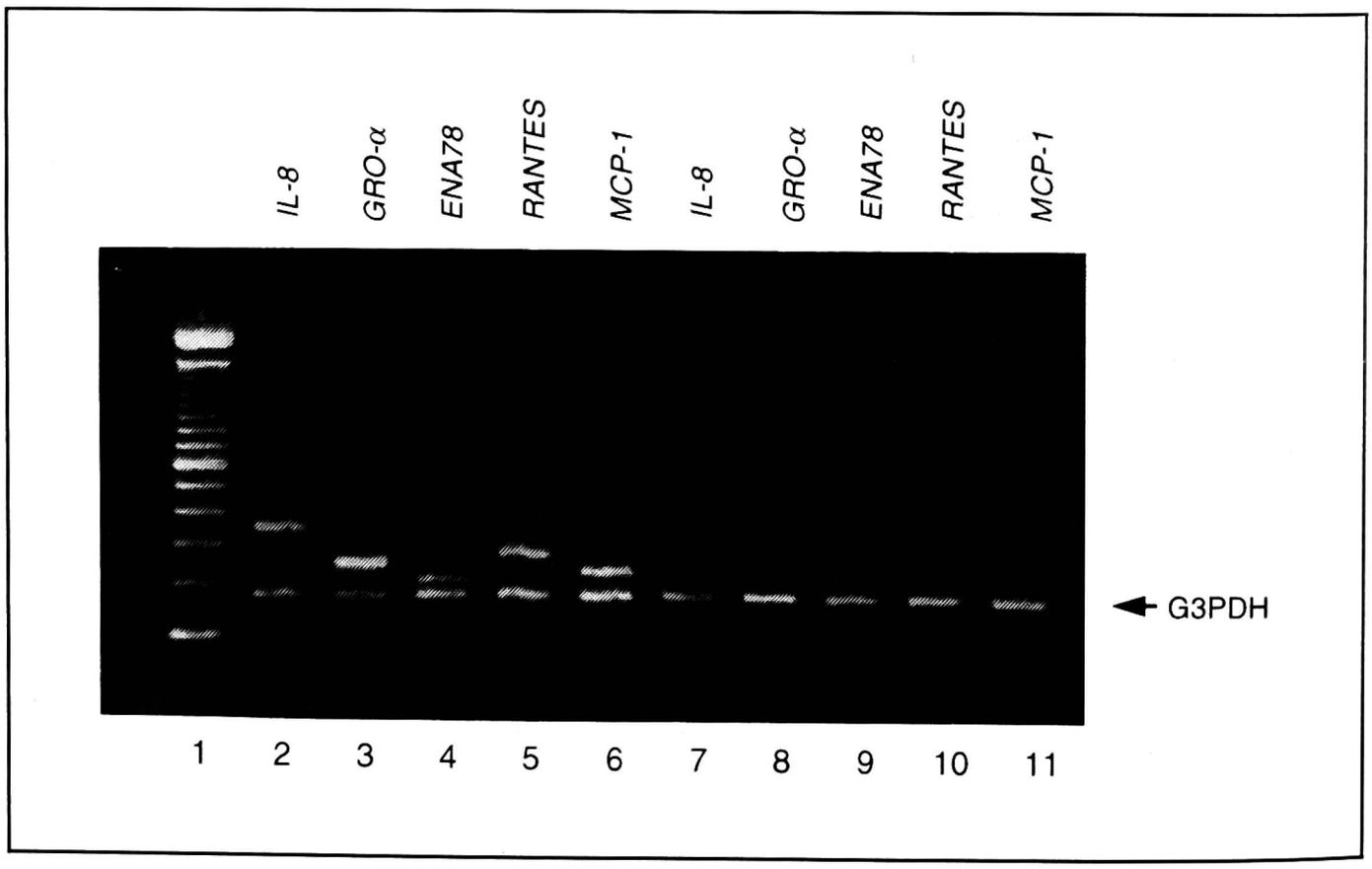

Fig. 1. Expression of IL-8, GRO-α, ENA-78, RANTES, MCP-1 mRNA in gastric antral mucosa. Gastric biopsy specimens from a *H. pylori* positive patient (lane 2—6) and a negative patient (lane 7—11) analysed for expression of IL-8, GRO-α, ENA-78, RANTES, MCP-1 and G3PDH (control) mRNA by RT-PCR. Lane 1 shows 100 bp DNA ladder

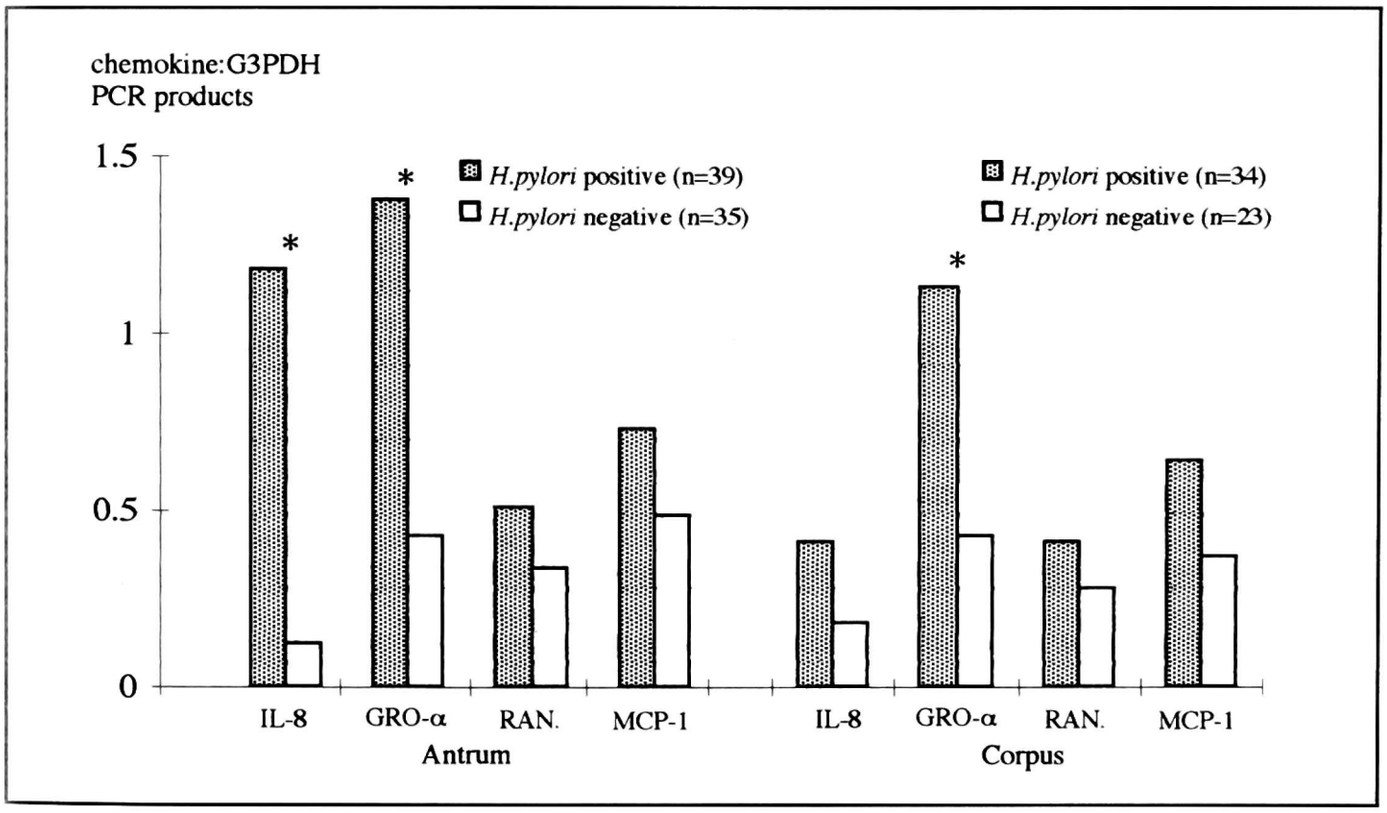

Fig. 2. Chemokine mRNA expression in *H. pylori* positive and negative gastric mucosa. The results are the mean ratio of chemokines to G3PDH RT-PCR product in gastric antral and corpus biopsies: * $p < 0.01$, difference is significant between *H. pylori* positive and negative mucosa. RAN = RANTES.

Recent studies show mRNA expression of other members of the chemokine family is increased in the gastric mucosa with *H. pylori* infection (*Fig. 1*). Semi-quantitative RT-PCR of chemokine mRNA expression in gastric biopsies shows that IL-8 and GRO-α mRNA expression is significantly higher in *H. pylori* positive patients than in *H. pylori* negative patients (*Fig. 2*). The expression of IL-8 and GRO-α mRNA is also significantly associated with polymorphonuclear activity suggesting that *in vivo* C-X-C chemokines primary play a role in active gastritis induced by *H. pylori* infection (27). The gastric epithelium is recognised as a source of chemokines and *H. pylori* infection is associated with enhanced IL-8 immunoreactivity in epithelial cells (28). In patients with duodenitis, increased immunoreactivity of IL-8 is also observed in the epithelium of the duodenal bulb mucosa (28). Many pathogens, both bacterial (11, 12, 29, 30) and viral (31), associated with enteritis stimulate intestinal cells to produce chemokines. Recent studies have focused on the ability of *H. pylori* to induce epithelial chemokines.

Gastric epithelial chemokine responses to H. pylori

In vitro studies show that *H. pylori* stimulates gastric epithelial cell lines to produce IL-8 (32—25) and GRO-α (36) which are known to be increased in gastric mucosa with *H. pylori* infection (2—4, 6). Proinflammatory cytokines

such as IL-1 and TNFα, which are known to be increased in the gastric mucosa in *H. pylori* infection (3, 5, 6) also stimulate chemokine secretion in gastric epithelial cell lines (37). Induction of IL-8 involves protein tyrosine phosphorylation (38) and NFκB activation (39, 40).

Considerable recent interest has focused on strain diversity of *H. pylori*. Many studies have shown that the infection with cytotoxin associate gene (*cag*)*A*-positive strains is highly associated with peptic ulcer disease (41), atrophic gastritis (42, 43) and gastric cancer (44, 45) and that infection of *cagA*-positive strains results in a more intense gastritis (2, 3, 41, 46). CagA-positive strain are associated with increased production of gastric IL-8 mRNA expression (2) and IL-8 protein *in vivo* (3). Our recent studies using semiquantitative RT-PCR show that patients infected with *cagA*-positive strains have higher C-X-C chemokine (IL-8 and GRO-α) mRNA expression than patients with *cagA*-negative infection whereas no differences were found in C-C chemokines (MCP-1 and RANTES) (unpublished observation). Thus infection of *cagA*-positive strains associates *in vivo* specifically with a C-X-C profile and enhanced polymorphonuclear infiltration in gastric mucosa.

Recent studies show that *cagA* is part of a 40 kb pathogenicity island (*cag* PAI) which contains over 30 genes (47, 48). *In vitro* studies have shown that the ability of *H. pylori* to induce chemokines in gastric epithelial cell lines varies. The response is restricted to strains with the CagA phenotype (32—34). Isogenic mutant strains have been used to assess the importance of gene products in the *cag* PAI in inducing epithelial chemokines. Deletion of *cagA* has no effect on IL-8 (34, 39) but deletion of multiple other genes in the 40 kb *cag* PAI abolishes the ability to stimulate IL-8 (47, 50, 51). These studies show that the CagA is a phenotypic marker for virulent strains and the epithelial chemokine responses is dependent on multiple genes in the *cag* PAI.

CONCLUSIONS

Both studies *in vitro* and *in vivo* show strain variation in mucosal inflammatory chemokine responses to *H. pylori*. Enhanced chemokine responses are induced by *cag* positive strains which have been clinically associated with more severe clinical outcome. Whilst infection with *cag* positive strains is associated with more severe disease, genetic variability in host chemokine responses may also contribute to disease outcome.

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