Helicobacter pylori and gastric inflammation

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H. pylori infection leads to gastric inflammation, characterised histologically by surface epithelial degeneration and infiltration of the gastric mucosa by acute and chronic inflammatory cells. H. pylori adherence, the production of a vacuolating cytotoxin and bacterial enzymes all contribute to epithelial damage. Recruitment and activation of immune cells in the underlying mucosa involves H. pylori chemotaxins, epithelial-derived chemotactic peptides (chemokines) such as IL-8 and GRO-α, and pro-inflammatory cytokines liberated by mononuclear phagocytes (TNFα, IL-1 and IL-6) as part of non-specific immunity. Antigen-specific cellular immunity results in a predominant Th1 lymphocyte response with an increase in IFN-γ secreting T-helper cells, whilst humoral responses lead to the production of anti-H. pylori antibodies and complement activation. The complex network of cytokines implicated in these inflammatory responses include counter-regulatory elements such as IL-10 which may serve to damp down inflammation. Molecular mimicry of host structures by H. pylori, with the generation of specific immunity directed against self-antigens may also contribute to host injury. Progress in molecular biology has revealed considerable genomic diversity amongst H. pylori strains, with cag+ bacteria being associated with increased chemokine and cytokine responses and more severe degrees of gastric inflammation. Strain heterogeneity may contribute towards the wide spectrum of disease manifestations encountered in clinical practice.

Gastric inflammation is an invariable finding in patients infected with H. pylori and represents the host immune response to the organism1. Histologically, H. pylori-associated chronic gastritis is characterised by surface epithelial degeneration, infiltration of the mucosa by chronic inflammatory cells (lymphocytes, plasma cells, and occasional eosinophils), and a characteristic but variable ‘active’ component consisting of neutrophils2. Qualitative or quantitative differences in H. pylori-induced gastric mucosal inflammation may play a pivotal role in determining the varied clinical outcomes of infection. This review focuses on the interactions between the organism and host cells which lead to mucosal inflammation.
Mechanisms of inflammation in *H. pylori* infection

When considering the mechanisms of *H. pylori*-induced mucosal inflammation, it is useful to consider the potential routes by which the organism may interact with host cells to initiate both direct cell injury and an immune response. *H. pylori* colonises the human gastric epithelium, living within the mucus layer in close proximity with the epithelial surface (to which it may adhere), but without invading the mucosa. There are two main mechanisms by which *H. pylori* (or its products) may produce gastric inflammation. Firstly, the organism may interact with surface epithelial cells, producing either direct cell damage or the liberation of epithelial-derived pro-inflammatory mediators (chemokines). Secondly, *H. pylori*-derived products may gain access to the underlying mucosa, thereby directly stimulating host non-specific and specific immune responses involving the liberation of a variety of cytokine messengers (Fig. 1, Table 1).

**Direct mucosal injury**

There are a variety of ways in which *H. pylori* may directly damage the surface epithelial layer, thereby contributing to changes in mucosal
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permeability and enhanced antigen exposure. Adherence of the organism to gastric epithelial cells is well recognised and is accompanied by loss of microvilli, irregularity of the luminal border, and intracellular changes including loss of cytoplasm, oedema and vacuolation. Surface epithelial degeneration correlates with the numbers of H. pylori in close contact with the epithelial plasma membrane, a finding that supports a direct toxic effect of bacterial products on epithelial cells.

Approximately 50% of H. pylori strains produce a heat-labile, protease-sensitive vacuolating cytotoxin which induces vacuole formation in cultured epithelial cells. Infection with cytotoxic strains has been positively associated with peptic ulceration and more severe degrees of gastric inflammation have been linked with infection with strains possessing the vacA gene signal sequence type s1a. Purified VacA has been shown to induce gastric ulceration and epithelial erosions in mice.

H. pylori expresses a variety of enzymes, some of which may have a role in mucosal injury thereby contributing to changes in mucosal permeability and facilitating the generation of specific immune responses. The most notable is urease, which by producing ammonia not only protects the organism from gastric acid but may also have toxic effects on the epithelium. Bacterial phospholipases may degrade the phospholipid components of the gastric mucosal barrier, and H. pylori alcohol dehydrogenase-mediated acetaldehyde production may also have toxic effects.

Epithelial chemokine responses

Peptides belonging to the chemokine family are involved in the recruitment and activation of specific immune cells (Fig. 1a). Different

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<thead>
<tr>
<th>Mediator</th>
<th>Usual actions</th>
<th>Reference(s)</th>
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<tr>
<td><strong>Cytokines</strong></td>
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<tr>
<td>TNFα</td>
<td>Pro-inflammatory (activation of leukocytes)</td>
<td>37,38,59,60</td>
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<tr>
<td>IL-1α/β</td>
<td>Pro-inflammatory (activation of leukocytes)</td>
<td>38,61,71</td>
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<td>IL-6</td>
<td>Pro-inflammatory, B- and T-cell activation/differentiation</td>
<td>38,61,71</td>
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<td>IL-7</td>
<td>T- and B-cell regulation</td>
<td>60,61</td>
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<td>IL-10</td>
<td>Immune down-regulation</td>
<td>59-61</td>
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<tr>
<td>IL-12</td>
<td>Stimulation of Th1 response</td>
<td>48</td>
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<tr>
<td>IFN-γ</td>
<td>Pro-inflammatory, especially cellular immunity</td>
<td>48,52</td>
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<td>GM-CSF</td>
<td>Pro-inflammatory, maturation factor</td>
<td>82</td>
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<td><strong>Chemokines</strong></td>
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<tr>
<td>IL-8</td>
<td>Neutrophil recruitment and activation</td>
<td>14-24,38,60,61</td>
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<td>GRO-α</td>
<td>Neutrophil recruitment and activation</td>
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<td>RANTES</td>
<td>Mononuclear cell recruitment and activation</td>
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<td>MIP-1α</td>
<td>Mononuclear cell recruitment and activation</td>
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Chemokines show marked target cell specificity with members of the C–X–C sub-family (e.g. IL-8, GRO-α) having specific chemotactic activity for neutrophils and members of the C–C family (e.g. RANTES, MIP-1α) having effects on monocytes and lymphocytes. A variety of studies have shown that the gastric epithelium is an important source of chemokines, which are released both in response to H. pylori and on exposure to endogenous pro-inflammatory mediators. Bacterial induction of epithelial chemokines involves a protein tyrosine pathway and NF-κB activation.

In vitro studies have shown that H. pylori induces gastric epithelial cell lines to increase both gene transcription and secretion of IL-8, which has neutrophil activating and chemo-attractant properties. Immunohistochemistry has confirmed gastric epithelial IL-8 immunoreactivity in H. pylori infection, and epithelial production of IL-8 may contribute towards the increased secretion observed from cultured whole antral biopsies. Recently, transcription of other members of the C–X–C chemokine family, as well as those with specificity for monocytes and lymphocytes (RANTES, MIP-1α) has been reported in H. pylori infected mucosa. The epithelial chemokine response may be particularly important in the early stages of H. pylori-induced inflammation, with the epithelium acting as a crucial first line of defense against microbial infection.

Non-specific immunity

The primary host defence mechanism to microbial attack usually involves a polymorph response. Although acute H. pylori infection in man has rarely been documented, limited histopathological studies suggest that there is indeed an initial strong neutrophilic response. Persistence of an active neutrophil component in chronic infection implies an important ongoing role for these cells in H. pylori-induced inflammation. In addition to the epithelial chemotactic cytokine response, diffusion of various bacterial products directly into the mucosa may result in neutrophil recruitment and activation (Fig. 1b).

In vitro, H. pylori is a potent source of factors capable of inducing neutrophil chemotaxis and activation. H. pylori water extract can induce adhesion and emigration of leukocytes from rat mesenteric venules, and in vitro exposure of human polymorphs to components of H. pylori leads to changes in adhesion molecule expression, chemotaxis, degranulation and the generation of reactive oxygen metabolites. A variety of bacterial products are implicated, including a 150 kDa H. pylori neutrophil activating protein (HP-NAP). Mononuclear phagocytes play a central role in early immune responses to bacteria, serving as an important source of pro-inflammatory cytokines.
mediators and as antigen-presenting cells involved in the initiation of specific immunity. H. pylori-derived soluble proteins (including a lipopolysaccharide-free extract) will activate peripheral blood monocytes in vitro, leading to increased expression of HLA-DR and interleukin-2 receptors, production of the inflammatory cytokines interleukin-1 and tumour necrosis factor-α (peptide and mRNA), and secretion of superoxide anion. Recent studies suggest the LPS-independent cytokine production may be mediated by the urease enzyme. In vitro culture of endoscopic antral biopsies has shown increased secretion of tumour necrosis factor-α, interleukin-6 and interleukin-1β in H. pylori infected subjects, all three peptides being predominantly macrophage-derived cytokines with a wide range of pro-inflammatory actions involved in leukocyte recruitment and activation.

As well as cytokines, the cells of the natural immune system are involved in the secretion of bio-active lipids which further contribute to recruitment and activation of phagocytic cells. Leukotriene B₄ (LT-B₄) induces neutrophil adhesion, chemotaxis, and degranulation in vitro, and is found in increased amounts in the mucosa of H. pylori infected subjects, particularly where there is active gastritis. Data are at present conflicting as to whether mucosal prostaglandin levels are altered in H. pylori infection.

Specific immunity

The presence of T-lymphocytes and plasma cells in the inflammatory infiltrate in H. pylori chronic gastritis suggests antigen-specific cellular and humoral immune mechanisms are important (Fig. 1c). The finding of increased numbers of CD45RO⁺ (antigen-committed) memory-type and CD25 positive T cells in the mucosa further supports this view. H. pylori infection results in the development of organised gastric lymphoid follicles which gradually decline in number after eradication of the organism, suggesting that specific H. pylori antigenic stimulation is required. Localisation of accessory cells (macrophages and follicular dendrites cells) in this acquired gastric lymphoid tissue resembles patterns in lymphoid tissue elsewhere in the gastrointestinal tract suggesting these cells facilitate antigen delivery from the gastric lumen. However, the mechanism of initial antigen uptake from the gastric luminal surface in vivo is unclear, but could involve passive absorption of soluble products, direct epithelial endocytosis of shed bacterial antigens or the passage of antigen through disrupted epithelial tight junctions.

CD4⁺ ('helper') T cells play a pivotal role in antigen-specific immune responses and infection is associated with an increase in gastric CD4⁺ T cells. H. pylori specific CD4⁺ lymphocyte clones have been obtained
from peripheral blood and gastric biopsies. T-helper cells are classified into 2 main types, depending on characteristic cytokine profiles (originally described in mice). Th1 lymphocytes secrete IL-2 and interferon-γ and mediate cellular immune responses, whereas Th2 cells produce IL-4, IL-5 and IL-10 which help in B-cell activation and antibody production including mucosal IgA responses. Most antigens probably induce a mixture of responses but, over time, some chronic infections are characterised by a response that is either Th1 or Th2 in nature. Cytokines produced early in infection are likely to polarise the specific T cell response. H. pylori has been reported to induce IFN-γ production by peripheral lymphocytes, increase IFN-γ producing cells (but not IL-4) in the gastric mucosa, and increase mucosal mRNA expression of IFN-γ and IL-12 which all strongly suggest a Th1 response predominates. The majority of H. pylori reactive T-cell clones generated from antral biopsies of infected patients exhibit a Th1 cytokine profile on stimulation by H. pylori antigens. This Th1 predominant lymphocyte response, favouring cell-mediated immunity, may not promote the elimination of H. pylori (as the bacterium is non-invasive), and may lead to cytotoxic damage of the epithelium.

Although a Th1 response predominates in H. pylori infection, there is little doubt that humoral immunity also plays a role. Gastric H. pylori specific T cell clones express antigen-dependent helper function for B cell proliferation and immunoglobulin secretion. H. pylori-specific immunoglobulins A and M (IgA and IgM) antibodies are detectable by enzyme-linked immunoassay in gastric juice in about one-third of subjects with H. pylori gastritis, and short-term biopsy cultures have subsequently confirmed local production of H. pylori specific IgG and IgA. Coating of the bacteria lining the epithelium by antibodies and activated complement components has been demonstrated using immunohistochemistry. Hence, the plasma cell infiltrate seen in H. pylori gastritis appears to represent a local humoral response to the organism. Mucosal IgA may serve to inhibit antigen uptake, block bacterial adherence or neutralise toxins. IgA does not activate complement efficiently, though recent work suggests that complement activation may be important in H. pylori gastritis perhaps mediated through mucosal immunoglobulins of the IgG class.

**Anti-inflammatory activity**

Whilst a wide range of pro-inflammatory mediators have been implicated in H. pylori-induced inflammation, much less is known about the natural antagonists of these cytokines. The balance between pro-inflammatory and immunosuppressive cytokines is likely to be a
critical determinant of the severity of H. pylori-associated inflammation. One explanation for the persistence of chronic H. pylori infection (despite apparent activation of cellular and humoral immunity) is immune down-regulation. In vitro studies have suggested that H. pylori may possess some immunosuppressive actions. H. pylori extracts have been shown to inhibit mitogen-induced proliferation of peripheral blood mononuclear cells and a whole inactivated H. pylori preparation induced lower proliferative responses in blood mononuclear cells from H. pylori-infected subjects than from uninfected patients. Increased levels of IL-10 (both secreted protein and mRNA) have recently been reported in H. pylori-infected mucosa, with levels of IL-10 increasing with the severity of gastritis. IL-10 is a potent inhibitor of both phagocyte and lymphocyte responses and is produced by a variety of human cells, notably mononuclear phagocytes.

IgA antibodies specific for the cytokine IL-8 are produced by cultured antral biopsies in infected patients, and IgA:IL-8 immune complexes have been reported in gastric juice, though it is presently unclear whether such anti-cytokine antibodies have immunoregulatory functions in H. pylori infection.

Strain variation in the inflammatory response

Although H. pylori infection appears to be invariably associated with long-term chronic gastritis, the severity of mucosal inflammation induced by the organism and the occurrence of clinically significant gastroduodenal lesions is highly variable, with only a minority of infected subjects developing serious disease. Differences in the pathogenicity of infecting H. pylori strains may be one mechanism involved in this diversity of clinical outcome.

Molecular analysis has allowed the identification of two groups of H. pylori based on the presence or absence of the cagA gene (cytotoxin-associated gene A), which encodes an immunodominant 120–140 kDa protein, and associated genes in the cag pathogenicity island (cagPAI). Systemic and mucosal humoral recognition of the CagA protein has been linked to peptic ulceration in some studies, and mucosal IgA recognition of CagA has been associated with the degree of mucosal neutrophil infiltration (‘activity’) and the extent of surface epithelial degeneration. Studies of the relationship between systemic humoral recognition of CagA and gastritis activity have not consistently found an association, though this may reflect differences between mucosal and systemic humoral responses or differences in ethnicity of study populations. Some microbiological studies of bacterial cagA
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expression have provided further evidence that duodenal ulcer patients are more frequently infected with cagA-positive strains \(^{69,71}\), whilst other studies have not \(^{72}\). However, mixed infections with cag\(^+\) and cag\(^-\) strains \(^{73}\) may explain discordant results, particularly as early studies generally relied on single, rather than multiple, bacterial isolates from a given patient \(^{70}\).

Molecular analysis of gastric mRNA has produced evidence that infection with cag\(^+\) strains is associated with increased transcription of IL-8 \(^{60}\), IL-1\(\alpha\) and IL-1\(\beta\) \(^{71}\) compared with those with cag\(^-\) infection. The mechanism of increased inflammation in cag\(^+\) bacterial infection thus may reflect strain-specific mucosal cytokine responses. There is evidence that IL-8, a potent neutrophil chemokine, may be particularly important. A number of in vitro studies using gastric epithelial cell lines have shown that the epithelial IL-8 response is observed specifically with strains of the CagA phenotype \(^{14,15,17}\). The CagA protein per se is not the direct inducer of epithelial chemokine responses \(^{16,17}\), but mutational studies have shown several genes in the cagPAI are essential for induction of epithelial chemokines \(^{65,74,75}\).

### Autoimmunity

Molecular mimicry of host structures by bacteria can lead to the generation of specific immunity directed against self-antigens and subsequent immunologically mediated host injury. Sequence homology between a number of H. pylori polypeptides and those of the host have been demonstrated, including gastric H\(^+\)/K\(^+\)-ATPase \(^{76}\). The finding that monoclonal antibodies directed against H. pylori could recognise an epitope on the gastric epithelium of mice and humans, and that administration of these antibodies produced gastritis in mice, led to speculation about a possible role for autoimmunity in H. pylori-associated mucosal inflammation \(^{77}\). Autoreactive IgG antibodies present in patient’s sera will bind to gastric corpus mucosa, and pre-absorption of these sera with H. pylori cells abolishes the cross reaction \(^{78}\). Recent interest has focused on the finding that H. pylori lipopolysaccharide expresses Lewis x (Le\(^x\)) and Lewis y (Le\(^y\)) human blood group antigens \(^{79,80}\) (which are found on various host cells, including human gastric mucosa). Interestingly, Le\(^x\) and Le\(^y\) expression is more frequently observed in cag\(^+\) strains \(^{81}\). Humans infected with H. pylori produce anti-Le\(^x\) antibodies \(^{80}\), and immunohistochemistry has shown that H. pylori induced anti-Lewis monoclonal antibodies react strongly with human gastric mucosa \(^{80}\). The question remains as to whether autoreactive antibodies in H. pylori infection are induced by the presence of H. pylori antigens, or whether the immune response is actually directed against
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the host cells themselves which have been rendered immunogenic by mucosal damage\textsuperscript{76}. The resolution of gastritis after bacterial eradication suggests autoimmune responses, whether against LPS determinants or other cross-reacting antigens such as heat shock proteins\textsuperscript{76} have no long term effect on mucosal integrity.

**Conclusion**

The immune response to *H. pylori* is co-ordinated by a complex network of inflammatory mediators, including chemokines and pro-inflammatory and immunosuppressive peptides, which are derived from the gastric epithelium and underlying mucosal immune cells. Strain-specific variations in the magnitude and characteristics of these cytokine responses are an important factor in determining the degree of chronic gastric inflammation, and may contribute to the broad spectrum of clinical manifestations observed.

**Acknowledgements**

The work of JEC is supported by the Yorkshire Cancer Research Campaign, European Commission (contract number IC 18CT95002A), The British Digestive Foundation, and Northern & Yorkshire Regional Health Authority.

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