

Review

Naturally acquired human immune responses against *Helicobacter pylori* and implications for vaccine development

Preface

Helicobacter pylori has been identified as a causative agent of gastroduodenal pathology. Vaccination studies with mouse models have shown that immunisation with bacterial antigens can provide protection against infection, indicating that it may be possible to design vaccines which terminate colonisation by *H pylori* or prevent it from taking place. Here, we review critically current knowledge of naturally acquired human humoral and cellular immune responses to *H pylori* with the aim of delineating questions which should be tackled in order to permit a rational and directed approach to the development of an effective vaccine. We have also reviewed the literature and identified candidate vaccine antigens.

Introduction

H pylori is a Gram negative flagellated bacterium which lives both in the mucus gel layer that coats the gastric mucosa and between the mucus gel layer and the apical surfaces of gastric mucosal epithelial cells. Although it seems to be largely extracellular, some invasion of gastric cells has been reported.¹ In Western countries, *H pylori* infection increases from a low prevalence in childhood to about 20% of people below 40 years of age,² with a steep rise in infection rates to 50% at age 60, attributed to increased infection during the Second World War. In underdeveloped countries, acquisition of infection occurs in 10% of children per annum so that 90% are infected by their teenage years.

There is a strong association between gastric *H pylori* infection and gastroduodenal disease.³ The presence of the bacterium invariably causes the surrounding mucosa to become inflamed,³⁻⁵ and the degree of gastritis present is positively correlated with the extent of *H pylori* colonisation.³⁻⁶ Although most people infected with *H pylori* remain asymptomatic, in some the gastritis progresses to more severe forms of gastroduodenal pathology. Atrophy, characterised by distortion and destruction of the glands, can develop. Furthermore, *H pylori* induced epithelial cell degeneration can result in ulceration⁴ and *H pylori* is implicated in 92 and 70% of all duodenal and gastric ulcers, respectively.² In addition, the presence of *H pylori* confers a sixfold increased risk of gastric adenocarcinoma and accounts for half of all gastric cancers.² *H pylori* is also strongly implicated in the development of gastric B cell mucosa associated lymphoid tissue (MALT) lymphomas, as shown by their absolute concurrence with *H pylori* colonisation,^{2,7} and by tumour regression upon eradication of *H pylori*.^{3,8} The development of peptic ulcers, atrophy and MALT lymphomas seems to occur independently, and is mutually exclusive in the case of atrophy and ulcers. However, atrophic gastritis may progress to gastric adenocarcinoma.⁹

The number of neutrophils and eosinophils, normally never found in lamina propria of the stomach, and lymphocytes and plasma cells, which are usually sparse, increases profoundly on infection.^{5,10} The density of mononuclear cells, but not neutrophils, in infected antral gastric mucosa correlates with the density of *H pylori*

colonisation.¹¹ The presence of lymphoid follicles in *H pylori* induced gastritis further shows that a strong lymphocytic response is elicited by *H pylori*. These follicles are absent in the healthy stomach and in other gastritides^{12,13} and consist of B cells surrounded by clusters of mostly CD4+ T cells.^{14,15} The degree of the lymphoid follicle hyperplasia correlates with the density of bacterial colonisation.^{13,16} Gastric B cell MALT lymphomas most likely originate from such follicles.¹⁷

Despite the vigorousness of this immune response, however, the infection in most cases is not eradicated. As *H pylori* actively secretes chemotactic products and effectively stimulates pro-inflammatory responses,¹⁸⁻²³ it may be that the bacterium benefits somehow from the tissue damage caused by the immune response.^{24,25} *H pylori* may have evolved so as to generate a balance between pro- and anti-inflammatory immune responses, inducing and sustaining a steady state of gastritis which is asymptomatic in the majority of hosts. In a small percentage of the population, however, this balance is not achieved, owing perhaps to genetic variation in both the bacterium and host and/or environmental factors, and thus pathology ensues as a result of the excessive activity of the macrophage, neutrophil and eosinophil effectors of mucosal damage.¹⁸⁻²³

Specific antibody responses may be critical for protective immunity against extracellular bacteria like *H pylori*, and T cells are well known for their capacity to induce and modulate the functions of effector cells (e.g., B cells, macrophages, neutrophils, and eosinophils) important in the control or eradication of extracellular bacteria. An effective vaccine against *H pylori* should thus aim to induce a specific, non-pathogenic humoral and T cell response. This article reviews critically current knowledge of naturally acquired human immunity to *H pylori*. Attention will be focused on areas of controversy or uncertainty which should be resolved to allow delineation of the appropriate responses to be induced by vaccination. The antigen specificity of the humoral response is also assessed and candidate vaccine antigens are identified.

Helicobacter pylori specific B cell responses

H PYLORI INDUCES LOCAL AND SYSTEMIC ANTIBODY RESPONSES

H pylori specific IgG, IgM and IgA antibodies are present at high titres in inflamed gastric mucosae of infected patients^{6,26,27} and antibody coated bacteria can be seen in virtually all biopsy samples of gastritis associated with infection.⁶ The degree of opsonisation correlates with the frequency of local plasma cells.^{28,29} Specific serum IgG is also produced, and is the most sensitive marker of infection (>90% sensitivity and specificity).³⁰ A drop in titre is a reli-

Abbreviations used in this review: APC, antigen presenting cell; CTL, cytotoxic T cell; Hsp, heat shock protein; IFN, interferon; IEL, intraepithelial lymphocytes; IL, interleukin; LFA, leucocyte function associated antigen; LPL, lamina propria lymphocytes; MALT, mucosa associated lymphoid tissue; NUD, non-ulcer dyspepsia; PBMC, peripheral blood mononuclear cells; TCR, T cell receptor; TGF, transforming growth factor.

able indicator of successful eradication.^{31–32} *H pylori* specific IgG and IgA responses are not, however, skewed towards a particular isotype.³³ A single report also found significant levels of *H pylori* specific IgE in serum samples and on basophils of infected, but not uninfected, patients with chronic gastritis.³⁴

SPECIFIC ANTIBODIES MAY LIMIT BACTERIAL GROWTH AND REDUCE BACTERIAL TOXICITY

Oral administration of antibodies directed against *H pylori* eradicates infection in animal models³⁵ and limited evidence exists suggesting that IgA antibodies in particular may contribute to controlling the bacterial population in humans. Infants of mothers with high titres of anti-*H pylori* IgA have a significant delay in acquisition of infections.³⁶ In addition, in a study of asymptomatic subjects, the density of *H pylori*, disease activity score, and rate of peptic ulceration were all significantly increased in subjects with low specific serological IgA titres compared with those with high titres.³⁷ Furthermore, although IgA deficient individuals are no more susceptible to *H pylori* infection,³⁸ they have a notably increased risk of developing gastric malignancies,³⁹ which may be a consequence of *H pylori* overgrowth unchecked by IgA. Opsonisation by IgG and IgM also promotes in vitro phagocytosis and killing by neutrophils,⁴⁰ and antibodies may modulate bacterial pathogenicity by neutralising essential virulence factors.⁴¹

SPECIFIC ANTIBODIES MAY CONTRIBUTE TO PATHOGENICITY

More evidence exists suggesting that *H pylori* induced antibody responses might be pathological. Specific mucosal IgG in the presence of soluble bacterial antigen promoted neutrophilic cytotoxicity to cultured Vero cells.⁴² Moreover, serum samples from most infected patients specifically crossreact with gastric mucosa but not with other tissues.⁴³ Mice immunised with *H pylori*, but not other bacteria, also developed such antibodies.⁴³ In addition, mice bearing a hybridoma secreting a *H pylori* specific monoclonal antibody shown to be crossreactive with both human and murine gastric mucosa developed stomach pathology, whereas mice bearing non-crossreactive hybridomas did not.⁴⁴ Autoantibody levels correlated strongly with histological inflammatory scores.

H pylori derived lipopolysaccharide, which contains Lewis antigens also occurring on human gastric epithelium, may induce some autoreactive antibodies.⁴⁵ Gastric inflammatory scores and associated atrophy correlated significantly with the expression of Lewis antigens on autologous *H pylori* strains.⁴⁴ Furthermore, the bacteria were significantly more likely to express the same type of Lewis antigen as their host,⁴⁶ suggesting that bacteria with alike Lewis phenotypes are selected in vivo. *H pylori* specific autoantibodies may also be directed against protein epitopes expressed on gastric parietal cells and intrinsic factor.^{47–48} Such autoantibodies are also typically found in human autoimmune gastritis.⁴⁹

SPECIFICITIES OF THE HUMORAL IMMUNE RESPONSE

Table 1 summarises studies investigating the specificity of local and systemic humoral responses to *H pylori* antigens by immunoblot, ELISA or ELISPOT.^{26–50–63} Most immunoblot studies did not identify the subject antigens so these are grouped according to molecular weight and possible identity. Donors are grouped according to disease status: gastroscopy (unspecified complications), peptic ulcer, non-ulcer dyspepsia (NUD), gastric carcinoma, and asymptomatic. The term dyspepsia refers to discomfort in the upper abdominal area which may be caused by various pathologies. All groups are subdivided according to *H pylori* carriage. With the exception of two studies^{54–57} which

did not distinguish between isotypes, all studies looking at systemic responses focused on IgG, whereas both IgA and IgG responses in gastric mucosae were examined.

A single study examining gastric mucosal humoral responses by ELISPOT found *H pylori* specific B cells in infected but not in uninfected individuals.⁵⁵ Serum and mucosal antibodies from infected subjects, regardless of disease status, also always exhibited more frequent responses to *H pylori* antigens. ELISA and ELISPOT studies revealed very low reactivity in uninfected subjects, but, notably, immunoblot studies often showed considerable responsiveness in these people—for example, to p46/47 and heat shock protein (Hsp) 60. Protein denaturation during immunoblotting may reveal cryptic epitopes recognised by crossreactive antibodies.

Several antigens seem to be reproducibly immunodominant in infected people, including three unidentified proteins of approximately 19, 25 and 35 kDa, urease A and B subunits, flagellin, Hsp60, flagellar sheath protein, and CagA. These proteins or a combination thereof are thus interesting candidate antigens for vaccines in humans, particularly those which can induce protective immunity in animals, namely urease,^{64–70} Hsp60⁷¹ and CagA.^{69–72}

One study, not included in table 1 because frequencies of antigen recognition were not assessed, used two dimensional immunoblotting followed by N-terminal sequencing of spots recognised by pooled IgG of infected endoscopy patients but not by uninfected donors.⁷³ This study identified over 30 *H pylori* proteins specifically recognised by infected patients, demonstrating the power of this technology. Most of those proteins were expressed by all eight strains analysed. In another study, although substantial antigenic diversity in *H pylori* isolates was found, the 25, 31, 56, 60 and 84 kDa proteins were highly conserved.⁷⁴

Several reports have attempted to associate recognition of *H pylori* proteins with disease. One protein, CagA, is strongly implicated in ulcer disease as it is recognised more frequently in patients with ulcers than in those with NUD.⁵² In addition, 75% of patients with peptic ulcer, NUD or gastric carcinoma, but only 25% of asymptomatic infected subjects, recognised Hsp60.⁵⁷ In contrast, lower serum responses to p33, p59 and p66 by *H pylori* positive patients with gastric carcinoma have been found.⁵⁶ These data implicate particular antigenic *H pylori* phenotypes in disease. However, whether the antibody responses to the pathology associated antigens contribute to the pathology itself is not known. With regard to CagA, it is now understood that CagA+ strains induce more interleukin (IL) 8 than CagA– ones (reviewed by Crabtree and Farmery⁷⁵), and thus CagA– specific antibodies are more likely to simply reflect the presence of CagA+ bacteria. Moreover, immune responses to bacterial Hsps could be protective rather than pathogenic.⁷⁶

T cell responses to *H pylori*

EPITHELIAL CELLS AND PROFESSIONAL ANTIGEN PRESENTING CELLS MAY ACTIVATE *H PYLORI* SPECIFIC T CELLS

The conditions in the inflamed stomach support the activation of T cells as the molecules required for T cell activation are increased on epithelial cells, monocytes, and dendritic cells. Although gastric epithelia do not normally express HLA-DR,⁷⁷ chronic gastritis in general is associated with a rise in epithelial HLA-DR expression, with significantly higher expression in infected than in uninfected gastric mucosae.^{5–78–79} The HLA-DR positive areas occur in close proximity to mononuclear cell infiltrates.⁸⁰ Human gastric epithelial cell lines and freshly isolated epithelial cells from gastric biopsy samples also constitutively express both B7–1 and B7–2 and can provide T cell costimulation in vitro.⁸¹ B7–2 is upregulated by stimulation with *H pylori*,

Table 1 Recognition of *Helicobacter pylori* antigens by different patient groups and asymptomatic donors

Antibody isotype/method/location	MW (kDa)	Identity	Per cent specific recognition by subjects ^a								Reference			
			Gastroscopy		Peptic ulcer		NUD		Gastric carcinoma			Asymptomatic		
			Hp+	Hp-	Hp+	Hp-	Hp+	Hp-	Hp+	Hp-		Hp+	Hp-	
IgG/ELISA/S	13	HspA	40	0										50
IgG/IB/S	14	NI	97	49										51
IgG/IB/S	14	NI	37*	12										52
IgG/IB/S	15	NI	85 ^b	60										53
IgG/IB/S	16	NI	49*	2										52
IgG/IB/S	19	NI	59	0										51
IgG/IB/S	19	NI	64***	5										53
All/IB/S	19	NI	100	0										54
IgG/IB/S	25	NI	54	0										51
IgG/IB/S	25	NI	45**	5										53
All/IB/S	25	NI	100	0										54
IgA/ESP/L	26	NI			A:33						A:67	A:0		55
IgA/ESP/L	26	NI			C:0						C:50	C:0		55
IgG/ESP/L	26	NI			A:0						A:17	A:0		55
IgG/ESP/L	26	NI			C:0						C:17	C:0		55
IgG/IB/S	27	NI	67***	5										53
IgG/IB/S	28	NI							66 ^b	45	65 ^b	37		56
IgG/IB/S	30	NI	82***	15										53
IgG/IB/S	30	NI	63*	30										52
IgG/IB/S	30	U/A	91	0										51
IgA/ESP/L	30+67	U			A:64						A:91	A:0		55
IgA/ESP/L	30+67	U			C:67						C:78	C:0		55
IgG/ESP/L	30+67	U			A:22						A:64	A:0		55
IgG/ESP/L	30+67	U			C:56						C:56	C:0		55
IgG/IB/S	67	U/B	90	0										51
IgA/IB/L	31	NI			80 ^b		75							26
IgG/IB/S	33	NI							61 ^b	54	100†	37		56
IgG/IB/S	35	NI	63*	11	100‡		48							52
IgG/IB/S	36	NI	76***	5										53
IgG/IB/S	42	NI	59*	7										52
IgG/IB/S	43	NI							33 ^b	16	39 ^b	12		56
IgG/IB/S	46	NI	54*	14										52
IgG/IB/S	47	NI	82*	45										53
IgG/IB/S	47	NI							68 ^b	45	78 ^b	62		56
IgG/IB/S	48	NI	34*	7										52
IgG/IB/S	50	NI	82***	30										53
IgA/ESP/L	51	Fl			A:91						A:91	A:0		55
IgA/ESP/L	51	Fl			C:78						C:100	C:0		55
IgG/ESP/L	51	Fl			A:44						A:45	A:0		55
IgG/ESP/L	51	Fl			C:0						C:33	C:0		55
IgG/IB/S	51	Fl	78	14										51
IgG/IB/S	54	NI	83*	18										52
IgG/IB/S	56	NI	85*	50										53
IgG/ELISA/S	58	HspB	76	0										50
IgG/IB/S	59	NI							73*	45	100†	50		56
IgG/IB/S	60	NI	94**	60										53
All/IB/S	60	Hsp60			75 ^c		75 ^c		75 ^c		25	0		57
IgA/ELISA/S	60	Hsp60	High ^{b,d}	Low										58
IgG/ELISA/S	60	Hsp60	High****d	Low										58
IgA/IB/L	61	NI			96 ^b		98							26
IgG/IB/S	62	NI							38 ^b	41	65§	25		56
IgG/IB/S	66	FSP	94	16										51
IgG/IB/S	66	NI	46*	16										52
IgG/IB/S	66	NI							47 ^b	29	73†	25		56
IgG/IB/S	74	NI	34*	11										52
IgG/IB/S	75	NI	75	0										51
IgG/IB/S	75	NI	85**	40										53
IgG/IB/S	85	NI							68*	29	61	37		56
IgG/IB/S	87	NI	61*	19	83‡		48							52
IgA/ELISA/S	87	VacA	High****d	Low										58
IgG/ELISA/S	87	VacA	High****d	Low										58
IgA/IB/L	90	NI			88 ^b		80							26
IgG/IB/S	90	NI	71	0										51
IgG/IB/S	120	NI	97¶	35										53
IgG/IB/S	120	NI							55 ^b	33	69 ^b	50		56
IgA/IB/L	120	CagA			100††		63							26
IgG/IB/S	120	CagA	86	0										51
IgG/IB/S	125	NI	71*	19	83‡		62							52
IgG/IB/S	128	CagA			100††† (DU)		60				61	6		59
IgG/ELISA/S	25	CagA fr			83 ^b (GU)									60
IgG/ELISA/S	25	CagA fr			100 ^c ;‡‡		75 ^c							61
IgG/ELISA/S	25	CagA fr			59 ^b						44			62
IgG/ELISA/S	25	CagA fr					66	0						61
IgG/IB/S	150	NI	85 ^b	70					78	8	54	8		53
IgG/IB/S	180	NI	68	0										51

^aWhere unnoted, no comparative statistics were performed. ^bNot significantly different from corresponding *H pylori* negative (Hp-) subjects. ^c*H pylori* carrier status not determined. ^dPer cent responders not specified.

*p<0.05, **p<0.01, ***p<0.001 v corresponding Hp- subjects.

†p<0.05 v uninfected healthy individuals and infected cancer patients; ‡p<0.05 v patients with non-ulcer dyspepsia (NUD); §p<0.05 v *H pylori* positive (Hp+) patients with cancer, but no different from Hp- asymptomatic subjects; ¶p<0.0001 v Hp- gastroscopy patients; ††p<0.001 v infected patients with NUD; †††p<0.0001 v infected patients with NUD and asymptomatic individuals; ‡‡p<0.0005 v patients with NUD.

A, antrum; C corpus; DU, duodenal ulcer; ELISA, enzyme linked immunosorbent assay; ESP, ELISPOT; Fl, flagellin; fr, recombinant fragment; GU, gastric ulcer; IB, immunoblot; L, local; MW, molecular weight; NI, not identified; S, systemic; U, urease; U/A, urease A; U/B, urease B.

and is also expressed at higher levels in infection in vivo. Gastric epithelial cells may be able to present bacterial antigens to T cells as during infection their endocytic-endosomal system has been shown to contain *H pylori* antigens and expression of the antigen processing enzyme cathepsin E is higher.⁵ Thus, gastric epithelial cells may be important antigen presenting cells (APCs) for CD4+ T cells in *H pylori* infection. With regard to more conventional APCs, monocyte numbers are increased in the lamina propria in *H pylori* infection⁵ and cathepsin E+ macrophages are concentrated in the lamina propria immediately underlying the *H pylori* colonised epithelium. HLA-DR is also upregulated on macrophages.⁵ In addition, gastric mucosal dendritic cell numbers are increased in infection relative to normal and uninfected dyspeptic gastric mucosae.⁵

Secreted products from *H pylori* such as urease induce expression of HLA-DR and B7-2 on monocytes and gastric epithelial cells,^{18 81 82} but interferon (IFN) γ also has this effect.^{81 82} Either T or natural killer cells can produce IFN- γ . Although natural killer cell numbers did not seem to be increased in *H pylori* infection in one report,⁸³ another did show an increase.⁸⁴

T CELLS IN *H PYLORI* ASSOCIATED INFLAMMATORY INFILTRATES HAVE A DISTINCTIVE PHENOTYPE AND ARE ACTIVATED

A number of studies have characterised the T cell component of the inflammatory infiltrate by immunohistochemical staining of gastric biopsy samples. The biopsy sample donors were always patients undergoing endoscopy for upper gastrointestinal complaints. As a consequence of this random recruitment, the donors tended to be heterogeneous in their degree of gastritis and clinical picture. Comparisons of *H pylori* positive gastric mucosae were made with histologically normal, uninfected and/or gastritic but uninfected mucosae. Sometimes these control groups were combined.

When compared with normal gastric mucosae, *H pylori* positive samples had increased numbers of predominantly CD3+CD4+ and also some CD8+ T cells in the lamina propria in all^{78 79 83 85} but one study, which found more CD8+ than CD4+ T cells.⁸⁴ With regard to intraepithelial lymphocytes (IELs), although two of three reports suggested that they did not seem to increase in number,^{78 85 86} an increased CD4:CD8 ratio was nevertheless also observed.^{78 83 85 86} In the lamina propria, the increase in CD4+, but not CD8+, T cells correlated with increasing grades of gastritis, activity and density of bacterial colonisation ($r = 0.87, 0.92$ and 0.80 , respectively).⁸⁶ This increase in the CD4:CD8 ratio did not occur in the gastric mucosal lamina propria of uninfected patients with dyspepsia of unspecified aetiology.⁷⁸ To make the point that the gastric mucosa is capable of distinct immune responses, the chronic gastritides associated with pernicious anaemia and coeliac disease are characterised by distributions and phenotypes of T cells different from those in *H pylori* positive gastric mucosae. Coeliac disease is characterised by a profound infiltration into the gastric epithelium of CD8+ cells with cytolytic potential.⁸³ In contrast, gastric mucosae of patients with pernicious anaemia are characterised by infiltrates of B cells and there is no alteration in the CD4:CD8 ratios,^{87 88} consistent with the concept that gastric mucosal damage in pernicious anaemia is mediated primarily by humoral mechanisms.

The T cells infiltrating into both the lamina propria and the epithelium of infected gastric mucosae seemed to be mainly T cell receptor (TCR) $\alpha\beta$ +.⁸⁶ Two reports suggested that there was no absolute increase in TCR- $\gamma\delta$ + T cells in either compartment,^{86 89} whereas a third found a

significant increase in numbers relative to normal gastric mucosae in both compartments.⁷⁸ This was also found in uninfected patients with gastritis, indicating it is not specific to *H pylori* infection. TCR- $\gamma\delta$ + T cells may therefore not play a significant role in *H pylori* induced gastritis.

In infection, T cells from the lamina propria, but not the epithelium,⁹⁰ were CD45RO+ and IL-2R+^{78 85} and thus have an activated/memory phenotype. Other activation markers are suggestive of some activation of IELs as there was a small but significant increase in cells expressing CD69 (an early T cell activation marker) as well as a significant decrease in leucocyte function associated antigen (LFA) 1 positive cells in *H pylori* positive gastric mucosae relative to both normal and uninfected dyspeptic gastric mucosae.⁹⁰ A reduction in LFA-1 expression has been previously reported upon activation of intraepithelial T cells.⁹¹

T CELLS RESPOND BY PROLIFERATION AND IFN- γ RELEASE TO *H PYLORI* ANTIGENS

Several studies have analysed in vitro T cell responses to *H pylori* antigens by infected and uninfected subjects.⁹²⁻¹⁰⁴ Nearly all studies recruited patients undergoing endoscopy for dyspepsia and divided them according to infection status. Occasionally healthy donors serologically positive for *H pylori* infection were grouped with the dyspeptic patients, and thus the degree of gastritis and the clinical picture of the infected and uninfected groups were heterogeneous.

Crude preparations of *H pylori* induced proliferation and secretion of IFN- γ and soluble IL-2R in vitro by both peripheral blood mononuclear cells (PBMC) and lamina propria lymphocytes (LPLs) of *H pylori* infected donors.⁹²⁻¹⁰¹ Whether IL-2 and IL-4 are secreted is not clear as in one study neither was induced⁹³ whereas both were induced in another.¹⁰⁰ Purified urease, Hsp60, and p25 also induce PBMC proliferation,^{98 102} and Hsp60 stimulates IL-10 but not IFN- γ release by PBMC from infected subjects.¹⁰² Proliferation in response to *H pylori* antigens, at least in PBMC, was due mainly to CD4+ T cells.⁹³ The response was not caused by *H pylori* superantigens or mitogens^{95 96 98} and multiple HLA-DR molecules seemed to be involved in T cell recognition.⁹⁴ The cloning efficiency of *H pylori* specific mucosal T cells compared with their precursor frequency in the blood suggests that these cells accumulate in gastric tissue.¹⁰³

Surprisingly, there seem to be very few differences in the quality of T cell responses to *H pylori* by uninfected compared with *H pylori* positive subjects in all criteria assessed so far.⁹²⁻¹⁰¹ Responsiveness to *H pylori* by uninfected donors resembles an analogous situation in malaria, where T cells from blood donors who have never lived in or visited malarious countries vigorously respond by proliferation and production of IFN- γ to malaria antigens.¹⁰⁵⁻¹⁰⁷ T cell clones reactive to malaria proteins were also able to respond to crude extracts of many common microorganisms,^{107 108} and it is thus possible that the uninfected donor response to *H pylori* reflects prior encounter with microorganisms with common T cell determinants. Unlike malaria, however, there is a paradoxical quantitative difference between *H pylori* infected and uninfected subjects in that PBMC and lamina propria cells from *H pylori* positive individuals showed a statistically significant reduced proliferative response and lower IFN- γ release.⁹²⁻¹⁰¹ The reduced response seemed to occur on challenge with the cytoplasmic fraction and urease but not the membrane fraction of *H pylori*.⁹⁶ Thus *H pylori* may induce immune responses which downregulate proliferation and IFN- γ secretion. Notably, the only difference between infected and uninfected donors in their in vitro T

cell responses to *H pylori* detected to date was a small but significant increase in the population of IL-2R+CD8+ cells in *H pylori* positive donor PBMC along with a significant increase in soluble CD8 release.⁹³ The CD8+ T cell response may reflect the induction of specific responses which do not occur in uninfected individuals and that could regulate IFN- γ secretion and proliferation by either regulatory cytokines or anti-idiotypic mechanisms.

As the studies reporting this phenomenon investigated dyspeptic patients only, it is not known whether the reduced immune response to infection also occurs in asymptomatic donors. The fact that there are pre-existing T cell responses to *H pylori* should not be ignored. It is possible that such responses could skew reactivity to the bacterium at the initial encounter in such a way that an adequate defence cannot be mounted, as has been postulated for malaria¹⁰⁵ and viruses.¹⁰⁹ Redirecting the immune response, possibly towards epitopes unique to *H pylori*, could be an important consideration in vaccine design.

Although *H pylori* infection clearly elicits a T cell response, there is no evidence that it is protective. This may, however, reflect the fact that infected but asymptomatic donors, or donors who may have had transient infections which they were apparently able to resolve, have never been examined. The assessment of mucosal T cell responses by such donors would be highly informative.

ROLE OF T CELLS IN GASTRODUODENAL PATHOLOGY AND THE TH1/TH2 PARADIGM

Much more evidence exists suggesting that T cells are involved in the induction of gastroduodenal pathology. Ulcerogenesis may be mediated by T cells, since, as estimated by FACS, patients with peptic ulcer have a significantly higher proportion of IL-2R+CD4+ gastric mucosal T cells than patients with NUD.¹¹⁰ Furthermore, the development of MALT lymphomas seems to be dependent on the presence of *H pylori* specific T cells, as shown by the fact that tumour cells from such lymphomas proliferate in the presence of non-neoplastic tumour infiltrating CD4+CD45RO+ T cells¹¹¹ and *H pylori*, but not of *H pylori* alone.^{112 113} The activation and proliferation of the neoplastic B cells in low grade lymphomas depends on CD40, which interacts with its counterpart CD40L on T cells to induce B cell maturation. The role of T cells in high grade lymphomas is less clear as tumour cell proliferation in these lymphomas is supported by CD40 binding in one report¹¹⁴ and independent of tumour infiltrating T cells in another.¹¹² A proportion (7–24%) of the tumour infiltrating T cells in low grade lymphomas express CD40L.¹¹¹ One study also showed that low grade tumour cell proliferation is assisted by IL-4 and IL-10, but not IFN- γ and IL-2, whereas high grade lymphomas are assisted by all four cytokines.¹¹⁴ Thus, T cells specific for *H pylori* and secreting IL-4 and IL-10 may be responsible for the initiation and perhaps progression of MALT lymphomas.

CD4+ T helper (Th) cells can be functionally polarised into either IFN- γ secretors (Th1) or IL-4, IL-5, IL-10, and IL-13 secretors (Th2),^{115 116} with T cells expressing cytokines of both patterns being termed Th0 T cells. Th1 and Th2 cells reciprocally inhibit each other, and there is widespread speculation that *H pylori* predominantly induces a Th1 response which is normally kept in check by moderate Th2 responses.¹⁰⁴ It is believed that disease occurs upon dysregulation of the inhibitory Th2 responses, causing overproduction of IFN- γ which results in increased activation of macrophages, neutrophils and eosinophils. These effector cells may then damage epithelial integrity by release of oxygen radicals, nitric oxide, proteolytic enzymes, and induction of apoptosis.^{10 18 117–120}

This hypothesis is important for vaccine development because it implies that a vaccine which upregulates Th2 responses may be protective. However, as we will show, the evidence supporting the hypothesis is weak or circumstantial and open to alternative interpretations. There are three lines of evidence: firstly, IFN- γ and IL-12 (Th1 cytokines), but not IL-4 (a classic Th2 cytokine) are thought to be present in the gastric mucosae of *H pylori* infected dyspeptic patients. Secondly, LPLs from *H pylori* positive patients do not bear CD30,⁸⁵ which is a putative marker for Th2 cells.¹¹⁵ Thirdly, the presence of ulcers has been reported to be associated with IFN- γ secreting CagA specific Th1 cells.^{103 104} Each of these points will be addressed in turn.

Th1 and Th2 cytokines in biopsy material

Table 2 summarises the studies investigating the presence of Th1 and Th2 cytokines in biopsy material.^{84 85 103 121–128} Cytokines tested only once are not shown. Comparisons of *H pylori* positive biopsy samples were usually made with normal gastric mucosae and occasionally also with gastric mucosae from uninfected dyspeptic patients. With the exception of two studies with defined patient groups,^{103 127} gastritic groups combined dyspeptic patients with a wide spectrum of gastritis severity and disease. A number of methodologies were used and more attention is focused here on studies quantifying cytokine protein production than on studies measuring cytokine mRNA.

The classic Th1 cytokines are IFN- γ and IL-12. Most biopsy specimens from *H pylori* positive patients had high numbers of IFN- γ + T cells^{85 122 126} and the frequencies of IFN- γ secreting T cells were significantly higher in infected than in normal gastric mucosae.^{124 127} However, samples from uninfected dyspeptic patients had many more IFN- γ secreting cells than the infected samples,¹²⁴ suggesting that the apparent increase in IFN- γ during infection is not pathologically significant. Supporting this is the observation that there was no difference in numbers of IFN- γ + cells in samples from infected peptic ulcer and asymptomatic donors.¹²⁷ The case for IL-12 is not clear because although infected samples were more frequently positive for IL-12 mRNA than those from normal and uninfected dyspeptic donors,^{103 122 125} a single report measuring IL-12 protein in biopsy material found similar amounts in both *H pylori* positive gastric mucosae and normal gastric mucosae.¹²²

Another marker of a Th1 phenotype is IL-2, an autocrine growth factor for T cells produced both by Th1 and Th2 cells but more abundantly by Th1 cells.¹¹⁵ Infected gastric mucosae had high numbers of IL-2+ LPL,⁸⁵ biopsy specimens secreted the cytokine in culture,⁸⁴ and half of the specimens were positive for IL-2 mRNA in one study.¹²¹ In the latter study, uninfected gastritic biopsy specimens were significantly less frequently positive for IL-2 mRNA than infected samples but examination by ELISA showed that both produced equivalent amounts of IL-2.⁸⁴ mRNA of another Th1 cytokine, IFN- α , which induces Th1 responses,¹²⁹ was present at equivalent frequencies in *H pylori* positive and uninfected gastritic mucosae and normal gastric mucosae (about 64%).¹²³

IL-4 is the classic Th2 cytokine. It is an autocrine Th2 growth factor¹¹⁵ which promotes Th2 cell development. Five studies found no or very low levels of IL-4 mRNA or IL-4+ cells in gastric mucosae of normal, infected, or uninfected dyspeptic donors.^{85 103 122 123 126} Immunohistochemical, ELISPOT and biopsy ELISA studies did detect IL-4 production but no significant quantitative differences between infected and normal or uninfected dyspeptic donors were found.^{84 124 127}

IL-10 is produced in higher quantities by Th2 cells and it suppresses Th1 cells.¹¹⁶ It is also produced by cells other

Table 2 Review of cytokine analysis of gastric mucosal biopsy specimens of *Helicobacter pylori* infected and uninfected gastritic and histologically normal patients

	Reference ^a													
	123	121	122	103	125	127	124	126	122	85	122	131	84	128
RT-PCR (% positive samples)						IHC (% positive samples (median MNC/mm ²))	ESP (median no spots)	FACS (median positive cells)			ELISA (median pg/ml)	ELISA (median values)		
	Whole biopsy				Biopsy MNC	Whole biopsy	LPL & IEL	CD4+ LPL & IEL	CD3+ LPL	Whole biopsy	Culture supernatant			
<i>IFN-γ</i>														
Hp+ IGM	83 ^b	12 ^b		100 (PU)	85 ^b	90% PU (5)**	145*	35	86	91				
Hp- IGM	100 ^b	13		0 (NUD)	75	90% AS (6)	371†							
NGM	83				71	10% (0.1)	20		64					
<i>IL-12</i>														
Hp+ IGM			100	100		78§§§					34 ^b			
Hp- IGM				0		55								
NGM			33			43					42			
<i>IL-2</i>														
Hp+ IGM	0	53‡‡								91			19 ^b ng/mg	
Hp- IGM	0	13											19.5 ng/mg	
NGM	0													
<i>IL-4</i>														
Hp+ IGM	0			0		90% PU (4.5) ^b	90 ^c	0	5	<2			8.4 ng/mg‡	
Hp- IGM	0			0		90% AS (3)							5.4 ng/mg	
NGM	0					80% (1)	40		5					
<i>IL-10</i>														
Hp+ IGM	22§	55¶	80			85††					0.2 ^b			411 pg/g/24 h‡‡
Hp- IGM	13 ^b	31				58								78
NGM	0		100			100					0.7			75
<i>IL-6</i>														
Hp+ IGM	17 ^b	25†††				90% PU (5)‡‡‡							106 pg/ml§§	16.2 ng/mg¶¶
Hp- IGM	0 ^b	0				100% AS (5)								8.25 mg/ng
NGM	0					50% (0.1)							25 pg/ml	
<i>TGF-β</i>														
Hp+ IGM		0				100% PU (9)‡‡‡								
Hp- IGM				0		100% AS (10)								
NGM				0		100% (3)								

*Significantly different from *H. pylori* negative (Hp-) inflamed gastric mucosae (IGM) (p<0.05) and normal gastric mucosae (NGM) (p<0.05). **Peptic ulcer (PU) and asymptomatic (AS) combined significantly different from NGM (p<0.01) but not each other. †Significantly different from NGM (p<0.01). ‡Significantly different from Hp- IGM (p=0.01). §Significantly different from uninfected IGM (p=0.06). ¶Trend towards significant difference from uninfected IGM (p=0.11). ††Trend towards significant difference from uninfected IGM (p=0.08). ‡‡Significantly different from NGM (p<0.01) and Hp- IGM (p<0.05). †††Significantly different from Hp- IGM (p=0.06). ‡‡‡PU and AS combined significantly different from NGM (p<0.05) but not from each other. §§Significantly different from NGM (p<0.001). ¶¶Significantly different from Hp- IGM (p<0.001). §§§Significantly different from NGM and Hp- IGM grouped together (p=0.054). ¶¶¶Where unnoted, no comparative statistics were performed. ^bNot significantly different from NGM and/or uninfected IGM. ^c*H. pylori* positive (Hp+) and Hp- IGM combined, and not significantly different from NGM.

IFN, interferon; TGF, transforming growth factor; RT-PCR, reverse transcription polymerase chain reaction; IEL, intraepithelial lymphocytes; IHC, immunohistochemistry; LPL, lamina propria lymphocytes; ESP, ELISPOT; ELISA, enzyme linked immunosorbent assay; MNC, mononuclear cells.

than T cells. Biopsy material from infected donors secreted significantly more IL-10 than that from normal or uninfected dyspeptic donors,¹²⁸ and a significant correlation between IL-10 secretion and the chronic inflammation score ($r = 0.4$) was found.¹²⁸ Notably, measurements of IL-10 mRNA repeatedly indicated that gastritic mucosae from uninfected samples were significantly less frequently positive than infected samples ($p=0.11$,¹²¹ 0.06,¹²³ 0.08,¹²⁵ and 0.05¹²⁸).

Like IL-10, IL-6 is produced in greater amounts by Th2 cells^{116, 130} and also by non-T cells. Biopsy samples from infected donors secreted significantly more IL-6 in culture than samples from either normal¹³¹ or uninfected dyspeptic donors.⁸⁴ Furthermore, *H. pylori* positive gastric mucosae had significantly more IL-6+ cells than normal samples.¹²⁷ In the latter study, however, no difference between infected asymptomatic and peptic ulcer donors was found, suggesting that IL-6 may not play a role in ulcerogenesis.

Transforming growth factor (TGF) β may polarise the T cell response towards a Th2 response.¹³² There were significantly more TGF-β+ cells in gastric mucosae from infected than from normal donors, although again there was no difference between infected peptic ulcer and asymptomatic donors.¹²⁷

Thus, relative to normal controls, IFN-γ, IL-2, IL-10, IL-6, and TGF-β seem to be significantly upregulated in *H. pylori* positive dyspeptic gastritis, whereas uninfected gastritic patients showed an increase in IFN-γ, IL-2 and

possibly IL-6 but not IL-10. There were quantitative differences between infected and uninfected gastritic mucosae in that less IFN-γ and more IL-10 and IL-6 were produced in infection.

With regard to the Th1/Th2 paradigm, although the upregulation of IFN-γ in infection supports the hypothesis, the crucial comparison between infected asymptomatic and peptic ulcer subjects indicates that the apparent increase in IFN-γ is not pathologically significant, at least for ulcerogenesis.¹²⁷ In support, an association between the frequency of IFN-γ expressing cells and the presence of ulcers was not found.¹²⁴ Furthermore, although IL-4 is not produced, there is evidence of Th2 activity in dyspepsia associated with infection. IL-10, a major regulatory Th2 cytokine, seems to be upregulated in infected gastric mucosae of dyspeptic patients, and its expression correlates with disease activity. Indeed, as uninfected dyspeptic patients produced IFN-γ but not IL-10 whereas infected subjects produced both, the presence of IL-10 (but not IFN-γ) may be specific to gastritis induced by *H. pylori* and suggests that IL-10 is not just produced in an attempt by the host's immune system to control IFN-γ production. The Th2 cytokines IL-6 and TGF-β are also upregulated in infected patients, although they did not correlate with disease parameters. IL-13, which mimics IL-4 in many functions, has not been investigated. There is also indirect evidence of Th2 activity in the gastric mucosa in that most infected dyspeptic patients have, in their plasma, *H. pylori*

specific IgE,³⁸ whose production is dependent on IL-4,¹¹⁵ and MALT lymphomas seem to be initiated by Th2 T cells¹¹⁴ (see earlier). Thus, the measurement of cytokines in biopsy material does not at this point provide evidence of a pathogenic IFN- γ dependent Th1 response.

Presence of CD30, a Th2 marker, on gastric T cells

The second line of evidence supporting the hypothesis is that CD30, a possible Th2 marker, does not occur on LPL from infected patients.⁸⁵ However, lamina propria cells from uninfected gastritis patients also did not express this molecule.⁸⁵ In addition, there is some controversy as to whether CD30 really does discriminate between Th1 and Th2 cells.¹³³

Th1 clones in infected gastric mucosa

Mucosal T cell clones have been generated from biopsy material from *H pylori* positive patients with peptic ulcer.^{94 103 104} The clones generated were all CD4+TCR- $\alpha\beta$ + and proliferated in response to crude extracts of *H pylori*, CagA, VacA, urease, or Hsp60.^{94 103} Analysis of the cytokine release by *H pylori* specific mucosal clones showed that most secreted IFN- γ but not IL-4 nor IL-5¹⁰³ whereas the remainder were of the Th0 phenotype. It was thus suggested that the T cell response to *H pylori* in patients with peptic ulcer predominantly follows a Th1 profile. It should be noted, however, that the clones were generated with IL-2, which is known particularly to support Th1 cell growth.¹³⁴ In a subsequent study, however, it was found that infected patients with peptic ulcers had significantly more Th1 clones than infected patients with NUD,¹⁰⁴ and that these recognised CagA more frequently, whereas most of the Th0 clones from both sets of patients responded to either VacA, urease or Hsp.^{103 104}

Although these studies provide the most direct evidence of the role of Th1 cells in *H pylori* induced pathogenesis, it should be noted that the Th1 clones were defined on the basis of a lack of IL-4 and IL-5 production, and other Th2 cytokines (e.g., IL-10, IL-13) were not examined. Furthermore, it was not indicated in the study whether all six patients with peptic ulcer had clones reacting to CagA, or whether the CagA specific clones were derived from just one or two patients. If the latter was the case, this would clearly make the study unrepresentative.

The remaining evidence for the hypothesis is that studies in animal models have shown that immunisation with *H pylori* antigens can provide Th2 mediated protection against *H pylori* colonisation.¹³⁵ Another study, however, showed that induction of a fine balance between both Th1 and Th2 responses was necessary for protection.¹³⁶ In any case, there are doubts that the mouse system adequately models the human situation,³⁵ which is probably one reflecting millennia of co-evolution. Thus, whether an imbalance in Th1/Th2 responses really is responsible for *H pylori* associated gastroduodenal pathology in humans requires more investigation. Indeed, the cytokine analyses of biopsy material question whether the inflammation induced by *H pylori* infection is Th1 type in the first place, and more importantly, it indicates that IFN- γ does not play a central role, at least in ulcerogenesis. The simplistic distinction between pathogenic IFN- γ producing Th1 and protective IL-4 producing Th2 cells may not be pertinent in this infection.

CYTOLYTIC T CELLS MAY CONTRIBUTE TO EPITHELIAL DESTRUCTION

Of the classic Th1/Th2 cytokines discussed earlier, the only one correlating with disease parameters was IL-10.¹²⁸ In addition, IL-7, whose mRNA was found more frequently in infected (83%) than in either uninfected dys-

peptic (33%) or normal gastric mucosae (38%) ($p < 0.05$), has been shown to correlate significantly with disease severity ($p < 0.05$).¹²³ IL-7 is a pleiotropic cytokine (not produced by T cells) which stimulates the proliferation and differentiation of mature CD4+ and CD8+ T cells. Its role in modulating Th1 and Th2 function is undetermined; in some systems IFN- γ release is enhanced by addition of IL-7,^{137 138} whereas in others it is reduced¹³⁹ or unchanged¹⁴⁰ with or without a concomitant change in IL-4 production.^{137 139 140} Its role in augmenting cytotoxic T cell (CTL) cytotoxicity is, however, quite well established,^{137 138 140 141} and it is possible that it may be responsible for the increased cytolytic character of the T cells found in *H pylori* infected stomachs as shown by a statistically significant increase in granzyme B+ T cells in both the lamina propria and epithelium of infected dyspeptic patients.⁸³ CTL lysis of the epithelium could thus contribute to the epithelial erosions associated with infection. This is supported by the observed increase in infection of gastric mucosal intraepithelial T cells in patients with¹⁴ compared with those without ulcers,^{78 81} indicating that the cytolytic cells may be in direct contact with epithelial cells.

Concluding comments

Increasing interest has been shown in the possibility of developing a vaccine against *H pylori*. Two types of vaccines are potentially possible: a prophylactic vaccine, which prevents new infections, and a therapeutic vaccine, which eliminates an existing infection. Current antibiotic eradication of *H pylori* infection is reliable but costly and there is the danger of increasing antibiotic resistance in bacterial strains.¹⁴² A cheap and potent therapeutic vaccine which eradicates infection and associated disease and simultaneously secures long lasting immunity is thus highly desirable.

Regarding prophylactic vaccines, which would allow global eradication of *H pylori*, some caution may need to be exercised. The vast majority of *H pylori* infected individuals never develop disease symptoms⁹ and colonisation with *H pylori* might even be beneficial for the human host¹⁴³ as recent studies have suggested that *H pylori* infection may protect against reflux oesophagitis¹⁴³⁻¹⁴⁵ and that colonisation by CagA positive strains may reduce the risk of developing cancer of the oesophagus and gastric cardia.¹⁴⁶ Thus, eradicating *H pylori* might reduce the risk of developing peptic ulcers, MALT lymphomas and cancers of the distal stomach while increasing the risk of developing reflux oesophagitis and cancers of the oesophagus and proximal stomach. These observations require thorough confirmation in large scale longitudinal studies.

This review shows that strong antibody and T cell responses are elicited by *H pylori*. That these responses apparently cannot, however, clear the infection probably reflects the plethora of immune escape mechanisms which the bacterium has evolved so as to thrive in its host. Understanding the nature of these mechanisms and also how a normally benign coexistence can result in pathology might lead to rational approaches to vaccine design. As most studies to date have examined infected donors with signs of *H pylori* associated pathology, attention has thus focused on how T cells and antibodies could induce this pathology. It may be argued that inducing immune responses which oppose pathological T and B cell activity should be a major goal of vaccination as such responses could modulate disease and possibly also result in the elimination of the bacterium. The popular hypothesis that a vaccine should induce Th2 immunity which suppresses pathological Th1 responses is in line with this goal. This review has shown, however, that the basis of this hypothesis

can be questioned and should be investigated more thoroughly. Furthermore, immune deviation is not the only T cell regulatory mechanism, and the possibility that—for example, a defect in the anti-idiotypic network contributes to pathology should be investigated. The role of cytotoxic CD8+ T cells in pathology, in particular ulcerogenesis, should also be assessed further.

It is possible that the different pathologies associated with *H. pylori* infection could be promoted by quite different mechanisms, and thus a vaccine opposing ulcerogenic immunity may be ineffective (or even pathogenic) in an immune context which leads to atrophy or MALT lymphoma. Other diseases not yet unequivocally associated with *H. pylori* infection, such as autoimmune gastritis,⁴⁹ may also be triggered by this bacterium by quite distinct mechanisms. Thus, it is essential that clearly defined patient groups are studied. In addition, infected but asymptomatic donors should be investigated to allow an understanding of the immune mechanisms permitting an apparently harmonious coexistence between host and bacterium.

We have also highlighted several candidate vaccine antigens, including urease, flagellin, flagellar sheath protein, Hsp60, CagA and the unidentified antigens p19, p25 and p35 which are recognised frequently by antibodies from infected subjects. Several antigens are recognised more frequently by subjects with *H. pylori* associated disease, namely CagA, p35 and p87/VacA. All of these antigens may be important candidate vaccine antigens.

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