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# *HELICOBACTER PYLORI* AND LONG TERM SURVIVAL IN THE GASTRIC MUCOSA: IS IMMUNOMODULATION THE KEY?

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## SUMMARY

The discovery of *Helicobacter pylori,* a bacterium that inhabits the mucus and epithelium of the stomach, revolutionised the discipline of gastroenterology and resulted in a paradigm shift in the management of gastroduodenal disease. *H. pylori* causes 95 % of duodenal ulcers, 60-70% of gastric ulcers, 60-70% of gastric adenocarcinomas and most if not all gastric B cell lymphomas. These diseases are relatively new human afflictions and it is suggested that in earlier times *H. pylori* actually evolved as a member of the normal microbial flora just as many other commensals evolved to inhabit the ecological niches provided by the mucus of the lower intestinal tract. Symptomatic disease is a consequence of recent environmental changes in the human host including possibly an increased acid output in the stomach. As with other normal flora, *H. pylori* inhabits its chosen niche, the gastric mucosa, for life and thus must have acquired sophisticated mechanisms to survive. These include an ability to withstand gastric acidity via the enzyme urease. The immune responses against the bacterium are evaded by a number of possible strategies including antigen mimicry via the manufacture of the blood group antigens Lewis x and y on the bacterial surface, antigen variation and a shedding of decoy bacterial antigens into the lamina propria. Immune responses are likely to be modulated in a number of ways including the production of a lipopolysaccharide of reduced biological activity. The Th lymphocyte profiles may also be modulated with infection directing the lymphocyte profile towards a predominantly inflammatory Th1 phenotype with the inflammation not only being unable to remove the mucosal coloniser but actually benefiting the bacterium. The move away from a Th2 response which would be more likely to result in removal of the *H. pylori* is a deliberate strategy. Understanding of these mechanisms of immunomodulation may have relevance beyond gastric disease. The mucus surfaces of most animals and probably some human populations are now known to be colonised with other *Helicobacter* species. The impact of these bacteria on lower bowel disease has yet to be determined.

## INTRODUCTION

In Perth in 1982, an event occurred which was to revolutionise the field of gastroenterology. Plates were removed from an incubator after a prolonged in-

cubation over the Easter long weekend (*Marshall* et al., 1984). These plates had been inoculated with material from gastric biopsies in an attempt to grow bacteria which had been observed by an histopathologist, Robin Warren, who was convinced they were associated with gastritis, inflammation of the gastric mucosa (*Warren*, 1983). The culture had been coordinated by a young medical registrar, Barry Marshall, who had teamed up with Warren as part of a short research project. The first Perth culture attempts incubated for shorter periods had failed, yet now the plates revealed small round colonies and so the bacterium we now know as *Helicobacter pylori* was born (*Goodwin* et al., 1989). Marshall and Warren then tried to convince the world that this bacterium was not only a cause of the gastritis but was indeed associated with peptic ulcer disease. Given the current wisdom was that ulcers were caused by acid, the suggestion that a bacterium could somehow be involved in ulcer formation was greeted with sceptiscm and frank disbelief by the community of gastroenterologists. While accepting the association, it was considered the bacterium was no more than an opportunistic coloniser attracted by the damaged gastric mucosa. However, over the years the evidence has accumulated to support the role of *Helicobacter pylori* in gastroduodenal disease and this has resulted in a paradigm shift in the management of these diseases. Anti *Helicobacter* therapy is now a mandatory component in the treatment of peptic ulcer disease (*Yamada* et al., 1994).

## *H. PYLORI*, A MAJOR GLOBAL PATHOGEN

Following ingestion of *Helicobacter pylori*, an acute infection most likely occurs in the gastric mucosa with infiltration of large numbers of polymorphonuclear leukocytes. In those where early infection has been monitored e.g. Marshall himself who courageously swallowed the organism in an attempt to fulfil Koch's postulates, Arthur Morris an intrepid New Zealander who did the same and a number of individuals accidentally infected at endoscopy, acute symptoms were observed such as nausea vomiting etc. (*Debongnie* and *Bouckaert*, 1993; *Marshall* et al., 1985; *Morris* et al., 1991) However in most infected persons, this early episode of symptoms is uninvestigated as the gastritis progresses to what was previously called type b gastritis. This is an asymptomatic inflammation of the gastric mucus associated with the infiltration of both polymorphs and mononuclear cells, an active/chronic gastritis (*Dixon* et al., 1996). Importantly for the thesis being generated in this article, most persons with *H. pylori* infection remain asymptomatic for life although if biopsied all would show gastritis. In one subset of infected individuals, the bacterium may move into the duodenal bulb where it infects small areas of gastric type metaplastic tissue, induces a duodenitis and causes duodenal ulcer as a result of excess acid coming in from the stomach. In others, the gastritis in the stomach proper induces damage such that the mucosa becomes susceptible to acid attack and a gastric ulcer results (*Graham*, 1996). The involvement of acid in peptic ulceration was proven when it was found that the ulcers completely healed if patients were given acid suppressive therapy such as the $H_2$ receptor antagonists or the proton pump inhibitors. However, after cessation of acid suppression the ulcers recurred in 80% of cases after one year. The major contribution of the underlying gastritis to ulceration was proven when it was

demonstrated that successful cure of *H. pylori* infection with antimicrobial drugs resulted in resolution of the inflammation and the ulcers did not come back, i.e. for the first time peptic ulcer disease could be cured (*Bell* et al., 1996). Thus the symptomatic *H. pylori*-associated diseases are essentially immunopathologies. While peptic ulcer disease results in significant morbidity and mortality, the major global impact of *H. pylori* infection comes from another consequence of long term inflammatory damage i.e. gastric malignancy. By a series of indirect and possibly direct effects on the gastric mucosa, the bacterium is responsible for at least 60-70% of gastric adenocarcinomas (*Goldstone* et al., 1996). This form of gastric cancer remains one of the world's major tumours, killing up to one million per year. *H. pylori* is also responsible for the majority of another gastric cancer, low and high grade B cell MALT lymphoma (*Wotherspoon*, 1996).

## *H. PYLORI* AS PREHISTORIC NORMAL FLORA OF THE GASTRIC MUCOSA

Our interest in microbial ecology began decades ago when one of us (AL) became fascinated with the bacteria that inhabit the mucus of the intestinal tract (*Lee*, 1985). This mucus provides a niche for a highly adapted group of spiral shaped bacteria in conventional mice and a range of other animal species. The caecal and colonic crypts are packed with these bacteria. We started to study *H. pylori* soon after its discovery because we reasoned it might be closely related to these lower bowel bacteria, as the also spiral/helical shaped organism occupied a similar ecological niche in the human stomach. Interestingly, many of these lower bowel bacteria have subsequently been shown to be *Helicobacter* species (*Lee* et al., 1992). Early on we described *H. pylori* as "almost normal flora", commenting that as with the commensals of lower bowel mucus the bacterium had evolved to inhabit gastric mucus. However, in this case the *H. pylori* always caused inflammation which we felt might be beneficial to the organism (*Hazell* et al., 1986). While not doubting the role of *H. pylori* as a gastric pathogen, we have more recently commented that the bacterium did indeed originate as a harmless commensal and it is only recently that it has become a pathogen (*Lee*, 1997). The *H. pylori*-associated symptomatic diseases, peptic ulcer disease and gastric cancer are relatively recent with ulcers only becoming common in the last two centuries. We have hypothesised that prior to 1700, gastric cancer was rare because people rarely lived to the age when it commonly occurs. Ulcer disease did not happen because acid output was lower than it is today due to co-infection with parasites which reduce acid production, nutritional and other unknown factors. Thus we claim that historically *H. pylori* is normal flora of the human body, and that it is only due to changes in environmental factors that it became able to cause symptomatic disease. From the large proportion of *H. pylori* infected persons in the world who are asymptomatic, we know that active/chronic gastritis of itself is not a debilitating condition. Ulcer disease and cancer are almost accidental consequences of infection. The bacterium certainly did not evolve to cause ulcer or malignancy as these conditions provide no advantage to the organism. Significantly nearly all other animal species examined have their own highly adapted gastric *Helicobacter* species which appear to colonise for life without

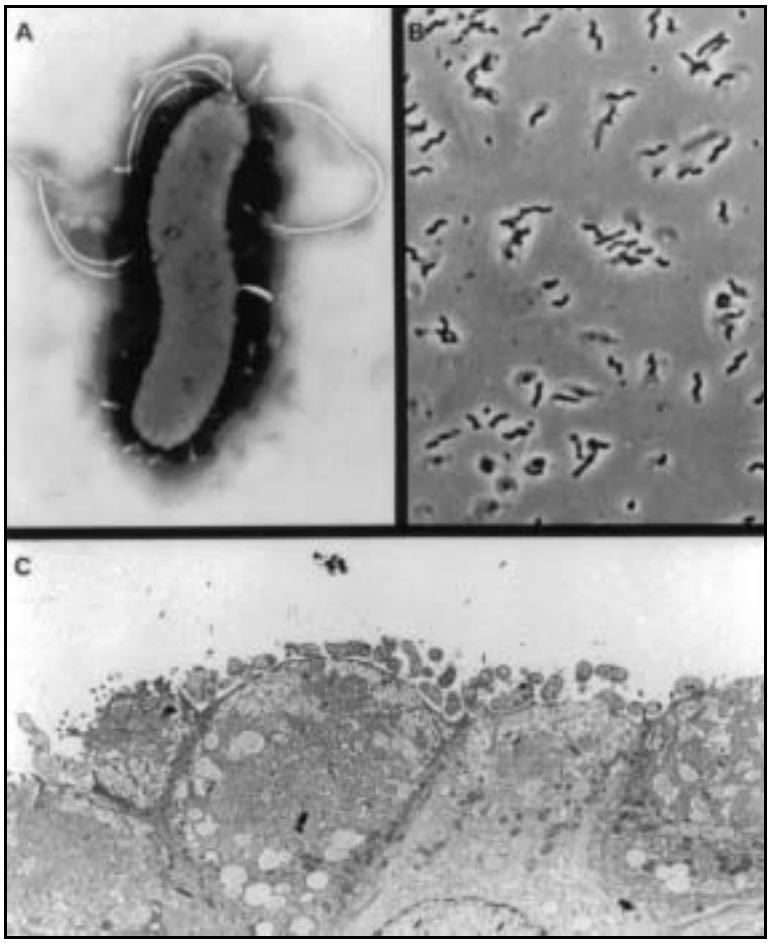


**Figure 1:**
**A** : Negative stain of *H. pylori* showing its spiral morphology and multiple polar flagella (x 18000).
**B** : Phase contrast view of a pure culture of *H. pylori* (x 1100).
**C** : Transmission electron micrograph showing H. pylori aligning along, and in some instances attaching directly to, the epithelial surface (x 3200).

significant consequence and which therefore fulfil the normal criteria for an autochthonous microflora (*Lee* and *O'Rourke*, 1993).

These concepts are particularly relevant to the topic of this symposium which aims to develop knowledge on the interactions between the immune mechanisms of the intestinal tract and the microorganisms of human and animal gut ecosystems. Understanding how this bacterium survives in the stomach despite a massive immune response mounted against it will provide information not only relevant to the colonising ability of other bowel flora but also might provide insights as to how other environmental factors might impact on the normal microbial flora of the lower bowel resulting in the appearance of symptomatic inflammatory conditions. Thus, the goal of this paper is to consider those factors that allow *H. pylori* to colonise the stomach whereas no other bacterium can. Possession of the enzyme urease is the characteristic

that allows it to survive gastric acidity via the breakdown of endogenous urea in the gastric mucosa thus providing ammonia to neutralise the local acid (*Lee* et al., 1996; *Meyer Rosberg* et al., 1996; *Mobley*, 1996).

Of more relevance here are the factors that permit long-term survival. *H. pylori* is acquired in early childhood and the infection remains for life (*Hazell* et al., 1994). This is despite an intense cellular response against the bacterium which manifests as the active/chronic gastritis which is also present lifelong and which sometimes increases in severity setting the scene for gastric cancer. A major humoral immune response also accompanies infection. Serology is an excellent predictor of active infection due to the high levels of IgG which are continually present (*Mendall*, 1997). The bacteria have been shown to be coated with IgA in the gastric mucosa (*Wyatt* and *Rathbone*, 1988). The working hypothesis of our group is that long term survival of *H. pylori* in the human gastric mucosa is due to immune evasion and immunomodulation. The aim of this chapter is to consider the strategies this highly evolved gastric bacterium has acquired over the millennia to evade and modulate the immune responses mounted against it.

## STRATEGIES FOR IMMUNE EVASION

### Antigen mimicry

The lipopolysaccharide (LPS) of the *H. pylori* cell wall has been shown to contain structures identical to host cell antigens and these will differ in different strains of the organism. Thus analysis of the chemical structure revealed that the LPS O antigen and core polysaccharide regions from three different *H. pylori* were all distinct (*Aspinall* et al., 1996). In each case, the O chain terminated in either Lewis y or Lewis x antigens. These antigens are structures commonly found on erythrocytes of persons of a certain blood group but are also expressed in the human gastric and other mucosae; it is possible that the expression of these antigens at the surface of the bacteria may act as a form of molecular mimicry - disguising the organism with the hosts own antigens to evade detection from the immune system. Two fucosyltransferases have been identified which may play a role in the molecular mimicry of Lewis antigen by *H. pylori* LPS (*Chan* et al., 1995). With possible relevance to the pathogenesis of *Helicobacter*-related diseases, some *H. pylori* infected patients have been found to produce autoantibodies directed against $Le^x$ antigens (*Negrini* et al., 1991) and sera from *H. pylori* infected pigs and humans were shown to contain autoantibodies reactive against carbohydrate and peptide epitopes expressed on both the gastric pump and human intrinsic factor (*Appelmelk* et al., 1997). Perhaps the production of these autoantibodies is an inadvertent side effect of an attempt by the bacterium to avoid detection, but one which is costly in the long run for the host.

A study of 152 clinical strains of *H. pylori* from various geographical locations showed 12 different serotypes based on the expression of Lewis antigens; 85% of strains could be typed based on their expression of one or more of $Le^x$, $Le^y$ or the related H1 antigen, with 77% of all strains expressing Lewis x (*Simoonssmit* et al., 1996). Interestingly, the majority of the Lewis non-typeable strains were of Chinese origin; given the high incidence of gastric cancer in this country, if fewer Chinese strains of *H. pylori* genuinely express Lewis antigens than those of Western countries, this does not support

the concept that molecular mimicry and/or production of autoantibodies targeted to Lewis antigens play a role in the progression from chronic *Helicobacter*-induced gastritis to more serious pathology. However, the non-typeability may have been due to loss of the O side chain as can occur following a number of *in vitro* passages (*Mills* et al., 1992).

**Host binding: Another disguise?**

Studies on the surface of *H. pylori* reveal that it is coated with a number of large protein molecules such as the enzymes urease and catalase (*Phadnis* et al., 1996). Both these proteins have been shown to be protective antigens in animal vaccine studies also confirming their surface location. It has been suggested that they originate from the lysis of other *H. pylori* cells. This would suggest that the bacterium has a particularly "sticky" surface for protein. In the protein rich milieu of gastric mucus it is not unlikely that host proteins could coat the organisms, once again hiding it from immune surveillance.

**Protein shedding: Evasion by decoy antigens**

The extreme lability of *H. pylori* which results in the release of proteins such as urease could also benefit the bacterium in a different way. In an important paper, Mai demonstrated urease antigen deep in the lamina propria of gastric biopsies from *H. pylori*-infected patients, far removed from the bacteria themselves (*Mai* et al., 1992). Given that the urease molecule has been shown to be chemotactic, it is possible that this and other antigens could act as a decoy stimulating the influx of phagocytic cells to a site removed from the actual site of infection. This is consistent with the observations that the zone of peak inflammation is often far removed from the bacteria which tend to be located in the outer mucus or attached to the outer surfaces of the gastric pits (*Fiocca* et al., 1994).

**The *H. pylori* genome: Evidence for antigenic variation**

A major leap forward in the study of *H. pylori* has recently occurred with the release of the complete genome sequence (*Tomb* et al., 1997). It is still much too early for all the implications of this genome to be determined but several observations have already been made. Firstly, as might be expected, there are many similarities between *H. pylori* and other Gram-negative bacteria such as *Escherichia coli*. However, there are also some significant differences which probably reflect functional modification. Most relevant to the current theme was the detection of a range of nucleotide sequences, which based on comparison with other well defined systems, indicates that *H. pylori* is equipped with a sophisticated machinery for extensive antigenic variation.

Alterations in antigenic epitopes is an important mechanism by which pathogens evade the immune system. A classical example of this occurs in the influenza virus. Two major antigens of this virus are the envelope expressed proteins neuraminidase (NA) and haemagglutinin (HA) which occur in several subtypes. It is mainly against these virulence factors that the immune response is mounted and infection with one strain of influenza confers protection against other strains possessing the same subtype of antigens. However periodically every 10-20 years, HA and less frequently NA undergo antigenic shift often leading to a new pandemic as all previous vaccines and acquired immunity become ineffective. Bacteria showing antigenic variation include *Neisseria meningitides, Neisseria gonorrhoeae* and *Haemophilus influenzae* (*Deitsch* et al., 1997).

The mechanisms of adaptive antigenic variation suggested by the *H. py-*

*lori* genome are listed below:

i) Slipped strand mispairing:
The genome sequence of *Helicobacter* has revealed a number of repeated sequences, some of which reside within open reading frames. This suggests that slipped strand mispairing may occur which could lead to the generation of phenotypic variation in molecules which have critical interactions with the host including surface structures such as pilins, lipoproteins or enzymes responsible for the production of LPS.

ii) Phase variation:
Synthesis of LPS involves glycosyltransferases which are enzymes involved in the transfer of saccharide moieties. Analysis of the genome has indicated that several of these may be subject to phase variation (*Tomb* et al., 1997). In phase variation, the bacteria possesses two or more highly homologous but not identical genes at different sites which encode antigenically distinct products; only one of these is expressed at any one time. An example of this occurs with the flagellin of *Salmonella* spp. where a gene encoding one flagellin protein is associated with a repressor for a second flagellin gene located at a separate site (*Simon* et al., 1980). When the first gene is transcribed, the second gene is repressed. During phase variation, a point mutation occurs in the promotor region of the first gene which is therefore not transcribed; this removes the repression on the second gene which is thus expressed.

iii) Another property possessed by *H. pylori* which can lead to antigenic variation is that of recombination events which result in mosaic organisation of a single loci. This means that different strains of *H. pylori* possess one type of allele which may influence the encoded proteins biological activity, e.g. the vacA protein (*Atherton* et al., 1995).

All of the above mechanisms have the potential to cause modifications which can induce antigenic variation to assist the *Helicobacter* in immune evasion. A more detailed discussion of the above mechanisms in other bacteria may be found in an excellent review published recently (*Deitsch* et al., 1997).

## IMMUNOMODULATION

### Down regulation of cellular activity

*i) Lymphocyte stimulation by Helicobacter antigens*

*Helicobacter* antigens have mitogenic capability; *in vitro* culture of isolated lymphocytes with antigens from *H. pylori* stimulate cells to undergo increased proliferation and produce elevated levels of cytokine. Several studies have reported that the response of human PBMC (*Karttunen* et al., 1990; *Karttunen*, 1991) and gastric T lymphocytes (*Fan* et al., 1994), from *H. pylori*-infected persons were stimulated significantly less *in vitro* by fixed *H. pylori* or bacterial sonicate respectively, than cells isolated from non-infected persons. In these cases, the responses to other non-*Helicobacter* mitogens such as Concanavalin A or pokeweed mitogen were unaffected. There was also a reported increase in the numbers of CD8+ T-cells in infected persons and T-lymphocytes expressing the CD8 molecule can possess suppressor activity. The induction of oral tolerance (a systemic non-responsiveness to an orally delivered antigen) is believed to be mediated by CD8+ cells by a mechanism involving the production of the cytokine, transforming growth factor β (*Miller* et al., 1992).

Thus it was suggested that the reduced stimulation following oral infection with *H. pylori* may have been caused by the production of antigen-specific suppressor cells.

Another group found that *in vitro* proliferation to both *Helicobacter* antigen and non-related mitogen was inhibited by infection with *H. pylori* which they associated with an activity of the cytoplasmic fraction (CF) of the bacteria on both monocytes and isolated T-cells (*Knipp* et al., 1994). During mitogenic activation of purified T-cells, expression of the receptor for the cytokine Interleukin 2 (IL-2) was down-regulated on the cell surface if stimulation occurred in the presence of a CF of *H. pylori*. These authors related these observations to a bacterial protein with an apparent molecular weight of 100±10 kDa which inhibited cell-proliferation (*Knipp* et al., 1996).

These studies propose that *Helicobacter* infection in some way downregulates lymphocyte proliferative responses in an antigen-specific manner. However, the majority of these studies looked at cells from the periphery and the few investigations of cells isolated from the actual site of infection presented unconvincing data. Thus it is still unclear as to the effect of *Helicobacter* infection on the immune response at the mucosal level, but the heavy inflammatory response that occurs is perhaps the best indicator that immune suppression is not actually occurring. Perhaps alternative explanations are that during infection there is a change in the frequency of antigen-specific cells in the peripheral blood as they are localised at sites of infection or there is a qualitative change in the recirculating lymphocytes which alters their responsiveness to mitogens; terminal effector cells for example would be expected to respond to stimulation in a different manner than a naive cell. This may not explain the observed reduction in response to other mitogens which has been reported by some workers.

*ii) The LPS of H. pylori shares properties with the lower bowel normal microflora*

The surface expression of LPS is an important factor in the pathogenesis of Gram-negative bacteria. LPS is the signal which the human immune system has evolved to recognise as the first sign of bacterial invasion and its presence stimulates the non specific and specific defences such as the inflammatory response. However with well adapted pathogens which cannot be quickly removed from the host, responses to excess LPS can actually contribute to the disease process. Thus endotoxin causes abortion of animals with brucellosis, the characteristic fever of typhoid, and the syndrome of septic shock which occurs during systemic infection with many Gram-negative bacteria. Binding of LPS to the host surface molecule CD14, a receptor found on the surface of monocytes and polymorphonuclear granulocytes leads to cell activation. Macrophages are stimulated to secrete lysosomal enzymes and proinflammatory cytokines such as IL-1 and Tumour Necrosis Factor alpha (TNF-$\alpha$). Neutrophils undergo lysosomal degranulation with release of kallikrein (which causes formation of mediators of acute inflammation such as bradykinin), proteases which act on complement to produce anaphylatoxins, and cationic proteins which stimulate release of histamine from mast cells. These all contribute to the generation of an inflammatory response by Gram-negative bacteria.

In the case of *Helicobacter* infection of the stomach, evidence suggests that it may be of advantage to the bacteria to tone down the stimulatory activity of its endogenous LPS. Infection of various strains of mice with *H. felis*, showed that certain strains of mice give an in-

tense, inflammatory response with atrophy (*Sakagami* et al., 1996). Mice, such as SJL, which suffer severe atrophic gastritis actually have a reduction in their bacterial infection; this is probably due to atrophy causing a decrease in acid secretion with resulting changes in the microenvironment, creating a habitat less favourable for Helicobacter colonisation. Thus an overzealous inflammation can be detrimental to bacterial survival. We have recently reported that whereas the C3H/He mouse responds to *H. felis* infection with a strong atrophic gastritis, infection of its derivative C3H/HeJ strain, which has a well characterised mutation in the LPS-response gene, produced no atrophy and little or no inflammation (*Sakagami* et al., 1997). This suggests, therefore, that the *Helicobacter*-associated gastritis is LPS-driven, although this is partly challenged by the evidence of *Mai* et al., (1991) who found that soluble *H. pylori* surface proteins with no detectable LPS could activate human monocytes *in vitro*.

If severe gastritis leads to a reduction in colonisation and LPS is a major factor leading to this gastritis, then it would be advantageous to the bacteria to reduce the immunomodulating activity of its endotoxin and this indeed does appear to be the case. Several studies have shown that LPS from *H. pylori* has much lower biological activity than that of other Gram-negative bacteria such as *E. coli* (*Birkholz* et al., 1993).

These observations may be explained by numerous biochemical studies which have revealed some unusual structural properties of *H. pylori*-LPS compared with endotoxin from other bacteria. *Helicobacter pylori* was originally called *Campylobacter pylori* but was eventually excluded from the *Campylobacter* genus; a major reason for this was the unusual fatty acid profile of *H. pylori* and *H. mustelae* (*Goodwin* et al., 1989). Interestingly, *C. jejuni* - a common cause of human gastroenteritis - also has LPS with low biological activity (*Moran*, 1995). The biological activity of LPS is related to its lipid A component which consists of chains of fatty acids; changes in these fatty acids would therefore be expected to alter the activity of the LPS and this has been proposed for the reduced activity of LPS from *Rhodopseudomonas sphaeroides* (*Henricson* et al., 1992); interestingly, *Geis* et al. (1990) have also reported unusual fatty acid substitutions in *H. pylori* - LPS. Relevant to the concept of *H. pylori* as almost normal flora is the observation that the low biological activity of LPS is also a feature of the LPS of *Bacteroides* species, members of the normal flora of the human lower bowel (*Lee* and *Moran*, 1994).

Another mechanism by which *H. pylori*-LPS may be modified to reduce its proinflammatory activity involves CD14. The attachment of LPS to the CD14 receptor is facilitated by a host serum protein termed Lipopolysaccharide Binding Protein (LBP) which increases in concentration during an infection. It has been shown that activation of CD14-transfected cell lines by *H. pylori* -LPS requires the presence of LBP but again had lower activity than that of LPS from *E. coli*, being unable to stimulate IL-8 secretion by an epithelial cell line (*Kirkland* et al., 1997). Kinetic studies have shown that the transfer of *H. pylori*-LPS to CD14 by LBP is greatly reduced compared with other bacterial LPS and this is likely due to poor binding of *H. pylori*-LPS to LBP (*Cunningham* et al., 1996). Additionally, expression of CD14 by monocytes was downregulated following incubation with a cytoplasmic fraction of *H. pylori* (*Knipp* et al., 1994). Both the percentage of CD14 positive cells as well as the density of CD14 expression on those cells were downregulated. This effect was not mediated by *H. pylori*-LPS. This is very similar to

what has been reported for *Mycobacterium avium-M. intracellulare* complex induced inhibition of T-cell proliferation to mitogen and antigen, which also demonstrated a reduction in CD14 expression with no effect on other important immunological surface molecules such as MHC-class II (*Tsuyuguchi* et al., 1990).

CD14 is a key molecule involved in the recognition of bacteria by the innate immune system and it is tempting to speculate that it has evolved in the host particularly to detect LPS-expressing Gram-negative bacteria. If so, then modification of the LPS by bacteria which survive chronically in the host could be an evolutionary step to reset the balance in the constant battle between host and pathogen.

In conflict with this oft quoted and appealing hypothesis of the low biological and proinflammatory activity of the *H. pylori* LPS is the fact that in reality infection is nearly always associated with a marked inflammatory response that is the active/chronic gastritis.

*iii) Other LPS related effects*

As mentioned above, *H. pylori* may manufacture a molecule which mimicks the Lewis x antigen and the fact that in the human host this molecule appears to play some regulatory role in immune and inflammatory responses raises some fascinating paradoxes. During inflammation, IL-1, TNF and/or LPS induces an increase in the surface expression of a range of adhesion molecules on local endothelial cells, including E selectin (*Bevilacqua*, 1993). E-selectin is a membrane glycoprotein mainly expressed by endothelial cells which interacts with structures containing sialyl Lewis x and its expression following cytokine activation has been linked to increased adhesion of blood neutrophils, monocytes, some memory T-cells and possibly eosinophils and basophils (*Bevilacqua*, 1993). It is normally only transiently expressed but this expression can be increased by IFN-γ, a ubiquitous cytokine at sites of *Helicobacter* infection. Similarly, L-selectin is constitutively expressed on most circulating lymphocytes, neutrophils and monocytes, is involved in cell adhesion and also appears to interact with sialyl Lewis x.

Sialyl Lewis x is obviously structurally different from Lewis x; however, a pentasaccharide containing Lewis x can block the interaction of a platelet form of selectin (P-selectin) with its sialyl Lewis x expressing ligand. In addition, it has been suggested that the immunosuppressive property of an endometrial protein, glycodelin, may be related to its expression of a Lewis x analogue. It was proposed that the glycan moiety may block B-cell activation via the CD22 receptor (*Dell* et al., 1995)

Finally, binding of the T-cell receptor CD2 (another structure involved in cell adhesion) to its ligand on monocytes and neutrophils is inhibited by monoclonal antibodies specific for Lewis x, plus the same antibody has been shown to inhibit the killing of target cells by NK cells (*Warren* et al., 1996) This means that in addition to a role in controlling the trafficking of leukocytes, Lewis x associated structures may also be important in the effector functions of immune cells.

At a recent meeting in Lisbon, the complexity of the relevance of Lewis antigens in *Helicobacter* associated diseases was made even more apparent with a report that infected subjects who lacked anti- $Le^x$ antibodies actually had a higher incidence of atrophic gastritis than those with antibodies (*Kuipers* et al., 1997). However, it is not clear whether the observed difference was due to the absence of anti-Lewis x antibodies in the infected host or bacterial strain variation; perhaps strains of Helicobacter which do not express Lewis x produce greater atrophy. All

this evidence implies a significant role for different forms of the Lewis x antigen in many different aspects of the host's inflammatory and immune response. Thus, when this molecule is expressed at the surface of a pathogen, as is the case with *H. pylori*, it raises the possibility of a mechanism of immunomodulation.

## Complement and *H. pylori* associated inflammatory effects

*Helicobacter* localising in the pit openings of the gastric mucosa have been found to be coated with activated complement, whereas those located within the foveolae were generally not (*Berstad* et al., 1997). This suggests the bacteria can evade the activity of complement by its localisation. This could be related to the exposure of the bacteria in their various niches to mucosally secreted antibodies as has been shown by *Wyatt* et al. (1986), although *in vitro* studies have shown that *H. pylori* can also activate complement in the absence of antibodies (*Bernatowska* et al., 1989). Urease is an important molecule released by the bacteria during infection and can be found in the gastric mucosa. Recently, evidence has been presented which suggests that urease from *H. pylori* inhibits the alternate pathway of complement activation *in vitro* (*Rokita* et al., 1997), which may imply an immunomodulatory role.

## Immunomodulation via manipulation of Th phenotype towards Th1

The host's acquired immune response has many varied components which can give a variety of responses, mainly controlled and directed by Helper T-lymphocytes. The production of T-cell clones revealed that these cells can be broadly divided into two subsets based on the cytokines that they produce (*Mosman* and *Coffman*, 1989). The Th1 cells produce Interferon gamma (IFN-γ) and IL-12, amongst others, which drives the immune response towards a cell mediated profile. This would be the response mounted against an invading microorganism that lives in an intracellular location. In contrast, Th2 cells produce IL-4 and IL-10 leading to a more humoral response with production of an antibody based reaction. The Th2 response, for example is responsible for the production of IgA-secreting plasma cells, the main effectors of mucosal immunity which localise in the lamina propria and secrete antibodies into the lumen of mucosal surfaces such as the intestine. This is the mechanism of defence against bacterial pathogens which invade mucosal surfaces and would be most likely to confer protection against an organism living in the stomach in mucus and on the epithelial surface i.e. a location inaccessible to cellular immunity.

The type of Th response generated *in vivo* in response to an infection can be vital to the outcome of the struggle between host and parasite and this is well illustrated in the classic oft quoted example of *Leishmania* infection in inbred strains of mice. C57BL/6 mice infected with *L. major* mount a Th1 type cell-mediated response which is effective against the intracellular parasite; the mice survive and have long lasting immunity. In stark contrast, challenge of the *Leishmania*-sensitive BALB/c mice induces a Th2-type response and these mice succumb to the infection and die (*Heinzel* et al., 1989; *Hill* et al., 1989).

It is therefore of great interest that infection with *H. pylori* actually produces an ineffective Th1 response (*Bamford* et al., 1997). *Kartunnen* et al. (1995) found that there is a predominance of IFN-γ secreting cells in lymphocytes isolated from the gastric mucosa of *Helicobacter*-infected persons and elevated levels of messenger for the Th1-promoting cytokine IL-12 (*Karttunen* et al., 1997) when compared to non-in-

fected controls. Recently presented data also showed that both IgG subtype responses and T-cell clones produced by antigenic restimulation of cells isolated from infected persons indicated a Th1-type profile (*Bamford* et al., 1997; *Bamford*, 1997). We would submit that this predominant Th1 phenotype is not just coincidence, but a deliberate action of the bacterium to circumvent the effective branch of the host's immune response.

This hypothesis is supported by the observation that effective protection against Helicobacter infection is indeed possible in mice, following immunisation with various bacterial products plus an adjuvant such as cholera toxin or heat labile toxin from *E. coli* (*Czinn* et al., 1993; *Ferrero* et al., 1995; *Lee* et al., 1995; *Michetti* et al., 1994; *Nedrud* et al., 1997; *Radcliff* et al., 1996). If immunisation can work then evidently the host's immune system has the capability to eject the pathogen but is incapable of doing so without some outside help.

The mechanisms of how immunisation actually achieves protection is still unknown, but the current favoured hypothesis is that oral exposure of the antigen in the presence of adjuvant causes a switch towards a Th2-type response (*Ernst* et al., 1996). Several studies have supported this, such as the adoptive transfer of *Helicobacter*-specific cloned Th1 cells into infected mice which led to an exacerbation of the gastric inflammation, whereas transfer of Th2 clones actually produced a reduction in bacterial colonisation (*Mohammadi* et al., 1996). However, it is becoming clear that the situation is much more complex than a simple switch to a Th2-type response. From current understanding of mucosal immunity, the most likely effector mechanism for clearance of a *Helicobacter* infection would involve an IL-4-dependent (Th2) antibody responses mediated via the secretion of IgA into the lumen of the stomach. Studies using oral pretreatment of mice with monoclonal IgA (*Czinn*, et al., 1993) and infection of knock-out mice lacking IL-4 (*Radcliff*, et al., 1996) certainly seem to support this. But this is where things get more complicated. Although the IL-4 deficient mice had reduced protection following immunisation, there was still a certain degree of protection even in the apparent absence of Th2 driven immunity. In addition, knock-out mice incapable of producing IgA (the main effector of Th2/mucosal immunity) were as well protected as wild-type mice following immunisation, indicating that protective immunity is possible in the absence of IgA (*Nedrud*, et al., 1996). Recent data showing that infection of mice lacking receptors for IFN-γ are not protected following immunisation have suggested an important role for Th1 cytokines, thus it may be that a mixed Th2/Th1 is necessary for effective protection (*Radcliff* et al., 1997).

If an immune response can be mounted which clears infection then why is over half the population of the world chronically infected with *H. pylori* ? To many, this clearly suggests that the bacteria manipulates the Th profile to its own advantage. This may not only be of benefit with regard to protection from immunity, but it has been shown that the Th1 cytokine IFN-γ can increase the membrane permeability of intestinal epithelial cells (*Madar* and *Stafford*, 1989) and this may be of benefit to the bacteria by allowing the release of nutrients into the stomach lumen. We have previously proposed that as inflammation is likely to benefit the organism, the move to an inflammatory Th1 response would thus be a benefit (*Hazell* et al., 1986).

Other infectious organisms have been shown to actually modify the hosts immune response to its own advantage. An example of this is the obligate intracellular protozoan *Toxoplasma gondii*. During the acute phase of infection with

this parasite there is a downregulation of the proliferative response of PBMC to both parasite antigen and non-specific mitogenic activation (*Chan* et al., 1986).*T. gondii* preferentially infests macrophages and it has been shown that during acute infection in mice, the parasite induces the macrophage to secrete IL-10 and nitric oxide which inhibits the cell mediated immunity which is so effective against intracellular pathogens (*Khan* et al., 1995). Eventually, over a number of weeks the host immune system overcomes this immunomodulation and clears peripheral tachyzoites - the multiplying, invading form of the pathogen. However, this window allows the parasite to multiply in the host and when effective immunity kicks in, it converts to the bradyzoite, or cyst form and hides in immunoprivileged sites such as the brain.

A similar story occurs with the causative agent of Chagas disease, *Trypanosoma cruzi*. During acute infection there is suppressed immunity in both humans and in animal models with reduced proliferative responses to antigen and mitogen plus diminished numbers of T-cells in the spleen. In human PBMCs, this has been related to a parasite-induced suppression of expression of the receptor for the crucial cytokine IL-2 on T-cells (*Beltz* et al., 1988). Without this receptor, T-cell activation is severely impaired.

Possibly the most intriguing precedent for manipulation of an immune response by a pathogen is shown in animal studies of schistosomiasis as yet again it features the Lewis x antigen, a molecule that has figured prominantly in the discussion above. A Lewis x trisaccharide has been found expressed on a surface antigen from the eggs of the parasitic worm, *Schistosoma mansoni* (*Velupillai* and *Harn*, 1994). This Lewis x antigen stimulated a B-cell enriched population of spleen cells to secrete IL-10 (a Th2 cytokine) and prostaglandin E2 which both downregulate Th1 cell mediated immunity. This is exactly the opposite to what would be advantageous to *Helicobacter* and our hypothesis, yet it demonstrates the potential immunomodulating capability of Lewis molecules. Could it be that in humans the Lewis x does the opposite to what it does in the *Shistosoma*-infected mice?

## CONCLUSION

Diseases caused by *H. pylori* remain one of the world's major killers. Thus, in a recent article on the causes of death of any type in the world, gastric cancer was ranked 14th for the year 1990 (*Murray* and *Lopez*, 1997). Moreover, due to the age profile of the globe, it was predicted that this *H. pylori* linked malignancy would move up the ladder of death to 8th in the year 2002. Better understanding of the mechanisms of survival of this pathogen in the gastric mucosa as discussed above should point the way to novel approaches to both prevention and cure. Understanding the interaction of this organisms in its mucus niche with the host's immune system may also provide pointers to other diseases lower down in the bowel. Unlike in the stomach where there is only one bacterial inhabitant, in the lower bowel a consequence of high species diversity is that the complex microflora play a protective role against invading bacterial pathogens. In animals, the intestinal mucus is inhabited by a myriad of spiral bacteria that are closely related to *H. pylori*. Could it be that these bacteria use similar evasive and immunomodulatory mechanisms? Could these mechanisms be protective of the intestinal mucosa? This may not

appear relevant to human disease as we have no lower bowel spirals, but remember the world is losing *H. pylori* from its gastric mucosae. In the developed world, children no longer become infected with *H. pylori* and it is likely that even if there were no intervention strategy the bacterium would be lost from whole populations in 100 years. Could not the same have happened with the lower bowel spirals? There is anecdotal evidence in some underdeveloped countries such as India there still may be many spiral bacteria on the lower bowel surface. (*Mathan* and *Mathan*, 1985) What would be the consequence of the removal of these potentially immunmodulatory commensals from the surfaces of the lower bowel? Could this be relevant to the observation that inflammatory bowel disease (IBD) is a new disease of the developed world? Recent animal experimentation has showed that other *Helicobacter* species can, as pure cultures, induce IBD (*Ward* et al., 1996). We need to learn much more about the interaction of the host with the mucus associated microbiota of our mucosal surface.

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