Among people infected with *Helicobacter pylori*, the virulence of the infecting strain is a major determinant of who develops disease. Strains producing vacuolating cytotoxin activity are more commonly isolated from people with peptic ulcers than without. The gene encoding the toxin, *vacA*, varies between strains, especially in its signal sequence and mid regions. *vacA* genotype influences cytotoxin activity, and signal sequence type correlates closely with peptic ulceration. Infection with strains possessing *cagA* (cytotoxin associated gene A) is more common among people with peptic ulceration or gastric adenocarcinoma than without. *cagA* is a marker for the *cag* pathogenicity island, which includes genes necessary for the enhanced inflammation induced by pathogenic strains. Serological detection of infection with *cagA*+ strains is at present the best practical test for virulence. However, before a strategy of screening and selective treatment can be considered, it is important to assess whether *cagA*− strains are entirely non-pathogenic.

*Helicobacter pylori* infection is the main cause of duodenal and gastric ulceration and a major risk factor for gastric adenocarcinoma and lymphoma. However, these diseases only occur in about 15% of infected persons. Of those infected, who will develop disease is influenced by the virulence of the infecting *H. pylori* strain, the genetic susceptibility of the host and environmental co-factors. Of these, bacterial virulence factors are the most studied. Bacterial virulence factors are characteristics present in some bacteria which enable them, rather than others, to cause disease. For *H. pylori*, it is still unclear whether all or only some strains are pathogenic. All strains cause a long-lasting histological gastritis characterised by lymphocytic and some degree of neutrophilic infiltration, although this gastritis *per se* is clinically silent. Whether all strains can potentially cause peptic ulceration or gastric carcinoma is unclear, although the risks associated with some are certainly much greater than those associated with others. Certain characteristics are present in only some strains and have been linked with disease. These are, firstly, vacuolating cytotoxin production and certain types of *vacA* (vacuolating cytotoxin gene A) alleles; secondly, CagA (cytotoxin associated gene product A) and genes in the *cag* (cytotoxin associated gene) pathogenicity islands.
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island; and thirdly, the ability to strongly and rapidly stimulate neutrophils to degranulate. Host susceptibility may be relevant both in determining who becomes infected with H. pylori and who amongst those infected develops disease. There is growing evidence that the host’s HLA type, and blood group antigen type and expression are important, at least as genetic markers of susceptibility. Important environmental factors include childhood living conditions (the strongest environmental correlate of who becomes infected), smoking (a major risk factor for duodenal ulceration amongst H. pylori-infected persons) and dietary factors (important risk factors for gastric adenocarcinoma).

Many characteristics of H. pylori are necessary for disease and thus are potential therapeutic targets and important subjects for research. Gastric colonisation is a prerequisite for H. pylori-associated disease, and for this both motility (derived from flagella) and urease have been shown to be important: mutant strains lacking these features cannot establish infection in animal models. Following colonisation, H. pylori must acquire nutrients in the gastric mucus niche, and as for all bacterial parasites, acquisition of iron from the host is a particular challenge. Infection is virtually lifelong in the absence of treatment, implying that evasion of the host response is efficient, and this is discussed in the accompanying paper on inflammation and autoimmunity. Adhesion is important for the enhanced inflammatory response seen for some pathogenic strains: for example, these strains only stimulate cultured epithelial cells to produce the pro-inflammatory cytokine interleukin 8 if they are allowed to adhere. Other H. pylori components are also pro-inflammatory: urease is an important stimulant of both local and systemic immunity as are many other surface expressed and cytoplasmic proteins. However, none of these shared factors can explain differences in disease outcome unless they differ qualitatively or quantitatively between strains, and in the rare instances where differences have been shown, these have not correlated with disease. Characteristics which are not shared between all strains and which have been correlated with disease are discussed below.

The vacuolating cytotoxin and vacA polymorphism

In 1988, Leunk et al showed that some H. pylori strains produced a protein in culture supernatant which induced vacuolation in a variety of cultured epithelial cell lines. Several groups have subsequently shown that these toxigenic strains are more commonly isolated from patients with peptic ulcers (Table 1) or with atrophic gastritis than from patients without these conditions. However, this does not explain
Table 1  Studies showing the proportion of patients with and without ulcers infected with a toxigenic strain of H. pylori.

<table>
<thead>
<tr>
<th>Study</th>
<th>Peptic ulcer</th>
<th>No ulcer</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>% Toxigenic *</td>
<td>(n)</td>
</tr>
<tr>
<td>Figura 198914</td>
<td>24</td>
<td>67%</td>
<td>53</td>
</tr>
<tr>
<td>Goosens 199217</td>
<td>41</td>
<td>49%</td>
<td>89</td>
</tr>
<tr>
<td>Rautelin 199418</td>
<td>24</td>
<td>46%</td>
<td>30</td>
</tr>
<tr>
<td>Tee 199519</td>
<td>93</td>
<td>66%</td>
<td>53</td>
</tr>
<tr>
<td>Atherton 199520</td>
<td>23</td>
<td>61%</td>
<td>33</td>
</tr>
<tr>
<td>Rautelin 199621</td>
<td>61</td>
<td>49%</td>
<td>19</td>
</tr>
<tr>
<td>Zhang 199622</td>
<td>45</td>
<td>67%</td>
<td>31</td>
</tr>
<tr>
<td>Weel 199623</td>
<td>76</td>
<td>57%</td>
<td>79</td>
</tr>
</tbody>
</table>

\(n\) = number of subjects of this type studied.

*Toxigenic \textit{in vitro}, as shown by \textit{in vitro} induction of vacuoles in cultured epithelial cells by culture supernatant.

n.s. = not significant.

entirely why only certain people develop disease. For example, toxigenic strains are frequently isolated from patients without disease, and non-toxigenic strains from people with disease (Table 1), and this latter point is especially telling: if the toxin is the cause of ulcers, patients with non-toxigenic strains should not get them. Despite this, the association between toxigenic strains and disease has been consistent across studies and has potential clinical importance.

\textit{Characterisation of the vacuolating cytotoxin and its interaction with epithelial cells}

Following purification of the vacuolating cytotoxin\textsuperscript{26}, the gene encoding it (\textit{vacA}) was cloned and sequenced simultaneously by 4 groups\textsuperscript{27-30}. The predicted cytotoxin precursor is about 1300 amino acids, the exact length varying between strains, and as for most exported bacterial proteins, it has a short N-terminal signal peptide (or signal sequence) which is recognised and cleaved during export of the toxin across the bacterial cytoplasmic membrane (Fig. 1). Further cleavage of the C-terminal third of the toxin precursor occurs during passage across the outer membrane: this region is thought to form a pore through which the rest of the polypeptide passes\textsuperscript{9} before being cleaved to leave an 87 kDa secreted polypeptide. The 87 kDa polypeptide consists of two domains separated by a loop which is predicted to be surface-exposed, and further cleavage may occur here to yield two subunits, which, however, remain closely associated\textsuperscript{28}. The polypeptides aggregate into flower-shaped hexamers and heptamers, which represent the mature
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The protein, VacA

The gene, vacA (3.9 kb)

Signal sequence allelic types
s1a
s1b
s2

Mid region allelic types
m1
m2

Data from literature.

active toxin. The mature toxin is peculiarly suited to the gastric environment in that it is activated by acid (even if the pH is subsequently neutralised) and in its activated form it not only causes more profound epithelial changes than before activation, but also becomes resistant to subsequent damage by acid and pepsin.

Epithelial cell vacuolation is not a marked finding in vivo in the stomach, but its relevance is supported by the observation that vacuoles are visible underneath adherent bacteria by electron microscopy, and by the finding that primary gastric epithelial cells in culture are even more susceptible to the toxin than immortal epithelial cell lines. The toxin binds to epithelial cells through its larger subunit and is slowly internalised to a perinuclear distribution. Exactly how it causes vacuole formation is unclear, but the vacuoles form from late endosomes and the process is dependent on, but not entirely caused by, stimulation of a small GTPase called rab. The vacuoles are acidic, and vacuolation is potentiated by weak bases, such as ammonia and nicotine.

Fig. 1 Schematic of the vacuolating cytotoxin protein (VacA) and the gene which encodes it, vacA. For the protein, the signal sequence cleavage site has been determined experimentally but the C-terminal cleavage site is estimated from the size of the secreted polypeptide. For the gene, alleles in individual strains may consist of any combination of signal sequence and mid region except s2/m1. kDa = kilodaltons. kb = kilobase pairs. Data from literature.
As well as its effect on epithelial cells in vitro, the purified toxin causes gastric epithelial damage when given intragastrically to mice. Sonicates of toxigenic strains cause similar effects, whereas sonicates of non-toxigenic strains or previously toxigenic strains rendered non-toxigenic by artificial disruption of vacA do not. It is notable in these studies that the toxin causes epithelial damage, but appears to have little effect on inflammatory cell infiltration. The toxin is expressed in vivo in human infection as evidenced by a specific antibody response, and the link between toxigenicity and gastroduodenal disease suggests that it is important in pathogenesis.

Polymorphism in vacA and the link between specific vacA allelic types and disease

Although only about 40% of H. pylori strains are toxigenic, all have vacA, the gene encoding the toxin. However, vacA alleles vary between strains, particularly in the region which encodes the second half of the signal sequence, and the mid region which encodes the C-terminal portion of the final processed polypeptide (Fig. 1). Three signal sequence types are found, s1a, s1b and s2, and two mid region types, m1 and m2. Each vacA allele can consist of any combination of signal sequence and mid region except s2/m1. This implies that some alleles have arisen by recombination between strains in vivo (swapping of DNA between strains in a manner analogous to sexual reproduction in eukaryotes). This has important implications for H. pylori evolution as it provides a potential mechanism for spread of favorable characteristics, such as virulence and antibiotic resistance determinants. The final structure of a vacA allele is also important for toxin activity: strains with vacA m1 alleles are more toxigenic than those with m2 alleles (which are mildly or non-toxigenic), and within the m1 group strains with s1a/m1 alleles are more toxigenic than those with s1b/m1 alleles.

vacA genotype is important in vivo: in the human stomach, strains with vacA m1 alleles are associated with higher levels of epithelial damage than those with m2 alleles. This is to be expected, as vacA mid region type is closely associated with in vitro toxin activity. Surprisingly however, the same study showed vacA signal sequence type rather than mid region type to be associated with the level of mucosal inflammatory cell infiltrate; strains with vacA s1a alleles were associated with more inflammation than those with s1b or s2 alleles. In this study, strains with vacA s1a alleles were also most commonly isolated from patients with ulcers (89% of patients with s1a strains had past or present ulcers) whereas strains with vacA s2 alleles were uncommonly isolated from ulcer patients (20% of those with s2 strains had ulcers). The study was performed in a predominantly elderly male population in
Helicobacter infection

Table 2  Studies showing the proportion of patients with and without peptic ulceration who were seropositive for CagA

<table>
<thead>
<tr>
<th>Study</th>
<th>Peptic ulcer</th>
<th>No ulcer</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% CagA*</td>
<td>n</td>
</tr>
<tr>
<td>Cover 1990</td>
<td>43</td>
<td>95%</td>
<td>74</td>
</tr>
<tr>
<td>Crabtree 1991</td>
<td>25</td>
<td>100%</td>
<td>51</td>
</tr>
<tr>
<td>Xiang 1993</td>
<td>22</td>
<td>100%</td>
<td>37</td>
</tr>
<tr>
<td>Cover 1995</td>
<td>57</td>
<td>83%</td>
<td>39</td>
</tr>
<tr>
<td>Ching 1996</td>
<td>297</td>
<td>82%</td>
<td>145</td>
</tr>
<tr>
<td>Graham 1996</td>
<td>100</td>
<td>59%</td>
<td>77</td>
</tr>
<tr>
<td>Weel 1996</td>
<td>76</td>
<td>93%</td>
<td>79</td>
</tr>
</tbody>
</table>

n = number of subjects of this type studied.

n.s. = not significant.

The USA, and it will be interesting to see if the same results are obtained for other populations. Why the vacA signal sequence type should be important is unclear, but preliminary studies suggest that it may be a marker for the level of cytotoxin production.

cagA and the cag pathogenicity island

CagA was first described as a protein which was expressed more commonly by toxigenic than non-toxigenic strains. Later studies showed that most people with peptic ulcer disease mounted a systemic or local antibody response to CagA, and although CagA+ strains are common even amongst those without ulcers, their prevalence in ulcer patients is consistently higher (Table 2). The systemic antibody response to CagA is easy to detect and many studies have assessed the link between CagA seropositivity and gastroduodenal disease. Both patients with duodenal ulcer and those with gastric adenocarcinoma are more likely to be CagA seropositive than those without those conditions. One study has shown that, in Hong Kong, CagA+ strains are more common in patients with non-ulcer dyspepsia than in otherwise similar asymptomatic control subjects, but these data still need to be confirmed in other populations.

Characterisation of CagA

The gene cagA was cloned and sequenced simultaneously by two groups and was found to encode a predicted protein of about 1200 amino acids, the exact size varying between strains and depending on the number of direct repeat regions in the gene. In contrast to vacA, strains which...
did not express the CagA protein were found to lack the gene. The function of CagA is unclear, the only clue being that its transcription is enhanced at mildly acidic pH\(^\text{52}\). The name \textit{cagA} (cytotoxin associated gene A) is misleading as disruption of \textit{cagA} does not affect production or activity of the vacuolating cytotoxin; the relationship between \textit{cagA} and the cytotoxin is discussed below. \textit{cagA}\(^+\) strains are associated with higher levels of inflammation and of pro-inflammatory cytokine expression \textit{in vivo} in the human stomach than are \textit{cagA}\(^-\) strains\(^\text{53}\). \textit{In vitro}, they stimulate a gastric epithelial cell line to produce higher amounts of the neutrophil-attracting cytokine interleukin 8 (IL-8)\(^\text{12}\), but this property too is unaffected by disruption of \textit{cagA}\(^\text{54}\). The implication of this is that \textit{cagA} is a marker for something else on which this epithelial cell stimulation is dependant.

\textit{The cag pathogenicity island}

Nucleotide sequencing around \textit{cagA} has shown that the gene is at one end of a collection of about 40 genes which are usually present or absent as a group (Fig. 2)\(^\text{55}\). Disruption of some of these genes reduces the ability of \textit{cagA}\(^+\) strains to induce interleukin 8 production by cultured epithelial cells, explaining the link between \textit{cagA} and inflammation\(^\text{55,56}\). By comparing the nucleotide sequences of these genes near \textit{cagA} with those of other known bacterial genes, it appears likely that at least part of the \textit{cag} region encodes a type IV secretion system which might allow surface expression or export of substances through the outer bacterial membrane\(^\text{55}\). One hypothesis is that this secretion system transports a factor, or factors, which stimulate epithelial cells to produce IL-8. Disruption of some genes in the \textit{cag} region abolishes or reduces the ability of \textit{H. pylori} strains to induce formation of epithelial cell pedestals, phosphorylation of tyrosine and changes in the cytoskeleton\(^\text{57}\). Disruption of other genes in the \textit{cag} region reduces the ability of \textit{H. pylori} to induce haemolysis and affects its growth rate \textit{in vitro}\(^\text{57}\).

The \textit{cag} region has much in common with pathogenicity islands in other bacteria: it appears to be involved in virulence, has a different nucleotide composition to other \textit{H. pylori} genes, is flanked by direct nucleotide repeats, and is relatively genetically unstable\(^\text{58}\). Like other bacterial pathogenicity islands, it is thought to have been acquired relatively late in evolution from an external source, perhaps a bacteriophage or plasmid. In many strains it is present in its entirety, but in others it is re-organised or even partially deleted following interruption by a novel insertion sequence (IS605) which is thought to be involved in chromosomal reorganisation\(^\text{55}\). Strains with partial deletions have not yet been fully phenotypically characterised, but some
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Fig. 2. Schematic of the cag pathogenicity island showing the genes at one end in detail (named cagA to cagM). The bar chart shows the result of artificially interrupting selected genes on the ability of H. pylori to stimulate the gastric epithelial cell line KATO-III to produce IL-8. The undisrupted parent strain is shown on the far right as a positive control. kb = kilobase pairs. Adapted from Censini et al."55.

Interleukin 8 secretion by KATO-III cells (% of that induced by parent strain)

Gene in the cag region which has been disrupted

of these appear to lack features found in typical cag+ strains55, and should prove valuable for research into pathogenic mechanisms.

Direct neutrophil activation by H. pylori

As well as stimulating epithelial cells to produce the cytokine IL-8, H. pylori also activates neutrophils directly in vitro and strains may be divided into 2 distinct types based on the strength of this effect18. About 50% of strains induce a rapid, strong neutrophil oxidative burst, and the remaining 50% a slower and weaker burst. Strains inducing a rapid, strong burst have been found more commonly in patients with peptic ulcer disease than in those without18,22, although this finding has not been universal21. Vacuolating cytotoxin activity has been assessed for the same strains, and while strains which strongly activated neutrophils were more often toxigenic, the correlation was not absolute implying that the toxin was not the cause of the neutrophil activating effect. Furthermore, nontoxigenic strains from patients with ulcers tended to activate neutrophils more strongly than non-toxigenic strains from patients without ulcers, although this did not reach statistical significance18,22. cagA status was not assessed in any of these studies, so the importance of genes in the cag region for direct neutrophil activation is unknown.

Although the basis of direct neutrophil stimulation by H. pylori is uncertain, a protein which may be at least partially responsible has been
identified and purified, and named HP-NAP (H. pylori neutrophil activating protein). HP-NAP is a 150 kDa protein made up of 15 kDa subunits, the gene for which (napA) has been cloned and sequenced. The main evidence supporting a role for HP-NAP in virulence is the ability of the purified protein to activate neutrophils in a dose-dependent manner so that they produce oxygen free radicals and adhere to cultured endothelial cells. Adherence to endothelial cells can also be induced by water extracts of H. pylori strains, and the effect can be partially blocked by specific anti-HP-NAP antibodies. The ability of H. pylori water extracts to stimulate neutrophils to stick to endothelial cells varies widely between strains, but strains from peptic ulcer patients are no different as a group to those from patients without ulcers. Furthermore, the nucleotide sequence of napA has homology with genes encoding cytoplasmic proteins which are not usually thought to be involved in neutrophil stimulation. Thus, further evidence is needed before HP-NAP can be accepted as an important bacterial virulence factor.

**Association between H. pylori virulence determinants**

The interrelationship of the three virulence factors discussed above is poorly understood, making it difficult to define their relative importance. The link between direct neutrophil activating status and the other two virulence factors is outlined in the preceding section, but the reasons for the association between strong neutrophil activation and toxigenic status are unknown. The link between cagA and vacuolating cytotoxin activity has a genetic basis: strains which are $cagA^+$ usually have the $vacA$ s1a or b genotype, and such strains are often toxigenic whereas strains which are $cagA^-$ usually have the $vacA$ s2 genotype, and such strains are non-toxigenic. The relationship is not invariable, but exceptions are rare. Although it is clear that the relationship between cagA and certain vacA genotypes is genetic, it is unclear why this genetic association exists. For bacterial species with clonal population structures, unrelated features may be associated purely because they are both present in a particular clone. However, the population structure of H. pylori appears not to be clonal from various lines of evidence. Firstly, as already discussed, the mosaic structure of vacA implies recombination between strains in vivo, and recombination has been demonstrated between co-cultured strains in vitro. Secondly, analysis of multiple proteins by multi-locus enzyme electrophoresis has shown that there are no consistent groupings by electrophoretic pattern, implying that none of the proteins studied are clonal markers. One alternative hypothesis is that there is a functional linkage between the
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cag island and the vacA s1 genotype such that one of these features would be advantageous only in the presence of the other: for example, a protein encoded in the cag region could be important for facilitating toxin export. The strong association between vacA genotype and cag status makes it difficult to deduce which is more important in pathogenesis. One study showed cagA status to be a better marker of peptic ulceration than toxigenic phenotype, although among cagA⁺ strains there was a non-significant trend for toxigenic strains to be more closely associated with ulcers than non-toxigenic strains²³. Another study showed that among cagA⁺ strains, those with the vacA s1a genotype were significantly more likely to be associated with ulcers than those with the vacA s1b genotype⁴¹. This suggests that vacA genotype is of at least additional importance to cagA, and may be more important. Thus, these studies suggest that both the cag region and vacA type are important in pathogenesis, as might be expected if the two are functionally linked.

Potential clinical importance of H. pylori virulence factors

Current medical practice is to treat H. pylori infection after it has caused clinical problems in an attempt to prevent their recurrence, and a US consensus meeting has made sensible recommendations about whom to treat on this basis⁶³. Using this approach, assessing H. pylori virulence has few potential indications since infections are treated after they have declared themselves as virulent by causing disease. However, strain typing could be useful in screening pre-endoscopy; for example, endoscopy might be unnecessary in young dyspeptic patients without ‘alarm symptoms’ for carcinoma who are infected with non-virulent strains. It might be even better to treat young dyspeptic patients infected with virulent strains without performing an endoscopy. Finally, it would be better still, if logistically possible, to treat patients likely to develop ulcers or gastric malignancy before those conditions arose. However, the major unknown factor is whether successful treatment of H. pylori infection reduces the risk of subsequent gastric malignancy and, if it does, by what age treatment is needed to have an effect. If treatment did reduce the risk, one possibility would be to screen for and treat all H. pylori-infected persons. Problems associated with such an approach would include expense, morbidity from drug side effects, and induction of antibiotic resistance both in H. pylori not successfully treated and in other pathogens. Also, it has been suggested that, since humans have co-evolved with H. pylori, they might derive some, as yet obscure, benefit from them⁶⁴. Certainly man adapts physiologically to infection during his lifetime and treatment can lead to problems such as reflux oesophagitis⁶⁵.
In view of these real and potential problems, it would seem preferable to screen for and treat only strains which are known to cause disease. The extent to which the vacuolating cytotoxin and CagA might be clinically useful as targets for screening is discussed below. Because direct neutrophil activation by *H. pylori* is still poorly characterised, this is not discussed further.

**Vacuolating cytotoxin activity, VacA serology and vacA genotype as useful clinical markers of virulence**

Vacuolating cytotoxin activity, as assessed by *in vitro* vacuolation of cultured cell lines, is unlikely to be a clinically useful virulence marker because gastric biopsy culture is necessary. Furthermore, many apparently non-toxigenic strains are isolated from patients with peptic ulcers, so treating only subjects with toxigenic strains would miss many potentially ulcerogenic infections. Using VacA serology would be an improvement as it is a non-invasive test, but there are uncertainties about its interpretation. If the purified toxin from a *vac A* s1a/m1 strain is used as the ELISA antigen, patients infected with toxigenic strains give a higher mean optical density value than those infected with non-toxigenic strains, but there is considerable overlap between the groups. If purified toxin from a *vacA* s2/m2 strain is used as the antigen, non-toxigenic strains give a higher mean value, but again with considerable overlap. Therefore, it is unclear what individual VacA ELISA values represent, although it is likely that it is a combination of VacA production *in vivo* and structural similarity to the toxin used as the ELISA antigen. Thus although it may be possible to adapt VacA ELISAs for clinical use, they still need further assessment and development.

*vacA* signal sequence type performs better as a marker of peptic ulceration than does *in vitro* cytotoxin activity. However, although infection with *vacA* s1a strains is more commonly associated with peptic ulceration than infection with *vacA* s1b strains, both types may be associated with ulcers and thus both would need treatment. Further preliminary studies in several countries have confirmed that *vacA* s2 strains are rarely associated with ulceration, but have also shown these strains to be rare in some populations, such as the Japanese who predominantly have strains with the most toxigenic *vacA* genotype, s1a/m1. In this situation, *vacA* heterogeneity cannot explain why some people develop ulcers and others do not, but it might explain why the Japanese have a high prevalence of *H. pylori*-associated disease such as gastric adenocarcinoma. Finally, at present, gastric biopsy is necessary before *vacA* genotype can be determined, so *vacA* genotyping cannot be used in non-invasive screening strategies. Thus, like toxigenic phenotype, it is currently best regarded as a research tool.
CagA serology as a clinically useful marker of virulence

CagA serology is easy and reliable to perform, and CagA seropositivity reflects the presence of cagA together with all or part of the cag pathogenicity island. Strains lacking part of the island may be avirulent, but more likely have an intermediate virulence, and thus should probably be treated. One problem with using CagA serology as a virulence marker is that infection with cagA+ strains is common (Table 2), so screening for and treating CagA seropositive subjects might result in unnecessary treatment. However, the large group of CagA seropositive subjects without ulcers has not been followed prospectively to see if they develop disease and this group may be at particularly high risk of developing gastric adenocarcinoma: CagA seropositive infection confers an increased risk over infection per se48,49, and those who develop duodenal ulcers are at reduced risk71, leaving those infected with cagA+ strains who do not develop duodenal ulcers logically at greatest risk of developing carcinoma. The second problem with using CagA serology is that avoiding treatment for CagA seronegative subjects would be safe only if they did not later develop malignancy which could have been prevented if their H. pylori infection had been treated earlier. A recent nested case control study estimated the excess risk of developing gastric adenocarcinoma for subjects infected with CagA- H. pylori over uninfected subjects as being low (odds ratio 2.2, 95% confidence interval 0.9–5.4)49, but further, larger studies are needed. If any significant risk is confirmed, it may be difficult to avoid treatment of these patients with cagA- H. pylori. The third main problem is that as for vacA s2 strains, cagA- strains may be rare in some populations. In these circumstances, it may be preferable to treat all those infected with H. pylori, and thus avoid the uncertainties involved with leaving those with cagA- strains untreated. Thus, for CagA serology to be a viable test for selecting strains to treat, three criteria must be fulfilled: firstly, there must be evidence that treatment reduces the risk of subsequent H. pylori-related malignancy; secondly, excess disease (especially carcinoma) in CagA seronegative subjects over uninfecteds must be considered negligible; and thirdly, the CagA seronegative group must be sufficiently large in the population to be screened to make avoiding treatment for this group worthwhile.

Vaccination using antigens involved in bacterial virulence

A logical improvement to screening for and treating virulent strains of H. pylori would be to vaccinate against them. This would avoid the specific problem of not knowing the age by which intervention is necessary to prevent gastric carcinoma. The possibility of such a strategy...
has been raised because vaccination of mice with purified vacuolating cytotoxin has been shown to protect them from subsequent experimental infection with toxigenic, but not non-toxigenic, strains of *H. pylori*. Although there are general concerns about vaccination against *H. pylori*, vaccinating to prevent infection by virulent strains whilst allowing gastric colonisation by avirulent strains is an attractive idea. However, the specific concerns remain concerning the residual pathogenicity of non-toxigenic or CagA− strains.

**Acknowledgement**

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**References**

36 Papini E, de Bernard M, Milia E et al. Cellular vacuoles induced by Helicobacter pylori originate from late endosomal compartments. Proc Natl Acad Sci USA 1994; 91: 9720–4
37 Papini E, Satin B, Bucci C et al. The small GTP binding protein rab7 is essential for cellular vacuolation induced by Helicobacter pylori cytotoxin. EMBO J 1997; 16: 15–24
49 Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk factor for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut 1997; 40: 297–301
Helicobacter infection


64 Blaser MJ. Not all Helicobacter pylori strains are created equal: should all be eliminated? Lancet 1997; 349: 1020–2


67 Perez-Perez GI, Tham KT, Peek RM, Atherton JC, Blaser MJ, Cover TL. Serologic responses to type m1 and m2 H. pylori VacA antigens. Gut 1997; 41: A110


69 Queiroz DMM, Mendes EN, Rocha GA et al. Gastric carcinoma (GC) strains of H.pylori present vacA sequence that allows to differentiate them from duodenal ulcer (DU) and chronic gastritis (CG) strains. Gut 1996; 39: A66

70 Atherton JC, Karita M, Gonzalez-Valencia G et al. Diversity in vacA mid-region sequence but not in signal sequence type among Helicobacter pylori strains from Japan, China, Thailand and Peru. Gut 1996; 39: A73

