Leading article

The clinical relevance of strain types of *Helicobacter pylori*

Helicobacter pylori infects about 30% of the population of western Europe and the United States, and about 80% of the population of many developing countries. Although it is the main cause of peptic ulcer disease and an important risk factor for gastric adenocarcinoma[1 2] and lymphoma,[3] most people with the infection do not develop these conditions. Who develops disease depends on the virulence of the infecting *H pylori* strain, the susceptibility of the host and environmental co-factors. Research into bacterial virulence factors has concentrated on determining whether certain strains of *H pylori* are more virulent than others, and if so, whether they can be identified easily. This work has led to several non-conserved bacterial virulence factors with a logical role in pathogenesis being associated with disease. These include in vitro production of vacuolating cytotoxin activity, the presence of certain genotypes of *vacA* (the gene encoding the vacuolating cytotoxin), possession of the *cag* (cytotoxin associated gene) complex, and the ability to activate neutrophils directly.

There are several reasons why the link between *H pylori* virulence determinants and peptic ulcer disease is unlikely to be absolute. Perhaps most important are the potential contributions of host and environmental factors to pathogenesis. Host factors remain ill-defined, but one possible example is the raised gastrin stimulated acid output seen in patients with duodenal ulcer disease. This remains elevated a year after treatment which may imply that it is host determined.[4] The best defined environmental factor is smoking, which increases the risk of duodenal ulceration for those infected with *H pylori*.[5] That such factors are important implies that pathogenic bacteria are necessary but not always sufficient to cause disease. Thus, although disease is to be expected only in people harbouring pathogenic strains, not everyone infected with a pathogenic strain is expected to develop disease. The relation between *H pylori* virulence factors and disease also may be obscured by the natural and drug-modified history of peptic ulceration. Peptic ulcer disease is a relapsing and remitting condition and a sufferer may be free of ulceration at the time of endoscopy. This problem is exacerbated by the widespread prescription of acid suppressing drugs, and makes underdiagnosis of ulcer disease common. The reverse problem, overdiagnosis of *H pylori* induced ulcers, is less common but may occur owing to undisclosed use of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs). These features will add to the number of patients infected with pathogenic strains who seem not to have ulcer disease, and to a lesser extent to the number with non-pathogenic strains who have ulcers.

About 50% of *H pylori* strains produce an active vacuolating cytotoxin[6–9] and evidence for its role in pathogenesis is good. The toxin causes vacuolation in a variety of cultured epithelial cell lines[7] and causes gastric epithelial damage when given to mice.[10] Its structure[11] and method of inducing vacuolation[12] are becoming better understood, and it seems well suited to the gastric environment, being activated by low pH, and then becoming resistant to acid and pepsin.[13] All strains have the gene encoding the toxin, *vacA*, but the structure of this varies, especially in the mid-region (which may be type m1 or m2) and the region encoding the signal sequence (which may be type s1a, s1b or s2).[14] The final structure is a mosaic, and all combinations of signal sequence and mid-region types are found except s2/m1.[15] A strain's *vacA* structure determines its in vitro cytotoxin activity, with type m1 *vacA* strains being more active than type m2, type s1a being more active than type s1b, and type s2 not producing detectable activity.[14 15] The link between the ability of a strain to induce epithelial cell vacuolation in vitro and peptic ulcer disease in vivo is consistent but not striking, and in particular 30–40% of patients with ulcers do not harbour toxigenic strains.[7 9] Conversely, *vacA* genotype seems to be a good predictor of ulcer disease; in a study from the USA over 90% of patients with duodenal ulcer disease had *vacA* s1 strains. Patients infected with *vacA* s1a strains were more likely to have ulcer disease than those with s1b strains, and those with s2 strains were no more likely to have ulcer disease than uninfected patients.[15] Likewise, in a preliminary UK study, all patients with ulcer disease had *vacA* s1 strains; no *vacA* s2 strains were associated with ulcers (although s2 strains were uncommon in this study population).[16] Why *vacA* genotype should be a better predictor of ulcerogenic potential than in vitro phenotype is unclear. It may be that testing for in vitro toxin activity is less accurate than genotypic testing, that toxin activity in vitro poorly reflects toxin activity in vivo, or that some property of the toxin other than its vacuolating ability is important in vivo (especially as epithelial cell vacuolation is not a striking finding in gastric biopsy specimens).

In contrast to *vacA*, the gene *cagA* is present in only 60–70% of *H pylori* isolates.[8 17–20] However, almost all *cagA*+ strains produce the CagA protein, and almost everyone infected with a *cagA*+ strain produces a detectable local and systemic antibody response.[18] Thus serological testing makes *cagA* easy to study, and indeed, *cagA* was first identified as potentially important because of the link between anti-CagA antibodies and peptic ulcer disease.[8 17–20] Over 80% of patients with ulcers harbour *cagA*+ strains, but such strains are common and about 60% of patients in endoscopic series without ulcers also have *cagA*+ strains.[17–20] Several studies have suggested that *cagA* status may be important in other *H pylori* associated conditions. Japanese Americans in Hawaii infected with *cagA*+ strains were found to be more likely to develop gastric adenocarcinoma than those with *cagA*– strains.[21] In The Netherlands, patients infected with *cagA*+ strains developed atrophic gastritis (thought to be a precursor of gastric adenocarcinoma) more quickly than those infected with *cagA*– strains.[22] A large Hong Kong study recently reported that patients with non-ulcer dyspepsia were more likely to harbour *cagA*+ strains than were asymptomatic

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controls (56% *v* 29%).[20] The implications of these studies are discussed later.

Although the function of *cagA* is unknown, *cagA*+ strains are associated with increased gastric inflammation in vivo, perhaps partly through induction of the pro-inflammatory cytokine interleukin 8 (IL-8).[19] Disruption of *cagA* does not affect IL-8 release from epithelial cell lines infected with *H pylori*, but disruption of two nearby genes, *picA* and B (permit induction of cytokine genes A and B) reduces it to near background levels.[23] *picA* and *B* are invariably linked with *cagA*, and it now seems that *cagA* is a genetic marker for a larger group of genes which has been termed the *cag* pathogenicity island. These genes have a different nucleotide ratio from other *H pylori* genes, and were thus probably originally acquired from another bacterial species. Some have sequence homology with genes involved in transmembrane trafficking in other organisms.[23] One hypothesis is that they induce enhanced inflammation by exporting an uncharacterised factor or factors which stimulate cytokine release.

vacA and the *cag* complex are the best characterised *H pylori* virulence determinants, but may not be the only ones. *H pylori* strains can be divided into two groups on the basis of direct activation of neutrophils during co-culture; one group induces rapid, strong neutrophil oxidative bursts, whereas the other produces delayed, weak activation.[24] In a Finnish study, 59% of patients with ulcers harboured strongly neutrophil inducing strains compared with 25% of non-ulcer patients.[24] The genetic basis of this phenomenon is unknown, and at present it does not offer a viable typing system. Another potential virulence factor is the newly described gene *iceA* (induced by contact with epithelium gene A) which, as the name suggests, was identified because its transcription is switched on following contact with cultured epithelial cells.[25] Like *vacA*, *iceA* is always present but exhibits notable variation. In a preliminary study from the USA, one form, type 1, was isolated from 67% of patients with ulcers, compared with only 23% of those without.[25] These findings are encouraging, but the association between *iceA* and ulcer disease must be confirmed, and a pathogenic link determined. *H pylori* strains differ quantitatively in various other features which may have pathological importance, including adherence, motility and urease expression. Thus, ongoing research may reveal new disease determinants on which further pathogenically relevant typing systems can be based.

H pylori virulence determinants are often associated with each other[14 16 25–27]—for example, *vacA* s1 strains are usually toxigenic and tend to be *cagA*+.[14] However, there are many exceptions,[14 16 25–27] and the suggested division of *H pylori* into the two broad categories of type I and type II bacteria[26] seems premature. The relation between virulence determinants makes it difficult to elucidate their relative importance for ulcer disease; the available data suggest that *vacA* genotype is an independent marker[15] and that *cagA* status is a better marker than in vitro cytotoxin activity.[27] Why virulence determinants are often associated with each other is unclear, particularly as those described are distant on the bacterial chromosome.[28] One possibility is that they are clonal markers, although the population structure of *H pylori* does not seem to be predominantly clonal.[29 30] The presence of mosaic genes[14] in particular implies genetic recombination in vivo: one strain acquires DNA from another and swaps it into its chromosome. This mixing of genetic elements, which has been likened to sexual reproduction in higher animals, means that specific alleles are unlikely to be good strain markers. Perhaps some form of functional linkage is more likely, whereby, for example, *vacA* s1 strains have survival advantage if they are *cagA*+.

Infection with a *cagA*+ strain increases the risk of both duodenal ulceration and gastric adenocarcinoma, yet patients with duodenal ulceration are at reduced risk of developing gastric adenocarcinoma in later life.[31] Thus, infection with *cagA*+ strains increases the likelihood of disease, but other factors seem to determine whether that disease is ulceration or carcinoma. One hypothesis is that host factors determine which disease develops: many people with *H pylori* infection have increased acid production (possibly predisposing to duodenal ulceration) but a subset remain hypochlorhydric and it has been suggested that these may be at risk of developing gastric adenocarcinoma.[32] Epidemiological evidence suggests that age at infection has some importance: children infected at younger ages are at increased risk of developing carcinoma in later life, although there is no evidence that later infection increases the risk of duodenal ulceration.[33] The environment seems to play a further role in that certain dietary factors including high salt and low antioxidant intake are risk factors for gastric carcinoma.[34] Conceivably, these factors could protect against ulcer disease by leading to early development of gastric atrophy and hence hypochlorhydria.

To predict the future place of strain typing in patient management it is essential that we define the exact importance of *vacA* genotypes and *cag* status for the development of disease. The data so far suggest that *vacA* s2 strains are minimally or non-ulcerogenic,[14–16] that *vacA* s1a strains commonly cause ulcers, and that s1b strains cause them less commonly.[15] The distinction between s1a and s1b strains may be largely academic: in practical terms, all these *vacA* s1 strains are potentially ulcerogenic. The main problem with *vacA* genotyping is that the methods used, PCR or DNA probe hybridisation, are usually considered research techniques. Detecting antibodies to toxin is simpler but its relevance is unclear; serum antibody production does not correlate well with the in vitro toxin activity of the infecting strain[35] and its relation with *vacA* genotype is unknown. Conversely, detecting anti-CagA antibodies shows infection with a *cagA*+ strain.[18] *cagA*+ strains can be thought of as potentially pathogenic, whereas *cagA*– strains are rarely associated with disease. The important remaining question is whether *cagA*– strains have low or no pathogenicity.

To date, the main reason for typing *H pylori* using potential virulence determinants has been to explore pathogenetic mechanisms. However, once the relation between *H pylori* virulence factors and disease is better clarified, testing for such factors could form a part of management strategies in several situations. It would probably make least difference for *H pylori* associated ulcer disease as this is a clear indication for *H pylori* eradication. Despite this, finding a non-pathogenic strain could lead one to suspect other aetiologies—for example, undisclosed aspirin or NSAID use. Treatment of non-ulcer dyspepsia could be advanced if the finding from Hong Kong that *cagA*+ strains are more common amongst sufferers than amongst asymptomatic controls is confirmed.[20] The hope here is that non-ulcer dyspeptics with *cagA*+ strains may represent a subgroup for whom *H pylori* eradication would be beneficial. Investigation of dyspepsia in the community, without knowledge of ulcer status, is another field in which a serological test for pathogenic potential could become important. Strategies have been suggested for deciding who to endoscope based on *H pylori* status—for example, only endoscoping patients under 45 years of age if they have positive serology.[36] Only performing endoscopies on those with CagA+ serology would reduce workload further. More radically, an argument could be made for treating *H pylori* in CagA seropositive dyspeptic patients

without knowledge of ulcer status. Currently, cancer prevention is not considered an indication for *H pylori* treatment as there is no direct evidence that treating *H pylori* reduces cancer risk. However, research is moving fast and a time may come when the evidence to support such a strategy is considered sufficient. If this situation arises, management of *H pylori* infection may be guided not only by grading the ulcerogenic potential of infecting strains, but also by grading their potential for carcinogenesis.

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John Atherton is funded by a Clinician Scientist Fellowship from the Medical Research Council.

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