The chips are down for *Helicobacter pylori*

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Microarrays can be used to demonstrate differences in genetic content between *Helicobacter pylori* strains, giving a foretaste of how research may be conducted in the near future

The whole genome sequence of *Helicobacter pylori* strain 26695 was published in 1997 and that of a second strain, J99, in 1999.^{1,2} These publications moved *H. pylori* research forward by a quantum leap. Comparing *H. pylori* genes with genes of known function in other bacteria gave immediate insights into *H. pylori* metabolism, structure, adaptive mechanisms, and virulence. Research became more focused in that workers could identify and study genes that were known to be important in other bacteria and appreciate their whole genetic context in *H. pylori*. For example, they could immediately see where an enzyme might fit in a metabolic pathway or what proteins were likely to comprise the *H. pylori* flagella assembly. Now, several groups have taken another quantum leap forward. They have constructed whole genome microarrays—ordered arrays of all identified *H. pylori* genes on glass slides—allowing experiments to be performed on all *H. pylori* genes simultaneously. Such microarrays have already been used to assess differences between *H. pylori* strains³ but the paper by Israel *et al* shows how they can be applied to research into pathogenesis.

Israel *et al* took two strains of *H. pylori*, one from a patient with a duodenal ulcer (DU) and one from a patient with a gastric ulcer (GU), and used them to infect Mongolian gerbils. The *H. pylori* infected Mongolian gerbil is the best (but still an imperfect) animal model of human *H. pylori* infection. Initial strain typing by Israel *et al* had suggested that both strains were fully virulent in that they both possessed several of the well known virulence markers, including the gene *cagA* (cytotoxin associated gene A). However, in the gerbil infections, the strain from the GU patient caused a much more severe gastritis and in a few gerbils caused gastric ulcers and gastric atrophy. In vitro, the GU strain stimulated epithelial cells to produce high levels of the proinflammatory cytokine interleukin 8. In contrast, the DU strain caused only mild gastritis in gerbils with none in the gastric corpus and stimulated only low level interleukin 8 production by epithelial cells in vitro. The DU strain also caused less epithelial cell proliferation and apoptosis than the GU strain, both in the animal model and in vitro.

The next step was to assess differences between the DU and GU strains of *H. pylori*, and to do this Israel *et al* used

Israel DA, Salama N, Arnold CN, *et al*. *Helicobacter pylori* strain-specific differences in genetic content, identified by microarray, influence host inflammatory responses. *J Clin Invest* 2001;107:611–20.

Helicobacter pylori enhances the risk for ulcer disease and gastric cancer, yet only a minority of *H. pylori*-colonized individuals develop disease. We examined the ability of two *H. pylori* isolates to induce differential host responses in vivo or in vitro, and then used an *H. pylori* whole genome microarray to identify bacterial determinants related to pathogenesis. Gastric ulcer strain B128 induced more severe gastritis, proliferation, and apoptosis in gerbil mucosa than did duodenal ulcer strain G1.1, and gastric ulceration and atrophy occurred only in B128+gerbils. In vitro, gerbil-passaged B128 derivatives significantly increased IL-8 secretion and apoptosis compared with G1.1 strains. DNA hybridization to the microarray identified several strain-specific differences in gene composition including a large deletion of the *cag* pathogenicity island in strain G1.1. Partial and complete disruption of the *cag* island in strain B128 attenuated induction of IL-8 in vitro and significantly decreased gastric inflammation in vivo. These results indicate that the ability of *H. pylori* to regulate epithelial cell responses related to inflammation depends on the presence of an intact *cag* pathogenicity island. Use of an *H. pylori* whole genome microarray is an effective method to identify differences in gene content between *H. pylori* strains that induce distinct pathological outcomes in a rodent model of *H. pylori* infection.

microarrays. They hybridised DNA from their two strains with copies of genes from the two genome sequenced strains, 26695 and J99. Hence they were able to show which genes were common to the GU and DU strains and which were different. The most marked difference was that the GU strain lacked a run of genes in the *cag* pathogenicity island. The *cag* pathogenicity island comprises a collection of genes next to *cagA* which have previously been shown to be important in inducing epithelial cells to produce proinflammatory cytokines (such as interleukin 8) and thus cause inflammation.^{4,5} Israel *et al* went on to experimentally mutate the GU strain by inactivating and deleting its *cag* pathogenicity island. They infected gerbils with the GU strain and its *cag*[−] mutants. The *cag*[−] mutants of the GU strain behaved very like the DU strain in that they produced less inflammation than the wild-type GU strain in the antrum and virtually none in the corpus. They also behaved like the DU strain in vitro stimulating only low level interleukin 8 production from epithelial cells. However, there were slight differences from the DU strain, the most obvious of which was that the *cag*[−] mutants still induced a similar level of epithelial cell apoptosis to their wild-type GU parent strain. Taken together, this work implied that most of the differences between the

DU and GU strains, and in particular the severity and distribution of gastric inflammation, were due to disruption of the *cag* pathogenicity island in the original DU strain. However, some differences, such as the effect on apoptosis, were due to other differences between the strains.

This study has several important messages. It confirms and extends a recent report using a *cagE* mutant (*cagE* being an important component of the *cag* pathogenicity island) which showed that functional *cag* pathogenicity island genes are important in *H. pylori* induced gastric inflammation in the gerbil.⁶ Importantly, it adds clinical relevance to this finding by showing that a strain with a natural partial *cag* deletion has a similar effect. However, it does not help us to understand differences between *H. pylori* strains associated with GUs or DUs as only single candidate strains were studied. Indeed, the DU strain in this study is atypical in that most DU strains have an intact *cag* pathogenicity island like the GU strain studied here.^{7,8} The findings in this paper are important but perhaps the study's best aspect is that in using microarrays to demonstrate strain differences throughout the genome, it gives a foretaste of how research may be conducted in the near future. What is more, as the paper's discussion points out, whole genome microarrays will get better. The microarray used in this study

could only detect major differences in genetic content whereas in the near future microarrays will be able to detect small differences in individual genes. That will be the next leap forward.

ACKNOWLEDGEMENTS

John Atherton is funded by a Senior Clinical Fellowship from the Medical Research Council.

Gut 2002;**50**:293–294

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