Animal models for host-pathogen interaction studies

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There is no model of Helicobacter pylori infection that exactly mimics the human diseases. In a particular, there are no good models of ulceration or gastric adenocarcinoma. Patterns of gastritis induced in the animals tend to be lymphocytic and lack the neutrophil infiltration typical of H. pylori infection in the adult. However, the animal models are starting to provide valuable information with respect to factors involved in the colonisation of the gastric mucosa and the importance of host factors in the development of gastric atrophy, as well as making possible the screening of potential therapeutic agents and vaccine candidates. Models include gnotobiotic piglets, primates, cats, dogs, ferrets and a range of rodents. Recent advances in the mouse models mean that they will allow us to dissect bacterial host interactions in a novel manner due to the availability of a wide range of immunological reagents and numerous mutant or transgenic strains.

We are still remarkably ignorant as to the pathogenesis of Helicobacter-associated disease. In particular, those factors that induce ulcer formation and gastric malignancy. We are starting to appreciate how these bacteria colonise gastric mucosa and induce inflammation, but there is no clear consensus as to what are those factors that determine the very different patterns of symptomatic disease seen within and between individual populations. In part, this lack of knowledge is due to a lack of an animal model that mimics the human disease. While those of us working with animal models all claim equivalence to human conditions, in reality our models fall short of what is needed. As yet, we have no readily reproducible model of ulceration or gastric adenocarcinoma. We can induce gastritis in animals but, in most cases, the patterns of inflammation resemble the lymphocytic gastritis seen in H. pylori infected children rather than the active/chronic gastritis seen in the infected adult. This lack of a perfect model is not uncommon in infectious disease. Salmonella typhi will only infect humans, there is no good animal model of cholera or campylobacteriosis and to mimic leprosy we need an armadillo. Thus, we have to work with what we have and, despite these limitations, the animal models of H. pylori infection are starting to provide insights into...
bacterial factors involved in colonisation and to highlight the importance of host factors in gastritis. They have proved useful as screens for novel antimicrobials1,2 and have made possible the development of human vaccines as is described elsewhere in this issue3. Each of the models described below have disadvantages and limitations and it is important these be appreciated by those wishing to undertake animal experimentation or to interpret the current literature on pathogenesis.

Primates

Although expensive, the primate would seem a logical model of H. pylori infection. However, many colonies are infected with monkey-adapted gastric helicobacters which morphologically resemble Helicobacter heilmannii4. This makes interpretation of experiments difficult. In some instances, primate colonies have become infected with H. pylori; whether these bacteria originated from human handlers is uncertain. However, it has been claimed that naturally infected monkeys are a model for human infection5. Importantly, in those animals infected solely with H. heilmannii, although the bacterium colonises in very large numbers, the gastritis is mild, whereas in monkeys co-colonised or solely infected with H. pylori the grading of inflammation is significantly higher. In a study with wild Japanese monkeys, the natural helicobacters were removed by antibiotic therapy and the animals re-infected with human isolates of H. pylori; these animals are being studied long term and results will be interesting, although the impact of the prior infection is difficult to assess6. One advantage of the primate models is that they can be followed by endoscopy7.

Pigs

The gnotobiotic piglet was the first successful animal model that involved infection with a human isolate of H. pylori8,9. The bacterium colonised in high concentration (10^6-8 cfu/g) and in a similar pattern to that seen in the human, although the inflammation was mononuclear with few neutrophils, i.e. a lymphocytic gastritis. The cellular response to infection was found to be predominantly T cells. CD4-like cells were associated with lymphoid follicles while, within the submucosa, a continuous layer of CD8 positive cells was observed. Mucosal production of anti-H. pylori antibody confirmed the antigen dependent nature of the local gastric immune and inflammatory responses10. Importantly, an active/chronic gastritis was seen in animals previously immunised systemically with
formalin killed *H. pylori*. The gnotobiotic piglet was the first model used to identify bacterial factors of *H. pylori* responsible for virulence. Thus mutants of the human pathogen deficient in motility and urease activity would not colonise the pig gastric mucosa. The major limitation of the piglet model, apart from the cost, was that the animals could only be kept in isolators for 3 months and, thus, the impact of chronic infection could not be determined. There have been attempts to overcome this problem by infection of specific pathogen free (SPF) adult pigs, however, results have been disappointing.

In a search of archival tissue from infected gnotobiotic piglets, small ulcers were observed in 10/39 animals while none were seen in uninfected controls. In Brazil, pigs from an industrial abattoir were investigated and there appeared to be an association between gastric ulceration of the pars oesophagus and infection with *H. heilmannii*. Given this part of the stomach is not seen in humans, the relevance of this finding is difficult to assess.

**Ferrets**

The ferret provides the only opportunity for extensive experimental investigation of a natural gastric helicobacter. *Helicobacter mustelae* is found on the gastric mucosa of all adult ferrets in the USA, as nearly all the animals come from the same commercial supplier. In other countries, animals may or nor be infected as sources are much more variable. Colonisation of the ferret gastric mucosa is similar to that seen in the human. The bacterium firmly adheres to the mucosa, mainly in the antrum. Unlike the human situation, very few bacteria are seen free in the mucus layer and bacteria are often endocytosed into the gastric epithelial cells. Chronic gastritis develops in infected animals but, once again, the neutrophil component of the response is lacking or low. Ulcers have been reported in adult animals, although not at a frequency high enough to allow experimental investigation of the lesions. Experimental investigation with the ferret model has been limited, however, infected animals do seem to be highly susceptible to chemical mutagens, suggesting a role for the bacterium in gastric carcinogenesis. Treatment of animals with acid suppressive therapy resulted in the excretion of live bacteria in the stools, an observation with tantalizing implications with respect to understanding the epidemiology of the human infection. The establishment of an *H. mustelae*-free ferret colony by Fox and colleagues opens up the way for studies of pathogenesis. Thus, experiments with isogenic mutants in the various flagella genes have provided interesting insights into the importance of motility in colonisation.
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**Cats and dogs**

Two single studies with germ-free dogs reveal that infection is possible with both *H. pylori* and *H. felis*. While these studies are unlikely to be repeated, they do emphasise an important aspect of bacterial host interaction. Namely, that the active component of the host response to infection with *Helicobacter* spp. is host dependent, as only a mononuclear response was seen in these young and, therefore, immunologically immature animals.

A chance occurrence in a laboratory colony of SPF cats has resulted in a model with great potential to dissect further these host factors. This colony, which is free of the natural feline helicobacters *H. heilmannii* and *H. felis*, became infected, presumably by human contact, with a bacterium with all the properties of a human *H. pylori* isolate. Subsequently, helicobacter-free SPF animals have been inoculated with a known human isolate and good colonisation was observed. The bacterium colonised mainly the antrum and cardia and induced an IgG serum response. In one animal, an active chronic gastritis was observed, but in others the inflammation was mainly mononuclear. The feline model has great potential for detailed investigation of host responses to *H. pylori* infection.

**Mouse models**

Mice are the most convenient animal model for any infection due to the economy of size and the availability of numerous immunological reagents and mutant strains of animals. However, the inability to colonise mice with human isolates of *H. pylori* restricted use of the rodent early on. Recent developments in mouse models ensure that they are likely to make an increasing contribution to our understanding of host bacterial interactions.

*Helicobacter felis*

*H. felis* is a feline gastric helicobacter that was shown to colonise the gastric mucosa of laboratory mice. Experiments in germ-free Swiss mice revealed not only excellent colonisation but also a florid active/chronic gastritis that mimicked the human pathology. Subsequent studies with other non germ-free strains of mice have revealed different patterns of inflammation. Indeed, it is the remarkable variations in pathology following infection of different strains of mice...
with the same culture of *H. felis* that provide the strongest evidence of the importance of host factors in *Helicobacter*-induced gastritis. Two groups have reported extensively on this phenomenon which we have termed host-dependent gastritis. Some mouse strains, (BALB/c and CBA) showed virtually no inflammation even after infection for up to 2 years, nearly the life span of the animal. In contrast, in SJL, C3H/He, DBA/2, and C57BL/6 infected mice, a severe to moderate gastritis was observed only in the body of the stomach, which increased in severity over time with specialised cells in the body glands being replaced. Studies using major histocompatibility complex (MHC)-congenic mice revealed probable contributions by both MHC and non-MHC genes to *Helicobacter*-induced inflammation. It is important to stress that the pattern of inflammation seen in these mouse strains was not typical of the active/chronic gastritis seen in the human. Rather, it has been suggested that this inflammation represents an important subset of the human pathology, namely atrophic gastritis. In mice, the severe pathology, which can completely destroy the mucosa, is confined to the body or acid secreting glandular tissue. Interestingly, this is not the area most heavily colonised with bacteria. Indeed, *H. felis* infection in mice is most commonly confined, almost exclusively, to the gastric antrum. Thus, the area of intense inflammation is removed from the area of heavy infection, the antrum, which remains histologically normal despite the presence of very large numbers of bacteria. Interestingly, as the atrophy in the body mucosa of infected mice increases in severity, the numbers of bacteria in the antrum decline. This parallels the decline in *H. pylori* infection seen in humans with gastric atrophy. Taken together, these observations suggest some type of autoimmune response as contributing to atrophy, a phenomenon likely to provide important insights into the human disease especially with respect to the precursor lesions of gastric cancer.

The basis of these cellular responses in *Helicobacter* infected mice is now being unravelled in an elegant series of experiments by the Czinn and Nedrud group. Adoptive transfer experiments with specific T cell clones from infected animals have shown that the severity of the inflammation is exacerbated by transfer of Th1 type CD4 T cells. One of the more fascinating hypotheses as to how *H. pylori* can survive in the host for such long periods of time, despite a vigorous cellular and systemic response against it, is that the bacterium somehow directs the T cell response towards the Th1 phenotype. The bacterium is thus exposed to a response more likely to impact on an intracellular parasite and which would be ineffective against a mucosal coloniser. These early mouse studies would suggest that this aspect of bacterial colonisation will be unravelled in these models.

The other main contribution of the *H. felis* mouse model, other than as a screening model for antimicrobial compounds or vaccine development,
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has been an increased understanding of those ecological factors that influence gastric colonisation, in particular the impact of the local acid environment\(^*\). Thus, in normal BALB/c mice, \textit{H. felis} colonises almost exclusively the antrum and cardia, \textit{i.e.} the non acid-secreting epithelia. When animals are put on acid suppression, the patterns of colonisation change and bacteria can now be seen in the normally acid-secreting body mucosa. The more complete the acid suppression, the more dramatic the effect. Thus, the proton pump inhibitor, omeprazole not only results in body colonisation and deep penetration down into the parietal cells, but the number of bacteria in the antrum decreases. This is exactly what happens to the patterns of gastritis seen in human \textit{H. pylori} infected patients on long term proton pump inhibitors\(^{37,38}\). The gastritis in the body increases while that in the antrum declines. These mouse studies, plus extensive analysis of the early literature on gastroduodenal disease, have resulted in the author and others proposing a major hypothesis on local acid output as one of the major determinants of \textit{H. pylori}-associated disease\(^{39}\). Recent \textit{in vitro} studies have implicated the urease enzyme as contributing to these effects\(^{40,41}\). It will be the animal models that will add further to these important concepts of bacterial-host interaction.

\textit{Helicobacter pylori}

Early studies showed that mice could not be infected by clinical isolates of \textit{H. pylori}\(^{28}\). The Japanese were the first to suggest that it may be possible to use mice infected with the human bacterium as useful animal models in their studies with nude and athymic and germ-free mice\(^{42}\). However, the colonisation levels appeared low and the model was not expanded by others. Marchetti and colleagues then reported on a mouse model using human isolates that they claimed mimicked the human infection\(^{43}\). This study was important as it showed immunisation could work against \textit{H. pylori} infection. However, close inspection of the data revealed serious deficiencies in this model for those interested in studying the interaction between bacteria and host. The levels of infection were remarkably low and bacteria could not be seen in tissue sections. Also, the figures of pathology were unconvincing and did not seem to resemble human pathology. Thus, while the possibility of a mouse-adapted \textit{H. pylori} model was established, there was a need for an alternative. At a meeting in Lausanne in 1996, a set of criteria were defined that listed what was required of such a mouse model\(^{44}\). Recently, we published our results on such a mouse-colonising strain, which we have called the ‘Sydney strain’ of \textit{H. pylori}, that appears to fulfil the Lausanne criteria\(^{45}\). This strain has been made widely available in an attempt to allow standardisation in mouse model work. Many groups
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around the world are using the Sydney strain and appear satisfied with its properties. It is a cagA, vacA positive strain that originated from a patient with a previous duodenal ulcer and a family history of serious gastric disease. Colonisation is variable but constant depending on the mouse strain used. Thus infection in BALB/c mice is low; however, in C57BL/6 mice, levels of $10^{6-7}$ cfu/g are obtained that remain constant for more than a year following infection, although a slight decline is seen. The bacterium colonises mostly the antrum and cardia and clearly adheres to the gastric mucosa with typical pedestal-like association (Fig. 1). Acid suppression will modify patterns of colonisation (S Van Zanten and A Lee, unpublished data) and immunisation will prevent infection or clear existing infection (Buck, Radcliff and Lee unpublished data). The model has been very successfully used in treatment trials (A. Lee and J O’Rourke, unpublished data).

However, it needs to be stressed that, as with all the animal models reported in this article, the pathology following infection with the Sydney strain does not truly mimic the human disease. In some mice strains, an atrophic gastritis is seen after several months of infection, but an early active/chronic gastritis is lacking. Given the high density of colonisation and the virulent nature of the isolate, this is disappointing. Indeed, these results strongly suggest that there is something intrinsically human-specific with respect to the induction of an active gastritis that we cannot mimic in animal models. Use of a wide range of genetically modified animals may reveal the basis of these differences.
The final use of the rodent models will be in investigation of gastric malignancy. No adenocarcinomas have been induced in long-term infected mice despite administration of chemical carcinogens (A Lee unpublished data). But many other long term-studies are presently underway. In contrast, there are very good models of Helicobacter-induced lymphomas. Long-term infection of BALB/c mice results in the appearance of lymphoid aggregations that are morphologically and histopathologically indistinguishable from the H. pylori associated low grade B cell lymphomas with characteristic lymphoepithelial lesions\textsuperscript{46}. Most lesions regress on anti-helicobacter therapy but we have noted progression to high grade tumours (Enno, unpublished observations). While, in its infancy and despite the long time required for the development of lymphomas, this model has clearly great potential with respect to identifying those critical factors needed for the genesis and maintenance of antigen driven tumours\textsuperscript{47}.

Other models

There are other reports from individual laboratories of other models of helicobacter infection. Thus, rats can be infected with either H. \textit{felis} or H. \textit{pylori}\textsuperscript{48}. Rodents infected with H. \textit{heilmannii} do appear to develop ulcer-like lesions more readily than H. \textit{pylori} or H. \textit{felis}\textsuperscript{49}. Recently, remarkable lesions have been reported in the Mongolian gerbil infected with human strains of H. \textit{pylori} despite an apparent low level of infection\textsuperscript{50-52}. This is a model that deserves extensive follow-up as the lesions appear very aggressive with a high degree of neutrophil activity.

Conclusion

While the cat and ferret models have the potential to provide information most relevant to the human disease, they are only available to a few. It is the increasing awareness of both the H. \textit{felis} model and the availability of the H. \textit{pylori} mouse models, in particular the Sydney strain (SS1), that is likely to produce most information in the next few years with respect to host-pathogen interactions. This is due to three reasons. Firstly, bacterial factors can be explored by use of isogenic mutants of mouse-colonising H. \textit{pylori} isolates. This will allow assessment of the importance of a myriad of physiological parameters in colonisation, thus providing opportunities for likely new therapeutic targets. Possession of the whole genome sequence of the bacterium will facilitate this process. Secondly, the availability of a multitude of genetically modified knockout
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or transgenic mice will allow a dissection, not only of factors responsible for colonisation and survival, but also of those factors necessary for induction of neutrophil activity or development of atrophy. Finally, these possibilities will be enhanced by increasing analysis of the cellular, humoral and cytokine responses of the human infection. These critical studies will lead to manipulations of the mouse models by, for example, cytokine enhancement or ablation that may at last mimic the true human pathology. This remains the ‘holy grail’ for all of us involved in animal experimentation.

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