Figure 1 A hierarchical stem cell model for the oesophagus. Stem cells in the epithelium (grey cells) can replenish the Barrett's or squamous epithelium in the absence of wounding. When the tissue-specific stem cell compartment is destroyed, it may be replenished by multipotent stem cells in the submucosal glands.

topic that is currently of intense interest in the cancer research community.

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Helicobacter bilis

Helicobacter bilis: bacterial provocateur orchestrates host immune responses to commensal flora in a model of inflammatory bowel disease

J G Fox

Helicobacter bilis can elicit heterologous immune responses to lower gut flora

The gastrointestinal tracts of mammals, including mice, are colonised with a diverse microecosystem. The caeca of normal conventional mice have been estimated to contain from 100 to

1000 individual species, and the number of bacteria can reach levels of up to 10¹¹ bacteria/g of faeces.^{1,2} These microorganisms provide essential nutrients for their host and also colonise mucosal niches,

which may partly influence the host response against microbial pathogens.^{2–7} By far, the greatest concentration and different types of bacteria comprising the gastrointestinal microflora of mice and humans reside in the caecum and colon.^{8–10} The fusiform-shaped bacteria, spirochetes and spiral-shaped bacteria were shown by histology to be almost exclusively located in the mucinous secretion overlaying the epithelium of the large bowel, and apparently in intimate association with the epithelial barrier.¹⁰ In the 1960s, Schaedler et al.¹¹ colonised germ-free mice with selected bacteria isolated from “normal” mice. He then supplied animal breeders with this series of microorganisms^{12,13} for use in colonising their mouse colonies. The one known as the Schaedler flora (SF) was the most popular. It contained eight anaerobic bacteria. In 1978, National Cancer Institute revised the SF of eight anaerobic bacteria to standardise the microbiota to be used in colonising axenic

(germ-free) rodents. The newly defined microbiota, now known as altered Schaedler flora (ASF), consisted of two lactobacilli, a *Bacteroides* sp, a spiral-shaped bacterium and four fusiform-shaped, extremely oxygen-sensitive bacteria.¹⁴

In the past, monitoring of the gnotobiotic animals relied on examining the morphology of the microorganisms with a limited evaluation of the organisms, biochemical traits and growth characteristics. Most published mouse studies evaluating the pathogenesis of inflammatory bowel disease (IBD) therefore simply refer to gut flora as conventional or specific pathogen-free (SPF) flora. Because of the limitations of the monitoring system used to identify the bacteria in ASF, as well as little or no characterisation of the flora in IBD mouse models, we recently characterised the phylogenetic positions of the ASF relative to known bacteria by using 16S rRNA sequence analysis¹⁵ and ascertained their distribution in the mouse gut using quantitative PCR.¹⁶ Importantly, this modified ASF is still being used by commercial breeders when establishing the gastrointestinal flora for their barrier-maintained SPF mice.

Although no animal model faithfully recapitulates all the features of IBD, rodent models that reproduce important hallmarks of the human disease are used extensively to investigate basic pathogenetic components of Crohn's disease and ulcerative colitis. Studies of the roles of endogenous microflora in IBD-like disease in mice are complicated by the fact that results depend on the management practices where the *in vivo* experiments are conducted. Under "conventional housing conditions", genetically engineered mice, particularly those with immune dysregulation, develop typhlocolitis when maintained in some facilities, but remain free of disease in others. The importance of the gastrointestinal flora is highlighted by the fact that germ-free mice, with this permissive IBD phenotype, do not develop IBD. More recently, investigators are using the newly identified murine pathogens, *Helicobacter hepaticus* and *H. bilis* to elicit IBD in these models.^{17, 18} *H. hepaticus* and *H. bilis* are endemic in most academic mouse colonies and have reliably induced disease in susceptible mouse strains in virtually all non-germ-free environments.^{19–24}

Jergens *et al.*²⁵ (see page 934) present interesting data using a variation of this model, which supports a role for *H. bilis* infection in eliciting typhlocolitis in defined ASF C3H/HeN mice, as well as an unanticipated finding that mice infected with *H. bilis* also develop an immune response to the commensal ASF, a response not observed in control mice nor in mice treated

with dextran sodium sulphate (DSS).²⁵ This model of IBD is intriguing and potentially very useful, given this inbred strain is an immunocompetent host, unlike the majority of IBD mouse models that have varying degrees of immune dysregulation. In their study, in addition to histological evaluation of intestinal tissues, the IgG1 (T helper cell 2 (Th2)) and IgG2a (Th1) serological immune responses to *H. bilis* and ASF in mice were measured using ELISA. Cytokine assessment of unfractionated single-cell suspensions of spleen and mesenteric lymph node (MLN) stimulated with concanavalin A (ConA) was conducted. ASF and *H. bilis* antigens were used to evaluate overall T cell responses.

Examination of the data indicated a robust IgG response to *H. bilis* in association with significant increases in cytokine responses, and revealed that the C3H/HeN strain had a balanced immune response to the helicobacter infection. Notably, both Th1- and Th2-associated cytokine responses to *in vitro* stimulation with *H. bilis* antigen were apparent. This is also reflected in histology data that demonstrated a mild to moderate inflammatory response one that is not so severe as to debilitate the host. The chronic inflammatory response to *H. bilis* that developed over a 10-week period may also explain why an immune response to ASF antigens was not observed in the acute DSS model.

The authors also measured antibody responses to each of the eight ASF species; however, they chose to present data as a mean of the composite antibody response for statistical comparisons. In aggregate, *H. bilis*-infected mice had statistically higher IgG levels (both Th1 and Th2) to ASF than controls. Although the data were not shown in detail, the authors stated that *H. bilis*-infected mice had higher overall IgG responses to four of the ASF (ASF 457, *Mucospirillum schaedleri*; ASF 500, low-content G+C-positive bacteria; ASF 502, *Clostridium* spp; and ASF 519, *Bacteroides distasonis*) than the other four ASF, two of which are *Lactobacillus* spp. C3H/HeJ Bir mice, which are reported to develop spontaneous colitis, also develop selective antibody reactivity to caecal bacterial antigens²⁶; these antibodies were composed primarily of IgG2a and, to a lesser extent, IgG1. However, unlike Jergens's findings, antibody reactivity was predominantly to antigens of aerobic and not to those of anaerobic bacteria. Interestingly, after rederivation of the C3H/HeJ Bir mice, likely to have been infected with enteric helicobacters and housed in SPF facilities (without helicobacters), there was a marked reduction in intestinal lesions.²⁶

The serological titre to *M. schaedleri* may, in part, be explained by the ability of this

organism to readily translocate across intestinal mucosa and colonise the liver of both conventional and mono-associated germ-free mice.^{27, 28} Whether the other three ASF-evoking systemic immune responses in the C3H/HeN mouse model have similar invasive properties is not known.⁷ Alternatively, *H. bilis*-infected mice may have activated dendritic cells that are more capable of processing luminal enteric bacterial antigens and/or may respond to antigens shared by *H. bilis* and select species of ASF.²⁹ It is tempting to speculate that lower antibody responses to lactobacilli, which may have probiotic anti-inflammatory activity, was reflected in *H. bilis*-infected mice that had only mild to moderate bowel inflammation and increased, but balanced, cytokine responses (interferon γ (IFN γ), tumour necrosis factor α (TNF α), interleukin 6 (IL6), IL10 and IL12) in splenocyte preparations.³⁰ It is important to determine whether the typhlocolitis observed in this model persists 10 weeks after *H. bilis* infection, the time point at which this study was concluded. If *H. bilis* had truly induced an ongoing lower bowel inflammatory response to ASF antigens, one would expect ongoing, chronic colitis. Alternatively, the host immune response may change the colonisation dynamics between *H. bilis* and ASF as well as between individual species of ASF as reported previously.^{31, 32} Future experiments are probably being planned by the authors to ascertain whether *H. bilis* perturbs the colonisation density or distribution of ASF in the intestinal tract in a manner similar to *H. hepaticus* in SW mice or *H. troglontum* infection in IL10^{-/-} mice.^{31, 32} These data may provide insight into the host's varying immune response to ASF after *H. bilis* infection and may help explain why serological IgG responses and inflammatory tissue responses (severity of lesion) decreased after an initial peak activity.

H. bilis infection also has had an unanticipated impact on primary gastric helicobacter infection in mice. Previous studies in our laboratory demonstrated that concurrent helminth infection modulated inflammation and gastric immune responses and reduced helicobacter-induced gastric atrophy.³³ With this in mind, we recently investigated the ability of *H. bilis* to modulate the pathogenesis of experimental *H. pylori* infection in C57BL/6 mice. Interestingly, mice coinfecting with *H. bilis* and *H. pylori* had significantly less gastric inflammation at both 6 and 11 months after infection compared with mice monoinfected with *H. pylori*. Serological analysis of IgG2c and IgG1 titres to *H. pylori* was predictive of gastritis scores at 6 and

11 months after infection, revealing significantly higher Th1-associated *H. pylori*-specific IgG2c responses to *H. pylori* in mice monoinfected with *H. pylori* than in mice coinfecting with *H. pylori* and *H. bilis*. Cytokine analysis also indicated that *H. pylori*-monoinfected mice had significantly increased levels of IFN γ and TNF α at both 6 and 11 months after infection, and significantly increased IL1 β and the aforementioned cytokines at 11 months when compared with mice coinfecting with *H. pylori* and *H. bilis* and control mice. These data suggest that concurrent infection with *H. bilis* can ameliorate gastric helicobacter pathology with concomitant attenuation of *H. pylori* Th1-mediated gastric and systemic immune responses.³⁴ Clearly, further studies are needed to determine the mechanisms for the protective effects of apparent heterologous immunity to *H. bilis* against helicobacter-associated gastric disease and to understand how *H. bilis* can elicit a heterologous immune response to lower gut flora, and whether, as Jergen *et al* suggest, this immune response to normal ASF flora plays a role in the initiation and progression of IBD. In this context, patients with Crohn's disease have immune responses to intestinal microflora.^{35–36} These provocative findings suggest that it is no longer sufficient to ascribe the initiation, progression or attenuation of gastrointestinal disease simply to the presence of undefined indigenous flora eliciting chronic inflammation after the loss of immune tolerance. Whether humans with IBD have key provocateur bacterial species orchestrating the host's response to the gastrointestinal microflora and the development or attenuation of chronic gastrointestinal disease remains an unsolved and largely unexplored hypothesis.

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