# Host non-responsiveness does not interfere with vaccine-mediated protection against gastric *Helicobacter* infection

Stacey Harbour[1], Hazel Mitchell[2] and Philip Sutton[1,3*]

[1]Centre for Animal Biotechnology, School of Veterinary Science, University of Melbourne, Parkville, Victoria 3010, Australia

[2]School of Biotechnology and Biomolecular Sciences, University of New South Wales, NSW 2052, Australia

[3]Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia

*Corresponding author:

Tel: +61 3 9936 6751; Fax: +61 3 9936 6528

Email: phil.sutton@mcri.edu.au

Keywords: *Helicobacter pylori*; vaccine; host responsiveness; gastritis;

Running head: Host non-responsiveness and vaccine-mediated protection against gastric *Helicobacter* infection

**ABSTRACT**

**Background:** *Helicobacter pylori* pathogenesis results from the inflammation induced by chronic infection. CBA mice are non-responsive to gastric *Helicobacter* infection, providing a useful model for examining host regulation of *Helicobacter*-induced gastritis. We examined whether gastric *Helicobacter* non-responsiveness impacts upon vaccine efficacy, and whether immune-mediated protection could occur in the absence of inflammation.

**Methods:** Mice were vaccinated prior to challenge with *H. felis* or *H. pylori*. Gastritis and *H. felis* colonization was evaluated histologically. *H. pylori* colonization was quantified by colony-forming assay.

**Results:** Immunizations protected CBA mice against challenge with either *H. felis* or *H. pylori*. Protection against *H. felis* was marked by a loss of non-responsiveness and development of an atrophic gastritis with mucus metaplasia. However vaccine-induced protection against *H. pylori* was only associated with cell infiltration into the gastric mucosa.

**Conclusions:** Non-responsiveness to gastric *Helicobacter* infection did not interfere with vaccination-induced protection. Vaccine-induced protective immunity against *H. pylori* was linked with the induction of cellular infiltration, but importantly not atrophic gastritis.

## INTRODUCTION

In some individuals, the persistent gastritis caused by infection of the gastric mucosa results in the development of *Helicobacter pylori*-related diseases, including peptic ulcer disease and gastric adenocarcinoma [1, 2]. However an estimated 80-85% of *H. pylori*-infected persons do not develop these associated pathologies, despite the presence of a bacterial-driven gastritis that lasts many decades. The reasons why some infected individuals develop disease while the majority remain asymptomatic multifactorial, and includes environmental as well as bacterial virulence factors [3, 4].

Human host genetic factors are also clearly linked to the susceptibility to *Helicobacter*-associated pathologies, in particular regulators of host immunity, and the pro-inflammatory cytokine IL-1ß [5]. The influence of host genetics on *Helicobacter* disease susceptibility was first demonstrated in mice, when it was shown that gastritis severity varied in inbred mouse strains infected with identical strains of bacteria. While C57BL/6, C3H/He, DBA/2 and SJL mice were responsive to the highly inflammatory gastric helicobacter, *H. felis*, others (BALB/c and CBA/Ca) developed no significant inflammation [6, 7]. Variability between mouse strains thus provides a useful tool for the study of host response in *Helicobacter* pathogenesis.

Further studies revealed that the non-responsiveness of CBA mice also applies to *H. pylori* infection, is dominantly inherited (the phenotype was passed on to 100% of offspring when crossed with three different responsive parents) and is associated with the production of IL-10 [8, 9]. The finding that this is a dominant trait indicates an active immunoregulatory process, rather than an immune defect, is responsible for the lack of gastritis observed in *Helicobacter* infected CBA mice.

While vaccinations against *H. pylori* can provide some reduction in colonization in mice, reliable vaccine-mediated sterilizing immunity remains elusive, as does a

complete understanding of the mechanism by which vaccinations reduce *H. pylori* colonization levels [10]. Significantly, immune-mediated protection against *Helicobacter* infection may be linked with the development of an exacerbated gastritis [11-13].

We have used the *Helicobacter* non-responder CBA mouse model to pose some key questions. First, to evaluate whether a host that is poorly responsive to *Helicobacter* infection has a defective vaccine-mediated protective immune response. Second, given the non-responsive nature of CBA mice, whether we could induce vaccine-mediated protection in the absence of gastric inflammation. Finally, whether *H. pylori* vaccination would impact upon the minimal *Helicobacter*-induced gastritis present in a resistant host.

## MATERIALS AND METHODS

### Animals

Mice used in these studies were age-matched females obtained from the Walter and Eliza Hall Institute, Melbourne, or the Animal Resources Centre, Perth, Australia. All animal experiments were approved by University Animal Ethics Committee.

### Immunization

*Helicobacter* lysates were prepared from *H. felis* strain CS1 [14], and *H. pylori* strain SS1 [15], as previously described [16, 17], protein content determined using the DC protein assay (BioRad, Regents Park, Australia) and then stored at -70˚C. Mice were immunized orogastrically with 4 weekly doses of 100 µL of PBS containing 1 mg of *Helicobacter* whole cell lysate adjuvanted with 10 µg cholera toxin (CT; Sigma Chemical Co., St Louis, MO.).

Four weeks after the last dose, mice were challenged with live bacteria. *Helicobacter* cultures were harvested and suspended in BHI and the final concentration adjusted to $10^9$ bacteria/mL. Mice were inoculated intragastrically once with 0.1 mL of bacterial suspension ($10^8$ bacteria).

Four weeks after challenge, stomachs were opened along the inner curvature and then divided into two halves. One half was fixed in neutral buffered formalin for histological assessment of gastritis and *H. felis* colonization where relevant. In the *H. pylori* experiment, the other half was used to quantify bacterial levels by colony-forming assay.

**Histological assessment of gastritis**

For assessment of gastric histopathology, blinded sections comprising strips of gastric tissue from the forestomach limiting ridge through the body to the antrum were stained with hematoxylin and eosin. Gastric inflammation was graded on the basis of cellular infiltrate (from 0-6) and mucus metaplasia and atrophy (from 0-3) as described in detail previously [18].

**Quantification of *Helicobacter* colonization**

For *H. felis*, fixed stomachs were embedded in paraffin, and 4 μm thick sections cut and stained by the modified Steiner silver method before quantification of bacterial colonization by histological assessment, as described previously [9]. *H. pylori* colonization levels were quantified by standard colony-forming assay [16].

**Statistics**

Statistical analyses were performed using the Statistical Package for the Social Sciences software, version 21.0. Differences in *H. felis* colonisation and gastric histopathology were assessed for significance by non-parametric Mann-Whitney (M-W) analysis. Differences in *H. pylori* colonisation was analysed by One-way ANOVA on log-transformed data.

## RESULTS

### Immunization of CBA mice confers protection against *Helicobacter felis* and overcomes non-responsiveness to infection

In two separate experiments, CBA mice were vaccinated against *H. felis*. In the first, CBA mice were compared with C57BL/6 mice as a control responder strain known to be protected by vaccinations and which develops gastritis when infected. The second study also included C3H/He mice as an extra control, also responsive to *Helicobacter* infection despite being MHC-identical to CBA mice. For both experiments, mice were immunized with *H. felis* lysate antigen plus CT adjuvant, while controls were sham vaccinated with antigen alone. Mice were subsequently challenged with *H. felis* before removal of stomachs for histological assessment of bacterial colonization and gastric pathology.

As expected, control CBA mice were non-responsive to *H. felis*, demonstrating a high level of infection but no evidence of gastritis (Figure 1). In both studies, immunization of CBA mice induced a significant protection against *H. felis* challenge that was equivalent to the control strains, despite the former's inherent non-responsiveness to gastric *Helicobacter* infection (Figure 1). However, this induction of protective immunity abrogated the non-responsive phenotype of these CBA mice, which presented with a typical gastric inflammation, including cellular infiltration,

mucus metaplasia and atrophy (Figure 1). Typical examples of the inflammatory response generated in these mice are shown in Figure 2.

The increased severity of gastritis evident in the stomachs of C57BL/6 mice (Figure 1) was a classic example of the documented phenomenon of post-immunization gastritis where immunization exacerbates gastritis following subsequent bacterial challenge [11, 12].

**Immunization of CBA mice confers protection against *Helicobacter pylori* and induces cellular infiltration**

We next evaluated whether the same effect occurred with *H. pylori*. As for *H. felis*, vaccination of CBA mice induced significant protection against subsequent *H. pylori* challenge, similar to that induced in C57BL/6 mice (Figure 3a). This vaccine-mediated protection in CBA mice was associated with a low level infiltration of immune cells into the gastric mucosa that was absent in the unvaccinated, infected controls. (Figure 3b). However, apart from this cell infiltrate, there was no evidence of pathological gastritis as marked by mucus metaplasia or atrophy in either the CBA or C57BL/6 mice (Figures 3c,d).

## DISCUSSION

It was first reported by Ermak *et al*. that effective prophylactic immunization against *Helicobacter* infection can result in an exacerbation of gastritis [11]. While this exacerbation could be removed by the antimicrobial eradication of residual bacteria [11] and was later shown to be a transient phenomenon [12], it raised the possibility that protective immunity may be intrinsically linked with severe inflammation. As gastritis is the key etiological factor in *H. pylori* pathogenesis, any potential association between vaccine-induced protection and inflammation is important.

We have used the CBA mouse as a model to evaluate the potential association between protective immunity and *Helicobacter*–induced gastritis, from a baseline of zero inflammation. Unlike other strains, CBA mice do not develop gastritis when infected with either *H. felis* or *H. pylori*, a dominantly inherited phenotype associated with production of the immunoregulatory cytokine, IL-10 [6, 8, 9]. BALB/c mice have also been described as minimal or non-responders due to their low inflammatory response to gastric *Helicobacter* infection [6, 7]. However, unlike CBA mice, the poor response of BALB/c mice is not dominantly inherited and an intermediate phenotype develops when they are crossed with C57BL/6 mice [19]. Hence the CBA mouse is probably the most un-responsive animal to gastric *Helicobacter* infection yet identified.

In this current study we have shown that the dominant non-responsiveness of CBA mice to *Helicobacter* infection in no way interferes with their ability to mount a protective immune response when vaccinated. In all three experiments presented in this study, the level of vaccine-induced protection achieved in CBA mice was similar to those of control responder strains of mice. This provides reassurance that individual variations in the host response to infection may not impact on the efficacy of a *H. pylori* vaccine if one were developed for humans.

A notable consequence of inducing protective immunity in the CBA mouse was a loss of their non-responsiveness. In the case of *H. felis* this involved a marked cellular infiltration into the gastric mucosa plus the development of severe mucus metaplasia and atrophy in most vaccinated/challenged CBA mice (Figures 1 and 2). This gastritis development in vaccinated CBA mice appeared effectively the same as the post-immunization gastritis reported in C57BL/6 mice [11]. This is only a short-term effect in C57BL/6 mice with the difference disappearing by 3 months post-challenge [12],

and we believe vaccinations induce a memory response that accelerates the initial development of gastritis upon subsequent *Helicobacter* challenge. There was no significant difference in gastritis between vaccinated CBA and C57BL/6 mice at one month post-challenge in this study. While we have not done longer term studies, we would predict that the post-immunization gastritis in CBA mice, as reported with C57BL/6 mice [12], would disappear over time.

With *H. pylori* however, there was no development of markers of severe gastric pathology, namely mucus metaplasia and atrophy, although a low level cellular infiltration did occur (Figure 3). This was also true for C57BL/6 mice in the *H. pylori* experiment reported here, although this has not always been the case in other studies [20]. Hence while severe post-immunization atrophic gastritis can be a feature of a protective immune response to *H. pylori* vaccination, it doesn't appear to be essential for the induction of protection. The appearance of cellular infiltration in the gastric mucosa of vaccinated but not unvaccinated, infected CBA mice is perhaps unsurprising, given any vaccine would be expected to induce an acquired immune response with the migration of immune cells into sites of active infection. Indeed, protective immunity against *H. pylori* is known to require a $CD4^+$ T-cell response, possibly involving Th17 cells [21, 22].

In conclusion, the vaccine-mediated induction of protective immunity against *H. pylori* was possible with no pathological gastritis and only a mild cell infiltration into the gastric mucosa. Most importantly, the successful immunization of CBA mice, the most non-responsive animal model available, suggests that the host response is unlikely to interfere with the development of a vaccine-induced protective immune response.

**ACKNOWLEDGMENTS**

This work was supported by the Victorian Government's Operational Infrastructure Support Program and by project grant GNT1002947 from the National Health and Medical Research Council of Australia.

## REFERENCES


1. Graham DY, Klein PD, Opekun AR, Boutton TW. Effect of age on the frequency of active *Campylobacter pylori* infection diagnosed by the [13C]urea breath test in normal subjects and patients with peptic ulcer disease. *The Journal of infectious diseases* 1988;157:777-80.

2. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. *Helicobacter pylori* infection and the development of gastric cancer. *The New England journal of medicine* 2001;345:784-9.

3. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995;55:2111-5.

4. Gaddy JA, Radin JN, Loh JT, Zhang F, Washington MK, Peek RM, Jr., Algood HM, Cover TL. High dietary salt intake exacerbates *Helicobacter pylori*-induced gastric carcinogenesis. *Infection and immunity* 2013;81:2258-67.

5. Snaith A, El-Omar EM. *Helicobacter pylori*: host genetics and disease outcomes. *Expert review of gastroenterology & hepatology* 2008;2:577-85.

6. Sakagami T, Dixon M, O'Rourke J, Howlett R, Alderuccio F, Vella J, Shimoyama T, Lee A. Atrophic gastric changes in both *Helicobacter felis* and *Helicobacter pylori* infected mice are host dependent and separate from antral gastritis. *Gut* 1996;39:639-48.

7. Mohammadi M, Redline R, Nedrud J, Czinn S. Role of the host in pathogenesis of *Helicobacter*-associated gastritis: *H. felis* infection of inbred and congenic mouse strains. *Infection and immunity* 1996;64:238-45.

8. Sutton P, Kolesnikow T, Danon S, Wilson J, Lee A. Dominant nonresponsiveness to *Helicobacter pylori* infection is associated with production of interleukin 10 but not gamma interferon. *Infection and immunity* 2000;68:4802-4.

9. Sutton P, Wilson J, Genta R, Torrey D, Savinainen A, Pappo J, Lee A. A genetic basis for atrophy: dominant non-responsiveness and helicobacter induced gastritis in F(1) hybrid mice. *Gut* 1999;45:335-40.

10. Sutton P, Chionh YT. Why can't we make an effective vaccine against *Helicobacter pylori*? *Expert review of vaccines* 2013;12:433-41.

11. Ermak TH, Ding R, Ekstein B, Hill J, Myers GA, Lee CK, Pappo J, Kleanthous HK, Monath TP. Gastritis in urease-immunized mice after *Helicobacter felis* challenge may be due to residual bacteria. *Gastroenterology* 1997;113:1118-28.

12. Sutton P, Danon SJ, Walker M, Thompson LJ, Wilson J, Kosaka T, Lee A. Post-immunisation gastritis and *Helicobacter* infection in the mouse: a long term study. *Gut* 2001;49:467-73.

13. Garhart CA, Redline RW, Nedrud JG, Czinn SJ. Clearance of *Helicobacter pylori* infection and resolution of postimmunization gastritis in a kinetic study of prophylactically immunized mice. *Infection and immunity* 2002;70:3529-38.

14. Lee A, Fox JG, Otto G, Murphy J. A small animal model of human *Helicobacter pylori* active chronic gastritis. *Gastroenterology* 1990;99:1315-23.

15. Lee A, O'Rourke J, De Ungria MC, Robertson B, Daskalopoulos G, Dixon MF. A standardized mouse model of *Helicobacter pylori* infection: introducing the Sydney strain. *Gastroenterology* 1997;112:1386-97.

16. Harbour SN, Every AL, Edwards S, Sutton P. Systemic immunization with unadjuvanted whole *Helicobacter pylori* protects mice against heterologous challenge. *Helicobacter* 2008;13:494-9.

17. Sutton P, O'Rourke J, Wilson J, Dixon MF, Lee A. Immunisation against *Helicobacter felis* infection protects against the development of gastric MALT Lymphoma. *Vaccine* 2004;22:2541-6.

18. Every AL, Ng GZ, Skene CD, Harbour SN, Walduck AK, McGuckin MA, Sutton P. Localized suppression of inflammation at sites of *Helicobacter pylori* colonization. *Infection and immunity* 2011;79:4186-92.

19. Walker MM, Worku M, Coggle S, Thursz MR. A novel method for assessing gastritis in the murine model demonstrates genetically determined variation in response to *Helicobacter felis* infection. *Helicobacter* 2002;7:265-8.

20. Chionh YT, Arulmuruganar A, Venditti E, Ng GZ, Han JX, Entwisle C, Ang CS, Colaco CA, McNulty S, Sutton P. Heat shock protein complex vaccination induces protection against *Helicobacter pylori* without exogenous adjuvant. *Vaccine* 2014;32:2350-8.

21. Delyria ES, Nedrud JG, Ernst PB, Alam MS, Redline RW, Ding H, Czinn SJ, Xu J, Blanchard TG. Vaccine-induced immunity against *Helicobacter pylori* in the absence of IL-17A. *Helicobacter* 2011;16:169-78.

22. Flach CF, Ostberg AK, Nilsson AT, Malefyt Rde W, Raghavan S. Proinflammatory cytokine gene expression in the stomach correlates with vaccine-induced protection against *Helicobacter pylori* infection in mice: an important role for interleukin-17 during the effector phase. *Infection and immunity* 2011;79:879-86.

## FIGURE LEGENDS

**FIGURE 1: Gastritis in immunized non-responder CBA mice, following challenge with *Helicobacter felis***

Mice were dosed with either *H. felis* lysate alone (Con) or *H. felis* lysate plus CT adjuvant (Vacc) before challenge with live *H. felis*. The effect of immunization on bacterial colonization and gastric pathology were assessed by histology. Data points show values for individual mice with bars representing group medians. P values show significant differences between vaccinated and control groups (M-W).

**FIGURE 2: Gastric morphology in immunized mice, challenged with *H. felis***

Non-immunized and immunized mice were challenged with *H. felis* and gastric pathology assessed 4 weeks post-infection. Images shown are gastric sections all taken under low power (x10 objective).

(A) An unimmunized, infected C57BL/6 mouse demonstrating a moderate cellular infiltration (CI) but no atrophy or mucus metaplasia.

(B) An immunized infected C57BL/6 mouse, with post-immunization gastritis marked by more severe CI, the loss of parietal cells and the development of mucus metaplasia (MM). A slightly reduced image is presented, in order to fit in the entire inflamed mucosa.

(C) An unimmunized, *H. felis* infected CBA mouse with normal morphology, indistinguishable from gastric tissues of uninfected mice (not shown).

(D) An immunized, *H. felis* infected CBA mouse with severe gastritis, including cellular infiltrate, severe mucus metaplasia and atrophy.

**FIGURE 3: Gastritis in immunized non-responder CBA mice, following challenge with *Helicobacter pylori***

Mice were dosed with either PBS alone (Con) or *H. pylori* lysate plus CT adjuvant (Vacc) before challenge with live *H. pylori*. The effect of immunization on bacterial colonization was assessed by colony-forming assay and on gastric pathology by histology. Data points show values for individual mice with bars representing group medians. P values show significant differences between vaccinated and control groups (a, ANOVA; b, M-W).

# Figure 1

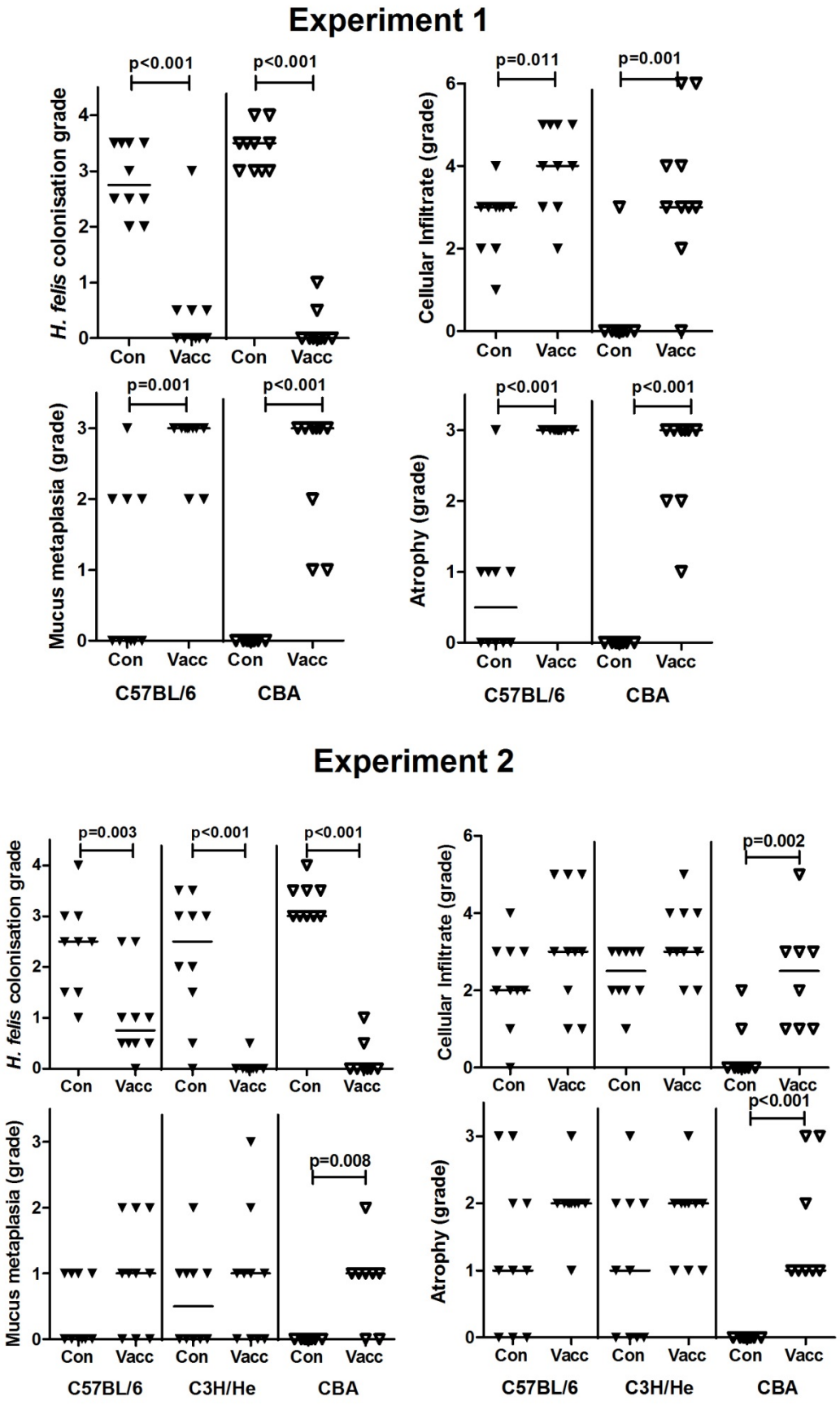
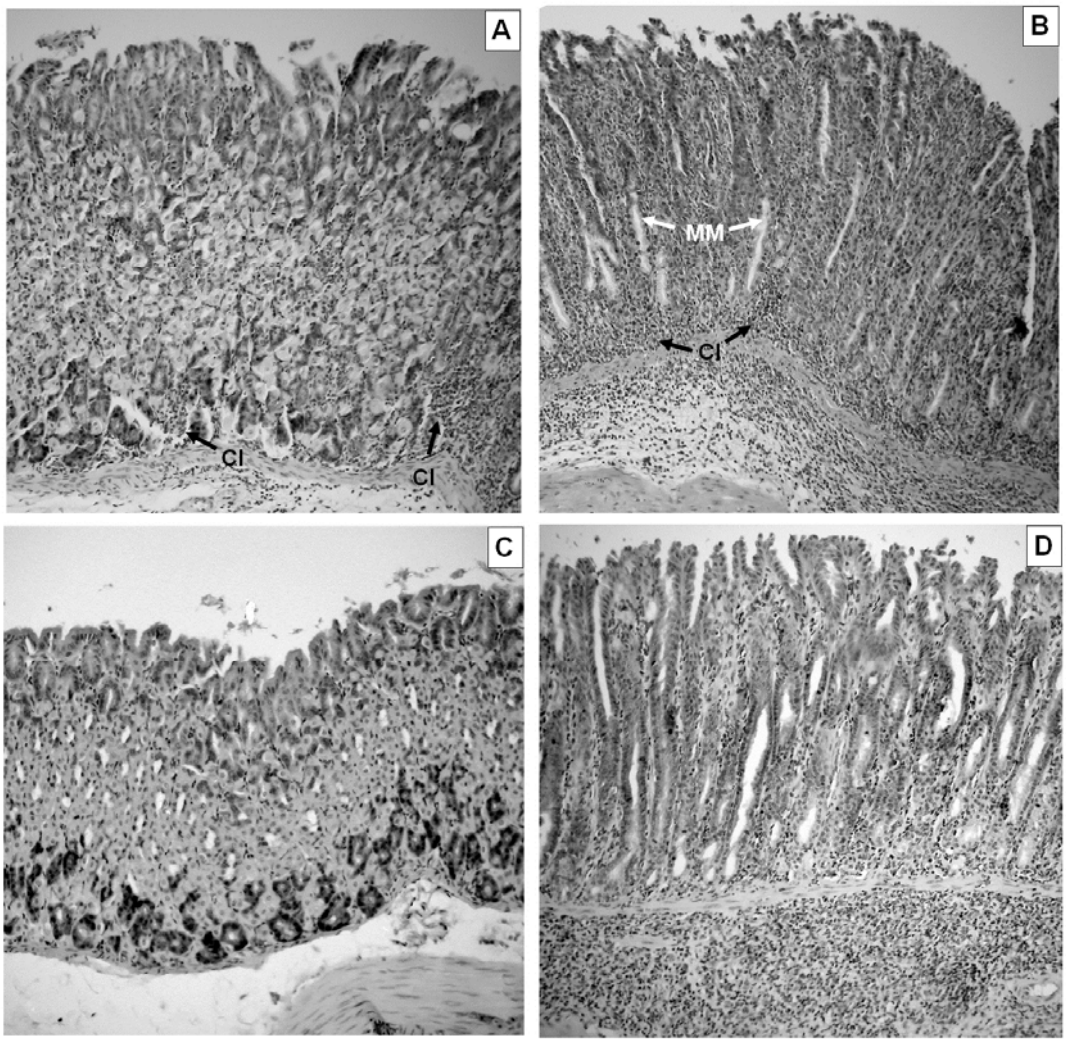
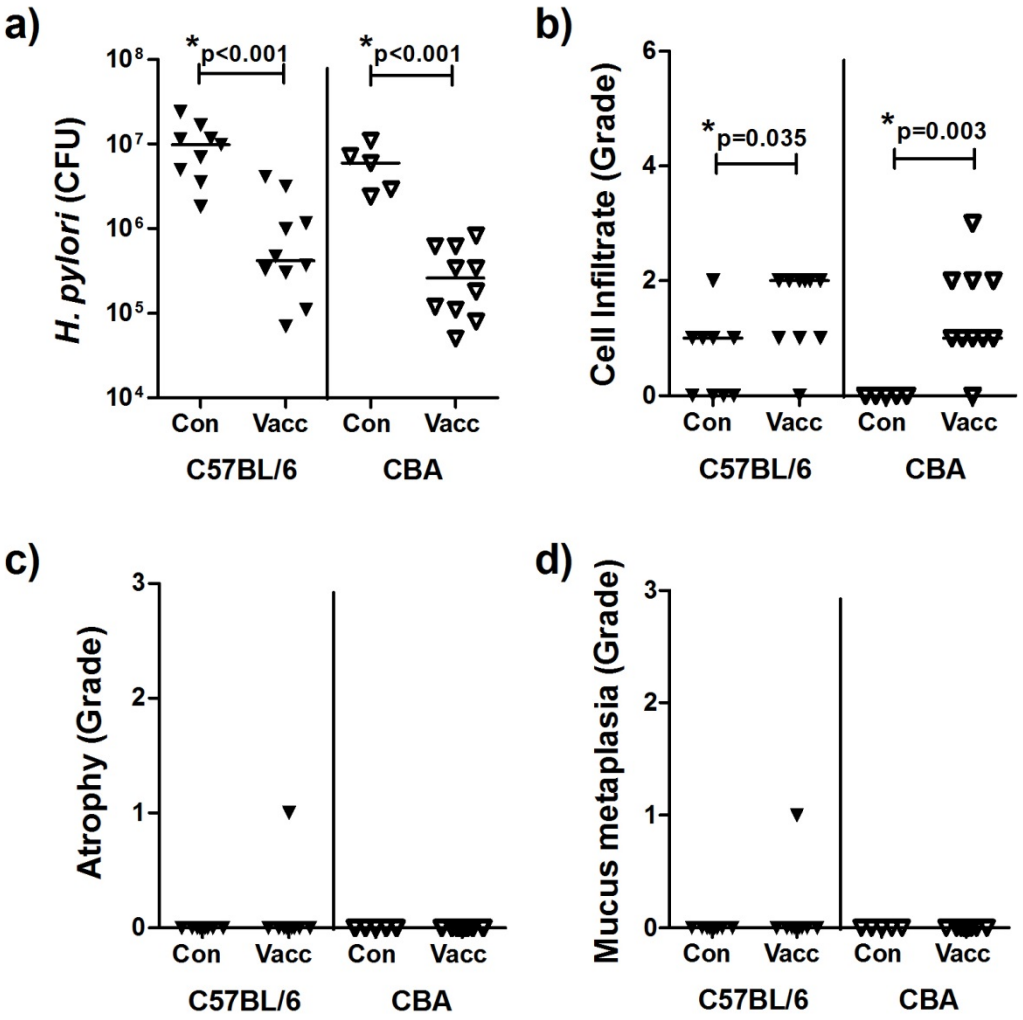

Experiment 1
H. felis colonisation grade
p<0.001
p<0.001
Cellular Infiltrate (grade)
p=0.011
p=0.001
Mucus metaplasia (grade)
p=0.001
p<0.001
Atrophy (grade)
p<0.001
p<0.001
Con
Vacc
C57BL/6
CBA
Experiment 2
H. felis colonisation grade
p=0.003
p<0.001
p<0.001
Cellular Infiltrate (grade)
p=0.002
Mucus metaplasia (grade)
p=0.008
Atrophy (grade)
p<0.001
C57BL/6
C3H/He
CBA

**Figure 2**



# Figure 3


a)
*p<0.001
*p<0.001
H. pylori (CFU)
10^8
10^7
10^6
10^5
10^4
Con
Vacc
Con
Vacc
C57BL/6
CBA
b)
*p=0.035
*p=0.003
Cell Infiltrate (Grade)
6
4
2
0
Con
Vacc
Con
Vacc
C57BL/6
CBA
c)
Atrophy (Grade)
3
2
1
0
Con
Vacc
Con
Vacc
C57BL/6
CBA
d)
Mucus metaplasia (Grade)
3
2
1
0
Con
Vacc
Con
Vacc
C57BL/6
CBA

**Author/s:**
Harbour, SN; Mitchell, HM; Sutton, P

**Title:**
Host Nonresponsiveness Does not Interfere With Vaccine-Mediated Protection Against Gastric Helicobacter Infection

**Date:**
2015-06-01

**Citation:**
Harbour, S. N., Mitchell, H. M. & Sutton, P. (2015). Host Nonresponsiveness Does not Interfere With Vaccine-Mediated Protection Against Gastric Helicobacter Infection. HELICOBACTER, 20 (3), pp.217-222. https://doi.org/10.1111/hel.12197.