DR PHILIP SUTTON (Orcid ID : 0000-0003-3129-1800)

Article type : Original Article

A lack of role for antibodies in regulating *Helicobacter pylori* colonization and associated gastritis

Umar Arshad¹, Sohinee Sarkar¹, Ghazal Alipour Talesh¹ and Philip Sutton^{1,2#}

¹ Mucosal Immunology, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia

² Department of Paediatrics, University of Melbourne, Parkville, Victoria 3010, Australia

#Correspondence:

Professor Phil Sutton,
Mucosal Immunology,
Murdoch Children's Research Institute,
Royal Children's Hospital,
Parkville, Victoria 3052,
Australia.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/HEL.12681](https://doi.org/10.1111/HEL.12681)

This article is protected by copyright. All rights reserved

phil.sutton@mcri.edu.au

Running Title: Antibodies do not regulate *H. pylori* colonization or associated gastritis

Author Manuscript

ABSTRACT

Background: *Helicobacter pylori* occupy a unique niche, located within the mucus layer lining the stomach, and attached to the apical surface of the gastric epithelium. As such, antibodies would be expected to play a major role in regulating infection and/or pathogenesis. However, experiments using antibody-deficient mice to study gastric helicobacter infection have yielded inconsistent results, although some pointed towards antibodies increasing colonization levels and decreasing gastritis severity. The variability in these studies is possibly due to their use of non-matched wildtype controls. This current study presents the first evaluation of the role of antibodies in *H. pylori* infection by comparing antibody-deficient mice with matched wildtype siblings. **Methods:** Matched wildtype and antibody-deficient μ MT mice were generated by heterozygous crossings. In two separate experiments, appropriately genotyped sibling littermates were infected with *H. pylori* for four months, then sera and stomachs collected. **Results:** There was no difference in *H. pylori* colonization levels between infected μ MT mice and sibling wildtype controls. Similarly, there was no significant difference in the severity of gastritis between these groups of mice, although there was a trend towards less severe gastritis in μ MT mice which was supported by a significantly lower *IFN* γ (Th1) gastric cytokine response. **Conclusions:** Comparing matched antibody-deficient and antibody-competent mice indicates that an antibody response does not influence *H. pylori* colonization levels. Contrary to previous studies, these results suggest antibodies might have a minor pro-inflammatory effect by promoting gastric Th1 cytokines, although this did not translate to a significant effect on gastritis severity.

INTRODUCTION

Chronic infection of the stomach by the bacterium *Helicobacter pylori* results in a long-term gastritis that is the main etiological factor in a number of pathological conditions, including gastric adenocarcinoma and peptic ulcer disease.^{1, 2} Infecting *H. pylori* are predominantly located within the mucus lining the gastric mucosa, with some of these bacteria attached to the apical surface of the gastric epithelium. By occupying this specialized niche, this important pathogen is effectively separated from direct contact with the cellular immune response and resides within the realm of mucosal immunity. Classically, the main component of the mucosal response to pathogenic infection within the gastrointestinal tract is considered to be a humoral response mainly involving IgA, although it is becoming increasingly recognized that IgG also plays an important role, being transported across the epithelium into the lumen of the gastrointestinal tract by the inaccurately named neonatal Fc receptor (FcRn).^{3, 4}

The positioning of *H. pylori* within the gastric lumen, combined with our current comprehension of the functionality of the gastrointestinal (GI) mucosal system has understandably resulted in an almost expectation that antibodies play an important role in regulating *H. pylori* colonization of the gastric mucosa as well as the ensuing inflammatory response that results from this infection. Such a view has indeed been investigated by a range of previous mouse studies that have used mice deficient in various aspects of humoral immunity to attempt to evaluate the impact of antibodies on *H. pylori* infection levels and associated gastritis.

However, the results generated by those studies have unfortunately been inconsistent and confusing. For example, two studies published in 1999 examined infection of *H. felis* (a close relative of *H. pylori* that provides a useful model for studying *Helicobacter*-induced gastritis)⁵ in antibody-deficient μ MT mice.⁶ While one study by Blanchard *et al* found no clear difference in *H. felis* colonization between infected μ MT mice and C57BL/6 wildtype controls,⁷ Roth *et al* found *H. felis* colonization of μ MT mice to be dramatically reduced, compared to wildtype controls, from 8 weeks post-infection.⁸ Counterintuitively with respect to these colonization findings, the Blanchard study found *H. felis* infected μ MT mice developed significantly more severe gastritis than infected controls in one of two experiments performed, while the Roth study found no difference in the severity of gastritis with the same infection between these same strains of mice.^{7, 8}

Starting around the same time, three other studies included experiments that involved antibody-deficient μ MT and control C57BL/6 mice infected with *H. pylori* strain SS1. While these studies were primarily exploring vaccinations against *H. pylori*, examination of the data within also presents contrasting results with respect to the effect of antibody deficiency in μ MT mice. While Ermak *et al.* found no difference in *H. pylori* colonization between infected μ MT mice and controls,⁹ a study by Akhiani *et al* from 2004 reported *H. pylori* colonization levels to be greatly reduced or lost in μ MT but not control mice, from 8 weeks post-infection,¹⁰ while the same authors did not find the same effect in a subsequent paper from 2006.¹¹ While Ermak *et al* didn't report on gastritis, both studies by Akhiani *et al* found that *H. pylori* infected μ MT mice exhibited an increased severity of gastritis compared to infected wildtype controls, despite the diverse effect of antibody deficiency on *H. pylori* colonisation.^{10, 11} This latter observation was similar to one of the two *H. felis* infection experiments performed by Blanchard *et al.*, but contrasted with the findings of Roth *et al.*^{7, 8}

Hence an overview of these studies reveals considerable variability, making it extremely difficult to draw meaningful conclusions as to the veracity of the data. Specifically, given this variability, despite numerous studies it remains unanswered as to whether antibodies do or do not play an important role in regulating colonization by *H. pylori* and/or, the severity of the resulting gastritis.¹⁰

In recent years, we have become increasingly aware of the powerful impact the microbiome, and in particular gut flora, plays in the host response to infection and inflammation, especially with regard to the GI tract.¹²⁻¹⁴ This means that subtle differences in the composition of the bacteria residing within the GI tract can have profound effects on pathogenic colonization, and resulting immune and inflammatory responses. In addition, there is increasing awareness of the potential impact of genetic drift within inbred mouse strains, whereby a line of an inbred mouse strain from one facility might possess genetic differences from the same inbred mouse strain housed elsewhere, such that these differences can exert a significant impact upon experimental outcomes.^{15, 16} These factors combined exemplify the importance of including perfectly matched control groups when evaluating genetic deficient mice, such as antibody-deficient μ MT.

The studies discussed above were performed up to twenty years ago, before we were aware of these complicating factors. It is therefore not surprising that, in those studies, the wildtype and μ MT mice were not matched appropriately as we would expect based on what is understood today. In three of the four studies, the wildtype and μ MT mice were obtained from different animal houses/suppliers, and so would be expected to have different microbiomes, as well as genetic differences. In the fourth study, while the wildtype and μ MT mice were bred within the same facility, they would still likely have considerable genetic differences and were not described as being co-housed, a recommended approach when attempting to minimize the effects of microbiome differences.¹⁷

Such differences in microbiome and genetic composition would readily explain the diverse results obtained by the studies performed previously evaluating the potential role of antibodies using μ MT mice. For this reason, we therefore undertook a new examination of the impact of antibodies on both *H. pylori* colonization and *H. pylori*-induced gastritis in mice. For this, and in contrast to previous reports, we performed a controlled study involving the infection of carefully matched littermates. In this evaluation, we found a deficiency in antibodies to have no effect on either *H. pylori* colonization or its associated gastritis.

MATERIALS AND METHODS

Mice

Animal experimentation was performed under institutional guidelines and with approval from the University of Melbourne Animal Ethics Committee. Mice were housed under specific pathogen free conditions with food and water *ad libitum*. Mice used in these experiments were generated by crossing heterozygous $\mu\text{MT}^{+/-}$ x $\mu\text{MT}^{+/-}$ mice with littermate wildtype and μMT mice identified by genotyping.

Genotyping of μMT and wildtype mice

DNA was obtained by incubating ear tags from 3-4 week old pups in alkaline lysis buffer containing 25 mM NaOH (Sigma-Aldrich, USA) and 0.2 mM EDTA (Merck Millipore, Australia) at 95°C for 30 minutes, after which neutralization buffer comprising 40 mM Tris-HCl (Roche Diagnostics, Australia) was added. Extracted DNA in supernatants were amplified by PCR using a T100™ Thermal Cycler (Bio-Rad, USA) using Promega Go Taq Green Master Mix, 0.2 μM forward and reverse primers, 2 μL of genomic DNA and 25 μL nuclease-free water. For genotyping, two separate PCR reactions targeting the wildtype allele and the neomycin gene inserted into the mutant allele were performed: Wildtype forward: 5' - CCG TCT AGC TTG AGC TAT TAG G -3'; Wildtype reverse: 5' -GAA GAG GAC GAT GAA GGT GG -3'; Knockout reverse: 5' -TTG TGC CCA GTC ATA GCC GAA T-3'. The PCR thermal profile was set to 94°C for 3 minutes, then 35 cycles of 94°C for 30 seconds, 66°C for 1 minute and 72°C for 1 minute, then 72°C for 2 minutes.

Helicobacter pylori infection

H. pylori strain SS1¹⁸ were grown on horse blood agar plates (7.6 g of Blood agar base No.2 (Oxoid, England) dissolved in 190 ml distilled water) in a 2.5L gas jar containing a Campygen gas pack (Oxoid) to generate microaerophilic conditions before cultivation in brain heart infusion (BHI) broth (Oxoid) containing 7.5% horse serum at 37°C under microaerophilic conditions in a shaking incubator (Excella E24, New Brunswick Scientific, Australia) at 150 rpm. Female mice¹⁹ were infected by a single oral gavage of 10⁷ *H. pylori* in 100 μL of BHI, with uninfected controls being sham dosed with 100 μL of sterile BHI.

Serum antibody response against *Helicobacter pylori* infection

The serum antibody response to *H. pylori* was measured by ELISA as previously.²⁰ 96-well immunoplates (Nunc, Denmark) were coated overnight at 4°C with 50 μL of 100 $\mu\text{g/mL}$ *H. pylori* lysate diluted in carbonate buffer, pH 9.6. Plates were blocked with 1% (w/v) bovine

serum albumin (BSA) in PBS, then sera (diluted 1:100 in 1% BSA/PBS) added to the wells. After washing, wells were incubated for 1 hour at room temperature with 50 μ L of goat anti-mouse IgG-HRP (1:10,000; Thermofisher, USA). Color was developed by addition of 100 μ L of 10 mg/mL 3,3',5,5-tetramethylbenzidine (TMB) (Sigma-Aldrich), and the reaction stopped with 2 mol/L H_2SO_4 . Absorbance was read at 450 nm on a Multiskan™ microplate spectrophotometer (Fisher Scientific, USA).

Quantification of *H. pylori* colonization levels in mice

Two mouse infection experiments were performed in this study. In the first experiment stomach homogenates were available, so *H. pylori* colonization was quantified by colony forming assay as described previously.²¹ In the second experiment, samples suitable for colony forming assay were not available, so *H. pylori* colonization levels were instead quantified by quantitative polymerase chain reaction (qRT-PCR). Longitudinally-halved mouse stomachs were homogenized in 1mL of TRIzol reagent (Invitrogen) using a PolyTron PT2100 (Kinematica AG) and genomic DNA (gDNA) and RNA extracted according to manufacturers' protocol (Invitrogen). gDNA was diluted 1:10 with nuclease-free water and used for the quantification of *H. pylori* SS1 genome copies (with primers targeting the 16S rRNA gene) relative to mouse *Gapdh* copies by quantitative real-time PCR (qPCR) in a Stratagene Mx3005P (Agilent Technologies). Reactions were set up in duplicates for each gene target in a total volume of 15 μ L containing 7.5 μ L of GoTaq® qPCR master mix (Promega), 5 μ L of diluted gDNA and 167 nM of each primer. Cycling conditions were as follows: initial denaturation at 95°C for 5 min, 45 cycles of denaturation at 95°C for 30s, annealing and extension at 60°C for 35s followed by a melt curve analysis. Primers used were: *H. pylori* 16S-rRNA F: CTATGACGGGTATCCGGC, R: ATTCCACCTACCTCTCCCA; mouse *Gapdh* F: TGCACCACCAACTGCTTAG, R: GGATGCAGGGATGATGTTC.^{22, 23}

Assessment of gastric pathology

The severity of gastric inflammation was performed as described previously.²⁴ Briefly, stomach sections stained with hematoxylin and eosin (H&E) were examined under light microscopy, in a blinded fashion, by two independent scorers (UA and PS). Stomachs were scored for three parameters, specifically cellular infiltration (CI), mucus metaplasia and atrophy. CI was graded by the presence of infiltrating lymphocytes and neutrophils: 1=mild multifocal; 2=mild widespread or moderate multifocal; 3=mild widespread and moderate multifocal; 4=moderate widespread or severe multifocal; 5=moderate widespread and severe

multifocal; and 6=severe widespread. Atrophy (loss of functional cells, i.e. parietal cells, in the gastric mucosa) and Mucus Metaplasia (appearance of mucus secreting cells in the gastric mucosa) was scored as 0, 1 (mild), 2 (moderate), or 3 (severe). Total gastritis scores were formulated by combining the scores of CI, atrophy and mucus metaplasia for an individual animal.

Quantification of cytokines by qRT-PCR

cDNA was reverse transcribed from the extracted RNA (1000ng RNA per reaction) using GoScript™ Reverse Transcription System (Promega) according to the manufacturer's instructions. Resultant cDNA (diluted 1:10) was used for the quantification of cytokines by qRT-PCR using 7500 fast real-time PCR system (Applied Biosystems). Reactions were set up in triplicates for each gene target per sample in a total volume of 15 µL containing 7.6 µL of GoTaq® qPCR master mix (Promega), 6.2 µL of diluted cDNA and 0.6 µL of 5µM stock of each primer. Cycling conditions were as follows: initial denaturation at 95°C for 3 min, 40 cycles of denaturation at 95°C for 30s, annealing and extension at 60°C for 30s followed by a melt curve analysis. Primers (Sigma) used were: *IFNγ* F: CAGCAACAGCAAGGCGAAA; R: CTGGACCTGTGGGTGTTGAC; *IL-17a* F: TCCAGAAGGCCCTCAGACTA; R: AGCATCTTCTCGACCCTGAA; *RPL32* F: ATCAGGCACCAGTCAGACCG, R: TGTGGACCAGGAAGTTGCGG. The average cycle threshold (Ct) values for each cytokine were normalized to the Ct of the housekeeping gene for the same sample.

Statistical analyses

Significances of differences between wildtype and µMT mice were determined by non-parametric analysis using the Mann-Whitney U-test and Graphpad Prism 7 software.

RESULTS

The role of antibodies in the control of *H. pylori* colonization and associated gastritis was evaluated in closely matched sibling animals. C57BL/6 and µMT mice (deficient in B cells and antibodies)⁶ were crossed to generate heterozygous µMT^{+/-} mice. Heterozygous µMT mice were then bred with other heterozygous µMT mice and all offspring genotyped to generate homozygous µMT mice and matching sibling littermate C57BL/6 wildtype controls.

Matched wildtype and µMT sibling littermates were infected with *H. pylori* in two separate experiments, with stomachs and sera collected for analysis at four months post-infection.

Four months was selected based on experience showing that this time point is ideal for observing a strong inflammatory response in the stomachs of infected C57BL/6 mice.

Sera were analyzed for the presence of antibodies against *H. pylori* in order to provide confirmation of the phenotype of the mice examined in these experiments. As expected, sera from infected wildtype but not μ MT mice contained antibodies against *H. pylori* antigens (Figure 1).

H. pylori colonization levels were quantified by colony-forming assay in the first experiment, and qRT-PCR in the second experiment. Irrespective of mode of quantification, the levels of *H. pylori* burden in wildtype and μ MT mice were similar and not significantly different (Figure 2).

To determine the effect of antibody-deficiency on *H. pylori*-induced gastritis, the severity of inflammation in the stomachs from these same mice were evaluated by histological examination of stained tissue sections. There was no significant difference in the severity of gastritis between *H. pylori* infected sibling littermate wildtype and μ MT mice, although the antibody-deficient mice trended towards lower levels of inflammation (Figure 3).

In order to further dissect this inflammatory response we analyzed the cytokine profiles of these tissues by qRT-PCR. As *H. pylori*-induced gastritis involves a mixed Th1- and Th17-type immune response, including in mice,²⁵⁻²⁸ we compared the levels of *IFN γ* (Th1) and *IL17A* (Th17) in the gastric tissues of sibling wildtype and μ MT mice. As would be expected, gastric tissues from infected WT mice had significant elevated levels of *IFN γ* and *IL17A* mRNA, as compared to uninfected wildtype controls (Figure 4), which is consistent with the increased severity of gastritis observed in these infected mice (Figure 3). However in μ MT mice, although mRNA levels of *IFN γ* and *IL17A* appeared higher in infected versus uninfected gastric tissues, this difference did not reach significance. Moreover, *IFN γ* mRNA levels in stomach from *H. pylori* infected μ MT mice were significantly lower than those of complimentary tissues stomach from infected sibling wildtype controls (Figure 4).

DISCUSSION

While antibodies are classically considered the primary defense against mucosal pathogens, including those within the GI tract, it remains unclear as to whether they play any role in protecting against *H. pylori* infection or associated gastritis. As discussed in the introduction, evidence from previous mouse studies using antibody-deficient mice have proven

inconsistent, possibly due to the use of non-matched control groups, which was standard practice at the time these earlier experiments were performed. With our improved understanding of the importance of matching genetic backgrounds and the major impact of the microbiome on host immunity, it is now important to revisit this question. This study provides the first evaluation of the role of antibodies in *H. pylori* infectivity and gastritis where antibody-deficient μ MT mice are compared with closely matching sibling littermate antibody-producing controls. By using matched sibling littermates, we are able to ensure the wildtype and antibody deficient mice used in these experiments are as genetically matched as possible, have no environmental differences during their derivation and antibody deficiency in μ MT mice does not overtly alter their microbiota.²⁹

While some early studies examining gastric *H. pylori* or *H. felis* infection in μ MT mice and unmatched C57BL/6 controls indicated no role for antibodies in controlling colonization, others suggested a lack of antibodies resulted in a reduction in *Helicobacter* colonisation.⁷⁻¹¹ Our comparison of matched sibling littermate wildtype and μ MT mice (as for all previous studies performed on a C57BL/6 background), found no significant effect of antibody deficiency on *H. pylori* infectivity, with *H. pylori* levels, if anything, being higher in μ MT mice. Hence antibodies do not appear to be important at controlling *H. pylori* infection in adult mice. This does not exclude the possibility that antibodies could play a role earlier in life; for example, antibodies in milk might still be protective, as suckling has been shown to protect the offspring of vaccinated mice.³⁰

A similar but opposite situation was evident from our evaluation of the effect of antibodies on *H. pylori*-induced gastritis. Several, though not all, previous studies involving unmatched μ MT and C57BL/6 controls suggested antibody deficiency leads to an increased severity of *H. pylori* induced gastritis.^{7, 10, 11} However, our comparison of matched littermates, not only found no significant difference in the severity of gastritis between wildtype and μ MT mice but had a possible trend towards a less severe *H. pylori*-induced gastritis in antibody deficiency. This trend towards a reduction in the severity of gastritis in the absence of antibodies was supported by our analysis of gastric pro-inflammatory cytokines, especially *IFN* γ which was significantly lower during *H. pylori* infection of μ MT mice. Interestingly, Akhiani et al (2006) reported that splenocytes from *H. pylori* infected μ MT mice also secreted less *IFN* γ in culture when stimulated with *H. pylori* lysate than did spleen cells from similarly infected wildtype cells.¹¹ While the μ MT and wildtype mice weren't matched, and no data from uninfected mice or unstimulated controls were presented,¹¹ this supports the

proposition that antibody-deficient mice produce a reduced Th1-type response to *H. pylori* infection.

The main conclusion to be drawn from the current study is that, when matched controls are used, an antibody response does not have a major effect on either colonization or gastritis. The observed effect of antibodies in some previous experiments was potentially due to minor genetic differences between the μ MT mice and unmatched C57BL/6 mice obtained from different colonies used in those studies, or variations in microbiome composition between those strains of mice, due to differences in housing or derivation.

An example of this is where one study obtained their μ MT mice from The Jackson Laboratories, and wildtype C57BL/6 controls from Taconic.⁷ Since that study was performed, it has been identified that the intestines of C57BL/6 mice at Taconic, but not Jackson, were colonized with segmented filamentous bacteria (SFB).³¹ These SFB were shown to have profound effects on the immune response of C57BL/6 mice to infection, especially in the GI tract and including an increased IL-17 and intestinal inflammatory response to bacterial infection.³¹ Hence differences in the source of mice and colonizing microbiota could clearly have impacted on the results of previous studies that compared mice obtained from these different suppliers, before such effects were known.

To summarize the findings presented in this paper, a comparison of *H. pylori* infection in appropriately matched antibody-deficient and antibody-competent mice has shown that, at least in mice, the presence of an antibody response does not appear to influence colonization levels of this important pathogen. There is an indication that antibodies might have a minor pro-inflammatory effect by promoting the gastric cytokine response, although this did not appear to translate to a significant effect on the severity of gastritis.

It is perhaps initially counterintuitive that antibodies are largely ineffective and almost irrelevant with respect to a major pathogen such as *H. pylori* that is predominantly non-invasive and resides on the luminal side of the gastric mucosal epithelium. However, this most likely reflects the highly evolved nature of *H. pylori* that by necessity have developed strategies to evade this important immune defense mechanism in order to facilitate an extremely chronic colonisation.³² In fact these observations effectively parallel those of the vaccine field, where it was initially assumed protection induced by immunization at the time was antibody mediated, until a number of vaccine studies involving knock-out mice convincingly demonstrated that vaccine-induced protection against *H. pylori* in these early

studies were also via a mechanism that was antibody independent.^{7, 9, 33} One of the earliest, but often forgotten studies, showed that *H. pylori* inhabit the gastric mucosa of chronically infected humans despite being coated with IgG, IgA and IgM antibodies,³⁴ which perhaps still remains the best demonstration of the general ineffectiveness of the natural humoral response to this pathogen.

ACKNOWLEDGEMENTS

This work was supported by grant GNT1120348 from the National Health and Medical Research Council of Australia and the Victorian Government's Operational Infrastructure Support Program. PS was supported by a fellowship from the DHB Foundation.

CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflict of interest.

FIGURE LEGENDS

FIGURE 1: A lack of serum antibody response in μ MT mice

Sera were collected from sibling littermate C57BL/6 wildtype (WT) and μ MT mice after 4 months infection with *H. pylori*. Anti-*H. pylori* IgG levels in the sera were quantified by ELISA. The boxplot presents the median (horizontal bar), interquartile range (box) and range (bars). Sera from infected wildtype but not μ MT mice contained antibodies against *H. pylori* lysate (*Mann-Whitney).

FIGURE 2: *Helicobacter pylori* colonization levels in antibody-deficient μ MT mice

H. pylori levels colonization in sibling littermate C57BL/6 wildtype (WT) and μ MT mice, at four months post-infection, were quantified by either colony-forming assay (Experiment 1; n=9 per group) or qRT-PCR (Experiment 2; n=6 per group). The boxplots present the median (horizontal bar), interquartile range (box) and range (bars). No significant difference was detected in *H. pylori* colonization levels between WT and μ MT mice (*p>0.05; Mann-Whitney).

FIGURE 3: Severity of *Helicobacter pylori*-induced gastritis in antibody-deficient μ MT mice

Tissue sections from the stomachs of sibling littermate C57BL/6 wildtype (WT) and μ MT mice, infected with *H. pylori* for 4 months, were H&E stained then evaluated for gastritis by a reader blinded to the identity of the mice. Each data point presents a value from an individual mice, with the data presented combined from two experiments performed. No significant difference was detected in the levels of cellular infiltration, atrophy, mucus metaplasia or total gastritis score, between infected WT and antibody deficient μ MT mice (* $p > 0.05$; Mann-Whitney).

FIGURE 4: Pro-inflammatory cytokine levels in gastric tissues from *Helicobacter pylori* infected antibody-deficient μ MT mice

Cytokine levels in the stomachs of sibling littermate C57BL/6 wildtype (WT; $n = 6/7$ per group) and μ MT ($n = 8$ per group) mice, either sham dosed or infected with *H. pylori* for 4 months, were quantified by qRT-PCR. The boxplots present the median (horizontal bar), interquartile range (box) and range (bars). Significantly different cytokine levels (* $p > 0.05$; Mann-Whitney).

REFERENCES

1. Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. *The New England journal of medicine*. 2001 Sep 13;345:784-9.
2. Wotherspoon AC, Doglioni C, Diss TC, et al. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. *Lancet*. 1993;342:575-7.
3. Ben Suleiman Y, Yoshida M, Nishiumi S, et al. Neonatal Fc receptor for IgG (FcRn) expressed in the gastric epithelium regulates bacterial infection in mice. *Mucosal immunology*. 2012 Jan;5:87-98.
4. Giragossian C, Clark T, Piche-Nicholas N, Bowman CJ. Neonatal Fc receptor and its role in the absorption, distribution, metabolism and excretion of immunoglobulin G-based biotherapeutics. *Curr Drug Metab*. 2013 Sep;14:764-90.
5. Lee A, Fox JG, Otto G, Murphy J. A small animal model of human *Helicobacter pylori* active chronic gastritis. *Gastroenterology*. 1990 Nov;99:1315-23.

6. Kitamura D, Roes J, Kuhn R, Rajewsky K. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. *Nature*. 1991 Apr 4;350:423-6.
7. Blanchard TG, Czinn SJ, Redline RW, Sigmund N, Harriman G, Nedrud JG. Antibody-independent protective mucosal immunity to gastric *Helicobacter* infection in mice. *Cell Immunol*. 1999;191:74-80.
8. Roth KA, Kapadia SB, Martin SM, Lorenz RG. Cellular immune responses are essential for the development of *Helicobacter felis*-associated gastric pathology. *J Immunol*. 1999 Aug 1;163:1490-7.
9. Ermak TH, Giannasca PJ, Nichols R, et al. Immunization of mice with urease vaccine affords protection against *Helicobacter pylori* infection in the absence of antibodies and is mediated by MHC class II-restricted responses. *J Exp Med*. 1998 Dec 21;188:2277-88.
10. Akhiani AA, Schon K, Franzen LE, Pappo J, Lycke N. Helicobacter pylori-specific antibodies impair the development of gastritis, facilitate bacterial colonization, and counteract resistance against infection. *J Immunol*. 2004 Apr 15;172:5024-33.
11. Akhiani AA, Stensson A, Schon K, Lycke N. The nontoxic CTA1-DD adjuvant enhances protective immunity against *Helicobacter pylori* infection following mucosal immunization. *Scandinavian journal of immunology*. 2006 Feb;63:97-105.
12. Helmink BA, Khan MAW, Hermann A, Gopalakrishnan V, Wargo JA. The microbiome, cancer, and cancer therapy. *Nature medicine*. 2019 Mar;25:377-88.
13. Kamada N, Chen GY, Inohara N, Nunez G. Control of pathogens and pathobionts by the gut microbiota. *Nature immunology*. 2013 Jul;14:685-90.
14. Kamada N, Seo SU, Chen GY, Nunez G. Role of the gut microbiota in immunity and inflammatory disease. *Nature reviews Immunology*. 2013 May;13:321-35.
15. Reardon S. Lab mice's ancestral 'Eve' gets her genome sequenced. *Nature*. 2017 Nov 13;551:281.
16. Casellas J. Inbred mouse strains and genetic stability: a review. *Animal*. 2011 Jan;5:1-7.
17. Omary MB, Cohen DE, El-Omar E, et al. Not All Mice Are the Same: Standardization of Animal Research Data Presentation. *Gut*. 2016 Jun;65:894-5.
18. 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 Apr;112:1386-97.

19. Every AL, Ng GZ, Skene CD, et al. Localized suppression of inflammation at sites of *Helicobacter pylori* colonization. *Infection and immunity*. 2011 Oct;79:4186-92.
20. Stent A, Every AL, Chionh YT, Ng GZ, Sutton P. Superoxide dismutase from *Helicobacter pylori* suppresses the production of pro-inflammatory cytokines during *in vivo* infection. *Helicobacter*. 2018 Feb;23.
21. McGuckin MA, Every AL, Skene CD, et al. Muc1 mucin limits both *Helicobacter pylori* colonization of the murine gastric mucosa and associated gastritis. *Gastroenterology*. 2007 Oct;133:1210-8.
22. Riley LK, Franklin CL, Hook RR, Jr., Besch-Williford C. Identification of murine helicobacters by PCR and restriction enzyme analyses. *Journal of clinical microbiology*. 1996 Apr;34:942-6.
23. Menheniott TR, O'Connor L, Chionh YT, et al. Loss of gastrokine-2 drives premalignant gastric inflammation and tumor progression. *The Journal of clinical investigation*. 2016 Apr 1;126:1383-400.
24. Sutton P, Danon SJ, Walker M, et al. Post-immunisation gastritis and *Helicobacter* infection in the mouse: a long term study. *Gut*. 2001;49:467-73.
25. Eaton KA, Mefford M, Thevenot T. The role of T cell subsets and cytokines in the pathogenesis of *Helicobacter pylori* gastritis in mice. *J Immunol*. 2001 Jun 15;166:7456-61.
26. Sawai N, Kita M, Kodama T, et al. Role of gamma interferon in *Helicobacter pylori*-induced gastric inflammatory responses in a mouse model. *Infection and immunity*. 1999 Jan;67:279-85.
27. Algood HM, Gallo-Romero J, Wilson KT, Peek RM, Jr., Cover TL. Host response to *Helicobacter pylori* infection before initiation of the adaptive immune response. *FEMS immunology and medical microbiology*. 2007 Dec;51:577-86.
28. Shiomi S, Toriie A, Imamura S, et al. IL-17 is involved in *Helicobacter pylori*-induced gastric inflammatory responses in a mouse model. *Helicobacter*. 2008 Dec;13:518-24.
29. Marcotte H, Lavoie MC. No apparent influence of immunoglobulins on indigenous oral and intestinal microbiota of mice. *Infection and immunity*. 1996 Nov;64:4694-9.
30. Gorrell RJ, Robins-Browne RM. Antibody-mediated protection against infection with *Helicobacter pylori* in a suckling mouse model of passive immunity. *Infection and immunity*. 2009 Nov;77:5116-29.
31. Ivanov, II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009 Oct 30;139:485-98.

32. Sutton P, Chionh YT. Why can't we make an effective vaccine against *Helicobacter pylori*? *Expert review of vaccines*. 2013 Apr;12:433-41.
33. Sutton P, Wilson J, Kosaka T, Wolowczuk I, Lee A. Therapeutic immunization against *Helicobacter pylori* infection in the absence of antibodies. *Immunology and cell biology*. 2000 Feb;78:28-30.
34. Wyatt JL, Rathbone BJ, Heatley RV. Local immune response to gastric *Campylobacter* in non-ulcer dyspepsia. *Journal of clinical pathology*. 1986 Aug;39:863-70.

hel_12681_f1.jpg

Experiment 1 CFU

Experiment 2 qRT-PCR

hel_12681_f2.jpg

hel_12681_f3.jpg

hel_12681_f4.jpg

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Arshad, U;Sarkar, S;Alipour Talesh, G;Sutton, P

Title:

A lack of role for antibodies in regulating *Helicobacter pylori* colonization and associated gastritis

Date:

2020-02-23

Citation:

Arshad, U., Sarkar, S., Alipour Talesh, G. & Sutton, P. (2020). A lack of role for antibodies in regulating *Helicobacter pylori* colonization and associated gastritis. *HELICOBACTER*, 25 (2), <https://doi.org/10.1111/hel.12681>.

Persistent Link:

<http://hdl.handle.net/11343/275451>