Investigating The Role of IL-17A Signalling In Protection Against *Helicobacter pylori* Infection

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Helicobacter pylori causes chronic gastric disease and cancer

H. pylori are Gram negative spiral shaped, highly motile bacteria that can survive in the high pH of the stomach. *H. pylori* is one of the leading causes of chronic gastric disease and gastric cancer worldwide, it is becoming increasingly resistant to antibiotic intervention and the natural immune response causes inflammation leading to many of the symptoms suffered (5). The link between *H. pylori* and gastric ulcers discovered in the 1980s by Australians Barry Marshall and Robin Warren who won the Nobel prize for their work. *H. pylori* is categorised as a class 1 carcinogen by the World Health Organization.

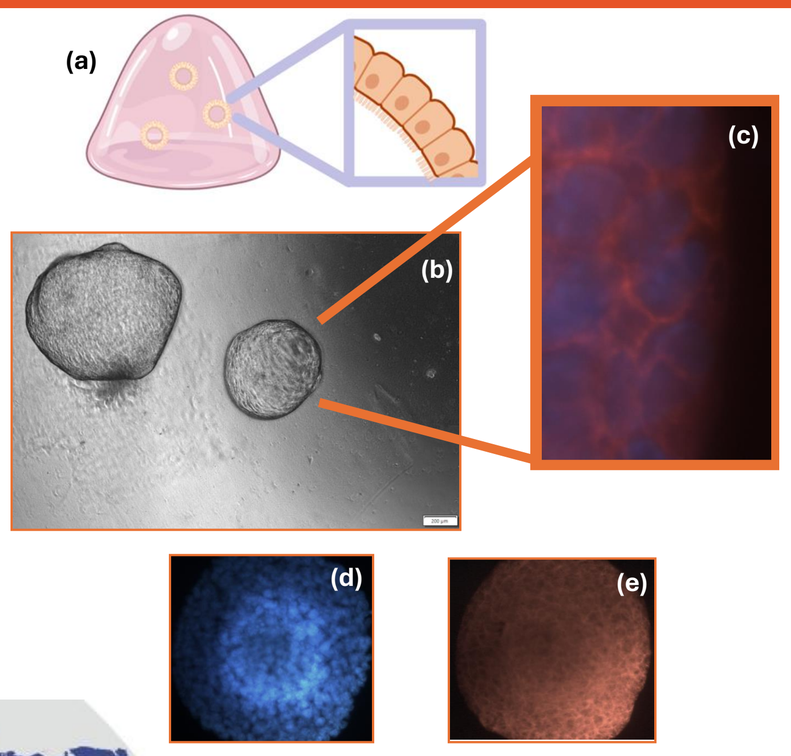


Figure 3. (a) Organoids in Matrigel showing cell structure produced (b) Mouse gastric organoids in Matrigel passage 6 after 7 days (c) DAPI and Phalloidin stain showing the correctly polarised cells (d) Phalloidin staining actin filaments of organoid at x40 magnification (e) DAPI staining the nucleus of organoid x40 magnification

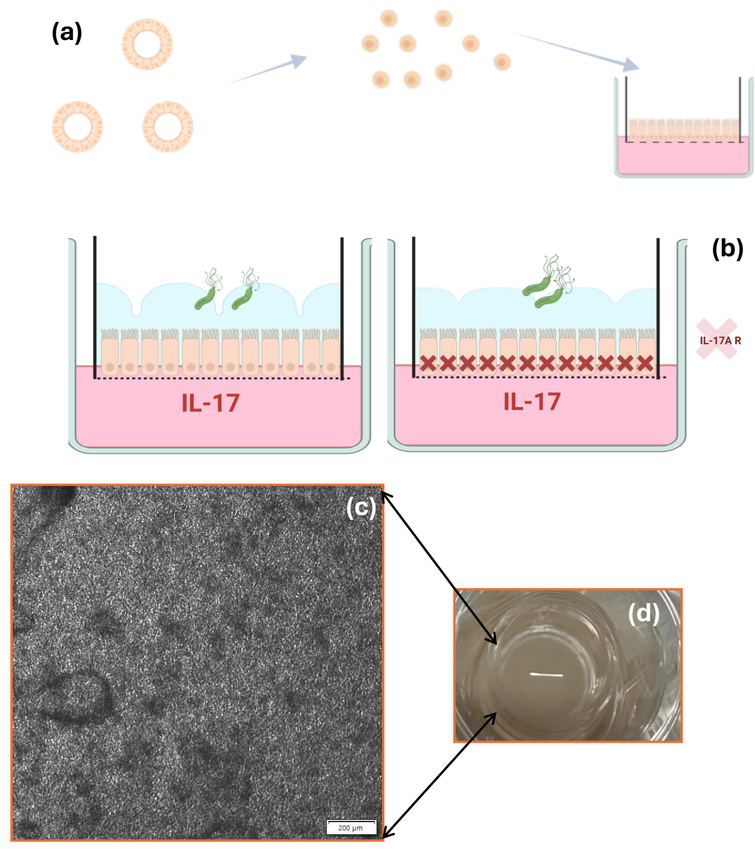
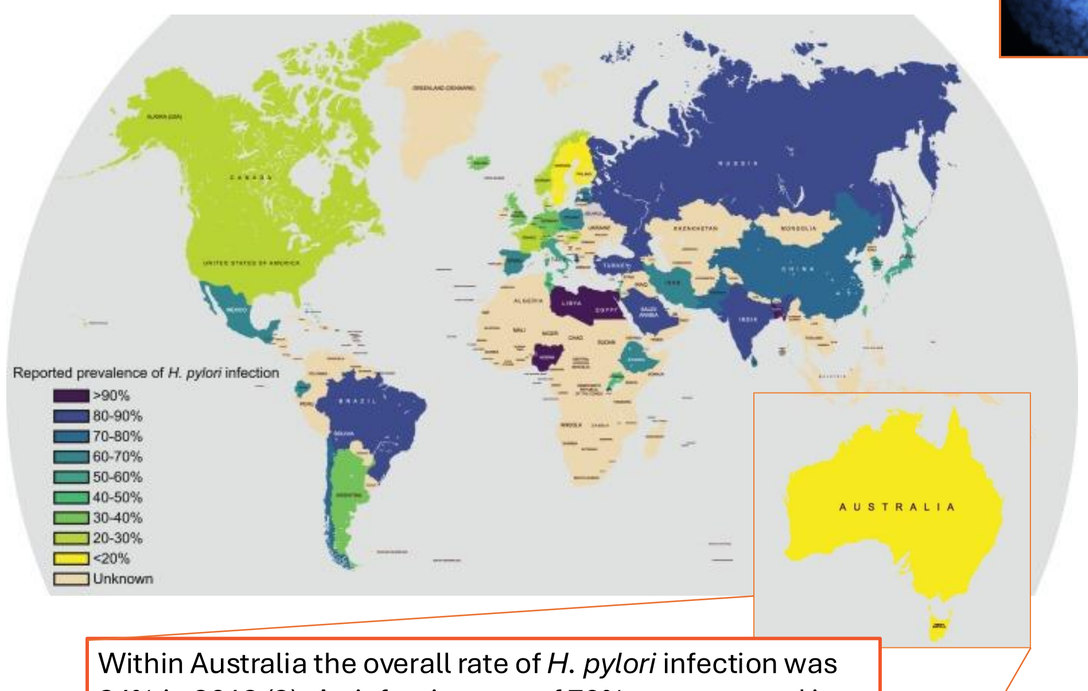


Figure 4. (a) Spheroids are trypsinised and used to seed trans well membrane with epithelial cells (b) Experimental design for air liquid interface challenged with *H. pylori CRISPR knockout of IL-17 receptor* (c) Trans membrane well with gastric epithelial cells in air liquid interface (d) Microscopic view of trans membrane air liquid interface well looking from above



Within Australia the overall rate of *H. pylori* infection was 24% in 2012 (2). An infection rate of 76% was reported in an Indigenous population in remote rural Western Australia in 2005 (2). High rates of infection have also been reported in refugees and vulnerable communities. **There is a lack of current data on current infection rates in Australia.**

Figure 1. Prevalence of *H. pylori infection* worldwide. The infection rates of *H. pylori* are as high as 85-95% in developing countries and approximately 30-50% in developed countries (1). Compounding the infection rate is the presence of antibiotic resistance to many first line antibiotics and increasing resistance reported globally (1).

Organoid models for *H. pylori* research

Traditionally, *H. pylori* research has been conducted in large part using animal models, organoid models offer a new approach allowing for the correctly polarised cells to be ethically, rapidly, and cost effectively investigated (5).

AIMS

- **To investigate the role IL-17A in protection against *H. pylori* infection**
  - **Knockdown of IL-17A Receptor using CRISPR Cas9 in a gastric organoid model**
  - **Investigate effect of IL17A R deletion in organoids challenged with *H. pylori***

Role of IL-17A Signalling In Protection Against *Helicobacter pylori* Infection

Interleukin-17 (IL-17) is a cytokine that plays a key role in immune responses to bacterial infections. This cytokine important in the regulation of the T-cell response (4). There is evidence that the role of IL-17 signalling is an important component in the protective mechanism for the host against *H. pylori*. The removal of IL-17 has been shown to eliminate protection from *H. pylori* in immunised mice (4,5). IL-17A is produced by activated T helper 17 (Th17) cells and is a proinflammatory mediator for inflammation immune responses.

Design of CRISPR Cas9 guides for IL-17 receptor knockout

Design guide RNA for CRISPR Cas9 knockout using computer assisted design (Benchling). Used to locate target site for knockout in the IL-17A receptor sequence

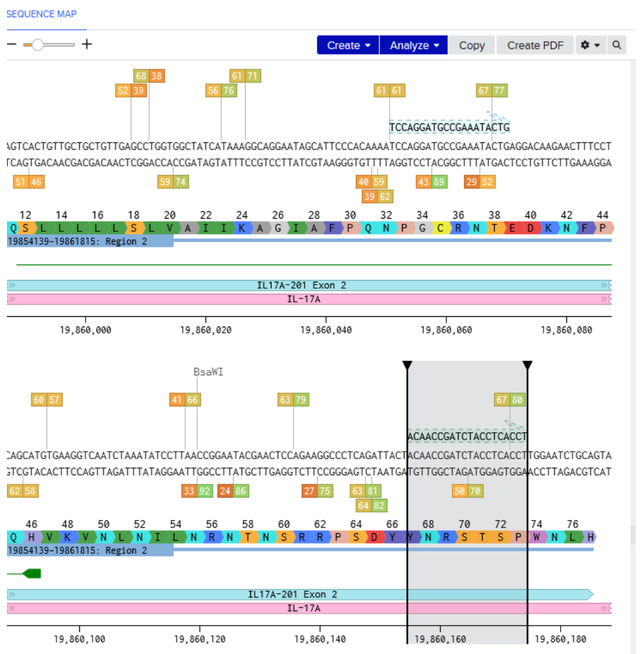


Figure 2. Shows interleukin-17A region in mouse DNA for potential target sites, the highlighted region shows the target sequence for the guide RNA.

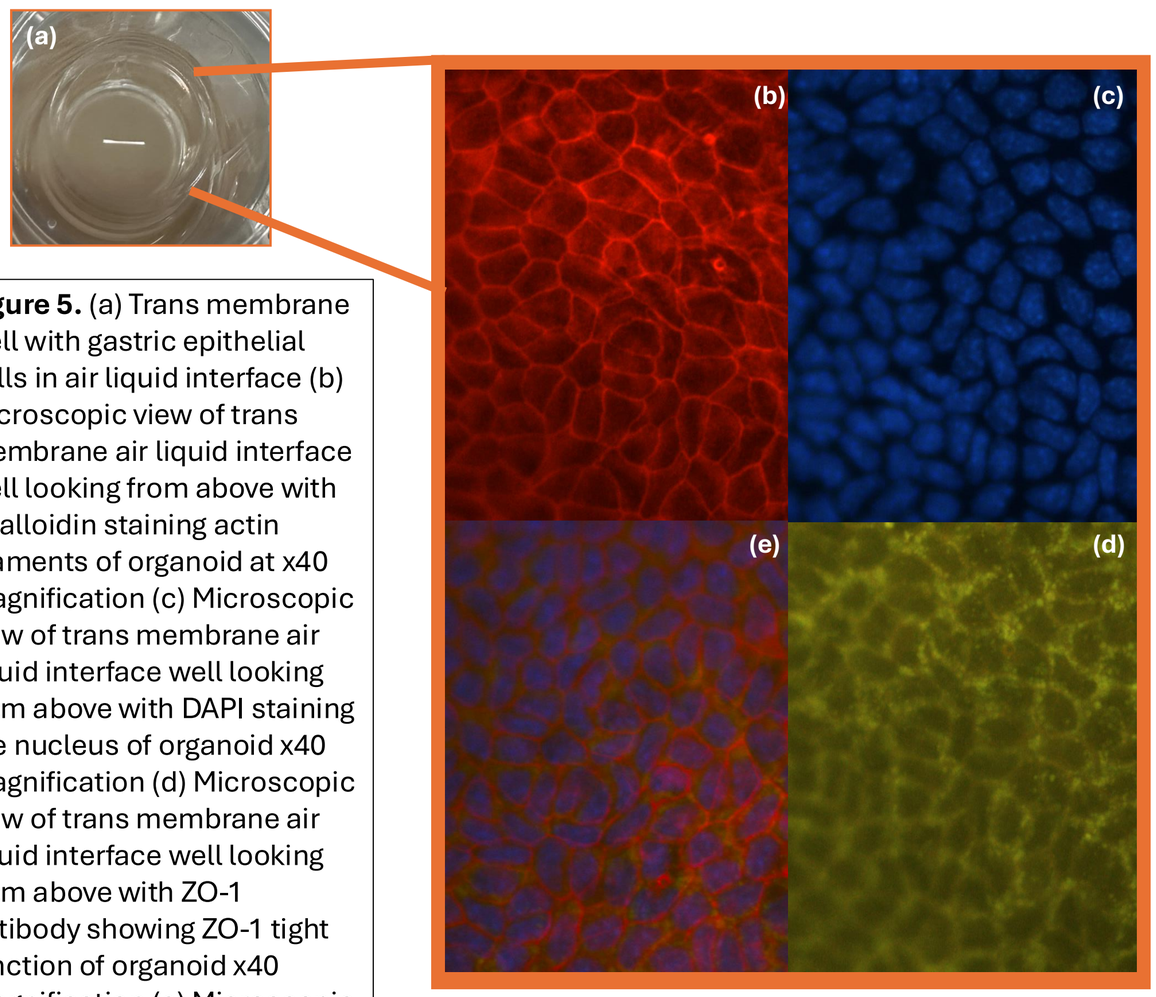


Figure 5. (a) Trans membrane well with gastric epithelial cells in air liquid interface (b) Microscopic view of trans membrane air liquid interface well looking from above with Phalloidin staining actin filaments of organoid at x40 magnification (c) Microscopic view of trans membrane air liquid interface well looking from above with DAPI staining the nucleus of organoid x40 magnification (d) Microscopic view of trans membrane air liquid interface well looking from above with ZO-1 antibody showing ZO-1 tight junction of organoid x40 magnification (e) Microscopic view of trans membrane well showing all three stains ZO-1 DAPI Phalloidin

References

1. Burkitt, M. D., Duckworth, C. A., Williams, J. M. & Pritchard, D. M. Helicobacter pylori-induced gastric pathology: insights from in vivo and ex vivo models. Dis Model Mech 10, 89–104 (2017).

2. Congedi, J., Williams, C. & Baldock, K. L. Epidemiology of Helicobacter pylori in Australia: a scoping review. PeerJ 10, e13430 (2022).

3. Idowu, S., Bertrand, P. P. & Walduck, A. K. Gastric organoids: Advancing the study of H. pylori pathogenesis and inflammation. Helicobacter 27, e12891 (2022).

4. Dixon, B. R. E. A., Hossain, R., Patel, R. V. & Algood, H. M. S. Th17 Cells in Helicobacter pylori Infection: a Dichotomy of Help and Harm. Infect Immun 87, e00363-19 (2019).

5. Walduck, A. K. & Raghavan, S. Immunity and Vaccine Development Against Helicobacter pylori. in Helicobacter pylori in Human Diseases: Advances in Microbiology, Infectious Diseases and Public Health Volume 11 (eds. Kamiya, S. & Backert, S.) 257–275 (Springer International Publishing, Cham, 2019). doi:10.1007/5584_2019_370.

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