Helicobacter pylori infection in pigs is dominated by Th1 and cytotoxic immune responses

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Introduction and Aims

Helicobacter pylori is the leading cause for peptic ulcer disease and gastric adenocarcinoma. While iTreg are required for long-term colonization without disease, Th1 and Th17 responses are associated with lower bacterial load at the expense of gastric pathology. We have developed computational models of *H. pylori* infection predicting a dominant Th1-response that results in lesion development in the gastric mucosa. In this study, pigs were infected with *H. pylori* strain SS1 or J99 to assess immune responses over time, as well as bacterial loads and gastric lesions after 2 months of infection.

Materials and Methods

Pigs were infected with 5x10^7 CFU *H. pylori* SS1 or J99 in sterile brucella broth by orogastric gavage on day 0 and 2 of the study. A non infected control group received brucella broth only. Lesions were assessed by histology and bacterial loads were determined by re-isolation on day 57 post-infection.

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood and immunophenotypically characterized by flow cytometry weekly. PBMC were stimulated *ex vivo* with 5µg/ml whole cell sonicated (WCS) SS1 and J99. Concanavalin A (ConA, 1 µg/ml) served as positive control and complete RPMI media (cRPMI) as negative control. After 4 days of culture proliferation was assessed by lymphoblastogenesis test measuring [^{3}H]-thymidine incorporation (2x10^{5}c/96 well) and flow cytometric analysis of cultured CFSE stained cells (2x10^{6}c/12 well).

Infection with *H. pylori* causes a predominant systemic Th1 response

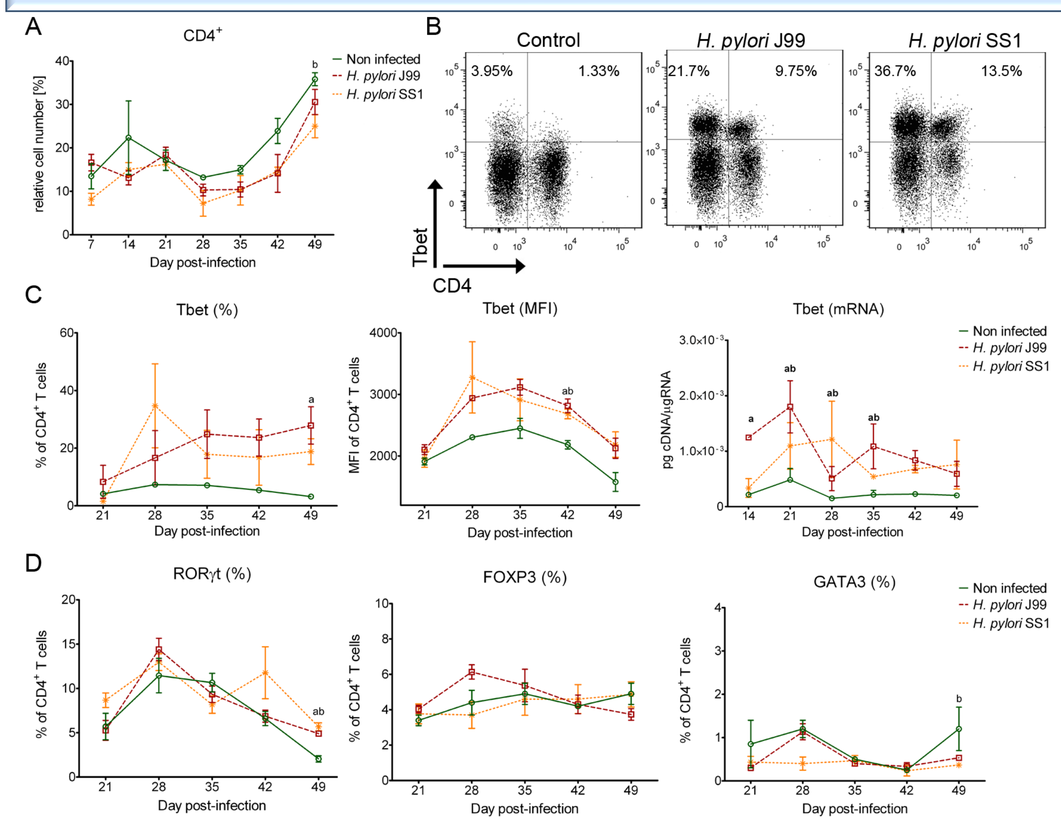


Figure 1. Highly elevated levels of Tbet-expressing CD4$^+$ T cells (B, C), and Tbet protein levels in CD4$^+$ T cells assessed by mean fluorescence intensity were detected in *H. pylori*-infected pigs, irrespective of the strain. In addition, Tbet mRNA levels (C) were increased in PBMC isolated from *H. pylori*-infected pigs as compared to PBMC obtained from the control group. *H. pylori* infection did not have any effect on the numbers of circulating CD4$^+$ RORgt$^+$ (i.e., Th17), CD4$^+$ FoxP3$^+$ (i.e., Treg) or CD4$^+$ GATA3$^+$ (i.e., Th2) cells (D), suggesting that, at least at the systemic level, the infection preferentially induces a dominant Th1 response. Letters indicate significant differences of *H. pylori* J99 (a) and SS1 (b) infected pigs to the non infected control, mean±SEM (n=2-3), p<0.05.

Expansion of cytotoxic T cells expressing Tbet upon *H. pylori* infection

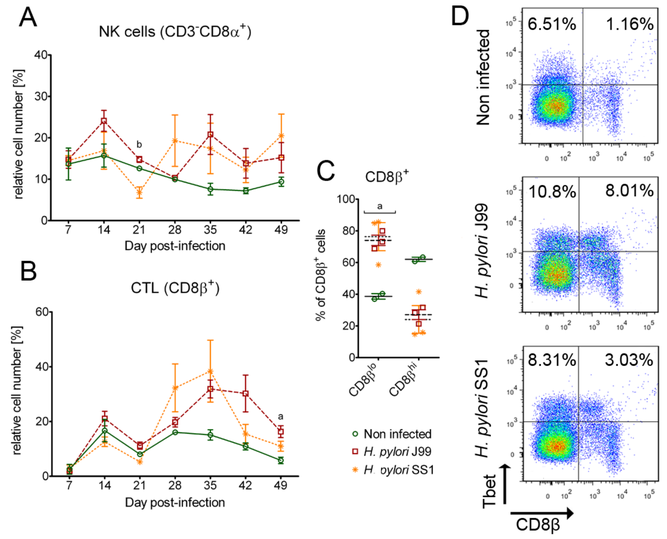


Figure 2. Upon infection CD3$^-$CD8α$^+$ NK cells accumulated in blood especially in the second month post challenge (A). By detection of CD8β, the most specific marker of cytotoxic T cells (CTL) in pigs, we could demonstrate that both *H. pylori* strains cause a pronounced increase in the relative number of circulating CTL (B). CTL expressing low levels of CD8β increased significantly due to infection (measured on day 42 post infection) (C). Furthermore, a CD8β^{lo} Tbet expressing cell subset was increased in PBMC due to infection (D), which was more pronounced in pigs infected with strain J99. Letters indicate significant differences of *H. pylori* J99 (a) and SS1 (b) infected pigs to the non infected control, mean±SEM (n=2-3, p<0.05).

Exposure to recall antigen only transiently induces proliferation in PBMC

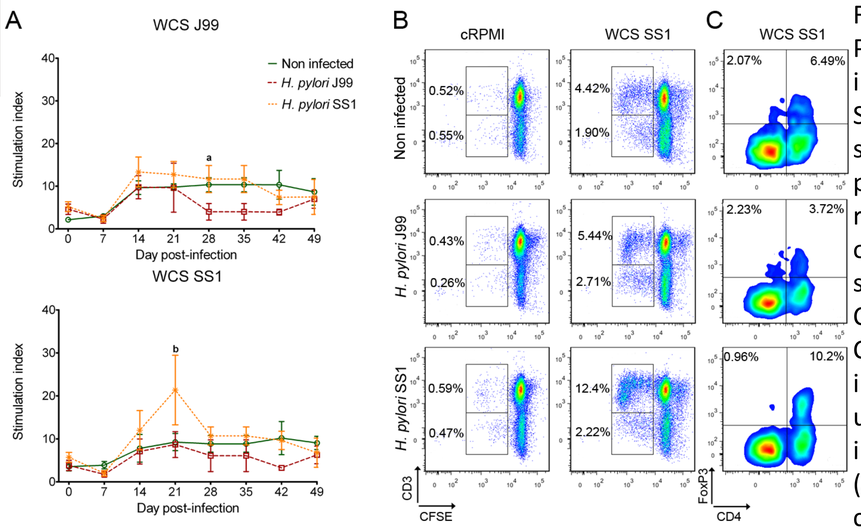
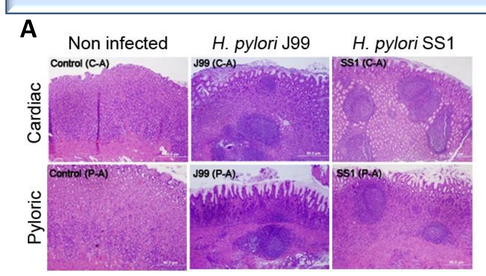


Figure 3. Significantly increased proliferation of PBMC was detected transiently on day 21 post-infection towards WCS SS1 antigens and only in SS1 - but not J99 infected pigs (A). *Ex vivo*, stimulation with WCS SS1 caused increased proliferation compared to cRPMI-treated cells regardless of the *in vivo* treatment. However, only cells from pigs challenged with *H. pylori* SS1 showed highly elevated levels of proliferating CD3$^+$ cells (B). Interestingly, elevated levels of CD4$^+$FOXP3 expressing cells were only detected in proliferating T cells from SS1 challenged pigs upon stimulation with antigen (C). Letters indicate significant differences of *H. pylori* J99 (a) and SS1 (b) infected pigs to the non infected control, mean±SEM (n=2-3, p<0.05).

Gastric lesions and strain-specific colonization capacity



B

Stomach region	*H. pylori* J99 # reisolates	*H. pylori* SS1 # reisolates
P-A	0/3	3/3
P-B	1/3	3/3
P-C	0/3	2/3
F-A	0/3	2/3
F-B	0/3	2/3
C-A	0/3	2/3
C-B	0/3	3/3
Total # reisolates	**1/21**	**17/21**
% Reisolation	**4.76**	**80.95**

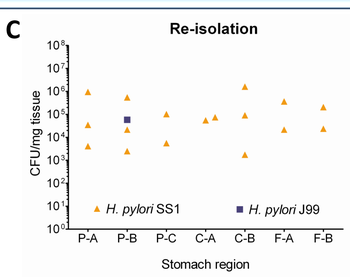


Figure 4. Infection with both *H. pylori* resulted in lesion development in different stomach regions (A). While H. pylori J99 could only be re-isolated from one stomach region after 57 days of infection, strain SS1 showed high colonization capacity (B) and bacterial loads (C) in all stomach regions .

Conclusion and Outlook

- ✓Both strains elicit predominant Th1 and cytotoxic immune responses
- ✓Weak and suppressed memory responses suggesting that specific virulence factors contribute to suppression of immune responses and chronic persistence
- ✓Strain J99 evoked a more dramatic acute response associated with undetectable bacterial loads
- ✓Strain SS1 showed long-term colonization capacity

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