Role of Muc1 in *Helicobacter pylori* Gastric Mucosal Inflammation

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Abstract

Rationale: *Helicobacter pylori* (Hp) is a Gram-negative, microaerophilic, emerging infectious disease agent that can inhabit various areas of the stomach and duodenum. Hp causes a chronic low-level inflammation of the gastric mucosa (gastritis) that is strongly linked with the sequential progression to atrophy, metaplasia, dysplasia, and gastric cancer. However, greater than 80% of individuals infected with Hp are asymptomatic, and it is currently unknown what factors (bacterial, host, and/or environmental) influence the incidence and character of gastric disorders associated with Hp infection. Our previous studies using a *Pseudomonas aeruginosa* lung infection model demonstrated that the epithelial Mucin-1 (Muc1) transmembrane glycoprotein down-regulates bacterial inflammation mediated through an NF-κB → IL-8 pathway. Therefore, we investigated the role of Muc1 in regulating inflammatory responses to Hp gastric infection.

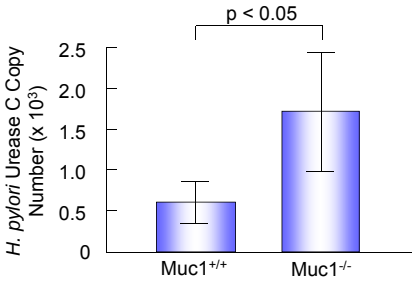
Methods: Muc1 wild type and knockout mice were infected with Hp strain SS1 by oral gavage and bacterial colony forming units as well as mRNAs encoding TNF-α and KC were measured in gastric tissues at 4 weeks post-infection. Human AGS gastric epithelial cells were transfected with pMUC1 expression vector or pcDNA empty vector, or with MUC1 siRNA or control siRNA, the cells were untreated or treated with Hp strain 26695, and IκBα phosphorylation, NF-κB activation, and IL-8 production were measured.

Results: Muc1 knockout mice exhibited increased bacterial colonization of the gastric mucosa and greater gastric inflammatory cytokine/chemokine responses compared with wild type mice following experimental Hp infection. Overexpression of Muc1 in AGS cells was correlated with decreased NF-κB activation and IL-8 production compared with cells expressing normal levels of Muc1. Conversely, knockdown of Muc1 expression in AGS cells was associated with increased activation of NF-κB and IL-8 production. Finally, inhibition of NF-κB activation with Bay 11-7082 blocked Hp-stimulated IL-8 production.

Conclusions: We conclude that, similar to *P. aeruginosa* lung infection, Muc1 plays an anti-inflammatory role in Hp gastric infection. Additionally, we hypothesize that decreased Muc1 expression in a subset of Hp infected individuals promotes persistent and elevated Hp-driven host inflammation that facilitates the development of gastric disorders such as chronic gastritis and adenocarcinoma.

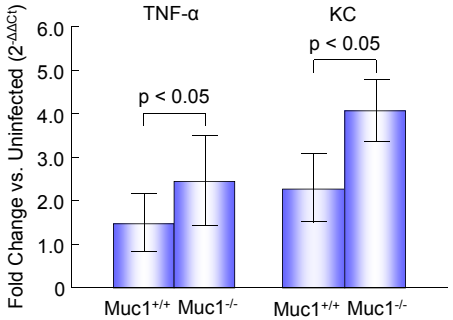
Results

Figure 1. Muc1 knockout mice exhibit increased gastric colonization by Hp.



Mice were infected with Hp strain SS1 by gastric intubation and bacterial urease C gene copy numbers in the stomach were quantified by PCR at 4 weeks post-infection.

Figure 2. Muc1 knockout mice display increased TNF-α and KC transcript levels following Hp gastric infection



Muc1$^{+/+}$ and Muc1$^{-/-}$ mice were uninfected or infected with 10^7 CFU/mouse of Hp strain SS1 and transcripts encoding TNF-α and KC were measured by quantitative RT-PCR in gastric tissue homogenates at 4 weeks post-infection.

Figure 3. Overexpression of Muc1 in AGS cells decreases Hp-stimulated NF-κB activation (A) and IL-8 production (B).

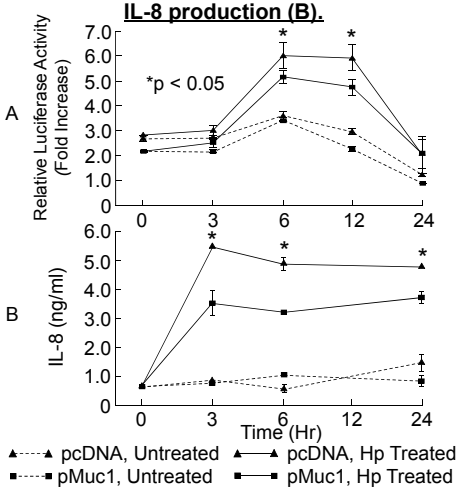


Figure 4. Knockdown of Muc1 in AGS cells increases Hp-stimulated NF-κB activation (A) and IL-8 production (B).

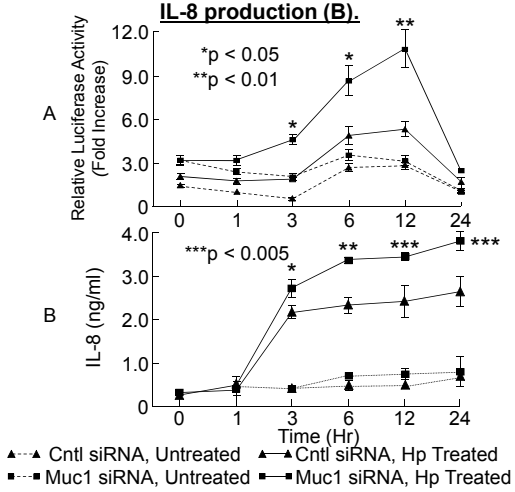
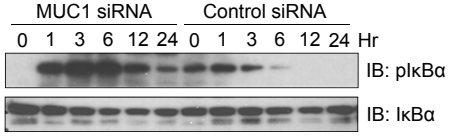
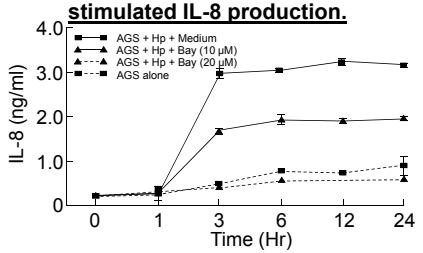


Figure 5. Muc1 siRNA increases H. pylori-stimulated IκBα phosphorylation.



AGS cells were transfected with Muc1 or control siRNAs, treated with Hp strain 26695, and phospho-IκBα (pIκBα) and total IκBα levels were determined by Western blotting.

Figure 6. Inhibition of NF-κB blocks Hp-stimulated IL-8 production.



AGS cells were pretreated with medium or Bay 11-7082 for 1 h, the cells were untreated or treated with Hp 26695, and IL-8 levels were determined by ELISA.

Summary

- Muc1 knockout mice exhibited increased bacterial colonization of the stomach and greater gastric TNF-α and KC levels compared with Muc1 wild type mice following experimental Hp infection.
- Overexpression of Muc1 in human AGS gastric cells was correlated with decreased NF-κB activation and decreased IL-8 production compared with cells expressing normal levels of Muc1.
- Knockdown of Muc1 expression in AGS cells was associated with increased NF-κB activation and increased IL-8 production compared with normal Muc1.
- Inhibition of NF-κB activation by Bay 11-7082 blocked Hp-stimulated IL-8 production.

Acknowledgments

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