The Contribution of T Cells to Intestinal Inflammation and Fibrosis

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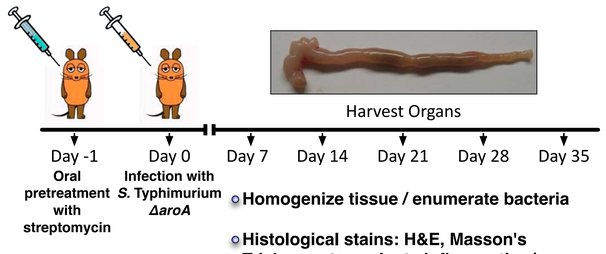
Introduction

- Instestinal fibrosis is a major complication in Crohn's Disease (CD) patients.[1]
- Mechanisms that lead to intestinal fibrosis and stricture formation are still poorly understood.
- The bacterium *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) causes food poisoning and gastroenteritis in millions of people each year.
- Mice pre-treated with the antibiotic streptomycin prior to infection with *S.* Typhimurium experience heavy colonization of the cecum and colon with significant colitis.[2]
- We recently showed that chronic infections with *S.* Typhimurium lead to severe intestinal fibrosis.[3]
- Here, we investigated the contribution of T cells to the development of intestinal fibrosis in mice caused by chronic *Salmonella*-induced colitis, and found that **T-cell deficient mice developed attenuated inflammation and fibrosis.**

Experimental Approach



Results

(1) *S.* Typhimurium *ΔaroA* chronically colonizes the cecum of B6 and Rag1$^{-/-}$ mice

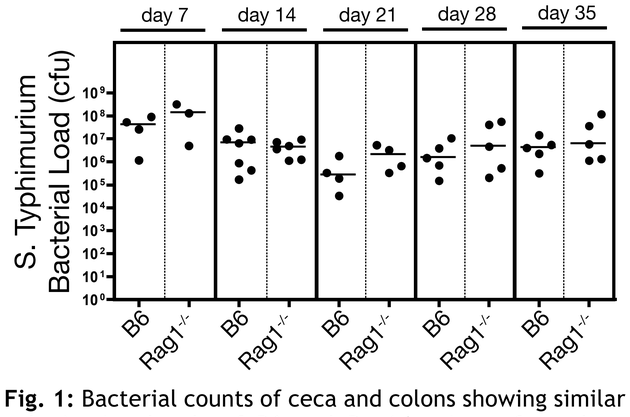


Fig. 1: Bacterial counts of ceca and colons showing similar colonization levels of B6 and Rag1$^{-/-}$ mice; cfu: colony forming units.

(2) *S.* Typhimurium *ΔaroA* is found primarily in the lumen of infected ceca

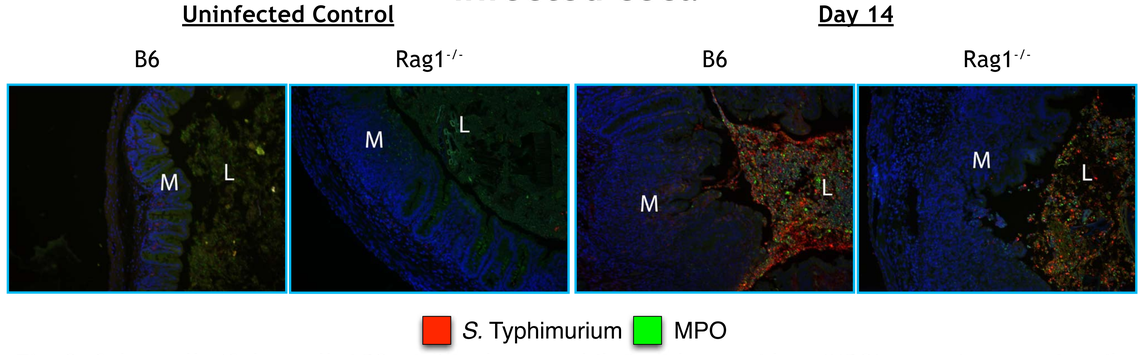


Fig. 2: *Salmonella* (*Salmonella* LPS, red) and neutrophils (myeloperoxidase (MPO), green) were primarily found in the lumen of ceca at 14 days post-infection (p.i.). Uninfected controls shown for comparison. DAPI staining of nuclei shown in blue. L = Lumen, M = Mucosa.

(3) Rag1$^{-/-}$ mice display attenuated inflammation and fibrosis

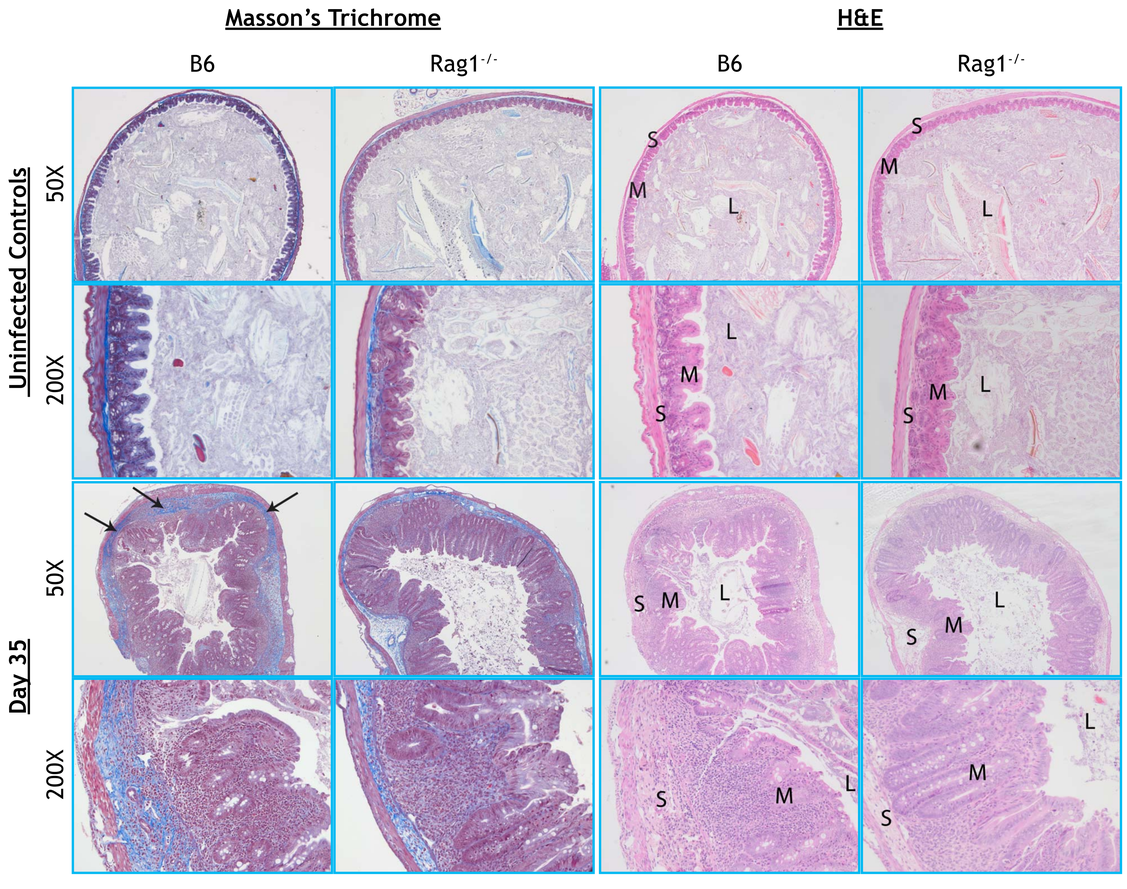


Fig. 3: Masson's Trichrome (MT) and Hematoxylin & Eosin (H&E) staining of mice ceca from uninfected controls and day 35 infected B6 and Rag1$^{-/-}$ mice. MT staining indicates position of collagen deposition (blue), while H&E staining reveals extent of inflammation and damage. L = Lumen, M = Mucosa, S = Submucosa. Note region of denser collagen deposition (black arrows) with greater edema in the submucosa of B6 ceca compared to Rag1$^{-/-}$ ceca.

(4) *S.* Typhimurium *ΔaroA* induces proinflammatory and profibrotic cytokines

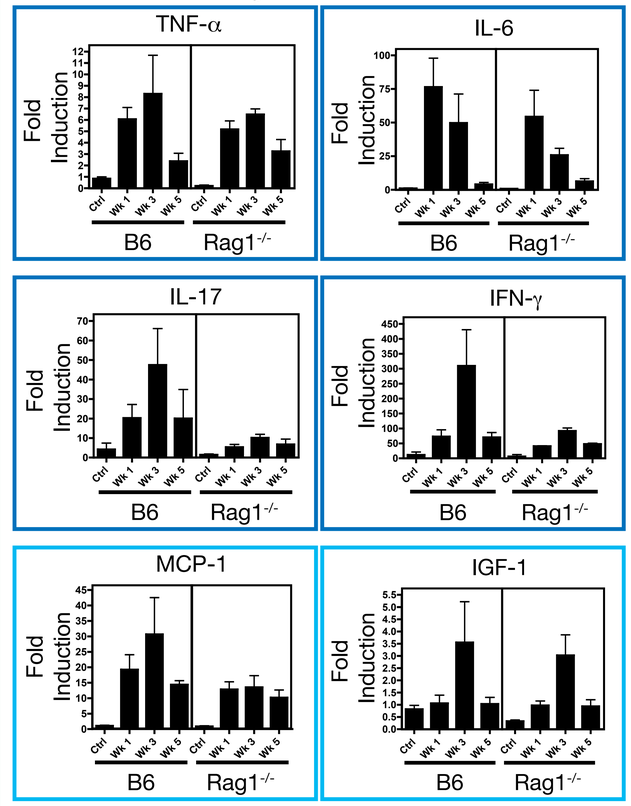


Fig. 4: Induction of proinflammatory (TNF-α, IL-6, IL-17, IFN-γ) and profibrotic (MCP-1, IGF-1) cytokines in infected ceca was determined by real-time PCR. Data were normalized to GAPDH levels and are relative to an arbitrarily selected uninfected control.

(5) B6 and Rag1$^{-/-}$ ceca have similar numbers of fibroblasts

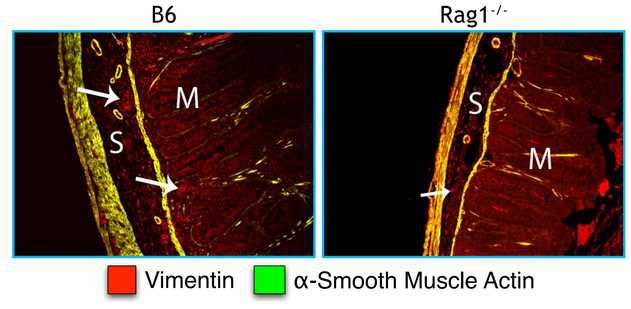


Fig. 5: Distribution of mesenchymal cell types in ceca of B6 and Rag1$^{-/-}$ mice infected with *S.* Typhimurium 35 days p.i., differentiated by staining for fibroblast-specific vimentin (V) and α-smooth muscle actin (A) shown at 400X magnification. Similar amounts of V^+A^- fibroblasts (white arrow) were found in the submucosa and mucosa of B6 and Rag1$^{-/-}$ ceca. S = Submucosa; M = Mucosa

Conclusions

- *S.* Typhimurium triggers severe fibrosis in the cecum.
- *Salmonella*-induced profibrotic and proinflammatory cytokines are maximally produced at week 3 post-infection.
- Fibroblasts and smooth muscle cells might be the predominant collagen-producing cells.
- T cells enhance intestinal inflammation and fibrosis

Acknowledgments

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Rising Stars of Research

References

1. Burke J. P., Mulsow J., O'Keane C. et. al. 2007. **Fibrogenesis in Crohn's disease.** Am. J. Gastroenterol. 102(2):439-48.
2. Coburn B., Li Y., Owen D. et. al. 2005. ***Salmonella enterica* serovar Typhimurium pathogenicity island 2 is necessary for complete virulence in a mouse model of infectious enterocolitis.** Infect. Immun. 73(6):3219-27
3. Grassl G. A., Valdez Y., Bergstrom K. S. B. et. al. 2008. **Chronic enteric *Salmonella* infection in mice leads to severe and persistent intestinal fibrosis.** Gastroenterology 134(3): 768-80.