

Examining the therapeutic potential of IL-22-induced adherent mucin production in *Clostridioides difficile* infection

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Introduction: *Clostridioides difficile* (*C. difficile*) is a toxin-producing enteric pathogen that causes severe diarrhea and inflammation. Intestinal adherent mucins are known to play a major role in epithelial protection and wound repair, but their levels during *C. difficile* infection are unclear. We aimed to characterize the levels of adherent mucins during *C. difficile* infection and identify the pathways that regulate these mucins. **Methods:** Antibiotic-treated mice were gavaged with PBS or *C. difficile* and monitored for 10 days. RNA sequencing was used to identify differentially expressed genes at days 3, 4, 5, 8, and 10 post-infection. Human and murine colonic cells and organoids were treated with IL-22 to determine the effects of IL-22 on adherent mucin expression *in vitro*. MNK3 (ILC3) cells and peritoneal macrophages were treated with *C. difficile* toxins, and IL-22 or IL-1 β levels were measured via ELISA. To evaluate the therapeutic potential of IL-22, antibiotic-treated mice were gavaged with PBS, *C. difficile*, or *C. difficile* and engineered *Limosilactobacillus reuteri* designed to release IL-22. Immunostaining and histological analysis were observed at day 3 post-infection in all mouse models. **Results:** Mice infected with *C. difficile* exhibited significant weight loss at day 3 post-infection. Also at day 3 post-infection, infected mice showed increased colonic MUC13 gene and protein levels compared to controls. MUC13 is known to be under the control of IL-22. Mirroring this, we also observed increased IL-22 expression in infected mouse colon. When treated with IL-22, colonic cells and organoids exhibited increased MUC13 expression. While *C. difficile* toxins did not stimulate IL-22 production in MNK3 cells, they did increase IL-1 β levels in peritoneal macrophages. Supernatant from these cells, as well as recombinant IL-1 β , stimulated IL-22 release from MNK3 cells. Mice treated with a combination of IL-22 producing *L. reuteri* and *C. difficile* exhibited less weight loss, reduced intestinal damage and inflammation, and an increased mucus barrier compared to *C. difficile* alone mice. **Conclusion:** Together these findings suggest that *C. difficile* toxins indirectly promote IL-22 production through macrophage-derived IL-1 β signaling and that IL-22 upregulation drives an increase in colonic adherent mucins and reduces *C. difficile* associated damage. This work reveals a potential therapeutic strategy to reduce intestinal damage during *C. difficile* infection.