Poor Disease Outcome from *C. difficile* Infection in the Aged Host: Role of Impaired Innate Immunity and Altered Intestinal Microbiota in a Mouse Model

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**Background**

*Clostridium difficile* is the most common pathogen to cause health-care–associated infections causing almost half a million cases in 2015, approximately 20,000 deaths, and 1.1 billion dollars or higher health-care cost in US hospitals. 1, 2 Not only are people aged 65 or older more susceptible to infection, they are also more likely to have poor outcome – more severe diarrhea, more deaths, more readmissions. Patients 75 and older were three times more likely to have severe disease from *C. difficile* infection (CDI) in a retrospective study in Strogin and Women's Hospitals. 3 While people 65 and older made up 50% of all reported total CDI cases, the deaths from CDI in this age group made up 83% of the total estimated deaths from CDI. In the most recent study in 2021 on the burden of CDI, People 65 and older were found to be too to ten times more likely to have recurrent infections depending on the study. 4-6 In this study, we aim to elucidate the effect of aging on outcome and immune response in CDI, including immune cell recruitment and cytokine production, using a mouse model.

**Methods**

Young (8 weeks old) and aged (18 months old) C57BL/6 mice were infected with *Clostridium difficile* strain VPI 10463 after broad spectrum antibiotic exposure. Uninfected mice served as controls. Mice were monitored daily for disease severity and survival. A separate group of mice was euthanized on day 2 and 5 to harvest blood and intestinal tissue. Peripheral blood was analyzed for differential blood cell counts. Histologic scoring was done on cecal and colonic tissue. Cecal tissue KC, IFN-γ, IL-6, IL-10, IL-17, TNF-α, G-CSF, and GM-CSF were measured using qPCR for gene expression and Luminesce for protein levels. Cellular components were analyzed using flow cytometry. Clositudal burden was quantified by qPCR for total gene and ELISA for C. difficile toxins A and B in the stool. To examine microbiota, qPCR was done with primers for Firmicutes and Bacteroidetes. In a follow-up experiment, dirty cage beddings were switched every other day between young and aged mice for one week prior to antibiotic exposure to determine the effect of microbiota on outcome of infection. The mice were then infected following protocol above and data was collected regarding clinical outcomes and immune response.

**Results**

**Clinical Outcome**

Mortality with CDI after 7 days was significantly worse in the aged group compared to the young group (83% vs. 17%). 1, 2 In addition, aged mice also showed higher clinical scoring and delayed but persistent weight loss compared with young mice.

**Neutrophil Count**

Peripheral blood and cecal neutrophil counts were similar between young and aged mice at baseline. On day 2 post-infection, neutrophil counts were significantly lower in the aged mice compared to the young mice in both blood and cecum. These differences were no longer apparent at day 5.

**Cytokines**

There were no significant baseline differences in cytokines tested between young and aged mice. At day 2 post-infection, protein levels of KC, IFN-γ, IL-6, IL-10, IL-17, TNF-α, G-CSF and GM-CSF were significantly lower in the aged mice. At day 5 post-infection, gene expression of KC and IL-10 were higher in the aged mice. IL-10 and IL-6 levels were not significantly different between the groups throughout the infection.

**Histologic Changes**

Histopathologic scores in the cecum were lower at day 2 but increased at day 5 post-infection in the aged mice.

**Neutrophil Count After Cage Bedding Switching**

There was a trend for lower neutrophil count at day 2 in aged mice for both peripheral blood count and cecal recruitment, but not statistically significant difference. At day 5 the aged mice neutrophil recruitment to cecum was not higher in the aged mice, in contrast to the previous experiment.

**Microbiota Before and After Switching Cage Bedding**

Microbiota was analyzed using qPCR of both before and obtained prior to infection. Primary for common DNA sequences of Firmicutes and Bacteroidetes, each a major phylum in the makeup of the intestinal microbiota, were used. Results showed significantly lower number of bacteria in the Bacteroidetes phylum in the aged mice before switching cage beddings while number of Firmicutes were not significantly different. Cage switching increased the numbers of both in aged mice.

**Discussion**

The mouse model of CDI in the aged host demonstrated significant effects of age on outcome, which is also observed in humans. Age-related reduced innate immunity in in vivo experiments with luminal cytokine responses following *C. difficile* infection. The pronounced sustained response at later stages of infection may be a potential mechanism for worse outcomes in the aged host. The early lower neutrophil recruitment and pro-inflammatory cytokine production may lead directly to adverse outcome or may lead to uncontrolled inflammation in later infection thus leading to worse outcome. The partial reversal of the effect of age by exchanging the intestinal microbiota suggests an interaction between the innate immune system and the microbiota. The findings on immune cell numbers and cytokine production suggest potential mechanisms by which aging may affect outcome and potential interactions between innate immune system and microbiota.

- Neutrophil recruitment to cecum was lower in day 2 in aged mice even after cage switching, but the increased neutrophil recruitment on day 5 disappeared with cage switching, suggesting that the increased inflammation leads to worse outcomes in the aged host.
- IL-10 levels were not different at first, but became lower at day 2 and higher at day 5 with aging when cage beddings were switched. This may be one of the potential mechanisms by which the microbiota may elicit an effect on outcome.
- Granulopoietic factors G-CSF and GM-CSF showed significant decreases in local levels during CDI with aging and lower systemic neutrophil counts. This may be a systemic mechanism in response to infection that is affected by aging and leading to a change in outcome with CDI.

**Conclusion**

In the mouse model of CDI, clinical outcome in the aged host is significantly worse despite similar infection burden compared to the young. Early stages of infection show lower neutrophil counts, both systemic and local, and lower production of cytokines (pro-inflammatory and neutrophil-related), with aging. Later stages of infection show a reversal of this trend, with higher levels of cytokines and neutrophils. Microbiota analysis showed lower numbers of Bacteroidetes in the aged mice.

Cage bedding switching was done to equalize intestinal flora between young and aged mice and led to an improvement in mortality in the aged mice. However, the weight change and clinical signs were still worse and prolonged in aged mice. The neutrophil counts and cytokine production patterns were similar, but the differences were not as great.

**References**