**What’s in the Box?**

**Investigation into Why Dirty Cages from Young Mice Can Save Aged Mice with C. difficile Infection**

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**Background**

Clostridium difficile is one of the most common health care-associated infections with almost half a million estimated cases per year and costing the healthcare system at least 1 billion dollars per year in the US.1,2 People aged 65 or older are more susceptible to infection and more likely to have poor outcomes – severe infections, death, and recurrences. Using a mouse model of Clostridium difficile infection (CDI), we have demonstrated the effect of aging.3,4 In this study we aim to elucidate the role of microbiota on outcome and immune response in CDI in the context of aging.

**Methods**

Young (8 weeks old) and aged (18 months old) C57BL/6 male mice were infected with Clostridium difficile strain VPI 10463 after broad-spectrum antibiotic exposure in two different experiments. In the first infection experiment, young and aged mice were infected and monitored daily for disease severity and survival. A separate group of mice was euthanized on days 2 and 5 to harvest blood and intestinal tissue. Peripheral blood was analyzed for differential blood cell counts. Histological sections were done on colon and intestinal tissues. Total tissue KC, MF-2, IL-10, IL-6, IL-17, TNF-a, IL-6, and GM-CSF were measured using qPCR for gene expression and Luminex for protein levels. Cellular components were analyzed using flow cytometry.

In the next cage switching experiment, dirty cage bedding was switched every other day between young and aged mice for one week prior to antibiotic exposure and infection and data was collected regarding clinical outcome and immune response. Stool samples were collected at baseline, after cage exchange, after antibiotic exposure, and after infection. Cigarette burden was quantified by qPCR for tG08 gene and ELISA for C. difficile toxins A and B in the stool. DNA was extracted from stool and the intestinal microbiota was analyzed in two ways. First, qPCR using common primers for major phyla was done, looking at Firmicutes, Bacteroidetes, and Proteobacteria, along with total 16S rDNA. A second method of analysis was sequenced using MiSeq Reagent Kit v3 after amplification of V1-V3 hypervariable regions of 16S RNA using broad range primers. Changes in the bacterial composition before and after antibiotics and before and after cage switch were analyzed using the statistical phylogenetic Principal Coordinate Analysis (PCoA).

**Results**

**Bacterial Population Diversity**

Diversity of the bacterial population within each individual mouse (alpha diversity) was measured using Shannon Diversity. However, in the aged vs young infection experiment, there was significantly lower diversity in aged mice compared to young mice at baseline, but after antibiotic diversity in both groups decreased and there was no significant difference between the groups. In the cage switching experiment also, there was significantly lower diversity in aged mice. This difference persisted despite cage switching and antibiotic exposure.

**Composition of Microbiome (Phylum Level - Illumina MiSeq Sequencing)**

The composition of the intestinal microbiota at the phylum level was analyzed using Illumina MiSeq. Baseline proportion of Bacteroidetes was significantly lower in aged mice compared to young mice, but after switching the cages, Bacteroidetes increased in the aged mice to be closer to the level in the young mice. Proteobacteria and Verrucomicrobia made up the most of the rest of the baseline microbiota, with more Firmicutes in the young mice and more Verrucomicrobia in the aged mice. Antibiotic treatment significantly decreased the proportion of Firmicutes while Proteobacteria increased to fit a significant portion of the microbiota, which continues with CDI.

**Clinical Outcome**

Mortality with CDI after 7 days was significantly worse in the aged group compared to the young group (83% vs 17%, p<0.05). Aged mice also showed higher clinical scoring and delayed but persistent weight loss compared with young mice.

**Discussion**

We were able to demonstrate both the detrimental effect of advanced age on outcome of CDI and the role of intestinal microbiota to reverse the age effect using a mouse model. There are clearly characteristics in the aged mouse microbiota that separates it from the young mouse microbiome, which seems to exist some effect on outcome. Most notable is the deficit in Bacteroides phylum, more specifically in the Bacteroides genus, in the aged mouse microbiome. In this model, the overall bacterial diversity did not seem to change with cage switching, which is different from conventional knowledge of higher diversity leading to better colonization resistance against CDI.4,5 These findings point to more complex effects from the microbiome rather than simple competition for resources. One striking finding from this study is that the difference in microbiome between young and aged mice and even changes in microbiome seen with cage switching were all diminished with antibiotic exposure, to the point of disappearance of statistically significant difference. However, the change in microbiome did lead to a difference in clinical outcome. This suggests the presence of changes in the host caused by the microbiome which are resistant to antibiotics. Our studies suggest an association with host immune response, which would be consistent even with antibiotic treatment. Further studies to elucidate this microbiome-related effect would be more illuminating for the study of CDI in aged hosts.

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**References**


