Host Susceptibility to Post-Influenza Secondary Bacterial Infections

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ABSTRACT

In both humans and in mice, increased susceptibility to super-infection occurs between Day 7 and 14 after influenza infection. Interestingly, the Apple lab here at Montana State University recently showed that if the super-infection happens earlier than day 7, specifically around day 3 after influenza infection, the mice are better able to clear the bacterial infection because of their activated innate immune response. However, we do not currently know whether super-infection with one bacteria that occurs during the pre-clinical influenza infection (and thus is potentially undetected) would prevent or ameliorate morbidity and/or mortality from another super-infection at day 7 post influenza. This work is important because during the pandemics that have affected the human population in the last century, almost 95% of those deaths involved secondary infections. The main secondary bacterial infections involved were *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Thus, in this study we sought to determine whether mice given a sub-clinical dose of H1N1 influenza on day 0, and *S. aureus* on day 3 would identify a time frame and infectious dose that would result in higher survivability. Here, we found that mice lost weight after Influenza infection and that super-infection of a subclinical dose of *S. aureus* (10^6) had no additional effect on weight loss or temperature. Interestingly, we also found that higher concentrations of *S. aureus* (10^6 or 10^5 CFU) had lower bacterial burden compared to lower concentrations (10^4 or 10^3 CFU). By better understanding how the host handles super-infections of varying degree during influenza infection, we may be able to prevent these secondary infections.

INTRODUCTION

Over the past century the human population has witnessed several well-documented influenza pandemics that have impacted not only the United States, but also the world. With the 1918 Spanish Flu, over 500 million people were infected, which resulted in over 50 million deaths. Within these 50 million individuals, approximately 95% resulted from secondary bacterial infections with either *Staphylococcus aureus* or *Streptococcus pneumoniae*. Pandemics also occurred in 1957, and 1968 and were caused by the H2N2 and H3N2 influenza viruses, respectively. Although during these times, a decrease in mortality rates were seen, due to the advancement in technology and medicine that introduced antibiotics, secondary bacterial infections with either *Staphylococcus aureus* (1957) or *Streptococcus pneumoniae* (1968) were still prevalent. The most recent influenza pandemic occurred in 2009 and was caused by a influenza H1N1 strain that underwent an antigenic shift in swine. Even with today's advancements, it is still difficult to determine the mortality rate globally, though it is estimated that the pandemic caused approximately 200,000 deaths, mainly due to respiratory issues. The Most common organisms found during the 2009 pandemic were both *S. aureus* and *S. pneumoniae*. These studies demonstrate that bacterial super-infections were associated with higher morbidity and mortality during the pandemics in the last century, thus signifying the importance in determining how to prevent them.

HYPOTHESIS

Our previous research demonstrated that at day 3 post influenza virus infection, susceptibility to secondary bacterial infections decreased compared to the increased susceptibility found at day 7 post-influenza. Therefore, we hypothesized that subclinical *S. aureus* infection at day 3 after influenza virus infection would allow the host to establish an immunological environment that would protect mice from increased susceptibility to *S. pneumoniae* super-infection on day 7.

METHODS

Both female and male Balb/c mice were bred at the Montana State University Animal Resource Center in Bozeman, Montana. The mice enrolled in experiments ranged in between the ages of 6-13 weeks old.

Mice were infected with 500 pfu (plaque forming units) of mouse-adapted influenza A virus PR8 strain on day 0 and/or challenged with 1x10^6 cfu (colony forming units) of *Staphylococcus aureus* (USA300 LAC strain) on day 3.

Mice were monitored daily and body weights and temperatures were recorded. At the time of sacrifice lungs were isolated, processed and plated on TSA plates to determine lung bacterial burden.

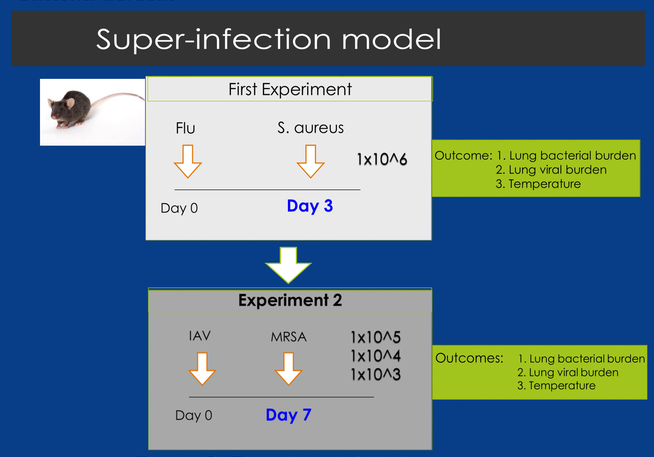

Figure 2: Experimental designs

RESULTS

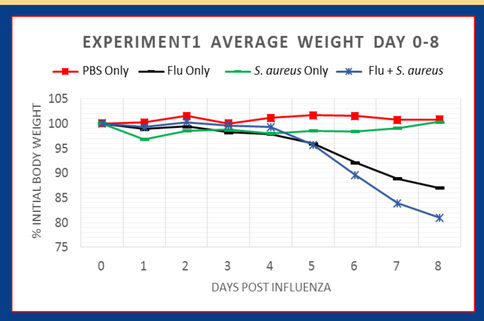

Figure 3. Increased weight loss with mice infected with Influenza. Secondary bacterial infection with subclinical dose (10^6 CFU) of *S. aureus* on Day 3 post-influenza infection causes body weight loss. Mice were intranasally infected with 500 PFU of influenza on Day 0 and with 1x10^6 CFU of *S. aureus* on day 3. The average body weights show that mice infected with both Flu, and Flu + *S. aureus* lost weight at a quicker rate than the mice infected with *S. aureus* alone. Data represent the mean body weights.

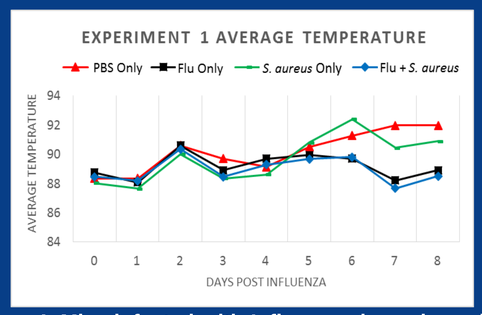

Figure 4. Mice infected with Influenza showed a reduction in average body temperature. Mice were infected as described in Fig. 3. Temperatures of the mice were recorded daily post influenza. There is no difference in temperatures until day 5. Data represent mean body temperatures.

RESULTS

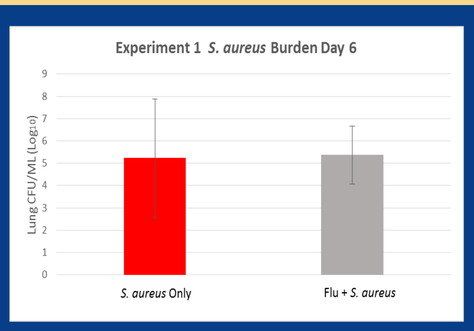

Figure 5. No difference in bacterial burden between secondary infection and *S. aureus* alone. Mice were infected as described in Fig. 3 legend. Lung bacterial burden was evaluated 8 days post influenza infection. Results show no significant differences in the lung bacterial burden between mice infected with Flu only and super-infection with the *S. aureus* on day 3. Data represent mean bacterial burden.

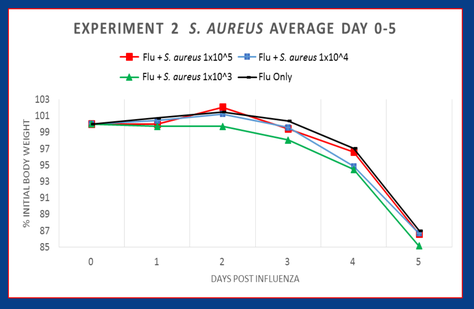

Figure 6. Low concentrations of *S. aureus* resulted in similar body weight loss as mice infected with influenza alone. Mice were infected with 500 PFU of influenza virus on Day 0, followed by the challenge with one of the three different concentrations of *S. aureus* on Day 3. the concentrations include 1x10^5 (Red), 1x10^4 (Blue), and 1x10^3 (Green) CFU as well as another group that received PBS (Black). The average body weights show that when mice are given a secondary infection of *S. aureus* at day 3, all of the "low" concentrations result in a similar body weight loss as mice infected with Flu only. Data represent mean body weight.

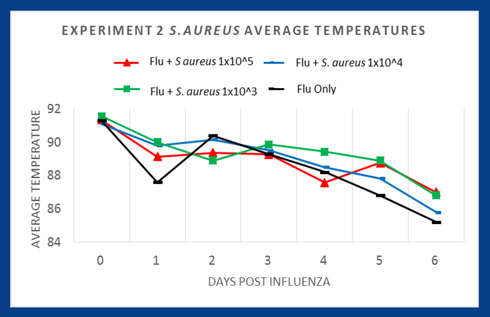

Figure 7. Day 3 post-influenza super-infected mice with low concentrations of *S. aureus* had no effect on body temperature compared to influenza only infected mice. Temperatures of the mice were recorded daily post influenza at a concentration of 500 PFU on day 0. the temperatures all decreased post influenza. *S. aureus* given on day 3 at concentrations of 1x10^5 (Red), 1x10^4 (Blue), or 1x10^3 CFU (Green), resulted in the same decline in average body temperature as Flu only. Data represent mean body temperatures.

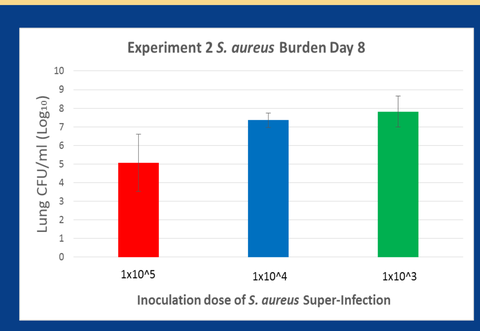

Figure 8. Bacterial burden in super-infected mice at the lower concentrations (10^3 and 10^4 CFU) of *S. aureus* had increased bacterial burden compared to the higher concentration (10^5 CFU). Influenza infection at a concentration of 500 PFU on day 0 to all mice. Bacterial burden in mice inoculated with *S. aureus* on day 3 with different concentrations: 1x10^5 (Red), 1x10^4 (Blue), and 1x10^3 CFU (Green) was determined. Data represent mean bacterial burden.

CONCLUSIONS

- The mice infected with a concentration of 1x10^6 CFU of *S. aureus* showed decreased weight and temperature when mice were infected with Influenza.
- At Day 3 post-influenza, super-infections with lower concentrations of *S. aureus* (1x10^5 ,1x10^4 , or 1x10^3 CFU) resulted decreased body weight and temperature compared to mice that received a larger dose of *S. aureus* (1x10^6 CFU)
- Higher bacterial burden occurred in mice that received the lower doses of *S. aureus* (1x10^4 and 1x10^3 CFU).

FUTURE WORK

Future research involving secondary bacterial infections post influenza has a very wide range of possibilities. We will further investigate why a higher concentration of *S. aureus* is less of a burden, compared to a lower concentration. Additionally, we will also determine how the introduction of a triple infection involving *S. pneumoniae*, at Day 7, will affect the host's susceptibility.

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