Determining whether type I interferon signaling affects composition of lung microbiota in the context of influenza virus infection

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ABSTRACT

For a long time, the lung was believed to be a sterile mucosal site and it was just recently that researchers realized microbial communities (microbiome) exist in the lung. However, it is still unclear how the composition of lung microbiome affects the immune responses to allergens and pathogens in the respiratory tract. Understanding this concept can provide us with important insights necessary to design tools for therapeutic interventions in the future.

Type I interferon (IFN) signaling in mammalian cells is known to be a very potent and early mechanism of anti-viral and some anti-bacterial responses. Consequently, mice lacking type I IFN signaling (Type I Interferon Receptor 1 knockout mice; IFNAR-/-) can be more susceptible to some infections than WT mice. Interestingly, even though type I IFN signaling is induced in the host within hours after viral and bacterial infection, different research groups have reported conflicting data on susceptibility of IFNAR-/- mice to both infections. Because type I IFN signaling is known to be induced by infections with both viruses and bacteria, it could also affect the composition of lung microbiota.

We hypothesized that upon influenza virus infection, the composition of microbiota in IFNAR-/- mice will be much different from that of WT mice.

MATERIALS & METHODS

Both C57BL/6 and IFNAR-/- mice were used in this study. IFNAR-/- mice are bred on C57BL/6 genetic background, and thus C57BL/6 mice were used here as WT controls. Both the C57BL/6 and IFNAR-/- mice are bred at Montana State University, Animal Resource Center, Bozeman MT. At 8–15 weeks of age, male or female mice (5/group) were enrolled in the inoculation experiments.

Mice were intratracheally inoculated with one of the following: 120pfu (plaque forming units) of influenza virus (mouse adapted H1N1 influenza virus strain; PR8), and 100 µL of Phosphate buffered saline (PBS), or were left untreated (naïve).

Mice were inoculated on day 0 and then their lung microbiota was evaluated on day 3 because this is the time when mice start showing symptoms of influenza virus infection. This is also time when the important changes occur in type I IFN signaling.

DNA was extracted from three parts of the respiratory tract; mouth (tongue, palate), upper respiratory tract (URT) (trachea, nasal bone), and lung (lung, bronchi). Then, DNA was amplified using set of specific primers that were to designed to recognize bacterial species-specific genes. Primers for 8 different bacteria (*Haemophilus influenzae, Lactobacillus plantarum, Moraxella catarrhalis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae*, Group A Streptococcus, Group B Streptococcus) that are commonly found in lung microbiota were tested. PCR was done using a thermal cycler (40 cycles). Then, 10 µl of PCR mixtures were run on a 2% agarose gel prepared in house and imaged under UV light.

RESULTS

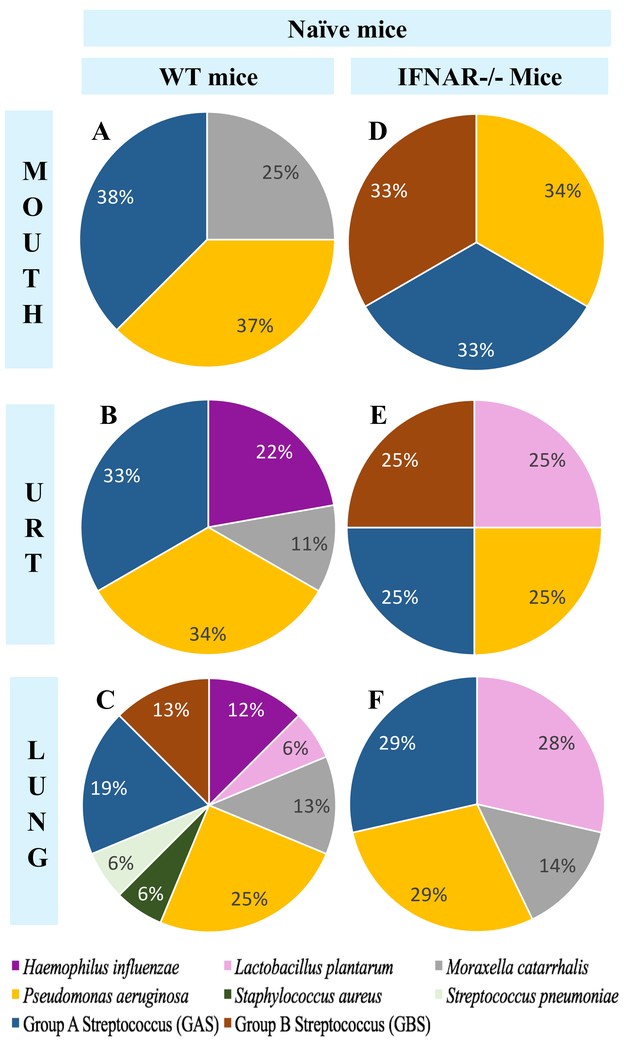

Figure 1. Bacterial species in mouth, URT, and lung in naïve WT mice and naïve IFNAR-/- mice. In both WT mice and IFNAR-/- mice *P. aeruginosa* and GAS were amplified from all parts of the respiratory tract. In mouth and URT, GBS (both mouth and URT; D/E) and *L. plantarum* (URT; E) were abundant in IFNAR-/- mice but not in WT mice. In mouth and URT, *M. catarrhalis* (both mouth and URT; A/B) and *H. influenzae* (URT; B) were abundant in WT mice but not in IFNAR-/- mice. *H. influenzae, S. pneumoniae, S. aureus*, and GBS were more abundant in lung of WT mice (C) when compare to IFNAR-/- mice.

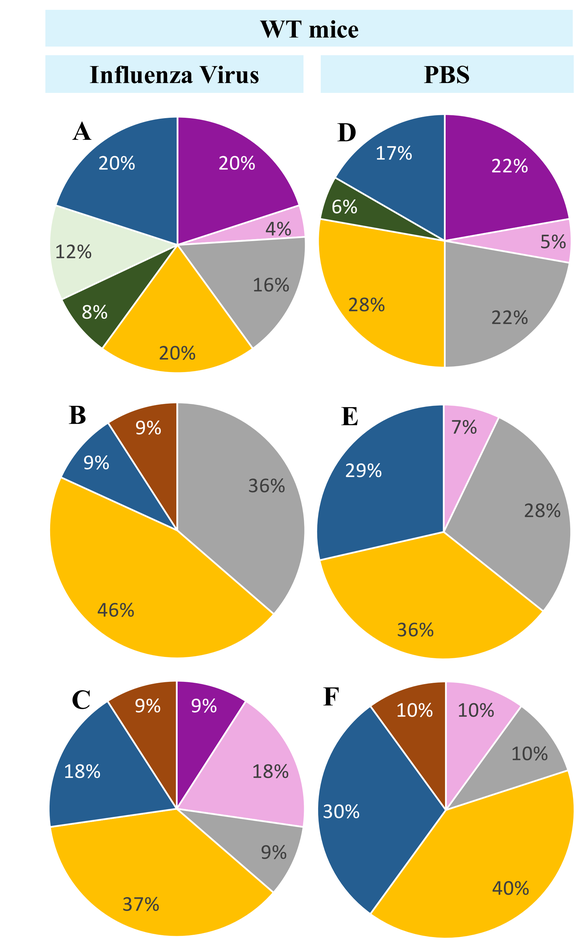

Figure 2. Bacterial species in mouth, URT, and lung in WT mice that were either infected with influenza virus or inoculated with PBS. GAS, *M. catarrhalis*, and *P. aeruginosa* were amplified from all parts of the respiratory tract of WT mice regardless of treatment. *H. influenzae* , *L. plantarum* , and *S. aureus* were abundant in mouth of mice inoculated with PBS (Fig.2D) but not in naïve mice (Fig.1A). In URT, *H. influenzae* was not amplified and *L. plantarum* was amplified in the mice inoculated with PBS (Fig.2E) compare to naïve WT mice (Fig.1B). *H. influenzae*, *S. aureus*, *S. pneumoniae* were abundant in lung of naïve mice (Fig.1C) but not in mice inoculated with PBS (Fig.2F). In mice that were infected with influenza virus, *S. pneumoniae* (Fig.2A), GBS (Fig.2B), *H. influenzae* (Fig.2C) were abundant but not in that of mice inoculated with PBS.

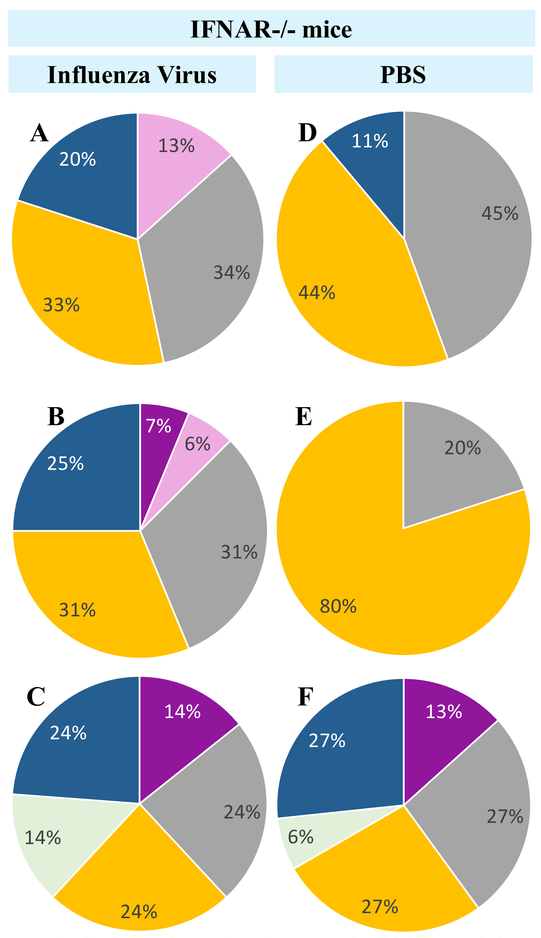

Figure 3. Bacterial species in mouth, URT, and lung in IFNAR-/- mice that were either infected with influenza virus or inoculated with PBS. *P. aeruginosa* and *M. catarrhalis* were amplified from all parts of the respiratory tract of IFNAR-/- mice that were inoculated with PBS and infected with influenza virus. GBS (Fig.1D/E), *L. plantarum* (Fig.1E/F) were abundant in naïve mice but not in mice inoculated with PBS. *H. influenzae* (Fig.3F) and *S. pneumoniae* (Fig.3F) were abundant in mice inoculated in PBS compare to the naïve mice. Furthermore, in mouth and URT, *L. plantarum* (Fig.3A/B) and *H. influenzae* (Fig.3B) were amplified in mice that were infected with influenza virus but not in mice inoculated with PBS. In lung, there were similar composition of bacterial species in both condition. *S. pneumoniae* (Fig3.C) was amplified and *L. plantarum* and GBS (Fig.2C) were not amplified in IFNAR-/- mice that were infected with influenza virus compared to the WT mice that were infected with influenza virus .

CONCLUSIONS

- Lungs of naïve WT mice appear to have higher abundance of bacterial species than lungs of naïve IFNAR-/- mice.
- *P. aeruginosa* and GAS are abundant in all parts of respiratory tract in both WT and IFNAR-/- mice regardless of the treatment.
- Inoculation of mice with PBS changes composition of lung microbiota.
- Influenza virus infection reduces abundance of bacterial species in the lungs of WT mice but increases the abundance of bacterial species in the lung of IFNAR-/- mice.

Genetic factors like type 1 IFN signaling can affect composition of lung microbiota

FUTURE WORK

In the future, I plan to determine whether type 1 IFN signaling affects composition of lung microbiota in the context of influenza virus infection by using more sensitive methods. The PCR provides a good initial screen, but it is not a quantitative method. Thus, there is a chance that I might have missed some bacterial species that were less abundant in these samples. In future, I will use more sensitive methods including deep sequencing followed by 16S qRT-PCR.

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