Interferon lambda signaling protects infant mice against lethal disseminating *Bordetella pertussis* infection

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INTRODUCTION

Infection of the respiratory tract by Bordetella pertussis causes whooping cough (pertussis) and can lead to lethality in susceptible populations, including infants prior to being fully vaccinated against disease[1]. Our group previously demonstrated a role for type 3 interferon (IFNλ) in exacerbating lung inflammation during B. pertussis infection in adult mice. However, IFNλ is not upregulated in infected infant mice[2]. In addition to those observations, our group previously showed that infected C57BL/6 mice inoculated at 7 days old (P7) succumb to infection whereas adult mice survive infection[3]. One of the major mechanisms by which IFNλ signaling affects outcomes of infections at the mucosal barriers is by changing barrier integrity[4]. We hypothesized that IFNλ plays an age-related role in *B. pertussis* infections where IFNλ signaling would be protective in infant infection by promoting lung epithelial barrier integrity.

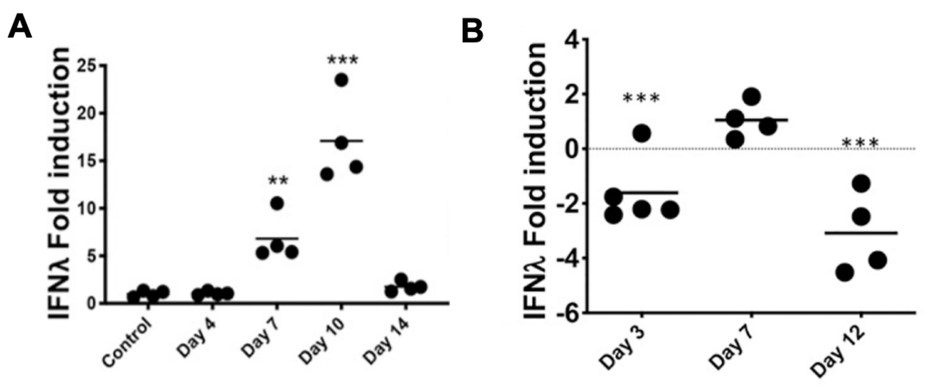

Figure 1. Ardanuy et al (2020), demonstrated that infected adult mice upregulated IFNλ over the course of infection (A), while animals inoculated at P7 and euthanized at any point post inoculation did not upregulate IFNλ.

METHODS

CC57BL/6 pups and IFNλ receptor knockout (IFNLR1KO) pups were infected with *B. pertussis* via aerosol at either P7 or P10. Pups were either euthanized 7 days post inoculation (dpi) (n = 7-11) or monitored for survival up to 21 dpi (n = 7-9). The lungs, spleen, blood, and a portion of the liver were collected from animals euthanized on 7 dpi. The tissue was homogenized and dilutions plated to calculate the bacterial burden in CFU/mL. RNA was isolated from flash frozen lung samples and gene expression was measured using qRT-PCR. P7 C57BL/6 pups were infected and treated intranasally with vehicle control (n=4) or purified IFNλ (n=4, 400 ng/animal/day) on 1, 3, 5, 7, and 9 dpi. Treated animals were monitored up to 21 dpi. The log-rank (Mantel-Cox) test was used to evaluate the significance between survival curves. A two-tailed t test was used to evaluate significant difference in bacterial burden between two groups.

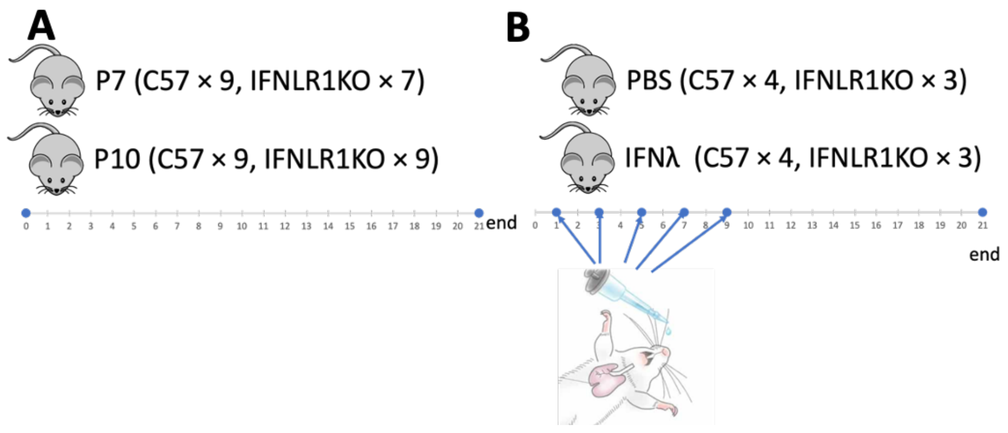

Figure 2. Survival Experimental Design. Mice were infected with *B. pertussis* at either P7 of P10 and then followed for survival for 21 days post inoculation for survival (A). P7 mice of the same litter were divided into two treatment groups, vehicle and IFNλ. Mice were anesthetized using isoflurane and received 400 ng of IFN λ dissolved in 1× PBS or its equivalent of vehicle intranasally once per day on days 1,3,5,7,and 9 post inoculation. Survival was recorded up to 21 dpi (B)

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RESULTS

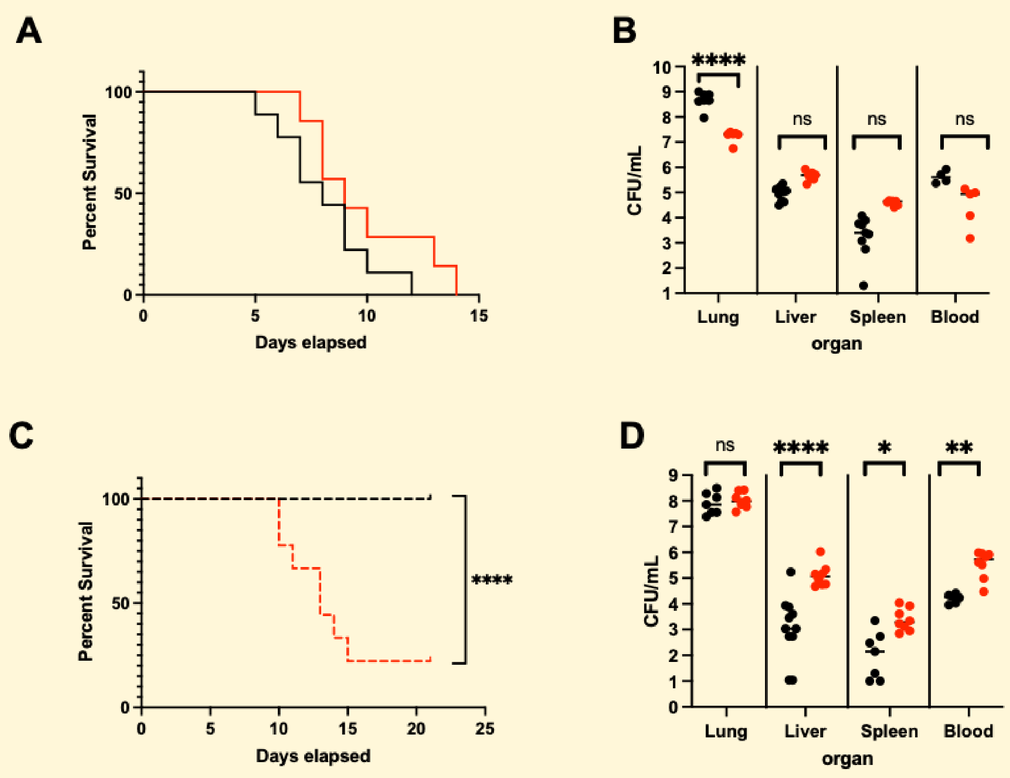

Figure 3. Bacterial dissemination correlates with lethality in P10 IFNLR1KO mice. C57BL/6 (black) and IFNLR1KO (red) pups inoculated at P7 showed no difference in survival between the two groups (p = 0.15) (A); however, for mice inoculated at P10, all of the C57BL/6 pups survived while only 22% of the IFNLR1KO pups survived until 21 dpi (p = 0.0008) (B). P10 IFNLR1KO pups showed significantly more dissemination to the liver, spleen, and blood (p<0.0001, p=0.04, and p=0.005 respectively) compared to P10 WT pups (D). P7 IFNLR1KO pups did not show any significant changes in dissemination from the lung (C).

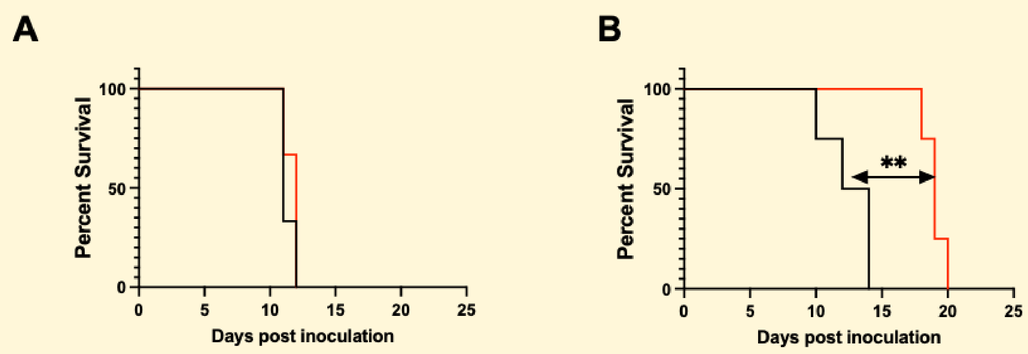

FIgure 4. Intranasal administration of IFNλ prolongs survival of P7 C57BL/6 mice. As a negative control, P7 IFNLR1KO mice were aerosol inoculated and treated as described in Figure 2B (A). P7 C57BL/6 mice were also were aerosol inoculated and treated as described in Figure 2B (p=0.0091) (B). p value is shown to be significant based on log-rank (Mantel-Cox) test. Red lines represent IFNλ treated animals and black lines represent PBS treated animals.

SUMMARY

1. **P10 IFNLR1KO mice are significantly more susceptible to lethality due to *B. pertussis* infection**
2. **P10 IFNLR1KO mice (and not P7 IFNLR1KO mice) demonstrate increased dissemination of bacteria from the lungs**
3. **Intranasal treatment of P7 C57BL/6 with recombinant IFNλ prolongs survival**

REFERENCES

1. Yeung, K. H. T., Duclos, P., Nelson, E. A. S., & Hutubessy, R. C. W. (2017). An update of the global burden of pertussis in children younger than 5 years: a modelling study. *The Lancet. Infectious diseases*, *17*(9), 974–980. https://doi.org/10.1016/S1473-3099(17)30390-0
2. Ardanuy, J., Scanlon, K., Skerry, C., Fuchs, S. Y., & Carbonetti, N. H. (2020). Age-Dependent Effects of Type I and Type III IFNs in the Pathogenesis of *Bordetella pertussis* Infection and Disease. *Journal of immunology (Baltimore, Md. : 1950)*, *204*(8), 2192–2202. https://doi.org/10.4049/jimmunol.1900912
3. Scanlon, K. M., Snyder, Y. G., Skerry, C., & Carbonetti, N. H. (2017). Fatal Pertussis in the Neonatal Mouse Model Is Associated with Pertussis Toxin-Mediated Pathology beyond the Airways. *Infection and immunity*, *85*(11), e00355-17. https://doi.org/10.1128/IAI.00355-17
4. Johnson, D and Carbonetti N. (2023). Roles and Effects of Interferon Lambda Signaling in the Context of Bacterial Infections[Accepted for publication in Journal of Interferon & Cytokine Research]