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Laboratory of Comparative Genomics

Modulation of Commensal Gut and Pulomnary Microbiomes Through Oral Microbial Inoculation and its Effects on Systemic Immune Response

K. Schachtschneider,[1] C.J. Yeoman,[2] B.A. White,[1,2] L.B. Schook[1,2]

[1]Department of Animal Sciences, [2]Institute for Genomic Biology, University of Illinois at Urbana-Champaign

Introduction:

The role of gastrointestinal microorganisms in developing and modulating the gastrointestinal immune response has been subject to investigation over the past few decades. However, early gastrointestinal tract stimulation and modulation of the systemic immune response is far from understood. Recent investigations in the area of immune responses and their relationship with the gut microbiota have opened the door to new approaches for disease treatment and control. This study explored the use of a non-pathogenic oral microbial inoculum to affect the composition of commensal microbiomes and subsequent systemic immune responses. Artificially raised piglets were divided into two groups: one group was orally inoculated (INOC) with non-pathogenic gut microbiota, and the second group was uninoculated (UNINOC). In order to determine the effects of changes in the microbiome on systemic immune response, allergic (type I) and delayed type (type IV) hypersensitivity responses were tested, as well as systemic immune responses via experimental infection with the respiratory pathogen *M. hyopneumoniae*. The 16S ribosomal RNA gene populations were amplified from DNA extracted from both fecal samples and nasal swabs collected temporally throughout the study. These amplicons were sequenced using 454 FLX-titanium technology to determine at great depth the effect of the oral microbial inoculum on both the gut and pulmonary commensal microbiomes.

Materials and Methods:

•All protocols for this experiment were approved and animals were cared for following the guidelines from the IACUC and IBC of the University of Illinois at Urbana-Champaign.

•Twelve piglets were artificially raised, fed bovine colostrum during the first 2 days of life, and medicated milk replacer thereafter until week 4. An automated feeding system was put in place in order to deliver milk replacer to piglets every 60 min at a daily rate of 360mg/Kg of live weight. A partial view of the automated feeding system is shown in Fig 1. Pigs were gradually introduced to a commercial dry feed formula, which eventually replaced the liquid feeding.

•The experimental design for the study is depicted in Fig 2. Piglets were split into 2 groups at weaning. One group was orally Inoculated (INOC) with a non-pathogenic inoculum 1x/day over 1 week. The second group was left uninoculated (UNINOC). The non-pathogenic inoculum consisted of a 1:1 mixture containing a fresh fecal slurry (obtained from a boar in a high health herd) and phosphate buffer saline. Four weeks after the oral exposure was completed, pigs were experimentally infected with 20 ml of 1x10^5 CCU/ml of *M. hyopneumoniae* strain 232 (Iowa State University, Ames, IA), using the intra-tracheal route of inoculation.

Fig. 1: Automatic feeding system for piglets.

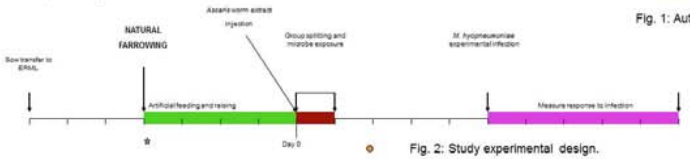

Fig. 2: Study experimental design.

•Severity of infection as well as systemic immune responses were recorded throughout the experiment, including antibody production, coughing levels, lung lesion scores, immune response gene expression levels and cytokine production.

•DNA was extracted from nasal swabs, bronchial swabs (BS), lung lavage (LL) and fecal samples using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA).

•The 16S ribosomal RNA gene populations were amplified from DNA extracted throughout the study.

•Apmlicons were sequenced using 454 FLX-titanium technology to determine at great depth the effect of the oral microbial inoculum on both the gut and pulmonary commensal microbiomes.

Results:

•Under the conditions of this study, pigs in group INOC had less respiratory clinical signs and lesions, and seroconverted earlier than pigs in group UNINOC, suggesting a beneficial effect of the oral non-pathogenic inoculum on the severity of mycoplasmal pneumonia. A summary of host responses for each treatment group can be found in table 1.

•Preliminary analyses of the sequencing results reveal compositional differences in the pulmonary microbiomes between treatment groups (figure 3). No preliminary analyses of the gut microbiome data is currently available.

•Bronchial swabs and lung lavage samples (Day 35) were shown to be compositionally different from nasal swab samples as shown in figure 4. Therefore day 35 samples were analyzed separately. This analysis revealed no compositional differences in the day 35 samples from the lungs of the two groups (figure 5).

•Analyses of the gut and pulmonary microbiomes is ongoing.

Parameter Measured	UNINOC	INOC	P-Value
DTH (1,000 Units)[a]	0.643	1.19	0.07
Cough Observations[b]	9.6	4.4	<0.005
Lung Lesions (looc area)[c]	42%	28%	0.07
Expression Level Variance (TLR2)[d]	2.8	0.48	0.04
Expression Level Variance (T-R6)[d]	2.44	0.25	0.01
TNF-alpha Variance[d]	2.41	0.43	0.0008

Table 1: Summary of systemic host responses from experimental groups.
[a]Ascaris antigen induced DTH- skin thickness (mm)
[b]Blinded coughing episodes per 30 mins following *M. hypopneumoniae* infection
[c]Area of lung lobe covered in lesions following *M. hypopneumoniae* infection
[d]Variance of the two groups for expression level and cytokine concentration

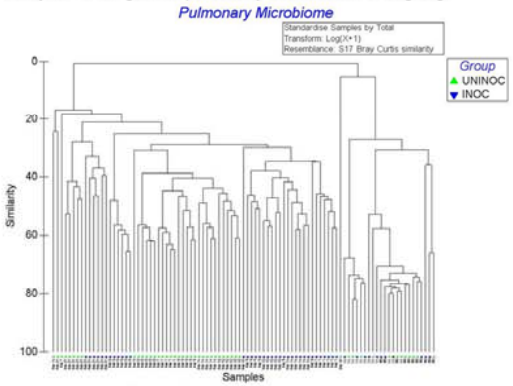

Figure 3: Clustering analysis of pulmonary microbiomes

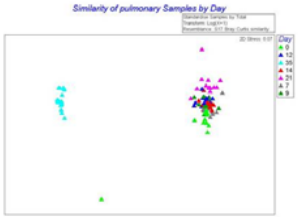

Figure 4: Clustering analysis of pulmonary microbiomes reveals compositional differences of BS and LL (day 35) samples from nasal swab samples

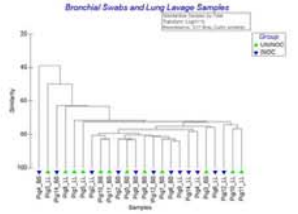

Figure 5: Clustering analysis of bronchial swabs and lung lavage samples

Conclusions and Implications:

In this investigation, we have tested the effect of microbiota manipulation on the gut and pulmonary microbiomes and subsequent systemic immune responses. Under the conditions of this study, pigs in group INOC had less respiratory clinical signs and lesions, and seroconverted earlier than pigs in group UNINOC, suggesting a beneficial effect of the oral non-pathogenic inoculation on the severity of mycoplasmal pneumonia. Preliminary sequencing results revealed compositional differences in pulmonary microbiomes between treatment groups, and analysis on both pulmonary and gut microbiomes is ongoing. These results suggest the oral microbial inoculum is influential to our commensal microbiomes, and has a significant effect on systemic immune responses.

Acknowledgements:

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