Comparison of the transcriptome response within the tracheobronchial lymphnode following infection with PRRSV, PCV2 or IAV

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Why Do We Need Bioinformatics?

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This investigation was a first in a series of *in vivo* comparative studies into the swine host immune response to several major porcine respiratory infections.

- Porcine reproductive and respiratory syndrome virus (PRRSV)
- Porcine circovirus type 2 (PCV2)
- Swine influenza virus (IAV)

Aim of this study was to acquire a better understanding of PRRS disease by comparing gene expression changes that occur in tracheobronchial lymph nodes (TBLN) of pigs infected these viruses.
• Experimental Design

• Pigs were allotted to one of 4 treatment groups:
  • sham inoculated control,
  • PRRSV-challenge (SDSU strain),
  • PCV2-challenge,
  • IAV-challenge.

• Pigs received an intranasal challenge with 2 ml of either sham or virus inoculum. Control pigs were sham inoculated with tissue culture supernatant.

• Five pigs from each group were euthanized and necropsied on 1, 3, 6, and 14 dpi.

• TBLN were homogenized and aliquots used for RNA extraction.

• Total RNA was pooled for each group within time point to make 16 libraries, for DGETP SAGE sequencing.
• SAGE (Serial analysis of gene expression)
  • Tag sequences obtained from 3’ end within cDNA that are long enough to uniquely identify each transcript.
  • Sensitive to low-abundant transcripts and small changes in gene counts.

• Caveats
  • However, older method of sequencing reliant on genome annotation.
  • Sequence tags need to be linked to known ID.
  • Identified tags can be normalized as counts.
1 main effect: Treatment (n=4)
  - Control, PRRSV(SDSU), PCV2, IAV (H1N1)
1 cofactor: Time (n=4)
  - 1 DPI, 3 DPI, 6 DPI, 14 DPI
Based on reduced model \( Y = \sim \text{Treatment} + \text{Time} + E \)
3 separate runs of Control vs. Virus
Fit-type parametric; FDR applied \( Q \leq 0.1 \)
  - Counts over 3000 genes
  - Tag sequences with 5 counts or less removed
  - Dispersion fit type: parametric
Results: Overlap of DEG for PCV2, PRRSV, and IAV infection

- **PCV2**
  - only 78 genes significant
  - 44% downregulated
  - 56% upregulated

- **PRRSV**
  - Total of 308 DEG
  - 43% downregulated
  - 57% upregulated

- **IAV**
  - Total of 215 DEG
  - 35% downregulated
  - 65% upregulated
### Results: List of DEG response shared across PRRSV & PCV2

<table>
<thead>
<tr>
<th>GENE NAME</th>
<th>PRRSV</th>
<th>PCV2</th>
<th>FUNCTION/PROCESS</th>
<th>PATHWAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRC1</td>
<td>-1.3</td>
<td>-0.91</td>
<td>Endocytosis of glycoproteins by macrophages</td>
<td>Phagosome, Adaptive Immune System</td>
</tr>
<tr>
<td></td>
<td>-1.62</td>
<td>-0.79</td>
<td>Reactive oxygen species metabolic process</td>
<td>Signal Transduction</td>
</tr>
<tr>
<td></td>
<td>-1.73</td>
<td>-0.51</td>
<td>Extracellular matrix organization</td>
<td>ECM proteoglycans, Integrin cell surface interactions</td>
</tr>
<tr>
<td>RAB11B</td>
<td>1.21</td>
<td>0.67</td>
<td>Exocytotic and endocytotic pathway regulation</td>
<td>AMPK signaling pathway</td>
</tr>
<tr>
<td>ME3</td>
<td>0.95</td>
<td>0.73</td>
<td>Oxidation-reduction process</td>
<td>Pyruvate metabolism</td>
</tr>
<tr>
<td>CAPNS2</td>
<td>2.21</td>
<td>0.79</td>
<td>Extracellular matrix disassembly</td>
<td>Degradation of the extracellular matrix</td>
</tr>
</tbody>
</table>

**Take away point #1:**
- PRRSV and PCV2 share ~ 1/3 (n=23) of the total number of DEGs observed for PCV2. More than twice the amount shared with IAV (n=9).

**Take away point #2:**
- Overlap in genes corresponded with studies that showed that DEGs during PRRSV & PCV2 infection effects ECM matrix, ECM receptor, and cytokine induction pathways.
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<thead>
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<tbody>
<tr>
<td>TNFRSF11B</td>
<td>-2.83</td>
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<tr>
<td>CXCL13</td>
<td>-2.64</td>
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<tr>
<td>AGO1</td>
<td>-2.34</td>
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<tr>
<td>VEGFA</td>
<td>-1.70</td>
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<tr>
<td>CAV1</td>
<td>-1.44</td>
<td></td>
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<tr>
<td>LY9</td>
<td>-0.95</td>
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<td>CSF3R</td>
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<td>SOCS3</td>
<td>1.44</td>
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<tr>
<td>IL1B</td>
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<td>IFI6</td>
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</tbody>
</table>

**Take away point #1:**
- Strong downregulation of CXCL13, which helps initiate the switch from innate to adaptive immune responses, may stall adaptive immunity to PRRSV.

**Take away point #2:**
- Strong upregulation of SOCS3, a suppressor of cytokine signaling, may spotlight a means of viral mediated anti-inflammatory induction within the host.
The red box containing the immune response genes is likely trying to increase inflammatory signaling.

Downregulation of the “hub” genes (red circles) is likely preventing downstream signaling to increase inflammation and/or signal adaptive immunity.
<table>
<thead>
<tr>
<th>GeneID</th>
<th>log2(FC)</th>
<th>G.O. Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCF2</td>
<td>-1.68</td>
<td>GO:0006955 immune response</td>
</tr>
<tr>
<td>LCN2</td>
<td>-1.51</td>
<td>GO:0006955 immune response</td>
</tr>
<tr>
<td>DOCK1</td>
<td>-1.45</td>
<td>GO:0038093 Fc receptor signaling pathway</td>
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<tr>
<td>SIGLEC5</td>
<td>-1.41</td>
<td>GO:0007155 cell adhesion</td>
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<tr>
<td>SLC1A5</td>
<td>-1.11</td>
<td>GO:0019058 viral life cycle</td>
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<tr>
<td>CD72</td>
<td>1.11</td>
<td>GO:0007155 cell adhesion</td>
</tr>
<tr>
<td>STAT5B</td>
<td>1.15</td>
<td>GO:0002443 leukocyte mediated immunity, GO:0080134 regulation of response to stress</td>
</tr>
<tr>
<td>SLAMF1</td>
<td>1.23</td>
<td>GO:0002443 leukocyte mediated immunity, GO:0080134 regulation of response to stress</td>
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<tr>
<td>CD247</td>
<td>1.26</td>
<td>GO:0007159 leukocyte cell-cell adhesion, GO:0080134 regulation of response to stress</td>
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<tr>
<td>CAMK2D</td>
<td>2.05</td>
<td>GO:0071345 cellular response to cytokine stimulus, GO:0080134 regulation of response to stress</td>
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<tr>
<td>ELMO1</td>
<td>2.14</td>
<td>GO:0038093 Fc receptor signaling pathway</td>
</tr>
<tr>
<td>CSNK2B</td>
<td>2.21</td>
<td>GO:0071345 cellular response to cytokine stimulus</td>
</tr>
</tbody>
</table>

**Take away point #1:**
- Downregulation of innate immune responses and upregulation of cellular-mediated immune responses.

**Take away point #2:**
- Upregulated adaptive immune responses related to CD247, CD72, and ELMO1, show that the IAV infected pigs were likely dealing with the cellular disadvantages to recovery.
The results showed that PRRSV, IAV and PCV2 viral infections followed a clinical course in the pigs typical of experimental infection of young pigs with these viruses.

The overlap of expressed genes between PRRSV and PCV2 uncovered an expanse of molecules that play roles in immune, redox, and structural functions that may elucidate co-infections.

For the PRRSV infected pigs, we mostly witnessed downregulation of genes related to signaling processes that can initiate adaptive immunity.

For IAV infected pigs, it is likely the differential expression observed is more closely related to oxidative and nutritive stress recovery of the host at later time points.
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