Avian Disease & Oncology Lab (ADOL) Research Update

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USDA-ARS-ADOL
Research Program at ADOL

Research Projects

• **Genomics (Cheng, Hunt, Zhang)**
  
  **Title**: Employing genomics, epigenetics, and immunogenetics to control diseases induced by avian tumor viruses

• **Tumor Virus (Dunn, Fadly, Heidari)**
  
  **Title**: Genetic and biological determinants of avian tumor virus pathogenicity, transmission, and evolution
Detecting Genes With Allele-Specific Expression In Response To Viral Infection

Compare ratios

uninfected

infected

A = C

A > C
Validation of GEBVs by Progeny Test

- 300 F7 roosters were genotyped and GEBVs (genomic estimated breeding values) calculated.

- The top 30 and bottom 30 roosters were each reciprocally mated to 6 random F7 hens to produce ~30 progeny per rooster.

- Disease incidence in progeny measured.
MD Incidence
(accuracy 61% higher than traditional BLUP)
Genetics and Vaccine Efficacy – MHC

Earlier studies showed:

- **MHC B** haplotypes affect host immunoresponse to MD vaccines.

- Chickens with **B*5** respond to serotype 2 vaccine better than serotype 1 vaccine.

- Chickens with **B*2, B*13, B*15, or B*21** haplotype(s) respond to serotype 1 vaccine better than serotypes 2 and 3.
Genetics and Vaccine Efficacy – non-MHC

- Significant differences between lines and between vaccines
What is a BAC clone?

Bacterial Artificial Chromosome
Trial 2

MD incidence

- JM/102W
- Md5
- pC12/130-10
- pC12/130-15
- pRB-1B-5 repaired(1232)

- None
- HVT
- HVT + SB-1

prototypes

unknown viruses
Trial 3

MD incidence

prototypes

unknown viruses

JM/102W
Md5
686
686-1 BAC
686-2 BAC

V
VV
VV+

v vv vv+
prototypes unknown viruses

None
HVT
HVT + SB-1
# Codon Table

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Codons</th>
<th>Amino acid</th>
<th>Codons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>GCT, <strong>GCC</strong>, GCA, GCG</td>
<td>Leu</td>
<td>TTA, TTG, CTT, CTC, CTA, CTG</td>
</tr>
<tr>
<td>Arg</td>
<td>CGT, CGC, CGA, CGG, AGA, AGG</td>
<td>Lys</td>
<td>AAA, AAG</td>
</tr>
<tr>
<td>Asn</td>
<td>AAT, AAC</td>
<td>Met</td>
<td>ATG</td>
</tr>
<tr>
<td>Asp</td>
<td>GAT, GAC</td>
<td>Phe</td>
<td>TTT, TTC</td>
</tr>
<tr>
<td>Cys</td>
<td>TGT, TGC</td>
<td>Pro</td>
<td>CCT, CCC, CCA, CCG</td>
</tr>
<tr>
<td>Gln</td>
<td>CAA, CAG</td>
<td>Ser</td>
<td>TCT, TCC, TCA, TCG, AGT, AGC</td>
</tr>
<tr>
<td>Glu</td>
<td>GAA, GAG</td>
<td>Thr</td>
<td>ACT, ACC, ACA, ACG</td>
</tr>
<tr>
<td>Gly</td>
<td>GGT, GGC, GGA, GGG</td>
<td>Trp</td>
<td>TGG</td>
</tr>
<tr>
<td>His</td>
<td>CAT, CAC</td>
<td>Tyr</td>
<td>TAT, TAC</td>
</tr>
<tr>
<td>Ile</td>
<td>ATT, ATC, ATA</td>
<td>Val</td>
<td>GTT, GTC, GTA, GTG</td>
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<tr>
<td>START</td>
<td>ATG</td>
<td>STOP</td>
<td>TAA, TGA, TAG</td>
</tr>
</tbody>
</table>

A 300-amino acid protein can be encoded $\sim 10^{151}$ ways
Marek's disease incidence (Trials 3 & 4)

Trial 3

Trial 4

*
Role of endogenous ALV and serotype-2 MDV in enhancement of spontaneous LL-like tumors

● Chickens of ADOL line 0 and transgenic line alv6 are known to develop spontaneous avian leukosis virus (ALV)-like lymphomas at two years of age or older.

● We have previously shown that Serotype 2 Marek’s disease virus can enhance the development of ALV and REV-induced bursal lymphomas in certain lines of chickens.
Spontaneous ALV-induced-like tumors in RFS chickens with or without ALV-E and vaccinated Serotype 2 (SB-1) MDV at hatch

<table>
<thead>
<tr>
<th>Lot</th>
<th>Inoculum</th>
<th>#chickens with tumors/#chickens at risk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBS</td>
<td>2/24 (8%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>SB-1</td>
<td>4/24 (17%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>AF-227</td>
<td>5/36 (14%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>AF-227 + SB-1</td>
<td>15/36 (42%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with different lower case letters are significantly different values (p < 0.05) as determined by Fisher’s exact test.
Protective efficacy of a recombinant BAC clone of a vvMDV containing a REV LTR insertion

Table 1. Pathogenicity Study

<table>
<thead>
<tr>
<th>Virus</th>
<th>Passage Level</th>
<th>MAB</th>
<th>MDV-Induced Lesions&lt;sup&gt;a&lt;/sup&gt; Total MDV/Total at risk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>-</td>
<td>0/13 (0%)&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>rMd5 REV-LTR</td>
<td>95</td>
<td>-</td>
<td>0/17 (0%)&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>rMd5 REV-LTR</td>
<td>90</td>
<td>-</td>
<td>0/17 (0%)&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>rMd5 REV-LTR</td>
<td>80</td>
<td>-</td>
<td>0/16 (0%)&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>rMd5 REV-LTR</td>
<td>70</td>
<td>-</td>
<td>0/17 (0%)&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>rMd5 REV-LTR</td>
<td>60</td>
<td>-</td>
<td>4/16 (25%)&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>rMd5 REV-LTR</td>
<td>40</td>
<td>-</td>
<td>7/17 (41%)&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>rMd5 REV-LTR</td>
<td>10</td>
<td>-</td>
<td>10/10 (100%)&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>rMd5</td>
<td>10</td>
<td>-</td>
<td>15/15 (100%)&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> MDV-Induced Lesions include bursa and thymic atrophy as a result of MDV pathology. Values with a different upper case letter indicate significantly different values (p < 0.05) as determined by Chi-square analysis.
Table 2. Protective Efficacy of rMd5 REV-LTR against vv+ MDV challenge in susceptible chickens without MDV maternal antibody

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Passage Level</th>
<th>MAB</th>
<th>Challenge Virus</th>
<th>MDV-Induced Lesions(^a) Total MDV/Total at risk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>-</td>
<td>None</td>
<td>0/34 (0%)(^A)</td>
</tr>
<tr>
<td>rMd5 REV-LTR</td>
<td>70</td>
<td>-</td>
<td>686</td>
<td>5/34 (15%)(^A)</td>
</tr>
<tr>
<td>rMd5 REV-LTR</td>
<td>90</td>
<td>-</td>
<td>686</td>
<td>24/37 (65%)(^B)</td>
</tr>
<tr>
<td>BACdelMEQ</td>
<td>19</td>
<td>-</td>
<td>686</td>
<td>20/34 (59%)(^B)</td>
</tr>
<tr>
<td>BACdelMEQ</td>
<td>50</td>
<td>-</td>
<td>686</td>
<td>3/32 (9%)(^A)</td>
</tr>
<tr>
<td>CVI988/Rispens</td>
<td>None</td>
<td>-</td>
<td>686</td>
<td>7/39 (18%)(^A)</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>-</td>
<td>686</td>
<td>37/37 (100%)(^C)</td>
</tr>
</tbody>
</table>

\(^a\)MDV-Induced Lesions include bursa and thymic atrophy as a result of MDV pathology. Values with a different upper case letter indicate significantly different values (p < 0.05) as determined by Chi-square analysis.
Effect of MDV infection on cecal tonsils

Cecal tonsils
H&E stain
(5 dpi)

Resistant
Line 63

Susceptible
Line 72

Uninfected

MDV-infected

20.0 μm

20.0 μm

20.0 μm

20.0 μm
Cecal tonsils
MDV pp38
(6 dpi)

Resistant
Line 6₃

Susceptible
Line 7₂

Uninfected

MDV-infected

A

B

C

D

[Images of histological sections showing differences between uninfected and MDV-infected cecal tonsils for resistant and susceptible lines, with annotations indicating scale in micrometers.]
Thank you for your attention