Eradication of Avian Metapneumovirus In Turkeys By Inducing Mucosal Immunity With Inactivated Virus

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Introduction

Avian metapneumovirus (aMPV), also referred to as avian pneumovirus or APV, causes an acute, highly contagious, upper respiratory tract infection in turkeys. Clinical signs of aMPV infection in turkeys consist of depression, coughing, sinusitis, airsacculitis, and mortality. aMPV is a negative sense, single-stranded RNA virus and is classified as a member of the genus Metapneumovirus in the family Paramyxoviridae. aMPV outbreaks in the United States were first reported in Colorado in 1996. Since 1997, the virus has become endemic in Minnesota and has spread to neighboring Midwestern states. The aMPV strain isolated in Minnesota (aMPV/MN/turkey/1-a/97) was identified as subtype C (aMPV/C) which had low sequence similarity with subtypes A or B endemic in Europe and other countries.

Acute infection with aMPV/C is characterized by viral replication in the ciliated epithelial cells of nasal turbinates and infraorbital sinuses, extensive lymphoid cell infiltration in the upper respiratory tract (URT) and immunosuppression (1, 2). Most turkeys recover from the acute phase of the disease although immunosuppressive effects may persist. The main economic loss due to aMPV/C is attributed to immunosuppression and poor flock performance. Current control measures for aMPV/C include the use of a modified live vaccine in endemic areas.

Because aMPV is an upper respiratory disease, the role of local immunity in the respiratory mucosa is of interest. Local immunity at the mucosal surface is the first line of defense that protects the host against infections. In general, secretory IgA is the major antibody type that lines the mucosal surface. IgA plays a critical protective role by binding and neutralizing viruses before they can enter and replicate in the mucosal target cells. The involvement of local immunity in the
pathogenesis of aMPV in turkeys has not been fully established. Previous studies have shown that large numbers of immune cells infiltrate the mucosa and the Harderian gland of turkeys during the acute phase of the disease (2). Also, the report with subtype B aMPV showed induced mucosal IgA in chickens.

The main objective of this project was to induce local and systemic immunity by immunizing turkeys with killed aMPV.

**Materials and Methods**

Two-week-old turkeys, lacking aMPV antibodies were inoculated with the 41\textsuperscript{st} passage of aMPV/C in Vero cells (aMPV41) via the oculonasal (O/N) route. At 7 days post inoculation, turbinate or spleen cells were collected and examined for relative proportions of CD4\(^+\) and CD8\(^+\) T cells by FACS analysis. The turbinate cells were examined for cytokine gene expression by real time RT-PCR. In vaccination experiments, turkeys were immunized with formaldehyde-inactivated aMPV41 (IV). Three consecutive inoculations of IV were given at 1, 10 and 17 days of age via the O/N route. Turbinate and spleen homogenates were examined for gene expressions of IFN-I, Cox-2 and iNOS by semi-quantitative RT-PCR. At 21 or 7 days after the final inoculation, each turkey was challenged by the O/N route with 5000 TCID\textsubscript{50} of aMPV41. Serum and tears obtained before the challenge were examined by ELISA for aMPV/C-specific IgG and IgA antibodies. Protection against the challenge virus was determined at 5 days post challenge. Immunized turkeys were examined for their ability to resist detectable lesion formations in the URT and the replication of the challenge virus in the respiratory mucosa. Virus replication was examined by quantitative real-time RT-PCR.

**Results and Discussion**

Exposure of turkeys to aMPV41 increased the relative proportion of mucosal CD8 T cells (Table 1). CD8 but not CD4 gene expression was upregulated in turbinate cells (Fig. 1). Upregulation of IL-10 gene expression (Fig. 2) indicated that the virus stimulated Th2 type immunity.

We have shown previously that poly(I:C) was an effective respiratory adjuvant that enhanced mucosal immune response to aMPV (Cha and Sharma, unpublished).
Also, immunization with IV increased the presence of IgA and IgA+ cells in the respiratory mucosa. Challenge experiments indicated that turkeys given three immunizations with IV were protected against challenge with live aMPV41 (Figs. 3 and 4). Vaccination also protected against T cell mitogenic inhibition caused by the virulent virus (Fig. 5). These results indicated that we were successful in inducing local respiratory immunity by applying killed virus to the respiratory mucous membranes of commercial turkeys lacking anti-aMPV antibodies.

**Implications for the Industry**

It may be possible to immunize turkeys against aMPV by applying killed virus to the respiratory tract (such as by spray). We have shown under laboratory conditions that respiratory vaccination with killed aMPV induced local immunity characterized by appearance of antibodies in the mucous membrane and protected turkeys against challenge with virulent aMPV.

**References**


Table 1 CD4- and CD8-positive T cells in the turbinate and spleen of aMPV exposed turkeys at 7 DPI.

<table>
<thead>
<tr>
<th>Experiment&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Organ</th>
<th>Treatment</th>
<th>CD4+ cells (%)</th>
<th>CD8+ cells (%)</th>
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<tr>
<td>Exp I&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Spleen</td>
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<td>aMPV41</td>
<td>34.43</td>
<td>37.80</td>
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<td></td>
<td>Turbinate</td>
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<td>33.63</td>
<td>25.50</td>
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<tr>
<td>Exp II&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>23.81±1.30</td>
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<tr>
<td></td>
<td></td>
<td>aMPV41</td>
<td>34.49±3.39</td>
<td>34.43±3.40&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Two-week-old turkeys were inoculated with aMPV41 via O/N route. At 7 days post inoculation, turbinate or spleen cells were collected to detect CD4+ and CD8+ T cells by FACS analysis.
2 n= one pool of 4-5 turkeys.
3 n= three pools of 3-5 turkeys.
4 Mean % ± standard deviation
5 Asterisk indicate statistically significant differences with untreated controls (P<0.05).
Figure 1 CD4 and CD8 gene expressions in the turbinate at 5 days post inoculation with aMPV41 (Exp I, n=3). Gene expressions were detected by real time RT-PCR. GAPDH were used as internal control.
Figure 2 IL-10 and IL-18 gene expressions in the turbinate at 5 days post inoculation with aMPV41 (n=3). Gene expressions were detected by real time RT-PCR. GAPDH were used as internal control.
Figure 3. Turkeys vaccinated with inactivated aMPV vaccine (IV) were protected against microscopic lesions induced by virulent aMPV. Different superscripts indicate significant differences (P<0.05).
Figure 5 Vaccination with inactivated aMPV vaccine (IV) protected against mitogenic inhibition of spleen cells induced by the challenge virus.
Figure 4. Turkeys vaccinated with inactivated aMPV vaccine (IV) were protected
against virus replication in the turbinate induced by virulent aMPV. At 5 days post challenge, choanal swab were collected for RNA extraction, viral load were tested by aMPV real time RT-PCR. Standard curve were established by known tittered virus. Different superscripts indicate significant differences (P<0.05).