Immune Evasion Strategies of Poultry Viruses

Abdul R Omar

Institute of Bioscience,
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
Serdang, Malaysia
Outline of the presentation

• Overview on viral-induced immunosuppression
• Indicators of immunosuppression
• Viral immune evasion
• Vaccines against immunosuppressive viral diseases
• Updates on NDV and IBDV of different virulence in modulating chicken immune responses
• Conclusion
Why viral diseases are difficult to control?

• Agent
  – Threats to various species of avian, some agents are zoonotic
  – Mutate constantly – variant, more immunosuppressive/virulent, extended host range, vaccine escape mutants

• Disease spectrum
  – Cause a wide range of diseases – mild to acute, persistence, concurrent infections, latent & tumor formation

• Management
  – Multi-age, intensive with high stocking density

• Control and preventive measures
  – Difficult to diagnose especially subclinical and persistent infection
  – Vaccination may complicate diagnosis
  – Poor vaccine induced immunity
Immunosuppression is a syndrome often associated with interaction of different factors.

**Noninfectious**
- Environment
- Management
- Nutritional
- Mycotoxin

**Infectious**
- Lymphotrophic viruses that destroy lymphocytes and/or macrophages
- Indirectly by releasing immunosuppressive mediators/cytokines

**Chemicals**
- Steroid
- Ammonia

**Toxin**
- proinflammatory cytokines

**Bacterial**
- REO
- IL-10

**Viruses**
- MDV
- CAV
- AIV
- IBDV
- REO
- CA
- ALV

**Increased susceptibility to infection**
- Poor vaccine induced immunity

**Immunosuppression**
- Transient or permanent
- Generalized or localized
Classification of the chicken immune system

- **Splenic NK cells**
- **Gut NK cells** (IEL NK cells)

**Innate immunity**
- NK cell
- Dendritic cell
- Macrophage

**Adaptive immunity**
- Naive T lymphocyte
- CD4+ T cell
- CD8+ T cell
- Plasma cell
- Memory cell

**Antigen presentation**
- Th Th1, Th2
- Tc
- Treg
- IFNγ
- IL-4
- IL-10

- Blood vessel
Viral immune evasion

- Block antigen processing and presentation
- Inhibit interferon production
- Induce apoptosis or autophagy
- Antigenic variants that outrun immune system
- Viral mimicry, eg. cytokines & their receptors
- Persistently infected cells (carrier)
- Latency & tumor development

Directly or indirectly affecting the immune system

Immunosuppression

# Avian lymphotropic viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Effects on the immune system</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B cell</td>
<td>T cells</td>
</tr>
<tr>
<td>CAV</td>
<td>-</td>
<td>Destroy</td>
</tr>
<tr>
<td>MDV</td>
<td>Destroy</td>
<td>Tumor development</td>
</tr>
<tr>
<td>REV</td>
<td>• Destroy</td>
<td>• Tumor development</td>
</tr>
<tr>
<td>IBDV</td>
<td>Destroy</td>
<td>• Refractory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reduced mitogen responses</td>
</tr>
</tbody>
</table>
Immunopathogenesis of chicken anemia virus

Immunopathogenesis of Marek’s disease virus

1. MDV
2. MDV replication
3. Cell death, apoptosis
4. v-IL-8
5. MDV antigens
6. Cytokine and immune responses
7. Latent MDV infection
8. Transformed T cells

Dev Comp Immunol 24 (2000) 201-221
Immunopathogenesis of Infectious bursal disease virus

B lymphocytes
- Necrosis
- Apoptosis
- Bursal atrophy
- Ab production

T lymphocytes
- Mitogenic response
- Influx of T cells in the bursa
- IFN-γ anti-viral responses

Mφ
- IL-8
- IL-6
- IFN-αβ
- Apoptosis

Acute phase

Recovery phase

Repopulation of B cells in bursal follicles
- Antibody production

Indicators of immunosuppression

- Increased mortality and poor performance
- Increased secondary bacterial infection
- Vaccination and medication failure
- Alteration in heterophil-lymphocyte (H/L) ratio
- Atrophy of lymphoid organs - thymus, bursa of Fabricius
- Depletion of specific population of lymphoid cells
- Detection of immune-related genes based on real-time PCR
- Functional genomics study of lymphoid tissues based on transcriptomic and proteomic analysis
Vaccines against immunosuppressive viral diseases

• Live attenuated and killed vaccines are been used to control of CAV in young birds.

• Live attenuated MDV vaccines of different serotypes able to induce protection against infection and tumour development. However, failed to induce sterilising immunity. In addition, vv + MDV able to break vaccine induced immunity. New gene delete MDV vaccines are currently under evaluation.

• Various types of IBDV vaccine – live attenuated of varying virulence, viral vector and immune complex are been used to control IBDV. However, emergence of variant and reassorted IBDV strains has been reported.
Updates on IBDV and NDV of different virulence in modulating chicken immune responses
What are the roles of different lymphocytes/monocytes during IBDV infection?
What are the roles of different lymphocytes/monocytes during IBDV infection?

- Dendritic cells
  - IL-8
  - IFN-γ
  - IL-12α
  - Apoptosis

- vvIBDV induced higher cell death and proinflammatory cytokines than vaccine strain

References:
Avian Pathol (2015) 44: 452-462
Avian Dis (2016) 60: 739-751
Interaction of IBDV with cellular targets induce apoptosis and immunosuppression
What are the roles of different lymphocytes/monocytes during IBDV infection?

Chicken Gastrointestinal associated Lymphoid Tissues (GALT)

- Populated by heterophils, Mφ, DC, NK cells, B and T lymphocytes
- Epithelial layers are populated with a specialized cells known as the *intraepithelial lymphocytes* (IEL).
- IEL population includes major subsets of NK cells and T cells
- In chickens, CD3-/CD8-/CD25+ IEL NK cells are found predominately in the gut

Percentage of different cell populations of chicken IEL

The majority of the cells are negative for CD3 and CD8
Majority of the CD3-/CD8- cells expressed CD25 (28.4+) and are considered as IEL-NK cells (gut NK cells).

Flow cytometry detection of IEL-NK cells. The histogram shows a representative result from control chickens. The value as the means ± SEM of three experiments.
IEL-NK cells in chickens inoculated with vaccine and very virulent IBDV strains

Normal CD3-/CD25+ IEL NK cells

IEL NK cells are refractory to IBDV infection

Dev Comp Immunol. 2018, 87:116-123
Many of the receptors and their analogues have been detected on chicken NK cells (Dev Comp Imm 2013 41:324-333)
Regulation of NK cell functions

The activating and inhibitory receptors of NK cells and their role in regulation of NK cells

Expression profiles of receptors
IEL NK cells in chicken inoculated with vaccine vs vv IBDV strains

- Isolate and purify the 28.4+/CD3-IEL NK cells
- In vitro activation of the cells with IL-18 and ionomycin/PMA
- Ex vivo analysis of the cells following vaccine and vvIBDV strain infection
- Expression profiles of selected receptors by real-time PCR
  - Inhibitory (B-NK)
  - Activating (CD69, B-Lec, NK-lysis)
  - Bifunctional (CHIR-AB1)
Expression profiles of receptors on IEL-NK cells following infection with vaccine IBDV

<table>
<thead>
<tr>
<th>Gene</th>
<th>Infection with vaccine strain of IBDV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>CD69</td>
<td>2.04±0.04*</td>
</tr>
<tr>
<td>CHIR-AB1</td>
<td>2.5±0.41*</td>
</tr>
<tr>
<td>B-Lec</td>
<td>1.03±0.01*</td>
</tr>
<tr>
<td>B-NK</td>
<td>4.83±0.22*</td>
</tr>
<tr>
<td>NK-Lys</td>
<td>2.22±0.14*</td>
</tr>
</tbody>
</table>

- Unlike infection with vvIBDV, the expressions of all the receptors at 1 dpi were not down-regulated.
- The expressions of all the receptors were up-regulated at 2 and 3 dpi, except for B-NK which showed a down-regulation at 3 dpi.

*Dev Comp Immunol. 2018, 87:116-123*
Expression profiles of receptors on IEL-NK cells following infection with vvIBDV

<table>
<thead>
<tr>
<th>Gene</th>
<th>Infection with very virulent strain of IBDV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>CD69</td>
<td>-7.69±0.09*</td>
</tr>
<tr>
<td>CHIR-AB1</td>
<td>-1.47±0.04*</td>
</tr>
<tr>
<td>B-Lec</td>
<td>1.54±0.31*</td>
</tr>
<tr>
<td>B-NK</td>
<td>1.40±0.20*</td>
</tr>
<tr>
<td>NK-Lys</td>
<td>-1.16±0.17*</td>
</tr>
</tbody>
</table>

- The expressions of CD69, CHIR-AB1 and NK-Lys were down-regulated at 1 dpi
- The expression of B-NK was up-regulated at 1 dpi, and down-regulated at 2 and 3 dpi

*Dev Comp Immunol. 2018, 87:116-123*
Differential expressions of receptors on gut NK cells following infection with IBDV

- vv strain of IBDV down-regulates the expression of activating receptors
- Vaccine strain of IBDV down-regulate the expression of inhibitory receptor
- Vaccine strain of IBDV stimulate gut NK cell functions
What are the roles of different lymphocytes/monocytes during IBDV infection?

IEL-NK cells modulate the expressions of activating and inhibiting receptors.
Effects of velogenic NDV infection on lymphoid cells in chickens

- Apoptosis and necrosis of IgM+ B cells
- Inhibit splenic T cell mitogen responses
- Increased production of proinflammatory cytokines, oxidative stress mediators (NO, MDA) and Th1-like cytokines primarily from macrophages
- Increased production of inflammatory mediators from mast cells
- The effects of different NDV strain infection on IEL-natural killer (gut NK) cells of chickens is unknown

BMC Vet Res 13 (2017):151
J Comp Pathol 145 (2011); 327-335
### Number of Intraepithelial lymphocytes (IEL) cell (control, 220 x 10^6 cells)

<table>
<thead>
<tr>
<th>NDV</th>
<th>IEL cells at different hpi</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Genotype VII</td>
<td>59 x 10^6</td>
</tr>
<tr>
<td>Genotype VIII</td>
<td>120 x 10^6</td>
</tr>
<tr>
<td>Vaccine</td>
<td>130 x 10^6</td>
</tr>
</tbody>
</table>

### Number of isolated CD3-/CD25+ IEL-NK cells cell (control, 70 x 10^6 cells)

<table>
<thead>
<tr>
<th>NDV</th>
<th>CD3-/CD25+ IEL NK cells at different hpi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Genotype VII</td>
<td>37 x 10^6</td>
</tr>
<tr>
<td>Genotype VIII</td>
<td>15 x 10^6</td>
</tr>
<tr>
<td>Vaccine</td>
<td>61 x 10^6</td>
</tr>
</tbody>
</table>

- Significant reduction in the number of CD3-/CD25+ NK cells in chickens infected with velogenic strains compared to lentogenic strain of NDV.
- Velogenic NDV causes apoptosis of IEL-NK cells (unpublished)

Expression profiles of immune-related genes associated with NK cell activity in IEL-NK cells following infection with different NDV strains

<table>
<thead>
<tr>
<th>NDV strains</th>
<th>Immune-related genes</th>
<th>Expression profiles in 28.4+ IEL-NK cells at different hours post inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>AF2240-I</td>
<td>CD69</td>
<td>-1.28±0.07&lt;sup&gt;a1&lt;/sup&gt;*</td>
</tr>
<tr>
<td></td>
<td>B-Lec</td>
<td>-2.01±0.17&lt;sup&gt;a1&lt;/sup&gt;*</td>
</tr>
<tr>
<td></td>
<td>B-NK</td>
<td>1.92±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>CHIR-AB1</td>
<td>22.02±0.07&lt;sup&gt;a1&lt;/sup&gt;*</td>
</tr>
<tr>
<td></td>
<td>NK-LYSIN</td>
<td>-1.03±0.08&lt;sup&gt;a1&lt;/sup&gt;*</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>-1.14±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IBS005/11</td>
<td>CD69</td>
<td>-2.04±0.34&lt;sup&gt;c1&lt;/sup&gt;*</td>
</tr>
<tr>
<td></td>
<td>B-Lec</td>
<td>-1.17±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>B-NK</td>
<td>1.48±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>CHIR-AB1</td>
<td>5.74±0.12&lt;sup&gt;a1&lt;/sup&gt;*</td>
</tr>
<tr>
<td></td>
<td>NK-LYSIN</td>
<td>-1.21±0.05&lt;sup&gt;c2&lt;/sup&gt;*</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>-3.63±0.23&lt;sup&gt;c12&lt;/sup&gt;*</td>
</tr>
</tbody>
</table>

LaSota

| CD69                  | 1.17±0.36<sup>1</sup> | 1.58±0.1<sup>2</sup> | 1.24±0.23<sup>3</sup> |
| B-Lec                | 1.24±0.2<sup>12</sup> | 1.56±0.57<sup>34</sup> | 1.38±0.05<sup>5</sup> |
| B-NK                 | 1.09±0.01             | 1.98±0.06             | 1.33±0.08<sup>1</sup> |
| CHIR-AB1             | 1.47±0.27<sup>1</sup> | 1.26±0.51<sup>2</sup> | 1.14±0.32<sup>3</sup> |
| NK-LYSIN             | 1.47±0.31<sup>12</sup>| 1.88±0.24<sup>34</sup> | 1.3±0.17<sup>5</sup> |
| IFN-γ                | 1.26±0.07<sup>2</sup> | 1.02±0.07<sup>4</sup> | 1.05±0.53<sup>5</sup> |

Differential expressions of receptors on gut NK cells following infection with NDV strains

• Velogenic NDV cause apoptosis of gut NK cells (IEL-NK cells)

• Velogenic NDV strain down-regulate the expression of the activating receptors

• Lentogenic NDV strain do not down-regulate expression of the NK cell receptors
Conclusion

- Immunosuppression is a syndrome often associated with interaction of non-infectious and infectious factors.
- Viruses have developed various strategies to induce immunosuppression directly or indirectly.
- Although IBDV is a B cell lymphotropic virus, very virulent strain able to target non B cells (T and NK cells) and inhibit their functions.
- Modulating on the expression of activating and inhibitory receptors of gut NK cells by IBDV of different virulence.
- Velogenic NDV is a pantropic virus that able to target various lymphoid cells including gut (IEL) NK cells.
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Thank You

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