Role of bursa Fabricius in Chicken’s Immunocompetence

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The structure of chicken’s immune system

PRIMARY LYMPHOID ORGANS:
- Bursa of Fabricius - responsible for B-cell development
- Thymus - responsible for T-cells, functions the same way as in mammals

SECONDARY LYMPHOID ORGANS:
- Spleen
- GALT (gut associated): Pyloric tonsil, Peyer’s patches, Cecal tonsil, Meckel’s diverticulum
- MALT (mucosal associated): Harderian gland, all mucosal tissues contain lymphoid cells throughout the body
Only found in avian species;
Part of the gut associated lymphoid tissues;
The lumen of bursa is directly communicates with the cloaca (antigens from the gut are continuously transported to the bursa and are necessary for normal B-cell development after hatch).
Why is the bursa necessary:

- Main role is to regulate B-cell development
- The microenvironment in the bursa allows regulated activation of Ig gene conversion process, that is a key differentiation event in avian B-cell development
- Without gene conversion the immunological capacity of B-cells is very limited such chickens survive only in sterile environment.
Bursa of Fabricius

Development of the bursa during embryonic age:
- ED4 \(\rightarrow\) bursa develops as a diverticulum from the cloaca.
- ED8 onward \(\rightarrow\) B-cell precursors colonize the bursa.
- ED15 onward \(\rightarrow\) follicles start to form.

- Ig gene conversion is first initiated at ED16; followed by extensive proliferation.
- From hatch the mature B-cells leave the bursa and colonize the secondary lymphoid organs.
Bursa of Fabricius

Bursal follicle

- Basic functional unit consists of $\sim 10^4$ cells (98% B-cells, that develop in a reticular epithelial supporting scaffold);

- Development of B-cells is regulated by FDC type of secretory dendritic cells that present antigens (yolk or external origin) in immune complex form for mature B-cells.

Development of the bursa after hatch:

- The follicles grow in size and numbers during the first weeks of live.
- All secondary lymphoid organs are fully colonized by **10-14 days of age**, with self sustaining B-cell population → the **loss of bursa after this age has minimal effect** on the immune competence of the affected animals.
- Maximum size of the bursa compared to body weight is reached at 3-4 weeks of age.
- After 8-10 weeks of age the bursa starts to involute.
- The dynamism of bursa growth and involution is significantly affected by breed, sex and growing conditions.
Seeding of bursal cells to the periphery:

- The **spleen** is the first organ to receive early post-bursal stem cells.
- IgM-secreting cells (B-cells) are already seen 3 days after hatching in the spleen and IgA and IgG-secreting cells by 6 days of age.
- *3-week old bursa contains 1 to 3 billion B cells, whereas there are approx. 6 billion B cells in the periphery.*
- Process is **completed by the 4th week** of age: mature B cell immune system is installed in the periphery.

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<table>
<thead>
<tr>
<th>Day after hatching</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>8</th>
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<tbody>
<tr>
<td>IgG-SC*</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>IgA-SC</td>
<td>0</td>
<td>0</td>
<td>38</td>
<td>125</td>
</tr>
<tr>
<td>IgM-SC</td>
<td>0</td>
<td>63</td>
<td>145</td>
<td>1,960</td>
</tr>
</tbody>
</table>

* Data expressed as IgG-, IgA-, or IgM-secreting cells (SC) per 10^6 mononuclear cells.


Effect of IBDV infection on Bursa of Fabricius

Differences in the pathogenesis of IBDV infection (acute phase):

- More virulent strains are characterized by:
  - Higher IBDV load in the bursa
  - Stronger T-cell influx into the bursa
  - Stronger induction of innate immune response
  - Similar level of macrophages, but higher level of pro-inflammatory cytokines and chemokines activation
  - Stronger inflammatory response in the bursa ➔ edema, haemorrhages, loss of follicle structure
  - Stronger extrabursal (systemic) response and replication:
    - Thymic T-cell necrosis and atrophy,
    - Splenic mitogenic response depressed (T-cell blastogenic activity ▼),
    - Haemorrhages in the muscles.
Differences in the pathogenesis of IBDV infection (chronic/recovery phase):

- More virulent strains are characterized by:
  - Delayed lymphocyte repopulation into bursa follicles
  - Loss of follicles or non-functional regeneration

- Differences in the recovery are the consequences of more severe bursal damage in the acute phase.
Consequence of IBDV infection on the immune system

Source: Sharma et al. 2000
Dev. Comp. Immunol. 24 223-
Other factors influencing the consequence of IBDV infection

- **Age of chickens**
  - **Young birds (< 2wks):**
    - mainly *immunosuppressive effect* (long-term),
    - less severe inflammatory reaction in the bursa, but longer regeneration phase (if any functional regeneration takes place)
    - usually no clinical manifestation
• Early infection (< 2 weeks of age) due to the absence of or low specific maternal immunity

• Strong, and persistent destruction of the bursa follicles

• Today, mainly due to «antigenic variant» IBDV strains
Age of chickens

**Older birds** (3-6 weeks the most susceptible):

- **Clinical disease** (depending on strain pathogenicity)
- stronger inflammatory reaction (cv and vv strains), T-cell accumulation and T-cell activation in the bursa and activation of macrophages are more pronounced; structure of follicles is destroyed due to the strong cellular immune response, but usually faster functional regeneration
- Only **transient immunosuppressive effect** due to the presence of extrabursal B-cells and faster regeneration
Maternally derived immunity

- Extent of MDA-effect is different according to the pathogenicity and antigenic characteristics of the strain.

Other factors influencing the consequence of IBDV infection

- Differences in pathogenicity
- Antigenic differences
Presence of other immunosuppressive agents

Most important is ICAV

- Pathogenicity of both IBDV and ICAV is increased
- More severe bursal lesions and immunosuppression
- Longer susceptibility period to ICAV-induced clinical signs
Do the bursa lesions caused by live IBD vaccines lead to immunosuppression?
Are bursal lesions necessary to protect chicken from IBDV challenge infection?

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The use of live vaccines for immunization against infectious bursal disease virus (IBDV) is always a tightrope walk between attaining a good protection against the field virus and a very low depletion of bursal lymphocytes by the vaccine virus itself; The question was if it is necessary for a good protection that the vaccine virus causes depletion of bursal lymphocytes. To address this issue, animal experiments using recombinant IBDV were performed. Recombinant IBDV showing different subtypes of virulence were used in these studies. During challenge experiments using the classical virulent Faragher 52/70 it became clear that in groups of chickens where the vaccine virus caused bursal lesions no lesions caused by the challenge virus were detectable. In contrast, only in the group of chicken were the vaccine virus induced no detectable lesions severe acute lesions were observed.
Bursa atrophy following vaccination with IBDV live vaccines can be observed in field and in experimental conditions;

Such an atrophy of bursa is linked with the “vaccine take” (vaccine virus replication in the bursa);

This bursa atrophy as a consequence of vaccine virus replication is necessary to get protection.
Effect of Live IBDV Vaccines on Bursa of Fabricius

• Appearance and wide distribution of very virulent IBDV field strains required the use of intermediar plus/hot vaccine strains, which cause strong lymphocyte depletion in the bursa during the acute phase of vaccine-take. This is followed by regeneration, but the normal size of the bursa will not be reached in the vaccinated birds.

• The dogma

  Chickens with small bursas are immuno-compromised.
Aim of the study:

To verify that the loss of bursa after 3-4 weeks of age (typical age when intermedier plus vaccines and Transmune are taken) does not result in significant immunosuppressive effect.

The method:

- evaluating the effect of bursectomy performed at 3 and 4 weeks of age on the humoral immune response to ND vaccination in commercial broiler chickens, and for comparison in parallel

- evaluating the effect of vaccination with intermedier plus vaccine (Cevac IBD L) in the same system
Broilers (Ross 308) with high level of MDA against IBDV

- Surgical bursectomy was performed at 21 or 28 days of age
- Vaccination with intermediate IBD vaccine (Cevac IBD L, 1 dose per os individually) at D25
- ND vaccination after bursectomy or at the peak of bursal lesions following IBD vaccine-take

### Table: Treatment and Samplings

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ND vaccination</th>
<th>Samplings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IBD vaccination</td>
<td>Bursectomy</td>
<td>D29</td>
</tr>
<tr>
<td>1/b</td>
<td>D25</td>
<td>-</td>
<td>D21 (n=5), D26 (n=5): 1/a</td>
</tr>
<tr>
<td>2/a</td>
<td>-</td>
<td>D21</td>
<td>D29 (n=4), D33 (n=7): 1/b</td>
</tr>
<tr>
<td>2/b</td>
<td>-</td>
<td>D28</td>
<td>D36 (n=5): 3/b</td>
</tr>
<tr>
<td>3/a</td>
<td>-</td>
<td>-</td>
<td>D27</td>
</tr>
<tr>
<td>3/b</td>
<td>-</td>
<td>-</td>
<td>D29</td>
</tr>
</tbody>
</table>

**After ND vaccination-serum samplings:**
- Pre-vaccination - on the day of vaccination (0 dpv)
- 1, 2 and 3 week post-vaccination
Influence of bursectomy and vaccine virus caused bursa damage on the immune competence in broilers

**Bursectomy**

1. Anaesthesia using pentobarbitone
2. Incision above the cloaca
3. Urether

Vaccinology Summit_April 2014, Lisbon
Influence of bursectomy and vaccine virus caused bursa damage on the immune competence of broilers

Bursectomy

Ligating using synthetic, absorbable, monofilament suture

Cautery

Bursectomized chickens were dissected at the end of the trial to check the lack of total bursa of Fabricius. No bursectomized chickens had residuum of bursa.
Vaccine virus caused lesions were observed four days post-vaccination in the IBD L vaccinated group.
Humoral immune response to ND vaccination

Influence of bursectomy and vaccine virus caused bursa damage on the immune competence in broilers.

No significant difference was observed among IBD vaccinated, bursectomized or control groups.

*: significantly different antibody level
Conclusion

- Even the total loss of bursa at three or four weeks of age (D21 or D28) did not result in immunosuppression.
- After 3 weeks of age the intermediate plus IBD vaccine-caused bursa lesion had no negative effect on the humoral immune response to ND vaccination performed at the peak of bursal damage.
- Small bursa observed after 3 weeks of age does not indicate that the birds are immuno-compromised.