Immunology of BVDV Vaccination

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Issues Regarding BVDV Vaccine Efficacy

- Cross protection
  - Between types 1 and 2
  - Within types due to antigenic variation
- Fetal protection
- Efficacy in distressed cattle
- Maternal antibody interference
- Onset and duration of immunity
- Efficacy when administered with other vaccines
Issues Regarding BVDV Vaccine Safety

• Immunosuppression by MLV BVDV
• Safety in distressed cattle
• Safety in pregnant animals
• Induction of mucosal disease
• Injection site lesions
  – Route of administration
  – Adjuvants
• Contamination with extraneous BVDV
<table>
<thead>
<tr>
<th>Pathogenic Mechanisms</th>
<th>Defense Mechanisms</th>
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<td>IgE</td>
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<td>Neutralizing antibody</td>
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<td>Th1 cytokines</td>
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Internal and external antigens
surrounding the viral genetic material

Roitt, I., Brostoff, J., Male, D. *Immunology*. 1985
Detection of Antigen Specific T Cell-Mediated Immunity to Bovine Respiratory Disease Viruses by Flow Cytometry
Cell-Mediated Immunity (CMI)

Functional T-cell subsets

- $\gamma\delta$ T
- $\alpha\beta$ T
- CD4
- CD8
- Th1
- Th2
T Cell Activation

Unstimulated

Stimulated

Antigen

stimulated

Cell surface marker

CD25 (IL-2R)

Cytokine
Five-color Flow Cytometry

- Simultaneous labeling of 3 T cell subset markers (CD4, CD8, $\gamma\delta$ TCR), activation marker CD25 and intracellular IFN$\gamma$.
- Detects co-expression of double positive cells, e.g. CD8 and $\gamma\delta$ TCR.
- Identifies all T cell subsets that express CD25 and/or IFN$\gamma$ in the same well.
CD25 Data Tabulation

• Percentage of the T cell population that is CD25+ from both unstimulated and antigen-stimulated cells

• Mean fluorescence intensity (MFI) of CD25 expression

• CD25 Expression Index calculation

\[
\text{CD25 Expression Index} = \frac{(%\text{CD25+ of stimulated cells})(\text{MFI})}{(%\text{CD25+ of unstimulated cells})(\text{MFI})}
\]
Monitoring T Cell Responses by CD25 and IFNγ Expression Analysis

- Immunize calves
- Collect blood samples from vaccinated and control calves
Monitoring T Cell Responses by CD25 and IFN$\gamma$ Expression Analysis

- Isolate peripheral blood mononuclear cells (PBMC)
- Incubate PBMC *in vitro* with antigens in microtiter plates
- Stain cell surface markers and activation markers
Influence of Maternal Antibody on Development of Memory T cells after Exposure to BVDV

Janice Endsley¹, Julia Ridpath², John Neill², James Roth¹

¹Iowa State University,
²USDA ARS National Animal Disease Center
Hypothesis

Calves infected with Bovine Viral Diarrhea virus in the presence of passive antibody will develop CD4, CD8, and γδ T cells specific for BVDV, without a detectable antibody response and will be protected from subsequent challenge.
Experimental Design

- Pooled colostrum from BVDV hyper-immunized cows was fed to 12 calves.
- Six of 12 calves were inoculated with BVDV type 2 (strain 1373) at 6 to 20 days of age.
- Three calves received no colostrum and no BVDV inoculation.
- Three calves received no colostrum and were challenged at 6 to 20 days of age (all died).
- All surviving calves were challenged with BVDV type 2 (strain 1373) at 8 to 9 months of age.
Temperature (After 1\textsuperscript{st} Inoculation)

![Graph showing temperature changes after first inoculation with and without colostrum.](#)
Neutralizing Antibody Responses to BVDV Type 2

Month after first BVDV inoculation

 SVN Titer (log10)
Activation of CD4 T Cells by BVDV Type 2

Expression Index

Weeks after first BVDV inoculation

- 0-10 wks
- 11-20 wks
- 21-32 wks

Colostrum
Colostrum, BVDV

P = 0.07
P = 0.04
Activation of CD8 T Cells by BVDV Type 2

Expression Index

Weeks after first BVDV inoculation

0-10 wks
11-20 wks
21-32 wks

P = 0.008
P = 0.10
Activation of $\gamma\delta$ T Cells by BVDV Type 2

Weeks after first BVDV inoculation

- 0-10 wks: $P = 0.04$
- 11-20 wks: $P = 0.006$
- 21-32 wks: $P = 0.04$

Expression Index

- Colostrum
- Colostrum, BVDV
IFN$_\gamma$ Production (ELISA)

**Chart Description:**
- X-axis: Time intervals: 0 - 10 Weeks, 11 - 20 Weeks, 21 - 32 Weeks
- Y-axis: IFN$_\gamma$ ng/ml
- Bars represent IFN$_\gamma$ production levels in Colostrum samples with and without BVDV:
  - Lighter shade: Colostrum, No BVDV
  - Darker shade: Colostrum, BVDV

**Observation:**
- IFN$_\gamma$ production is highest in the 21 - 32 Weeks interval, especially in the presence of BVDV.
- The absence of BVDV in Colostrum shows lower IFN$_\gamma$ levels across all time intervals.
Temperature (After Challenge)

- **Temp (F)**
  - 107
  - 106
  - 105
  - 104
  - 103
  - 102
  - 101
  - 100
- **Days post challenge**
  - Days 0 to 20
- **Challenge**
- **Colostrum + Virus**
- **Colostrum**
- **No Colostrum**
## Virus Isolation from Buffy Coats (After Challenge)

<table>
<thead>
<tr>
<th>Group</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum + BVDV</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Colostrum</td>
<td>3/6</td>
<td>4/6</td>
<td>6/6</td>
<td>6/6</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>No colostrum</td>
<td>2/3</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>
Mean SN Titers to BVDV 1373 After Challenge

Colostrum + Virus
Colostrum
No Colostrum
Induction of Antigen Specific T Cell Subset Activation to Bovine Respiratory Disease Viruses by MLV Vaccine

Experimental Design

- Vaccination with modified-live virus vaccine (BHV-1, BRSV, BVDV types 1 and 2, and PI3) (Vista vaccine – Intervet)
  - Week 0
- Blood collection for CMI assay
  - Weeks 0, 4, 5, 6, 8, 24, 25, 26, 27
- Challenge
  - BHV-1 Cooper strain on week 25 (2 ml of $10^8$ TCID$_{50}$/ml)
- Nasal secretion collection for virus titration
  - Days 0-14 post-challenge
CMI assay

*In vitro* stimulation of PBMC

- Unstimulated
- Mitogen stimulated
- Live virus stimulated
  - BHV-1 (Bovishield®)
  - BRSV (Bovishield®)
  - BVDV type 1 (TGAN-NADC)
  - BVDV type 2 (890-NADC)
BHV-1 Results

CD25 Expression Index

<table>
<thead>
<tr>
<th>Subset</th>
<th>All PBMC</th>
<th>CD4</th>
<th>CD8</th>
<th>gd</th>
<th>Non T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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Statistically significant ★ (p<0.01) ★ (p<0.05)

- Pre-vaccination
  - Week 0

- Post-vaccination
  - Weeks 4, 5, 6, 8

- Pre-challenge
  - Weeks 24, 25

- Week 1 post-challenge
  - Week 26

- Week 2 post-challenge
  - Week 27
## BRSV Results

### CD25 Expression Index

<table>
<thead>
<tr>
<th>Subset within Group</th>
<th>Control</th>
<th>Vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>All PBMC</td>
<td>CD4</td>
<td>CD8</td>
</tr>
<tr>
<td>gd</td>
<td>Non T cells</td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant $\star (p<0.01)$, $\bigstar (p<0.05)$

<table>
<thead>
<tr>
<th>Pre-vaccination</th>
<th>Post-vaccination</th>
<th>Pre-challenge</th>
<th>Week 1 post-challenge</th>
<th>Week 2 post-challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>Weeks 4, 5, 6, 8</td>
<td>Weeks 24, 25</td>
<td>Week 26</td>
<td>Week 27</td>
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BVDV Type 1 Results

CD25 Expression Index

Subset

- All PBMC
- CD4
- CD8
- gd
- Non T cells

Statistically significant

- (p<0.01)
- (p<0.05)

Pre-vaccination
- Week 0

Post-vaccination
- Weeks 4, 5, 6, 8

Pre-challenge
- Weeks 24, 25

Week 1 post-challenge
- Week 26

Week 2 post-challenge
- Week 27
BVDV Type 2 Results

CD25 Expression Index

Subset within Group

Pre-vaccination

Post-vaccination

Week 0

Weeks 4, 5, 6, 8

Week 24, 25

Week 26

Week 27

Control

Vaccinated

Statistically significant  ★ (p<0.01)  ★ (p<0.05)
BHV-1 Results

$\Delta \%\text{IFN}_\gamma +$

- Pre-vaccination: Week 0
- Post-vaccination: Weeks 4, 5, 6, 8
- Pre-challenge: Weeks 24, 25
- Week 1 post-challenge: Week 26
- Week 2 post-challenge: Week 27

Statistically significant: ★ (p<0.01) ★ (p<0.05)
BRSV Results

$\Delta \%\text{IFN}_\gamma +$

<table>
<thead>
<tr>
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<th>All PBMC</th>
<th>CD4</th>
<th>CD8</th>
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<td>Control</td>
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Pre-vaccination

Week 0

Post-vaccination

Weeks 4, 5, 6, 8

Pre-challenge

Weeks 24, 25

Week 1 post-challenge

Week 26

Week 2 post-challenge

Week 27

Statistically significant

☆ (p<0.01)

★ (p<0.05)
BVDV Type 1 Results

$\Delta \% \text{IFN}_{\gamma} +$

Statistically significant  ★ (p<0.01)  ★ (p<0.05)

 Subset within Group

Pre-vaccination

Week 0

Post-vaccination

Weeks 4, 5, 6, 8

Pre-challenge

Weeks 24, 25

Week 1 post-challenge

Week 26

Week 2 post-challenge

Week 27
BVDV Type 2 Results

Δ %IFNγ +

Subset within Group

Pre-vaccination

Post-vaccination

Week 0

Weeks 4, 5, 6, 8

Pre-challenge

Weeks 24, 25

Week 1 post-challenge

Week 26

Week 2 post-challenge

Week 27

Statistically significant

★★ (p<0.01) ★★ (p<0.05)
Virus Titration Results

Log virus titer

Day post challenge

Mean control group

Mean vaccinated group

Statistically significant

★ (p<0.01) ★ (p<0.05)
Post-challenge Body Temperature

Statistically significant

Control
Vaccinated

(p<0.01)  (p<0.05)
Efficacy of Killed BVDV Vaccines for Induction of T Cell Mediated Immunity?
Immunosuppression by BVDV and MLV BVDV Vaccine
Suppression of Neutrophil Iodination by Virulent BVDV

Roth et al, AJVR 42:244-250, 1981
Suppression of Neutrophil Iodination by MLV BVDV and ACTH

Roth and Kaeberle, AJVR 44:2366-2372, 1983
Goals for BVDV Vaccine

- Induce high and prolonged SN antibody titers
- Induce strong CD4, CD8, and $\gamma\delta$ T cell responses
- Induce active immunity in the presence of passive antibody
- Safe in pregnant cows
- Not suppress native or acquired immune defense mechanisms
- Serve as a marker vaccine to aid BVD eradication programs
Approaches to Improved BVDV Vaccines

• New adjuvants to enhance T cell responses
• Live vectored vaccines coding for protective BVDV antigens
• Identify and delete virulence and immunosuppressive genes from BVDV for new generation MLV vaccines
• All three of these approaches:
  – Should induce CMI
  – Should not be immunosuppressive
  – Could serve as marker vaccines