Overview of the Immune Response and it’s Relevance to Vaccine Efficacy

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What is the basis for protective immunity for the disease in question

- Circulating antibody?
  - IgM, IgG?
- Mucosal antibody?
  - IgA, IgE?
- Cell-mediated immunity?
  - Cytokine secretion ($T_{H1} \text{ vs } T_{H2}$)?
  - Cytotoxic T cells?
  - Gamma Delta T cells?
- What are the important antigens?
Pathogenic Mechanisms

- Adherence to Mucosa
- Invasive parasite
- Exotoxin/Endotoxin
- Viremia
- Septicemia
- Intracytoplasmic Growth
- Growth in phagosome
- Infect epithelial cells

Defense Mechanisms

- Mucosal Antibody (IgA)
- IgE
- Neutralizing Antibody
- Neutralizing Antibody
- Opsonizing Antibody
- Cytotoxic T cells
- $T_{H1}$ Cytokines
- Gamma Delta T cells
Functional T-cell subsets

γδT

αβT

CD4

CD8

TH1

TH2

<table>
<thead>
<tr>
<th>Lymphocyte Type</th>
<th>Antigen Recognition</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cells</td>
<td>Intact, unprocessed molecules</td>
</tr>
<tr>
<td>$T_{H1}$ and $T_{H2}$ cells</td>
<td>Exogenous peptides processed and presented on MHC II</td>
</tr>
<tr>
<td>Tc cells</td>
<td>Endogenous peptides processed and presented on MHC I</td>
</tr>
<tr>
<td>Gamma Delta T cells</td>
<td>Intact, unprocessed molecules</td>
</tr>
<tr>
<td>Lymphocyte Type</td>
<td>Response to Antigen</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>B cells</td>
<td>Produce Antibodies</td>
</tr>
<tr>
<td>$T_{H1}$ cells</td>
<td>Secrete cytokines which enhance phagocyte killing</td>
</tr>
<tr>
<td>$T_{H2}$ cells</td>
<td>Secrete cytokines which help antibody production (IgA and IgE)</td>
</tr>
<tr>
<td>Tc cells</td>
<td>Cytotoxic for cells synthesizing foreign proteins</td>
</tr>
<tr>
<td>Gamma Delta T cells</td>
<td>Secrete cytokines and/or cytotoxic for cells with abnormal surface antigens</td>
</tr>
</tbody>
</table>
Parham, P., The Immune System. 2000, Garland Publishing, Fig. 6.32
\[ T_{H0} \]

- IL-2
- IL-4
- IFN-\(\gamma\)

\[ T_{H1} \]

- IL-3
- GM-CSF

\[ T_{H2} \]

- IL-3
- GM-CSF

- IL-4
- IL-5
- IL-6
- IL-10

LT (TNF-\(\beta\))
Selection of effector mechanisms by TH1 and TH2 cells

TH1 inhibits production of IFNγ, which activates macrophage. IFNγ inhibits TH2 proliferation.

TH2 inhibits TH1 proliferation via IL-10. TH2 produces IL-4 and IL-5.

TH2 activates mast cell and eosinophil. Mast cell and eosinophil produce antibody, including IgE.
KILLERS AND THEIR HELPERS

T lymphocytes (right) destroy infected and foreign cells in the body and generally oversee most immunologic reactions—including many of the ones that cause autoimmune diseases. Cytotoxic ("killer") T cells attack their targets directly. Helper T cells produce a variety of substances that enhance the immune responses of the killer cells and other parts of the immune system. The T cell antigen receptor (below) is the key to the cells’ activity. The receptor consists of one alpha-chain protein and one beta-chain protein. The receptor is fussy: it can bind only with a single type of antigen, which must be held by specific major histocompatibility (MHC) proteins on the surface of cells.
Exogenous Pathway of Antigen Presentation

Abbas, et al. Cellular and Molecular Immunology, 1997
Endogenous Pathway of Antigen Presentation

Abbas, et al. Cellular and Molecular Immunology, 1997
ISCOMs Carry Peptides into the Cytoplasm

Allele-specific motifs in peptides eluted from MHC class II molecules

Roitt, I. et al. Immunology, 1996
Binding peptides from class I molecules

Roitt, I. et al. Immunology, 1996
**FIGURE 3-9. The nature of antigenic determinants.** Antigenic determinants (shown in gray) may depend upon protein folding (conformation) as well as upon covalent structure. Some linear determinants are accessible in native proteins, whereas others are exposed only upon protein unfolding. Neoantigens arise from covalent modifications such as peptide bond cleavage.
Bacterial Antigens

Viral Antigens
Internal and external antigens surrounding the viral genetic material

Roitt, I., Brostoff, J., Male, D. *Immunology*. 1985
Fig. 11.4 The molecules involved in the interaction between T cells and APCs. The various cytokines and their direction of action are also shown.
Detection of Antigen Specific T cell subset Activation
Flow Cytometer
Flow Cytometer

Mixture of cells is labeled with fluorescent antibody

Stream of fluid containing antibody-labeled cells

Laser

Green photomultiplier tube (PMT)

Red PMT

Side scatter

Forward scatter

Analysis of cells stained with labeled antibodies
Expression Index = \[
\frac{\text{(% T cells stimulated)(MFI)}}{\text{(% T cells unstimulated)(MFI)}}
\]
Methods

Modified live and inactivated vaccines

Immunize

Isolate PBMC from immune & naïve calves

Stimulate with Ag: live, inactivated, recombinant
Two-color flow cytometry

Monoclonal Ab staining

1) Anti - IL2R
2) Anti - CD4, CD8 or γδ TCR

CD4

CD8

γδ TCR

IL2R (CD25)
Flow cytometry output

CD4

Negative Response

Positive Response

CD25
Induction of T Lymphocytes Specific for BVD Virus in Calves With Maternal Antibody

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John Neill\textsuperscript{2}, James Roth\textsuperscript{1}

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Endsley et. al., Viral Immunol, 17:13-23, 2004
Objective

To determine if infection with virulent BVDV in the presence of maternal antibody will induce antigen specific T cells and protective immunity without inducing antibody
Experimental Design

- Pooled colostrum from BVDV hyper-immunized cows fed to 12 calves.
- Six calves inoculated with BVDV 1373 at 6 to 20 days of age.
- All calves challenged with BVDV 1373 at 8 months of age.
- Monitor antibody and T cell subset responses
Serum Neutralizing Antibody to Type 2 BVDV

Months after first BVDV challenge

SN Titer (log_{10})

- Colostrum
- Colostrum, BVDV
Activation of CD4⁺ T cells by BVDV2

Expression Index

Weeks after first BVDV challenge

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

Colostrum
Colostrum, BVDV

P = 0.07
P < 0.05
Activation of CD$8^+$ T cells by BVDV2 890

Weeks after first BVDV challenge

Expression Index

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

Colostrum, BVDV

P < 0.01

P = 0.10
Activation of γδ T cells by BVDV2 890

![Chart showing activation of γδ T cells by BVDV2 890.](chart)

- **Expression Index**
- **Weeks after first BVDV challenge**
- **0-10 wks**: P < 0.05
- **11-20 wks**: P < 0.01
- **21-32 wks**: P < 0.05

- **Colostrum**
- **Colostrum, BVDV**
Interferon Gamma in Supernatants From Mononuclear Cells Stimulated with BVDV 890

Weeks after first BVDV challenge

<table>
<thead>
<tr>
<th>Weeks</th>
<th>IFNγ ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10 wks</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>11-20 wks</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>21-32 wks</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

Colostrum, no BVDV  | Colostrum, BVDV
Rectal Temperature After BVDV 1373 Challenge at 8 Months of Age

Days After Infection

Degrees Fahrenheit

Previously Challenged
Not Previously Challenged
Total Leukocyte Counts After BVDV 1373 Challenge at 8 Months of Age

Platelets

Days After Infection

Previously Challenged

Not Previously Challenged
Isolation of BVD Virus from Buffy Coat After Second Challenge

<table>
<thead>
<tr>
<th>DAYS POST CHALLENGE</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previously Challenged</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>Not Previously Challenged</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>
</tbody>
</table>
Conclusions

When challenged with virulent BVDV, calves with high maternal antibody titers to BVDV:

- Did not produce an antibody response to BVDV
- Did develop memory CD4⁺, CD8⁺, and γδ T cells
- Did produce an anamnestic antibody response upon subsequent challenge
- Are protected from BVDV challenge in the absence of detectable serum neutralizing antibody
T Cell-Populations Responsive to Bovine Respiratory Syncytial Virus in Seronegative Calves

Matt Sandbulte and James Roth

Vet Immunol Immunopathol, 84:111-123, 2002
Two-Fold Testing for BRSV Immunity in Beef Calves

- Calves tested for virus-specific neutralizing antibody and T cells
- 16 identified as seronegative
- Among these, T cell responses were detected in several.

![T Cell Responses to BRSV in Seronegative Calves](chart.png)
Why T Cells Without Antibody?

**Hypothesis:**

Those calves were previously infected with BRSV in the presence of maternal antibody. The humoral response was blocked, but T cells were primed.

**Test:**

Vaccinate naïve calves and T cell-positive calves with BRSV. Does the latter group have immunologic memory, i.e. develop superior antibody and T cell responses?
CD4+ T Cell Responses

CD25 Expression Index

- Week -4
- Week -2
- Week 0
- Week 2
- Week 4
- Week 6

Group 1
Group 2
Group 3

†‡

*
CD8+ T Cell Responses

CD25 Expression Index

Group 1
Group 2
Group 3

†‡
‡  ‡
†‡  ‡

†‡
‡‡
‡
‡
†
*
Gamma delta T Cell Responses

CD25 Expression Index

- Group 1
- Group 2
- Group 3

Week -4 | Week -2 | Week 0 | Week 2 | Week 4 | Week 6
---|---|---|---|---|---
†‡ | †‡ | †‡ | * | †‡ | †‡
Neutralizing Antibody Responses

- * Group 2 > Group 1
- † Group 3 > Group 1
- ‡ Group 3 > Group 2

Graph showing the Log(2) SVN Titer from Week -8 to Week 6 for Naïve / No treatment, Naïve / Vaccine, and CMI(+) / Vaccine groups.
Limitations to Technology for Detecting T Cell Subset Activation

- Day to day and animal to animal variability require that large numbers of animals be used and that they be sampled repeatedly.

- Expensive; labor and equipment intensive.

- Lymphocytes should be set up the same day as collected.

- Valid comparisons can only be made on lymphocytes assayed in parallel.