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

Pathogenic Mechanisms	Defense Mechanisms
Adherence to mucosa	Mucosal antibody (IgA)
Parasites	IgE
Exotoxin/Endotoxin	Neutralizing antibody
Viremia	Neutralizing antibody
Septicemia	Opsonizing antibody
Intracytoplasmic growth	Cytotoxic T cells
Virus replication	Types 1 and 2 Interferons
Growth in phagosome	Th1 cytokines
Infect epithelial cells	Gamma delta T cells

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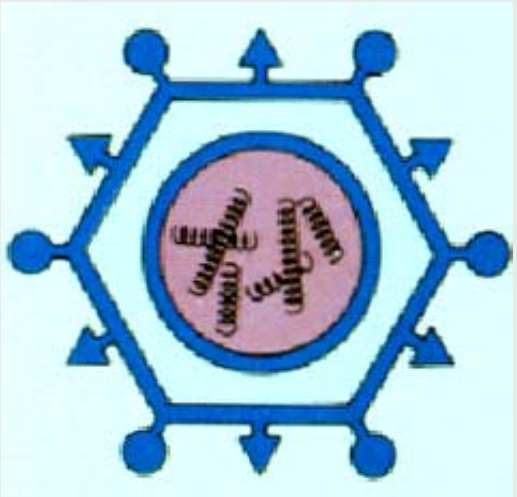
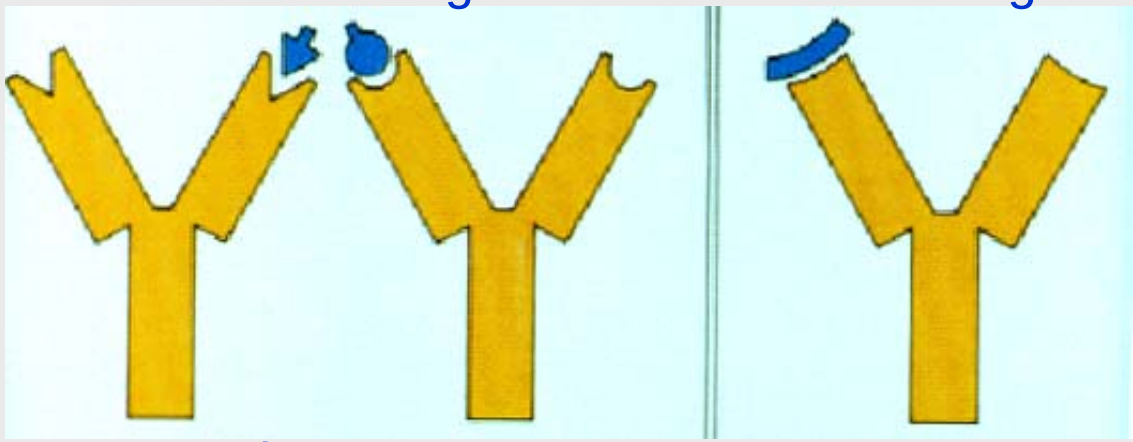
Internal and external antigens surrounding the viral genetic material



external antigens

internal antigens



non-protective

protective

non-protective

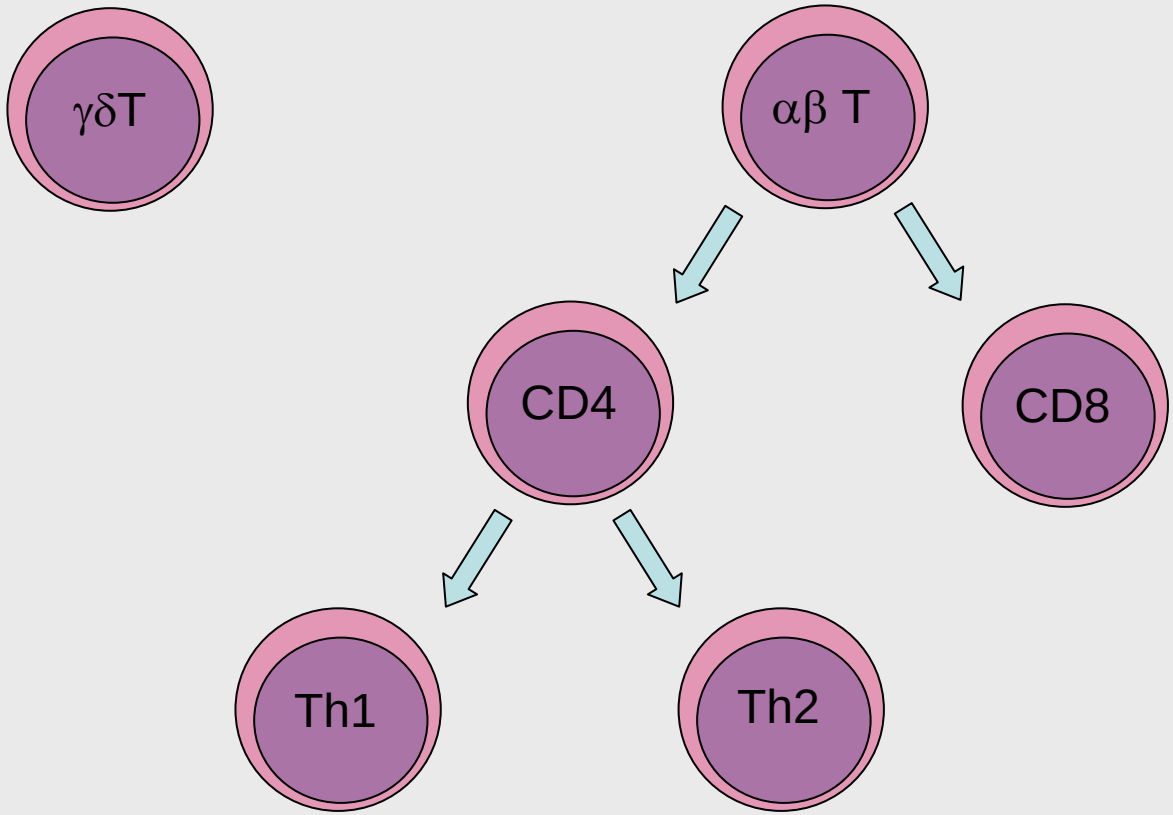
Roitt, I., Brostoff, J., Male, D. *Immunology*. 1985

the Center for Food Security & Public Health
IOWA STATE UNIVERSITY

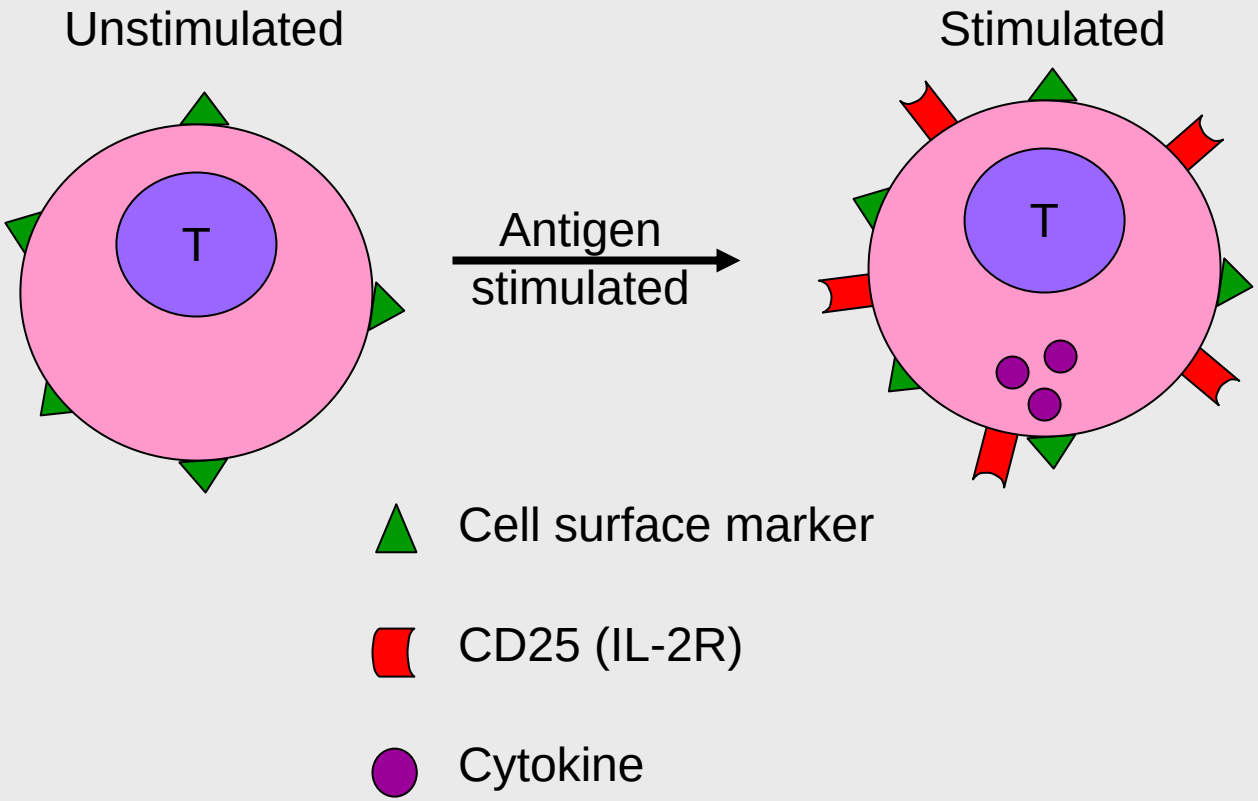
Detection of Antigen Specific T Cell-Mediated Immunity to Bovine Respiratory Disease Viruses by Flow Cytometry

Cell-Mediated Immunity (CMI)

Functional T-cell subsets

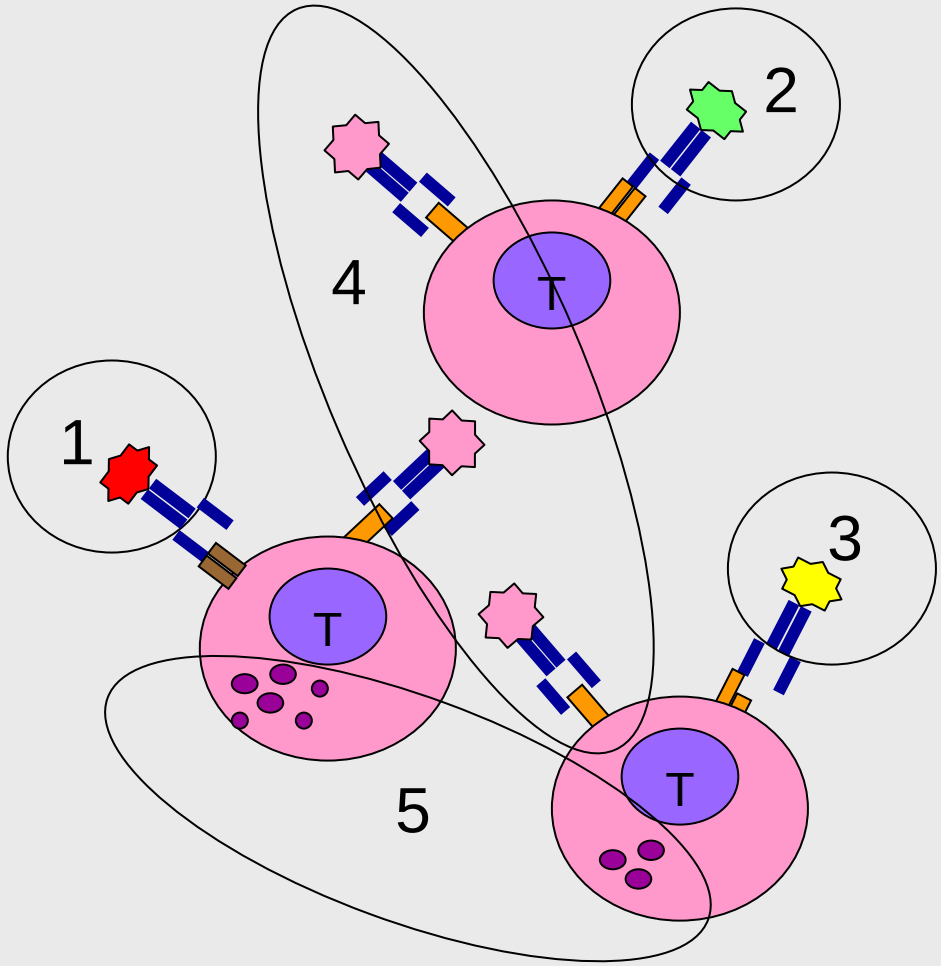


T Cell Activation



Five-color Flow Cytometry

- Simultaneous labeling of 3 T cell subset markers (CD4, CD8, γδ TCR), activation marker CD25 and intracellular IFNγ.
- Detects co-expression of double positive cells, e.g. CD8 and γδ TCR.
- Identifies all T cell subsets that express CD25 and/or IFNγ 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

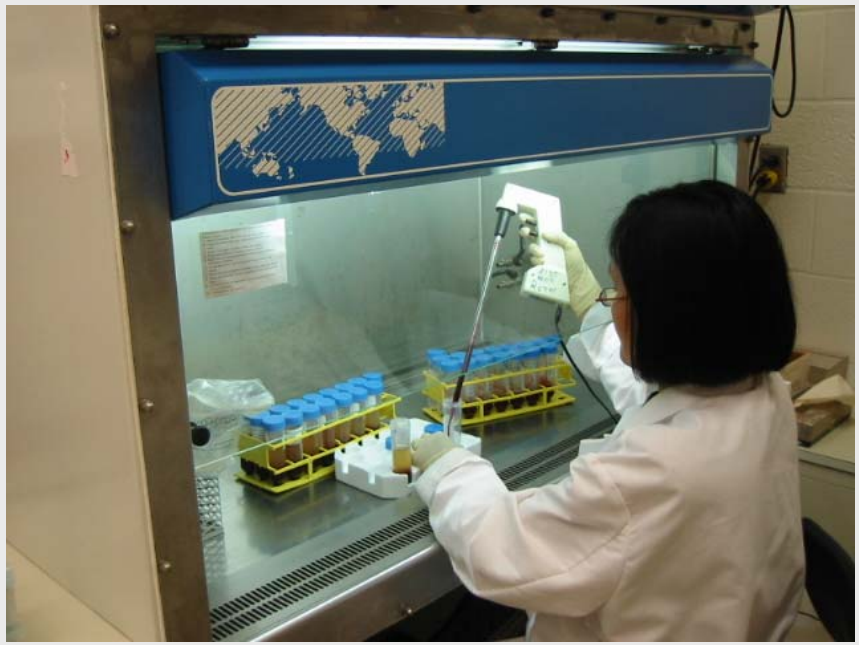
$$\text{CD25 Expression Index} = \frac{(\%\text{CD25+ of stimulated cells})(\text{MFI})}{(\%\text{CD25+ of unstimulated cells})(\text{MFI})}$$

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 IFNγ 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 Endsley[1], Julia Ridpath[2],
John Neill[2], James Roth[1]

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

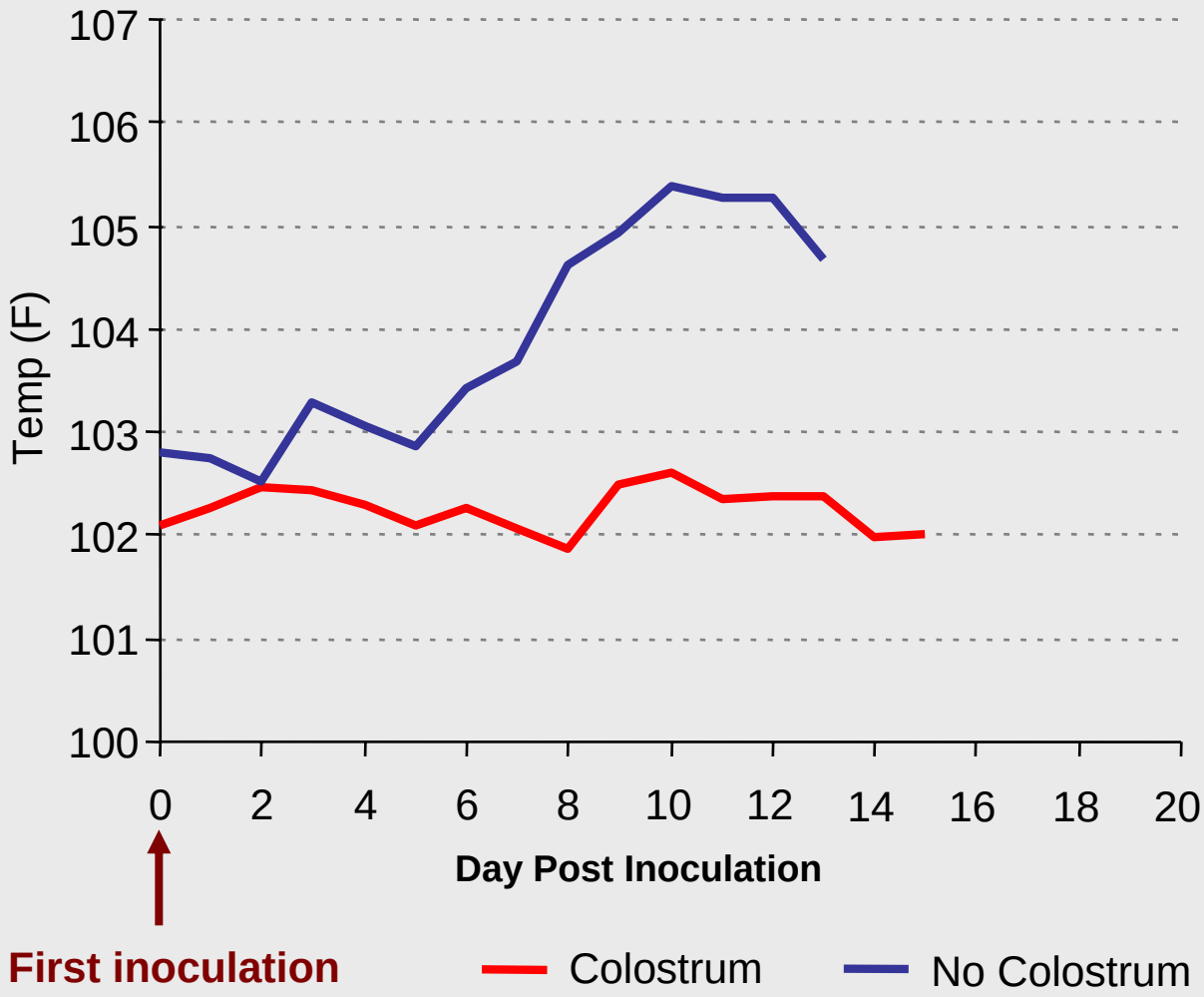
Hypothesis

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

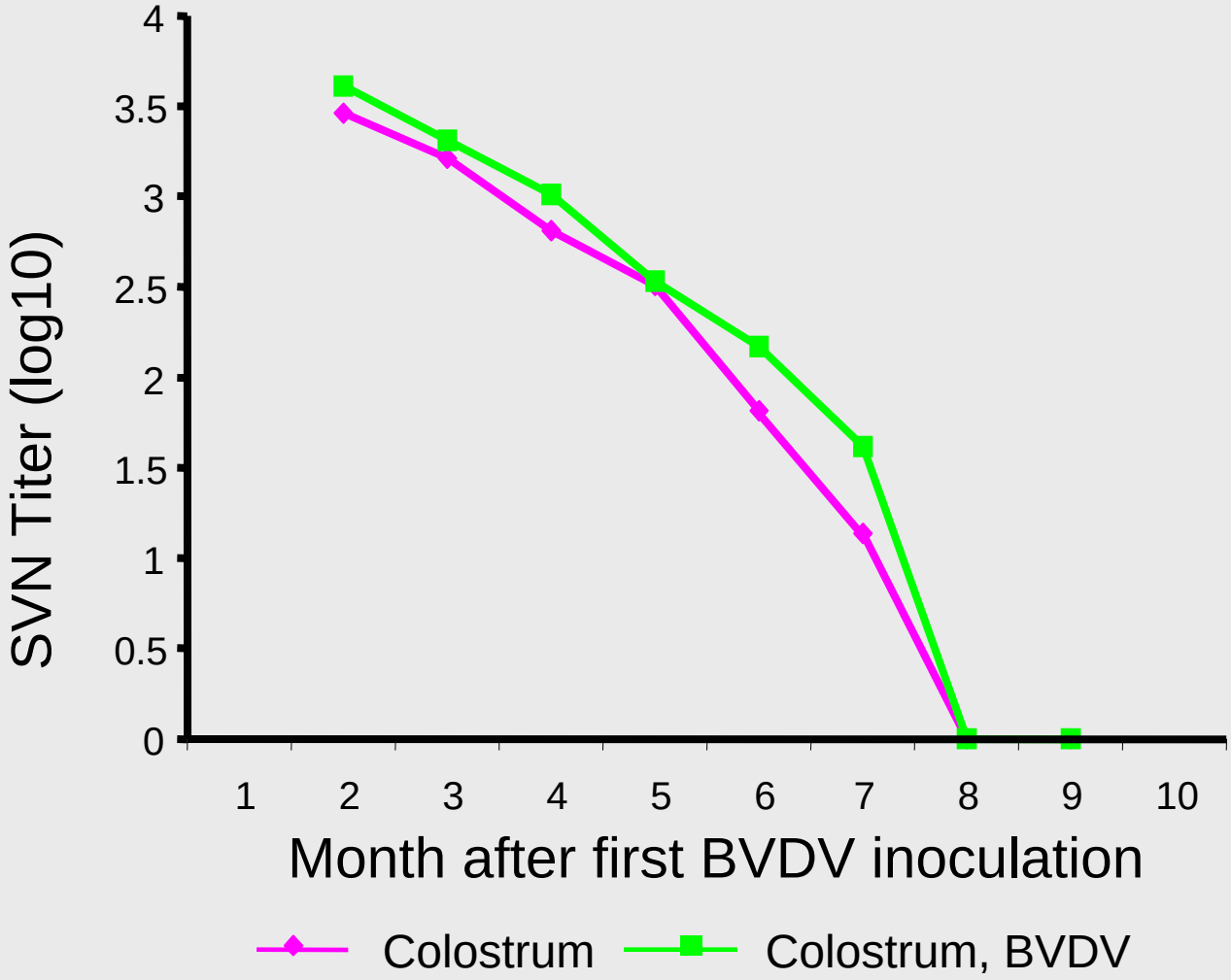
Experimental Design

- 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.

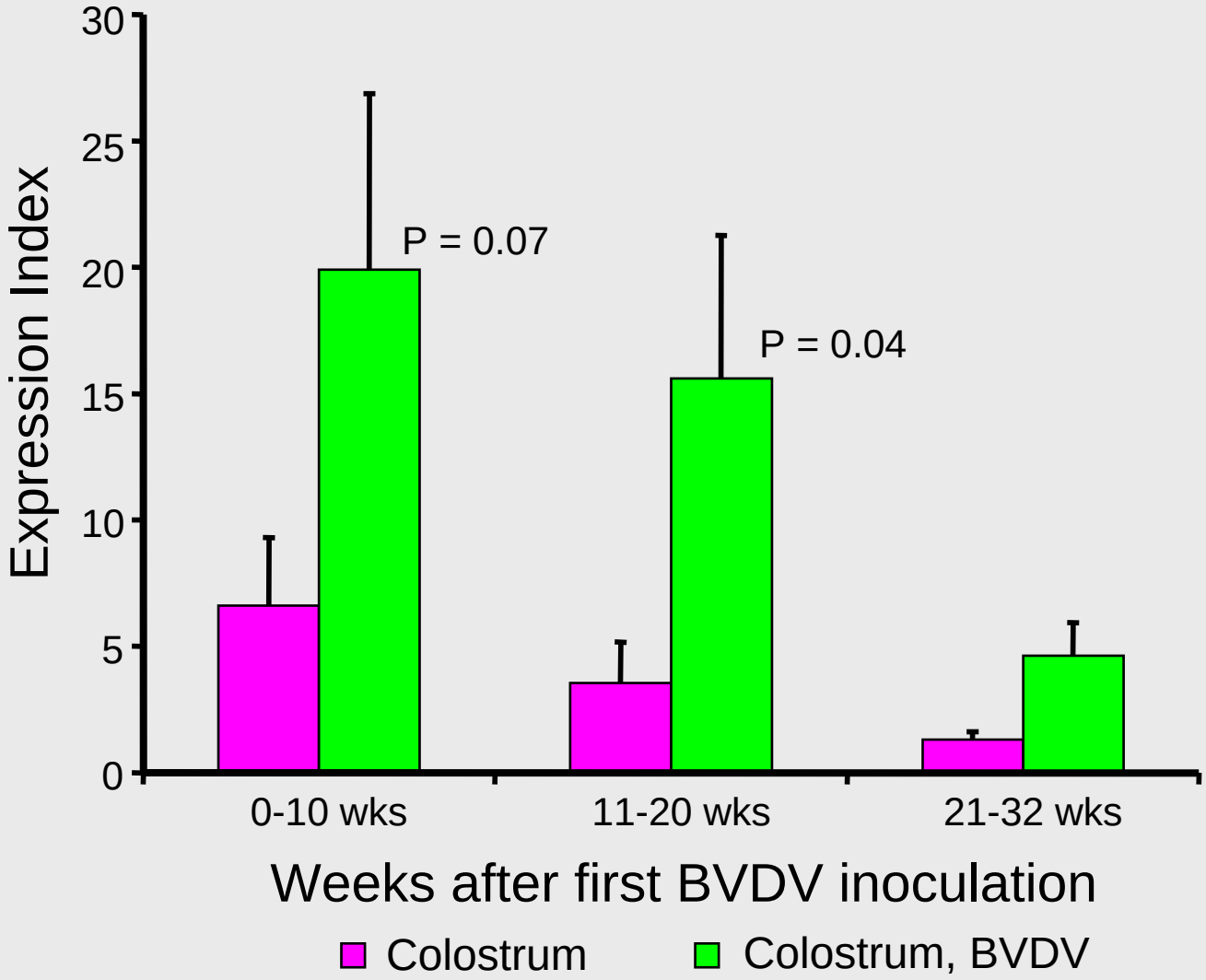
Temperature (After 1st Inoculation)



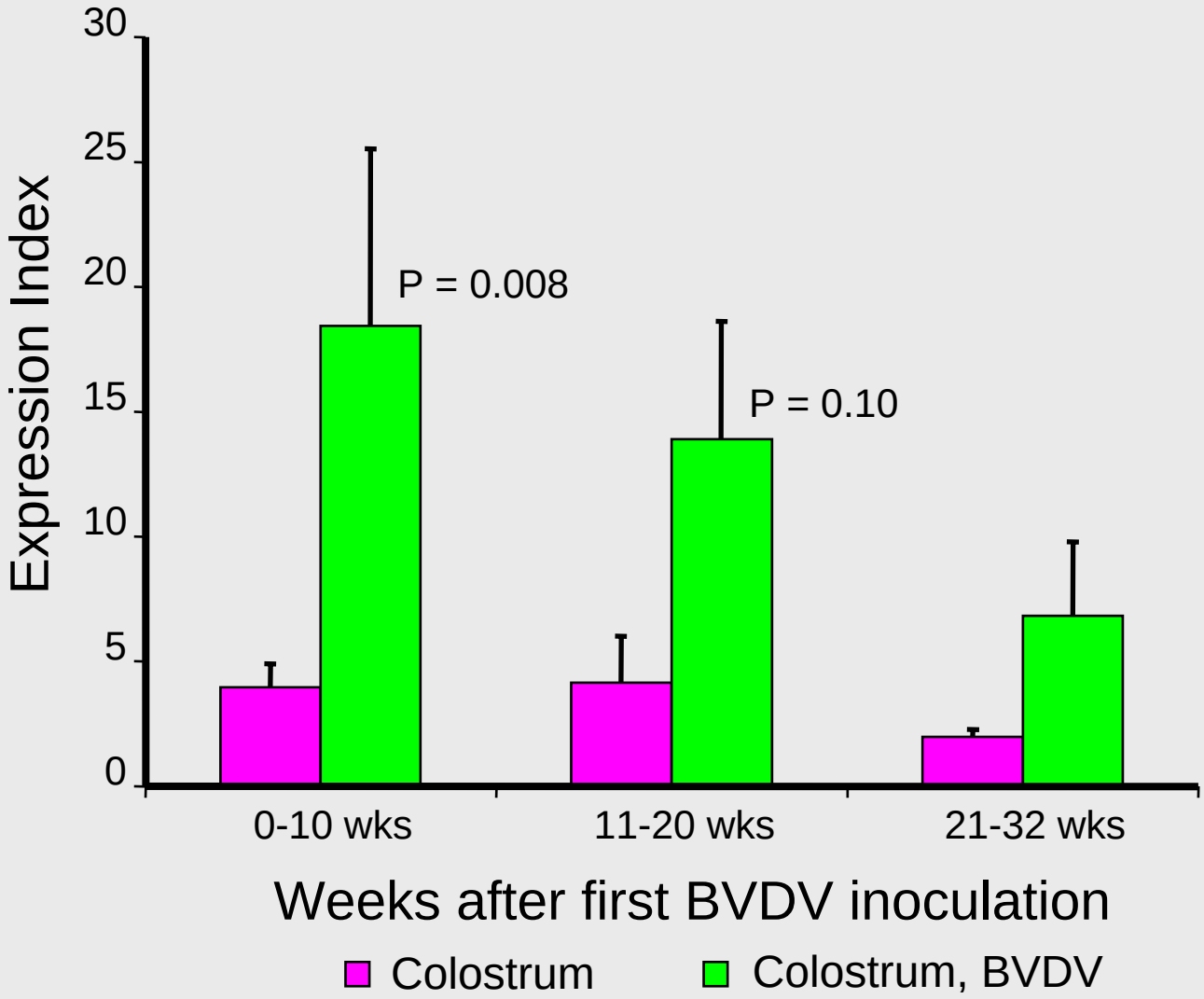
Neutralizing Antibody Responses to BVDV Type 2



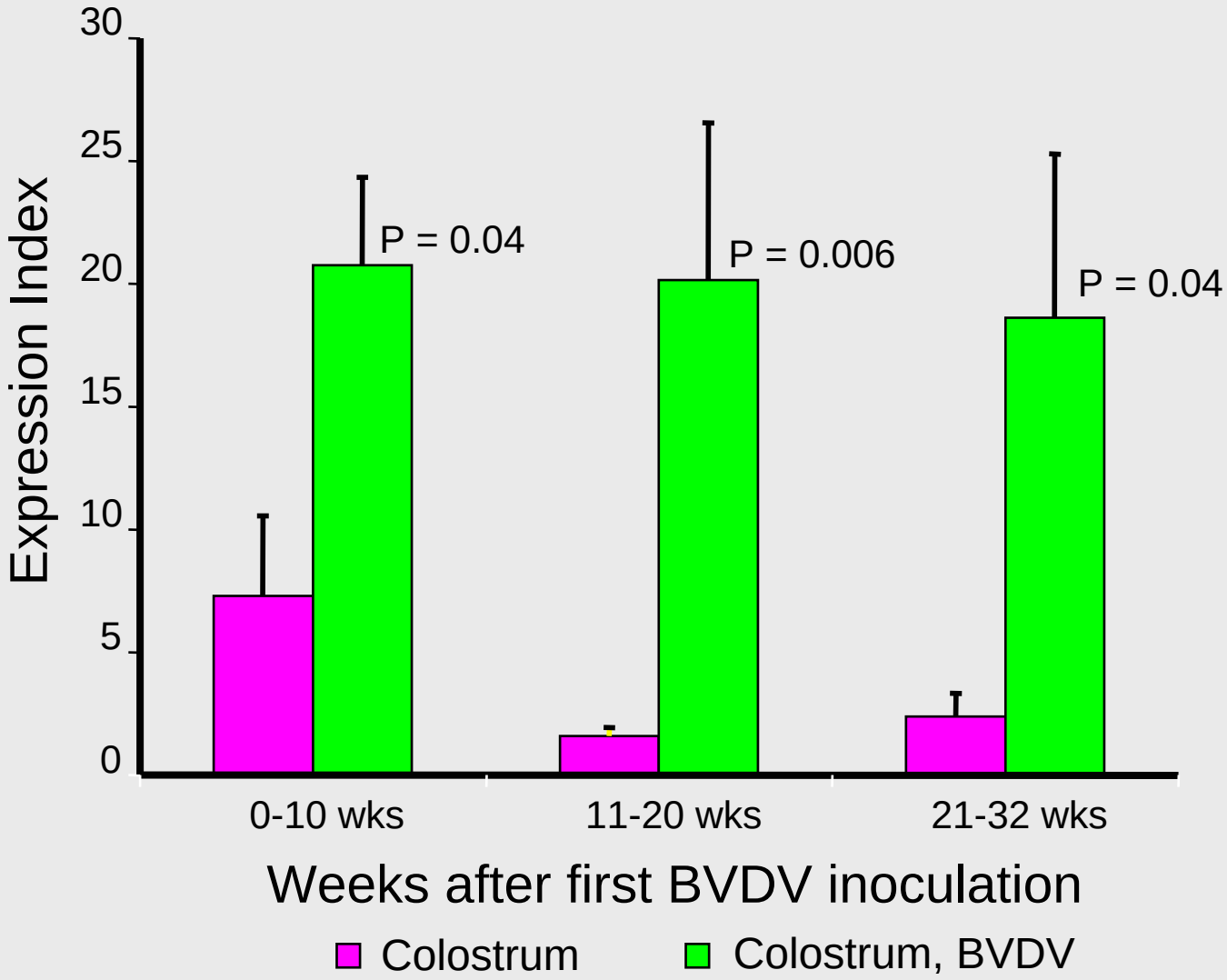
Activation of CD4 T Cells by BVDV Type 2



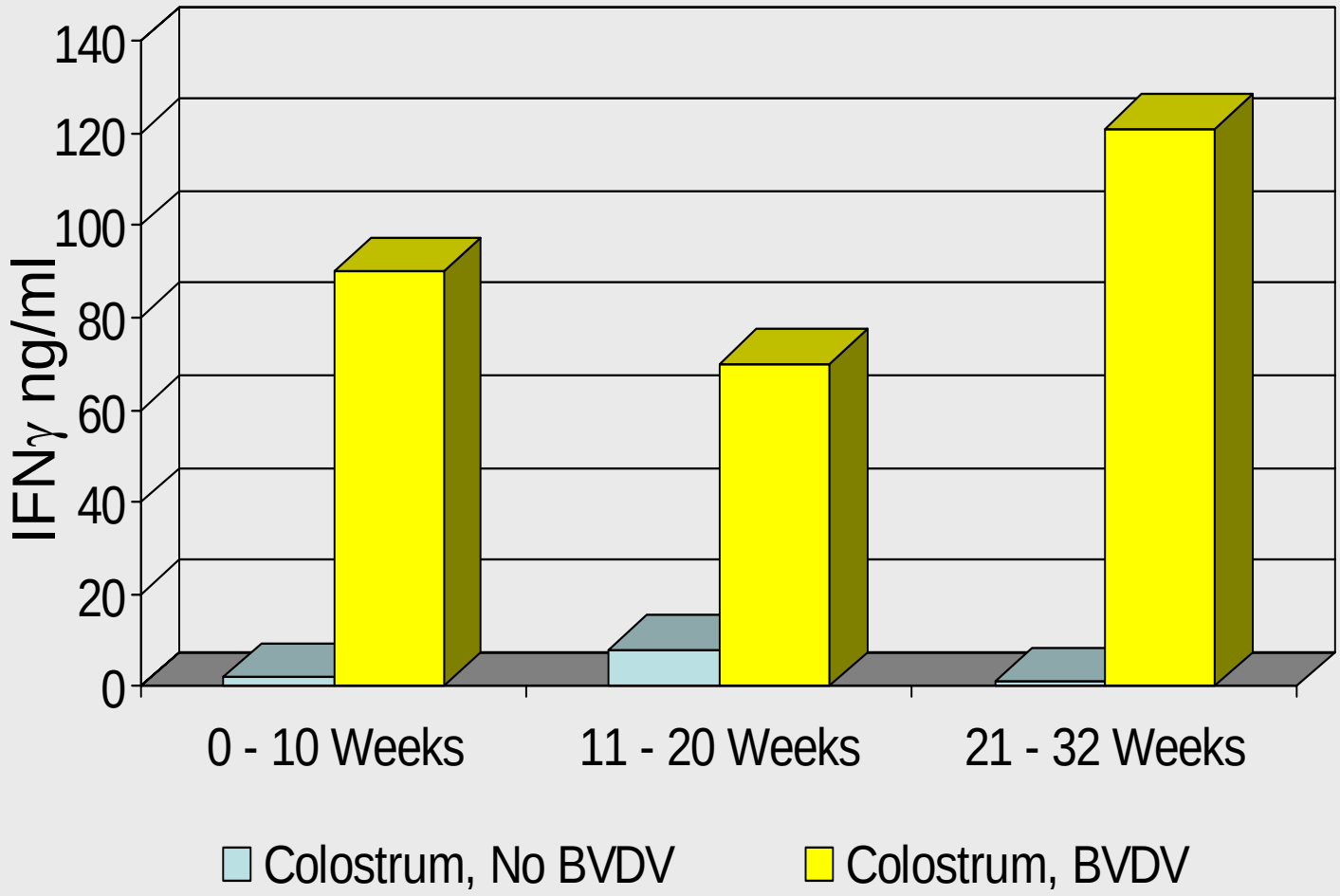
Activation of CD8 T Cells by BVDV Type 2



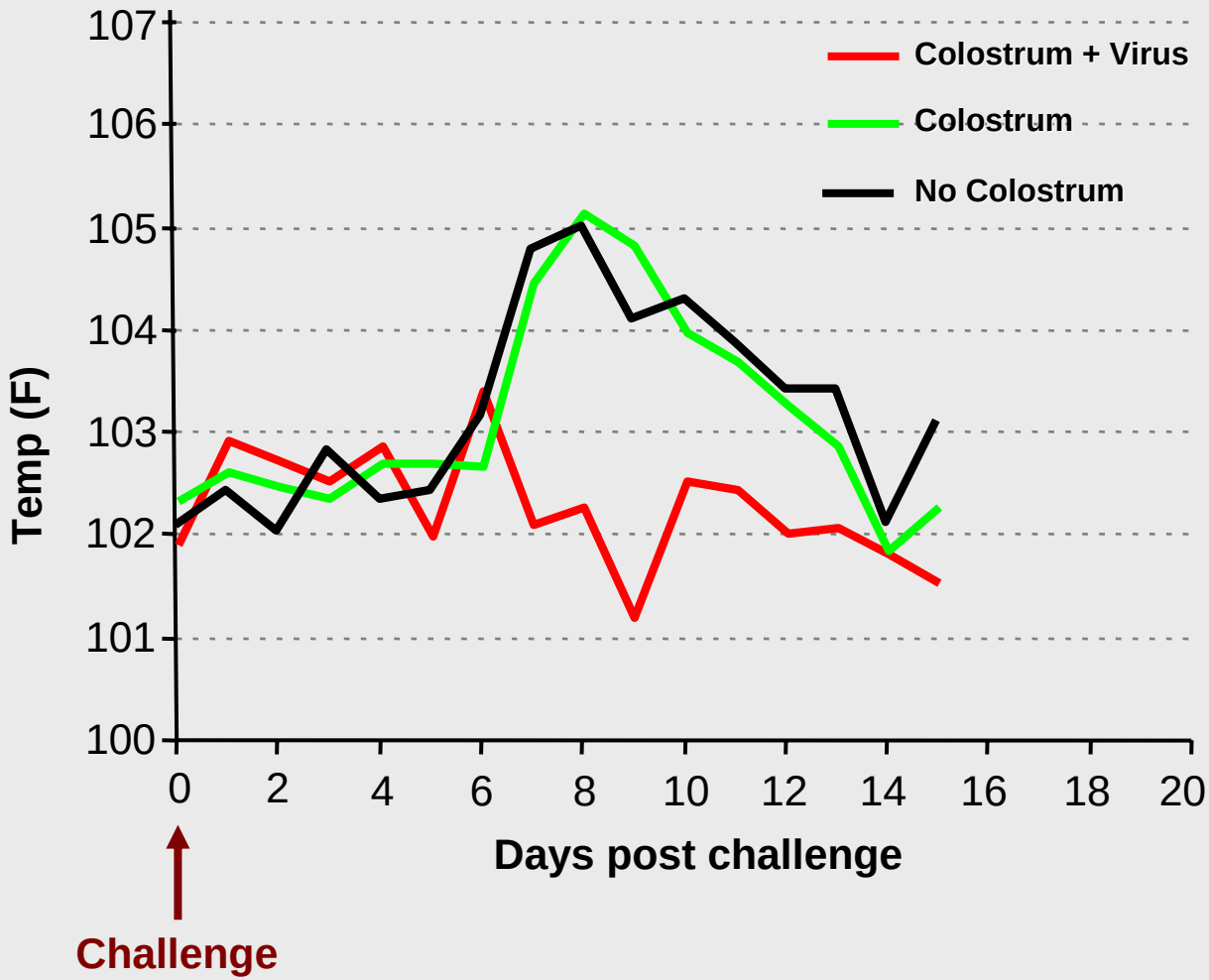
Activation of γδ T Cells by BVDV Type 2



IFNγ Production (ELISA)



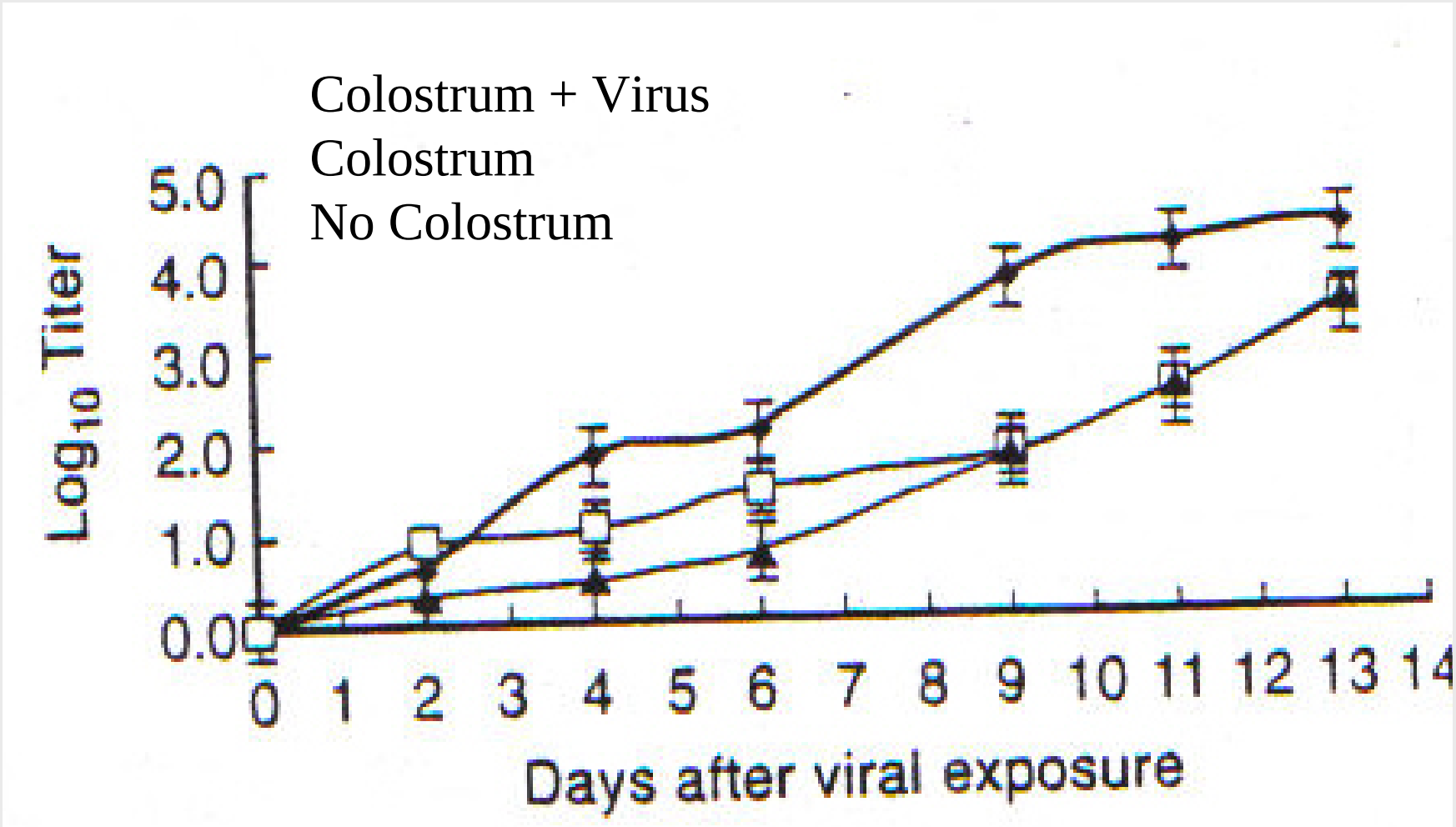
Temperature (After Challenge)



Virus Isolation from Buffy Coats (After Challenge)

	Days post challenge					
Group	2	4	6	8	10	12
Colostrum + BVDV	0/5	0/5	0/5	0/5	0/5	0/5
Colostrum	3/6	4/6	6/6	6/6	2/6	2/6
No colostrum	2/3	3/3	3/3	3/3	0/3	0/3

Mean SN Titers to BVDV 1373 After Challenge



Induction of Antigen Specific T Cell Subset Activation to Bovine Respiratory Disease Viruses by MLV Vaccine

Platt, R., W. Burdett, and J. A. Roth. 2006. Induction of antigen specific T cell subset activation to bovine respiratory disease viruses by a modified-live virus vaccine. Am J Vet Res, In press.

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

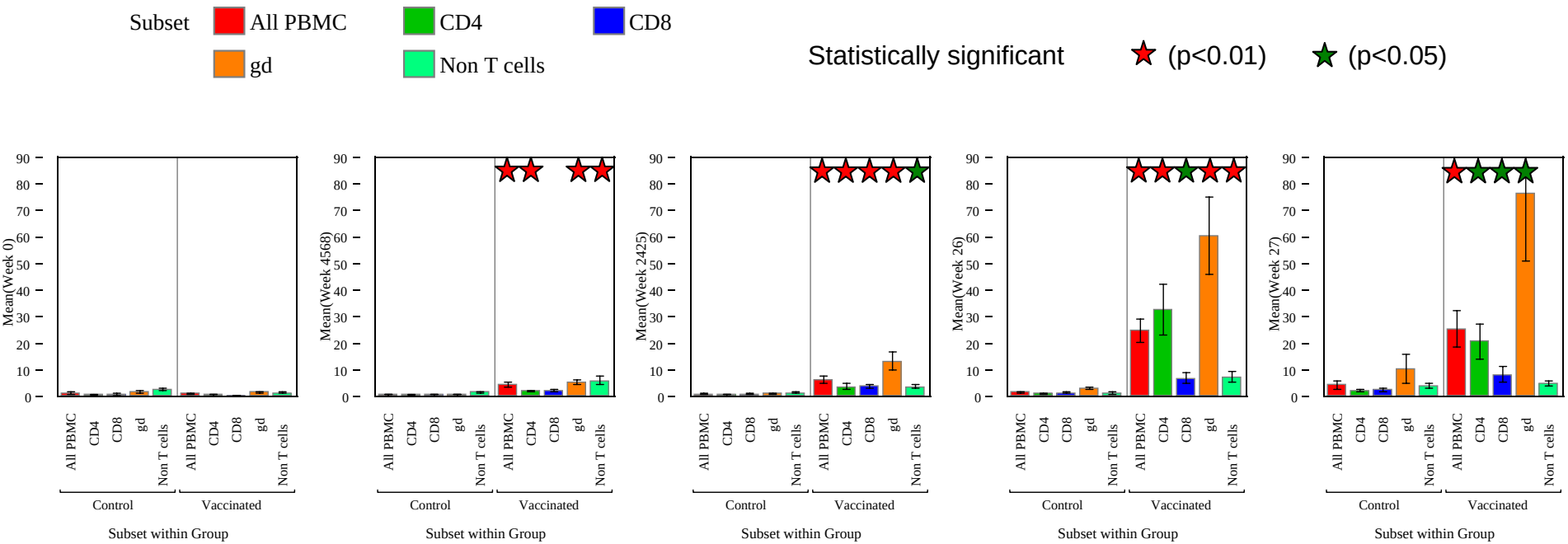
CMI assay

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

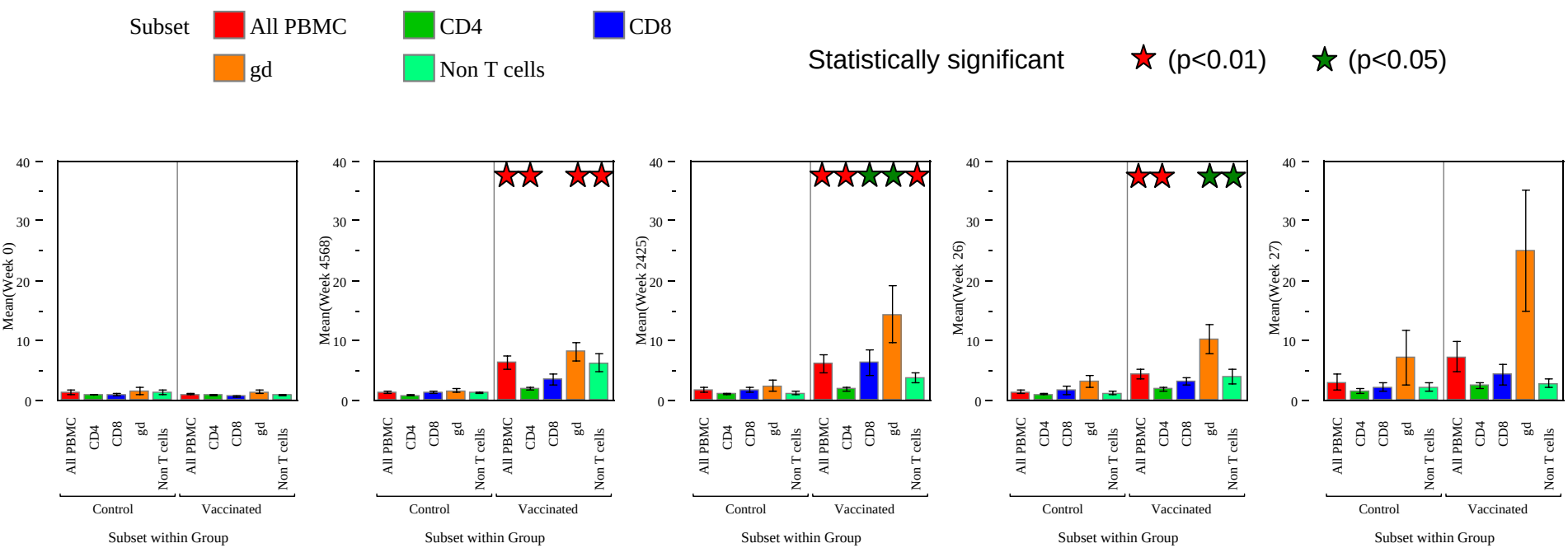
CD25 Expression Index



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

BRSV Results

CD25 Expression Index



Pre-vaccination	Post-vaccination	Pre-challenge	Week 1 post-challenge	Week 2 post-challenge
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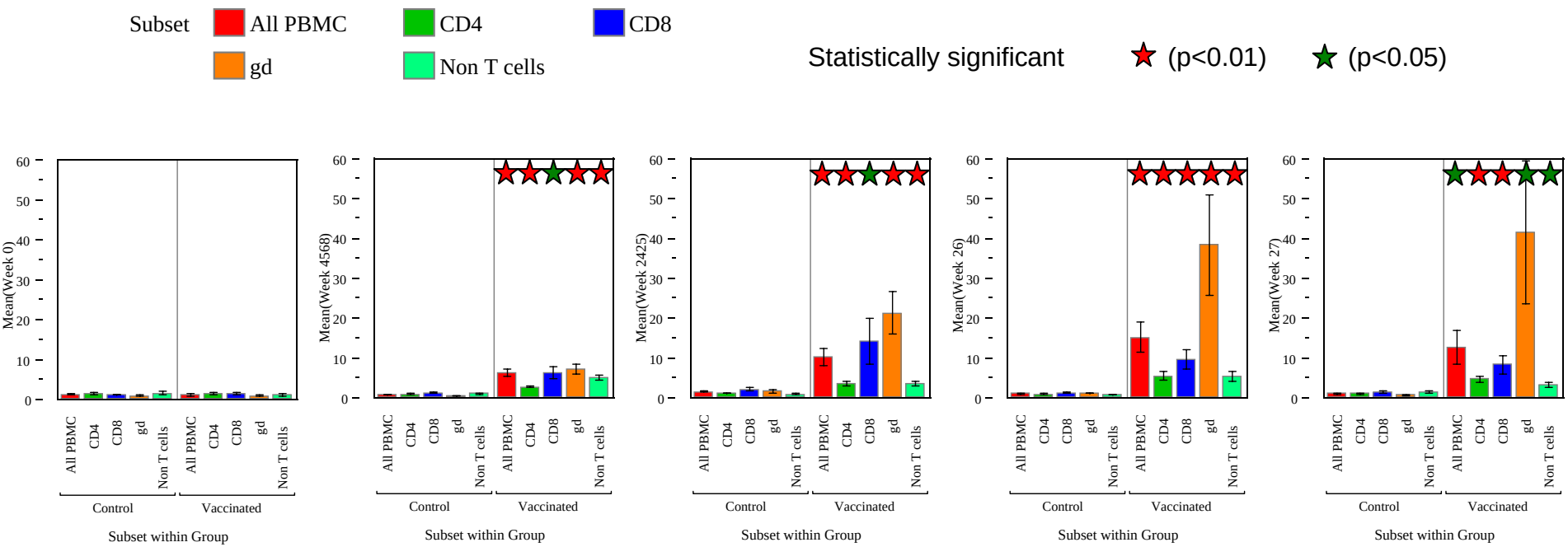
BVDV Type 1 Results

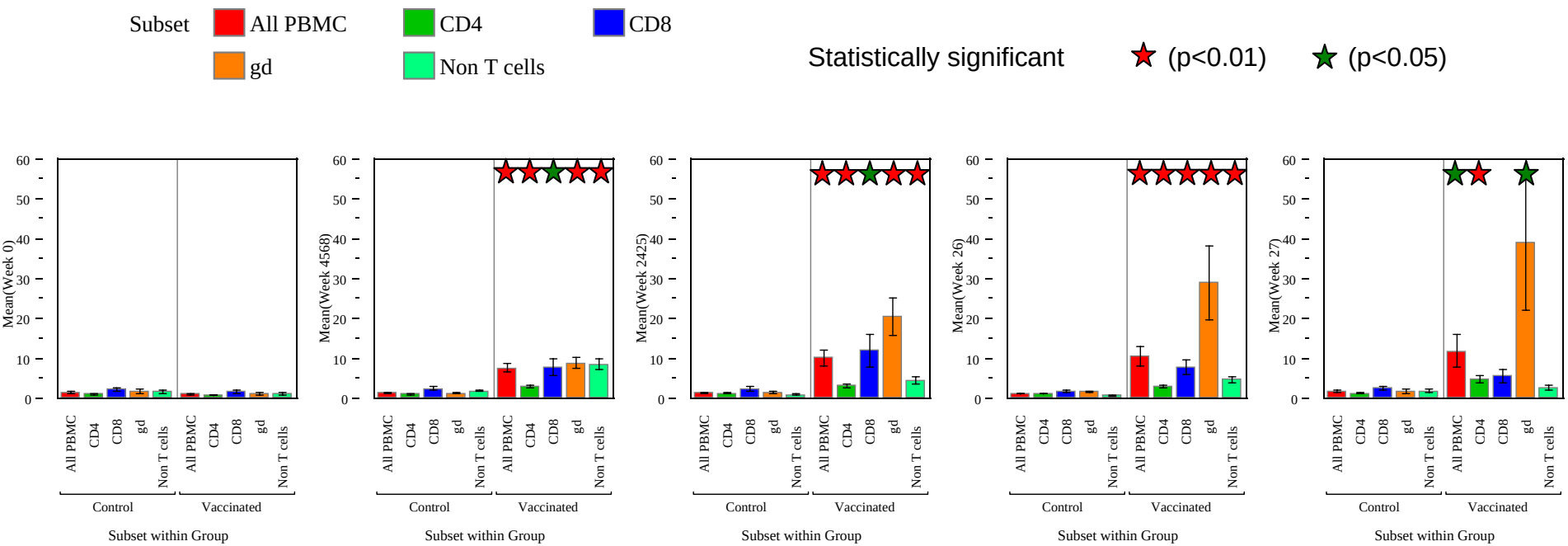
CD25 Expression Index



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

BVDV Type 2 Results

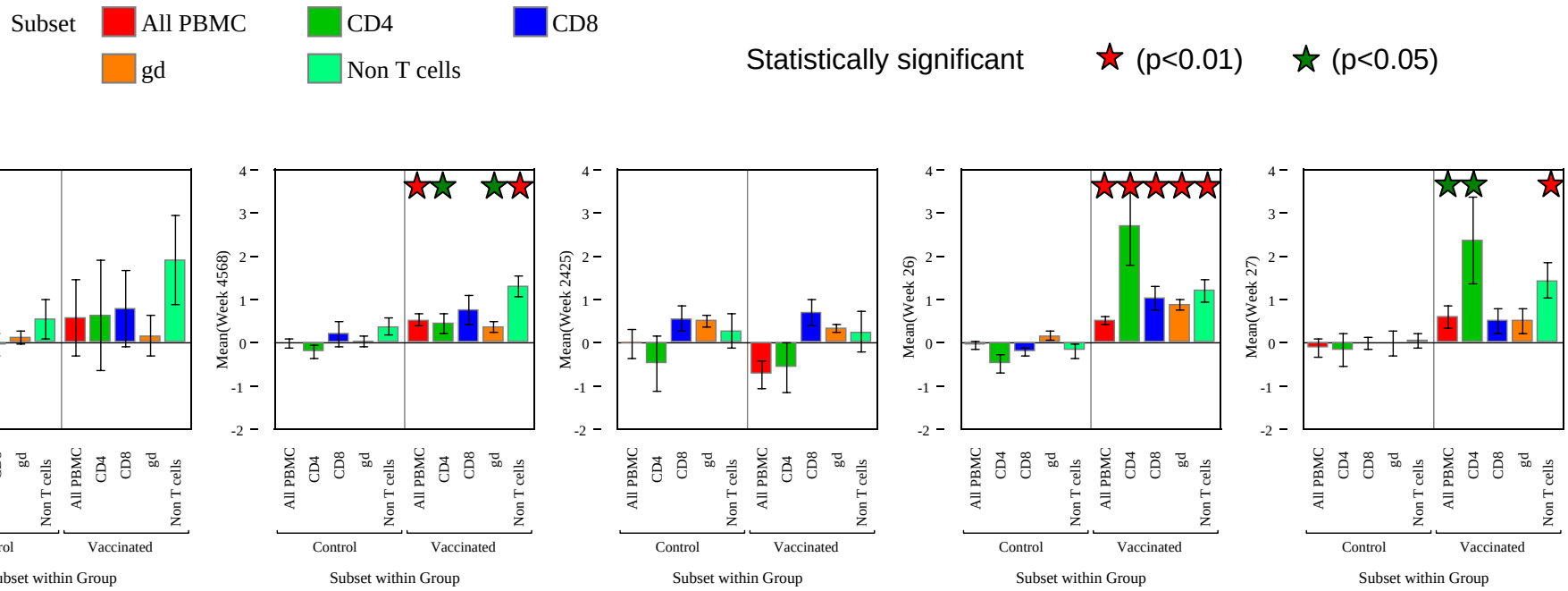
CD25 Expression Index



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

BHV-1 Results

Δ %IFNγ +



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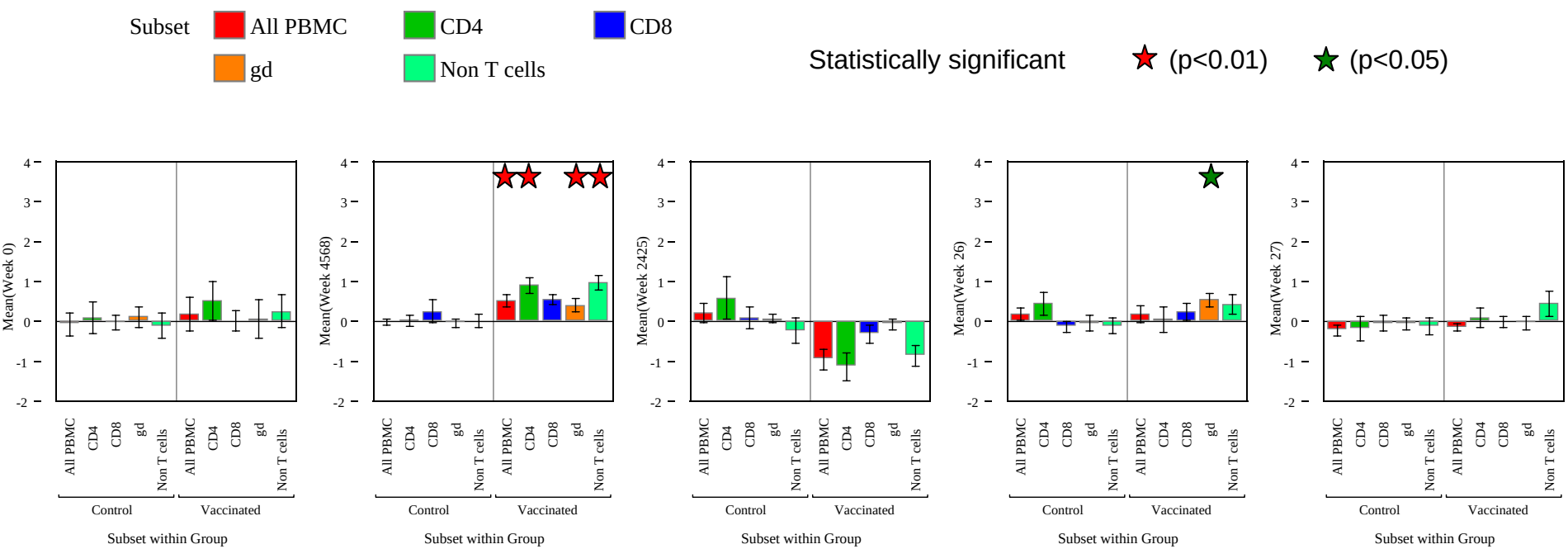
BRSV Results

Δ %IFNγ +



Pre-vaccination	Post-vaccination	Pre-challenge	Week 1 post-challenge	Week 2 post-challenge
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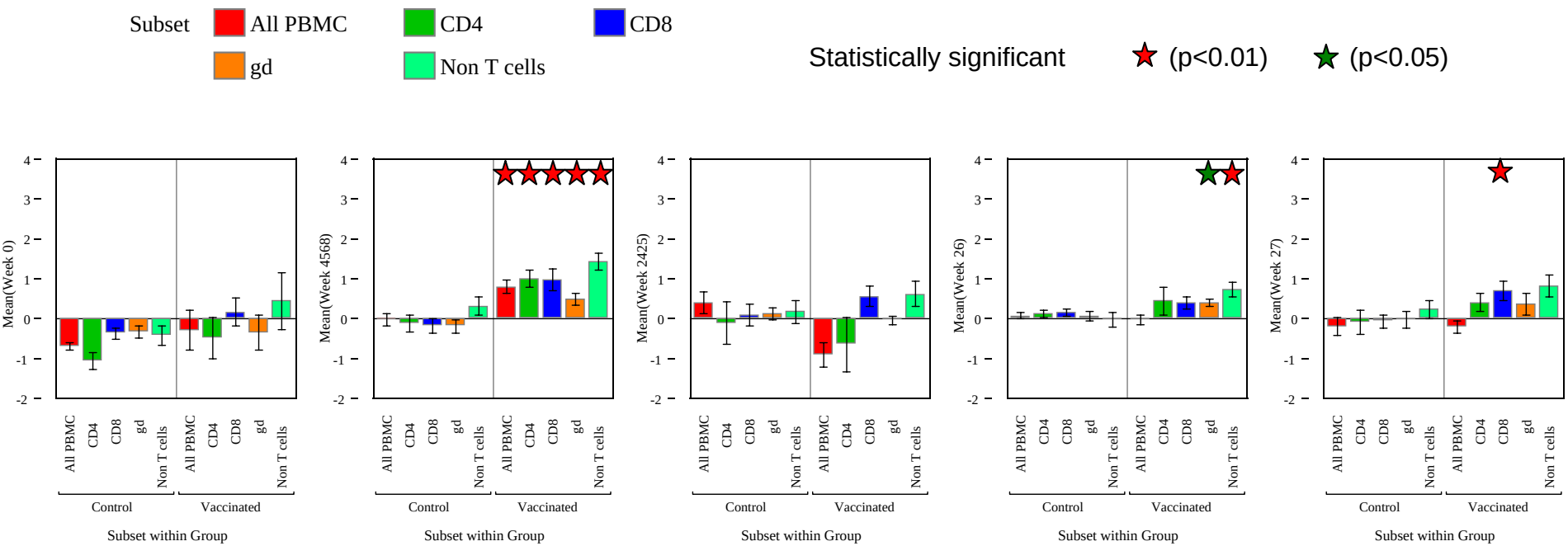
BVDV Type 1 Results

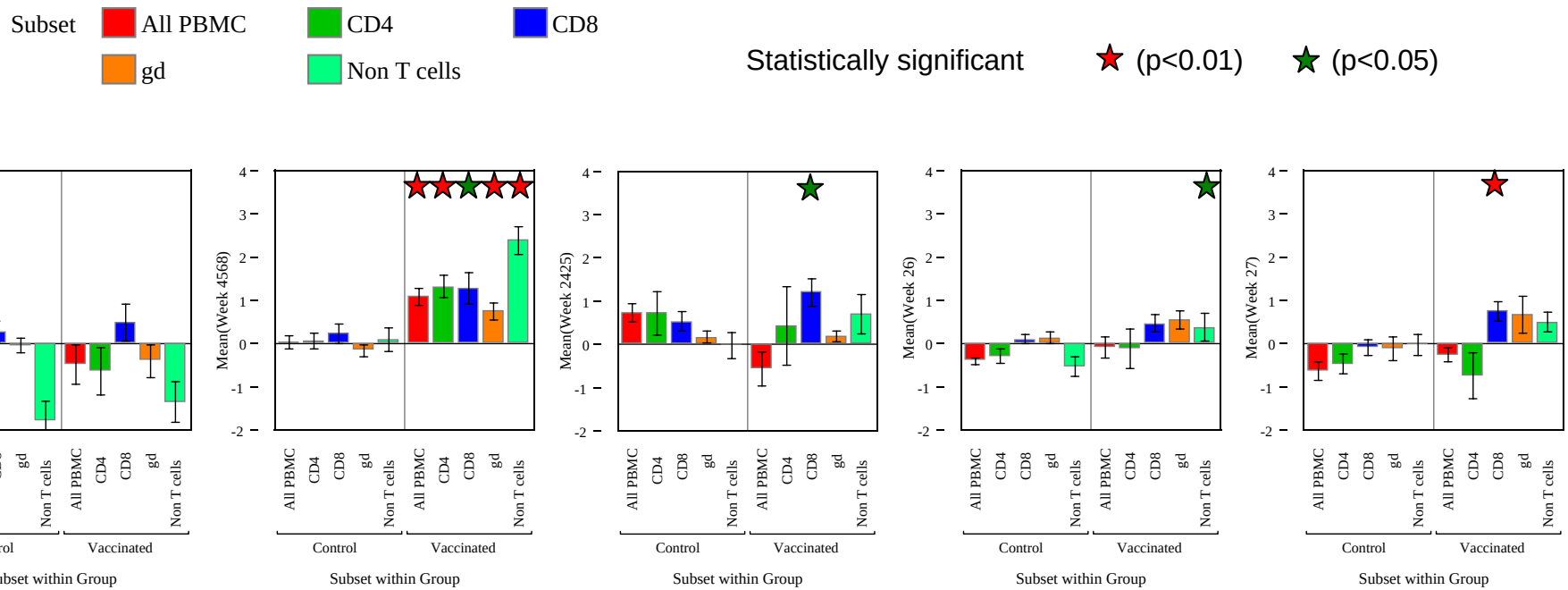
Δ %IFNγ +



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

BVDV Type 2 Results

Δ %IFNγ +



Pre-vaccination	Post-vaccination	Pre-challenge	Week 1 post-challenge	Week 2 post-challenge
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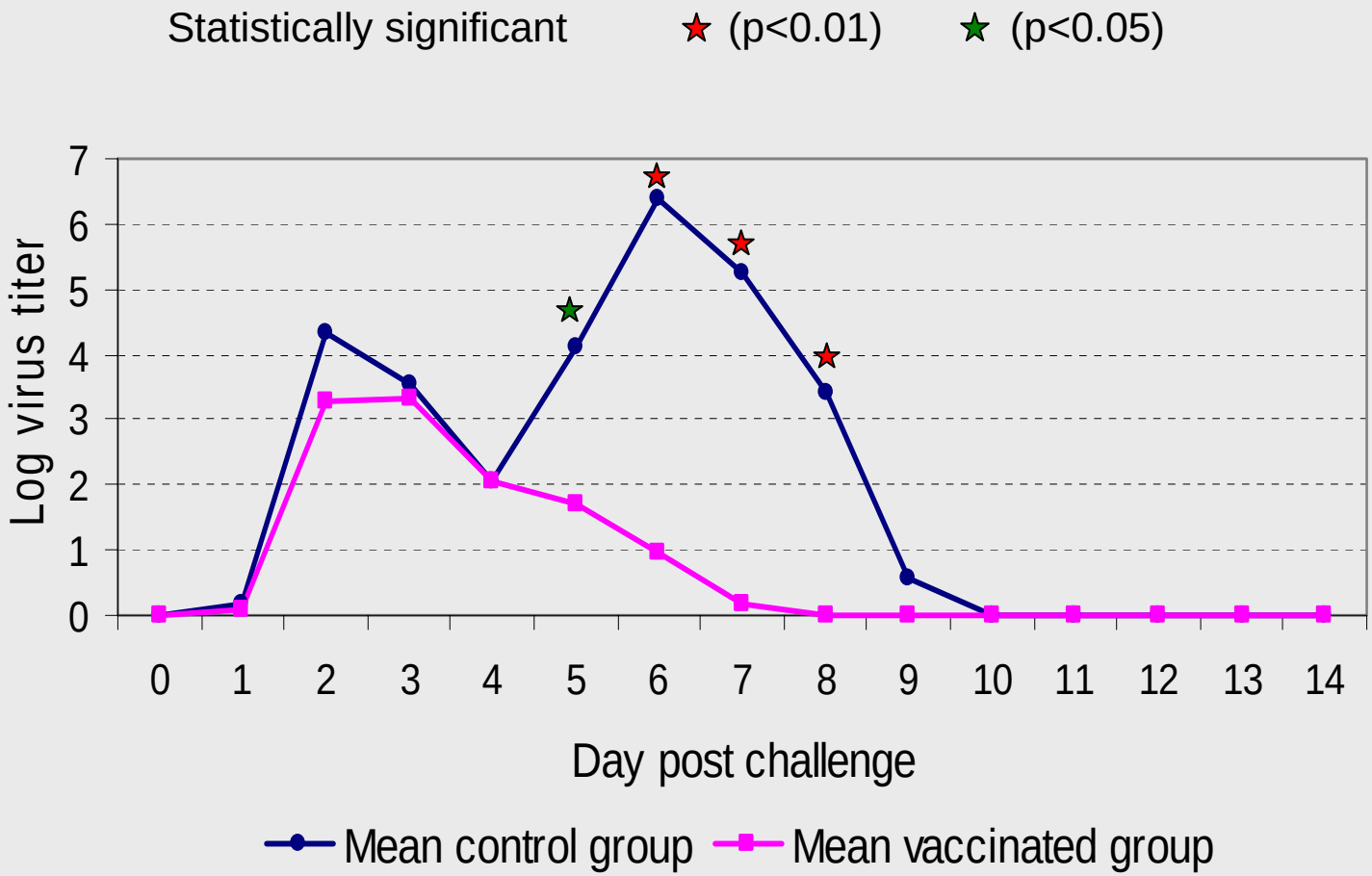
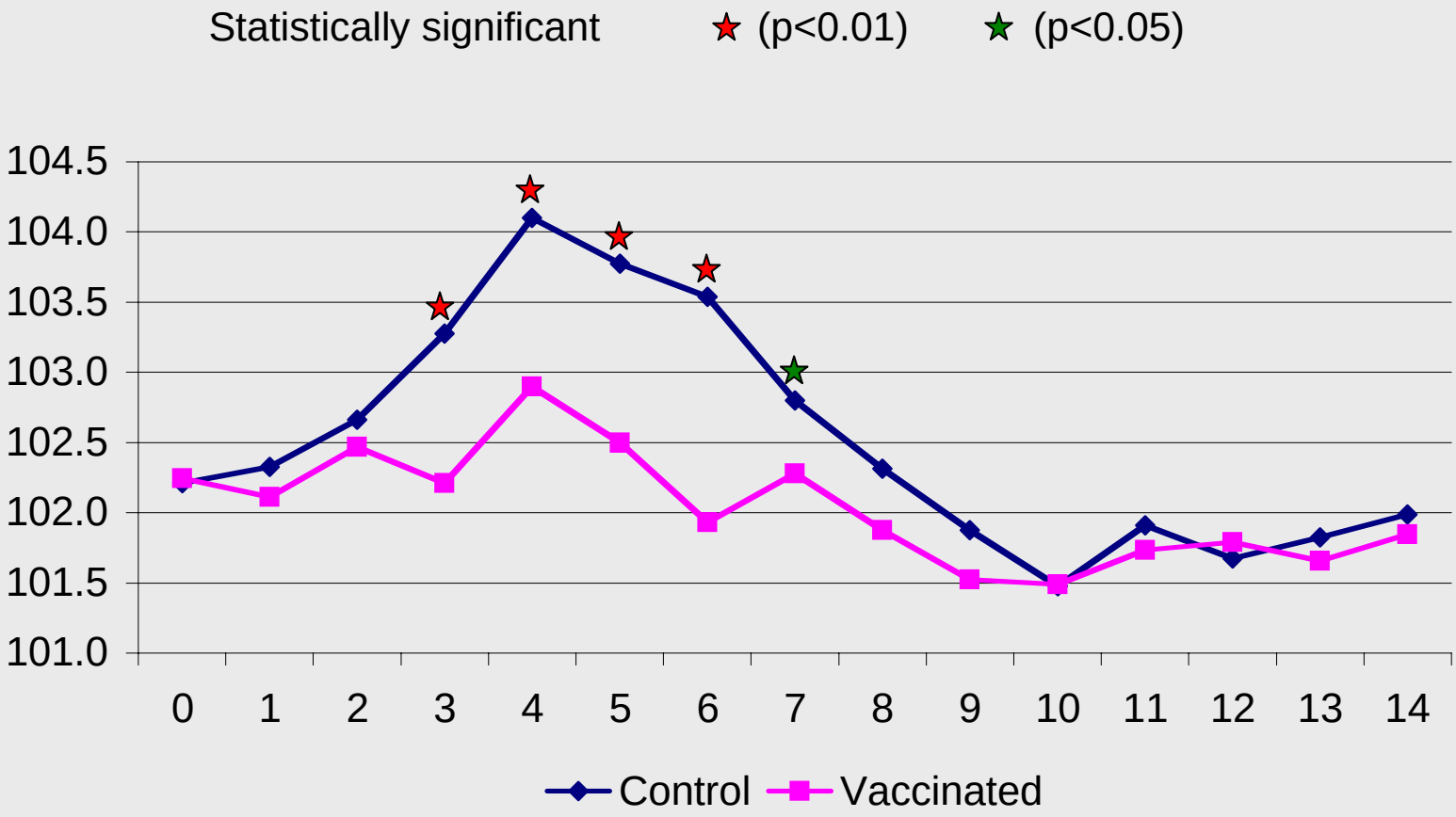
Virus Titration Results



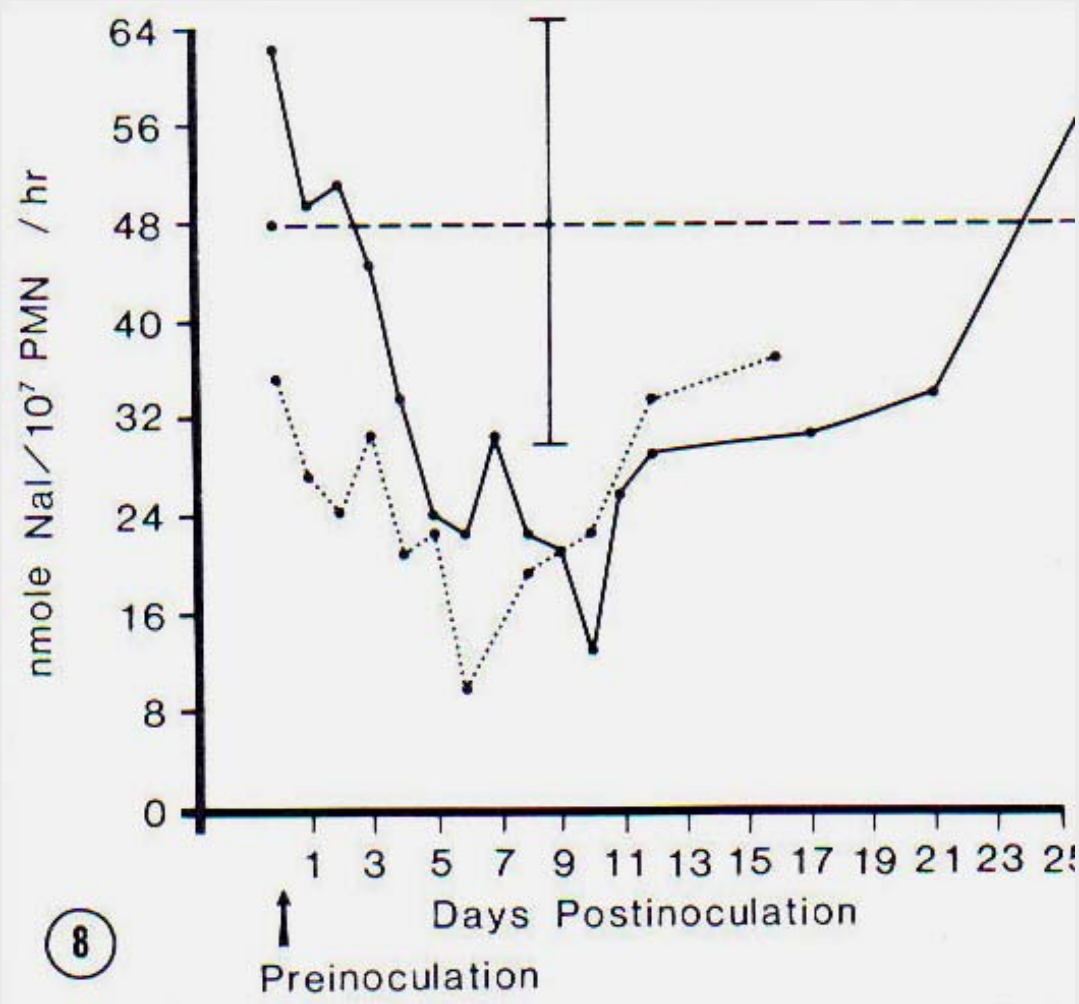
Post-challenge Body Temperature



Efficacy of Killed BVDV Vaccines for Induction of T Cell Mediated Immunity?

Immunosuppression by BVDV and MLV BVDV Vaccine

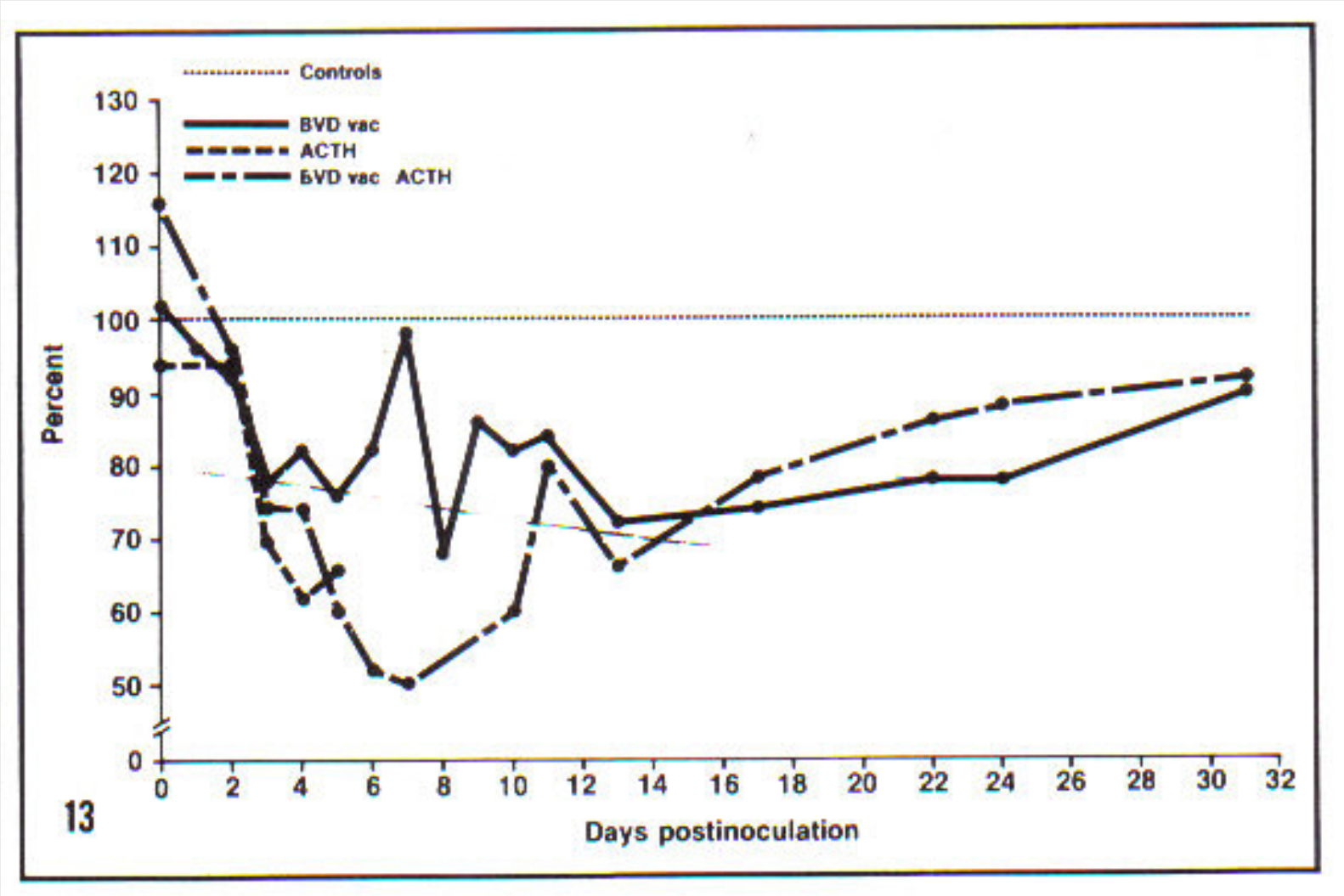
Suppression of Neutrophil Iodination by Virulent BVDV



Controls
NADL BVDV IN
1015-74 BVDV IM

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