Potential Application of Cell-Mediated Immunity Assays to Potency Testing of Veterinary Biologics

Nancy E. Clough, DVM, PhD
Center for Veterinary Biologics

James A. Roth, DVM, PhD
Institute for International Cooperation in Animal Biologics
Iowa State University
Humoral vs. Cell-Mediated Immunity

Both types important for immunity to most diseases, although one type may be more predominant for some.

Historically, humoral immunity has been studied more extensively because of easy sampling (serum) and technologically simpler assays.
Cell-mediated immunity

- **Nonspecific**
  - Neutrophils, natural killer cells, macrophages

- **Antigen-specific**
  - Lymphocyte populations proliferate in response to antigen-specific stimulation
  - Expanded cell populations effect a change directly on infected cells or via stimulation of other effector cells
    - Direct cell-cell interactions (membrane-mediated)
    - Release of effector molecules into the local environment
Types of CMI Assays

Broadly grouped by what is to be measured:

- Antigen-specific lymphocyte stimulation
- Effector activity
Types of CMI Assays: Antigen-Specific Lymphocyte Stimulation

- **In vivo**
  - Delayed-type hypersensitivity

- **In vitro**
  - Blastogenesis assays
    - Colorimetric, flow cytometry methods available
    - Can select cell population to test
  - Activation markers
    - Antigen-specific activation must be differentiated from nonspecific bystander activity
Types of CMI Assays:

Effector Activity

- **Cytotoxicity assays**
  - Traditionally done with $^{51}$Cr
  - MHC compatibility of target cells critical

- **Cytokine expression**
  - Protein assays
  - mRNA assays
  - Reagents, sequences for several veterinary species available

- **Other effector molecules** (e.g., perforin, granzyme A)
Potential Applications to Biologics: Why CMI Assays Can Be Useful

- CMI response more relevant than serological response to some diseases
- Is an important factor in immunity to nearly all diseases
- Possible to assay at local, rather than systemic, level
Challenges of CMI Assays

- Many assays qualitative or only semi-quantitative
- CMI responses can be highly variable; must compare control & subject animals in parallel on the same day.
- Proper in vivo & in vitro controls critical
- Histocompatibility issues in outbred populations
- Cells must be processed promptly
- Many assays require specialized equipment/reagents
- Capacity to process many samples
Potential Application:
Serial Release Potency Assay

Ideal potency assay: sensitive, specific, reliable, reproducible, simple, and quick to perform; demonstrates close correlation with clinical outcome (efficacy)

- Many current CMI assays not appropriate as a sole method for measuring vaccine potency
- May complement other assays
- May be more appropriate for in-process intermediates than final product
Potential Application: Reference Monitoring

- As part of a panel to characterize antigen stability, especially bio-functionality
- May be combined with other highly quantitative methods
Additional Reading
