Sponsors

University of Minnesota
College of Veterinary Medicine

College of Agricultural, Food and Environmental Sciences

Extension Service

Swine Center

Editors
W. Christopher Scruton
Stephen Claas

Layout
David Brown

Logo Design
Ruth Cronje, and Jan Swanson;
based on the original design by Dr. Robert Dunlop

Cover Design
Shawn Welch

Cell-mediated immunity in infectious diseases

James A. Roth, DVM, Ph.D., DACVM
Department of Veterinary Microbiology, College of Veterinary Medicine, Iowa State University

Cell-mediated immunity is a rather simple label for a broad and complex set of mechanisms that require T-cell activity. Effector T-cells are induced against pathogens in response to primary infection or vaccination; some activated cells eventually differentiate into memory T-cells that can expand and exert antimicrobial activity very rapidly in the event of secondary exposure.[1] Cytotoxic T-lymphocytes (CTLs), which express CD8 protein on their surface, have an important role against a wide range of intracellular pathogens.[2,3] These lymphocytes are programmed to kill infected cells when their T-cell receptors (TCRs) recognize pathogen-derived peptides that have been processed and displayed on major histocompatability complex (MHC) class I molecules via the endogenous pathway.[4,5] The endogenous pathway is most efficient at processing and presenting peptides which are synthesized in the cytoplasm of the infected cell. In contrast, the exogenous antigen-presenting pathway is most efficient at processing and presenting peptides that are outside of the cell and are internalized by phagocytosis or pinocytosis. Peptides processed by the exogenous pathway are presented on MHC class II molecules recognized by CD4+ helper T-cells.[5] Helper T-cells coordinate the overall adaptive immune response by modulating the activities of many other immune cells, both through direct cell-cell contact and secretion of cytokines.[6,7] Cytokines can be categorized by the many different functional activities they mediate, and any cytokine may have multiple effects.[7,8] Some cytokines activate innate immune cells such as phagocytes and natural killer cells and stimulate their participation in inflammatory processes, whereas others suppress or resolve inflammation. Several cytokines promote the activation of and antibody production by B-cells, as well as antibody class switching of B-cells. Certain cytokines have effects further upstream in the ontogeny of cells, in that they direct the maturation and mobilization of distinct classes of cells. Chemokines control immune cell traffic in at least two ways: They attract the specific sets of cells that bear corresponding receptors and activate the integrin molecules that mediate leukocyte adhesion to vascular endothelium around the site of an infection.[9]

In recent years, the balance of immune regulation has been one of the foremost topics in immunology.[8] Successful defense against the majority of infections requires an early T helper (Th) 1 cell-mediated inflammatory response (Type 1 response). Interferon-α, interleukin-2 (IL-2), and lymphotoxin-α are particularly important Type 1 cytokines. Type 2 responses (i.e., those mediated by Th-2 cells) are generally characterized by high antibody production and often by recruitment of eosinophil and mast cell activity. Of the Type 2 cytokines, IL-4, IL-10, and IL-13 are among the most dominant and best characterized. One purpose for Type 2 responses appears to be mediation of immunity against helminth infections. Also, a transition to predominantly Type 2 responses appears to be important for resolving inflammation after other kinds of infection. However, when Th-2 cell-driven mechanisms dominate early in infection, microbial replication may proceed unchecked. This, as well as antibody-mediated hypersensitivity, can enhance the severity of disease. Importantly, cytokines from Type 1 responses tend to suppress Type 2 responses, and vice versa, so that one or the other response predominates. Given the divergent properties of helper T-cell subsets, the Type 1 versus Type 2 theory has been used to explain striking divergences between protection and disease development in response to certain infections in humans and rodent species, such as leprosy and leishmaniasis.

Measurement of cytokine activity and T helper subsets has been and promises to be a useful measure of vaccine efficacy against infectious agents of animals. Much of the research that has shaped the Type 1 versus Type 2 theory has involved studies of mice. Human helper T-cell responses also polarize toward Type 1 or Type 2 profiles, though the definitions seem to be less rigid.[8] There is considerable evidence to support this paradigm in numerous domestic animal species, as well.[10–13] However, it is important to be aware that the effects exerted by a cytokine in 1 species may not be identical to those exerted by its homologue in another species.[10]

Gamma delta (γδ) T-cells are the other major T-cell subset.[14] The TCR for this subset of T-cells is a heterodimer comprised of a γ chain and a δ chain, which are structurally distinct from the α and β, chains that form the TCRs of CD4+ and CD8+ T-cells. Antigen recognition by γδ T-cells is not usually MHC-restricted. Certain host cell markers, such as stress-induced heat-shock proteins, can activate γδ T-cells. The functions of γδ T-cells in response

to pathogens are not well understood, but their prominent distribution in mucosal surfaces is consistent with a role in surveillance and rapid response to invaders. There is also evidence that γδ T-cells modulate inflammatory responses and promote tissue repair.[15]

Past efforts to evaluate immune responses in domestic animals typically focused on humoral immunity. One reason for this is the relative ease with which serum antibody responses can be monitored, by use of ELISAs and virus neutralization and hemagglutination assays. Immunological studies in recent years have highlighted the substantial roles of cell-mediated immunity (CMI) in defense against human and animal infections. This has accentuated the importance of examining CMI as part of any thorough effort to characterize an immune response to infection or vaccination.

When evaluating vaccines used in veterinary medicine, serum antibody titer is often cited as an important measure of vaccine efficacy. Yet, in a variety of situations, these data are insufficient to predict a positive or negative outcome for the vaccinated animal regarding development of disease:

- Specific antibodies to some infectious agents do not adequately protect the host from infection and disease.[16]
- Antibodies to certain infectious agents enhance disease. One mechanism for this is antibody-dependent enhancement[17], in which immunoglobulin-bound pathogens rely on Fc receptor-mediated phagocytosis for entry to host cells. Immune complexes can also contribute to the pathogenesis of infectious diseases by triggering complement activation and inducing a Type III hypersensitivity response.[18]
- Antibodies may not always be detectable following an adaptive immune response. With regard to several viral agents, adaptive CMI responses can be mounted in the presence of passively transferred maternal antibody, even while humoral responses are suppressed.[19,20] Thus, animals with no detectable antibody titers can be fully resistant to challenge.

Thorough evaluation of vaccine efficacy requires the evaluation of T-cell responses to vaccination in populations of animals over time. This requires assays that can be performed on large numbers of outbred animals. Some assays are very cumbersome to use for this purpose (e.g., CTL assays). Other CMI assays are inherently more practical for this research aim (e.g.,, in vitro tests for antigen-specific lymphocyte activation and proliferation, or cytokine production).

Lymphocyte responses to antigens are detectable in vitro by flow cytometric analysis of activation-induced cell surface markers. Fluorescent antibodies can be used to label these molecules. Activation markers that have been used for the purpose of detecting recall responses include CD25 (IL-2 receptor α subunit), CD69, and MHC class II.[21–24] The expression of such markers can be analyzed in parallel with T-lymphocyte subset markers to assess the level of responses by distinct T-cell populations having CD4, CD8, and/or γδ TCR on their surface. Levels of activation marker expression in a cell population can be measured in terms of two values: The percentage of cells that express the marker and the mean fluorescence intensity of staining for the marker. Monoclonal antibodies against porcine lymphocyte subset markers and activation markers are commercially available.

Cytokine-producing activity can be measured in distinct T-cell subsets by use of intracellular cytokine staining techniques and multiparameter flow cytometry.[25,26]. Two key requirements for the success of this approach are that cells retain the cytokine molecules they produce and that cells are permeable to antibody during the staining steps. The first requirement is met by treating cells with an inhibitor of the Golgi complex, such as brefeldin A, during culture. The second requirement is met by fixing cells and making their membranes permeable with a detergent solution before labeling. All of the reagents necessary for blocking secretion, fixation, and permeabilization can be purchased together in a kit. Data are acquired and analyzed by much the same approach as the aforementioned flow cytometric assays for T-cell activation. The main advantage of intracellular staining is its powerful capacity to trace cytokine production to specific populations of effector lymphocytes. However, the assay is complicated by the special requirements for inhibition of protein secretion and treatment of cells to induce membrane permeability. Altered cellular activity may distort normal antigen-driven processes, and cell-labeling procedures inevitably allow some leakage of cytokines. In addition, the technique is more time-consuming than flow cytometric assays for T-cell activation markers.

There are some important considerations regarding the interpretation of data from assays of lymphocyte activation and cytokine secretion. The demonstration of either type of response following exposure to antigen does not prove any particular effector function. If one subset of lymphocytes is better adapted to survive and proliferate in vitro, then the relative levels of antigen-driven proliferation or activation across subsets may be skewed in favor of that subset. In addition, it is possible that antigen-specific cytokine production by one T-cell population in an in vitro system might promote non-specific bystander responses by other T-cells. Ordinarily, the activation of a T-cell depends on antigen recognition by the T-cell receptor, and further stimulation by cytokines amplifies that antigen-specific response. However, memory T-cells can be sensitive to stimulation by certain cytokines in the absence of antigen.[27] Thus, it is plausible that an antigen-

specific T-cell response in vitro might lead to the activation of accompanying memory T-cells that do not share the same specificity. It has not been proven conclusively whether this causes measurements of antigen-specific T-cell activity to be overestimated. Of course, even bystander T-cell activation presumably depends on antigen-specific cells having made a recall response in the first place.

It is important to bear in mind that the results of any given assay must be interpreted cautiously, whether the assay evaluates antibody production or cellular activity; there is artificiality and variability in any in vitro test. Careful study is required to determine how well the readout from an assay correlates with immunity against the pathogen in question.

Acknowledgment

Some information in this presentation was extracted from M. R. Sandbulte and J. A. Roth, Methods for analysis of cell-mediated immunity in domestic animal species. *JAVMA*, in press (2004).

References

1. Seder R.A. and Ahmed R. 2003. Similarities and differences in CD4+ and CD8+ effector and memory T-cell generation. Nature Immunology 4:835-842.

2. Doherty P.C. 1996. Cytotoxic T-cell effector and memory function in viral immunity. Current Topics in Microbiology and Immunology 206:1-14.

3. Barry M. and Bleackley R.C. 2002. Cytotoxic T-lymphocytes: All roads lead to death. Nature Reviews Immunology 2:401-409.

4. Zinkernagel R.M. and Doherty P.C. 1979. MHC-restricted cytotoxic T-cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. Adv Immunol 27:51-177.

5. Germain R.N. 1994. MHC-dependent antigen processing and peptide presentation: providing ligands for T-lymphocyte activation. Cell 76:287-299.

6. Clarke S.R. 2000. The critical role of CD40/CD40L in the CD4-dependent generation of CD8+ T-cell immunity. J Leukoc Biol 67:607-614.

7. Paul E.P. and Seder R.A. 1994. Lymphocyte responses and cytokines. Cell 76:241-251.

8. Spellberg B. and Edwards J.E. 2001. Type 1/Type 2 immunity in infectious diseases. Clin Infect Dis 32:76-102.

9. Mackay C.R. 2001. Chemokines: immunology's high impact factors. Nature Immunology 2:95-101.

10. Brown W.C., Rice-Ficht A.C. and Estes D.M. 1998. Bovine Type 1 and Type 2 responses. Vet Immunol Immunopathol 63:45-55.

11. Gomes-Keller M.A., Nunez R., Schroff M., Oswald D., Willett B.J. and Lutz H. 2002. Up-regulation by feline interleukin-4 and down-regulation by feline interferon-gamma of major histocompatibility complex class II on cat B-lymphocytes. Vet Immunol Immunopathol 88:197-208.

12. Fischer T., Buttner M. and Rziha H.J. 2000. T helper 1-type cytokine transcription in peripheral blood mononuclear cells of pseudorabies virus (Suid herpesvirus 1) primed swine indicates efficient immunization. Immunol 101:378-387.

13. Woolums A.R., Singer R.S., Boyle G.A. and Gershwin L.J. 1999. Interferon gamma production during bovine respiratory syncytial virus infection is diminished in calves vaccinated with formalin-inactivated BRSV. Vaccine 17:1293-1297.

14. Hayday A.C. 2000. Gamma Delta Cells: A Right Time and a Right Place for a Conserved Third Way of Protection. Annu Rev Immunol 18:975-1026.

15. Carding S.R. and Egan P.J. 2002. Gamma Delta T Cells: Functional Plasticity and Heterogeneity. Nature Reviews Immunology 2:336-345.

16. Westenbrink F., Kimman T.G. and Brinkhof J.M. 1989. Analysis of the antibody response to bovine respiratory syncytial virus proteins in calves. J Gen Virol 70:591-601.

17. Tirado S.M. and York K.J. 2003. Antibody-dependent enhancement of virus infection and disease. Viral Immunol 16:69-86.

18. Murphy B.R., Prince G.A., Walsh E.E., Kim H.W., Parrott R.H., Hemming V.G., Rodriguez W.J. and Chanock R.M. 1986. Dissociation between serum neutralizing and glycoprotein antibody responses of infants and children who received inactivated respiratory syncytial virus vaccine. J Clin Microbiol 24:197-202.

19. Bradshaw B.J.F. and Edwards S. 1996. Antibody isotype responses to experimental infection with bovine herpesvirus 1 in calves with colostrally derived antibody. Vet Microbiol 53:143-151.

20. Ellis J.A., Hassard L.E., Cortese V.S. and Morley P.S. 1996. Effects of perinatal vaccination on humoral and cellular immune responses in cows and young calves. J Vet Med Assoc 208:393-400.

21. Caruso A., Licenziati S., Corulli M., Canaris A.D., De Francesco M.A., Fiorentini S., Peroni L., Fallacara F., Dima F., Balsari A. and Turano A. 1997. Flow cytometric analysis of activation markers on stimulated T-cells and

their correlation with cell proliferation. Cytometry 27:71-76.

22. Whist S.K., Storset A.K. and Larsen H.J.S. 2000. The use of interleukin-2 receptor expression as a marker of cell-mediated immunity in goats experimentally infected with Mycobacterium avium ssp. paratuberculosis. Vet Immunol Immunopathol 73:207-218.

23. Maecker H.T., Maino V.C. and Picker L.J. 2000. Immunofluorescence analysis of T-cell responses in health and disease. J Clin Immunol 20:391-399.

24. Quade M.J. and Roth J.A. 1999. Dual-color flow cytometric analysis of phenotype, activation marker expression, and proliferation of mitogen stimulated bovine lymphocyte subsets. Vet Immunol Immunopathol 67:33-45.

25. Maino V.C. and Picker L.J. 1998. Identification of functional subsets by flow cytometry intracellular detection of cytokine expression. Cytometry 34:207-215.

26. Pala P., Hussell T. and Openshaw P.J. 2000. Flow cytometric measurement of intracellular cytokines. J Immunol Methods 243:107-124.

27. Tough D.F., Sun S., Zhang X. and Sprent J. 2000. Stimulation of memory T-cells by cytokines. Vaccine 18:1642-1648.