

How Can We Measure a Relevant Immune Response?

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Abstract

Challenge of immunity by natural or experimental infection with a given pathogen is the most relevant test on naturally acquired or vaccine-induced immune responses. This approach is often not feasible due to logistic and economic constraints. In natural challenge situations there are potential problems with equal levels of pathogen exposure in the population at risk. In the case of many models of experimentally induced disease the method of administration and dose of pathogen raises questions regarding the relevance of the model to the disease as it occurs in nature. These problems with using challenge infections as the ultimate test of immunity have led to the development of numerous laboratory tests of immune function that are used as an adjunct to challenge and in assessing vaccine efficacy. When employing laboratory assays in an attempt to measure a relevant immune response several questions should be borne in mind: 1) Does this immune response mechanism that is being tested relate to prevention of infection or prevention of disease? 2) Is the population of cells or soluble mediators (antibodies and cytokines) being tested relevant to the anatomical site of disease? 3) Is the immune response mechanism being tested likely to be relevant to protection based on current knowledge about the pathogenesis of a given disease. Immune function testing as it relates to bovine respiratory disease is discussed in the framework of these questions, emphasizing cellular and antibody responses to bovine herpes virus-1 and bovine respiratory syncytial virus.

In summary, there are currently numerous laboratory tests to assess immune function. Given the multifactorial nature of immunity, in most instances a panel of tests is probably required to assess a relevant immune response. When possible laboratory test results should correlate with clinical indicators of protection derived from field trials and experimental models of disease.

Conventional wisdom and common sense both tell us that natural challenge of immunity, which assumedly occurs during the course of clinical trials or in any feedlot, is perhaps the only meaningful method to measure a relevant immune response. However, there are numerous factors to consider before accepting the results of clinical trials or the height of a "dead pile" as evidence that a relevant naturally acquired or vaccine-induced immune response has been reliably assessed.

Ribble (1990) suggests that those wishing to evaluate data from clinical trials assessing vaccine efficacy ask the following series of questions in order to determine the validity of the data and conclusions:

- How were the trial animals challenged?
- Were there concurrent groups of controls?
- Was the measure of outcome meaningful?
- Were the biology and epidemiology of the disease considered?
- Were there any biases in the study?
- How likely was the result a chance finding?

These questions indicate potential problems in assuming that clinical trials or "challenge" situations will necessarily lead to the measurement of a relevant immune response. Controllable and uncontrollable factors alluded to in these questions often confound clinical research, rendering the interpretation of results difficult. In addition, logistical and economic constraints often limit the undertaking of large scale clinical trials. These considerations have fostered the development and application of numerous laboratory tests to evaluate immune responses. Laboratory tests to evaluate the immune response are best used in conjunction with clinical trials or relevant models of disease; however, they are often used in place of clinical research, thereby raising questions about their relevance.

One consideration in evaluating the relevance of laboratory tests is, does the test measure an immune effector mechanism that is likely to be related to the prevention of infection (sterile immunity) versus reduction in the severity of disease once infection occurs. Most economically important diseases in veterinary medicine occur at mucosal surfaces in the respiratory and gastrointestinal tract. Therefore, according to the current dogma, measurement of IgA responses in body fluids collected from a mucosal site should provide the best indication of a response that is relevant to protection

from *infection*. In the case of virus-associated bovine respiratory disease (BRD) this type of a response would be expected to correlate with decreased viral shedding. Indeed, virus-specific mucosal IgA responses have been correlated with decreased shedding of viruses, such as bovine respiratory syncytial virus (BRSV), (Kimman *et al.*, 1989). Increasingly, investigators are reporting the isotype of antibody responses following immunization or experimental infection (Kimman *et al.*, 1989). This is accomplished with the use of isotype-specific ELISAs that employ monospecific antibodies that are raised against purified IgM, IgG, and IgA isotypes of the species in question. One potential problem with isotype specific ELISAs for IgA is that many are probably not specific for the J chain or secretory component of this immunoglobulin and therefore will not discriminate between locally produced IgA and IgA that exudes from the serum during an inflammatory process, as usually occurs during a viral infection. Anecdotal "vaccine breaks" are reported from the field following the on or off label intranasal administration of vaccines for respiratory viruses such as bovine herpes virus-1 (BHV-1). Does this indicate that measuring a mucosal response may not be the best indicator of an immune response that will reduce the severity disease resulting from an infection that breaches the first line of defense (IgA) at the level of the mucosa of the upper airways? Do we need to assess systemic IgG and T lymphocyte responses in addition to IgA to measure a response that is more relevant to protection from disease? What are the implications for vaccine design and delivery? Should we consider simultaneous mucosal and parenteral administration of vaccines and subsequent measurement of both local and systemic responses as the most relevant measurement of a protective response?

A second, related parameter to consider in measuring potentially important immune responses is the phenotype of the response at a site that is relevant to the biology of an induced immune response (vaccination), or to a disease process. The availability of leukocyte subset-specific monoclonal antibodies and recombinant cytokines has greatly improved our ability to dissect immune responses in ruminants to a level that was only possible in human beings and laboratory rodents until recently. Traditionally, immune function tests have examined responses in peripheral blood as an indicator of what may be happening in tissue sites of infection or in local lymphoid organs. Although the use of leukocyte subset-specific monoclonal antibodies and flow cytometric analysis to characterize changes in peripheral blood has aided our understanding of the systemic effects of various infections in ruminants, such as trypanosomiasis, bluetongue, and BVD (Ellis *et al.*, 1988), it is becoming increasingly evident that examination of the phenotype of immune responses *in situ* is necessary to measure a

more relevant immune response. The labor intensive technique of cannulation of afferent and efferent lymphatic vessels used in conjunction with flow cytometric analysis of leukocyte subsets and measurement of antibodies and cytokines has greatly improved our understanding of immune responses following experimental virus infections such as, BHV-1 (Godson *et al.*, 1992) and bluetongue virus (Barrat Boyes *et al.*, submitted), and is currently being employed in several laboratories to assess the development of immune responses following vaccination. In addition, the use of immunohistochemical identification of leukocyte populations and local cytokine responses can be used to characterize on ongoing immune response at lesion sites.

Finally, once we can measure a systemic and/or site-specific immune response, we should consider whether or not the response is appropriate for the pathogen in question. Relatedly, in the case of vaccines, we should consider what is the best way to deliver antigen to tailor the immune response to specific types of pathogens. Recent studies addressing differences in antigen processing at the molecular level has yielded new dogma regarding the induction of CD4 ("helper") versus CD8 ("cytotoxic") T lymphocyte responses (Monaco, 1992). One often-touted generality that has been derived from this work is that inactivated vaccines will not induce CD8 or cytotoxic lymphocyte responses, which are thought to be critical in the recovery from viral infections. Similarly, studies of the immune responses to respiratory syncytial viruses in human beings and cattle indicate that inactivation, in addition to altering antigen presentation, can alter functionally important epitopes in the viral envelop proteins, which results in the production of antibodies that fail to neutralize and may be pathogenic (Ellis *et al.*, 1992). These investigations also indicate that it is critical how one measures an antibody response. Although ELISAs lend themselves well to automation and to testing large numbers of samples, and may be a good indicator of exposure to vaccine or field virus, they may be a poor measure of a functionally important or protective (appropriate) antibody response. Studies such as these indicate that the form in which antigen is presented to the immune system dictates the phenotype of T cell and antibody responses and, moreover, whether a particular response may be appropriate or inappropriate (potentially disease-enhancing). There are, however, contrary data with regard to the induction of T cell responses, suggesting that the choice of adjuvants or antigen delivery systems, such as ISCOMS, may be more important than the nature of the antigen in determining the type of T cell response that is stimulated by vaccination. Another important consideration that has come to light primarily on the basis of studies of mycobacterial and leishmanial

infections in mice and human beings is that the dose of antigen is probably critical in determining whether a response will be predominately antibody or cell-mediated, and consequentially appropriate or inappropriate for a particular pathogen (Bretschger, 1992). Critical to understanding the mechanisms by which this occurs has been the characterization of two subsets of "helper" T cells, Th1 and Th2, which have been defined in mice on the basis of cytokine profiles. Whether or not similar phenomena are operant in immune responses in cattle and other domestic animals awaits clarification. Classically lymphocyte proliferation or blastogenesis following exposure to antigen *in vitro* has been used as an index of a T cell response subsequent to vaccination or infection. Beyond blastogenesis assays, the measurement of cytotoxicity mediated by cytotoxic lymphocytes (CTL) has been taken as the gold standard of a functional or relevant immune response for viral infections such as herpes in human beings and cattle. Some investigators, however, suggest that the classic CTL is a laboratory artifact, and it is locally produced cytokines, such as interferon, at the sites of viral replication, and not cytotoxic T cells, *per se*, that are the critical effectors in the resolution of viral infections (Ramsey *et al.*, 1993). Some studies of BHV-1 immunity in cattle tend to support this hypothesis (Campos *et al.*, 1989). The recent availability of reagents to specifically quantitate physiological concentrations of bovine cytokines should aid in determining which are the most relevant and appropriate cellular responses to measure in viral infections in cattle.

In conclusion, the veritable explosion of information in basic and applied immunology over the past few years, rather than simplifying matters, further indicates that immune responses to pathogens comprise a complex symphony of events. Identification and measurement of relevant immune responses should harmonize laboratory tests and clinical studies in order to better illuminate the overall biology of the disease and the role of the immune response in the process.

Summary

Challenge of immunity by natural or experimental infection with a given pathogen is the most relevant test of naturally acquired or vaccine-induced immune responses. This approach is often not feasible due to logistic and economic constraints. In natural challenge situations there are potential problems with equal levels of pathogen exposure in the population at risk. In the case of many models of experimentally induced disease the method of administration and dose of pathogen raises questions regarding the relevance of the model to the disease as it occurs in nature. These problems with using challenge infections as the ultimate test of immu-

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In summary, there are currently numerous laboratory tests to assess immune function. Given the multifactorial nature of immunity, in most instances a panel of tests is probably required to assess a relevant immune response. When possible laboratory test results should be correlated with clinical indicators of protection derived from field trials and experimental models of disease.

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