Immunity and diagnosis of PRRS in homologous and heterologous infection

Michael P. Murtaugh, PhD

Take-home message: The key to immunological control of PRRSV requires a better understanding of the mechanisms of immunological protection in a heterologous challenge model that measures outcomes that are most relevant to current and future management practices. A more thorough understanding of the anti-PRRSV immune response in both respiratory and reproductive disease conditions will inevitably lead to more sensitive, informative, and discriminating diagnostics.

The status of PRRS in the field today, from the perspective of a scientist working in the laboratory, is complex. The virus itself comprises a multitude of genetically variable forms including both North American and European genotypes. Within each genotype differences of up to about 20% in nucleotide sequence are observed. Field viruses that were identified years ago are not observed at present, indicating that the genetic variation is not only expanding, but also is shifting. PRRSV may be changing less rapidly antigenically and immunologically, insofar as commercially available ELISA diagnostic kits appear still to react with the universe of PRRSV field isolates, and vaccines that were derived from ancient field isolates still provide substantial experimental protection against challenge with current virulent viruses. Nevertheless, the problem of PRRS has not abated in the 10 years since the first licensed vaccine was marketed for immunological protection against disease, and more virulent and atypical forms of the disease continue to appear in the field.

Against this background a mountain of data has been amassed regarding the immunological responses of swine against PRRSV. The December, 2004, issue of Veterinary Immunology and Immunopathology, for example, is entirely devoted to PRRS immunology and immunopathology. While this information has not yet led to a solution to the problem of PRRS, it brings key questions and issues into better focus. As the parts of the problem become better identified the path to a solution hopefully will be revealed. Here I will lay out my personal opinions and observations on the nature of immunity to PRRSV as they relate to protection and diagnosis.

PRRS immunity

In the early days of PRRS, experiments to elucidate the nature of immune protection assumed that PRRSV caused a simple, acute infection that was resolved when viremia ended. After clearance of infection, circulating antibodies and memory T and B lymphocytes prevented reinfection. The intensity of the immune response correlated with the degree of protection. Immune response was measured by the assays available at the time. Antibody reactions with PRRSV-infected cell monolayers and neutralization of viral infectivity were followed by ELISAs with infected cell lysates or purified recombinant proteins coating the wells. All of these tests indicated an ongoing or previous exposure to the virus. The presence of neutralizing antibodies was evidence of protection based on the critical role of neutralizing antibodies in protection against many other viral diseases. Evidence of T cell or cell-mediated immunity to PRRS was slow in coming until the application of an interferon γ ELISPOT. In this assay, PRRSV-specific T cells are identified and enumerated by culturing lymphocytes, commonly in the form of peripheral blood mononuclear cells, in presence or absence of virus. Cells stimulated by PRRSV antigens secrete interferon γ, which binds to an antibody previously attached to the well, and is identified by reaction with a visible dye. The number of colored spots corresponds to the number of PRRSV-specific cells.

We now know that the early assumptions about PRRSV infection were largely wrong. PRRSV causes prolonged infections that persist for months after the virus disappears from the blood. Persistent infection implies viral subversion of immunity, and is a poorly understood process at this time. The interferon γ ELISPOT appears to have little, if any, predictive value of PRRS immune status, thus casting in doubt again the role of T cells in anti-PRRS immunity. Antibodies continue to be a valuable indicator of exposure, but the role of neutralizing antibodies in protection against PRRS is controversial. Because the mechanisms of immune resistance to PRRS have not been
determined, we lack a theoretical basis for using immune parameters as surrogate measures of protection. This is a problem since immunological correlates of protection are an important experimental and diagnostic tool for testing, monitoring and predicting immunity against PRRS. A substantial body of knowledge concerning anti-PRRS immunity has been amassed from the interpretation of immune response experiments. If the underlying assumptions that antibody and T cell responses being responsible for immunity are incorrect, then we must be cautious in the design and assessment of protection experiments, careful in interpretation of immunodiagnostics, and quick to develop more informative tools for immune assessment.

Homologous immunity

The substantial genetic variability in PRRSV, within the North American and European genotypes, inevitably results in antigenic differences among the viral proteins. Since any single vaccine or wild-type virus, or any single diagnostic test, is based on one or a very few genetically distinct viruses, it is possible that immunity to one virus may not protect against another virus, or that diagnostic immunoreactivity to one set of viral antigens may not be universal. However, two lines of evidence indicate that immune responses are broadly cross-reactive. Commercially available diagnostic kits appear to detect antibody responses to virtually all North American isolates and to many, if not all, European genotype isolates. Highly conserved antigens must therefore be present. Second, molecular genetic analysis of PRRSV isolates reveal that conserved amino acid sequences exist within all PRRSV proteins. The widespread existence of conserved amino acid sequences provides the rational expectation that an immune response to one PRRSV will provide immune resistance to other PRRSV isolates.

It is hardly necessary to point out that there are many opinions about the extent and characteristics of protective immunity to PRRSV. Nevertheless, the author believes that there is general agreement that a state of fully protective immunity is conferred on pigs to a second exposure of the same virus, or by an attenuated live vaccine to its parental virulent form. This homologous immunity is consistently, reproducibly total. Virus routinely cannot be reisolated from blood and clinical signs of disease are absent. However, surprisingly, there is a consistent absence of the anamnestic antibody response. Normally, conventional antigens produce a large and rapid rise in antibody titers in a previously immunized animal. This “booster” effect is an immunological correlate of an immune response to challenge. The absence of any detectable change in immune parameters occurs in the presence of complete protection against infection and against disease. An important and unfortunate conclusion is that immunological correlates of protection, especially increased antibody titers, cannot be used to predict protection. Thus, studies of immune protection and vaccine efficacy that utilize immune outcomes are subject to misinterpretation. Given the inability to identify immune measures of protection, the most reliable method for evaluating efficacy of protection is by clinical and pathogenic signs in challenge experiments.

Heterologous immunity

Heterologous protection against PRRSV ranges from fact to myth and everything in between, depending on the source of information. The reasons for such disparate views vary due to differences in outcome measures, expectations, experimental designs, and economic considerations. Immune protection, whether heterologous or otherwise, is determined experimentally by the response of individual pigs to virulent virus challenge at various times after the first exposure. Challenge of immune pigs with virulent viruses that are unrelated to the immunizing strain, i.e. the heterologous strain, consistently results in substantial protection from infection and from disease. Quantitative determination of viral loads in blood show a reduction of at least 100-fold and substantially reduced durations of viremia. Measures of disease consisting primarily of lung involvement scores, are significantly reduced. Moreover, the results are independent of nucleotide sequence similarity between immunizing and challenge viruses.

The problem here is that the preceding positive description of heterologous protective immunity is not consistent with the widespread perception that “the vaccine does not work.” PRRS vaccines by definition provide heterologous protection because the isolates from which they were derived are presumed to no longer exist in the environment. Thus all challenge exposures in the field are heterologous. Even in the case of purposeful infection in the form of serum therapy, the afforded protection will be heterologous if more than one virus is present or is acquired in the herd.
Expectations are another part of the perception problem. Reductions in infection and disease that demonstrably improve herd health may be judged as failures if protection is not absolute or if the degree of protection still results in economic injury. The solution to this problem requires better communication, but ultimately does not address the scientific and medical needs to improve heterologous immunity. A more central issue, at least for the author, is the relevance of experimental findings in individual pigs to the dynamics of current swine management practices. It is entirely feasible that profoundly effective immunity to PRRSV in individual pigs may not translate into highly reliable protection in the field if a rare infected individual is housed among a population of unprotected animals.

Genetic variation in host resistance will result in variable levels of protection in a uniformly vaccinated herd. The large size of commercial herds compared to the relatively small numbers of animals in experiments increases the likelihood of vaccinated pigs still at risk of PRRS in a herd, but not in a controlled experiment. Challenge of these animals could result in PRRS disease, but should represent only a minor proportion of the herd and would not be expected to cause widespread disease without additional spread of infection.

Possibly more important is the distinction between infection and disease. All sows are susceptible to PRRSV infection, but the reproductive disease outcome is dramatically different in late gestation versus early gestation or non-pregnancy. Similarly, late gestation sows appear to be a more sensitive indicator than young pigs of disease severity. Thus, various levels of protective immunity that are observed in the young pig respiratory disease model may translate in the field to lower levels of protective immunity in sows challenged late in pregnancy. As in the case of genetic variation, there is very little data to determine how much of the perception problem is due to extrapolation of respiratory disease experimental results to field applications in sow herds in which the underlying assumptions about level of protection may be incorrect.

Ignorance of herd management practices by researchers may contribute to discrepancies between experimental results and field experiences. For example, robust protection of sows that is nearly but not completely effective eventually will result in PRRS breaks in an unprotected nursery or grow-finish barn. A single infected sow that produces a litter of piglets that are viremic at birth or become infected before weaning can transmit PRRSV if they are transferred to a large population of susceptible animals after weaning. The challenge of preventing PRRSV in this situation can be formidable, and in practical terms may become an issue of disease control rather than viral elimination.

The current status of heterologous immunity and protection is obscured for the reasons cited above. Perhaps experiments that measure risk of transmission, that consider reproductive as well as respiratory disease models, and that are more relevant to current management practices will lead to improvements in the overall efficiency of heterologous protection. In addition, the importance of integrating vaccination procedures or other immunological control methods with all other management procedures as part of a comprehensive health management plan cannot be overstated.

**Immunodiagnosis of PRRS**

Routine monitoring for serum antibodies to PRRSV is a powerful diagnostic tool for determination of the exposure status of an animal. The ELISA is well-suited for monitoring since it is simple to perform, relatively inexpensive, easy to implement, and, thanks to commercialization, reliable and reproducible across laboratories. No other immunodiagnostic method offers all these advantages. The ELISA in all of its current forms is best at detecting exposure events and confirming vaccination, but it is not a reliable indicator of protection. No immune assay will effectively predict protection until the immunological mechanisms of protection are firmly established.

While ELISA tests are an excellent monitoring tool, several unmet needs in PRRS immunodiagnostics persist. Perhaps most critical is the inability to distinguish vaccinated from infected animals. Detection of PRRSV outbreaks earlier would facilitate more rapid intervention and improved control. More complete knowledge of the immune response to the full range of PRRSV proteins might help to resolve the immune status of seropositive animals that become seronegative over time or following repeated vaccination.

Differential diagnostics that discriminate between vaccinated and infected swine is feasible based on the presence of conserved and variable antigenic regions in PRRSV. Serological tests directed to unique antigenic sequences in
vaccine strains may have discriminating potential, whereas conserved antigenic regions would identify all animals that were exposed to PRRSV, as currently available tests now do. Knowing the kinetics of response to individual PRRSV proteins will facilitate the identification of antigenic sequences that elicit the earliest response and thus provide the first immunological warning of infection. Similarly, the patterns of decay or persistence of antibody levels to individual viral proteins will help to illuminate the duration of antibody responses and differences in response to individual proteins.

In summary, the key to immunological control of PRRSV requires a better understanding of the mechanisms of immunological protection in a heterologous challenge model that measures outcomes that are most relevant to current and future management practices. A more thorough understanding of the anti-PRRSV immune response in both respiratory and reproductive disease conditions will inevitably lead to more sensitive, informative, and discriminating diagnostics.

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