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Effector mechanisms of humoral immunity to porcine reproductive and respiratory syndrome virus

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Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) continues to afflict swine nearly 30 years after it was first discovered as the causative agent of “mystery swine disease”. Immunological tools of vaccination and exposure to virulent viruses have not succeeded in achieving control and prevention of PRRSV. Humoral immunity, mediated by antibodies, is a hallmark of anti-viral immunity, but little is known about the effector mechanisms of humoral immunity against PRRSV. It is essential to understand the immunological significance of antibody functions, including recently described broadly neutralizing antibodies and potential non-neutralizing activities, in the immune response to PRRSV. Here, we review recent research from PRRSV and other host-pathogen interactions to inform novel routes of exploration into PRRSV humoral immunity which may be important for identifying the immunological correlates of protection against PRRSV infection.

Keywords

PRRSV; B cell; humoral immunity; neutralizing antibody; antibody function

II. Introduction

Porcine reproductive and respiratory syndrome (PRRS) has plagued swine health and wellbeing for nearly 30 years. The etiologic agent of PRRS disease is an enveloped, positive-stranded RNA virus which is aptly named porcine reproductive and respiratory syndrome virus (PRRSV) for the late-term abortions, weak-borne piglets and growing pig pneumonia which it manifests. Within the PRRSV genome, genetic diversity was first apparent due to the simultaneous emergence of two vastly different genotypic populations, type 1 (European) and type 2 (North American), in the late 1980s. Both highly mutable genotypes subsequently radiated extensively and spread globally. Type 2 PRRSV accounts for the great majority of severe disease outbreaks, especially in North America and Asia.

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The ability of PRRSV to rapidly mutate its genome while retaining, or even enhancing, virulence makes it a formidable opponent for the naïve or even previously exposed porcine immune system. As a result, recent research has focused on understanding the immune response to PRRSV as well as identifying the factors necessary for the induction of protective immunity to genetically diverse viral strains. Research has concentrated on the humoral immune response since antibodies frequently play a critical role in control and prevention of viral infections [1, 2]. While cell-mediated immunity likely plays an important protective role as well, swine immunologists currently lack the necessary reagents for detailed investigations of the role of T cells in PRRSV immunity.

Continued viral genetic diversification, coupled with the current gap in knowledge of what constitutes a protective immune response to infection, has resulted in the present situation where PRRSV continues to evade immune countermeasures. Here, we review what is known about antibody effector mechanisms that may be relevant to control of PRRSV. Many recent reviews address other general aspects of cellular and humoral immunity to PRRSV for interested readers [3–5].

III. PRRSV neutralizing antibodies

The existence of neutralizing antibodies to PRRSV in the serum of previously infected pigs was discovered in the early 1990s [6]. Passive transfer studies verified that PRRSV specific antibodies were capable of protecting animals from homologous PRRSV challenge, and that the anti-PRRSV neutralizing immunoglobulin fraction was responsible for prevention of disease [7, 8]. These studies supported the concept that vaccination could prevent clinical PRRS disease. However, the impressive mutability and recombination capacity and, therefore, genetic variability, of the virus resulted in incomplete clinical benefit of live or inactivated PRRS vaccines [9, 10]. Recent efforts to develop alternative vaccination methods, such as virus-like particles, replicon vectors, and a nonpathogenic porcine circovirus type 1 virus vector to induce a neutralizing response against purported neutralizing epitopes have yet to demonstrate immunological protective efficacy [11–13].

Recent work focusing on the identification of animals with neutralizing antibodies against genetically diverse PRRSV strains has shown that mature pigs are capable of producing broadly neutralizing antibodies against disparate strains including both type 1 and type 2 PRRSV [14, 15]. In our experience, these broadly neutralizing capabilities are only found in a proportion of adult animals with similar PRRSV exposure histories (unpublished data). Interestingly, research findings in humans infected with HIV provide a conceptual model for individual variation in PRRSV neutralizing antibody patterns. In humans, broadly neutralizing anti-HIV antibodies also appear after extended periods of infection in a fraction of infected individuals [16–18]. This knowledge of HIV immunity has spawned considerable research which has since become a blueprint for the identification of broadly neutralizing antibodies against genetically diverse viruses.

Characterization of HIV neutralizing antibodies was accomplished by screening large cohorts of patients for breadth of neutralizing activity [19, 20]. Individual memory B cells were isolated from candidate individuals, including rare controllers, and differentiated into

antibody secreting cells *in vitro* [21]. Culture supernatants were then screened for neutralizing activity against panels of HIV isolates representing the breadth of genetic diversity [22, 23]. Heavy and light chain antibody mRNA were sequenced and cloned from memory B cells that displayed neutralizing activity [24, 25]. Study of these sequences revealed that broadly neutralizing antibodies have higher levels of somatic hypermutation compared to levels typically observed in antibodies specific to many other pathogens. Additionally, broadly neutralizing monoclonal antibodies against HIV have displayed the capacity to produce sterilizing immunity in macaques challenged with chimeric simian-human immunodeficiency virus (SHIV) [26–28].

The translation of this approach to the study of PRRSV specific memory B cells in swine is achievable. While there is no specific cellular marker to identify porcine memory B cells, the use of B cell tetramers, or fluorescently labeled protein, makes the functional identification and sorting of porcine memory B cells feasible. The differentiation of porcine memory B cells into antibody secreting cells and screening of culture supernatants for neutralizing activity can be accomplished with previously characterized methods [29]. Ideally, this work would identify specific heavy and light chain sequences which could be targeted through vaccination to elicit a broadly neutralizing protective antibody response.

IV. Non-neutralizing antibody functions against PRRSV

Several PRRSV structural and non-structural proteins are highly antigenic (e.g., N, nsp1, nsp2, nsp7, GP5) and result in a long lasting antibody response post-exposure [30–34]. However, these same proteins are not targets of neutralizing responses [29, 35]. Therefore, antibodies with affinity for these viral antigens have previously only been considered useful for determining previous exposure and seroconversion. This logic, of course, assumes that neutralization is the only effector function of an antibody. Recent studies in HIV immunity used adoptive transfer experiments to show that anti-HIV antibodies can clear infected cells *in vivo* by an FcγR-dependent mechanism [36]. Indeed, there are a variety of effector functions which both non-neutralizing and neutralizing antibodies may employ against pathogens.

Antibody dependent cell-mediated cytotoxicity (ADCC) relies on the binding of IgG to viral antigens presented on the surface of infected cells and to FcγR expressed on a variety of effector cells, most importantly NK cells. Recent research with both HIV and HSV has shown that ADCC can be directed against non-neutralizing viral antigen targets [37]. Furthermore, this research has suggested that ADCC may be important for prevention of HSV reactivation as well as clearance of HIV infected T lymphocytes [38–40]. Currently, there is no published research examining the role of ADCC in the clearance of PRRSV from infected macrophages. However, the migration of immune cells into PRRSV-infected endometrium suggested an increasing recruitment of NK cells into infected tissue, indicating that further investigation into their immunological function and significance in PRRSV immunity is warranted [41].

Antibody-dependent cellular phagocytosis (ADCP) involves the opsonization and phagocytosis of virally-infected cells by macrophages. This antibody effector function also

has been implicated in the clearance of HIV infected cells [42, 43]. ADCP has not been described in PRRSV immunity, possibly due to the fact that macrophages are the permissive cell for PRRSV infection.

Antibody-dependent complement-mediated cytotoxicity (CDC) is a potential method for destruction of PRRSV infected macrophages. CDC involves the binding of antibodies to infected cells and the subsequent fixing of complement via the classical pathway. CDC was shown to have no effect on cytotoxicity in a type 1 PRRS virus infection model [44]. However, this antibody effector function has not been examined in a type 2 PRRSV infection model or for extended periods of infection >12 h.

Antibody dependent complement mediated virolysis is yet another functional pathway that may be important for PRRSV immunity. There has been considerable research describing complement-mediated virolysis and the anti-virolytic evasion methods employed by many enveloped viruses, such as HCV, vaccinia virus, HCMV, and HIV [45–49]. Studies characterizing the role of antibody-dependent complement-mediated virolysis in PRRSV is lacking, perhaps due to the perception that, even if it is a mechanism for virion destruction, it is less important for immune protection than other antibody effector functions. Nevertheless, it is possible that virolysis slows the spread of infection in the host and accelerates viral clearance.

Not all effector mechanisms of antibodies are beneficial to the host. Antibody dependent enhancement (ADE) is a phenomenon by which virus is bound by non-neutralizing or sub-neutralizing concentrations of antibodies facilitating the entrance, via antibody binding to Fcγ receptor, and subsequent infection of myeloid cells such as macrophages, dendritic cells, and granulocytes. This blind spot of the immune system is known to be exploited by dengue virus. In a small proportion of dengue cases, severe clinical disease occurs in vaccination or re-infection of immune individuals [50–53]. it has been suggested that ADE plays a role in infection with other viruses, including Chikungunya, influenza, and Ebola [54–56]. However, research in support of these claims is restricted to *in vitro* studies and animal models. It has also been proposed that ADE plays a role in PRRSV infection and pathogenicity [8, 57–59]. However, the data are based on *in vitro* studies alone. The absence of confirmed cases of ADE in immune swine herds that are re-vaccinated or re-challenged with virulent field viruses, both of which are common occurrences in the swine industry, argues strongly that ADE is not a feature of PRRSV interaction with pigs. Therefore, the focus of future work investigating the association of ADE and PRRSV should be on reproducing this immunological phenomenon under conditions that result in more severe clinical disease in the pig. Further analysis of ADE and PRRSV can be found in the following reviews [4, 5].

V. Conclusion

PRRSV has devastated the U.S. swine industry for well over 25 years, and it has shown no sign of slowing down. Given that mechanisms of protection remain obscure more than 25 years after PRRSV was identified as the causative agent of “mystery swine disease,” all avenues of antibody functionality need to be examined to fully understand the role of

humoral immunity in the response to PRRSV. Vaccination and prior exposure provide uneven protection to virulent virus challenge, but the underlying immunological mechanisms needed for effective protection are not known. The effector mechanisms of humoral immunity to PRRSV infection include broadly neutralizing antibodies that are present in a portion of exposed animals. However, the conditions of virus exposure history and immunological variables necessary to elicit a broadly neutralizing antibody response are not yet known. The characterization of memory B cells from animals with broadly neutralizing activity against PRRSV, and cloning of their antibody molecules, will help to establish a benchmark for future vaccine development. However, the history of PRRS immunology teaches us to thoroughly investigate other antibody effector functions that may contribute to and more fully explain solid protective immunity. Finding answers to prevailing questions about neutralizing and non-neutralizing PRRSV specific antibodies will help to fill the gap in knowledge of what constitutes a protective immune response to PRRSV.

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V. References

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