Title: The contribution of adaptive immunity to Porcine Reproductive and Respiratory Syndrome virus infection - NPB # 13-187

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Scientific Abstract:
Porcine Reproductive and Respiratory System is an economically devastating disease, costing the pork industry millions of dollars in losses every year in the United States. Despite intensive research over the last twenty years, effective containment of the causative virus (PRRSV), and vaccination strategies against PRRSV have not yet been successfully developed. What has been established is that the virus has numerous viral evasion strategies, including the ability to subvert innate immunity, escape effective antibody responses, and may have the ability to recruit and manipulate immunosuppressive immune cells to dampen anti-viral responses. Additionally, vaccine development has been hampered by the extremely high mutation rate of the virus, resulting in ineffective long-term antibody-mediated protection.

The network of interactions between the PRRRSV and host’s immune responses is complicated, and effective models to study and dissect innate immunity and/or adaptive anti-viral responses have been lacking in pigs. Many other viruses have been successfully studied in mouse models, where components of the immune system can be selectively manipulated, either through genetic manipulation (such as transgenic, and knockout strategies), adoptive transfer of immune cell subsets, or with antibody-mediated cell depletion studies.

Recently, we have described a line of pigs with a spontaneous, naturally-occurring genetic defect that give rise to animals with severe combined immunodeficiency (SCID). These animals have almost no circulating T and B cells in peripheral blood, and lack normal T and B cell zones in lymph nodes, spleen, thymus, and gut. However, natural killer cells, granulocytes, and monocyte/macrophage population are present.

We have proposed to use this novel, SCID porcine biological model to understand the interaction of PRRSV and innate and adaptive immunity. Previous work has established that during acute infection, viremia levels in SCID pigs is lower that those of normal littermates. This suggested that cells of the adaptive immune systems were either modulating macrophages to a more PRRSV-permissive phenotype, or were dampening anti-viral responses during acute infection. Therefore, we initiated a comprehensive analysis of PRRSV infection in SCID (vs control) animals by infecting animals with a GFP-expressing virus. We proposed that we would be able to quantify the number of infected porcine alveolar macrophages (PAMs) in bronchoalveolar lavages (BALF) in these animals, and determine if higher numbers of macrophages were present in samples derived from normal animals. We also endeavored to assess whether there were significant differences in the anti-viral responses between the control and SCID piglets by assessing the phenotype of macrophages, and the production of cytokines.

This study effectively reproduced the lower viremia loads observed in a previous study; however, we were unable to quantify the number of infected macrophages, as the GFP signal was not detected. Of interest, infection levels among SCID animals varied, and animals with higher viremia loads had higher levels of PRRSV-induced immunopathology and higher anti-viral cytokine responses. Nearly half of the SCID, those with lower viremia, did not have any evidence of lung pathology, and had lower levels of anti-viral cytokines. As T cell-derived cytokines were nearly undetectable in normal animals, this suggested that PRRSV-related lung pathology might be caused by cytokines...
duced by cells of the innate immune system. However, these observations do not rule out the possibility that T cells are still playing a role in modulating innate immunity. Additionally, PAMs derived from uninfected SCID piglets demonstrated higher levels of activation, as measured by expression level of SLA-II. This more “activated” phenotype may render PAMs more resistant to PRRSV infection. Whether this phenotype is dependent on the presence of T cells will be an area of future investigation.

In order to directly investigate the role of T cells in PAM permissiveness and acute viral infection, a pig model where T cells can be selectively reconstituted is needed. Therefore, we sought to develop a strategy where we could adoptively transfer T cells from genetically-matched donors into recipient SCID pigs in order to reconstitute the T cell population. Initially, we sought to transfer mature T cells, using fluorescent activated cell sorting and purification of CD3+ T cells. However, this strategy was unsuccessful. For our subsequent experiment, we adoptively transferred unlabelled thymocytes from SLA-matched normal donors into SCID recipients. After 28 days post-transfer, we began to measure a small level of T cell engraftment. By 49 days, at the end of the experiment, significant levels of T cells could be detected in peripheral blood, lymph nodes, spleen, and gut tissues. Furthermore, all T cell subsets, including regulatory T cells, were represented in the T cell population. Also of note, recipients did not show evidence of contaminating B cells, validating that the adoptive transfer of thymocytes was a viable methodology to specifically establish T cell reconstitution. While other groups have successfully generated B and T cell reconstitution using bone marrow from normal animals, this was the first study to ever successfully selectively engraft the T cell population. This model can now be implemented into future studies to evaluate the contribution of T cells to PRRSV infection, their role in dampening anti-viral responses, modulating macrophage permissiveness, and ultimately, determine whether this population may be a novel target for vaccine development.