RESEARCH REPORT
SWINE HEALTH

Title: The Comparative T-cell Repertoire Response in PRRSV, SIV and PCV2 Infected Piglets
NPB #05-143

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Industry Summary

Viral infection exposes the host immune system to epitopes that are normally recognized by T cells and which leads to both cytotoxic responses against virus-infected cells and to B cells that make viral neutralizing (VN) antibodies. The anti-viral response to PRRSV is reported to be delayed, the response to porcine circovirus (PCV-2) is poorly understood but the response to swine influenza (SIV) is robust and classical and resolves the infection in 2-3 weeks. Therefore we wondered if the T cell receptor Vβ gene usage (important in specificity and an indicator of T cell superantigen activity) was unusual in PRRSV-infected piglets versus those infected with SIV.

In addressing our first objective we compared four major TCRVβ families; Vβ 4,-5,-7-, 12 in PRRSV, PVC-2 and SIV infected isolator piglets. Isolator piglets were used in an effort to remove other sources of immune pressure, e.g. other pathogens, gut flora, etc. We proposed and initially addressed TCRVβ usage by cloning and hybridization using Vβ gene family-specific probes but later developed a Cyber Green-based real-time PCR assay for quantitation of TCRVβ. The data remain incomplete but suggest: (a) there is little evidence for preferential TCRVβ usage in PRRSV, PCV-2 and SIV infections and (b) there is no T cell superantigen effect in any piglet we have examined.

In pursuing our second objective, we studied the affect of PRRSV, PCV-2 and SIV on B cell activity by quantifying IgG, IgM and IgA in serum and bronchial alveolar lavage and through adaptation of real-time PCR to measure the proportion of T- and B cells in lymphoid tissue of infected animals and sham controls. The latter

method failed to show that PRRSV preferentially causes B cell proliferation; a conclusion not supported by data on Ig levels and earlier histological studies. This raised our concern about the value and validity of the real-time PCR. Thus we are collaborating with Dr. Harry Dawson to compare two forms of real-time PCR with cloning and hybridization; a full report on these comparative studies will be available at year's end.

In summary the delayed appearance of VN in PRRS is unlikely to be due to some type of unusual T cell repertoire usage. Rather it is more likely to be a B cell immune dysregulation phenomenon (see NPB 05-174). This phenomenon may be the cause of the delayed protective response to PRRSV and the incomplete protection provided by current vaccines.

Scientific Abstract

T lymphocytes play important roles in viral immune responses. This study addressed this topic by comparing the proportional usage of four major TCRVβ families in isolator piglets infected with PRRSV, PCV-2 and SIV. We also compared the ratio of expression of B and T cell receptors in selected tissues of the same animals. Data to date indicate that there is no preferential usage of TCRVβ4,-5,-7 or -12 in any of the porcine viral disease studied but there are changes in some of these compared to sham controls. Surprisingly, real-time PCR indicated that these families comprised <20% of TCRVβ usage in the piglets examined which is inconsistent with preliminary results. This and peculiar data on T:B cell ratios obtained by the same method raises concerns about the real time PCR method; studies on this subject are ongoing. Assuming the real-time data to be reliable, none of the viral infections skew the Vβ family usage in the manner that would be expected for a T cell superantigen effect. Using real-time PCR we failed to demonstrate that B cells are preferentially expanded in PRRSV infections. This observation conflicts with previous data and data on Ig levels that indicate this infection is a B cell proliferation disorder. In this study B cells proliferation in PRRS was supported by 10-20 fold elevation of IgG, IgM and IgA in serum and bronchial alveolar lavage (BAL) when compared to infection with PCV-2 and SIV.

We believe these limited studies reject the notion of an unusual effect of the three porcine viruses we studied on the T cell repertoire while some data confirm earlier studies that PRRS is a B cell lymphoproliferation disorder in isolator piglets. Future studies should target the role of B cells in this disease and further test the validity of quantitative PCR.

Introduction

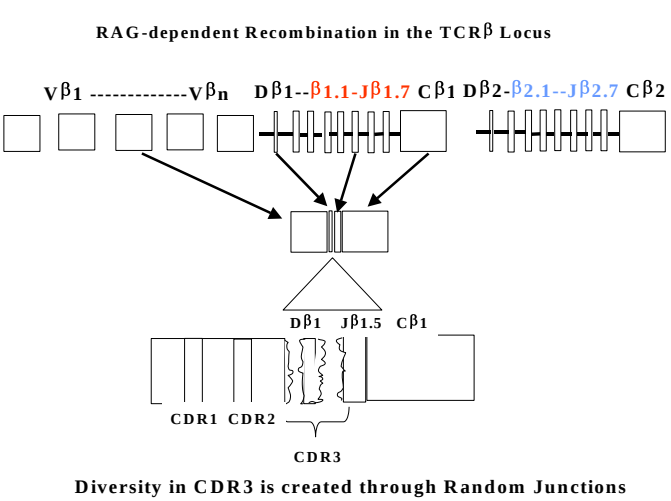
There are many factors that influence the bottom line for pork producers. Some of these are fixed or slightly variable costs, e.g., interest rates and feed. However, infectious disease can be a highly variable cost, e.g., one moment the sow herd is healthy and the next moment 50% of the sows can abort. The potential for dramatic increases in economic loss forces producers to design and develop disease control and prevention strategies. An important component of these strategies is the use of vaccines to prevent infection, or at the very

least, reduce the clinical disease associated with infection. If vaccines were perfect, pork producers would have few worries about infectious disease and this grant would never have been funded. However, since infectious disease is responsible for significant economic loss, vaccines must not be completely effective in preventing and controlling disease. This conclusion is not quite correct because there is still some uncertainty over the effectiveness of the vaccines for PCV2 and those for PRRSV and SIV do not induce cross-protection. In contrast, pseudorabies is an example of a disease in which an efficacious vaccine was developed using traditional methods and when properly used in an extensive national program, the disease was eradicated from the US swine herd. Unfortunately, there is reason to believe that traditional methods may not yield PRRSV and SIV vaccines that can achieve the same favorable outcome.

A major impediment to developing broad cross-protective PRRSV and SIV vaccines is a lack of knowledge about the host/pathogen immune response, or more specifically, how a pathogen might modulate the host immune response. We believe there is need to step back and gain a better understanding of the pathogen/host immune response. In an effort to gain a better understanding of the swine immune response we propose to conduct a study that primarily compares the swine T-cell response to PRRSV, SIV, and PCV2. These three viruses are selected because of their specific virus/host interaction and their economic importance to the US swine industry. PRRSV alone has been estimated to have an annual economic health cost of 600 million dollars. We hypothesize that each of these viruses will induce a unique immune response based on: (1) how quickly SIV is cleared from the pig in comparison to PRRSV and PCV2, (2) the tropism of PRRSV for antigen presenting cells compared to SIV and PCV2, and (3) the unique characteristics of the lymphadenopathy that develops following PRRSV and PCV2 infections when compared to SIV (Lemke et al 2004; Lager and Vincent unpublished data). We had hoped that the comparative knowledge derived from this proposed study will lay the foundation for a better understanding of the swine immune response and provide insight into how PRRSV, PCV-2 and SIV vaccines could be engineered to be as successful as the pseudorabies vaccine.

Acquired immunity to pathogens depends on two major types of lymphocyte, T- and B-cell. While differentiated B-cells secrete virus neutralizing antibodies, certain T-cells are the master controllers ("helpers") of these B cells. Other T-cells are cytotoxic cells that kill virus-infected cells; others control inflammatory cell function, e.g. macrophages, while regulatory T cells (Tregs) maintain immune homeostasis in the host. The acquired immune response is highly specific and T cells control this specificity. The T cell surface receptor that determines specificity is known as the T-cell antigens receptor (TCR). The mechanism that stimulates T cells to perform their preordained role involves the linkage of the TCR with a molecular complex on the surface of antigen presenting cells known as the MHC. The task of the antigen-presenting cell is to capture the pathogen and break it down into pieces and present one of these pieces in conjunction with the MHC. This presentation acts as the linkage that triggers the "go" signal for the T cell. T cell clones receiving this signal are activated and proliferate to provide enough cells with the correct specificity to eliminate or control the pathogen.

Therefore understanding the immune response to a viral pathogen entails knowing what T cells have become expanded, which requires recognizing them by their TCR and also determining whether they are the cytotoxic variety, the helper variety, Treg variety, etc. Understanding how T-cell clones are recognized requires understanding how the TCR is formed. During the generation of T-cells in thymus, somatic RAG-dependent rearrangement occurs which in the case of TCRβ, results in a gene comprised of gene segments from the Vβ, Dβ and Jβ regions of the genome (Fig. 1). Furthermore, as these segments are spliced, nucleotides are added or deleted so the final product for each T-cell is unique and this is reflected in the length of the CDR3 region. Figure 2A shows that for any one T-cell, there is only <u>one</u> functional CDR3 length (the second major band in Fig. 2A represents the non-productive TCRβ rearrangement that was also perpetuated during T-cell proliferation). The unselected repertoire of naive T cells displays a Gaussian spectratype (Fig. 2B) which becomes oligoclonal as certain clones are selectively expanded because of their specificity for specific viral antigens or T-cell superantigens.



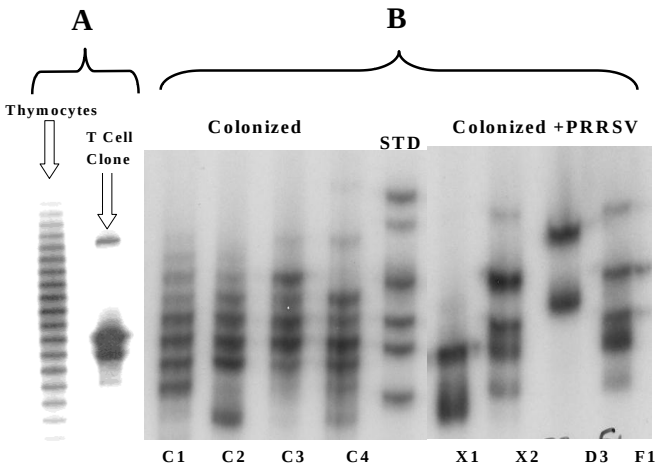


Figure 1. Diagrammatic illustration of the rearrangement events in the porcine TCRβ locus to form mature T-cell clones. The expanded depiction shows the junction of the rearranged TCRβ variable regions gene segments in an effort to illustrate the random and clone-specific formation of CDR3. Clones can be recognized by the length of their CDR3 using spectratyping (Fig. 2).

Figure 2. Use of spectratyping (clonotyping) to measure T cell expansion. Part A shows the polyclonal (Gaussian) spectratype of an unselected T-cell population and that of a single clone. Part B compares the spectratype for a porcine Vβ supergroup in four colonized isolator piglets and their PRRSV-infected littermates. Note the pronounced selection in the PRRS piglets including some animals with only two expanded clones. STD = length standards for CDR3.

Since T-cell numbers are tightly regulated, it means that expansion of certain clones diminishes the number of T-cell clones available to recognize other pathogens. This is especially well demonstrated in the case of so-called T-cell superantigens that recognize portions of framework region 3 of the TCR thus selecting T-cells not on their ability to recognize any particular antigen (which depends on CDR3) but because of sequences they share within Vβ families. This can result in "holes in the repertoire" but can also cause cytokine shock like

in the case of the Tampax incident of >20 years ago (Schlievert et al., 1981). Of course, specific viral antigen(s) can also greatly expand certain clones resulting in skewing of the repertoire like that shown in Fig. 2B. While both events skew the repertoire, at least the latter usually expands viral-specific clones whereas the former does not which can lead to unwanted immune responses that cause immunopathology. Since it has been reported that PRRS is associated with susceptibility to other pathogens in neonatal piglets (Feng et al., 2001; Wills et al., 1997). *either mechanism* could immunocompromise the piglet and could explain PRRS-associated morbidity.

Distinguishing between clonal expansion by viral antigen versus superantigen is straightforward since the latter expands clones based on their Vβ family sequence rather than their antigen specificity (Herman et al., 1991). To make this distinction, the expanded clones must be identified at the molecular level, i.e. by characterization of their Vβ usage. Since we have now characterized the Vβ repertoire in swine (Butler et al., 2005) and have developed methods for quantification of clonal usage (Fig. 3), this question can be answered in the proposed research.

Whether a viral antigen, a self antigen or a superantigen explain the expansion of certain T-cell clones seen in PRRSV-infected piglets, remains to be determined. Since α/β T-cells come in many flavors, i.e. $CD4^+$, $CD8^+$, $CD4^+$ $CD25^+$ etc., and each appears to have a different immunological function (see above), it is important to determine which subset of T-cells displays the selected repertoire. This can indicate whether cytotoxic T-cells are at work or whether they are helper cells, inflammatory cells or Tregs. Thus, separation of T-cells from various sources into subsets can be done prior to repertoire evaluation to provide additional insight into the events occurring during the host's immune response to viral pathogens. Obviously this short statement does not begin to explain the extremely complex nature of the immune response and the role of T- and B-cells. However, we have extensive experience in studying B and T development and their repertoires at the molecular level (Butler et al., 2000a,b; 2004; 2005; Sinkora et al., 2000) including with isolator piglets and conventional piglets experimentally infected with PRRSV (Lemke et al., 2004; McAleer et al., 2005).

Based on studies evaluating the immune response in other species, it is apparent that some pathogens have developed mechanisms that can evade, delay, divert, or lessen an acquired immune response. Obviously, this is good from the perspective of the pathogen and bad from the perspective of the host. It is assumed that swine pathogens also have similar survival mechanisms and this is what contributes to the frustrations faced by pork producers today in their battle against disease. Perhaps the best example of this frustration and the ability of a pathogen to circumvent a pig's immune response is PRRSV. It may be that PRRSV has some mechanism(s) that thwarts an efficient acquired immune response. How this occurs is not clear, but it is clear that traditional methods of preparing vaccines is not the complete answer when it comes to controlling and preventing PRRS. Others have also followed this logic and have studied cytokine levels and T cell subset distribution during PRRSV. However, the technology for studying the TCR repertoire has only become available following our characterization of the TCRβ genes (Butler et al., 2005). Our studies also established

the normal T-cell repertoire of uninfected swine. As yet no database has been established for the repertoire associated with viral infections. We have chosen an SIV infection to represent what we believe results in a typical anti-viral immune response, i.e., an immune response that is robust and clears the pathogen from the host quickly. The immune response to a third virus, PCV2, will also be evaluated since it is hypothesized that the immune response to this virus will be different when compared to PRRSV and SIV. The selection of these three viruses is also based on the fact that they are reported as cause of the three most important diseases concerning US pork producers. Information derived from this study may have application to each of these diseases. To understand the direct effect of a swine pathogen on the immune system it is paramount to begin by studying the effect in the most simple of models, the germ-free pig.

Objectives

First Objective

Characterize the TCRβ repertoire of neonatal isolator piglets infected with PRRSV, PCV2 and SIV and identify any unique or characteristic clonal expansion/selection of T cells belonging to the major TCRβ supergroups.

Second Objective

Determine whether SIV and PCV2 infections resemble PRRSV in causing hypergammaglobulinemia, lymphoid hyperplasia, autoimmunity or any other remarkable form of immunopathology.

Material and Methods

a. Animal studies: Piglets recovered by Caesarian from two gilts were distributed to isolator units in mixed fashion (Table 2). Surgery, rearing, inoculation and blood sample collection was as previously described (Lemke et al., 2004). All animals were be maintained bacteria-free and inoculated with either PRRSV, PCV-2 or SIV on day 7 and weekly blood samples collected. Blood samples were processed for recovery of plasma and leucocytes. The latter were placed in Tri-reagent and stored at –70C for subsequent preparation of total RNA. At necropsy (day 42, week 6) tonsil, BLN, MLN and spleen were collected in liquid nitrogen for eventual TCRβ repertoire analysis. Also at necropsy, tissue was collected for routine viral culture, for conventional histology and immunochemistry; the later are for detection of autoimmunity and immune complex deposition in the kidney. Bronchial alveolar lavage (BAL) was also collected at necropsy.

b. Recovery of cDNA Total RNA was prepared from blood leucocytes stored TriZol and from solid tissues collected in liquid nitrogen as previously described ((Butler et al., 2000a; 2005; McAleer et al., 2005). cDNA was prepared from total RNA using random hexamer oligos.

c. Recovery of transcripts for spectratypic analysis. Rearranged Vb genes in three major superfamilies were recovered using primer sets specific for each superfamily group. The first round PCR product obtained in this manner was then subjected to a nested second round PCR. The product was end labeled with ^{32}P and analyzed on a 6% acrylamide sequencing gel and the size distribution of CDR3 visualized by autoradiography (Butler et al 2000b; 2007; Fig. 2).

d. Recovery of transcripts for quantitative PCR. cDNA prepared as described above was the target for PCR amplification using primer sets specific for the TCRVβ 4,-5,-7 and 12 families. The quality and specificity of these products is described in section VI-e.

e. Vβ gene family usage determined by cloning and hybridization. TCRVβ rearrangements recovered by PCR for Vβ family supergroups were cloned into pCR4 TOPO (Invitrogen, Carlsbad, CA) and individual insert-containing clones transferred with toothpicks to individual wells of a 96-well microtiter plate. The clones were then grown up overnight and 50 μl of the culture supernatant transferred to the wells of a new plate. The bacteria were then processed *in situ* to recover their plasmid DNA which is then transferred to, and cross-linked on, a nylon membrane. Membranes were then sequentially hybridized with ^{32}P-DNA probes specific for each Vβ family and finally a pan-specific probe to determine how many clones contain VDJ inserts. Figure 3 provides a typical result. This method allows the proportion of each Vβ family used to be calculated. At least 50 clones per time point and per animal are being analyzed.

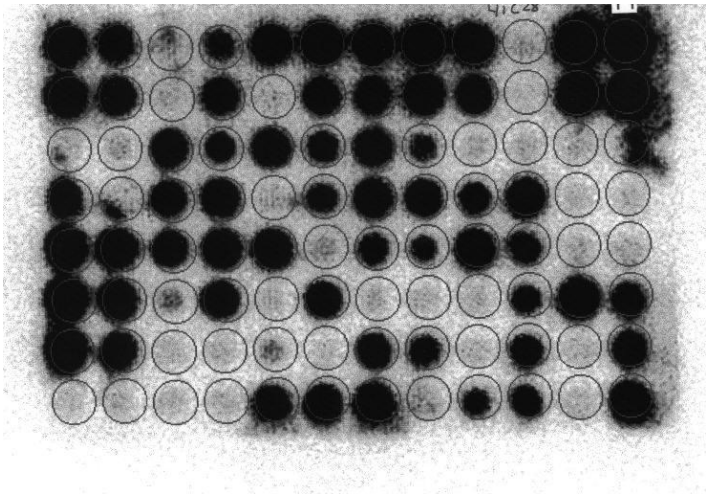


Figure 3. Hybridization of 96 porcine TCRVβ clones with a probe specific for TCRVβ5 (TRBV5; Butler et al 2005). Example data show that 60% of the clones are using this Vβ gene.

f. Real-time PCR : With the assistance of Kevin Knutson ("DNA Core") at the University of Iowa and Dr. Harry Dawson, USDA-ARS, Beltsville, we developed a Cyber Green-based assay to measure total expressed TCRVβ (pan) and to measure expression of TCRVβ 4,-5,-7 and -12. The specificity and linearity was

established using purified clones and independently verified by Dr. Dawson. We also developed real-time PCR for expression of pan VH as a rapid means to determining the B:T cell ratios in various hard tissues. cDNA from a B cell knockout pig served as a negative control (Mendicino et al 2008) . As housekeeping genes we used GUS (beta-glucuronidase) β-actin and HPRT (hypoxanthine-guanine phosphoribosyltransferase).

g. Sandwich ELISA: Immunoglobulin levels in blood and BAL were quantified by sandwich ELISA as previously described (Butler et al., 2000b; Lemke et al., 2004).

h. Data analysis. Spectratypic and autoantibody differences were assessed by inspection (see Fig. 2). Vβ usage between infected animals and controls were statistically analyzed with the assistance of Patrick Breheny, a Ph. D Biostatistics intern and by Dr. Kathryn Chaloner, Department of Biostatistics. For animals in any one treatment group and at any one time point, a minimum of 200 clones ere tested.

Results

First Objective We compared TCRVβ expression of Vβ 4,5,7,12 and total Vβ expression (TCRVβ pan) in tracheal bronchial lymph notes (BLN) tonsil, and spleen of SIV , PCV-2 and PRRSV infected isolator piglets and their sham controls. Initial studies utilized the cloning and hybridization method that we had previously described (Butler et al 2005; see Fig. 3). To expedite the analysis a Cyber Green-based real-time PCR assay was developed as described above in which specificity was established using plasmids containing each family. We determined that using 2.5 ng of cDNA was optimal for comparative studies. Regression plots showed that responses were linear over a 1 to 1 million copies for each Vβ gene. To determine the reliability of the system, we choose a tissue from four animals, conducted the assay in triplicate on each plate and then repeated the process three times. This was undertaken to determine if any apparent animal differences that we might observe could be due to assay variation. The results are presented graphically in Figure 4 and discussed in Section VIII.

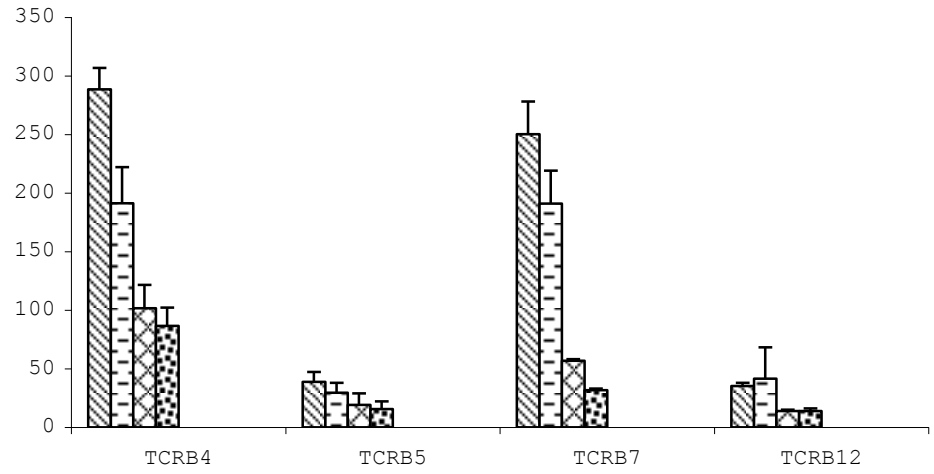


Figure 4. Graphic display of assay variation over nine replicates of the same tissue sample from four different animals (each bar is a separate piglet). ANOVA revealed no significant difference between replicates on a single plate or between three different plates. Error bars (when visible) represent the overall standard error of the mean. The maximum CV was < 20% in all tests except for TCRVβ in piglets # 2 (horizontal stipples)

These studies provided data showing that the proportional usage of TCRVβ–4,-5,-7 and-12 did not differ in BLN, spleen or tonsil of infected animals after infection with any of the three viruses (Fig. 5). In BLN and spleen proportional usage did not differ from sham animals but in tonsil all three viral infection resulted in a trend toward increased usage of Vβ4 and Vβ5 and a decrease in Vβ7 while data show differences in the usage of Vb 4,5,7 and 12, there is no evidence that PRRSV has a T cell superantigen effect as regards Vβ 4, 5, 7 or 12. When compared to pan Vβ, data indicated that these four Vβ families accounted for only 20% of total TCRVβ usage (data not shown). This surprising result could be methodological or biological and is being pursued. Thus, we provided Dr. Harry Dawson (ARS-USDA Beltsville) with the same samples and primers and he confirmed the specificity using a probe-based assay. A comparison of results obtained by the Cyber Green-based PCR, the probe-based PCR (Dr. Dawson) and conventional cloning and hybridization is currently being completed.

In data not shown, spectratypic analysis failed to provide a consistent pattern in which certain clones were expanded (Fig. 2) when different animals in the same treatment group were tested. Thus proliferation of certain T cell clones that share a common CDR3 length was not observed.

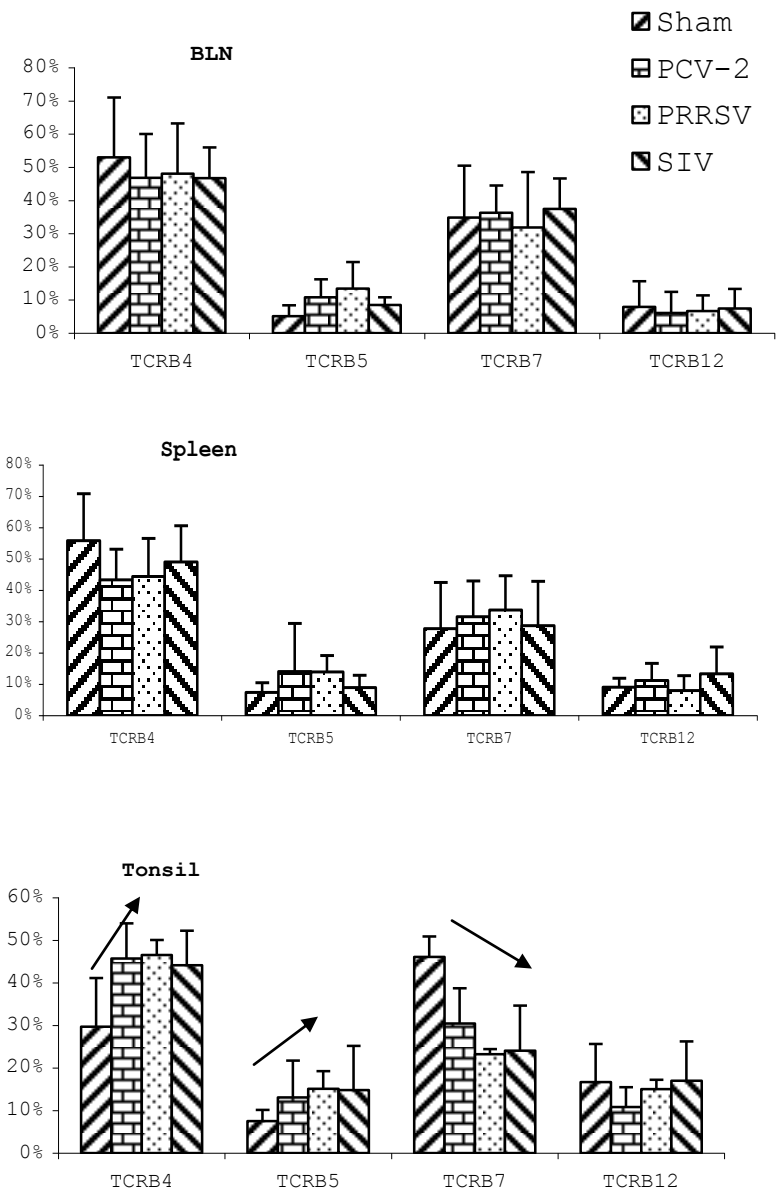


Figure 5: Proportional usage of four families of TCRVβ genes in isolator piglets infected with three different viruses versus sham controls. Legend on figure. Arrows indicate trends in Vβ usage.

Second objective. We compared the Ig levels in blood and bronchial alveolar lavage of isolator piglets infected with SIV, PCV-2 and PRRSV (Fig. 6). These data show that Ig levels were only elevated in PRRSV-infected piglets. Because of this outcome we did not test for autoantibodies or histologically examine lymphoid tissue for B cells or T cell numbers. Rather we developed a real-time PCR assay to quantify the level of VDJ rearrangement (a measure of total B cells) and pan TCRVβ (a measure of total mature T cells) in various lymphoid tissues. As a negative control for B cells, we used tissue from a B cell knockout piglet (Mendocino et al 2008) that lacks IgG, IgM and IgA transcripts and are totally agammaglobulinemia. This adapted PCR method allows the ratio of B:T cells in various tissues of virus-infected piglets to be readily measured. Results

are shown in Fig. 7. These data show considerable variation among animals in each group (error bars are standard error of the mean) while variation among replicates was negligible to the same extent as for the determination of TCRVβ usage (Fig. 4). Despite animal variation, sham controls expressed more TCR than BCR whereas the ratio was nearly the same for all virus-infected piglets (Fig. 7). Using this real-time PCR assay, no elevation of BCR transcripts over TCR transcripts was seen in any tissue including those from PRRSV-infected piglets.

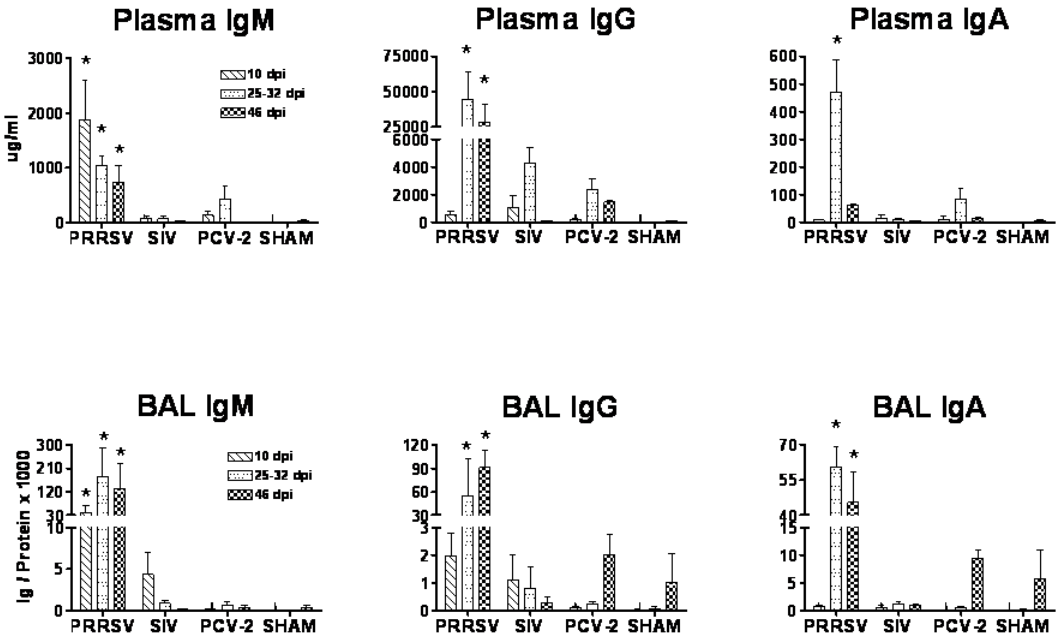


Figure 6. Levels of IgM, IgG and IgA in plasma and BAL of isolator piglets infected with PRRSV, PCV-2, and SIV or maintained as sham controls. In BAL the levels are expressed as the ratio of the Ig concentration to total protein (Ig/Protein X 1000). * = Concentration is significantly higher than for the same Ig in the plasma or BAL of other piglets.

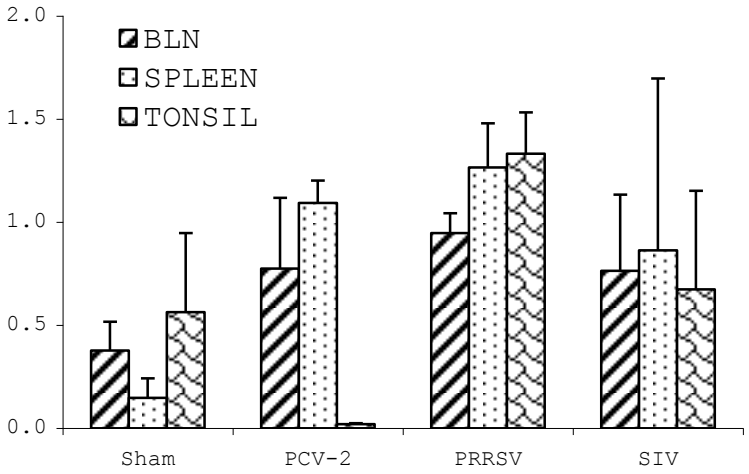


Figure 7 The ratio (Y-axis) of T cell receptor (TCR) to B cell receptor (BCR) expression in three tissues from animals infected with PCV-2, PRRSV , SIV versus sham controls. Studies were done on 5 sham animals, four infected with PCV-2, 3 infected with PRRSV and 7 infected with SIV. Error bars are standard error of the mean.

Discussion

Data obtained on TCRVβ family usage in four major families rejects the notion that PRRSV, PCV-2 or SIV causes preferential usage of Vβ genes in these families and therefore the notion that one of these may display a T cell superantigen effect. Furthermore, spectratypic studies (data not shown) failed to indicate that a certain viral infection can be characterized by the expansion of a particular T cell clone based on CDR3 length alone. Overall, pursuit of the first objective failed to show that any of the three viruses studied was associated with a particular effect on T cell clonal expansion. During the course of the study we choose to adopt real-time PCR as a method to determine TCRVβ family usage. This method allows many more samples to be analyzed compared to cloning and hybridization (the method proposed in the grant). However, the adoption of real-time PCR required "downtime" in order for method development. The specificity of the primers used and the dose response confirmed the validity of the system by using cloned TCRVβ genes from the four families selected. The specificity was also confirmed independently by Dr. Dawson.

While no preferential usage of Vβ genes from any family studied was seen, the method revealed that the combined usage of all four gene families comprised only 20% of total TCRVβ usage. This surprising result could be explained by: (a) a limitation of real-time PCR in this application, (b) a flaw in or original studies indicating that these were the major Vβ genes used by swine or (c) the hypothesis that the TCRVβ usage in the pre-immune repertoire (fetal and newborn piglets) is significantly different than in 5 week old animals. This observation precipitated a more detail study that is currently ongoing.

Preliminary histological studies addressing the second objective were unremarkable and labor intensive. However, quantitation of Ig levels in serum and BAL confirmed that PRRSV greatly elevated the level of IgG,

IgM and IgA production in both serum and BAL (Fig. 6). Therefore we decided to utilize real-time PCR to measure preferential B cell proliferation in solid tissues of virus-infected piglets. This method generated data that is both inconsistent with immunohistology (Lemke 2005) and with differences in Ig secretion between PRRSV-, SIV- and PCV-2- infected piglets (Fig. 6).This result challenges the validity of real-time (quantitative) PCR as an indicator of events in the immune system. Therefore we initiated a head-to-head comparison of two forms of real-time PCR and conventional cloning and hybridization. The results of this comparison will not be available until the end of 2008. For the present, use of real-time PCR to determine the proportion of B and T cells during a viral immune response must be questioned.

In summary, we found no evidence for selective T cell proliferation of TCRVβ from a particular family in any of the three viral diseases studied and found real-time PCR to give data that were higher consistent for any one sample. Therefore the variation we observed using this method between animals in any particular group is attributed to animal variation not assay variation (Fig. 5 and 7). However when quantitative PCR was adopted to determine T:B cell ratios, we found no preferential B cell proliferation in PRRSV-infected piglets; this finding is inconsistent with previous histological studies and with data on secreted Ig levels (Fig. 6). While these PCR data are troubling, we believe the bulk of the data indicate that PRRSV infection is a B cell disorder that may only indirectly effect T cell responses to viral antigens and thus delay resolution of the infection. See the final report for NPB grant 05-174 and Butler et al 2008.

Publications

Butler, J.E., P. Weber, N. Wertz and K.M. Lager 2008. Porcine reproductive and respiratory syndrome virus (PRRSV) subverts development of adaptive immunity by proliferation of germline-encoded B cells with hydrophobic HCDR3s J. Immunol. 180: 2347-2356

Butler, J.E., N. Wertz, P. Breheny and H. Dawson. A comparative study on TCRVβ usage measured by two forms of real-time PCR and conventional cloning and hybridization. (Manuscript in preparation)

Butler, J.E., N. Wertz, P. Breheny and K.M. Lager. TCRVβ usage and B:T ratios determined by PCR in virus-infected isolator piglets. (Brief communication in preparation).

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