RESEARCH REPORT
SWINE HEALTH

Title: Interaction of PRRSV and Porcine Dendritic Cells: Potential Role in Viral Persistence - **NPB# 04-196**

Investigator: Randy E. Sacco

Institution: National Animal Disease Center, USDA/ARS

Date Received: February 10, 2006

Abstract
Porcine reproductive and respiratory syndrome virus (PRRSV) may persist in lymphoid tissue of pigs for months. It is possible that a subset of porcine dendritic cells sequester the virus and transport it to the draining lymph node where the virus is able to persist within the dendritic cell network. We have provided the first isolation and characterization of porcine pulmonary dendritic cells (PDCs). These cells were phenotypically and functionally compared to monocyte-derived dendritic cells (MDDCs). Our data indicates that PDCs are phenotypically and functionally distinct when compared to MDDCs. Furthermore, the MDDCs were shown to be more susceptible to PRRSV infection than are PDCs. We next sought to determine whether we could track the trafficking of PDCs to the draining lymph node using the dye dye carboxyfluorescein diacetate succinimidyl ester (CFSE). We were unable to detect labeled PDCs in the draining lymph nodes by flow cytometry or by immunohistochemical staining of lung or lymph node tissues.

Introduction
Dendritic cells play an important role in immune surveillance and are strategically located in tissues at sites that make them an early target for pathogen contact. Some types of dendritic cells can trap viruses and retain them for months. For example, data indicates that human immunodeficiency virus can be retained by dendritic cells in lymphoid tissue, even in the absence of viral replication, and can serve as a long-term source of infectious virus (1). Since PRRSV may persist in lymphoid tissue for months (2, 3), we plan to test the idea that a subset of lung dendritic cells sequester PRRSV and transport the virus to the draining lymph node where the virus is able to persist within the dendritic cell network. Our first objective was to test whether PRRSV can actively infect dendritic cells. Secondly, we characterized porcine respiratory tract dendritic cells. This will allow us to identify these cells when they traffic to the draining lymph node. Finally, we attempted to trace the trafficking of dendritic cells to the lymph nodes by using the dye carboxyfluorescein diacetate succinimidyl ester (CFSE). In the future, we would propose to examine whether PRRSV sequestered by PDCs can serve as a source of long-term infectious virus.

Objectives

Objective 1. Determine whether PRRSV can replicate in porcine monocyte-derived dendritic cells (MDDCs).

Objective 2. Isolation and characterization of porcine respiratory tract dendritic cells.

Objective 3. Trafficking of porcine dendritic cells from lung to draining lymph nodes.

Results

Porcine MDDCs were derived from peripheral blood mononuclear cells. Briefly, mononuclear cells were isolated from peripheral blood by density gradient centrifugation. Monocytes were allowed to adhere to plastic overnight at 37°C. Fresh medium containing recombinant porcine granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin (IL)-4 were added and cells cultured at 37°C. Fresh medium containing GM-CSF and IL-4 was added every third day of the two-week culture period. Cells were be characterized as dendritic cells by electron microscopy (see Figure 1), and by flow cytometric analysis for cell surface markers and tracer endocytosis. Following the two-week culture period, dendritic cells were harvested, counted and 2 x 10^5 cells incubated in suspension with PRRSV strain NADC-8 at an MOI of 0.5 or 1.0. Two hours later, dendritic cells were washed five times and added to wells of tissue culture plates. The data indicates that PRRSV can infect MDDCs as determined by intracellular staining of PRRSV using FITC-conjugated monoclonal antibody SDOW17 (Figure 2). It should be noted that PRRSV induces massive cell death in these in vitro-derived cells within 40 hours of infection.

We have developed a procedure for the isolation of porcine pulmonary dendritic cells. The procedure involves enzymatic digestion of lung tissue and sorting of dendritic cells by flow cytometry based on expression of the cell surface molecule, CD11c. Figure 3 shows a representative electron micrograph of a porcine pulmonary dendritic cell (PDC). It was noted from the examination of numerous electron micrographs that the cells isolated display the size and morphology typical of dendritic cells, but have fewer cytoplasmic vacuoles compared to MDDCs (see Figure 1 and Figure 3).

Flow cytometric analysis was used to examine cell surface molecules on pulmonary dendritic cells. As shown in Figure 4, porcine PDCs and MDDCs exhibit differing expression of cell surface molecules such as major histocompatibilty complex (MHC) class I and I, CD16 (Fc gamma receptor) and CD80/86 (co-stimulatory molecules measured by CTLA4-Ig fusion protein binding). Functionally, the PDCs are readily able to inject antigens via receptor-mediated endocytosis (data not shown). Thus, based on our data, porcine PDCs seem to be in state of maturity that lies between immature and mature cells when compared to MDDCs. This is similar to previous reports regarding the isolation of pulmonary dendritic cells from other species.

Finally, we have found that pulmonary dendritic cells are less susceptible to PRRSV infection than are MDDCs. It has been suggested that MDDCs have characteristics of both macrophages and dendrtic cells. Given that macrophages at specific stages of development are susceptible to PRRSV infection, our data supports the idea that MDDCs may display some macrophage-like characteristics.

We next sought to determine whether we could track the trafficking of PDCs to the draining lymph node using the dye carboxyfluorescein diacetate succinimidyl ester (CFSE). We were unable to detect labeled PDCs in the draining lymph nodes by flow

cytometry or by immunohistochemical staining of lung or lymph node tissues. In the future, we will try additional cell labeling techniques with other fluochrome dyes (such PKH dyes) to determine whether we can label and track PDCs.

Discussion

A method for isolation of porcine PDCs has been developed and phenotypic and functional analyses have been conducted on the isolated cells. It is apparent that the cells isolated are a heterogeneous population of dendritic cells. Futher characterization of these subsets is required and will be dependent on the development of additional reagents against porcine dendritic cell surface molecules.

We have shown that PDCs are quite distinct phenotypically and functionally when compared to MDDCs. The implications of this research suggests that studies which compare the interaction of PRRSV with host dendritic cells should utilize the appropriate tissue-derived cells rather than in vitro-derived cells which likely have characteristics of both macrophages and dendritic cells. Moreover, studies are needed which show how interaction of PRRSV with tissue dendritic cells modulates the function of these dendritic cells. Given the critical role of DCs in regulating the early host response to infection, determining how PRRSV modulates PDC function should provide important clues as to how the virus might avoid early elimination.

Lay summary

Porcine reproductive and respiratory syndrome virus (PRRSV) may persist in tissues of pigs for months. How the virus persists in certain pigs remains to be determined. It is possible that a subset of white blood cells trap the virus and allow it to remain in host tissues undetected by the immune system. We have provided the first isolation and characterization of a specific type of white blood (called a dendritic cell) in the lung of pigs which may play a role in early contact with PRRSV. These tissue-derived cells were distinct compared to similar cells derived in the laboratory. Our data indicates that lung cells are less susceptible to viral infection that the in vitro derived cells. We next sought to determine whether we could track the trafficking of cells to the othe tissue in the body using the dye carboxyfluorescein diacetate succinimidyl ester (CFSE). We were unable to detect labeled cells in the tissues. The use of other appropriate dyes should be considered.

Figures

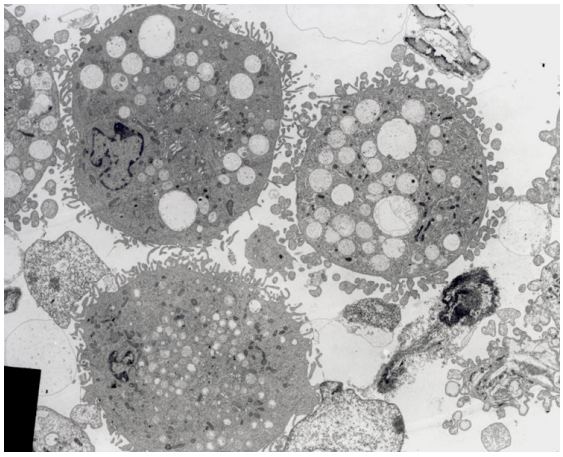

Figure 1. Representative transmission electron micrograph (TEM) of porcine monocyte-derived dendritic cells. Note the highly vacuolated cytoplasm of these in vitro derived cells.

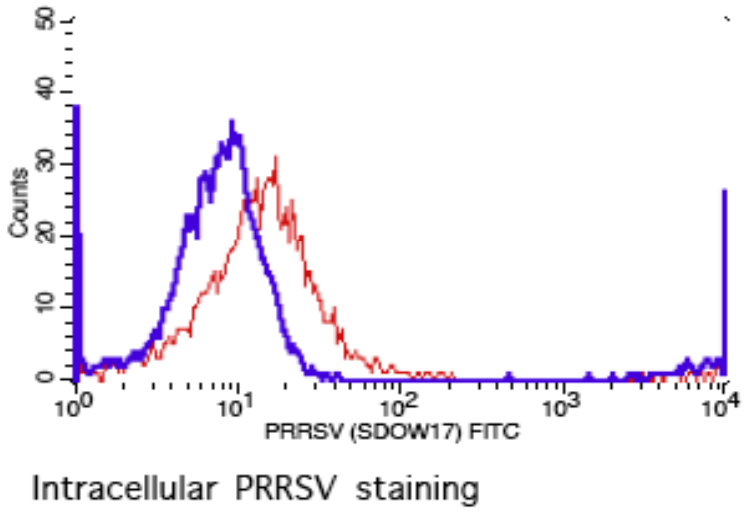

Figure 2. Intracellular PRRSV staining of porcine MDDCs using FITC-conjugated monoclonal antibody SDOW17 (red). The blue line shows the sham-infected control cell staining.

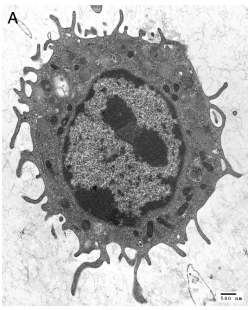

Figure 3. Representative TEM of a porcine pulmonary dendritic cell isolated by enzymatic digestion of lung and sorting on CD11c.

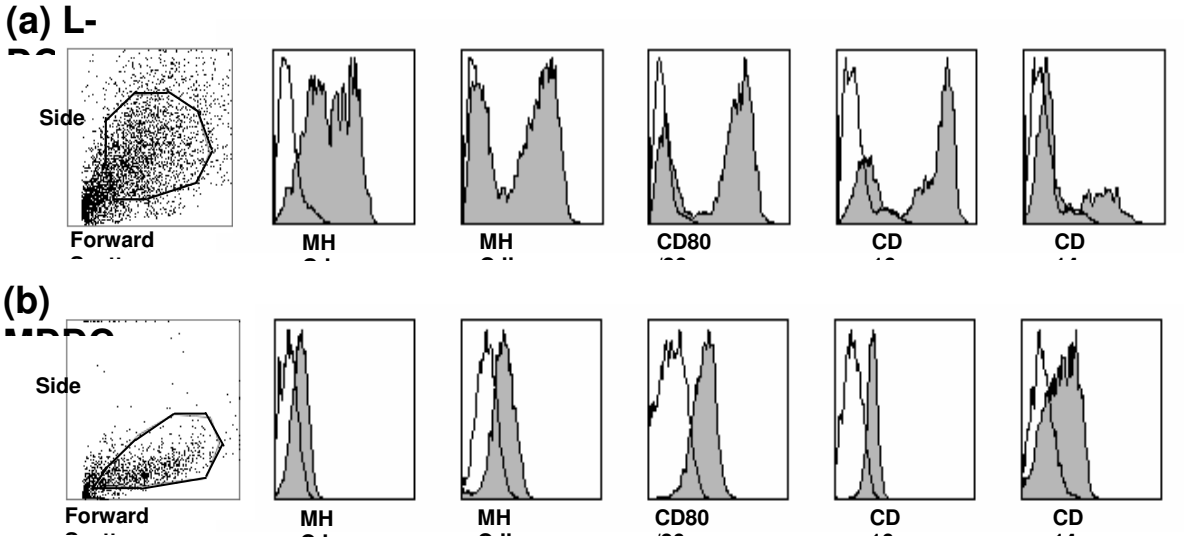

Figure 4**. Dendritic cell phenotype.** (a) CD11c^{+} cells from the lung-enriched fraction and (b) monocyte-derived dendritic cells were assessed for forward and side scatter properties and for the expression of indicated surface markers (grey histogram) by flow cytometry. Open histograms indicate isotype-controls.