Effect of PRRSV Infection on MHC Expression by Macrophages and Monocytes

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ASL-R1404

Summary and Implications

Porcine reproductive and respiratory syndrome virus (PRRSV) is a recent and widespread pathogen in the U.S. swine population. PRRSV infects cells of the macrophage/monocyte/dendritic lineages which are important antigen presenting cells (APCs) of the immune system. Using flow cytometric (FACs) analysis, we demonstrated that PRRSV infection decreases the expression of the major histocompatibility complex (MHC) glycoproteins on the cell surface of infected macrophages. This decrease in MHC protein expression may reduce the ability of the macrophages to present viral antigens to the appropriate lymphocytes. The potential lack of viral antigen presentation may play a crucial role in the persistent viremia observed in PRRSV-infected pigs.

Introduction

PRRSV, an *Arterivirus*, first appeared in swine herds in the United States in 1987. PRRSV causes reproductive failure and respiratory disease in susceptible pigs. In naive herds, reproductive failure, neonatal death loss, respiratory disease with secondary bacterial pneumonia, septicemia, and enteric disease may have devastating economic consequences.

Replication of PRRSV in macrophages is an important aspect of the pathogenesis of PRRSV and other viruses of the *Arterivirus* group. These viruses characteristically target macrophages and produce persistent or chronic infections. Immunocytochemistry, *in situ* hybridization, and virus isolation data suggest that PRRSV establishes a viremia, and is present within the tonsil follicles and pulmonary alveolar macrophages (PAMs) within 24 hours following infection. PRRSV can be detected in tonsils and lungs for up to 28 days post infection. Transmission of the virus by contact between pigs has been demonstrated for at least 100 days post infection.

Macrophages play an important role in the presentation of viral antigens to the immune system, therefore their infection may strongly influence the course of host immune responses during PRRSV infection. Antigen presenting cells (APCs) process and present various self and foreign antigens to the immune system by complexing the antigens to the MHC class I or II glycoproteins. The MHC-antigen complexes are transported to the cell surface where antigens are presented to the various cells of the immune system. Macrophages and dendritic cells possess both classes of MHC glycoproteins, but are especially rich in class II glycoproteins which bind exogenous peptides and present them to B and $CD4^+$ T lymphocytes. Infection of cells by viruses results in the association of viral peptides with the class I molecules on the cell surface, leading to the subsequent destruction of the virus-infected cell by $CD8^+$ cytotoxic T lymphocytes (CTL).

The objective of this study was to investigate the possible effect PRRSV may have on the expression of the MHC glycoproteins on infected macrophages. Using PAMs and monocyte-derived macrophages (MDMs), we demonstrated in the past year that PRRSV infection decreases the mean fluorescent intensity (MFI) of the MHC class I and II antigens on the cell surface as measured by FACs. In addition, a similar decrease in other macrophage surface proteins was detected. These findings will help improve understanding: 1) for the pathogenesis of PRRSV infection; 2) how the virus is able to successfully evade the immune; and 3) the mechanisms by which the virus persists in cells.

Materials and Methods

Crossbred pigs, 4- to 7-weeks-old, were used to obtain PAMs and peripheral blood monocytes (PBMCs). Peripheral blood was obtained by cardiac puncture of anesthesized pigs and the PBMCs were isolated over ficoll-hypaque differential centrifugation. The PBMCs were washed and contaminating erythrocytes lysed. The cells were resuspended in RPMI-1640 medium with 10% fetal bovine sera and antibiotics. The cells were placed in 100 m.m. tissue culture plates and the monocytes were allowed to adhere. After 24 hours, the non-adherent cells were washed off and media from L929 cells added to fresh media at a ration of 1-3 for a minimum of 24 hours. The medium from L929 cells have been shown to contain (GM-CSF) which stimulates the monocytes to become macrophages.

PAMs were obtained from the same pigs following euthanasia and removal of the lungs. The lavage media consisted of Eagle's minimum essential media with 10% fetal bovine sera and antibiotics.

A well characterized virulent strain of PRRSV, VR2385, was used to infect the macrophage/MDM cultures at a multiplicity of infection (MOI) of 1.

MHC class I and II antigen expression was determined using monoclonal antibodies and FACs analysis. To confirm the cells with decreased expression were PRRSV infected, an intracellular staining assay to detect PRRSV nucleocapsid protein was developed for FACs analysis.

Results and Discussion

Both PRRSV-infected MDMs and PAMs were shown to induce a decrease in the MFI of class I and II cell surface glycoproteins. Table 1 presents the MFI's of MHC class I and II over varying time periods.

PRRSV infection significantly decreased class I expression as early as 4 hours post infection in the MDM's of the 6-week old pigs. There was a less remarkable decrease in class II expression. The response of PRRSV-infection on PAM's also yielded a marked decrease in the MFI of class I and II expression.

To determine if the effect of PRRSV infection was MHC-specific, an antibody to an adhesion molecule on macrophages (74-22-15) also was measured. PRRSV-infection induced a decrease in the surface expression of this protein as well.

These results suggest that PRRSV may have a significant impact on the expression of cell surface proteins of macrophages. The consequences of this may potentially explain the ability of PRRSV to persist in pigs, even in the presence of circulating antibodies. By decreasing the expression of proteins on the surface of infected cells, the immune system will not be able to recognize viral infected cells and thus will not destroy them.

Table 1. Log fluorescent mean channel numbers showing intensity of MHC class I and class II (DR and DQ) of monocyte derived macrophages (MDM's) and pulmonary alveolar macrophages (PAM's) averaged from two 6-week-old pigs infected with PRRSV at different time intervals.

		Time Post Infection				
Cell Type	Negative	4Hours	6Hours	8Hours	12Hours	24Hours
			MHC Class I			
MDM	57	19	16	17	15	ND
PAM	42	ND	46	ND	ND	31
			MHC Class II (DR)			
MDM	33	25	20	26	21	ND
PAM	145	ND	135	ND	ND	53
			MHC Class II (DQ)			
MDM	22	19	16	17	16	ND
PAM	75	ND	62	ND	ND	38
			74-22-15			
MDM	299	ND	103	ND	ND	96.8
PAM	185	ND	92.7	ND	ND	84.2

*ND= Not Done