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ScienceDirect
Veterinary Microbiology 129 (2008) 281–293
veterinary microbiology
www.elsevier.com/locate/vetmic

Phenotypic and functional modulation of bone marrow-derived dendritic cells by porcine reproductive and respiratory syndrome virus

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Received 9 October 2007; received in revised form 30 November 2007; accepted 5 December 2007

Abstract

It is well documented that there is a delay in the development of effective immunity to porcine reproductive and respiratory syndrome virus (PRRSV) in infected and vaccinated pigs. This suggests that PRRSV might possess some inherent properties to evade host defense mechanisms during the early stage of infection. Dendritic cells (DCs) play a crucial role in the activation and control of T-cells in response to viral antigens. In this study, we investigated the phenotypic and functional property changes of bone marrow-derived immature DCs (BM-imDCs) that take place after infection by PRRSV. Results showed that BM-imDCs were permissive to PRRSV infection, as productive replication took place in these cells. A down-regulated expression of MHC I molecules along with an up-regulated expression of CD80/86 is observed at 48 h following infection. Also at 48 h following PRRSV infection, a significant increase of IL-10 secretion by BM-imDCs was noticed. Results suggest that the inhibited expression of MHC I and the enhanced secretion of IL-10 by BM-imDCs after PRRSV infection might be among the strategies used by the virus to evade the host immune defenses.

Keywords: Porcine reproductive and respiratory syndrome; Dendritic cells; Modulation

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically impacting diseases that affect the swine industry in most swine-producing countries. The disease is symptomatically characterized by reproductive failure in pregnant sows and gilts, and severe respiratory distress in piglets and growing pigs (Zimmerman et al., 2006). The causative agent, the PRRS virus belongs to the family Arteriviridae, a family which also includes the lactate dehydrogenase-elevating virus of mice, the equine

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0378-1135/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.vetmic.2007.12.002

arteritis virus, and the simian hemorrhagic fever virus. These viruses share similar properties, including the ability to replicate in macrophages and the induction of persistent infection in the host (Meulenberg et al., 1993).

Farms with animals known to have been infected with PRRSV had a noticeable increase in the number of secondary viral and bacterial infections throughout their animal population. These field observations suggest that PRRSV infection might induce immunosuppression in hosts, however, results from experimental studies have not fully confirmed this hypothesis. While some experiments have failed to demonstrate an increased severity of the disease in pigs co-infected with PRRSV and bacteria, other studies have shown that a primary PRRSV infection exacerbates the poor clinical outcome for a pig that is subjected to a secondary bacterial infection (Zimmerman et al., 2006).

Mechanisms of the interaction between PRRSV and secondary bacterial or viral invaders are unclear. *In vivo* and *in vitro* studies have shown that the principal target cells of PRRSV infection in pigs are cells of monocyte/macrophage lineage (Duan et al., 1997; Lawson et al., 1997). Infection of pulmonary alveolar macrophages (PAMs) and pulmonary intravascular macrophages (PIMs) with PRRSV resulted in the killing of these cells coupled with a reduced capacity to perform bactericidal activity (Thanawongnuwech et al., 1997). Blood-borne bacteria are mainly cleared by the lungs in pigs (Crocker et al., 1981), and thus, the detrimental effect of PRRSV infection on monocyte/macrophage lineage cells, including PAMs and PIMs, might render pigs more susceptible to secondary bacterial and viral infections.

Dendritic cells (DCs) are the primary professional antigen-presenting cells and are known to play essential roles in the initiation, stimulation, and regulation of the immune response to bacterial and viral infections (Ardavin, 2003). Myeloid-derived DCs share certain similarities to monocyte/macrophages including morphology, phenotype, phagocytic capacity, and cytokine production. In addition, the detection of PRRSV in interdigitating dendritic cells in tissues of PRRSV-infected pigs has been reported (Haynes et al., 1997). These data indicate that DCs might be one of the main target cell types for PRRSV infection, however, little is known about the precise effect of PRRSV infection on porcine DCs (Wang et al., 2007). In this study, we investigated the permissiveness of porcine bone marrow-derived immature DCs (BM-imDCs) to PRRSV and also the consequences of their interactions following viral infection. Immature DCs were generated from bone marrow haematopoietic cells (BMHCs), and viral replication, along with the associated morphological changes, maturation, and cytokine production in PRRSV-infected BM-imDCs were examined. The ultimate goal of this study was to clarify the factors that mediate the formation of antiviral immunity and also to elucidate the possible pathogenesis of PRRSV infection in pigs.

2. Materials and methods

2.1. Preparation of porcine BM-imDCs

The preparation and culture of BM-imDCs were done according to the method described previously (Carrasco et al., 2001; Chaung et al., 2005). Briefly, BMHCs were prepared by flushing the bone marrow of the humerus and femur of pigs which were 6–8 weeks old, cross-bred, and PRRSV-free with phosphate-buffered saline containing 0.3% ethylenediaminetetraacetic acid (PBS–EDTA). Collected cells were treated with ACK lysing buffer containing 0.15 M NH_4Cl, 10 mM $KHCO_3$ and 0.1 mM Na_2EDTA to lyse red blood cells. BMHCs were then washed with the PBS–EDTA solution twice, and cell concentration was adjusted to 4×10^6 cells/ml in RPMI-1640 medium supplemented with 20 ng/ml rpGM-CSF, 20 ng/ml rpIL-4 (R&D Systems; Wiesbaden, Germany) and 10% porcine serum (Gibco® BRL; Grand Island, NY, USA) (DC-RPMI), and cultured in 75 cm^2 cell culture flasks (Falcon, Becton Dickinson; Franklin Lakes, NJ, USA) at 37 °C in a 5% CO_2 incubator. At days 3 and 6, half of the medium was replaced with fresh DC-RPMI. At day 7, cells were collected, washed twice and adjusted to a concentration of 2×10^6 cells/ml in DC-RPMI and used as BM-imDCs. For induction of maturation, BM-imDCs were cultured in DC-RPMI containing 1 μg/ml lipopolysaccharide (LPS; Sigma–Aldrich; St. Louis, MO, USA) for either 24 or 48 h. Characteristics of the obtained BM-imDCs have been reported previously (Chaung et al., 2005).