PORCINE ARTERIVIRUS ENTRY IN MACROPHAGES

Heparan sulfate–mediated attachment, sialoadhesin-mediated internalization, and a cell-specific factor mediating virus disassembly and genome release

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1. INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) was first described as a new disease in pig herds in North America late in the 1980s and beginning of the 1990s in Europe and is characterized by respiratory disease in young piglets and late-term reproductive failure.1,2 The causative agent of the disease was identified in Europe in 1991 and in the US in 1992 as a virus, PRRS virus (PRRSV).2-5

PRRSV is classified with equine arteritis virus (EAV), lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus (SHFV) in the family Arteriviridae, which is grouped with the Coronaviridae and the Roniviridae in the order Nidovirales.6-8 The virus is an enveloped particle with a diameter of 50 to 65 nm and contains a polyadenylated, positive-strand RNA genome.9 The genome is about 15 kDa and encodes 9 open reading frames: ORF1a and 1b code for nonstructural proteins, ORF2a, 2b, 3, 4, 5 and 6 code for structural membrane proteins, and ORF7 codes for the nucleocapsid protein (N).10-13

Currently, PRRSV is present in most, if not all swine-producing areas of the world, including North and South America, Western and Eastern Europe, and Asia. As the virus is now enzootic in most countries, the number of acute disease outbreaks has diminished and infections are in general mild and subclinical.14 From an economical point of view, however, the virus still causes major losses and is considered as the most important pig disease worldwide.

Characteristic for all members of the Arteriviridae is not only that they have a very narrow host tropism, but also that they share a marked in vivo tropism for cells of the monocyte/macrophage lineage.14,15 Even in cell culture, arteriviruses have, with the exception of EAV, a very narrow cell specificity, only allowing replication in primary

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macrophages from their respective hosts and in a limited number of cell lines upon adaptation of the virus.\textsuperscript{16}

For PRRSV, it has been shown that \textit{in vivo}, the virus infects a subpopulation of resident macrophages present throughout the body, and that alveolar macrophages are primary target cells for the virus.\textsuperscript{17, 18} \textit{In vitro}, porcine alveolar macrophages (PAM) have been shown to efficiently sustain virus replication.\textsuperscript{5, 19, 20} Peripheral blood monocytes can also be infected \textit{in vitro} at very low levels, but only when they have been cultivated for 24 h.\textsuperscript{19, 21} Interestingly, peritoneal macrophages were shown to be refractory for PRRSV infection.\textsuperscript{19} Besides primary macrophages of porcine origin, only the African green monkey kidney cells MA-104, and cells derived thereof, such as Marc-145 cells, can sustain \textit{in vitro} virus replication.\textsuperscript{22} Although PRRSV has thus both \textit{in vivo} and \textit{in vitro} a very restricted cell tropism, it can replicate in several cell lines upon transfection of the genomic RNA, indicating that the restricted cell tropism is very likely the result of the presence or absence of specific receptors on the membrane of macrophages and of other macrophage-specific factors.\textsuperscript{23, 24}

In our laboratory, we have been studying for several years PRRSV entry in macrophages, and two receptors on macrophages have been identified: (1) heparan sulfate and (2) sialoadhesin. The glycosaminoglycan heparan sulfate was identified as a receptor because both heparan sulfate and heparin, an analogue of heparan sulfate, strongly reduce infection of macrophages with both European and American PRRSV strains when present during virus inoculation.\textsuperscript{25, 26} Other glycosaminoglycans, such as chondroitin sulfate A and dermatan sulfates had no effect on PRRSV infection, indicating that the observed effect was specific. Also, treatment of macrophages with heparinase I, an enzyme which destroys heparan sulfate, reduced infection of macrophages. Using flow cytometry and labeled PRRSV, it was observed that heparin strongly reduced virus attachment to macrophages.\textsuperscript{26} Heparan sulfate has also been proposed as receptor on Marc-145 cells, because heparin reduces PRRSV infection when present during infection, or when these cells are pretreated with heparinase I.\textsuperscript{27}

Sialoadhesin, a macrophage-specific protein belonging to the sialic acid-binding immunoglobulin-like lectin (Siglec) family, was identified as a PRRSV receptor on macrophages because (a) a sialoadhesin-specific monoclonal antibody (mAb 41D3) is able to completely block infection of macrophages, (b) mAb 41D3 reduces PRRSV attachment to macrophages, (c) PRRSV co-localizes with sialoadhesin on the surface of macrophages, (d) both the \textit{in vivo} and \textit{in vitro} PRRSV susceptible porcine cells express sialoadhesin, and (e) nonsusceptible cells internalize PRRSV upon expression of a recombinant sialoadhesin.\textsuperscript{28-30}

In this overview, our findings about the different steps of PRRSV entry in macrophages (attachment, internalization and genome release into the cytoplasm) will be discussed and these will be related to the findings of others on PRRSV entry in both macrophages and Marc-145 cells.

2. PRRSV ATTACHMENT: INITIATED BY AN INTERACTION WITH HEPARAN SULFATE AND FOLLOWED BY ATTACHMENT TO SIALOADHESIN

The role of heparan sulfate and sialoadhesin in PRRSV attachment to macrophages was first evaluated using flow cytometric attachment studies in the presence or absence