20th International Pig Veterinary Society Congress

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Durban
South Africa

We are delighted that the International Pig Veterinary Society Congress 2004, decided to select South Africa as the host country for the 20th IPVS Congress. The Pig Veterinarians of South Africa will ensure that this congress lives up to the best traditions of previous congresses; incorporating an interesting and topical scientific programme, fascinating accompanying persons tours and an excellent social programme, allowing delegates the opportunity to network with their overseas colleagues.

This, the first IPVS congress on the African continent, will undoubtedly be of enormous benefit in generating solutions to the emerging pig veterinary challenges, especially those related to exotic and changing viral diseases, decreased use of antimicrobials and nutritional advances. The congress is important to further pig veterinary science in South Africa, to encourage younger veterinarians to join the pig industry, as a vehicle to generate funds for research and to improve the pig industry in Southern Africa.

South Africa is a magnificent and beautiful country, and offers tourists value for money. Thus, pre and post congress tours will be a major attraction for delegates to come to South Africa. Durban, in KwaZulu Natal, is a vibrant multi-cultured city with magnificent beaches, easily accessible game parks, theme villages and a moderate winter climate making it an ideal tourist destination. We urge our colleagues throughout the world to use this opportunity to get a glimpse of the continent’s rich and fascinating wonders and to enjoy the hospitality of their African friends.

Dr Peter Evans
Chairman: Local Organising Committee: IPVS 2008
PROTECTION OF PRRSV-INFECTED MACROPHAGES AGAINST ANTIBODY-DEPENDENT, COMPLEMENT-MEDIATED CELL LYSIS DUE TO ABSENCE OF SURFACE EXPRESSION OF VIRAL PROTEINS

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Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) can cause reproductive failure in sows and is associated with the respiratory disease complex affecting pigs of all ages. The virus is able to evade from the host’s immune system, which results in prolonged virus replication for several weeks. Until now, mechanisms of PRRSV immune evasion are not well understood. A possible immune evasion strategy of an enveloped virus is the absence of surface expression of viral proteins in infected cells, since this prevents recognition by virus-specific antibodies (Ab) and consequent cell lysis by either the classical complement pathway or cell-mediated cytotoxicity. The first goal of this study was to investigate if viral proteins are expressed on the surface of PRRSV-infected primary macrophages, the in vivo target cells. Subsequently, the sensitivity of PRRSV-infected macrophages towards antibody-dependent, complement-mediated cell lysis (ADCML) was investigated.

Materials and Methods
To determine if viral proteins were expressed at the surface of PRRSV-infected cells, porcine alveolar macrophages were inoculated with PRRSV and at 0, 6, 9 and 12 h post inoculation, the cell surface was stained with biotinylated PRRS-specific polyclonal antibodies (pAb) and FITC-labeled streptavidin; afterwards, the cells were fixed, permeabilized and intracellular viral nucleocapsid proteins were visualized using nucleocapsid protein-specific monoclonal antibodies (mAb) and Alexafluor350-labeled goat anti-mouse IgG. The cells were then analyzed with a confocal microscope, macrophages were visualized using nucleocapsid protein-specific antibodies (1). The specificity of the assay was assessed by incubating PRRSV-infected macrophages with pAb at 9 h post inoculation, and after washing, the cells were incubated for 1 h with porcine complement. The dead infected cells were counted using fluorescence microscopy. The effectiveness of the porcine complement was confirmed with pseudorabies virus-infected macrophages and pseudorabies virus-specific antibodies (1). The specificity of the assay was assessed by incubating PRRSV-infected macrophages with pAb derived from a PRRSV-negative pig and complement.

To determine if PRRSV-infected cells were sensitive towards ADCML, an ADCML assay was performed. PRRS-inoculated macrophages were incubated for 1 h with PRRSV-specific pAb at 9 h post inoculation, and after washing, the cells were incubated for 1 h with porcine complement. The dead infected cells were counted using fluorescence microscopy. The effectiveness of the porcine complement was confirmed with pseudorabies virus-infected macrophages and pseudorabies virus-specific antibodies (1). The specificity of the assay was assessed by incubating PRRSV-infected macrophages with pAb derived from a PRRSV-negative pig and complement.

To investigate the effect of Ab and complement on in vivo infected macrophages, the ADCML assay was performed on macrophages obtained by bronchoalveolar lavage of a PRRSV-infected pig at 5 days p.i.

Results and Discussion
Using confocal microscopy and flow cytometry, viral proteins were not detected on the surface of in vitro or in vivo PRRSV-infected alveolar macrophages, indicating that viral proteins were not expressed on the surface of PRRSV-infected macrophages. To determine if the absence of surface expression of viral proteins was sufficient to protect infected cells against ADCML, an ADCML assay was performed on in vitro (Table 1) and in vivo (data not shown) PRRSV-infected macrophages. No significant increase in cell lysis was observed, indicating that both in vitro and in vivo PRRSV-infected macrophages were protected towards ADCML.

Table 1 The effect of antibodies and porcine complement on lysis of in vitro PRRSV-infected macrophages at 9 h post inoculation

<table>
<thead>
<tr>
<th>Polyclonal antibodies</th>
<th>Complement (% pig serum)</th>
<th>Lysed PRRSV-infected macrophages (%)</th>
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<tbody>
<tr>
<td></td>
<td>0 %</td>
<td>5 %</td>
</tr>
<tr>
<td>PRRSV-negative 0.5</td>
<td>10.2 ± 1.6</td>
<td>11.2 ± 2.9</td>
</tr>
<tr>
<td>1.0</td>
<td>nd*</td>
<td>nd*</td>
</tr>
<tr>
<td>PRRSV-specific 0.5</td>
<td>9.7 ± 1.4</td>
<td>9.6 ± 2.4</td>
</tr>
<tr>
<td>A</td>
<td>10.5 ± 2.4</td>
<td>10.7 ± 2.4</td>
</tr>
<tr>
<td>PRRSV-specific 0.5</td>
<td>11.2 ± 0.4</td>
<td>12.2 ± 1.0</td>
</tr>
<tr>
<td>B</td>
<td>12.8 ± 1.4</td>
<td>17.8 ± 6.0</td>
</tr>
</tbody>
</table>

*: not determined

Further analysis of PRRSV-infected macrophages showed that absence of viral proteins from the cell surface was not due to internalisation of the proteins from the plasma membrane, but was rather due to retention of the viral proteins inside infected cells. Putative endoplasmic reticulum and golgi retention motifs were found in two PRRSV envelope proteins (GP2 and GP3), but so far it is not known if these motifs are functional.

Conclusion: This study shows that viral proteins are not expressed on the surface of PRRSV-infected macrophages, which masks infected cells from PRRSV-specific Ab and protects them against antibody-dependent cell lysis, both in vitro and in vivo. PRRSV-specific Ab clearly have the potential to clear free virus from circulation, but they cannot eliminate PRRSV-infected cells, as shown in this study. Cell-mediated immunity may be a necessary component for the elimination of PRRSV-infected cells, and the importance of it will be investigated in the near future.

References