Increased proteolytic activity and matrix metalloprotease expression in lungs during infection by porcine reproductive and respiratory syndrome virus

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The local increase in the secretion of extracellular proteases, allowing cleavage of the extracellular matrix and thereby facilitating the infiltration of T cells, monocytes and neutrophils, is a hallmark of chronic inflammation and autoimmunity. In pulmonary genetic diseases, such as emphysema and cystic fibrosis, proteases can also favour the development of local immunodeficiency by degrading key regulators of the immune response, such as CD4, CD8, IgG, ICAM-1 and C3b receptors. Since several infectious agents can give rise to severe pulmonary disorders associated with opportunistic infections, we sought to determine whether an increase in proteolytic activity occurred during infection with porcine reproductive and respiratory syndrome virus (PRRSV), the causative agent of a new disease in swine characterized by severe respiratory problems in young pigs. Piglets were infected with the virus and bronchoalveolar lavages were collected at various times post-infection to measure the net proteolytic activity. It was shown that PRRSV infection leads to a significant increase in proteolytic activity in pulmonary fluids. Maximal activity was found at 7 and 14 days post-infection, with a return towards normal levels at day 42. Zymographic analyses showed a significant increase in the secretion of matrix metalloproteases (MMPs) 2 and 9, two enzymes involved in tissue remodelling. Histological analyses showed a correlation between the increase in proteolytic activity and the appearance of lesions that were characterized by massive lympho-mononuclear cell infiltration. These results suggest that virus infection of the lungs can lead to a transient increase in proteolytic activity that could favour opportunistic infection.

Introduction

Immunosuppression related to virus infections of the respiratory tract often predisposes a host to subsequent respiratory tract diseases (Wright, 1997). The molecular mechanisms that lead to immunosuppression by respiratory viruses are not well defined. In some cases, immunosuppression is believed to be due to the destruction of immune cells resulting from virus replication, such as the case of infections caused by human or simian immunodeficiency virus (reviewed by Graziosi & Pantaleo, 1998; Mankowski et al., 1998). In AIDS patients, destruction of CD4-positive immune cells is thought to compromise the local immune response severely, thereby favouring the establishment of opportunistic infections, most notably by Pneumocystis carinii and Mycobacterium tuberculosis (reviewed by Weeks, 1998; Glück et al., 2000; Murray et al., 2000). A similar situation has been described in cattle in the case of shipping fever syndrome (reviewed by Shoo, 1989). This syndrome of bronchopneumonia, which often extends to a true fibrinous pleuropneumonia, represents the most economically important health problem in feedlot cattle. It is caused by a group of respiratory viruses, including bovine herpesvirus-1, parainfluenza virus-3, bovine respiratory syncytial virus and bovine respiratory coronavirus, that contribute to the susceptibility of cattle to opportunistic bacteria such as Pasteurella haemolytica or Pasteurella multocida. These viruses are thought to interfere with bovine host-defence mechanisms by (i) altering macrophage and neutrophil functions in the lungs and airways, (ii) causing severe inflammatory damage to the epithelial surfaces of the alveoli and (iii) modifying the surface properties of...
respiratory epithelial cells, thereby favouring bacterial adhesion and growth (Babiuk et al., 1988; Storz et al., 1996; Wright, 1997).

A number of studies have shown that local production of extracellular proteases plays a key role in immune lung disorders (reviewed by Greenberger, 1997). The increased expression of extracellular proteases can affect the host’s immune response against opportunistic infectious agents. In cystic fibrosis, for instance, opsonization of *Pseudomonas aeruginosa* is ineffective because neutrophil-derived elastase released in the extracellular space cleaves immunoglobulins and digests the C3b receptor on neutrophils, thereby limiting phagocytosis of pathogens (Greenberger, 1997). The cellular immune response can also be altered whenever extracellular proteases cleave molecules involved in cell-mediated immunity, such as CD4 and CD8 (Döring et al., 1995), ICAM-1 (Champagne et al., 1998) and IL-2 (Ariel et al., 1998). The local production of extracellular proteases, most notably matrix metalloproteases (MMPs), can also alter the local tissue architecture directly by degrading the proteins of the extracellular matrix (Shapiro, 1994). High levels of MMP-2 and MMP-9 in the lungs have been shown to promote the infiltration of inflammatory cells and to exacerbate the symptoms associated with bronchial asthma (Kumagai et al., 1999). Although both MMP-2 and MMP-9 have been shown to cleave denatured collagen (gelatin) and, somewhat less efficiently, native collagen types IV and V, they can also degrade elastin (Senior et al., 1991), an important component of the lung architecture. Most of our understanding of the role of proteases in immunological lung disorders has, however, come from studies of specific genetic diseases. Whether proteolytic activity can be augmented in pulmonary virus infections remains unknown.

Porcine reproductive and respiratory syndrome (PRRS) is an emerging virus disease causing late-term reproductive failure and severe pneumonia in unweaned and weaned piglets (Bilodeau et al., 1991; Goyal, 1993). The causative agent, PRRS virus (PRRSV), is a member of the new family *Arteriviridae*, order *Nidovirales*, that replicates in lung alveolar macrophages, producing an influenza-like illness associated with respiratory distress (Snijder & Meulenberg, 1998; Dea et al., 2000). Respiratory disease in the nursery is indeed a common sign of PRRSV infection in a herd, with the presence of classical interstitial pneumonia, along with lesions typical of co-infections by common pulmonary agents such as *Mycoplasma hyopneumoniae, Mycoplasma hyorhinis, Pasteurella multocida* and *Streptococcus suis* (Goyal, 1993; Kobayashi et al., 1996). The presence of these pathogens in lungs of PRRSV-infected piglets has been taken as an indication that PRRSV infection can favour the establishment of opportunistic infectious agents (Dee & Joo, 1994; Molitor et al., 1997), although it is yet unclear whether PRRS does indeed lead to local immunosuppression (Drew, 2000; Samsom et al., 2000). One reason for this ambiguity is that the pathogenetic mechanisms of PRRS remain poorly defined. The purpose of the present study was to investigate the possible involvement of extracellular proteases in the pathogenesis of the immune lung disorders associated with PRRSV infection in pigs.

**Methods**

**Reagents and antibodies.** MMP-9 (gelatinase B) and MMP-2 (gelatinase A) were purified from supernatants of activated human monocytic cells (THP-1) by affinity chromatography on gelatin-Sepharose and their proteolytic activity was confirmed by fluorescence-activated substrate conversion (FASC), as described previously (St-Pierre et al., 1996). Coomassie brilliant blue G-250 was obtained from Bio-Rad. Gelatin (300 bloom, type A, from porcine skin) was purchased from Sigma. MAb IAF-K8, directed to a well-conserved epitope of the nucleocapsid protein of the North American IAF-Klop strain of PRRSV, was produced and purified from ascitic fluid of plasmone-primed mice inoculated with the secretory hybridoma (Dea et al., 1996). RNase H, Moloney murine leukaemia virus reverse transcriptase and Tag DNA polymerase, RT–PCR buffers, DNA ladders and cell culture media and antibiotics were all obtained from Life Technologies. RNA guard was obtained from Amersham Pharmacia Biotech.

**Animals.** Fifteen castrated crossbred F1 (Landrace × Yorkshire) specific-pathogen-free (SPF) piglets, 4–5 weeks old, were obtained from a breeding farm located in southern Quebec, Canada. Prior to shipping, the breeding stock and piglets were tested and proven to be seronegative for PRRSV, encephalomyocarditis virus, porcine parvovirus, haemagglutinating encephalomyelitis virus, transmissible gastroenteritis virus and *Mycoplasma hyopneumoniae*. Animals were also tested at the end of the experiment by multiplex PCR to confirm the absence of porcine circovirus type 2, *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis*, as described elsewhere (Ouadani et al., 1999; Caron et al., 2000). The piglets used in this study were from two different litters and were divided randomly into one control group and four experimental groups (three piglets per group) and kept in facilities equipped with a micro-organism-free, filtered in-flowing and out-flowing air systems. The animals were fed commercial feed and water ad libitum.

**Virus infection.** The Quebec cytopathogenic strain IAF-Klop of PRRSV (Mardassi et al., 1994), which has been propagated for 15–20 passages in MARC-145 cells, a clone of MA-104 cells highly permissive to PRRSV (Kim et al., 1993), was used in this study. Prior to experimental inoculation of SPF piglets, the tissue culture-adapted PRRSV strain was propagated twice in primary cultures of porcine alveolar macrophages (PAMs) to increase its virulence for pigs. PAMs were obtained by bronchoalveolar lavage (BAL), as described previously (Wensvoort et al., 1991). Virus infectivity titres of $10^7$ TCID$_{50}$/ml were determined for the virus stock used for inoculation of the 12 experimental piglets. These piglets were intratracheally under sedation with acepromazine/ketamine by using a laryngoscope and then monitored daily for clinical symptoms for a 3-week observation period before euthanasia. Their lungs, spleen, kidneys and mesenteric and thoracic lymph nodes were collected aseptically and processed for histopathology, RT–PCR and attempts at cultivation of PRRSV in MARC-145 cells. The three control piglets were mock-infected with virus-free culture medium.

**Bronchoalveolar lavage (BAL).** At days 3, 7, 14 and 42 post-infection (p.i.), three piglets were euthanized and their lungs were collected aseptically and filled with 100 ml PBS supplemented with 1% penicillin–streptomycin, 0.2% gentamycin, 1% anti-pplo (tylosin) and 0.4% amphotericin (fungizone). The PBS-filled lungs were then massaged and 35–45 ml of lavage fluid was obtained by applying gentle
Histological examination. Samples from thoracic and mesenteric lymph nodes were collected and kidney samples were also harvested for histopathological analysis. Infection; some were frozen, others were fixed in formalin. Spleen and specifically taken from dark-red, collapsed zones showing typical signs of centrifugation at 300 \( g \), for 20 min to remove cells. Lung samples were specifically taken from dark-red, collapsed zones showing typical signs of infection; some were frozen, others were fixed in formalin. Spleen and kidney samples were also harvested for histopathological analysis. Samples from thoracic and mesenteric lymph nodes were collected and frozen.

Histological examination. Thin sections (5 \( \mu m \) thick) of formalin-fixed, paraffin-embedded tissues from the lungs, spleen and kidneys of control and experimentally infected pigs were processed routinely for haematoxylin–eosin (H&E) staining, as described previously (Dea et al., 1991).

Measure of net proteolytic activity in BALs of PRRSV-infected pigs. The proteolytic activity of BALs was measured by FASC, as described previously (St-Pierre et al., 1996). The enzymatic reactions were performed at 37 °C for 18 h in a final volume of 100 µl serum-free RPMI as the reaction medium. Briefly, samples for analysis contained 88 µl BAL at various dilutions and 5 µl FITC-labelled substrate-coated microspheres. The volume was completed with serum-free RPMI – Tris–HCl (pH 9.5), 150 mM NaCl solution and the samples were kept on ice until analysis. To titrate the proteolytic activity of the BALs, serial dilutions ranging from 1/5 to 1/625 (v/v) were prepared. Affinity-purified human MMP-9 was used as a positive control. Flow cytometric analyses were performed on a Coulter XL-MCL using standard optics for detection of FITC fluorescence, as described previously (St-Pierre et al., 1996). The \( E_D_{50} \) corresponded to the dilution with 50% maximal proteolytic activity.

Virus titres are expressed as TCID\(_{50}\)/g tissue.

### Table 1. Detection of PRRSV genomic RNA in different organs by RT–PCR

Detection of genomic RNA is indicated as: +, RNA detected; ±, low levels of RNA detected; *+, RNA hardly detectable; –, no RNA detected.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Non-infected controls</th>
<th>PRRSV-infected piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days p.i.</td>
<td>7 days p.i.</td>
</tr>
<tr>
<td>Lung</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Spleen</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Kidney</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Thoracic lymph node</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

*ND, Not detected.

### Table 2. Virus titres in lungs and spleen

Virus titres are expressed as TCID\(_{50}\)/g tissue.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Non-infected controls</th>
<th>PRRSV-infected piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days p.i.</td>
<td>7 days p.i.</td>
</tr>
<tr>
<td>Lung</td>
<td>ND</td>
<td>( &gt; 10^{5.7} )</td>
</tr>
<tr>
<td>Spleen</td>
<td>ND</td>
<td>( 10^{4.2} )</td>
</tr>
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</table>

### Detection of MMPs by zymography. Gelatinolytic activity in the serum and BALs of PRRSV-infected piglets was determined by SDS–PAGE–gelatin zymography, as described previously (Aoudjit et al., 1997), with minor modifications. Briefly, aliquots of serum (diluted 1:10 in distilled water) or BALs (concentrated 20-fold by lyophilization) were mixed with 5 µl loading buffer (Bio-Rad, Laemmli loading buffer) and then analysed by electrophoresis on an 8% SDS–polyacrylamide gel containing 1 mg/ml denatured collagen. After electrophoresis, the gel was washed to remove SDS and incubated in a renaturing buffer (50 mM Tris, 5 mM CaCl\(_2\), 1% Triton X-100, 0.02% NaN\(_3\)) for 18 h at 37 °C. The gel was then stained with Coomassie brilliant blue and destained in 30:10:60 (by vol.) methanol/acetic acid/water. Gelatinolytic activity was identified as a clear band on a blue background. Quantitative analysis was carried out by using a computerized densitometric imager (model GS-670, Bio-Rad). Results were expressed as arbitrary scanning units.

### Virus isolation. Ten per cent homogenates of lung and spleen were prepared in serum-free DMEM, supplemented with 1% penicillin–streptavadin, 0.2% gentamycin and 0.4% fungizone, by using a Dremel MultiPro (model 395 type 5) apparatus. The homogenates were clarified by centrifugation at 3000 g for 30 min and then diluted further to 1/100, 1/1000 and 1/10000 in serum-free DMEM prior to inoculation onto confluent monolayers of MARC-145 cells in Limbro 24-well tissue culture plates. Following a 4 day incubation period at 37 °C, the virus was harvested by two freeze–thaw cycles of infected cultures. Aliquots of 200 µl clarified supernatant fluid were used for a subsequent passage on the same cell type and the cultures were then monitored daily for the presence of cytopathic effect. Virus titres were calculated by using the formula of Reed and Muench and expressed as TCID\(_{50}\)/g tissue.
Serological identification of PRRSV was obtained by indirect immunofluorescence staining with MAb IAF-K8, as described previously (Mardassi et al., 1994a).

- **RT–PCR and PCR experiments.** Total RNA was extracted from frozen tissue samples with the Tripure reagent (Roche), according to the manufacturer’s directions. Total RNA was resuspended in 20 µl diethyl pyrocarbonate-treated water and processed for RT–PCR as described previously (Mardassi et al., 1994b). The oligonucleotide primers used were VR7.1.1S (5’ ATGCCCAGCCAGTCATCA) and VR7.2.2AS (5’CGGATCAGGGCCAGTGAT), designed to amplify a 303 bp DNA fragment of the ORF7 gene of North American and European strains of PRRSV (Shin et al., 1998).

### Results

**Virus detection and isolation**

Before addressing the question of proteolytic activity during PRRSV infection, it was crucial to establish the presence of the virus in all organs taken from piglets that were inoculated with the virus and had experienced typical or atypical signs of PRRS. First, analyses were carried out by RT–PCR with primers amplifying the N protein gene sequence of the virus genome. Three mock-infected piglets were used as negative controls. The results showed that all infected piglets were positive for virus RNA at day 3 p.i. in all the organs tested (Table 1). The infection persisted throughout the following 2 weeks. At day 42 p.i., virus RNA was undetectable in specimens taken from most organs. It could still be detected, however, in the lungs and thoracic lymph nodes. Such a pattern of viremia is typical of PRRSV infection (reviewed by Albina, 1997).

In order to confirm the presence of PRRSV, two consecutive passages of lung and spleen homogenates from experimentally

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**Fig. 2.** Gelatinolytic activity in BALs (a–b) and serum (c–d) of PRRSV-infected piglets, as determined by zymography analysis. In panels (a) and (c), electrophoretic profiles in 8% SDS–polyacrylamide gels containing gelatin show two clear bands corresponding to the position of gelatinase A (MMP-2) and gelatinase B (MMP-9) in 20-fold concentrated BALs or serum from individual normal and PRRSV-infected piglets. In panels (b) and (d), densitometric analysis of SDS–polyacrylamide–gelatin gels indicates the levels of MMP-9 (shaded bars) and MMP-2 (open bars) in BALs. The controls used in this experiment were three mock-infected piglets. The two MMP-2 bands represent the pro-active and active forms of the enzyme. The single band in the MMP-2 controls represents the secreted pro-active form of human MMP-2 purified from THP-1 cells by affinity chromatography. The difference between the molecular mass of human and porcine pro-MMP-2 is due to species specificity.
infected piglets were performed on MARC-145 cells. Virus titres were expressed as the reciprocal of the highest dilution at which the presence of virus could still be detected by immunofluorescence. At 3 days p.i., the virus could be detected in lungs and spleen of all infected piglets (Table 2). It persisted with high titres for the following 2 weeks and then disappeared.
gradually. These results correlated with those obtained by RT–PCR.

**Proteolytic activity in BALs**

In order to determine whether PRRSV could modulate the repertoire of extracellular proteolytic enzymes in the lungs, serial dilutions of BALs ranging from $1/5$ to $1/625 \, (v/v)$ were prepared and cleavage of denatured collagen was measured by FASC, a method that allows quantitative measurements of the net proteolytic activity in biological fluids (St-Pierre et al., 1996). A mean of the values obtained for the three piglets at each day p.i. was calculated. The proteolytic activity found in the lungs of PRRSV-infected piglets at day 3 p.i. was similar to that of the controls (Fig. 1). A marked increase in net proteolytic activity was observed at days 7 and 14 p.i., however. The degradation of gelatin substrate was not inhibited significantly by elastase-specific inhibitors (data not shown). This increase in proteolytic activity was transient, as the level of activity progressively diminished and returned to normal at day 42 p.i. These results show that PRRSV infection increases the proteolytic activity significantly in the lungs.

**Measurement of collagenolytic activity**

Collagen is abundant in the lungs, as it forms the bronchovascular skeleton. It is also found in the alveolar septum and the lining of basal membranes. In order to determine whether the net increase in proteolytic activity observed could be correlated with an increase in collagenase activity, gelatin zymography was performed on BALs of PRRSV-infected piglets. The results indicated that, indeed, the levels of gelatinases A and B (MMP-2 and MMP-9, respectively) had increased significantly at days 7 and 14 p.i. (Fig. 2a–b). Densitometric analysis of the zymograms revealed that the collagenolytic activities at days 3 and 42 p.i. were comparable to those found in the mock-infected controls (Fig. 2a–b). The changes in MMP levels were restricted to the lungs, as no differences in MMP levels were detected in serum samples collected during the course of infection (Fig. 2c–d).

**Analysis of PRRSV-induced lesions**

In order to correlate the changes in proteolytic activity with the pathology of PRRSV-infected lungs, formalin-fixed, paraffin-embedded tissues were stained with H&E. PRRSV infection led to an infiltrant and proliferative pneumonia characterized by perivascular and peribronchiolar infiltrate, as has been commonly described (reviewed by Albina, 1997). Typical examples of the interstitial pneumonia caused by PRRSV infection are shown in Figs 3(d–f) and 4. In more advanced stages, the lungs were filled with lymphoid cells and adopted a glandular aspect (Fig. 4a). At such stages, pyknotic cells and oedema fluid could also be seen in the infiltrates (Fig. 4b; Table 3). Light interstitial pneumonia and light hyperplasia of type II pneumocytes was observed at days 3 and 7 p.i., the pneumonia and hyperplasia being most severe at day 14 p.i. A milder interstitial pneumonia was noticed at day 42 p.i., although it was still more severe than that found at day 3 p.i. Other signs of infection could also be observed at days 7 and 14 p.i. (Table 3). In the kidneys, the glomeruli were more cellular and proteinaceous debris was found in the tubules. Focal activation of lymphocytes could also be observed in various areas of the spleen. These results showed a correlation between the increase in proteolytic activity and the severity of histopathological manifestations of the disease.

**Discussion**

In the present study, we demonstrated that (i) intratracheal injection of PRRSV induces a significant increase in the proteolytic activity of cells in the pulmonary fluids of infected piglets. This increase correlates with the pathology of interstitial pneumonia caused by PRRSV infection. The results suggest that PRRSV-induced lesions are mediated by an increase in proteolytic activity, which may play a role in the pathogenesis of the disease. Further studies are needed to elucidate the molecular mechanisms underlying this process.
Table 3. Histological findings in infected organs

Severity of lesions is scored as: —, normal appearance; ±, light; +, moderate; ++, bad; ++++, severe.
A solidus represents a range of severity, e.g. +/++ indicates that severity of lesions ranged from moderate to bad. Abbreviations: alv., alveole or alveolar; NA, not available. Control piglets were normal for all criteria.

<table>
<thead>
<tr>
<th>Microscopic lesion</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>42</th>
</tr>
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<tbody>
<tr>
<td>Intersitial pneumonia</td>
<td>+</td>
<td>+/++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Pyknotic cells in septae</td>
<td>±</td>
<td>±</td>
<td>+/++</td>
<td>+</td>
</tr>
<tr>
<td>Protein-rich exudate (alv. and bronchioles)</td>
<td>−</td>
<td>−/++</td>
<td>±</td>
<td>−/++</td>
</tr>
<tr>
<td>Macrophages and debris (alv. and alv. ducts)</td>
<td>−</td>
<td>−/++</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Hyperplasia (pneumocytes type II)</td>
<td>+</td>
<td>+/++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Necrotizing bronchiolitis</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Macrophages and debris (bronchioles)</td>
<td>−</td>
<td>±</td>
<td>+/++</td>
<td>+</td>
</tr>
<tr>
<td>Hyperplasia (bronchiolar epithelium)</td>
<td>+</td>
<td>+/++</td>
<td>+/++</td>
<td>+</td>
</tr>
<tr>
<td>Peribronchioral infiltration</td>
<td>+</td>
<td>+</td>
<td>+/++</td>
<td>+/++</td>
</tr>
<tr>
<td>Perivasular infiltration</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Necrotizing bronchitis</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Macrophages and debris (large bronchi)</td>
<td>−</td>
<td>±</td>
<td>−</td>
<td>±</td>
</tr>
<tr>
<td>Enlarged glomeruli (kidney)</td>
<td>−</td>
<td>±</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Protein-rich exudate in tubules (kidney)</td>
<td>−</td>
<td>±</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Focal activation of lymphocytes (spleen)</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>NA</td>
</tr>
</tbody>
</table>

piglets, (ii) the increase in proteolytic activity is transient, being maximal between days 7 and 14 p.i., with a return towards normal levels at 6–7 weeks p.i., (iii) a significant increase in the secretion of both MMP-2 and MMP-9 is observed in the BALs, but not in the serum, of infected piglets and (iv) the increase in proteolytic activity is concomitant with the appearance of severe histological lung lesions in infected piglets.

The results showed clearly that both MMP-2 and MMP-9, but not leukocyte elastase, were upregulated in the lungs of PRRSV-infected piglets. This pattern of proteolytic activity was not unexpected, as elastase-rich BALs are mostly associated with neutrophil infiltration, as observed in emphysema and cystic fibrosis (Greenberger, 1997). In fact, the detection by 7–14 days p.i. of increased amounts of extracellular MMP-2 and MMP-9 proteolytic enzymes in BALs, two proteases commonly secreted by cells of the lymphocytic/monocytic lineage, corroborates recent observations by other investigators, who reported that the numbers of lymphocytes and monocytes increased considerably in the lungs of PRRSV-infected piglets from day 2 until day 21 p.i. (Shimizu et al., 1996; Samsom et al., 2000). Indeed, Beyer et al. (1998) have shown that there is an increase in the alveolar macrophage chemokine AMCFII in the lungs following PRRSV infection. The increased secretion of both MMPs was detectable only in pulmonary fluids and could not be detected in serum samples, indicating that modulation of the immune response during PRRS is only locally affected.

There have been some indications that PRRSV infection is associated with local immunosuppression that favours opportunistic infections (Dee & Joo, 1994; Molitor et al., 1997; Drew, 2000). One possible reason for this immunosuppression is the fact that the virus can compromise the immune system of infected pigs temporarily through its ability to infect alveolar macrophages (Oleksiewicz & Nielsen, 1999; Zhang et al., 1999; Chiou et al., 2000). At the molecular level, the effect of PRRSV on PAMs might result from the ability of the virus to alter the cellular transcriptome. This possibility is supported by the recent study by Zhang et al. (1999), who showed that four gene transcripts were induced following infection of PAMs by PRRSV, one of which was a gene encoding a ubiquitin-specific protease that regulates protein trafficking and degradation. Our data, showing that the virus infection induces a rapid increase in the proteolytic activity of BALs, reveal another mechanism that may compromise the pulmonary immune response of PRRSV-infected pigs for several days, since such proteolytic enzymes can lead to the cleavage of key molecules involved in development of the immune response (Döring et al., 1995; Champagne et al., 1998). Further experiments will be necessary, however, to determine whether such molecules are indeed cleaved upon PRRSV infection and to identify those that are more susceptible to the proteolytic activities of MMP-2 and MMP-9. The fact that the increased proteolytic activity of BALs could not be associated with upregulated elastolytic activity suggests, however, that local expression of molecules such as CD4, CD8 and ICAM-1 could be intact during the infection process (Champagne et al., 1998).

A number of studies have shown the presence of both PRRSV and mycoplasmas in the lungs of dyspneic piglets.
(Thacker et al., 1999). Although it can be postulated that the increase in proteolytic activity of BALs induced upon PRRSV infection may favour secondary mycoplasmal infection, further studies are required in order to determine whether mycoplasmal infections may also lead to increased proteolytic activity in the lungs that could potentiate the effects of PRRSV, as suggested recently following vaccination against both agents.

In conclusion, the results obtained in the present study provide preliminary evidence that PRRSV pulmonary infection can modulate local proteolytic activity, mainly a significant increase in the secretion of both MMP-2 and MMP-9 by cells of the lymphocytic/monocytic lineage associated previously with pulmonary dysfunction (O'Connor & FitzGerald, 1994; Kumagai et al., 1999). These results contribute to a better understanding of the molecular mechanisms underlying PRRS, particularly pulmonary dysfunction and transient immunodeficiency.

The authors wish to thank Hélène Drolet, Nicole Sawyer, Doris Legault and Marcel Desrosiers for their excellent technical assistance. Particular thanks go to Dr Edouard F. Potworowski for comments on the manuscript. This report was taken in part from a dissertation to be submitted by M.G. to the Centre de Recherche en Santé, INRS–Institut Armand-Frappier, in partial fulfilment of the requirements for the MSc degree. This work was supported by the Natural Sciences and Engineering Research Council of Canada (strategic grant STP0202083) and Biovet Inc., St-Hyacinthe, Quebec, Canada. M.G. was a recipient of a student grant from the Fondation Armand-Frappier. Y.S-P. is a scholar of the Fonds de la Recherche en Santé du Québec.

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Received 29 September 2000; Accepted 30 January 2001