Effect of cellular changes and onset of humoral immunity on the replication of porcine reproductive and respiratory syndrome virus in the lungs of pigs

Geoffrey G. Labarque, Hans J. Nauwynck, Kristien Van Reeth and Maurice B. Pensaert

Laboratory of Virology, Faculty of Veterinary Medicine, University of Gent, Salisburylaan 133, B-9820 Merelbeke, Belgium

Twenty-two 4- to 5-week-old gnotobiotic pigs were intranasally inoculated with $10^{6.0} \text{TCID}_{50}$ of porcine reproductive and respiratory syndrome virus (PRRSV) (Lelystad) and euthanized at different time intervals post-inoculation (p.i.). Bronchoalveolar lavage (BAL) cell populations were characterized, together with the pattern of virus replication and appearance of antibodies in the lungs. Total BAL cell numbers increased from $140 \times 10^6$ at 5 days p.i. to $948 \times 10^6$ at 25 days p.i. and remained at high levels until the end of the experiment. The number of monocytes/macrophages, as identified by monoclonal antibodies 74-22-15 and 41D3, increased two- to fivefold between 9 and 52 days p.i. with a maximum at 25 days p.i. Flow cytometry showed that the population of differentiated macrophages was reduced between 9 and 20 days p.i. and that between the same time interval, both 74-22-15-positive and 41D3-negative cells, presumably monocytes, and 74-22-15- and 41D3-double negative cells, presumably non-phagocytes, entered the alveolar spaces. Virus replication was highest at 7 to 9 days p.i., decreased slowly thereafter and was detected until 40 days p.i. Anti-PRRSV antibodies were detected starting at 9 days p.i. but neutralizing antibodies were only demonstrated in one pig euthanized at 35 days and another at 52 days p.i. The decrease of virus replication in the lungs from 9 days p.i. can be attributed to (i) shortage of susceptible differentiated macrophages, (ii) lack of susceptibility of the newly infiltrated monocytes and (iii) appearance of anti-PRRSV antibodies in the lungs. Neutralizing antibodies may contribute to the clearance of PRRSV from the lungs.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first described in the United States in 1987 as a new viral disease of swine (Hill, 1990) and appeared in Europe in 1990 (Terpstra et al., 1991). Since then, the virus has spread to all the major swine-producing countries worldwide. Porcine reproductive and respiratory syndrome virus (PRRSV), the causative agent of the disease, was first isolated in the Netherlands in 1991 (Wensvoort et al., 1991) and subsequently in the United States in 1992 (Collins et al., 1992). A PRRSV infection in swine is clinically characterized by reproductive failure in sows and gilts and respiratory distress in young pigs. However, overt respiratory signs are difficult to reproduce and in most experimental studies the infection is subclinical or a transient fever is the only clinical sign (Van Reeth et al., 1996). Based on the similarity in morphology, genomic organization and strategy of gene expression, PRRSV has been classified as a member of the family Arteriviridae (Cavanagh, 1997). All the viruses of this family have common features in so far that (i) cells of the monocyte/macrophage lineage are the primary or the only target cells in vivo and that (ii) they cause persistent infections in their respective hosts (Plagemann & Moennig, 1992).

PRRSV has a strong tropism for cells of the monocyte/macrophage lineage. However, replication of the virus in these cells is subject to several restrictions. It is confined to well-differentiated cells of the monocyte/macrophage lineage in lungs and lymphoid tissues and is not detected in progenitor cells such as bone marrow cells and peripheral blood monocytes (Duan et al., 1997b). This restricted cell tropism for PRRSV is partly due to the expression of the putative PRRSV receptor on the membrane of susceptible pulmonary alveolar macrophages (PAMs), which is not detectable on refractory peripheral blood monocytes (Duan et al., 1998b). Also, PRRSV replicates only at a certain stage of differentiation and maturation of the alveolar macrophages (Choi et al., 1994;
Duan et al., 1997b). The complex effect of phenotype on the susceptibility of alveolar macrophages probably forms the basis for the low percentage of PRRSV antigen-positive bronchoalveolar lavage (BAL) cells after infection, even in the phase when virus replication in the lungs is most extensive (Mengeling et al., 1995; Duan et al., 1997a).

PRRSV is able to persist in different organs. It can be isolated in lungs until 35 (end of the study) (Duan et al., 1997a) to 49 days after inoculation (Mengeling et al., 1995), in tonsils until 21 days after inoculation (Duan et al., 1997a), in semen until 92 days after inoculation (Christopher-Hennings et al., 1995) and in serum until 150 days after inoculation (Wills et al., 1997), and persistence occurs despite the onset of a PRRSV-specific humoral and cell-mediated immune response. The mechanism of persistence is unknown.

The purpose of the present experiments was to study a number of pathogenic events in detail in the lungs during a PRRSV infection in gnotobiotic pigs. The BAL cell population was examined, consisting of quantification of cells, determination of cell viability, morphological and phenotypical characterization of different subpopulations with specific attention to the population of cells of the monocyte/macrophage lineage and determination of presence of the putative PRRSV receptor, and these events were correlated with virus replication (titration and quantification of viral antigen-positive cells) and appearance of neutralizing and non-neutralizing antibodies. These studies show that a single PRRSV infection causes multiple marked changes in the lungs even though clinical signs remain absent.

Methods

- **Virus strain.** PRRSV (Lelystad) (Wensvoort et al., 1991) was used in the present study. Virus used for inoculation was at the fifth passage in PAMs, which had been obtained from 4- to 6-week-old gnotobiotic pigs.

- **Pigs and inoculation.** Twenty-nine caesarean-delivered colostrum-deprived (CDCD) pigs were used. They were housed in isolation facilities. Twenty-two pigs were intranasally inoculated at the age of 4 to 5 weeks with 10⁶ TCID₅₀ Lelystad virus in 3 ml PBS (1:5 ml in each nostril). The remaining seven pigs were left uninoculated and served as negative controls. One to three of the PRRSV-inoculated pigs were euthanized at 1 (4 weeks of age), 3. 5, 7 (5 to 6 weeks of age), 9, 14 (6 to 7 weeks of age), 20 (7 to 8 weeks of age), 25, 30, 35 (9 to 10 weeks of age), 40 and 52 (12 weeks of age) days post-inoculation (p.i.) by intraperitoneal injection with an overdose of barbiturates (sodium pentobarbital, 20%; IC KELA).

  The control pigs were euthanized at 4, 5, 6, 8 and 10 weeks of age. The right lung was used for bronchoalveolar lavage and samples from the left apical, cardiac and diaphragmatic lung lobes were collected for virological examinations (virus titration and quantification of viral antigen-positive cells).

- **BAL cell analysis.** The right lung was lavaged with 60 to 120 ml Dulbecco’s PBS without Ca²⁺ and Mg²⁺ via an 18 gauge blunt needle inserted through the trachea. The left main bronchus was cross-clamped to prevent lung lavage fluid from entering the left lung. About 75 to 90% of the initial volume of the lavage fluid was recovered. The BAL fluid was centrifuged (400 g, 10 min, 4 °C) to separate the cells and the cell-free lavage fluid.

  Fractions of the cell-free lavage fluid were either stored at —70 °C until virus titration on PAMs or concentrated 10 times by dialysis against a 20% (w/v) solution of polyethylene glycol (mol. mass 20000) and cleared of residual virus by ultracentrifugation at 100000 g (Van Reeth et al., 1999) for determination of anti-PRRSV antibodies.

  Cell pellets were resuspended in PBS and the total number of BAL cells was determined. Cell viability was assessed using trypan blue dye exclusion. Cytocentrifuge preparations of BAL cells were made by centrifuging at 140 g for 5 min. One preparation was stained with DiffQuik (Baxter, Düdingen, Switzerland) to determine the percentage of mononuclear cells and neutrophils; another was fixed in acetone for 20 min at —20 °C to determine the percentage of viral antigen-positive cells using a streptavidin–biotin immunofluorescence technique. Cells were incubated first with a pool of monoclonal antibodies (MAbs) against the PRRSV nucleocapsid protein (dilution 1/100 of WB1 and WB4-6) (Drew et al., 1995), subsequently with 1/100 biotinylated sheep antimmunofluorescence technique. Cells were incubated first with a pool of monoclonal antibodies (MAbs) against the PRRSV nucleocapsid protein (dilution 1/100 of WB1 and WB4-6) (Drew et al., 1995), subsequently with 1/100 biotinylated sheep antimouse antibodies (Amersham), and then with 1/100 streptavidin–fluorescein isothiocyanate (FITC) (Amersham). Finally, cells were washed, mounted in a glycerin–PBS solution (0-9:1; v/v) with 2.5% 1,4-diazabicyclo(2.2.2)octane (DABCO) (Janssen Chimica) and viral antigen-positive cells were counted by fluorescence microscopy (Leica DM RBE). The specificity was confirmed by absence of fluorescence in BAL cells of unoinoculated control pigs.

  Flow cytometric analyses of the BAL cells were done with a Becton–Dickinson FACS caliber equipped with a 15 mW air-cooled argon ion laser and interfaced to an Apple Macintosh Quadra 650 computer using BD Cellquest software. Three parameters were stored for further analysis: forward light scattering (FSC), sideward light scattering (SSC) and green fluorescence (FL1). At least 10000 cells were analysed for each sample.

  The specificity of identification of neutrophils and cells of the monocyte/macrophage lineage, MAb 74-22-15 (Pescovitz et al., 1984) was used. The percentage of cells of the monocyte/macrophage lineage was determined by subtracting the percentage of neutrophils determined by DiffQuik from the 74-22-15-positive cells. The percentage of BAL cells expressing the putative PRRSV receptor on their cell membrane was determined using MAb 41D3 (Duan et al., 1998a). The reactivity of both MAbs against the specific cell-surface determinants was flow cytometrically evaluated by an indirect immunofluorescence technique. To determine the number of 74-22-15- and 41D3-positive BAL cells, 5 × 10³ cells were incubated with 1/300 74-22-15 or 1/1000 41D3 respectively for 1 h at 4 °C. Subsequently, the BAL cells were incubated with 1/30 FITC-labelled goat anti-mouse IgG (Molecular Probes) for 1 h at 4 °C. Three washings were done with cold PBS before and after each incubation. BAL cells which were incubated with FITC-labelled goat anti-mouse IgG only were included as controls.

- **Virological examinations of lung tissue and BAL fluid.** Twenty percent suspensions of lung tissue were made with cold PBS. The suspensions were clarified by centrifugation. Fifty µl of tenfold serial dilutions of the supernatants of the lung suspensions and of the cell-free lavage fluids was inoculated on 1 day cultured PAMs, which were obtained from 4- to 6-week-old pigs from PRRSV-negative farms. After incubation for 1 h at 37 °C, the samples were replaced by medium. After 72 h at 37 °C, the PAMs were washed once with PBS and further stained using an immunoperoxidase monolayer assay (IMPA) as described by Wensvoort et al. (1991).

  Tissue samples from the lungs were embedded in methylene cellulose medium and frozen at —70 °C. Cryostat sections (5 to 8 µm) were made and fixed in acetone for 20 min at —20 °C. A streptavidin–biotin
immunofluorescence technique, similar to that described for BAL cells, was used to stain and localize the viral antigen-positive cells in the lung tissue.

**Serological examination.** Anti-PRRSV antibody titres were determined in sera and BAL fluids using the IPMA technique described by Wensvoort et al. (1991). IPMAs with MARC-145 cells were set up to determine the immunoglobulin (Ig) isotypes of the PRRSV-specific antibodies. Briefly, MARC-145 cells were seeded in 96-well cell culture plates, inoculated with 50 µl of a Belgian isolate of PRRSV (94V360) and incubated for 18 h at 37 °C (5% CO₂). Then, the culture medium was removed, and cells were washed in PBS and dried at 37 °C for 1 h. The plates were kept at −70 °C until use. Plates were thawed and then fixed in 4% paraformaldehyde for 10 min. The paraformaldehyde was removed, the cells washed twice with PBS and a solution of 1% H₂O₂ in methanol was added. Plates were washed twice with PBS and serial fourfold dilutions of the sera and BAL fluids were added. Sera and BAL fluids were incubated for 1 h at 37 °C. Plates were washed three times with PBS plus 1% Tween 80 and 50 µl of 1/10 mouse anti-swine IgG1, 1/10 mouse anti-swine IgG2, 1/100 mouse anti-swine IgM and 1/100 mouse anti-swine IgA (Van Zaane & Hulst, 1987) respectively were added per well, incubating at 37 °C for 1 h. Plates were washed three times and 50 µl of 1/3000 goat anti-mouse antibodies conjugated with peroxidase (Dako) was added per well, incubating at 37 °C for 1 h. Plates were washed three times and 50 µl of a substrate solution of 3-amino-9-ethylcarbazole in 0.05 M acetate buffer, pH 5, with 0.05% H₂O₂ was added to each well, incubating at room temperature for 20 min. Then, the reaction was blocked by replacing the substrate by acetate buffer and the results were determined by examination with a microscope.

Neutralizing antibodies were determined in sera and BAL fluids using a virus neutralization test on MARC-145 cells as described by Swenson et al. (1994). A MARC-145-adapted Lelystad strain was used in this assay.

### Results

#### BAL cell analysis

Total BAL cell numbers of unoinculated control pigs ranged between 114 and 383 × 10⁶. BAL cells consisted of 97 to 98% of cells of the monocyte/macrophage lineage, 1% of neutrophils and 1 to 2% of non-phagocytes, presumably lymphocytes (BAL cells – 74-22-15-positive cells). Cell viability of BAL cells ranged between 93 and 98%. BAL cell populations in PRRSV-infected pigs are shown in Fig. 1. Their number and composition were similar to those of the uninoculated control pigs during the first 5 days p.i. Mean BAL cell numbers increased from 140 × 10⁶ at 5 days p.i. to 948 × 10⁶ at 25 days p.i. and then remained at high levels until the end of the experiment with numbers ranging between 642 and 782 × 10⁶. The absolute number of cells of the monocyte/macrophage lineage, as identified by MAb 74-22-15, increased two- to fivefold between 9 and 52 days p.i. with a maximum at 25 days p.i. BAL cells consisted of 55 to 92% of cells of the monocyte/macrophage lineage, 1 to 15% of neutrophils (33% in one pig euthanized at 9 days p.i.) and 6 to 31% of non-phagocytes. Cell viability of BAL cells ranged between 84 and 98%.

In the uninoculated control pigs, the percentage of BAL cells with the putative PRRSV receptor (41D3-positive cells) varied between 93 and 95%. In PRRSV-infected pigs, their percentage was similar to that of the controls during the first 3 days p.i. At 5, 7, 9, 14, 20 and 25 days p.i. respectively, 41D3-positive BAL cells represented 83 ± 1, 63 ± 5, 38 ± 14, 41 ± 9, 53 ± 0 and 75 ± 4% of total BAL cells. From 30 days p.i., the percentage of 41D3-positive BAL cells reached the percentage of cells of the monocyte/macrophage lineage.

The different pattern of light-scattering characteristics [cell size (FSC) and granularity (SSC)] of the BAL cell population throughout a PRRSV infection is presented in Fig. 2 (dot-plot diagrams). In the uninoculated control pigs, the majority of BAL cells were flow-cytometrically recognized as large (high FSC value) (population P1) and small (low FSC value) (population P2) cells with a strong granularity (high SSC value). Based on their light-scattering properties and their surface expression of a specific monocyte/granulocyte marker (recognized by MAb 74-22-15) and of the putative PRRSV receptor (recognized by MAb 41D3), these two cell populations were characterized as cells of the monocyte/macrophage lineage. The particles with the lowest light-scattering (population P3) were characterized as non-phagocytes (74-22-15- and 41D3-double negative cells) and fragments of cells of the monocyte/macrophage lineage (74-22-15-single positive and 74-22-15- and 41D3-double positive). The changes of the number of cells of BAL cell populations P1, P2 and P3 throughout the infection are presented in Fig. 3. In PRRSV-infected pigs, the light-scattering characteristics of the BAL cell population were similar to those of the uninoculated control pigs during the first 5 days p.i. Between 9 and 20 days p.i., the number of 41D3-positive cells of the monocyte/macrophage lineage in populations P1 and P2 was reduced. From 7 days p.i., an increase of very small particles with low light-scattering was observed (population P4) and these particles stained positive for both 74-22-15 and 41D3.

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**Fig. 1.** Quantification and characterization of BAL cells throughout a PRRSV infection. * Cells of the monocyte/macrophage lineage = 74-22-15-positive cells — neutrophils. ** Lymphocytes = BAL cells — 74-22-15-positive cells.
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Fig. 2. Different patterns of light-scattering characteristics [FSC (cell size) and SSC (granularity)] of the BAL cell population throughout a PRRSV infection.

present in the BAL fluid. They exceeded the maximal FSC and SSC values shown on the dot-plot diagrams in Fig. 2 (population P5). During the same time interval, populations of small cells were entering the alveolar spaces. The slightly granulated (low SSC value) cells (population P6) were characterized as, on the one hand, 74-22-15-positive and 41D3-negative cells, representing probably monocytes, and on the other hand 74-22-15- and 41D3-double negative cells, representing probably non-phagocytes (presumably lymphocytes). The strongly granulated cells (high SSC value) were only observed at 9 and 14 days p.i. (population P7). These cells were also 74-22-15-positive and 41D3-negative. From 25 until 52 days p.i., the majority of BAL cells were recognized as 74-22-15- and 41D3-positive cells of the monocyte/macrophage lineage and 74-22-15- and 41D3-double negative cells, presumably lymphocytes.

Virological examinations of lung tissue and BAL fluid

The results of virus titrations of lung tissue and BAL fluid are presented in Fig. 4. All uninoculated control pigs were negative for PRRSV. In the inoculated pigs, PRRSV titres of apical, cardiac and diaphragmatic lung lobes were similar and the pattern of virus titres in lung tissue resembled that in BAL fluid. The highest virus titres were reached in BAL fluid at 7 days p.i. \((10^{7.3} \text{ TCID}_{50}/\text{ml})\) and in lung tissue at 9 days p.i.
Pathogenesis of PRRSV infection in lungs

Fig. 3. Changes in the number of cells of BAL cell populations P1, P2 and P3 throughout a PRRSV infection.

Fig. 4. Course of PRRSV titres in lung tissue (log\textsubscript{10} TCID\textsubscript{50}/g) and BAL fluid (log\textsubscript{10} TCID\textsubscript{50}/ml) throughout a PRRSV infection.

(10\textsuperscript{7.1} TCID\textsubscript{50}/g). Afterwards, virus titres decreased slowly during the next 5 weeks. Virus was not detected in the pig euthanized at 52 days p.i.

Viral antigen-positive BAL cells were first observed at 1 day p.i., increased to a maximum of 3% at 9 days p.i., decreased to 0.5% at 14 days p.i. and remained at levels of 0.1 to 0.2% until 40 days p.i. The quantification of viral antigen-positive cells and/or foci in lung tissue throughout a PRRSV infection is presented in Fig. 5. Single viral antigen-positive cells were observed from 3 until 35 days p.i. with a maximal number of 45 cells/100 mm\textsuperscript{2} lung tissue at 7 days p.i. Viral antigen-positive foci were defined as areas in lung tissue consisting of groups of viral antigen-positive cells and cellular debris. Viral antigen-positive foci were found from 3 until 14 days p.i. with a maximal number of 37 foci/100 mm\textsuperscript{2} lung tissue at 9 days p.i.
The intact viral antigen-positive cells in lung tissue and BAL fluid were morphologically recognized as macrophage-like cells. Viral antigen-positive cells were not observed in lung tissue and BAL fluid of uninoculated control pigs.

**Antibodies in sera and BAL fluids**

The antibody titres against PRRSV in sera and BAL fluids of PRRSV-inoculated pigs are presented in Fig. 6 (on a logarithmic scale). PRRSV-specific antibodies in sera and BAL fluids were first detectable by the IPMA at 9 days p.i. IPMA titres in serum rose to 10240 ($2^{10}$) at 20 days p.i., whereas IPMA titres in BAL fluid rose to 2560 ($2^{11}$) at 25 days p.i. The distribution of the immunoglobulin isotypes of PRRSV-specific antibodies in sera and BAL fluids throughout the infection is presented in Fig. 7 (on a logarithmic scale). The first detectable IPMA antibodies in sera and BAL fluids of PRRSV-inoculated pigs were antibodies of the IgM and IgG isotype (IgG1 subclass). IgM antibodies in sera and BAL fluids were detected only at 9 and 14 days p.i., whereas IgG antibodies were detected until 52 days p.i. IgG1 IPMA titres in sera and BAL fluids followed the curve of total IPMA titres. The curve of IgG2 IPMA titres in sera and BAL fluids was similar to that of IgG1 IPMA titres, but was at a lower level. IgA antibodies in sera were detected starting from 14 days p.i., increased to a maximum of 1280 ($2^{10}$) at 25 days p.i. and were detected until 35 days p.i. IgA antibodies in BAL fluids were highest at 14 days p.i. ($2^{10}$) and were detected until 35 days p.i.

Neutralizing antibodies in sera were detected from 25 days p.i. The titres remained at a low level ($2^{1-3}$) until the end of the study. Neutralizing antibodies in BAL fluids were only detected in two pigs, one euthanized at 35 days and one at 52 days p.i.

**Discussion**

The present study showed clear relations between PRRSV replication, morphological and phenotypical changes in the BAL cells and onset and presence of non-neutralizing and neutralizing antibodies.

The most striking morphological and phenotypical changes in the BAL cell population consist of (i) the reduction of the population of susceptible well-differentiated macrophages and (ii) the massive influx of both 74-22-15-positive and 41D3-negative cells of the monocyte/macrophage lineage, probably monocytes, and 74-22-15- and 41D3-double negative cells, probably non-phagocytes.

Between 9 and 20 days p.i., a reduction of the population of
41D3-positive cells of the monocyte/macrophage lineage was observed. The reduction of this cell population is probably caused by a combination of cell lysis due to virus replication and apoptosis. The highest virus titres and number of viral antigen-positive cells in lungs and BAL fluids were indeed detected at 7 to 9 days p.i. and apoptosis in the lungs between 1 and 10 days p.i. was demonstrated earlier by Sur et al. (1998) and Sirinarumitr et al. (1998). These authors reported that apoptotic cells were pulmonary alveolar and intravascular macrophages and mononuclear cells in the alveolar septa and showed that they were more abundant than viral antigen-positive cells. The increased number of very small and slightly granulated particles, which stained positive for both 74-22-15 and 41D3, in the BAL cell population from 7 days p.i. on, may represent an increase of lysed infected macrophages and apoptotic bodies from macrophages in apoptosis. The very large and strongly granulated cells, found in the BAL fluid from 7 until 20 days p.i., may represent strongly activated macrophages phagocytizing the apoptotic bodies. The biological significance of apoptosis in the pathogenesis of a PRRSV infection remains to be clarified.

Throughout the PRRSV infection in the lungs of gnotobiotic pigs the total number of BAL cells continuously increased from 5 days p.i. until 25 days p.i., mainly due to an influx of both 74-22-15-positive and 41D3-negative cells, probably monocytes, and 74-22-15- and 41D3-double negative cells, probably non-phagocytes (lymphocytes). This observation is supported by the flow cytometric analysis of the BAL cell population, evaluating the changes of size (FSC), granularity (SSC) and presence of markers on the cell surface throughout the infection. The mechanism by which PRRSV induces the specific influx of monocytes is not yet known, but Van Reeth et al. (1999) suggested that chemotactic cytokines produced by PRRSV-infected macrophages mediate the influx of new cells of the monocyte/macrophage lineage.

Despite the continuous increase in the number of cells of the monocyte/macrophage lineage from 9 until 25 days p.i., the number of viral antigen-positive cells in lung tissue and BAL fluid decreased from 9 days p.i. This is probably the result of (i) shortage of susceptible well-differentiated lung macrophages (see above), (ii) lack of susceptibility of the newly infiltrated blood monocytes (Duan et al., 1997b) and (iii) appearance of anti-PRRSV antibodies in the lungs. It is remarkable that viral antigen-positive cells and cellular debris were mainly localized in foci in lung tissue until 14 days p.i. and that, thereafter, only single viral antigen-positive cells were observed. It is possible that infected cells in foci are destroyed by antibody-dependent cell lysis. Single viral antigen-positive cells, morphologically recognized as macrophage-like cells, were observed until 35 and 40 days p.i. in lung tissue and BAL fluid respectively. These single viral antigen-positive cells were the source of the virus detected in lung tissue and BAL fluid until 40 days p.i. Why these single viral antigen-positive cells are able to persist despite the presence of the humoral immunity is not known, but may be attributable to either the cell or the virus. Therefore, a full phenotypical characterization of this specific subpopulation of cells will be performed. An alternative explanation may be the appearance of so-called quasispecies throughout the PRRSV infection. A recent study by Rowland et al. (1999) revealed the emergence of a distinct PRRSV subpopulation during infection of pigs, identified by a single amino acid change in the ectodomain of glycoprotein 5. Studies using lactate dehydrogenase-elevating virus of mice, another member of the family Arteriviridae, demonstrated how a small number of mutations in the ectodomain of the ORF 5 protein can alter the cell tropism and the interaction of the virus with neutralizing antibodies, favouring persistence of the virus in its host (Plagemann, 1996; Chen et al., 1997).

Immunoglobulin M, A, G1 and G2 (IgM, IgA, IgG1 and IgG2) antibodies were all involved in the specific humoral immune response to PRRSV. IgM antibodies appear rather late during a PRRSV infection, which is in contrast with infections with other respiratory viruses such as influenza virus (Lee et al., 1995) and Aujeszky's disease virus (Rodak et al., 1987), where IgM antibodies were detected as early as 3 and 7 days p.i. respectively. The kinetics of the isotypes of anti-PRRSV antibodies in BAL fluids were similar to those in sera, indicating that these antibodies are the result of a leakage from systemic antibodies.

Clearance of PRRSV from the lungs coincided with the appearance of neutralizing antibodies in sera and BAL fluids. However, since low amounts of PRRSV remain in the lungs in spite of the presence of neutralizing antibodies in sera and BAL fluids, other immune factors or mechanisms such as cell-mediated immunity are probably involved in the complete elimination of the virus at this site. Why neutralizing antibodies appear so late in infection and remain at rather low levels (titres ranging between 2 and 12) is not yet known.

Based on the results of the present study, a hypothetical model of the series of events in the pathogenesis of a PRRSV infection in the lungs of gnotobiotic pigs can be made. After inoculation, primary replication of the virus takes place in well-differentiated lung macrophages. Subsequently, a reduction of the resident macrophages takes place, which is accompanied with an influx into the lungs of new cells of the monocyte/macrophage lineage, which are initially refractory to PRRSV. Lack of susceptible cells and the appearance of PRRSV-specific antibodies cause a decrease in the number of viral antigen-positive cells starting at 9 days p.i. Nevertheless, a low number of single viral antigen-positive cells persists in the lungs for several weeks. Clearance of PRRSV from the lungs coincides with the appearance of specific neutralizing antibodies. Most likely, other mechanisms such as cell-mediated immunity, are necessary for complete elimination.

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