BACKGROUND PAPER

THE APPEARANCE OF THE PORCINE RESPIRATORY CORONAVIRUS HAS CREATED NEW PROBLEMS AND PERSPECTIVES

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In 1984, the appearance of a new porcine coronavirus related to TGEV was suspected based on an unexpected but widespread prevalence of antibodies to TGEV found in the swine population of several European countries. Most remarkable was the observation that this widespread occurrence of TGEV antibodies was seen in the absence of previous outbreaks or epizootics of diarrhea and also the fact that such findings were made in countries, e.g. Denmark, which had never experienced TGEV infection previously. The ease with which the virus spread, not only within a country, but also across national borders, pointed towards long distance virus transmission, almost certainly by the aerogenic route.

The virus involved was isolated in 1986 from the respiratory tract of swine and was found to be a coronavirus very closely related to the well-characterised TGEV. It was called porcine respiratory coronavirus (PRCV). Soon after its isolation, comparative antigenic studies between PRCV and TGEV were performed with the use of monoclonal antibodies which had been prepared against different epitopes of the Purdue strain of TGEV. It was found that in the TLM strain of PRCV three peplomer S epitopes, present in TGEV, are lacking or have been modified. Monoclonal antibodies directed against these epitopes have no virus-neutralizing capacity. Therefore, infection with TGEV or PRCV in swine induces neutralizing antibodies which are similar in titre and differentiation cannot be made by the sero-neutralisation test. An examination for the presence or absence of the differentiating epitopes on TGEV strains and PRCV strains showed that 6 virulent field TGEV strains, and 8 different TGEV attenuated strains (used as vaccine) bound the differentiating monoclonal antibodies while none of the 4 PRCV field strains did so. These studies permitted the conclusion that PRCV was not an attenuated TGEV which had "escaped" and been able to circulate in the swine population. This possibility had to be considered since it has been published that some TGEV strains, concomitantly with artificial attenuation, gradually acquire a tropism for the respiratory tract.

The slight difference found in epitope structure provided a means for serological differentiation between pigs which have been infected with TGEV and those which have been infected with PRCV. There is a definite need for such differentiation particularly in countries which export swine to countries which require that the imported animals are free from a previous infection with the enteropathogenic virulent TGEV. A differentiating ELISA test has been developed for this purpose but more data will be reported in this chapter on the molecular aspects of PRCV compared to TGEV and their possible use in differentiation.

The slight difference in molecular structure and particularly in the external glycoprotein composition has markedly altered the virus-host interactions of PRCV compared to that of TGEV. With TGEV, the pathogenesis in neonatal pigs is straightforward. After oral uptake of the virus, a massive replication occurs in the absorptive cells on the small intestinal villi with cell degeneration, desquamation and watery diarrhoea as a consequence. With PRCV, however, oronasal inoculation in neonatal pigs leads to virus replication to very high titers (up to $10^{8.3}$ TCID₅₀ per gram lung tissue) in the respiratory tract. Replication occurs in nasal, tracheal, bronchial, bronchiolar and alveolar epithelial cells and also in alveolar macrophages. Tonsillary tissue also supports virus replication.

One of the key points from a pathogenetic point of view is whether PRCV had kept its tropism for the intestine or not. Upon aerosol inoculation of 1 week old colostrum-deprived pigs, virus replication can be found in the small intestine, but the intestinal infection evolves totally differently from that of an enteric virus. Replication starts in the ileum and gradually spreads towards the duodenum. Immunofluorescence remains limited to a few cells located at crypt or villus sites. It is not clear which type of cell is involved and research is under way to identify it. Infected cells are found only on some villi and their number remains limited to one or a small group. They are often difficult to find. It can be assumed that they are not common villous enterocytes since one might then expect them all to be of equal sensitivity for the virus. In that case, the infection would be expected to run a normal progressive course with more cells infected at the peak of virus replication. That the intestine is not very sensitive to PRCV virus can be concluded from the finding that virus titres in the intestine remain low (maximally $10^{3.7}$ TCID₅₀). Also, more than 10^3 TCID₅₀ of PRCV are needed to consistently start the intestinal infection by direct virus inoculation in the lumen of the small intestine. PRCV certainly does not behave as an enteric virus since virus transmission does not occur through faeces. The amount of virus excreted via the faeces is probably insufficient to initiate an intestinal infection in a contact animal. A respiratory infection is needed so that sufficiently high quantities of virus can be ingested to initiate the intestinal infection.

An important practical question is whether PRCV infection, as it occurs in the field, induces an immunity to TGEV. In the field, PRCV regularly persists on farms and pigs usually become infected between the ages of 5 and 8 weeks. At that time, the respiratory infection occurs in the presence of relatively low titres of maternal antibodies in the blood. Whether or not intestinal replication also occurs in pigs infected at that age and in the presence of passive serum antibodies is not known. This aspect may be important from an immunological point of view as will be explained elsewhere in this chapter.

The PRCV-TGEV tandem opens perspectives for future research on virus-cell interactions and immunity mechanisms:

1. It is remarkable that a slight change in molecular arrangement in the E2 peplomer protein creates a virus which exhibits a totally different cell tropism. A prototype highly enteropathogenic TGEV has turned into a respiratory virus which has lost tropism for common villous enterocytes. Apparently the altered molecular site must be crucial for determining virus-cell interactions.
2. The tandem may present an ideal model for studying mucosal immunity and to test the concept of a common mucosal immune system in pigs. A PRCV infection with immunological stimulation in the respiratory tract can be used to find out if an immunological effect occurs against TGEV in the intestine. Vice versa, an intestinal infection with TGEV can be employed to examine a possible immunological reaction in the respiratory tract against PRCV.
3. It is known that an enteric viral infection in sows leads to a stimulation of the gut-mammary link with appearance of IgA in the milk (lactogenic immunity). However, TGEV neutralizing IgA is also found in the milk after an (exclusively?) respiratory infection with PRCV as will be reported further. It will be interesting to find out if IgA in the milk also can be obtained via a respiratory-tract-mammary gland link and if lactogenic immunity can be afforded in the intestine by this respiratory-induced IgA.