Establishment of an *In Vitro* System for the Prediction of the Degree of Virulence of Classical Swine Fever Virus Isolates

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**Background and aim**

The world organisation for animal health continuously registers numerous outbreaks of classical swine fever (CSF), a highly contagious disease of pigs caused by the CSF virus (CSFV). The economic consequences of such outbreaks are important, mainly due to the stamping out policy and restrictions in meat trade. A major problem is the persistence of CSFV in wild boar, the natural reservoir in which disease often remains unapparent. In domestic pigs the different CSFV isolates cover a continuous spectrum of virulence. Infections with highly virulent strains are efficiently shed by infected pigs and spread particularly fast. On the other hand low virulent strains – often diagnosed later – represent a particular problem for disease control. Hence, it is important to define the virulence of the CSFV isolates that are circulating in the wild boar reservoir and pose a constant threat of reintroducing CSF into the domestic pig population. Currently, the only possibility of classifying the virulence of a CSFV strain is by animal experimentation using pigs, which is ethically problematic. Previous work has suggested that virulence is determined by multiple genetic elements. Simple comparison of genome sequences of strains differing in virulence does not yet permit the prediction of a particular strain phenotype. Again, research in this area is currently pursued with experiments involving pigs. Therefore, the aim of the present study is the reduction and finally the replacement of experimental infections of pigs by a cell culture methodology enabling a prediction of the virulence of a particular CSFV isolate.

**Method and results**

During the past decade, we have cloned infectious genomes of CSFV strains with defined virulence (Ruggli et al., 1996; Mayer et al., 2003) and have made significant progress in understanding the interaction of CSFV with various primary cell systems of the porcine immune system, representing natural target cells for the virus (Carrasco et al., 2004; Balmelli et al., 2005). Based on these findings, we will systematically compare CSFV strains differing in virulence in terms of virus replication characteristics at different stages of the virus life cycle and in terms of their capacity to influence the activation of cells of the innate immune system.

We will proceed in four steps:

1. Establish a primary porcine cell culture system for virus stock production. Previous work has shown that propagation of CSFV on certain permanent porcine cell lines rapidly selects for adaptive mutants with an attenuated virulence phenotype. Therefore primary cells will be used.

    ![Fig. 1: Porcine endothelial cells visualised under native conditions.](image1)

    ![Fig. 2: CSFV-infected primary porcine fibrocytes.](image2)

    The viral nonstructural protein NS3 detected with a specific monoclonal antibody is visualised in blue. The red-stained microfilaments show the spindle-shaped morphology of the fibrocytes.
2. Determine the relationship between virulence and in vitro virus replication in selected primary porcine cell systems (e.g. endothelial cells, fibrocytes and monocyte-derived dendritic cells; Fig. 1, 2 and 3). We have previously obtained evidence that the ratio of cell-associated to released virus can vary with strains of different virulence (Mittelholzer et al., 2000).

3. Determine the relationship between virulence and in vitro parameters of the innate immune response using primary porcine cells (e.g. conventional and plasmacytoid dendritic cells). This is based on our previous work showing that virulence correlates with levels of IFN-α and pro-inflammatory cytokines in vivo (Summerfield et al., 2006).

4. Evaluate the accuracy of the in vitro model for correlation with virulence.

Conclusions and relevance for 3R

Correlates between the in vivo virulence phenotype and in vitro characteristics of CSFV isolates will allow feasible and ethically acceptable CSFV diagnostic and research processes to be applied. An in vitro system for predicting the degree of virulence would significantly reduce the overall number of animals employed for experimental infections. The major refinement will be the gain of knowledge in terms of molecular basis of the pathogenic characteristics of the virus, reducing the number of animal experiments to a minimum. Although cell culture models will only permit a prediction of the virulence of a particular virus isolate, in most cases this prediction will suffice to replace animal experiments. The knowledge created in this project will not only be useful in CSFV diagnostics but may also be applied towards generating safer live virus vaccines.

References


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