Towards a sequence-based diagnostic procedure to evaluate the virulence of classical swine fever viruses

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Key words
Classical swine fever virus, CSFV, pestivirus, virulence, reverse genetics, *in vitro* assays, differential transcriptomics

Aim of the study
For appropriate classical swine fever virus (CSFV) surveillance and control measures to be applied, it is essential to possess *in vitro* tests to predict the virulence of emerging CSFV wild boar isolates in domestic pigs. Therefore, the present project was aimed at identifying *in vitro* markers of CSFV virulence. The parameters considered were (i) viral genetic elements that determine replication efficiency and evasion from the antiviral state of IFN-γ-stimulated macrophages, and (ii) cellular genes that are up- or down-regulated in IFN-γ-stimulated macrophages in response to infection with high and low virulent CSFV strains.

Material and methods
In vitro correlates of CSFV virulence were studied using experimental systems developed in the 3R-project 105-06 and in the former BLV project 1.10.13. In particular: (a) CSFV genes controlling replication efficiency in PEDSV.15 cells and in IFN-γ-stimulated macrophages were investigated using chimeric low virulent CSFV. This was performed in close collaboration with T. Tamura, visiting PhD student from the laboratory of Y. Sakoda, Hokkaido University, Japan; (b) Replication and viral protein expression related to virulence was assessed in IFN-γ-stimulated macrophages using flow cytometry (FCM) and in PEDSV.15 using a novel in-cell ELISA as a simple alternative to qRT-PCR; (c) the transcriptomic response of IFN-γ-stimulated macrophages to CSFV of different virulence was determined by mRNA sequencing at the Next Generation Sequencing platform (Prof. T. Leeb) in close collaboration with Dr. R. Bruggmann from the Interfaculty Bioinformatics Unit at the University of Bern.

Results and significance
(a) The NS4B gene of CSFV controls viral replication efficiency, which is related to virulence. Using reverse genetics, we demonstrated that the NS4B and E2 genes carry virulence determinants. In particular, by introducing selected amino acid codons from the high virulent vEy-37 virus in the low virulent GPE$^-$ backbone, we obtained gain of virulence as measured in the different assays. (b) CSFV virulence can be related to viral replication efficiency using FCM and in-cell ELISA. Extensive evaluation of 13 well-characterized CSFV field isolates validated the use of FCM and of a novel in-cell ELISA to predict virulence *in vitro*. Kinetics of viral protein expression as determined by FCM early after infection provided the most reliable correlates of virulence. (c) Differential transcriptomic responses of IFN-γ-stimulated macrophages to CSFV infection cannot be exploited to predict virulence. Cellular mRNA sequencing revealed a general low transcriptomic response to CSFV infection, irrespectively of the virulence of the isolates. Only 37 out of approximately 10'000 annotated genes were significantly differentially regulated between high and low virulent CSFV in IFN-γ-stimulated macrophages, with less than 2-fold differences only, which does not allow reliable differentiation.

Publications, posters, and presentations
Töpfer, A. et al (2013) Sequencing approach to analyse the role of quasispecies in virulence of classical swine fever virus. Virology 438, 14-19 *(BLV 1.10.13)*
Leifer, I. et al (2013) Approaches to define the viral genetic basis of classical swine fever virus virulence. Virology 438, 51-55 *(BLV 1.10.13)*
Liniger M. et al. Highly virulent classical swine fever virus isolates can be differentiated from low virulent isolates in cell culture. *In preparation*
Tamura, T et al. A N-terminal amphipathic helix in NS4B of classical swine fever virus contributes to pathogenicity in pigs. *In preparation*
Liniger, M. et al. Differential transcriptomic response of porcine monocyte-derived macrophages to low and high virulent classical swine fever virus. *In preparation.*

Project *1.12.20 (follow-up of 1.10.13)*

Project duration *1 October 2012 – 30 September 2013 (no researcher on the project from 1.1. to 31.3.2013)*