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Section **Fields (of activity)**

Study of African swine fever immunopathogenesis in domestic pigs

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Aim of the study
The aims of this project were (1) to characterize the phenotype of selected recent genotype II ASFV isolates from Europe in Swiss Large White pigs of different hygiene and immune status, (2) to study the targets, functions and type of B- and T-cell responses in the context of ASF immunopathogenesis and of protection against infection, (3) to construct recombinant specific gene-deleted ASFV expressing marker genes, and (4) to identify the cellular factors that restrict the replication of ASFV to porcine macrophages.

Material and methods
In order to address aims 1 and 2, we performed extensive comparative immunopathogenesis studies in Large White specific pathogen-free (SPF) pigs bred at the IVI *versus* conventionally-bred Large White pigs obtained from a local farm and from Agroscope in Posieux. We analyzed (i) the clinical outcome and the host immune responses to highly virulent (Armenia 2008 and Georgia 2007) and attenuated (Estonia 2014) ASFV field isolates, (ii) the antibody and T-cell responses, (iii) the cytokine and transcriptome profiles and (iv) the microbiota composition. We performed also a genome-wide B-cell epitope screening using a synthetic ASFV protein library and immune sera from pigs that survived the infection. For aim 3, we replaced selected ASFV genes with luciferase (NLuc) or EGFP reporter gene cassettes using three different approaches: (i) assembly of the full-length ASFV genome in a yeast artificial chromosome (YAC) using transformation-associated recombination (TAR) in cooperation with Prof. Jörg Jores from the Institute for Veterinary Bacteriology, Vetsuisse Faculty of Bern, (ii) CRISPR/Cas9-assisted recombination and (iii) classical homologous recombination. Finally, we addressed aim 4 using stringent differential transcriptomic analyses of non-permissive porcine blood monocytes and of the corresponding differentiated and permissive monocyte-derived dendritic cells.

Results and significance
Host factors influence the severity of disease caused by attenuated African swine fever virus. The two types of pigs differed significantly in their baseline immune status and microbiota composition and responded differently to ASFV infection, depending on the virulence of the isolate. With highly virulent ASFV Armenia 2008, all animals showed severe disease and had to be euthanized within 5 to 7 days post-infection, with no significant difference in clinical and immunological parameters between the two types of pigs. In contrast, during infection with the attenuated Estonia 2014 field isolate, SPF pigs presented a milder and shorter clinical disease with full recovery and seroconversion, whereas farm pigs had a more severe and prolonged disease with typically 50% lethality. Farm pigs showed higher production of inflammatory cytokines, whereas SPF pigs produced anti-inflammatory cytokines early after infection. Altogether, our data indicate that the immune status and the hygienic background have a major impact on the severity of ASF. Importantly, a higher baseline innate immune activity promotes immunopathological inflammatory cytokine responses and delays lymphocyte proliferation in response to attenuated strains, which can cause acute to chronic ASF. Such effects are of high significance for live attenuated ASF vaccine development (Radulovic *et al.*, 2021a, 2021b, 2022a, 2022b, 2022c).
***Host factors influence the protective immunity conferred by attenuated African swine fever virus*.** Next, we studied protective immunity conferred by attenuated Estonia 2014 ASFV in the two types of pigs. All pigs that recovered from infection with the attenuated virus seroconverted by day 21 post-infection while ASFV was

still detected in the blood for up to 4 months. After challenge with the virulent Armenia 2008 strain, SPF pigs showed complete protection from disease with no clinical nor hematological symptoms and only mild viremia, while farm pigs developed a severe clinical disease with high fever, high viremia and a strong thrombocytopenia and leukopenia. These findings show that SPF pigs are a promising model to identify innate immune and microbial factors associated with resilience to ASFV infection and will help identify protective adaptive immune responses (Mehinagic *et al.*, 2022; Radulovic *et al.*, in preparation).

Identification of host factors that lead to protection against ASF. The experiments described above were exploited (i) to perform a genome-wide screen for immunogenic B-cell epitopes of ASFV with convalescent sera from the protected pigs, and (ii) to identify correlates of protection by differential analyses of immunological parameters and transcriptome profiles in protected *versus* non-protected pigs. We identified 25 different ASFV proteins capable of eliciting antibody responses, 10 of which had never been identified as such before.

Genetic engineering of ASFV from clinical material. We assembled the entire 190-kbp genome of ASFV Georgia 2007 isolated from blood of infected pigs in YAC (Labroussaa *et al.*, 2021). We developed a method for CRISPR/Cas9-assisted recombination and targeted eight genes for deletion and replacement with the EGFP or NLuc reporter genes. The ASFV promoter-driven NLuc reporter gene was exploited to implement a diagnostic assay for rapid detection of live virus in porcine macrophages (Mehinagic *et al.*, in preparation).

***Permissive permanent cell lines for the isolation and amplification of ASFV field isolates*.** Differential transcriptomic analyses of permissive *versus* non-permissive primary porcine cells revealed 50 cellular genes (coding essentially for membrane-bound proteins) that may be required for ASFV permissiveness. However, we did not succeed yet in producing a permissive cell line using selected gene expression. As an alternative, we obtained an immortalized porcine kidney macrophage (IPKM) cell line (Masujin K. *et al.*, 2021, Sci Rep 11:4759) that supports efficient replication of ASFV field isolates of different genotypes.

Output. Besides peer-reviewed publications, this project generated knowledge for the FSVO, veterinarians, pig producers and public in form of didactic videos (clinical signs and pathology), talks at veterinary symposia (SVSM, TVL), and different articles in specialized magazines (SAT, Schweizer Bauer, SNF Horizonte).

Publications

Labroussaa, F.; Mehinagic, K., Cippa, V.; Liniger, M.; Akarsu, H.; Ruggli, N.; Jores, J. (2021) In-yeast reconstruction of the African swine fever virus genome isolated from clinical samples. STAR Protoc 2(3):100803.

Radulovic, E.; Mehinagic, K.; Wüthrich, T.; Hilty, M.; Posthaus, H.; Summerfield, A; Ruggli, N.; Benarafa, C. (2022a) The baseline immunological and hygienic status of pigs impact disease severity of African swine fever. PLoS Pathog 18(8): e1010522.

Posters and oral presentations

Mehinagic, K. (2020) ASP im Hausschwein: Auswirkungen von Virulenz und Hygienestatus auf das klinische und pathologische Erscheinungsbild. 23. Seminar der Schweizerischen Vereinigung für Schweinemedizin (SVSM), oral presentation, 3 – 5 September 2020 in Engelberg.

Ruggli, N. (2021) Erkenntnisse aus experimentellen ASPV Infektionen im Hausschwein mit hoch- und mittelgradig virulenten Isolaten. Frühjahrstagung Tierärztliche Vereinigung für Lebensmittelsicherheit, Tiergesundheit & Tierschutz (TVL) zum Thema ASP, oral presentation, 8 April 2021 online.

Radulovic, E. (2021a) The immunological and hygienic status of pigs define disease severity of African Swine Fever. VIA meeting, Study group veterinary immunology, German Society of Immunology, oral presentation, 21 May 2021 online.

Radulovic, E. (2021b) The immunological and hygienic status of pigs define disease severity of African Swine Fever, 7th European Veterinary Immunology Workshop, oral presentation, 29-31 August in Zagreb, online.

Radulovic, E. (2022b) Host factors influence the severity of disease caused by attenuated African swine fever virus. 14th annual meeting EPIZONE, oral presentation, 18 – 20 May 2022 in Barcelona.

Mehinagic, K.; Radulovic, E.; Posthaus, H.; Beer, M.; Blome, S.; Summerfield, A; Ruggli, N.; Benarafa, C. (2022) Protective immunity conferred by attenuated African swine fever virus infection is strongly dependent on host factors. 14th annual meeting EPIZONE, poster, 18 – 20 May 2022 in Barcelona.

Radulovic, E. (2022c) Innate and adaptive immunity against African swine fever virus infection are modulated by host factors in domestic pigs. Global African Swine Fever Research Alliance meeting, oral presentation, 24-27 May 2022 in The Dominican Republic.

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