Vaccines

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Generation of a Novel Dual Swine Influenza Vaccine expressing both H1 and H3

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Swine influenza viruses (SIV) cause significant economic losses in agriculture and are a permanent risk for human public health. For control, commercial inactivated mono-, bi- or tri-valent vaccines containing antigens of SIV H1N1 and H3N2 subtype are administered on swine farms.

The simultaneous expression of both H1 and H3 antigens by one influenza A virus was made possible by rewiring of the gene segment packaging signals resulting in the incorporation of an additional ninth segment (Gao et al., J Virol 84(16):8062-8071 (2010) doi:10.1128/JVI.00722-10). Each of the eight gene segments possesses segment-specific packaging signals at both ends overlapping the non-coding and coding regions. In that study, a virus was rescued that contained a modified PB1 segment in which its packaging signals were replaced by those of the NA segment. Because the PB1 protein is strictly essential for virus growth and the complete set of segment-specific packaging signals is required, the virus is forced to incorporate both the modified PB1 segment plus the additional RNA segment flanked by the PB1 packaging signals. On the basis of the pandemic strain A/Bayern/74/09 (H1N1), we generated by reverse genetics a vaccine candidate which, in addition to the H1 HA, expresses the H3 HA from A/Swine/IDT1864/2003 (H3N2). First results of the in-vitro characterization demonstrate the presence of the modified PB1 plus the additional H3 gene segment and sufficient virus yield in cell culture.

With its highly variable head domain, the HA is considered to be the most relevant antigen whereas in particular the internal proteins are conserved at a considerably greater extent. Therefore, such a nine-segment virus may serve as backbone allowing the periodic replacement of two HA antigens. Furthermore, combining the two main antigens into one vaccine strain offers a valuable strategy to reduce production and costs, particularly, if the number of co-circulating influenza lineages would increase. Overall, we aim to investigate the suitability of such dual-HA viruses to serve as platform for inactivated and attenuated live influenza vaccines in swine.

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Comparative binding analysis of HIV-1 envelope variants to neutralizing antibodies by flow cytometry, microscale thermophoresis and SPR- spectroscopy

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Binding studies are an essential tool to characterize new identified anti HIV-1 envelope antibodies. Different methods exist to quantify their binding depending on the production strategy of the envelope protein. While membrane bound envelopes on the surfaces of cells or viral particles facilitate native like structures affinity measurement has to be done by suitable methods like flow cytometry. By the use of truncated and soluble envelope variants other methods like SPR-spectroscopy or microscale thermophoresis can be applied but compromises have to be made with regard to the overall protein conformation.

To compare the different strategies three envelope mutants based on the C-clade isolate 16055 were generated with point mutations within the V3-region. The mutations were identified out of a high throughput screen indicating two mutations with higher binding and one with reduced binding. Proteins were produced for each mutant as either membrane bound gp145, soluble gp140 and soluble gp120 in human HEK293 cells. Their binding affinities were analysed against the V3-antibodies HGN194 and 447-52D by the respective method. The calculated dissociation constants showed that differences in binding affinity between the three tested variants can be detected across the different methods. By comparing absolute binding affinities differences were observed depending on the form of the produced protein and the method which was used. By the use of antibody 447-52D the measured affinity constants were $0.18e^{-9}$ M in flow cytometry analysis compared to 25.4 e^{-9} M in microscale thermophoresis and $3.34e^{-7}$ M in SPR- analysis.

Taken together the calculated dissociation constant is strongly dependent on the method and the envelope protein conformation. Differences in antibody recognition to restricted epitopes like the V-3 region can be detected across all tested platforms.