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Characterization of an egg-culture derived, neuraminidase-negative variant of highly pathogenic avian influenza virus H5N1

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Influenza viruses typically escape from a certain immunogenic pressure by antigenic drift and antigenic shift.To investigate this phenomenon, highly pathogenic avian influenza virus (HPAIV) of subtype H5N1 was forced to replicate under the immunogenic pressure of a neutralizing, polyclonal, chicken-derived serum. After 50 egg-passages, the resulting escape mutant ("EscEgg50" or "H5N1del") revealed major changes in the neuraminidase-encoding segment. Extensive deletions and rearrangements were detected inthe new segment 6, and further 13 amino acid substitutions distributed over the other segments could be identified.Nevertheless, the cleavage site of the hemagglutinin of "EscEgg50" was still polybasic, and electron microscopy analysis did not show any unusual accumulation of virus particles at the cell surface. Interestingly,the new segment 6 resulted from a complex sequence shuffling and insertion of a very short sequence fragment from segment 3. Characterization of the escape variant confirmed the loss of neuraminidase activity. By using reverse genetics a recombinant virus consisting of the HPAIV H5N1 backbone and the new segment 6 could be generated, while attempts to generate a virus without the segment 6 sequence failed. Comparativein vivocharacterization defined the escape variant as low-pathogenic influenza virus, while the control virus was still highly virulent after intravenous or oronasal application.
The described virus mutant is to our knowledge the first "neuraminidase-negative" influenza virus generated by immune-escape without external neuraminidase supplementation. In addition, the virus variant showsan unusual sequence composition in the new segment 6.

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