Engineered avian influenza viruses: towards identification of molecular markers of virulence for rapid characterisation of new virus isolates

Matthias Liniger, Manuela Ocaña-Macchi, Kenneth C. McCullough, Jon Duri Tratschin, Artur Summerfield, Nicolas Ruggli
Institute of Virology and Immunoprophylaxis, Sensemattstrasse 293, CH-3147 Mittelhäusern, Switzerland

Key words
Avian influenza virus (AIV), high pathogenic, low pathogenic, reverse genetics, virulence, vaccine, chicken

Aim of the study
The objective of this research project was to provide the IVI with modern genetic tools to study viral and host factors governing virulence of avian influenza virus (AIV), in order to support the prediction of the pathogenic potential of emerging AIV for domestic poultry, and to guide the development of novel vaccines.

Material and methods
Reverse genetics was established for high pathogenic (HP) and low pathogenic (LP) AIV. Various amino acid and genome segment-exchange mutant AIV were generated and analysed in vitro and in vivo. Novel assays were developed to study the interaction of AIV with the chicken type I interferon (IFN) system. Chicken genes encoding the signalling elements of the type I IFN induction pathway were identified and used to examine the antiviral type I IFN responses of avian cells upon stimulation with viral pathogen-associated molecular patterns (PAMP). Knock-down of selected chicken genes in cell culture was applied to understand gene functions.

Results and significance
A critical role of AIV hemagglutinin in the pathogenesis of AIV in chicken was demonstrated: the viral load and the cleavability of HA enabling systemic spread of the virus are two major factors to consider for the prediction of the outcome of an infection with a particular isolate. The chicken (ch)MDA5, chCARDIF and chLGP2 were identified as signalling components capable of sensing AIV PAMP to induce type I IFN in chicken cells in the absence of RIG-I that is missing in chicken as opposed to mammals und ducks. These findings contribute to understand the difference in susceptibility to AIV observed between chicken and other species. They also led to the development of a novel genetic adjuvant for the vaccination against AIV. Vaccinated chicken were protected against a lethal challenge with a HPAIV. A possible patent is under evaluation. With reverse genetics, the viral NS1 and PB2 of HPAIV but not of LPAIV were found to control efficiently the type I IFN induction in HD-11 cells. This permitted the development of a cell-based in vitro assay for the discrimination of HPAIV and LPAIV. Finally, the study of genetic reassortants of porcine and AIV in porcine dendritic cells revealed a role of avian PB2 for enhanced activation of cells of the innate immune system

Publications (posters and presentations at scientific meetings are not listed)
Ocaña-Macchi M., Bel M., Guzylack-Piriou L., Ruggli N., Liniger M., McCullough K.C., Sakoda Y., Isoda N., Matrosovich M., Summerfield A. 2009. Hemagglutinin-dependent tropism of H5N1 avian influenza virus for human endothelial cells. J. Virol. 83:12947-12955.

Moulin H.R., Liniger M., Python S., Guzylack-Piriou L., Ocaña-Macchi M., Ruggli N., Summerfield A. 2010. High interferon type I responses in the lung, plasma and spleen during highly pathogenic H5N1 infection of chicken. Vet. Res. 42:6.

Liniger M., Summerfield A., Zimmer G., McCullough K.C., Ruggli N. (2012) Chicken Cells Sense Influenza A Virus Infection through MDA5 and CARDIF Signaling Involving LGP2 J. Virol., 86(2):705.

Liniger M., Summerfield A., Ruggli N. MDA5 can be exploited as an efficacious genetic adjuvant for a DNA vaccine against lethal H5N1 influenza virus infection in chicken. Submitted for publication.

Liniger M., Moulin H.R., Sakoda Y., Ruggli N., Summerfield A. (2012) Highly pathogenic avian influenza virus H5N1 controls type I IFN induction in chicken macrophage HD-11 cells: a polygenic trait that involves NS1 and the polymerase complex. Virology Journal, 9:7.

Ocaña-Macchi M., Python S., Gsell Albrecht M., Stech J., Stech O., Summerfield A. (2012) Avian influenza A virus PB2 promotes interferon type I inducing properties of porcine Influenza A Virus in porcine dendritic cells. Virology 427, 1–9.

Project *1.07.09*

Project duration *April 2007 – May 2011*