Flu away

Dr Gert Zimmer summarises his project to develop an avian influenza viruses vaccine using the innovative platform of RNA replicons to open up the possibility of large-scale immunisation of domestic poultry

DR GERT ZIMMER

Could you begin by explaining your research goals and what needs your current project addresses?

In Europe, the general prophylactic vaccination of poultry against avian influenza viruses (AIV) of subtypes H5 and H7 is currently not allowed. This policy is based on two arguments: first, conventional inactivated influenza virus vaccines may protect animals from disease, but may not completely prevent virus replication and excretion; second, conventional vaccines do not allow for serological discrimination between infected and vaccinated animals (DIVA), which leads to the implementation of export bans of animals and animal products into other countries. Our goal is to develop a safe and efficacious vaccine that fully complies with the DIVA concept and will be able to prevent virus shedding from vaccinated animals.

For those who are unfamiliar, what are RNA replicons and what makes them ideal for use in a general vaccine platform?

RNA replicons are disabled RNA viruses that lack at least one essential gene. These vectors can be propagated on helper cells that express the deleted viral gene. The complemented replicon particles are infectious, delivering the recombinant RNA genome containing an antigen of choice with high efficiency into the cytosol of a target cell. The autonomous replication and transcription of the viral genome guarantees that the recombinant antigen is expressed at high levels, stimulating both humoural and cellular immune responses. Importantly, progeny viruses are not released as the deletion is not complemented by normal cells. Replicon vectors are not only non-pathogenic, but also unable to revert to virulence (a risk always associated with live-attenuated vaccines).

What methods have you used to develop and test your vaccine? In what ways is your approach unique to previous efforts in this area?

A key achievement was the generation of a transgenic helper cell line that allowed us to propagate the replicon particles. The cell culture supernatant containing a defined infectious dosis of replicon particles can be directly used for immunisation. Any inactivation and purification processes or formulation with adjuvants (required for conventional inactivated vaccines) are not necessary. Another clear advantage of our replicon vector is that it is not a natural avian pathogen, thus excluding any pre-existing immunity which may reduce the efficacy of the vaccine.

What have been some of your most important achievements so far?

We found that a single immunisation of chickens with recombinant RNA replicons expressing H5 hemagglutinin resulted in induction of broadly neutralising antibodies that conferred full protection against challenge infection with antigen-drifted H5 highly pathogenic AIV and minimised virus shedding, leaving sentinel birds in the same cage uninfected. The vaccine allowed simple serological differentiation between infected and vaccinated animals.

Are there any side effects associated with your vaccine? What do you say consumers who may be concerned about eating poultry that comes from vaccinated chickens?

Side effects due to vaccination have not been observed in our animal trials. As the replicon particles do not need to be formulated with

adjuvant (which is mostly the case with inactivated vaccines), any side effects due these compounds can be excluded. The RNA replicon vector does not integrate into the host chromosomal DNA, nor does it persist in the cell. Thus, when the consumer eats the meat, the RNA replicon has been gone for a long time. By the way, poultry is commonly vaccinated with conventional vaccines (except influenza), very often using live-attenuated viruses.

What are the next steps in your research and when do you believe your vaccine will be made widely available for use?

We have developed a safe and efficient marker vaccine that works very well in experimental settings. Next, field trials have to be performed in order to evaluate the vaccine with a larger number of animals.

Are there any other areas of your work you wish to mention?

We have another project running in our lab that is dedicated to the adaptation of H5 and H7 low-pathogenic AIV isolates from waterfowl to domestic chickens. Taking advantage of recombinant AIV reassortants and mutants, we are studying the molecular determinants responsible for efficient replication, excretion and transmission. The plasticity of these viruses and their ability to change are incredibly large.

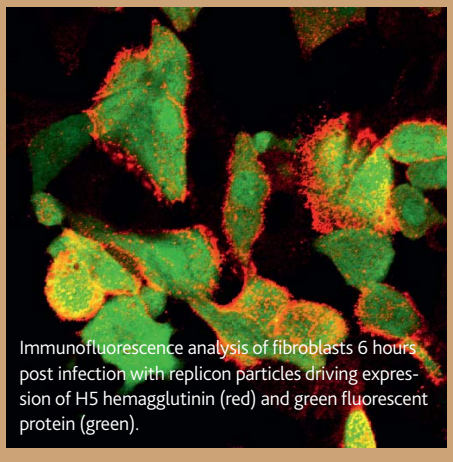
DR GERT ZIMMER

Fortifying a defence against bird flu

As public interest in avian influenza wanes with time passing since the last pandemic, a project at the **Institute of Virology and Immunoprophylaxis** in Switzerland aims to produce an innovative vaccination technique for poultry to prevent future outbreaks

AVIAN INFLUENZA VIRUSES (AIV) pose a continual threat both to domestic poultry and, through zoonotic transmission, to humans. These viruses are endemic in aquatic birds worldwide, and though they are well adapted to their natural hosts and do not normally cause disease, they are shed into the environment in large quantities. This facilitates transmission to both domestic poultry and mammals, including man. There are an estimated 50 billion domesticated chickens raised each year worldwide, as well as numerous other forms of poultry. In 1997, a particular deadly strain of AIV of the H5N1 subtype emerged in China. This virus has been continuing and repeatedly causing severe outbreaks of disease in poultry with 100 per cent mortality, and has crossed national boundaries across Asia, and could eventually reach Europe and Africa. Several million birds have been killed either by infection or slaughter. Importantly, H5N1 has also infected man and, in most cases, it is the result of direct exposure to dying poultry. Such high pathogenic forms of AIV (HPAIV) develop from low pathogenic AIV (LPAIV) through the development of multibasic cleavage sites that are cleaved by ubiquitously expressed subtilisin-like proteases. The process of increasing pathogenicity is thought to happen within domestic birds, and does have deadly consequences. In effect, vaccination of poultry may help prevent a pandemic. The most catastrophic example where AIV is thought to have played a role is the 'Spanish flu' of 1918, caused by a H1N1 strain. It is estimated that this pandemic caused 50 million deaths worldwide.

Despite the deadly potential, most European countries do not allow general vaccination of poultry against HPAIV. There are two principal reasons for this: firstly, currently available inactivated influenza virus vaccines do not allow for serological discrimination between infected and vaccinated animals, and may lead to the unnoticed spread of AIV; secondly, whilst these traditional vaccines protect against signs of the disease, they are not efficient at producing sterilising immunity. Infected birds are still able to shed considerable amounts of a virus, a process which may even encourage mutations or reassortants. The latter can be a mixing of avian with porcine or human influenza virus gene segments. The 2009 H1N1 outbreak, which started in Mexico and California, included genetic material from all three. Similarly, it has been theorised that the Spanish flu may have passed through a mammalian species before reaching humans. More clear information is available for later pandemics, such as the Asian flu (H2N2 in 1957) and the Hong Kong flu (H3N2 in 1968), which certainly contained



Immunofluorescence analysis of fibroblasts 6 hours post infection with replicon particles driving expression of H5 hemagglutinin (red) and green fluorescent protein (green).

RNA REPLICONS ADDRESSING THE REQUIREMENTS OF HIGH-QUALITY INFLUENZA VIRUS VACCINES FOR POULTRY

OBJECTIVES

- Develop safe and efficacious marker vaccines for protection against Avian influenza viruses, Porcine reproductive and respiratory syndrome virus (PRRSV), Bluetongue and African horse sickness virus, Japanese encephalitis virus
- Analyse the mechanisms of how vaccines trigger the immune response in animals

KEY COLLABORATORS

Gerd Pluschke, Swiss Tropical and Public Health Institute, Basel, Switzerland

PoRRSCon consortium, Hans Nauwynck, University of Ghent, Belgium

PREDEMICS consortium, Sylvie van der Werf, Institute Pasteur, Paris, France

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GERT ZIMMER received a Doctoral degree in Sciences from the University of Marburg and worked as a Senior Lecturer at the Veterinary School Hannover, Germany. He presently holds a position as Head of Virology at the IVI in Mittelhäusern, Switzerland. His work focuses on influenza and other RNA viruses with a particular importance given to vaccine development.

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gene segments from both avian and human influenza, demonstrating how reassortants may have pandemic potential. An efficacious AIV vaccination which allows for easy differentiation between infected and vaccinated animals and prevents viral shedding thus has the potential not only to protect poultry but also to prevent future influenza pandemics.

A NOVEL SOLUTION

The project led by Dr Gert Zimmer seeks to produce a vaccine which will fulfil these criteria. In order to do this, the project focuses not upon traditional vaccine forms, but instead upon negative-strand RNA virus replicons. These are single cycle viral vectors that are able to deliver foreign genes to cells with great efficiency. Infectious viral progeny cannot be produced because of the lack of essential structural proteins. Consequently, RNA replicons have the potential to be excellent vaccines, combining the safety of inactivated vaccines with the efficacy of live attenuated virus vaccines. The difference to conventional inactivated influenza virus vaccinations is clear: "In contrast to our replicon system, previously used vaccine vectors were mostly based on propagation-competent viruses, which may mutate and regain virulence," he explains.

> The process of increasing pathogenicity is thought to happen within domestic birds and does have deadly consequences

Vaccines produced from RNA replicons will not allow shedding of viral material, and therefore open up the chance for immunoprophylaxis through the immunisation of domesticated birds. Yet this goal, which the project is moving towards, is also problematic. Zimmer is aware that the success must be tempered: "RNA replicon vaccines work perfectly when applied intramuscularly, whereas the nasal/intratracheal or oral routes proved to be less effective". The conditions of commercial poultry farming mean that intramuscular vaccinations are time consuming and labour intensive. In order for the vaccination to be practically applicable on a large scale, it would have to be easily used by poultry farmers. An ideal vaccine would be applied within drinking water or through sprays, routes of application for which RNA replicons are not yet effective. "We are currently trying to improve our vaccine vector in this regard," he affirms.

Yet this is not the whole scope of RNA replicon vaccinations. Though the project is currently focused on AIV, the research is pertinent for both human and porcine influenza as well.

A non-vaccinated chicken that died following infection with highly pathogenic AIV (H7N1). Note the hemorrhages at the comb.

"In principle, any antigen of interest can be expressed with the RNA replicon vaccine platform," Zimmer explains. "The antigens may be derived from viral, bacterial, or parasitic pathogens." Through collaboration, the project has specific aims to move beyond influenza to porcine reproductive and respiratory syndrome virus, Bluetongue virus, African horse sickness virus and Japanese encephalitis virus. At the present time, he is also collaborating with the Swiss TPH Institute in Basel on evaluation of RNA replicons that express antigens of a mycobacterial pathogen. Mycobacteria cause serious diseases in mammals, including leprosy and tuberculosis, and Mycobacterium ulcerans, which is being examined, causes Buruli ulcer, the third most common mycobacteriosis in humans.

INTERNATIONAL APPLICATION

The projects have been funded by the Swiss National Science Foundation and the Swiss Federal Veterinary Office, as well as the European Community's Seventh Framework Programme (FP7). Collaborators include a member of the Swiss Tropical and Public Health Institute and consortiums from Europe. Making the vaccine widely available is dependent upon industrial and political priorities, and Zimmer is aware of this: "We have to attract a company to be interested in our vaccine and it has to be officially approved. Not to forget, the authorities have to be convinced that general prophylactic vaccination of poultry against H5 and H7 AIV should be allowed with high-quality vaccines".

Most European countries do not allow general vaccination of poultry against HPAIV, but this is in contrast to countries such as China, Egypt, Vietnam or Pakistan. Each of these has had numerous problems with H5 AIV and started vaccination campaigns, predominantly using inactivated influenza virus vaccines, but were mostly unsuccessful. Such a course actually assisted the emergence of mutated strains and expedited virus evolution. The problems experienced in these countries are able to be addressed by RNA virus replicons, and Zimmer hopes for a mutually beneficial venture: "We would be very interested in any sort of collaboration with vaccine manufacturers in these countries to provide a solution for this problem".