Evaluation in chickens of a live attenuated NS1 mutant avian influenza virus (AIV) vaccine

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Low pathogenic avian influenza (LPAI) virus causes serious economic losses to the poultry industry and has the potential to mutate to highly pathogenic AI (HPAI) strains. In recent years, H5 and H7 subtype viruses have been shown to infect humans during outbreaks of AI virus in commercial poultry. The direct transmission of this virus to humans without an intermediate host and the lack of immunity in the human population to these virus subtypes makes them good candidates for the next pandemic virus. A proper vaccination program in commercial poultry can play a critical role in the prevention and control of AI and therefore safeguard public health. Due to trade implications, differentiation of infected from vaccinated animals (DIVA) is an important issue in the control of AI. In this study we describe the development of a live attenuated vaccine for poultry, with DIVA properties using an NS1 deletion mutant H5N3 virus.

Influenza virus NS1 protein plays a major role in blocking the host antiviral response. Using an eight-plasmid reverse genetics system, we have recovered moderately virulent parental (rH5N3) and NS1 mutant (rH5N3/NS1-143) viruses. rH5N3/NS1-143 expresses only the first 143 amino acids of the NS1 protein compared to the 232 of the parental rH5N3. The growth properties of rH5N3 and rH5N3/NS1-143 were compared in cell culture and in different age embryonated chicken eggs. Our results confirm that NS1 is involved in down regulation of interferon as shown by IFN-ß mRNA expression analysis and by the inability of rH5N3/NS1-143 to efficiently grow in older age, interferon competent, chicken embryos. To evaluate the potential use of rH5N3/NS1-143 as a live attenuated vaccine, 6-week old chickens were vaccinated with rH5N3 and rH5N3/NS1-143 viruses. rH5N3/NS1-143 vaccinated chickens showed partial attenuation, when compared to rH5N3 vaccinated chickens, as determined by histological lesions of trachea and lungs. In addition, rH5N3/NS1-143 infected chickens developed a strong antibody response to NP and/or M1 but not to NS1. Interestingly, rH5N3 challenge of rH5N3/NS1-143 vaccinated chickens, indicated that the NS1 mutant virus confers protection to rH5N3. Regardless of the strong immune response to NP and/or M1, the challenge virus was able to replicate in rH5N3/NS1-143 vaccinated group.

The rH5N3/NS1-143 virus can be used as a DIVA vaccine. The vaccine is attenuated in its replication in chickens and yet, protects from pathology caused by rH5N3 and partially against virus replication. Studies are in progress to determine if a killed, inactivated rH5N3/NS1-143 vaccine confers better protection and can be used as a DIVA vaccine.

The use of AI vaccines with DIVA properties will play an important role in the control of AI in commercial poultry in the USA, will protect the industry from trade

blocks and will protect the human population from infection with AI virus by reducing the viral load in commercial poultry.