Haemagglutinin activation by human transmembrane protease serine 2 or human airway trypsin-like protease is necessary for production of high titre influenza A virus pseudotypes for the evaluation of pandemic potential

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INTRODUCTION

Influenza A viruses are classified using the antigenic and phylogenetic characteristics of the envelope glycoproteins haemagglutinin (HA) and neuraminidase (NA). Currently 17 different haemagglutinin subtypes are recognized and are divided into two lineages: Group 1 and Group 2 (Figure 1).

The haemagglutinin is the trimeric envelope glycoprotein necessary for viral entry into susceptible cells. Each HA monomer is produced as a single polypeptide precursor, HA0, that during maturation is cleaved into two subunits, HA1 and HA2. This process is necessary for the virus to acquire infectivity. When multiple basic amino acid residues (lysine and arginine) are situated immediately upstream of the cleavage arginine, the haemagglutinin is cleaved by ubiquitously expressed subtilisin-like endoproteases such as furin. This determines the virulence and lethality for several avian species observed in the H5 and H7 highly pathogenic avian influenza (HPAI) strains [1]. On the other hand, cleavage of low pathogenic avian influenza (LPAI) strains, human and mammalian influenza viruses is thought to be mediated by tissue-specific proteases.

Recently, human lung proteases belonging to the serine protease family that are able to cleave the haemagglutinin of human influenza strains were identified: Transmembrane Protease Serine 2 (TMPRSS2) and Human Airway Trypsin-like protease (HAT) [2]. To study HA activation, viral entry mechanisms and as surrogate for serological antigens for the measurement of antibody response directed against the influenza haemagglutinin, pseudotype lentiviral particles are a suitable approach [3].

MATERIALS AND METHODS

Lentiviral pseudotypes were produced as described previously [4, 5]. Briefly, HIV gag-pol plasmid p8.G, firefly luciferase expressing plasmid pcSFLu, HA expressing plasmid and pCAGGS-TMPRSS2 or pCAGGS-HAT [2] were co-transfected into human embryonic kidney HEK293T/17 cells. As a control, transfections without the protease plasmids were performed. After 24 hours incubation, recombinant neuraminidase from Clostridium perfringens (Sigma) was added (Figure 2) to facilitate pseudotype exit from the producer cells. 48 hours post-transfection supernatant was harvested, filtered through 0.45μm filters and stored at -80°C.

RESULTS

We successfully obtained high-titre viral particles expressing the haemagglutinin (Figure 4 and Figure 5). The pseudotype titres were compared to the controls: pseudotype particles produced without the envelope protein (dennovo) and influenza HA pseudotypes produced in the absence of the protease (aprotease), before and after TPCK-Trypsin treatment to obtain HA activation. An H5 HPAI influenza strain (A/Vietnam/1194/05) is also shown as additional control.

CONCLUSIONS

In a prior study with Group 2 pseudotypes and using TMPRSS2 [6] and in this study we have shown that:

- TMPRSS2 and HAT function in effecting the activation, presumably by cleavage, of influenza A virus haemagglutinins to produce high-titre influenza pseudotypes.
- Influenza pseudotypes can be employed to determine which proteases are involved in haemagglutinin activation of individual subtypes.
- Influenza pseudotypes can be used as surrogate antigens in neutralization assay.

FUTURE WORK

- Use pseudotypes as tools for studying the antibody response induced by vaccination and after experimental and natural infection (see poster P2-534).

REFERENCES


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