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).

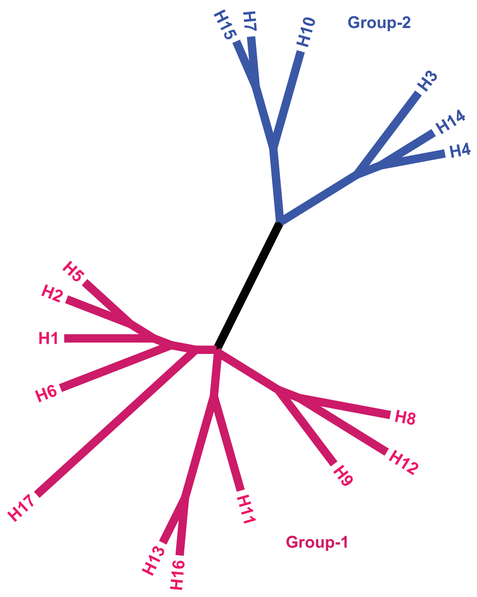

Figure 1: Phylogenetic relationship of influenza A haemagglutinin subtypes

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 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 p8.91, firefly luciferase expressing plasmid pCSFLW, HA expressing plasmid and pCAGGS-TMPRSS2 or pCAGGS-HAT [2] were co-transfected into human embryonic kidney HEK293T/17 cells. As 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.

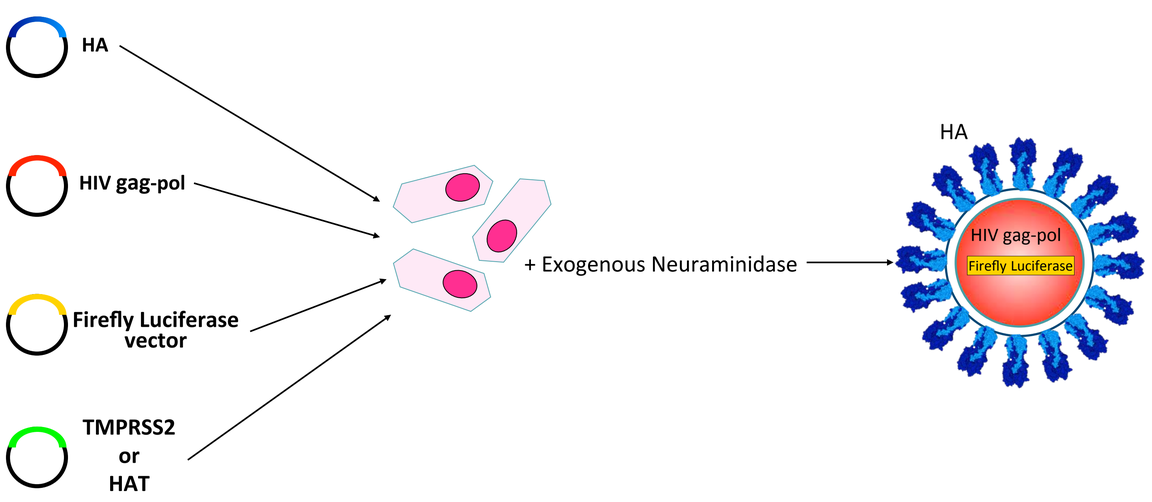

Figure 2: Schematic representation of influenza pseudotype production

To test the ability of TMPRSS2 and HAT to activate haemagglutinin, HEK293T/17 cells (10^4 cells/well) were transduced with the pseudotype particles produced (Figure 3). After 48 hours, luminescence of cell cultures (in relative luminescence units) was evaluated by luminometry using the Bright-Glo assay system (Promega, UK). The pseudotype titres then were expressed as RLU/ml. As a control pseudotypes produced in the absence of proteases were tested for the ability to transduce HEK293T/17 cells before and after TPCK-Trypsin (Sigma) treatment at a final concentration of 50 µg/ml.

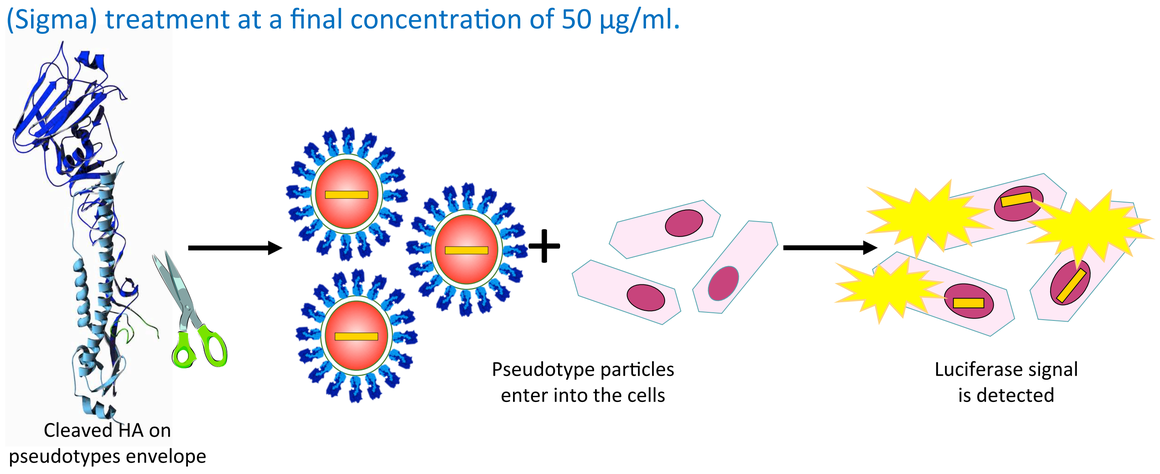

Figure 3. Pseudotypes transduce HEK293T/17 cells after HA cleavage and activation

Pseudotype particles were used as surrogate antigens in neutralization assay using reference OIE hyperimmune antisera or C179 monoclonal antibody (Takara) for H1 and H2 pseudotypes.

RESULTS

We successfully obtained high-titre viral pseudotype particles harbouring the haemagglutinin (Figure 4 and Figure 5). The pseudotype particle titres were compared to the controls: pseudotype particles produced without the envelope protein (Δenvelope) and influenza HA pseudotypes produced in the absence of the protease (Δprotease), 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.

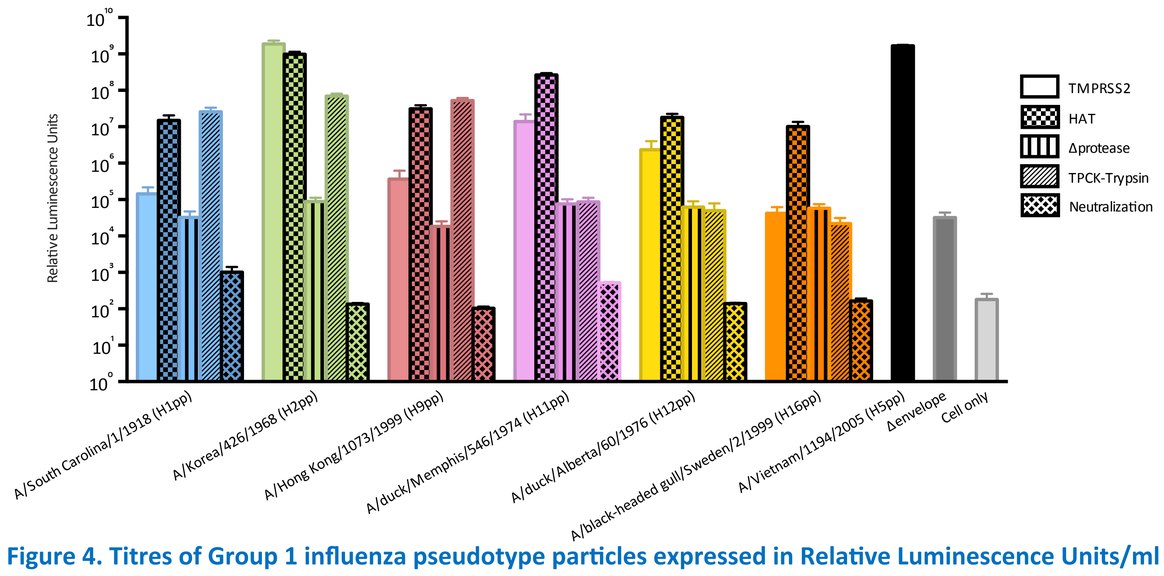

Figure 4. Titres of Group 1 influenza pseudotype particles expressed in Relative Luminescence Units/ml and pseudotypes neutralization by reference antisera and C179 monoclonal antibody (H1 and H2) expressed in Relative Luminescence Units

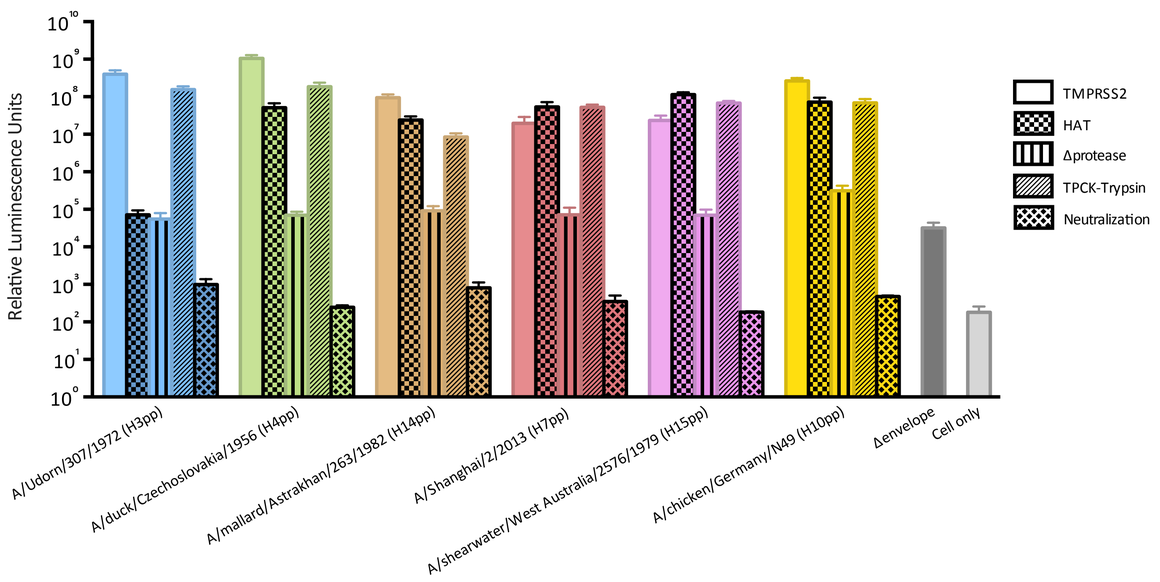

Figure 5. Titres of Group 2 influenza pseudotype particles expressed in Relative Luminescence Units/ml and pseudotypes neutralization by reference antisera expressed in Relative Luminescence Units

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).

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