Neuraminidase-dependent entry of influenza A virus is determined by haemagglutinin receptor-binding specificity

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Introduction

Influenza A viruses (IAVs) like other respiratory viruses, target the airway epithelium of the respiratory tract which varies in cell composition and is covered in a **mucus** layer; a viscous gel predominantly comprised of heavily-glycosylated mucins that provides the first barrier of defence against pathogens. Thus IAVs must be able to cross this barrier, binding to and cleaving decoy receptors present on mucins via the **haemagglutinin (HA)** and **neuraminidase (NA)** proteins respectively, in order to reach their **sialic acid (Sia)** receptors on the underlaying epithelial tissue, which are found on the termini of glycan chains. [1] Conflicting results have been reported regarding the role of NA in IAV entry.

AIM: to dissect the interplay between HA, NA and (decoy) receptors. We analysed the importance of NA activity for virus entry in relation to the receptor-binding specificity of HA, cell surface receptor repertoire and the presence of mucus.

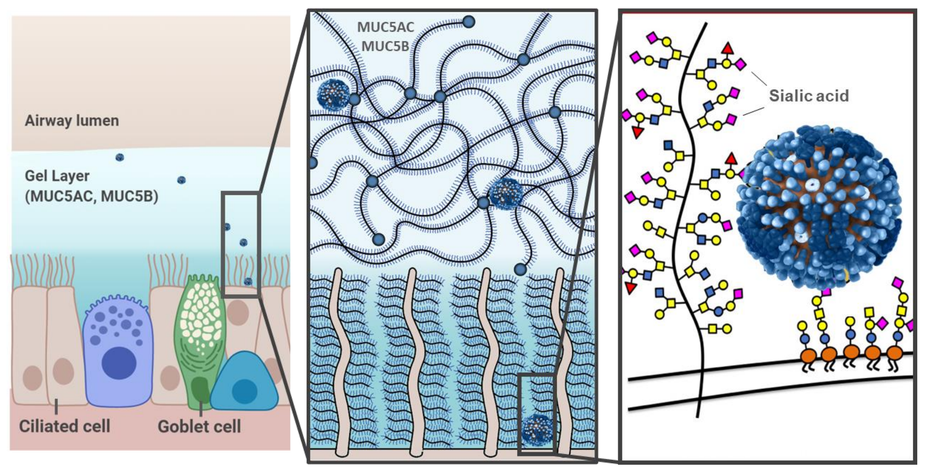

***Fig. 1**. Influenza A virus crossing the respiratory mucus barrier.*

Results

IAVs differ in their NA-dependence for entry

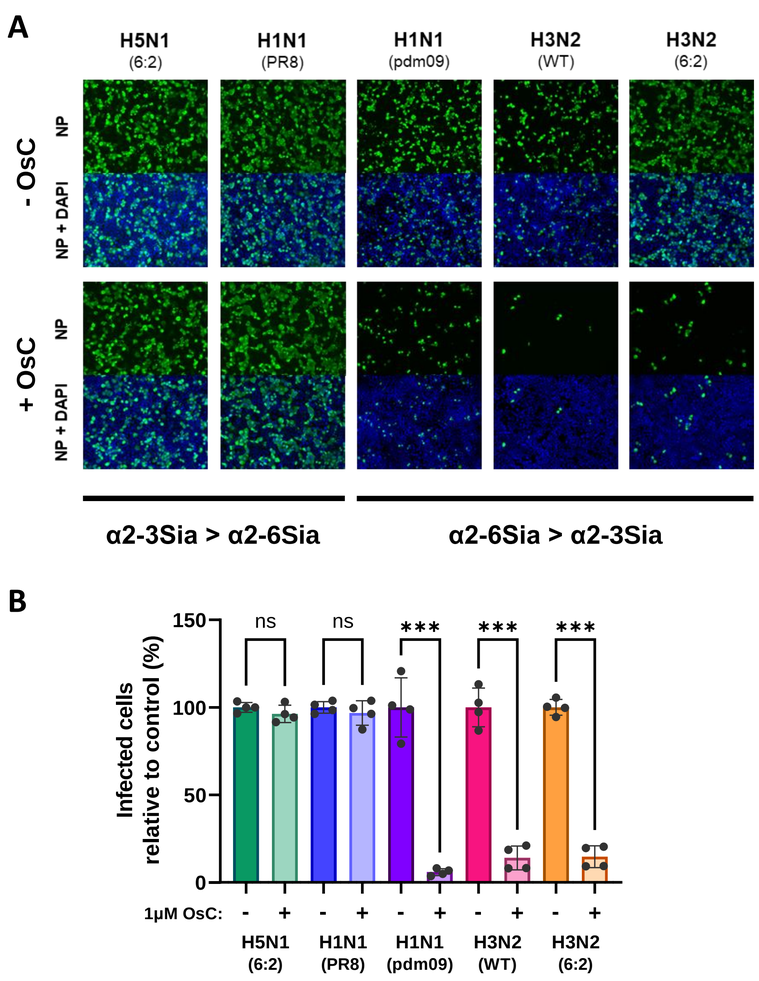

***Fig. 2. (A)** Representative images of single-round IAV infections in MDCK-II cells with/without 1 μM OsC. At 19 hours post-infection (hpi), cells were stained for IAV nucleoprotein (NP) and nuclei with DAPI. NP-positive and total cells quantified and shown in **(B)**. Binding preferences determined by biolayer interferometry (data not shown). Bars represent mean ± SE.*

Differences in IAV OsC sensitivity depends on HA and not NA

***Fig. 3.** Single-round IAV infections with/without 1 μM OsC. **(A)** 19 hpi, cells stained for NP and nuclei, and quantified. **(B,C)** Cells infected (C) 24 h or (B) 48 h post-transfection of Gaussia luciferase (GLuc) reporter plasmid and luminescence read 16 hpi. Bars represent mean ± SE.*

Cell lines with different receptor repertoires affect IAV OsC sensitivity

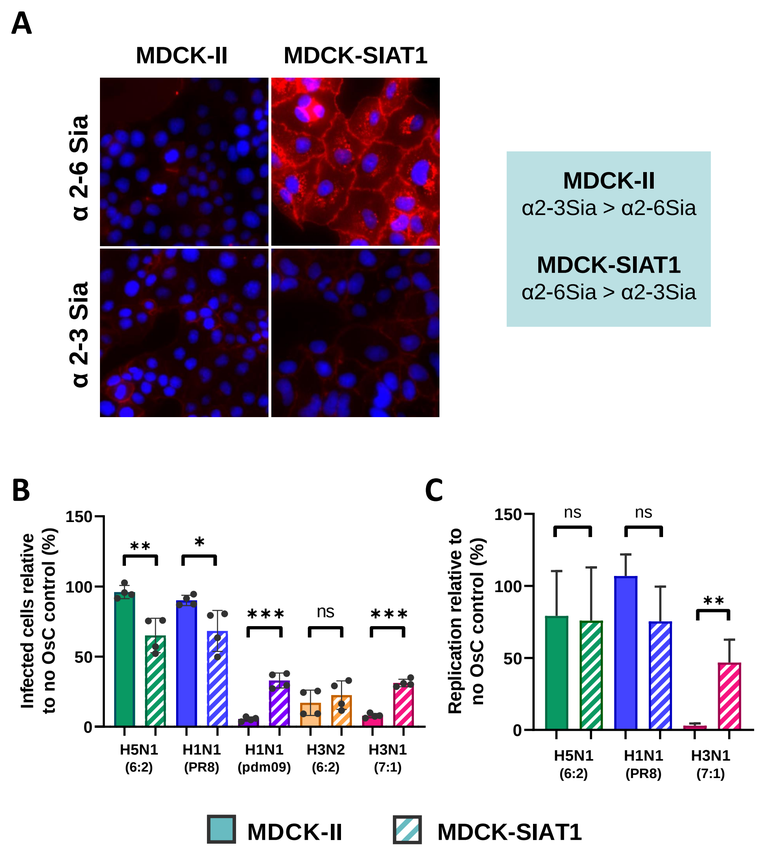

***Fig. 4. (A)** Lectin stains showing α2-6Sia (SNA) and α2-3Sia (MALI) abundance in MDCK-II and MDCK-SIAT1 cells. **(B)** Single-round IAV infections on MDCK-II and MDCK-SIAT1 cells with/without 1 μM OsC. At 19 hpi, cells stained for IAV NP and nuclei, and quantified. **(C)** Single-round IAV infections in MDCK-II and MDCK-SIAT1 cells transfected with GLuc reporter plasmid, with/without 1 μM OsC. Cells were infected 48 h post-transfection and luminescence read 16 hpi. Bars represent mean ± SE.*

Mucus inhibition of entry is receptor repertoire- and virus-dependent and reflects OsC sensitivity

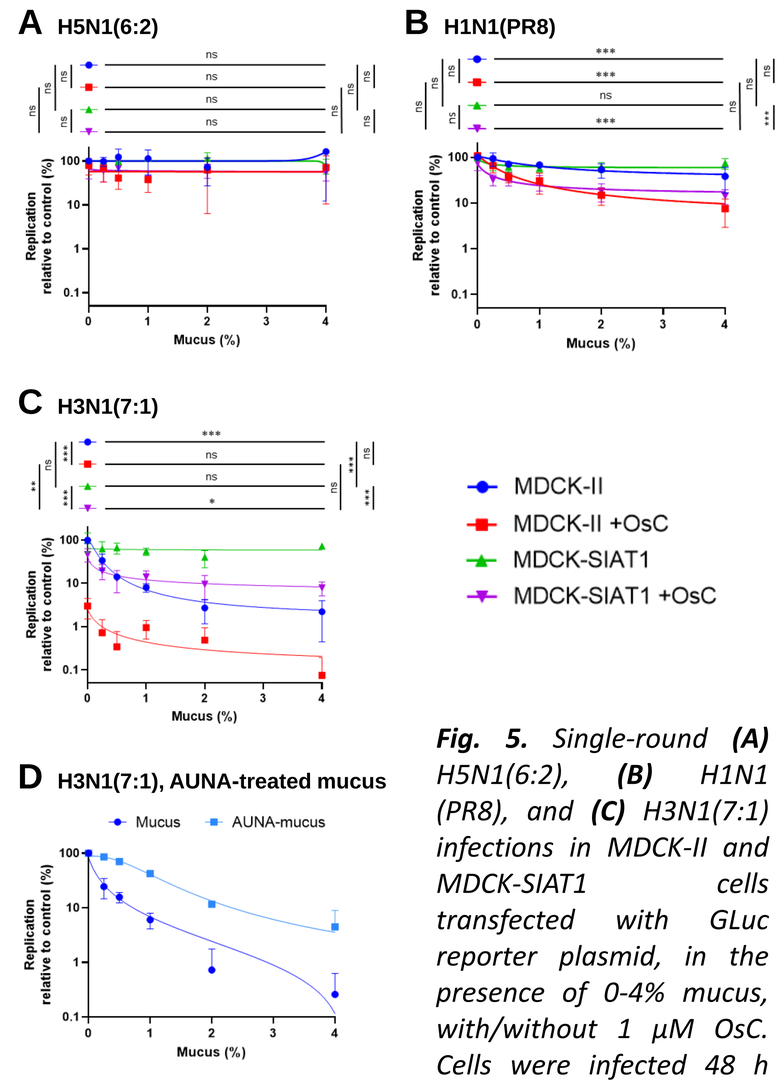

***Fig. 5.** Single-round **(A)** H5N1(6:2), **(B)** H1N1 (PR8), and **(C)** H3N1(7:1) infections in MDCK-II and MDCK-SIAT1 cells transfected with GLuc reporter plasmid, in the presence of 0-4% mucus, with/without 1 μM OsC. Cells were infected 48 h post-transfection and luminescence read 16 hpi. **(D)** Single-round H3N1(7:1) infections in MDCK-II cells transfected with GLuc reporter plasmid, in the presence of 0-4% mucus or AUNA-treated mucus. Plots represent mean ± SE.*

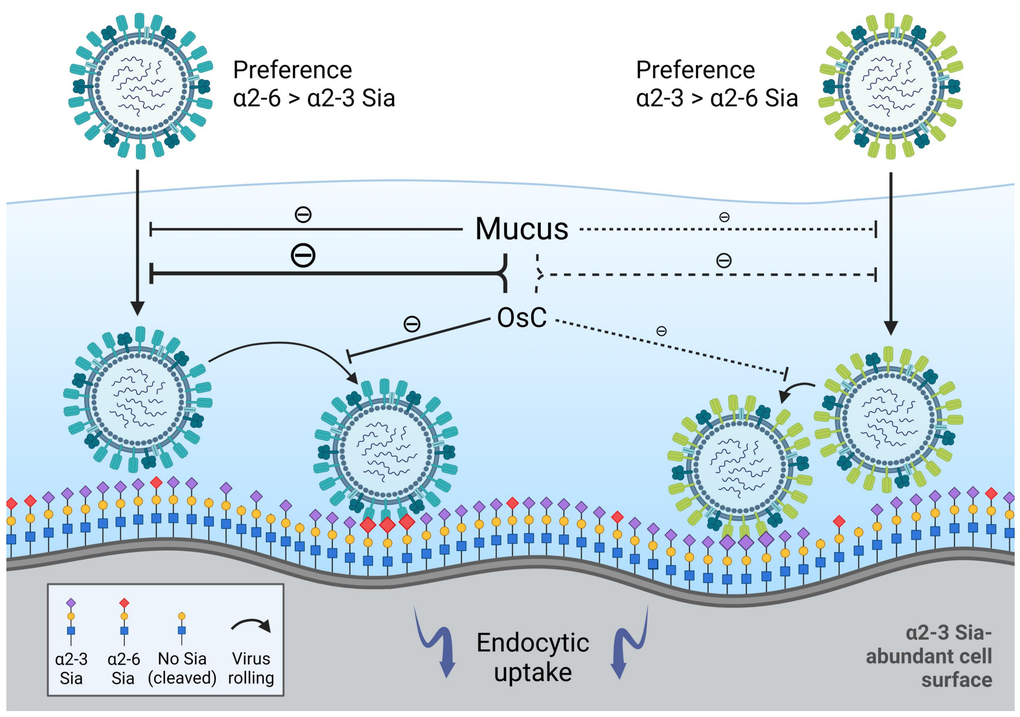

***Fig. 6**. IAV entry inhibition by OsC and/or mucus depends on IAV HA receptor-binding preference and preferred receptor abundance.*

Conclusions

- A virus' sensitivity to entry inhibition by OsC appears to correlate with its HA receptor-binding preference and receptor abundance. If there is not a sufficient abundance of a preferred receptor, NA inhibition results in greater IAV entry inhibition.
- Entry inhibition by OsC correlates with inhibition by mucus; both interfere with functional virus-receptor interactions.
- Results will determine the design of future mucus-virus interaction studies.

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[1] **Wallace *et al.* (2021) Respiratory mucus as a virus-host range determinant. *Trends Microbiol.* DOI: 10.1016/j.tim.2021.03.014**. [2] Cohen *et al.* (2013) *Virol. J.* DOI: 10.1186/1743-422X-10-321.