Replication Dynamics, Pathogenicity, and Evolution of Influenza Viruses in Caco-2 Cells

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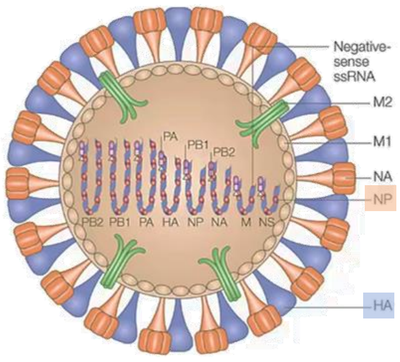
BACKGROUND

Seasonal influenza viruses continue to cause **epidemics** each year, with considerable morbidity and mortality worldwide. Influenza virus has a segmented genome consisting of 7 or 8 single stranded negative-sense **RNA** molecules that encode for up to 12 viral proteins including the two main surface glycoproteins **hemagglutinin** (HA) and **neuraminidase** (NA). The HA and NA protein are responsible for virus entry and release of cells, respectively, through their interaction with **sialic acid** receptors present in host cell[1].

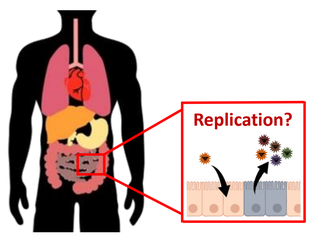


Structure of Influenza virus particle

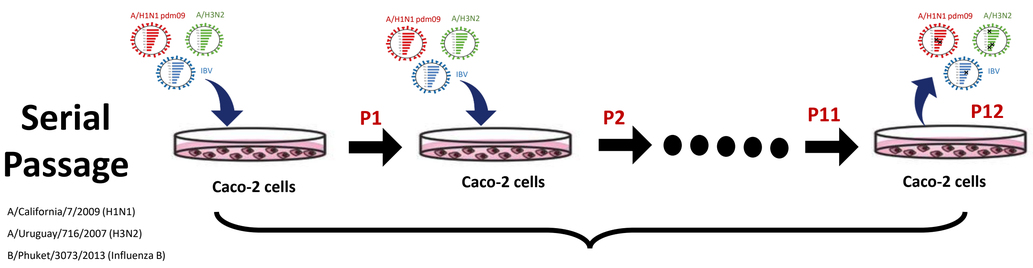
In human, influenza virus mainly replicate in the respiratory tract causing **respiratory** and occasionally systematic symptoms. However, **gastrointestinal** (GI) symptoms such as nausea, diarrhea, vomiting and abdominal pain are not a rare manifestation of influenza infection [2], particularly the pandemic influenza A(H1N1) in 2009 [3,4]. Moreover, viral RNA has been detected in **fecal samples** of up to 20% of influenza-infected patients [2]. Therefore, possibility of influenza virus replication is suspected, but the mechanism of this replication remains to be further investigated.

OBJECTIVE

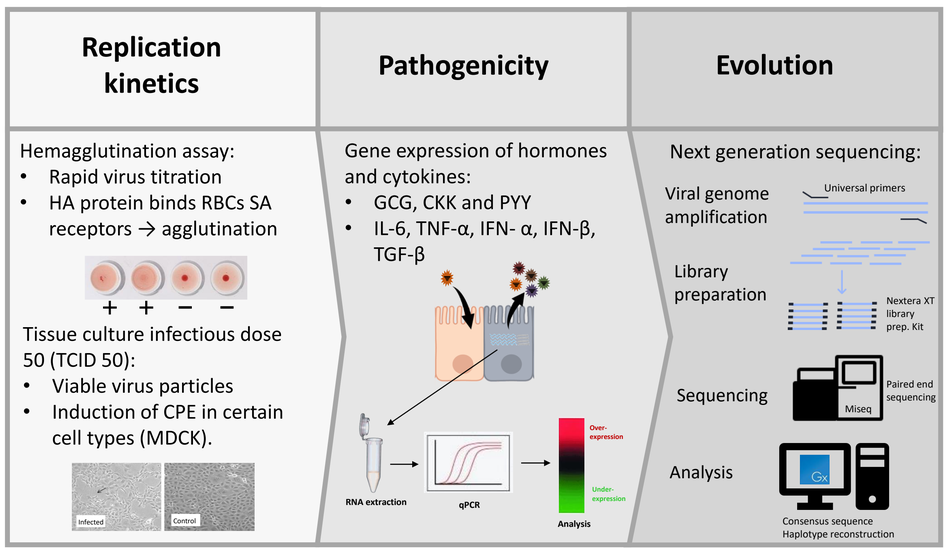
To understanding the adaptation capability of influenza viruses in Human Caucasian colon adenocarcinoma cells (Caco-2) cells by evaluating viral replication kinetics and changes in viral genome and quasispecies diversity as well as elicitation of hormones and cytokines following sequential virus infection.



METHODS



Collect virus supernatant after each passage at 4 days post infection



RESULTS

Replication kinetics of influenza viruses while adapting to Caco2 cell line

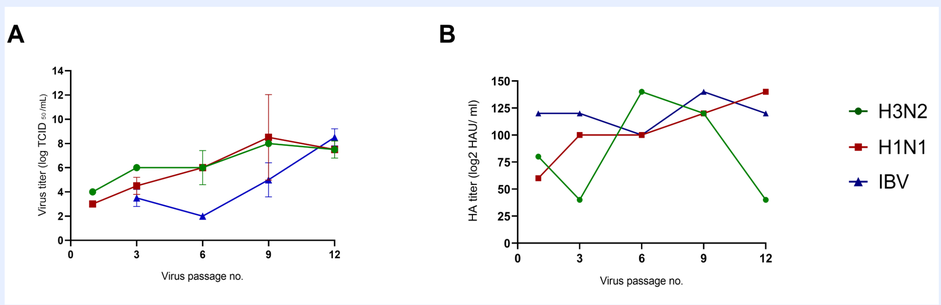


Figure 1. Influenza A subtypes: H1N1 and H3N2 and influenza B viruses were passaged for 12 times in Caco2 cells. For each virus, the titer was quantified by (A) hemagglutinating assay and (B) Virus titer quantified by TCID50 for virus passages 1, 3, 6, 9 and 12. Virus titers (TCID 50) of IBV passage 1 is not reported because clear cut CPE was not observed.

HA protein consensus sequences alignment

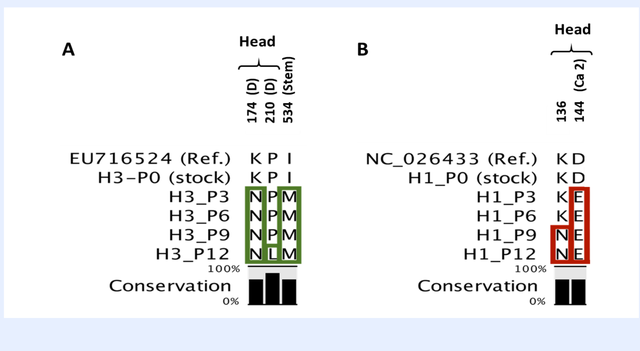


Figure 2. Amino acid substitutions introduced into HA during adaptation process are boxed H3N2 (A), H1N1 (B). Location of identified amino acids substitutions in HA protein; head, stem, or antigenic sites as indicated in parentheses. No amino acids substitutions were detected for IBV Carried out using CLC genomic workbench v.11.

Diversity and frequency of HA haplotypes

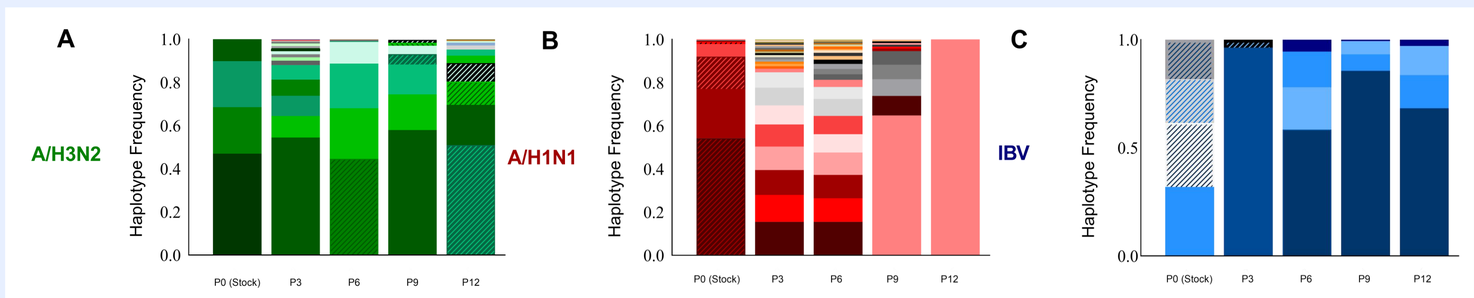


Figure 3. HA haplotypes of (A) H3N2, (B) H1N1, and (C) IBV at different passages in Caco2 cells**.** Number of HA haplotypes were estimated for the original stock virus, passages 3, 6, 9 and 12 using the conservative mode of QuasiRecomb tools. For all HAs, full coding region with at least 1000x coverage was used to reconstruct the haplotypes. Estimated frequency of each haplotype is shown for each assembled haplotype. Shared haplotypes are indicated with the same color; while uniquely colored bars represent haplotypes that appeared once.

CONCLUSIONS

- After 12 passages the three influenza viruses used in this study maintained their infectivity and became more adaptive, represented by yielding highest titer at passage 12.
- Overall, three amino acid substitutions were found in HA protein (K174N, P210L,and I534M) for H3N2 virus and two substitutions for H1N1 virus (K136N and D144E).
- Sequencing analysis of HA haplotypes revealed higher number of haplotypes in H3N2 and H1N1 viruses than in B virus, and some certain HA haplotypes maintained with increasing frequency along the passaging of the virus signifying their potential role in virus adaptation to Caco-2 cells.
- Influenza virus variants that emerge following passaging in intestinal cells might influence viral transmission (fecal-oral rout), adaptation to GI tract, and viral ecology or evolution.
- The expression of hormone and cytokines in Caco-2 cells was considerably different between the passages of H3N2 and H1N1 viruses.
- This study provides insights into possibility of direct infection of intestinal cells with influenza viruses explaining the GI complication during influenza infection.
- Whether intestinal tract could be a site for influenza replication and hence transmission via fecal shedding need to be further explored.

Cytokines and hormone responses following H3N2 and H1N1 infection in Caco-2 cells

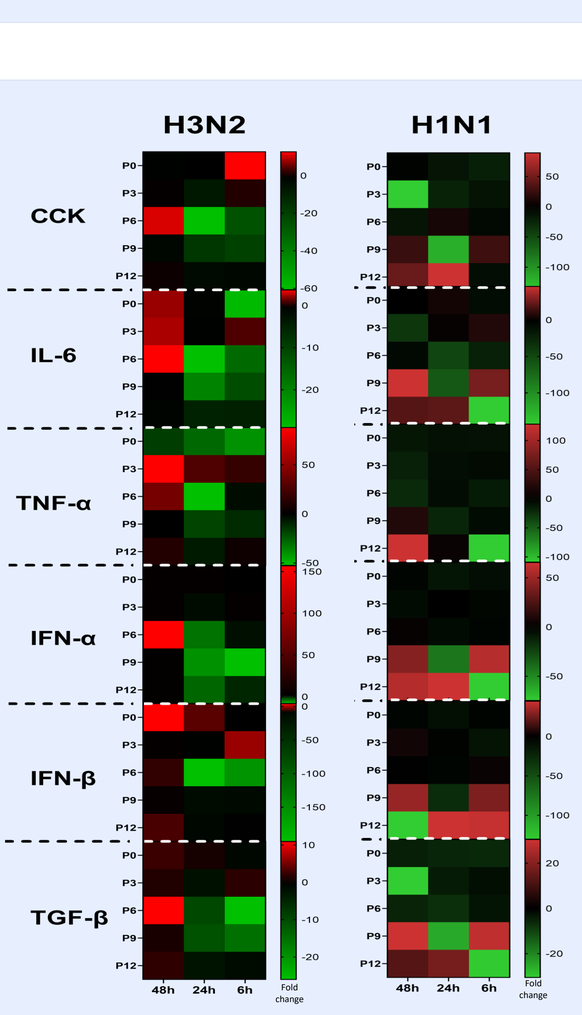


Figure 3. Levels of CCK, IL-6, TNF-α, IFN-α, IFN-β, and TGF-β. levels mRNA were quantified at 6-, 24- and 48-hours post infection by RT-qPCR. The gene expression normalized to β-actin mRNA was shown as fold change of expression values over negative control. The data were analyzed using the 2-DCt method. Experiments were done in duplicates.

References

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Acknowledgments

This Project was supported by grant number NPRP11S-1212-170092