A(H2N2) and A(H3N2) influenza pandemics elicited durable cross-reactive and protective antibodies against avian N2 neuraminidases

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Background

Cases of human infection with avian influenza viruses (AIV) have shown distinct age-specific incidence and disease burden[1]. Epidemiological pattern suggests that children are more susceptible to more severe A(H9N2) infections than adults[2]. **We investigated the reactivity of N2 antibodies against A(H9N2) AIV to determine whether antibodies to NA play a role in protection against zoonotic influenza diseases.**

Objectives

1. To assess the age-stratified seroprevalence of cross-reactive N2 antibodies in the population.
2. To determine the protective efficacy of cross-reactive N2 antibodies.

Methods

1. We used serum samples from healthy individuals in Guangzhou to measure antibody titers against representative A(H9N2) and A(H3N2) viruses selected based on evolutionary analysis[3]. Pooled serum samples were used in passive transfer and A(H9N2) challenge experiments in mouse models.
2. We used EPI-HK cohort to evaluate whether the high avian N2 cross-reactivity was related to the 1957 pandemic exposure
3. We used pre and post-A(H3N2) infection serum samples from CARES cohort to demonstrate whether the cross-reactive antibodies extended to other avian NA subtypes.

Results

Cross-reactive and protective anti-N2 antibodies against A(H9N2) AIV were present in the human population in an age-dependent manner.

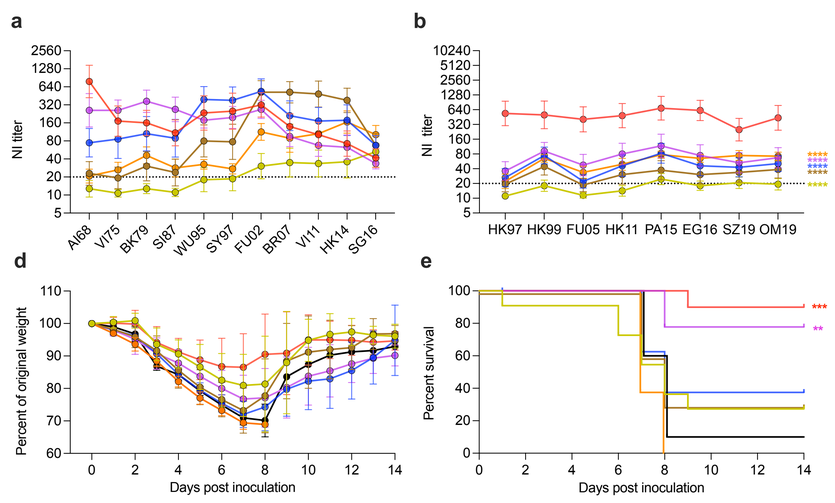

Fig.1 Age-stratified NA inhibition (NI) antibody profiles against (a) A(H3N2) and (b) A(H9N2) influenza viruses; (c) NI-titers against the 1968 pandemic strain A/Aichi/2/1968 (H3N2)(AI68); (d) Weight loss and (e) survival curves of mice inoculated with pooled human sera from respective age-groups. Serum samples from healthy individuals in Guangzhou.

Exposure to the 1968 pandemic N2, protected against A(H9N2) challenge in mice.

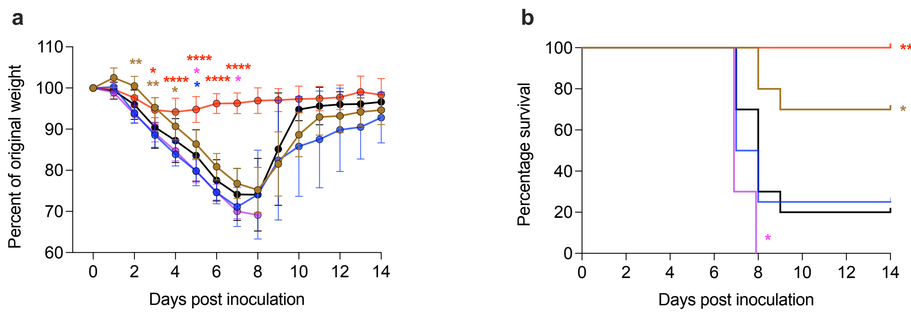

Fig.2 (a) Weight loss and (b) survival of mice inoculated with pooled sera from mice previously immunized with A(H6Nx) viruses containing the NA of AI68(H3N2) or SG16(H3N2) or the control MI15(H1N1) and ZJ19 (H9N2) or PBS.

Individuals born before 1957 have higher anti-AIV N2 titers.

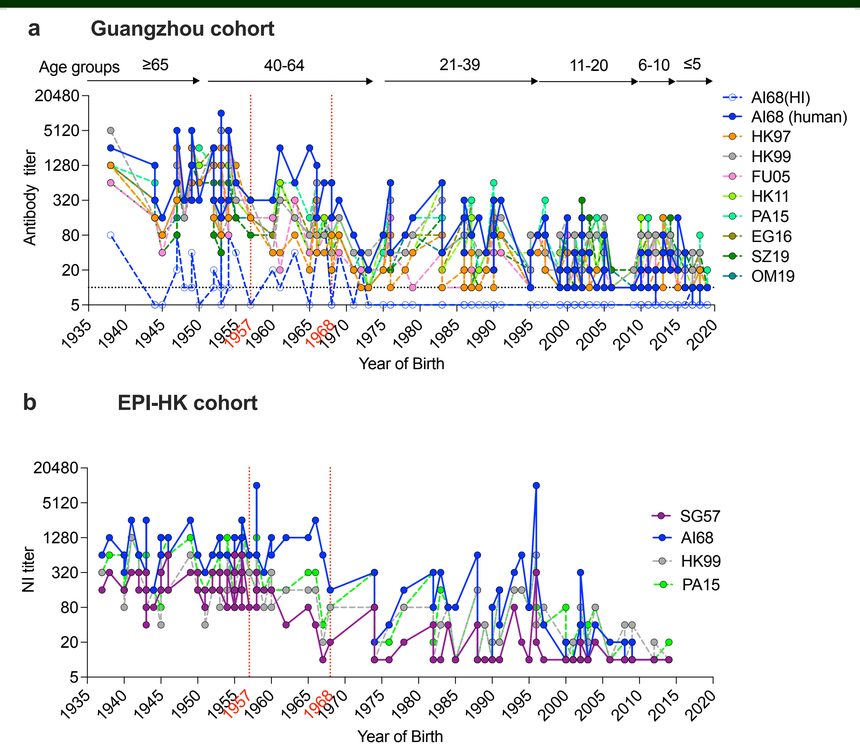

Fig.3 (a) The NI antibody profile against the NA of AI68 and avian A(H9N2), and the HI antibody profile against AI68, as determined for Fig.2, when stratified by year of birth; (b) The NI antibody profile of the EPI-HK participants against N2 of pandemic strains; SG57 and AI68, and avian N2; HK99 and PA15.

Compared to young people, old adult have higher anti-NA antibodies against other avian NA subtypes.

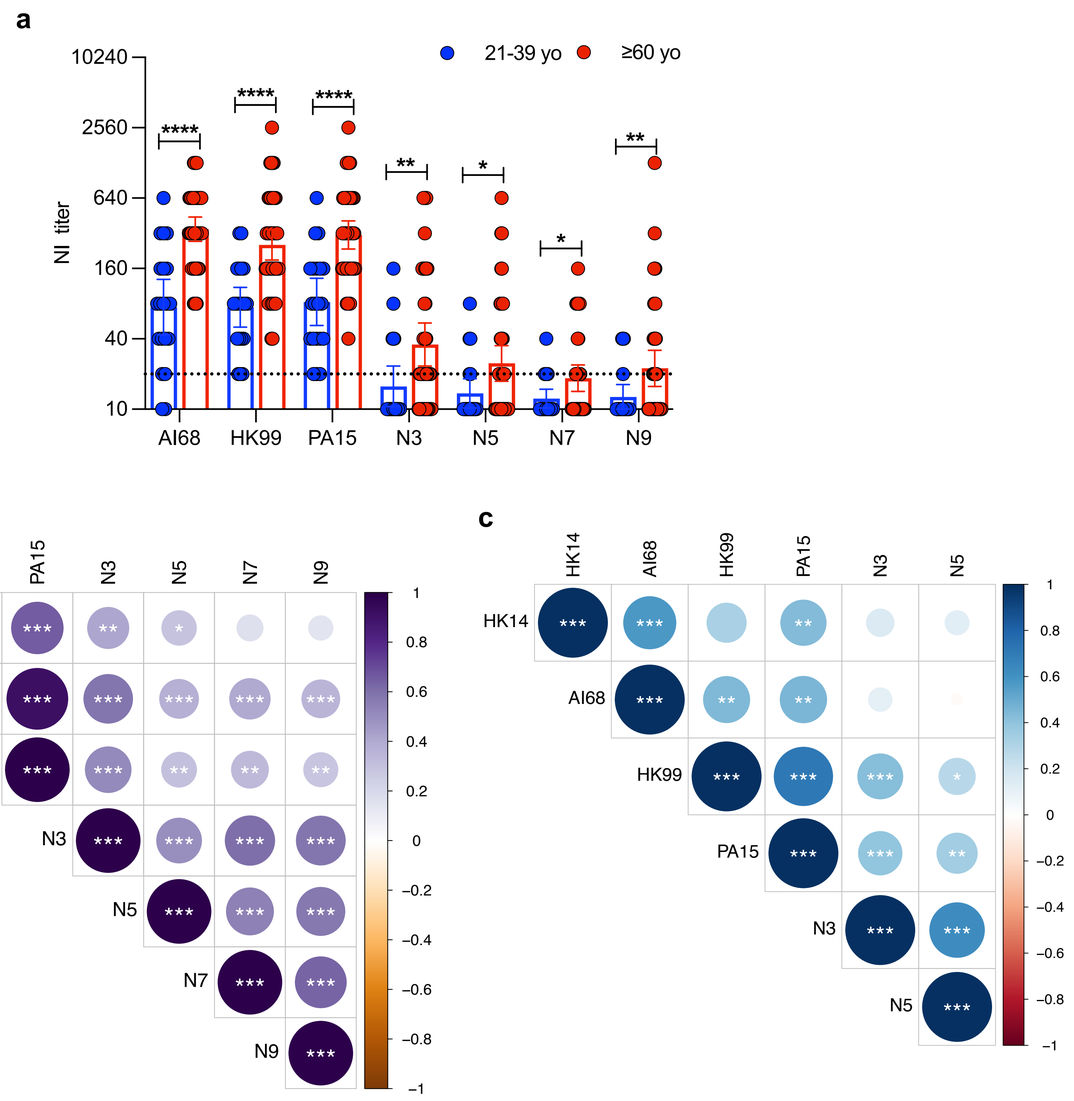

Fig.4 (a) NI antibody titers to the human N2 of AI68 (H3N2), avian N2s from HK99 and PA15, and other NAs; (b) Correlation of baseline NI antibody titers between different NA subtypes and (c) correlation of seroconversion post-infection with recent A(H3N2) influenza viruses between different NA subtypes. Serum samples from CARES cohort.

Conclusion

1. Cross-reactivity to avian N2 was likely due to prior exposure to early human N2s which shared high similarity to avian N2s.
2. Our findings highlight the importance of NA-based immunity.
3. NA immunity should be included in risk-assessments of zoonotic influenza viruses.

References

1. Philippon DAM, Wu P, Cowling BJ, Lau EHY. 2020. Avian Influenza Human Infections at the Human-Animal Interface. J Infect Dis 222: 528-37
2. Peacock THP, James J, Sealy JE, Iqbal M. 2019. A Global Perspective on H9N2 Avian Influenza Virus. Viruses 11
3. Liang Z, Lin X, Sun L, Edwards KM, Wong SS, et al. 2024. A(H2N2) and A(H3N2) influenza pandemics elicited durable cross-reactive and protective antibodies against avian N2 neuraminidases. Nat Commun 15: 5593

Acknowledgements

This work was supported by the Theme-based Research Scheme (Project No. T11-712/19-N) of the Research Grants Council of the Hong Kong SAR Government, the Hong Kong's Research Grants Council General Research Fund (GRF) (No. 17114023) and the State Key Laboratory for Respiratory Diseases Open Project (SKLRD-OP-202006).