Evaluating the abundance of cross-reactive influenza hemagglutinin and neuraminidase-targeted memory B cells

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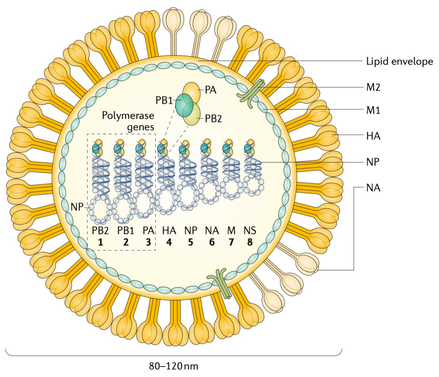
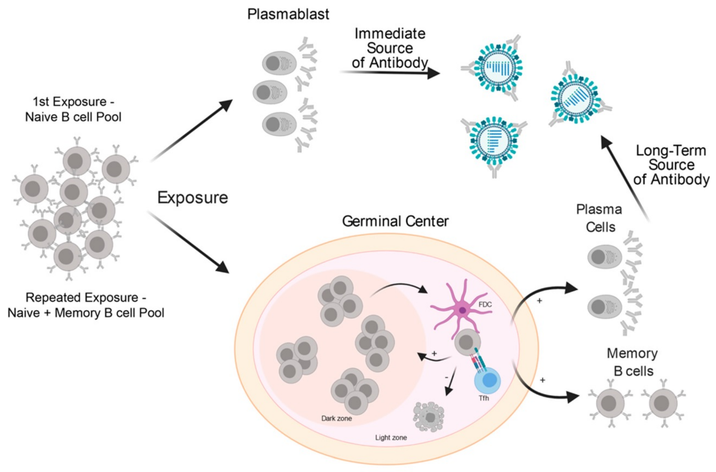
Introduction

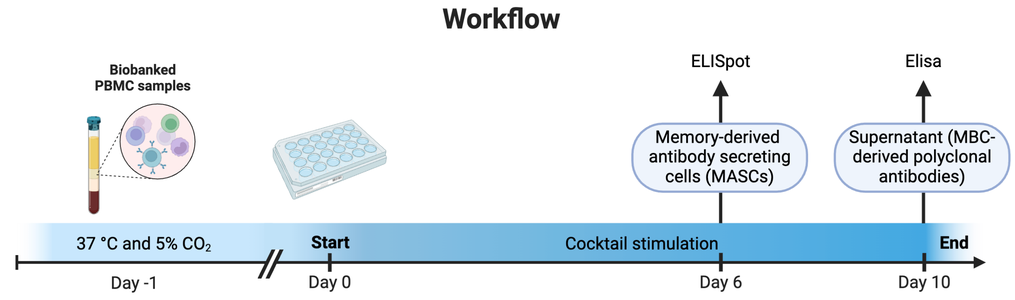
Influenza is a highly infectious respiratory disease, leading to significant global morbidity and mortality. According to the World Health Organisation (WHO), influenza causes approximately **3–5 million** influenza severe cases and **290,000 – 650,000 deaths** per year worldwide. The two glycoproteins, namely **haemagglutinin (HA)** and **neuraminidase (NA)**, are key targets for the immune response and play essential roles in viral entry and release from host cells.





However, the relationship between plasma antibodies and circulating **memory B cell (MBC)** profiles in the human body remains unclear. Additionally, the different antibody profile patterns and immune responses after influenza infection between children and the elderly groups also raise questions about the influence of **age** on the evolution of MBC compartments.

Methods



The biobanked human peripheral blood mononuclear cell (PBMC) samples from four individuals were selected and induced by a stimulation cocktail to **form memory-derived antibody-secreting cells (MASCs)**. After 6 days of stimulation, some of the MASCs were collected and the frequency was analyzed by **ELISpot assay**. The supernatant secreted by the MASCs was collected as **MBC-derived polyclonal antibodies (MPAbs)** on day 10. The titers of the MPAbs in the supernatant and the antibodies in the plasma samples of the same individual were determined by **ELISA**. In the ELISpot and ELISA assays, a total of **11 influenza A strains** were selected as targets based on prototypical strains of geographical and temporal importance. Statistical analyses were conducted after collecting the experimental data, with significance determined by a two-tailed test at $p < 0.05$.

This study received ethical approval from the Institutional Review Board of the University of Hong Kong (Ref: UW 23-268).

Results

1 High H3N2 specificity in derived MASCs (done by Dr. Lin)

- All MASC samples from the four individuals indicated strong cellular specificity towards **H3 and N2 strains**, suggesting a dominant MBC population targeting H3N2 across these individuals (Fig. 1, 2).

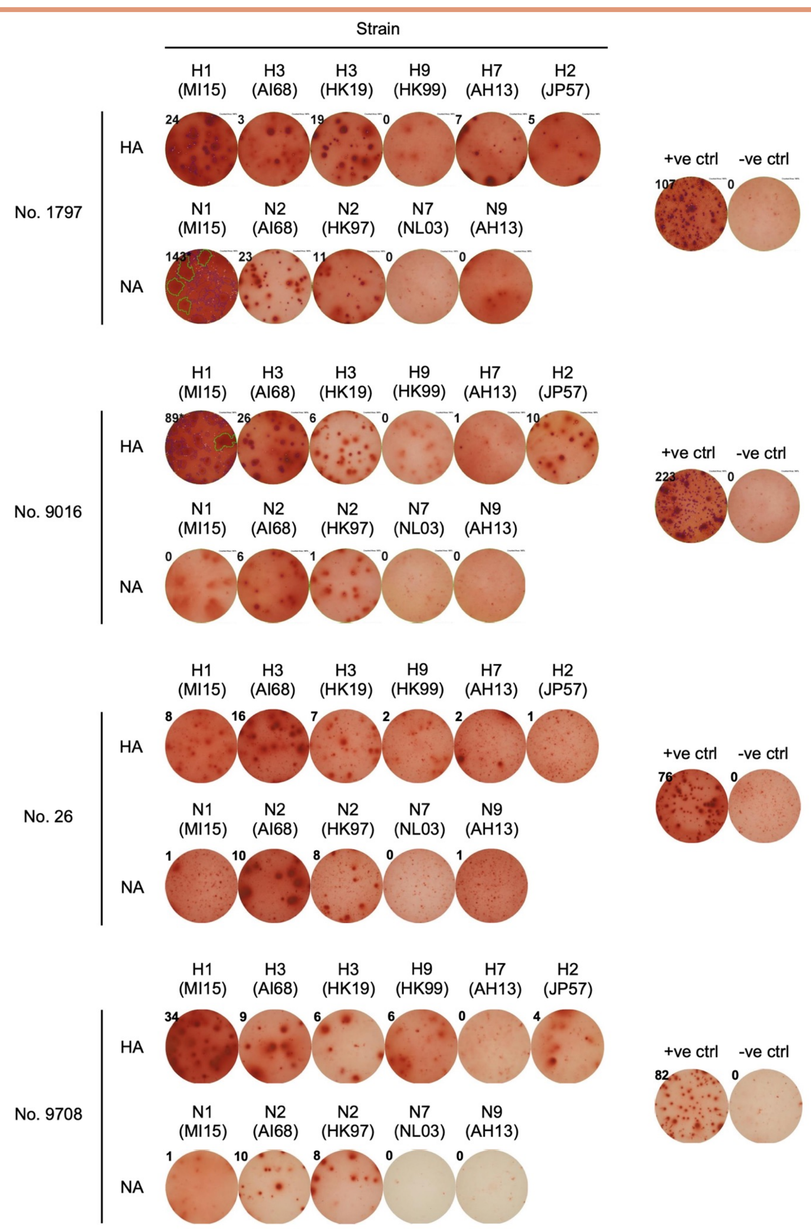


Fig. 1. ELISpot results from MASCs against 11 strain antigens.

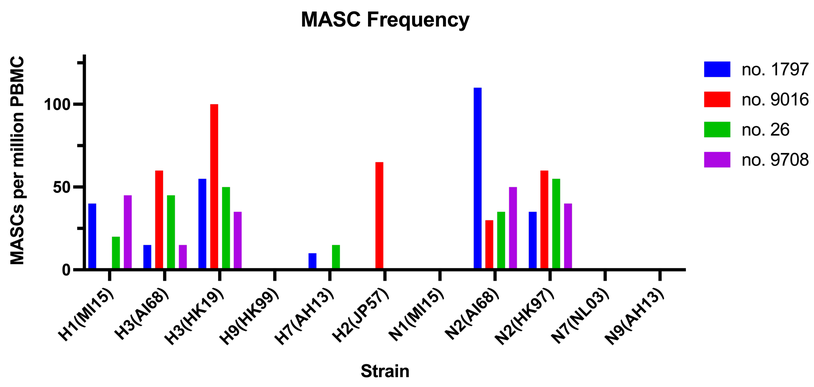


Fig. 2. The frequency of MASCs derived from per million PBMC.

2 Positive correlation of the plasma antibody titers and MPAb titers

- The antibody profiles were **similar** between plasma and supernatant samples, although individual no. 1797 and no. 9708 showed high plasma titers for N2(AI68) and N2(HK97) with undetectable MPAb titers.
- Spearman correlation analysis revealed a **significant positive correlation** in individual no. 9016, indicating a strong relationship between plasma and MPAb titers (r=0.9738, p<0.0001) (Fig. 3).

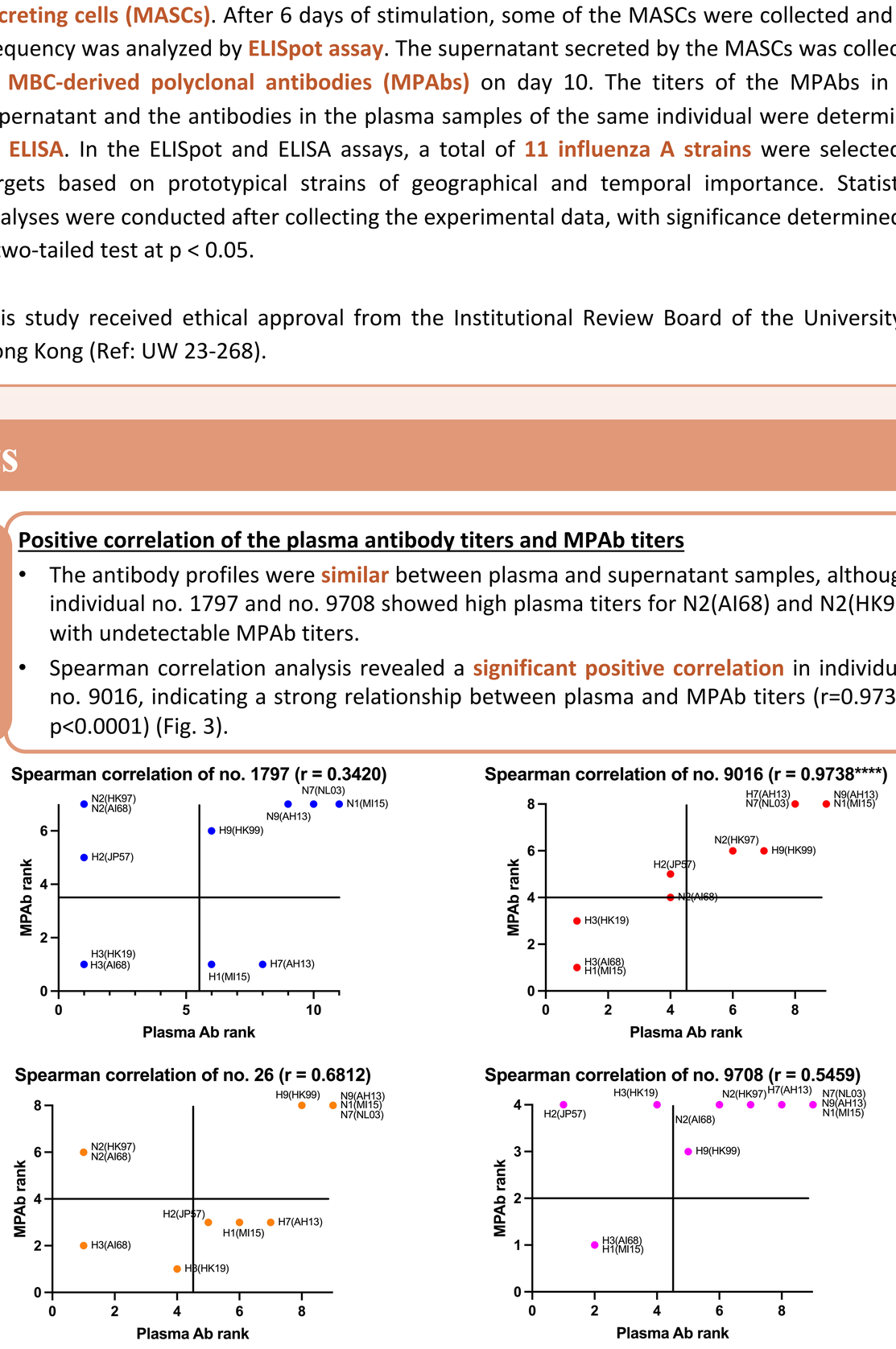


*Fig. 3. Spearman correlation of the rankings of plasma Abs and MPAbs titers. The highest titer was ranked as 1 while lowest titer was ranked as 11. **** refers to p (two-tailed) <0.0001.*

3 The magnitude of MBC reactivity to HA and NA antigens closely parallels to the plasma antibody profiles

- **Significant positive correlations** between MASC specificity and both plasma Ab levels and MPAb levels, with a stronger association observed with **plasma Ab titers** (Fig. 4).
- MASC populations targeting certain antigens did not always result in elevated antibody production, indicating potential **limitations** in MASC functionality in producing specific antibodies.

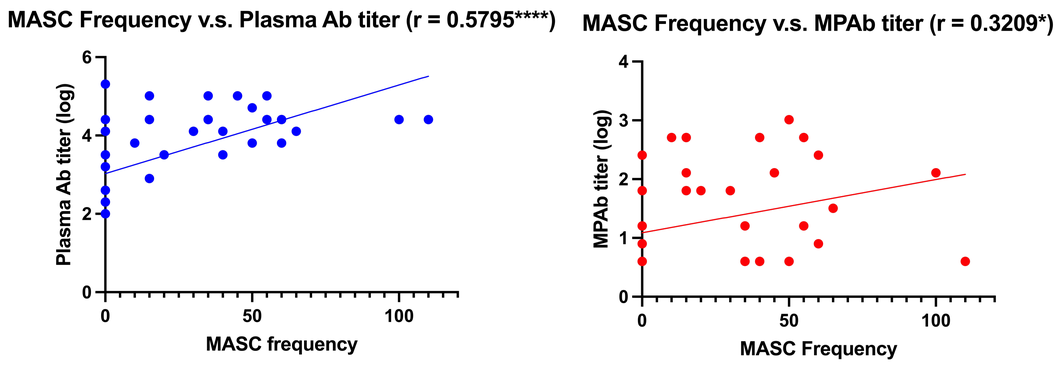


*Fig. 4. Relationship between MASCs frequency, plasma Ab titers and MPAb titer. * refers to p (two-tailed) <0.05, **** refers to p (two-tailed) <0.0001.*

Reference list

1. Chen, X., Liu, S., Goraya, M. U., Maarouf, M., Huang, S., & Chen, J.-L. (2018). Host Immune Response to Influenza A Virus Infection. *Frontiers in Immunology, 9*(320). https://doi.org/10.3389/fimmu.2018.00320
2. Krammer, F., Smith, G. J. D., Fouchier, R. A. M., Peiris, M., Kedzierska, K., Doherty, P. C., Palese, P., Shaw, M. L., Treanor, J., Webster, R. G., & García-Sastre, A. (2018). Influenza. *Nature Reviews Disease Primers, 4*(1). https://doi.org/10.1038/s41572-018-0002-y
3. Liang, Z., Lin, X., Sun, L., Edwards, K. M., Song, W., Sun, H., Xie, Y., Lin, F., Ling, S., Liang, T., Xiao, B., Wang, J., Li, M., Leung, C.-Y., Zhu, H., Bhandari, N., Varadarajan, R., Levine, M. Z., Peiris, M., & Webster, R. (2024). A(H2N2) and A(H3N2) influenza pandemics elicited durable cross-reactive and protective antibodies against avian N2 neuraminidases. Nature Communications, 15(1). https://doi.org/10.1038/s41467-024-49884-9
4. World Health Organization. (2023). Influenza (Seasonal). Who.int; World Health Organization: WHO. https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal)

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