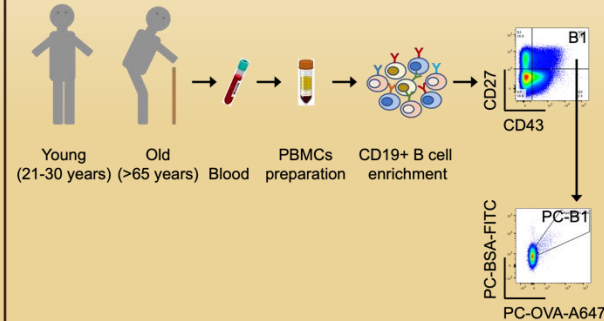


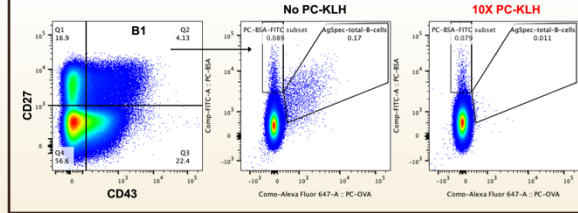
ABSTRACT

B1 cells, a distinct lineage of B cells originally identified in mice and subsequently in humans, produce natural antibodies in the absence of external stimulation such as infection or vaccination. However, with advancing age, B1 cells decline in number, reduce antibody secretion, and lose antigenic diversity. Phosphorylcholine (PC) is a ubiquitous antigen expressed on several microbial pathogens including *Streptococcus pneumoniae* and apoptotic cells. PC-specific natural antibodies are particularly important in providing protection against pathogens such as *Streptococcus pneumoniae*. While younger individuals (ages 5–49) typically maintain robust PC-specific natural antibody levels, older adults exhibit a marked reduction in these antibodies. In this study, we investigated the behavior of PC-specific human B1 cells. Initial attempts to identify PC-specific B1 cells using PC-BSA-FITC led to non-specific recruitment due to binding to the carrier protein or FITC fluorophore. To overcome this, we developed a dual-staining method using PC-BSA-FITC and PC-OVA-A647, gating cells based on diagonal staining. These cells were further validated using PC-KLH to confirm specificity. Analysis of B cells from the peripheral blood of young and older healthy volunteers revealed that PC-specific B1 cells decline with age, paralleling the overall decline in B1 cells. Our findings highlight that the age-related decline in B1 cells and B1 cell-derived natural antibodies contributes to the increased susceptibility of older adults to pneumococcal infections. These insights provide a foundation for developing strategies to restore natural antibody levels and improve immune defenses in aging populations.

METHODS

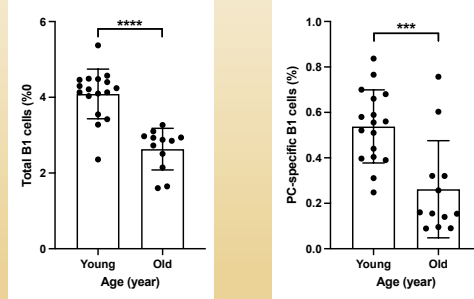


PC-KLH inhibits PC-BSA-FITC and PC-OVA-A647 binding

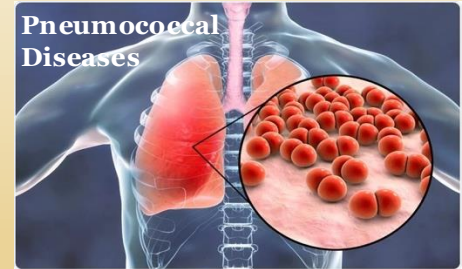
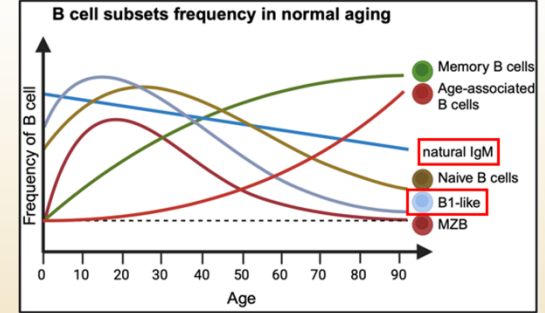


RESULTS

Human B1-like and PC-B1-like B Cells decline significantly with advancing of age



CONCLUSION



REFERENCES

Rodriguez-Zhurbenko, N, et al. 2019. *Front Immunol*
Mol, J, et al. 2021. *Front Immunol*

ACKNOWLEDGEMENT

We gratefully thanks to our Flow and Imaging core manager Michael Clemente for his excellent support with the Flow cytometry work. This work was supported by NIH R01 (AI142004) to Prof. Rothstein.