Engineering B-Cell Lines to Investigate the Evolution of Broadly Neutralizing Antibodies Against Influenza A Virus

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Influenza A viruses (IAV) exhibit high genetic diversity due to frequent mutations and genomic reassortment during co-infections, allowing IAV to become endemic and potentially cause pandemics through antigenic shifts. To address pandemic risks, research has focused on universal vaccines targeting conserved regions of the hemagglutinin (HA) protein, specifically the stem domain. Stem-directed broadly neutralizing antibodies (sbnAbs) often originate from particular heavy chain V-segment germline genes and initially target either group 1 or group 2 IAV. Over time, B cells expand the breadth of sbnAbs to recognize both IAV groups. This study examines the development of sbnAbs using hypermutating B-cell lines expressing the germline precursors of previously characterized sbnAbs. Cell lines were engineered to express unmutated common ancestor (UCA) Fab sequences, which were introduced into RA1 B-cells. Serial passaging and Fluorescence-Activated Cell Sorting (FACS) analysis were used to monitor somatic hypermutation and antibody evolution. Understanding the evolutionary pathways of sbnAbs can guide the design of universal vaccines by focusing on key interactions that occur early in the maturation process of B cell receptors, particularly those derived from germline genes with a higher propensity for sbnAb development. Furthermore, this knowledge can offer insights for the design of therapeutic antibodies targeting the HA stem region of IAV.