DEVELOPMENT OF ANTIGEN SPECIFIC ELISPOT ASSAYS FOR SINGLE CELL DETERMINATION OF ANTIBODIES ELICITED BY DIFFERENT INFLUANZA VACCINES

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Each year the influenza virus infects 5-10 % of adults and 20-30 % of children. Of these infections, 3-5 million lead to severe illness and in 250,000- 500,000 cases the infections lead to death. When an influenza virus enters the host body, the host's immune system recognizes the foreign invader and instructs B-cells to produce and secrete antibodies (Abs) that will bind to the hemagglutinin (HA) proteins that coat the surface of the virus. The structure of a HA molecule includes a helical stem domain that is attached to the plasma membrane of the virus, and a globular head domain that mediates binding to target host cells. Most Abs created by B-cells in response to a normal influenza infection or vaccine are aimed at the HA glycoproteins and typically bind to immunodominant epitopes on the globular head. On occasion, rare Abs are made that bind to *conserved* epitopes on the stalk of the flu HA and can neutralize diverse types of influenzas. The antibodies which recognize these *conserved* epitopes are "cross-reactive" and very desirable. Currently-available trivalent influenza vaccines cannot protect against future flu viruses with new mutations that are constantly occurring. An appropriate and accurate assay to detect antibody production can help determine the efficacy of an influenza vaccine. The purpose of this research was to use an enzyme-linked immunospot (ELISPOT) assay to determine the breadth of immune response from influenza vaccinations. By fine tuning the concentration of antigen bound to the base of the ELISPOT assay wells and the concentration of cells placed in the wells, we were able to develop assays with optimal results. The assays showed various extents of antibody cross-reactivity, indicating that the antibodies can neutralize multiple influenza virus strains. Cross-reactive antibodies provide broader protection against influenza viruses. The ability to encourage the immune system to produce such cross-reactive antibodies could lead to the development of a universal influenza vaccine. The assay developed here can be used to test immune response to more broadly protective vaccinations.