May 20th, 12:30 PM

Isolation of Human Antigen-Specific Antibodies from Memory B-Cells Nearly Two Years Post Vaccination

Stuart Nelson
University of Massachusetts Medical School

Andrew Crowley
University of Massachusetts Medical School

William D. Thomas Jr.
University of Massachusetts Medical School

See next page for additional authors

Isolation of Human Antigen-Specific Antibodies from Memory B-Cells Nearly Two Years Post Vaccination

Stuart Nelson[1], Andrew Crowley[1], William Thomas[1] and Colby A Souders[1]

MassBiologics of the University of Massachusetts Medical School, Department of Product Discovery[1], 460 Walk Hill St, Boston, MA 02126.

Corresponding Author: Colby.Souders@umassmed.edu, (617)-474-4050

Abstract:

Isolation and production of therapeutic human monoclonal antibodies (mAbs) traditionally utilizes a handful of techniques including antibody engineering, phage display, hybridoma generation from transgenic mice or EBV immortalization of B-cells. Over the past decade a new approach has emerged that attempts to extract antigen-specific memory B-cells from the peripheral blood of individuals vaccinated or infected with the target. Initial attempts focused on culturing B-cells and inducing differentiation to plasmablasts for analysis of antibody-antigen specificity, but results were largely mixed due to difficult culture conditions and/or rarity of target cells. With advancing technology in cell sorting, single antigen-specific memory B-cells can be identified and sorted with fluorescently labeled antigens. This method has produced virus-specific mAbs from HIV-infected patients and tetanus-specific mAbs within weeks after Tdap immunization. Many other studies claim to have found antigen-specific mAbs months to years after immunization or clearance of an infection; however, these studies fail to provide direct evidence of antibody specificity by cloning and expressing the mAbs from B-cells.

Here we report the efficient isolation of tetanus-specific mAbs from a subject Td-immunized almost two years prior to blood draw. Initially, the total B-cell population was isolated from peripheral blood mononuclear cells enriched by negative selection, then stained to identify tetanus-specific memory B-cells. These cells were individually sorted and PCR was performed to amplify heavy and light chain variable regions of the B-cell's antibody mRNA. After sequencing, 15 of 42 samples produced both heavy and light chain antibody sequence and 11 mAbs were cloned and transiently expressed. ELISA analysis indicated 5 of the 11 mAbs bound the Hc protein fragment of tetanus toxin and 3 were specific for Hc. We plan to extend this initial success to additional targets and longer gaps between vaccination and B-cell isolation to identify functional therapeutic human antibodies.