World Health Organization

The WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention Atlanta, GA: 2007 report on preliminary characterization of a collection of monoclonal antibodies generated against expressed vaccinia proteins and their recognition of variola virus

Victoria Olson, Scott Smith, Kevin Karem, Charles Whitbeck, Roselyn Eisenberg, Gary Cohen, Inger Damon

Background: Orthopoxviruses have two infectious forms of virus particles, mature virus (MV) particles and enveloped virus (EV) particles. Each form differs in its abundance, structure, and function. MV particles are the most numerous type of virion, possess one membrane, release from host cells upon lysis, and are believed to be responsible for transmission between hosts. EV particles make up only a fraction of overall infectious virions, possess two membranes, release from host cells through exocytosis, and are believed to be responsible for transmission within a host. Because EV undergoes extra membrane wrapping, they contain a different assortment of proteins than MV. Proteins from both membranes have been shown to be targets for neutralization by antibodies, and, in addition, vaccination with proteins from both membranes has been shown to elicit protective immunity in various animal models. The most commonly studied membrane proteins for potential use as vaccines are vaccinia virus A33R and B5R from EV, and A27L and L1R from IMV. These proteins may also be important in development of diagnostic strategies such as antigen capture assays. Because of the interest in these proteins as vaccine or therapeutic targets, abundant sources of monoclonal antibodies are now available for study. Here we characterize the *in vitro* ability of monoclonal antibodies generated against these proteins to functionally recognize variola virus as evidenced by neutralization assays.

Methods: All assays with variola virus were performed in the maximum containment laboratory while observing biosafety level 4 procedures at the Centers for Disease Control and Prevention. Plaque reduction neutralization tests (PRNTs) were performed to measure the ability of each antibody to neutralize variola virus. Antibodies were mixed with variola virus and incubated for 3 hours prior to their addition to confluent layers of BSC-40 cells for 3 days. Antibodies were tested in duplicate at concentrations ranging from 10ug to 0.0032ug. Controls included virus only wells, naïve serum, and rabbit anti-variola (Bangledesh) pooled sera. Neutralization was measured by comparison to virus only wells and endpoint titers were reported at 50% and 90% levels of plaque reduction.

Comet reduction assays were performed to evaluate the ability of antibodies to recognize variola EV as evidenced by the inhibition of variola EV release measured by comet tail formation. Confluent monolayers of BSC-40 cells were inoculated with 50 pfu/well and incubated for one hour. After removing and washing inoculums, cells were incubated for 4 days with growth media containing antibody in duplicate at concentrations ranging from 25ug to 0.008ug. Controls included rabbit anti-variola (Bangledesh) pooled sera and virus only wells. Comet tail formation was observed by immunohistochemistry staining.

Results: Many of the antibodies that were raised against vaccinia virus membrane proteins cross reacted with variola virus as shown by reductions in comet tail formation and plaque number. However, our data, in comparison to previous studies with vaccinia virus by Aldaz-Carroll et al. (J. Virol. 81 (15): 8131-8139), demonstrate differences in reactivity of some of the antibodies with variola virus. These characteristics may be exploited to develop diagnostic assays. Additionally, in order for novel smallpox vaccines or immunotherapeutics to achieve a maximal level of protective efficacy, they may need to be optimized by incorporating antigenic epitopes of variola virus proteins.