

The WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention Atlanta, GA: 2006 report on support for development of second and third generation smallpox vaccines

Kevin Karem, Victoria Olson, Scott Smith, Inger Damon

Vaccine trials are underway to assess the safety and efficacy of novel second generation smallpox vaccines in human subjects. As part of the assessment, samples were submitted to the Centers for Disease Control and Prevention for testing the ability to neutralize Variola virus in vitro. Plaque reduction and neutralization assays (PRNTs) were performed on trial sera against a Variola strain originally isolated from Bangladesh. Controls for each assay included neutralization of Variola with positive control sera (multiply vaccinated with Dryvax) and Vaccinia ImmunoGlobin (VIG) as well as naïve human sera samples. PRNT levels were determined by comparison to virus only controls and reported as the endpoint titer achieving 50% or 90% plaque reduction. PRNT assays, which target the mature virus (MV) forms of orthopoxviruses, using variola, represent one method of efficacy testing for protective antibody responses and may provide a method to compare developing vaccine candidates. Additional methods (comet reduction or extracellular enveloped virus (EEV) titration) will focus on the ability to neutralize EEV, implicated in the dissemination of orthopoxviruses within the host.

Previous observations have suggested that there is not a 1:1 correlation in PRNT assays when vaccinia (VAC) and variola (VAR) are used as the virus targets for neutralization. One of the early reports was from Downie AW and McCarthy J Hyg. 1958. During the development of the ACAMBIS 1000 vaccine, studies performed at CDC and at ORAVAX additionally demonstrated there was not a one to one correlation. The following data is extracted from Weltzin et. al. Nat. Med. 2003.

# primates	vaccine	take	VAC >50% PRNT ≥1:40	VAR >50% PRNT ≥1:40
6	ACAM1000	6/6	5/6	2/6
6	Clone 4	6/6	1/6	2/6
6	Dryvax	6/6	4/6	3/5

Although all non-human primates developed a “take,” presumed to be a correlate of protection, the numbers of primates which developed the ability to neutralize variola at a $>50\%$ PRNT $\geq 1:40$ was not the same as the number with the ability to neutralize vaccinia at a $>50\%$ PRNT $\geq 1:40$. Those primates immunized with one of the clonal derivatives were less likely to be able to neutralize variola at a titer of $\geq 1:40$, although those immunized with ACAM1000 were able to efficiently neutralize vaccinia. Given the fact that historic smallpox vaccines, of a polyclonal nature (i.e. DryVAX) were not 100% effective to prevent smallpox in individuals, and the clonal vaccine regimens (i.e. ACAM1000) appear to less effectively neutralize variola, we felt it would be of benefit to do a more comprehensive evaluation (given the very small sample size reported in the Weltzin et. al. study) in order to see if the difference in VAR and VAC neutralization titers is significant.

Method. We have negotiated with NIAID and the VTEU system to evaluate a subset of human sera derived from one of the vaccine trials. The sera are obtained from individuals vaccinated with the control (DryVAX), and one of the less-reactogenic vaccines in development (MVA). Paired sera (before and after vaccination) from 30 individuals vaccinated with DryVAX, and 30 individuals vaccinated with MVA will be compared at “peak” immunologic response times 30 days post multiple puncture vaccination with DryVAX or 30 days post the second of two i.m. vaccinations with MVA per the “standard” doses for each respective vaccine. Sera, with appropriate negative and positive controls, will be evaluated for the ability to neutralize variola, using the same protocol used to establish PRNTs versus vaccinia at the VTEU (Newman et. al. JID 2004). The assays will be done in a blinded fashion, and the results passed to the NIAID counterparts for unblinding. Statistical support to compare geometric mean titers, or other analytic comparisons, will be performed by NIAID and CDC statisticians.

Results Variola neutralization assays were performed in the BSL-4 facility at the CDC using the same methodologies used by the VTEU. This data has been sent to an independent contractor who is comparing the anti-vaccinia and anti-variola neutralization results.