NAb pool yielded 84% neutralization. A significantly higher number of NSI subtype E viruses were sensitive as compared to SI E viruses (p=0.009); no association between viral phenotype and sensitivity to NAb was observed for subtype B viruses (p=0.856). Strikingly, concurrent CD4 T cell numbers were significantly lower in subtype E infected patients whose isolates were more resistant to NAb, both in the overall study group (p<0.001), as well as in the 22 patients with NSI isolates (p=0.013). **Conclusions:** The finding that NSI subtype E viruses are more sensitive to NAb than SI E viruses suggests that differences may exist between subtypes B and E regarding phenotype and susceptibility to neutralization. Furthermore, in subtype E-infected patients with disease progression and lowered CD4 cell numbers, the culturable viruses appear to be less sensitive to heterologous NAb. This implies that NAb sensitivity may play a role in viral selection during pathogenesis, and that both SI and NSI viruses are subjected to this immune selective pressure. Characterization of the evolution of the biologic properties of both B and non-B HIV-1 subtypes will provide a clearer understanding of the repertoire of antibodies required for a vaccine to be effective against all phenotypes and subtypes.


**HIV-1 PRIMARY ISOLATE NEUTRALIZATION USING HIV+ OR OGP160-VACCINEE SERUM IN A SENSITIVE, INTRACELLULAR P24 FLOW CYTOMETRIC ASSAY AND A SINGLE-ROUND INFECTION ASSAY**


**Background:** Standardized and reproducible measurement of vaccine-induced primary isolate neutralizing antibodies (NAb) has remained a challenge to HIV vaccine developers. Sensitive assays will be required to effectively detect NAb elicited by potentially effective subunit vaccines, such as oligomeric env proteins. We previously reported a sensitive PBMC neutralization assay utilizing intracellular (IC) p24 detection by flow cytometry and a format that allows for incubation of NAb with cells and virus during culture. This approach should measure antibodies that block both cell-free and cell-cell transmission. We hypothesize that some vaccines may elicit NAb that specifically inhibit entry, while others may induce NAb that block cell-cell transmission and fusion; quantitation of these NAb may require specific assay formats. **Methods:** Serum from recipients of o-gp160 in US B-B and Thai B-E prime-boost trials, as well as HIV+ sera, were tested against subtype A, B, and E isolates. An extracellular (EC) p24 reduction assay or the IC p24 flow cytometry assay with antibody added back during culture was used. The ViroLogic, Inc. Phenosense single-round infection assay is being utilized to assess inhibition of virus entry. **Results:** One to two log increases in NAb titers for matched clade HIV+ sera were observed using the IC p24 flow cytometry assay with NAb added back, as compared to the standard EC p24 antigen capture based assay. Five to ten-fold increases in cross-clade HIV+ serum neutralization titers were also observed using the IC p24 assay and B and E clade reagents. Using both pooled and individual sera from o-gp160 MN vaccinees, 5 to 10-fold higher titers were observed against
the US-1 subtype B primary isolate when the ICp24 assay was used. Using pooled serum from recipients of the ogp160 E vaccine, >50% neutralization of the vaccine homologous TH023 virus and the heterologous M066 clade E primary isolates was observed using the IC p24 assay, but not the EC p24 assay. **Conclusions:** Neutralizing antibodies should be assessed at multiple mechanistic levels, to include precise measurement of inhibition of entry, as well as inhibition of cell-cell transmission within the cellular population that is producing new virions. These approaches will allow for the assessment of NAb in the setting of both sterilizing and non-sterilizing vaccine-induced immunity to HIV.


**HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 PRIMARY ISOLATE NEUTRALIZATION RESISTANCE IS ASSOCIATED WITH THE SYNCYTIIUM-INDUCING PHENOTYPE AND LOWER CD4 CELL COUNTS IN SUBTYPE CRF01_AE-INFECTED PATIENTS**

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A number of human immunodeficiency virus type 1 (HIV-1) non-B-subtype products have been developed for present or future vaccine trials; in Thailand, several studies using subtype B and/or CRF01_AE vaccines have been conducted. To better characterize the biologic properties of these subtypes, 70 HIV-1 subtype B and E isolates were phenotyped as syncytium-inducing (SI) or non-syncytium-inducing (NSI) isolates and assessed for sensitivity to neutralizing antibody (NAb). A significantly higher number of NSI subtype E viruses were neutralization sensitive than SI subtype E viruses ($P = 0.009$), while no association between viral phenotype and sensitivity to NAb was observed for subtype B ($P = 0.856$), suggesting a difference in the neutralization patterns of subtypes B and E. Strikingly, concurrent CD4 T-cell numbers were significantly lower for subtype E-infected patients whose isolates were more resistant to NAb, both for the overall study group ($P < 0.001$) as well as for the 22 patients with NSI isolates ($P = 0.013$). Characterization of the evolution of biologic properties of both B and non-B HIV-1 subtypes will provide a clearer understanding of the repertoire of antibodies that must be elicited for a vaccine to be effective against all phenotypes and subtypes.

**Journal of Virology 2003; 77(15): 8570-6.**