# Epitope Specificity and Polyfunctional CD4+ Responses in HIV-1 Highly Exposed Seronegative Versus Infected Female Commercial Sex Workers

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## ABSTRACT

A long standing study of low social economic status commercial sex workers, in the Majengo cohort of Nairobi has demonstrated variable susceptibility to HIV-1, where some of the highly exposed subjects remain persistently seronegative, indicative of resistance to HIV -1. There has been intense interest in understanding the mechanism responsible for this phenomenon. Understanding the specific immune responses conferring protection from infection in individuals exposed to HIV-1 is critical for vaccine design. However, if both HIV -1 infected and resistant individuals have HIV-1 specific T helper responses, what is unique about the later group that protects them from infection? This study evaluated the protective immunity in light of polyfunctional immune responses due to specific selective peptide recognition by in *vitro* stimulation of the subjects. The peptide pools were designed from the entire HIV-1 Clade -A genome giving 778 overlapping peptides grouped into 20 peptide pools. Each pool had 40 peptides apart from pool 20 which had 18 peptides. The immune responses were measured by a 4 laser 10 color LSR II flow Cytometer from a total of 104 subjects. The Peripheral Blood Mononuclear Cells were obtained by ficoll centrifugation method of cell separation. PBMCs were stimulated with peptide pools of HIV -1 and the responses measured for pro-inflammatory cytokines production at day three and for proliferation at day six of incubation at $37^{o}C$ and 5% $CO_{2.}$ The data was analyzed to ascertain polyfuctionality due to epitope specifity done using the chi squared and Student t tests. The resistant group showed significant levels of IFNγ, TNF –α, IL-2 production and proliferation, especially in response to peptide pools 1, 12, 13 and 14 *(*Env, P24, P31 and P2P7P1, P6 P7, Protease and REV peptide pools). The difference was significant at 95% CI with a p value of 0.0001. There was no correlation between IFN-y production and

proliferation using students T test at  a P value 0.0001. Pool numbers 8 (gag and nef) and 17(Rt and Tat) showed polyfunctional immune responses while in the initial screening the two did not show high IFN-γ production thus the need to repeat the polyfunctinality capturing the entire genome to capture all epitopes that are specific. It is evident that there could be selective preferential epitope recognition in the resistant group that could be responsible for the unique polyfuctionality. Future directions include breaking down the specific peptide pools with unique responses confirming polyfuctionality due to epitope specificity. Polyfuctionality and epitope specificity correlates could be used to determine efficacy of HIV-1 vaccine by mimicking the effective responses of those who appear resistant.