HIV/AIDS continues to be one of the most important health problems in the world despite the progress made in developing highly active antiretroviral therapy (HAART). These therapies target HIV progression that is defined by CD4 depletion and increase in HIV-RNA counts. The activation of T cells appears to be one of the main causes of CD4 count reduction (1). The focus in recent years has been on the patient population termed, Elite Controllers (EC). These individuals have been able to control their HIV infection in the absence of antiretroviral therapy for over 10 years and make up approximately 5% of all chronically infected HIV individuals. The criteria for EC is the following, 1) serologically positive for HIV-1 infection, 2) CD4 counts above 500 cells/µl, 3) undetectable plasma viremia using commercial assays (<50 HIV-RNA copies/ml), and 4) absence of HIV-related symptoms for an extensive period of time (>10 years) (1). The existence of these individuals provides hope that effective vaccines against HIV may be developed.

Research has shown the immunological profile of EC individuals contains HIV-specific CD8+ T cells which have a superior capacity to proliferate and have enhanced effector functions (cytokine secretion, expression of lytic molecules such as perforin and granzyme B). These polyfunctional HIV-specific CD8+ T cells result in an effective granzyme B-mediated killing of HIV-infected cells (2). Thus, producing a vaccine that recapitulates the EC response pattern remains a challenge.

We have developed an HIV-based DNA vaccine, GenePro® (∆4SHIVKU2), which expresses 7 proteins of the virus. This vaccine is a molecule of DNA that induces strong and potent immunity against HIV-1. Because several genes from HIV are deleted, this DNA does not integrate into the genome of the host and does not produce infectious particles or persist in the vaccinated host, however, they are recognized by the body's immune system as an infection, and a significant cellular and antibody immunological response develops. The viral proteins in this DNA-based therapy are produced within myocytes, a muscle cell, for a period of several weeks. This results in a potent stimulation of the immune system, including a cell-based immune response that contains the virus replication and retains a memory of it in case HIV resurfaces (3).

**Figure 1:** The DNA composition for GenePro®. The SIV rt, int, vif genes have been deleted and the 3’LTR region replaced with SV40 PolyA rendering the backbone noninfective, while maintaining its potency
In our mouse study (4) we performed kinetic analyses on splenocytes of BALB/c mice that were immunized with a single high dose (200 µg) injection of GenePro®. The cell-mediated response was measured using multiparametric FACS analysis. The analysis showed CD8+ T cells that were specific for all HIV Ags, had robust proliferation abilities mostly without IFN-γ production, and contained granzyme B. Longitudinal characterization also showed that CD8+ T cells underwent expansion (within 2 to 4 weeks post immunization), contraction, and memory generation (14 to 20 weeks post immunization) which was maintained throughout the lifespan of the animal (more than 63 weeks post immunization).

The results seen in the mouse study were replicated in the nonhuman primate (rhesus macaques) study (5). Again, a single high dose (30 mg total, 10mg DNA vaccine/kg body weight from the ratio that has been successfully used in mice) intramuscular injection of GenePro™ was given to the animals and the immune response was measured using multiparametric FACS analysis similar to the mouse study. Interestingly, all animals developed broad and polyfunctional HIV-specific T cell immune response that persisted for months with a reemergence in the blood following the initial decline but in the absence of antibody response. The majority of vaccine-specific CD4+ and CD8+ T cells lacked gamma interferon production but showed high antigen specific proliferation capacities. Proliferative CD8+ T cells expressed the lytic molecule granzyme B.

**Polyfunctional CD8+ T Cell Responses to HIV**

*Initial gating on live lymphocytes or splenocytes CD3+ CD4+ (blue) or CD8+ (orange)*

Gated on CD3+ CD8+
Altogether, our results in the mouse and macaque studies strongly indicate that a single injection of GenePro® does not simply elicit broadly reactive CD8+ T cell immunity but also evoke CD8+ T cell immunity that remains broadly reactive even after an extensive absence of viral antigen expression. In general, the ability to vigorously proliferate after infection results in the generation of a large pool of secondary effector T cells and is believed to be central to providing optimal protective immunity.

Importantly, the proliferative results seen in both the mouse and nonhuman primate studies are similar to those observed in HIV-infected EC (6,7). Beside functional defects, several studies of HIV chronically infected patients have shown that the HIV-specific CD8+ T cell pool is characterized by an absence of central memory T cells identified by specific surface markers (CCR7+, CD62L+ phenotype) (8). These results support the idea that CD8+ T cell differentiation is incomplete or arrested in such patients. In contrast, EC contain a discrete subset of HIV-specific circulating CD8+ T cells which display central memory phenotype (6). These findings have important implications for vaccine development and suggest that a successful vaccine should support all stages of differentiation and maturation. Importantly, phenotypic analysis carried out in our mouse study, indicate that a single injection of GenePro® elicit HIV-specific CD8+ T cells with central memory phenotype (4). Drawing comparisons between HIV DNA vaccinated and naturally infected EC groups, help to provide direction for future research and hope that an effective vaccine can be developed against AIDS.

References


