**Background:** Understanding the quality of immune responses induced by different vaccine regimens is essential for improving the prospects of AIDS vaccines.

**Methodology:** To identify optimal regimens, we compared vaccination protocols that used DNA, protein, or a combination of DNA and protein using a classical prime/boost strategy or delivered in a co-immunization regimen combining the two vaccine components. Magnitude, breadth, longevity and mucosal dissemination of anti-Env humoral responses were monitored in vaccinated macaques. Rhesus macaques were vaccinated using electroporation with either DNA, DNA/protein co-immunization, or DNA prime followed by protein boost or by protein alone. As protein source, we used AT-2 inactivated SIV particles or HEK293 produced HIV or SIV Env. Humoral (including neutralizing, linear epitope and V1V2 antibodies) and cellular responses were followed over time. Some of the vaccinated macaques were challenged to determine immune correlates of protection.

**Results:** Protein only vaccination induced robust humoral responses, which rapidly declined. In contrast, co-immunization with DNA (IM delivered by needle) and protein induced long-lasting responses. We expanded on these observations and implemented several vaccine improvements, i.e. better env DNA plasmids, inclusion of IL-12 DNA as adjuvant and improved in vivo DNA vaccine delivery by intramuscular injection followed by in vivo electroporation (IM/EP) and inclusion of protein as boost or co-immunization regimen. Although DNA only vaccination achieved strong humoral responses, protein boost after DNA vaccination greatly increased Ab levels. Importantly, a co-immunization strategy of DNA/protein injected in the same muscle the same time induced highest and broad humoral responses. Similar data were obtained using either purified Env proteins or inactivated viral particles. Inclusion of DNA in the vaccine promoted persistence of plasma antibody levels for greater than 2 years. SIV DNA/protein vaccination induced: higher SIV Env-specific IgG in saliva; more responders with higher SIV Env-specific IgG in rectal fluids; higher and longer-lasting plasma bAb and Nab to homologous and heterologous Env; and Ab to V1/V2. Systemic and mucosal vaccine-induced Ab responses against SIVsmE660 correlated with slower virus acquisition upon challenge. In addition, vaccinated macaques showed strong protection against chronic viremia compared to controls. Similar to SIV, HIV DNA/protein (purified Env) vaccination also induced higher Ab levels compared to DNA only, including significantly higher levels of V1/V2 Abs.

**Conclusions:** DNA/protein co-delivery increases the magnitude and longevity of systemic and mucosal humoral immune responses in immunized rhesus macaques.