Abstract

A key goal in development of preventative HIV-1 vaccines is the ability to elicit broadly neutralizing antibodies (bNAbs) following vaccination. The target of these bNAbs is the only exposed protein on the surface of the HIV-1 virion, the trimeric HIV-1 envelope glycoprotein (Env). Our research group previously developed a modified two-domain CD4 molecule (2dCD4^{S60C}) that binds with greater affinity to HIV-1 Env glycoproteins through the formation of a stabilizing disulphide bridge. This allowed us to generate stable immunogen complexes suitable for immunizations in animal models. We previously showed that rabbits immunized with Env-2dCD4^{S60C} complexes elicit potent bNAbs targeting clinically relevant tier-2 HIV-1 pseudoviruses *in vitro*. These neutralizing responses were generated regardless of the form (monomeric and trimeric Env) or viral subtype (subtype B and C) of the Env glycoprotein in complex with 2dCD4^{S60C} and were noted to be anti-CD4 in nature. This study describes immunogenicity testing of the Env-2dCD4^{S60C} complex in a non-human primate model (NHP), the Chinese-origin rhesus macaque.

Trimeric HIV-1 Env glycoproteins based on a consensus sequence of subtype C founder viruses (gp140$_{FVC}$GCN4t(+)) and 2dCD4^{S60C} were expressed using mammalian and bacterial cell expression systems. The gp140$_{FVC}$GCN4t(+) and 2dCD4^{S60C} proteins were purified using lectin-affinity, nickel-affinity and size-exclusion chromatography. Immunogen complexes were then generated under reducing conditions. Immunogenicity of gp140$_{FVC}$GCN4t(+), gp140$_{FVC}$GCN4t(+)-2dCD4^{S60C} (Env-2dCD4^{S60C}) and 2dCD4^{S60C} was evaluated in genetically characterized Chinese-origin rhesus macaques (n=7) following a six-dose, four-month vaccination regimen. Breadth and potency of vaccinated macaque sera was evaluated in a TZM-bl pseudovirion neutralization antibody assay. Fourteen pseudoviruses, including the twelve tier-2 HIV-1 Env Global Panel viruses, and an infectious tier-2 SIV/HIV-1 chimeric virus (SHIV-1157ipd3N4), were tested against. Basic mapping of the target of the neutralizing responses generated in NHPs was done using various recombinant CD4 molecules to deplete sera of anti-CD4 antibody responses, and then tested in the TZM-bl pseudovirion neutralization antibody assay. Two years following the vaccination regimen, the NHPs were vaccinated with a booster immunization and subsequently challenged using a low-dose intra-rectal SHIV challenge using SHIV-1157ipd3N4.

Env-2dCD4^{S60C} complex immunized NHPs (n=4) developed bNAb responses by day 74 of the vaccination regimen following four immunizations, neutralizing 11/12 of the HIV-1 Env Global Panel of pseudoviruses with titres above 1:100, with a geometric mean titre of 1:356 for the entire group against the panel. In contrast, a single macaque that received gp140$_{FVC}$GCN4t(+)-only, developed no neutralizing titres against the HIV-1 Env Global Panel, while the 2dCD4^{S60C}-only immunized macaques (n=2) displayed similar breadth to the complex group but with lower potency (1:60). Mapping of the target of the responses of the sera using recombinant CD4 proteins to deplete sera of anti-CD4 antibodies resulted in a reduction in neutralizing potency of sera from

Env-2dCD^{S60C} immunized NHPs, with a complete loss of neutralizing potency in one of the sera tested.

Two years later, neutralizing titres were no longer detectable in the NHPs, but a booster immunization returned antibody titres to neutralizing levels after two weeks, to levels previously seen. Following booster immunizations, the NHPs were administered ten low-dose intra-rectal SHIV inocula over a 10-week period. CD4+ T-cell counts were monitored using flow cytometry and SHIV viral RNA loads were monitored using RT-PCR. Infection with, or protection from the SHIV-1157ipd3N4 challenges, remained inconclusive.

The unique Env-2dCD4^{S60C} complexes elicit potent and broad neutralizing antibody responses in Chinese-origin rhesus macaques against clinically relevant tier-2 HIV-1 pseudoviruses. These neutralizing antibody responses are primarily anti-CD4 in nature, with the precise mechanism of antibody mediated neutralization requiring further delineation. Future work includes assessing whether these elicited anti-CD4 antibody responses are immunotoxic in the vaccinated subject, as well as further proof of concept SHIV-challenge studies (using vaccinated NHPs), to support the advancement of these Env-2dCD4^{S60C} immunogens into human clinical testing.

Keywords

HIV-1; SHIV; Vaccine; Envelope glycoprotein; CD4; Immunogen; Non-human primate; Rhesus macaque; Broadly neutralizing antibody; CD4i