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Research Paper

Dose-Response Relationship of DNA and Recombinant Fowlpox Virus Prime-Boost HIV Vaccines

Implications For Future Trials

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KEY WORDS

vaccine, immunologic dose-response relationship, body surface area, prime-boost

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ABSTRACT

Estimating effective doses of novel HIV vaccines is challenging. Dose-response analyses of DNA and fowlpox virus HIV vaccines showed that 1 mg of DNA vaccine and 5×10^7pfu of fowlpox virus booster was immunogenic in macaques. However, this dose was poorly immunogenic in humans. When adjusted for body surface area, the human dose studied was equivalent to a poorly immunogenic lower dose in monkeys. These data provide a rationale for guiding dosing in future trials of HIV vaccine technologies.

Asian macaque models have provided extensive guidance for HIV vaccine development. Several features underlie its utility: molecular and functional similarities of macaque MHC with human HLA, the outbred nature of the model, and rigorous challenge systems using simian immunodeficiency virus (SIV) infection.[1] Recent vaccine development has focused on T-cell-stimulating vaccines, based on observations that HIV-specific T-cell immunity is linked to partial clearance of initial viremia in humans.[2] Heterologous DNA-prime/viral vector-boost vaccines that induce this type of immune response have been shown to provide partial protection in monkeys from SIV-HIV chimeric viruses.[3] However, recently reported phase I clinical trials of DNA/poxvirus prime/boost HIV vaccines have been poorly immunogenic.[4,5]

Preclinical macaque studies typically use vaccine doses that are not scaled to animal size, often the same dose proposed for clinical trials.[6-8] Using comparatively high vaccine doses in nonhuman primates produces robust safety, immunogenicity and efficacy data that facilitates progression into clinical trials. For novel vaccines where there is no clinical precedent, dose selection is often empiric and based on immunogenic and efficacious doses in the primate model, tempered by practical, ethical and financial considerations associated with clinical manufacture.

We have conducted five separate trials of DNA and rFPV prime-boost HIV vaccines expressing multiple HIV-1 or SHIV antigens at various doses in 25 pigtail and 16 cynomolgous macaques and an initial clinical trial in 18 humans (Fig. 1).[3,9-11] All vaccines were administered intramuscularly without adjuvants, with 2 or 3 priming DNA vaccines at 3–4 weekly intervals followed by 1 or 2 rFPV boosters at 4-week intervals. Eight macaques received HIV-1 vaccines at the lowest dose of 0.15 mg DNA/7.5 x 10^6pfu of rFPV. Twenty-two received 1 mg DNA/5 x 10^7pfu rFPV doses of HIV or SHIV vaccines (16 HIV-1 vaccines and 6 SHIV vaccines),8 received 2 mg DNA/10^8pfu rFPV of HIV-1 vaccines and 3 received HIV-1 vaccines at the highest dose of 4.5 mg DNA/2 x 10^8pfu rFPV. In each trial, the ratio of DNA and rFPV doses was maintained within a narrow range (4.4–5.0 x 10^7pfu of rFPV/mg of DNA vaccine).

Immunological responses were observed in almost all macaques at the three highest doses of DNA/rFPV studied (1 mg/10^7pfu, 2 mg/10^8pfu and 4.5 mg/2 x 10^8pfu, respectively) with positive responses of 95%–100% by IFNγ ELISpot, 79%–100% by enzyme immunoassay (EIA) and 100% by western blot (WB) (Fig. 1A). T-cell responses were confirmed by ICS for the 1 mg/10^7pfu and 4.5 mg/2 x 10^8pfu doses. [3,9,10] However, the lowest dose studied (0.15 mg/10^7pfu) was poorly immunogenic, eliciting responses in only 2/8 animals by ELISpot and 1/8 animals by WB and EIA. This suggests that the minimal effective dose of the combination of vaccines is between 0.15 mg/7.5 x 10^6pfu and 1 mg/5 x 10^7pfu in macaques.

An initial clinical trial at the 1 mg/5 x 10^7pfu dose was recently performed in 18 human subjects.[12] Although the vaccines were well tolerated at this dose, T-cell responses by IFNγ ELISpot were only observed in 4/18 and antibody responses by EIA and WB in 1/18 subjects. Given the similarity in vaccine construction and manufacture, mode of administration

and efficacy measures, the difference between the human and nonhuman primate data raises the question of the adequacy of the dose used in humans. Other novel HIV vaccines candidates have also failed to reliably elicit T-cell immunity in clinical trials despite promising results in preclinical trials.[4,7] Guiding principles to normalize doses of novel T-cell-based vaccines from nonhuman primates to humans are likely to be helpful.

For many drugs, dose calculations for extrapolating differences in size and weight from preclinical to clinical trials are made using estimated body surface area (BSA).[13,14] BSA is a good predictor of many physiological parameters, in particular the elimination of injected compounds via the circulation.[15] Clearance rates of injected materials are linked more closely to BSA-based normalization than using total or lean body weight (mg/kg)-based calculations.[15] We hypothesised that a BSA-based normalization could be a useful guide for dosing T-cell based vaccines (such as DNA and poxvirus vaccines) that require periods of time within the host (prior to elimination) to express the inserted genes. This hypothesis is less likely to apply to protein vaccines with adjuvants that promote retention within tissues and readily stimulate B cell responses at low doses. Similarly, dosages of live-attenuated vaccines are relatively independent of host and size since replication within the host occurs.

When the dose of the DNA and rFPV vaccines across macaques and humans is normalised for BSA, doses of DNA vaccine greater than 3.9 mg/m^2 of BSA (1 mg/5 x 10^{7}pfu) were immunogenic in macaques (Fig. 1B). The poorly immunogenic dose of 0.8 mg/m^2 BSA (0.15 mg/7.5 x 10^{6}pfu) in macaques was similar to the poorly immunogenic dose in the clinical trial (0.7 mg/m^2, 1 mg/5 x 10^{7}pfu). On the basis of BSA, there was approximately a 6-fold difference in the DNA and rFPV vaccine doses used in the human study compared with the minimal immunogenic dose in macaques. We hypothesise that dose is a factor and that taking into account BSA-based calculations could potentially guide investigators to more relevant doses of novel T-cell-based HIV vaccines for humans.

The impact that vaccine dose can have on immunogenicity of DNA prime/viral vector boost vaccine modalities has been highlighted in two recent clinical trials. DNA and MVA vaccines encoding malarial antigens were administered at various doses and dosing regimes in order to optimize vaccine performance.[16] Higher doses (2 mg DNA vaccine/1.5 x 10^{8}pfu MVA) were found to be more immunogenic than low doses in humans (1 mg/3 x 10^{7}pfu). Lower doses of SIV-based DNA/MVA vaccines were previously demonstrated to induce strong T cell immunogenicity in rhesus macaques.[6,17-19] Recent human DNA vaccine trials conducted at the NIH Vaccine Research Center also suggest that 4–8 mg of DNA vaccine may be required to efficiently stimulate T-cell immunity.[20] This is in line with our predictions that up to 6-fold increases in the dose of DNA and rFPV vaccines we studied in the clinical trial may be required to efficiently stimulate T-cell immunity (Fig. 1B). The human population studied may also need to be taken into account since many Asian populations will have significantly lower BSA levels and, in some instances responses to drug therapies, compared to Caucasian populations.[21]

As further animal and human data are published on novel HIV vaccine vectors, it will be possible to evaluate whether our observations on DNA/rFPV vectors are confirmed and can be generalized to other vector-based vaccines. Conference reports suggest than adeno-associated virus vectors have also demonstrated suboptimal immunogenicity in humans despite promising macaque immunogenicity reported with SIV vectors.[22,23] Alternatively, adenovirus

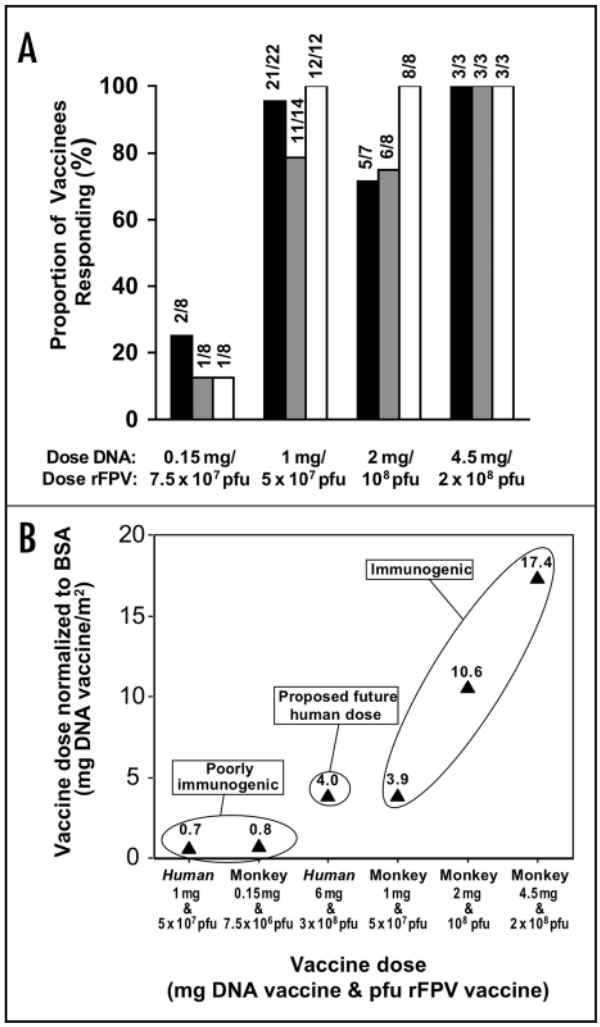

Figure 1. Dose Response of DNA prime/rFPV boost vaccines. a) T-cell immunogenicity was assessed by IFNγ ELISpot assay to overlapping HIV or SIV Gag peptide pools or inactivated HIV-1 particles 1-5 weeks after the final rFPV boost and plotted as a proportion of positive responders ■ (> 50 sfu/10^6 PBMC). Humoral immunity was measured by EIA ■ and increased p24 band density in western blot □. b) Body surface area-normalized comparison of vaccine exposure. Four vaccine doses from macaque trials, one clinical trial and a proposed clinical trial, expressed as BSA. Dose for DNA vaccine only is shown on the vertical axis - the rFPV vaccine dose is closely scaled to the DNA vaccine dose in all trials (4.4 - 5.0 × 10^7 pfu/mg DNA vaccine). BSA (m^2): Human = ([Height(cm) × Weight(kg)]/ 3600)$^{1/2}$, Monkeys = (K_n [11.8 for monkeys] × Weight(kg)$^{0.67}$)/100.

serotype 5 vectors have been immunogenic in both rhesus macaques using SIV vectors and, at conference presentations, in humans with HIV-1 vectors, although the dose that is required for optimal immunogenicity is high (often ≥10^9 virus particles).[24,25] Larger doses of Canarypoxvirus vectors (without DNA priming) did not improve T cell immunogenicity in a human trial and were associated with unacceptable reactogenicity.[26] For DNA and poxvirus vectors, scale up of doses much beyond 5mg and 5 x 10^8 pfu respectively is likely to be expensive, require larger volumes of injection or multiple injections, and associated with more reactogenicity.

Alternate biologic explanations of the differences between humans and nonhuman primates in immunogenicity of DNA/poxvirus vectors are also possible and should be further explored. It is possible that differences in entry and expression of vaccine vectors

within target APC populations exist between macaques and humans but are not yet well understood. This may be a particular problem for avian viral vectors such as rFPV that undergo an abortive replication cycle in mammalian cells. The stage at which replication of avian poxvirus vectors are blocked may however, be different between various mammalian species and influence immunogenicity.[27,28]

An additional limitation of camparing some macaque and human HIV vaccines studies is that few other groups have reported the immunogenicity of the same HIV-1 vectors in both macaques and humans. Differences in the immunodominance T cell epitopes between SIV and HIV-1 will exist. Some macaque vaccine studies have primarily selected animals with particular MHC alleles that present immunodominant SIV epitopes such as the CM9 SIV Gag epitopes in rhesus macaques.[6,17,19,29] This will skew the immunogenicity towards a positive response in SIV studies in comparison to studying more outbred populations with HIV-1 vaccines.

Extensive dose-ranging studies of novel T-cell-based HIV vaccines in clinical trials involve considerable time, effort and resources. The dose of novel vaccines chosen has important implications for performing toxicology experiments for regulatory requirements and practicality and cost of scaling up the required doses for human use. More predictive measures of vaccine dose from preclinical models, such as using BSA-based calculations, could assist streamlining studies of effective doses.

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