Candidate HIV-1 gp140ΔV2, Gag and Tat vaccines protect against experimental HIV-1/MuLV challenge

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Abstract

Pre-clinical HIV-1 vaccine protocols, using multiple vaccine modalities and a potent adjuvant were assessed for vaccine efficacy in an experimental HIV-1 challenge model. C57Bl/6 mice were immunized with DNA plasmids encoding HIV-1 gp140, Gag and Tat alone or in combination with the corresponding recombinant proteins formulated in the adjuvant MF59. HIV-1 DNA alone or a DNA prime protein boost schedule resulted in complete protection against challenge with HIV-1/MuLV-infected murine cells. Although HIV-1 protein immunization in combination with MF59 resulted in partial protection, the DNA priming seemed to be crucial for obtaining full protection against the challenge. It is likely that the partial protection seen after immunization with protein alone is, to a certain extent, due to effects of the adjuvant since some animals that received the adjuvant MF59 alone were protected from the challenge. For the most part, antigen-specific cell-mediated immune responses as detected in the spleen (in contrast to responses detected in peripheral blood) of immunized animals appeared to be associated with protection in this study.

Keywords: Vaccination; AIDS; T cells; Antibodies; Challenge; Small animal model

1. Introduction

There is an urgent need for a vaccine against the human immunodeficiency virus type 1 (HIV-1). A factor that hampers this quest is the lack of suitable small animal models for studying HIV-1 pathogenesis and vaccine efficacy. So far no one has been able to develop an experimental small animal model where complete replication of HIV-1 can occur, likely due to multiple differences in host cell factors between man and other species. The HIV-1/murine leukemia virus (HIV-1/MuLV) model is based on a pseudotyped virion containing the full HIV-1 genome with the MuLV envelope. We have repeatedly shown that in this model, immunization with HIV-1 components can confer protection against this experimental HIV-1 infection [1,2].

A number of reports have shown promising induction of T lymphocyte immunity after priming with antigen-encoding DNA and boosting with protein subunit vaccines or recombinant viral vectors [3,4]. The potential importance of the breadth of immunity, supposedly achieved by delivering multiple immunogens, has also been explored [5–7]. Here,
we evaluate the potential of combining the HIV-1 antigens gp140, Gag and Tat, delivered as either plasmid DNA or as the corresponding proteins formulated in the potent adjuvant MF59 [8–10]. The capacity to eliminate an engraftment of HIV-1-infected cells was finally tested by intraperitoneal injection of HIV-1/MuLV-infected syngeneic splenocytes, followed by recovery of remaining infectious HIV-1. The results show that a mixture of HIV-1 gp140, Gag and Tat DNA alone and the same immunogens as proteins (together with MF59) could mediate protection against the experimental HIV-1 challenge. DNA prime followed by a protein boost also resulted in a high level of protection, whereas, interestingly, the reverse order of delivery (protein followed by DNA) did not result in significant protection.

2. Materials and methods

2.1. Animals and immunogens

The HLA-A2 transgenic C57Bl/6 mouse strain has the β2 microglobulin and H-2Db knocked out and instead expresses a chimeric HLA-A2, covalently attached with the human β2 microglobulin chain (C57Bl/6.A2.H-2Db−/−B2m−/−) [11]. Animal housing and care were in compliance with the provisions and general guidelines of the Swedish Animal Welfare Agency. The genetic immunogens used were HIV-1 DNA plasmids encoding an oligomeric form of the HIVSF162 gp140 lacking the V2 region (pCMVLink.gp140dV2SF162), Gag (pCMVKM2, gagMod.SF2) and Tat (pCMVKM2, TatHXB2) [12]. As protein immunogens, we used the corresponding recombinant (r) HIV-1 proteins; ro-gp140 (O-gp140dV2 SF162) [13] and rGag (SF2) were produced in CHO and yeast cells, respectively, and rTat (IIIB, clone BH10) was expressed in E. coli and purified in a way that preserves the biological activity of the protein [14,15]. The MF59 adjuvant (Novartis Vaccines and Diagnostics, Inc.) is an oil-in-water emulsion employed in commercial influenza vaccines.

2.2. Immunizations and experimental HIV-1 challenge

Mice were immunized with the three DNA plasmids, the three recombinant proteins or prime–boost combinations of the two modalities (Fig. 1). Each DNA immunization consisted of 20 μg gp140 encoding DNA delivered in the right tibialis muscle and 20 μg each of HIV-1 Gag and Tat DNA delivered together in the left tibialis muscle. The protein vaccine component consisted of 20 μg ro-gp140 delivered in the right tibialis muscle and 20 μg rGag and 5 μg rTat delivered in the left tibialis muscle. The proteins were formulated in 50% MF59 solution. Mock treatment consisted of MF59 adjuvant alone or empty DNA plasmid alone. The experimental challenge has been described elsewhere [2,16–19]. Briefly, 3 weeks after the final immunization (week 12), the animals were injected intraperitoneally with 1 million syngeneic splenocytes infected with HIV-1/MuLV of subtype B (LAI). Ten days later (week 13), mice were sacrificed and cells were recovered from the peritoneal cavity and co-cultured with activated human PBMC or Jurkat-Tat cells. Culture supernatants were screened for HIV-1 p24 antigen every 3–4 days (in-house p24 ELISA [20]). The animals were regarded as unprotected if culture supernatants from at least one of the two permissive cell systems were p24 positive at two or more time points during the cultivation.

2.3. Cytokine ELISpot

Group-wise pooled mouse blood was purified over 5 ml Ficoll-Paque [21] (Amersham Biotech, Uppsala, Sweden) at weeks 11 and 13. The ELISpot assays were set up with 200,000 cells/well in 96-well ELISpot plates (Millipore MAIPN4510, Bedford, MA, USA) coated with anti-IFN-γ or anti-IL-2 monoclonal antibodies (Mouse ELISpot Plus Kits, MabTech, Stockholm, Sweden). Cells were stimulated ex vivo in duplicates with two HIV-1 gp160 peptide pools covering amino acids (aa) 1–388 and 378–849, respectively (National Institutes of Health AIDS Reagent Depository; catalog no. 7100); a p55Gag peptide pool (NIH catalog no. 7098) or a Tat peptide pool (NIH catalog no. 7101). All peptide pools were used at 1.25 μg/peptide/ml. Recombinant HIV-1 proteins rp5160 (ProteinSciences, Meriden, CT, formerly MicroGeneSys), rp55Gag (Novartis Vaccines and Diagnostics, Inc.), rTat (described above) and hepatitis B virus surface antigen (rHbsAg) were used for ex vivo stimulation at a final concentration of 1 μg protein/ml. Cultures were kept for 36 h at 37 °C with 5% CO2. Splenocytes were similarly purified

![Fig. 1. Immunization schedule. Six groups of mice were immunized with three plasmids encoding gp140, Gag and Tat antigens (DNA) or the same immunogens delivered as recombinant proteins (Protein). One group was given two DNA immunizations followed by one protein boost (DNA:Protein) and one group received one protein prime followed by two DNA boosts (Protein:DNA). An additional two groups were immunized with either empty plasmid alone (MockDNA alone) or adjuvant alone (MF59 alone). Three weeks after the final immunization, all animals were inoculated with 1 million HIV-1/MuLV-infected syngeneic splenocytes (week 12). † = time of bleeding; †† = time of sacrifice.](image-url)
10 days after challenge and analyzed group-wise using the same reagents and assay conditions. The number of spot-forming cells (SFCs) was quantified by using an AID ELISpot reader (Autoimmun Diagnostika GmbH, Germany) and net spot-forming cells (NetSFC) were calculated as the number of SFCs following stimulation with HIV-1 antigens less the number of SFCs in wells of unstimulated cells.

2.4. Serology

Antibody IgG ELISA was performed essentially as previously described [6,22]. Briefly, ELISA plates (Nunc Maxisorp, Denmark) were coated with 1 μg/ml of ro-gp140, rGag or rTat, respectively. Ten-fold serial dilutions of serum from each individual mouse were added to the plates. Reactive antibodies were detected with goat anti-mouse IgG antibodies conjugated to horseradish peroxidase (HRP) (DAKO PO447, Denmark). Plates were developed with O-phenylene diamine (OPD) and the optical density (OD) was read at 490 nm. The mean OD plus three standard deviations of pre-immunization sera was used as cut-off for a positive response.

2.5. Plaque reduction assay

The GHOST(3) cell line-based plaque assay is a single cycle infectivity assay for HIV and SIV, where green fluorescent protein (GFP) expression indicates infection and green fluorescent plaques are counted as plaque-forming units (PFU) [23–25]. For the neutralization assay, heat-inactivated group-wise pooled sera and virus (HIV-1 LAI/IIIB) were diluted to give two final serum dilutions (1:20 or 1:40) and a virus dilution to yield between 20 and 100 PFU/well. The neutralizing property of the serum was calculated as percent neutralization is 30%, that is, values below 30% reduction of plaque formation are considered to be negative for neutralization.

2.6. Statistics

The statistical software SPSS 13.0 was used for analyses of protection and development in antibody titers. The criterion for statistical significance was \( p < 0.05 \). The log rank survival analysis was used for comparison of time to viral culture positivity in ascites from the challenged mice, both in the overall test and in the post hoc pairwise comparisons between groups. The combined group of MockDNA- and MF59-immunized animals served as control group. The development of specific IgG titers (anti-Env, Gag and Tat; weeks 11–13) within the different immunized groups was analyzed with Wilcoxon’s signed ranks test. The pre-challenge HIV-1-specific IgG titers were compared between the different immunized groups using Student’s \( t \)-test.

3. Results

3.1. Protection against experimental HIV-1 challenge

Four groups of mice were immunized against HIV-1 by DNA, proteins or combinations of the two (Fig. 1). The experimental challenge consisted of HIV-1/MuLV-infected syngeneic splenocytes that were injected intraperitoneally into the immunized or naïve mice. Ten days later, the peritoneum was washed and the peritoneal cells were analyzed for the presence of viable virus. We then compared results from the individual vaccine groups with those obtained from the two control groups either separately (i.e. MockDNA alone or MF59 alone) or combined into one group (Fig. 2B). The overall log rank survival analysis shows a statistically significant difference between all immunized groups of animals and control animals in the time to infection \( (p < 0.001) \). As expected, the empty DNA plasmid control did not induce any protection (5/5 animals HIV-1 positive from post-isolation day 9).

Pair-wise post hoc log rank analyses revealed that, with the exception of the animals immunized with protein followed by DNA, all HIV-1 immunized groups were better protected than the combined control groups \( (p < 0.05, \text{ Fig. 2}) \). In addition, animals immunized with either DNA alone, protein in MF59 or DNA followed by protein were all better protected than the group receiving protein followed by DNA \( (p < 0.01, \text{ Fig. 2A}) \). The DNA priming seemed to be crucial for obtaining full protection against the experimental cell-based challenge. Both the DNA alone-immunized mice and those primed twice with DNA and boosted once with protein were fully protected, with eight out of eight animals protected in both groups \( (\text{ Fig. 2A}) \). In contrast, the animals receiving the reverse prime-boost protocol, with one protein immunization followed by two DNA immunizations (protein:DNA), were significantly less capable of clearing the HIV-1/MuLV engraftment (only one out of eight animals was protected).

The group of mice immunized only with the proteins formulated in MF59 also displayed a high level of protection \( (5/6 \text{ animals, Fig. 2A}) \). However, since immunization with the adjuvant MF59 alone induced some protection \( (2/5 \text{ animals}) \), this indicates that a part of the protective effect of the protein alone or of the DNA prime protein boost might be attributed to effects of the adjuvant. Although the correlates of protection against this inoculum are not completely resolved, evidence suggests that HIV-specific T cells \([2]\) and cells of the innate immune system \( (\text{i.e. natural killer cells}) \) are of importance.

3.2. Cellular immunity in blood and spleen

After challenge, both IFN-γ and IL-2 ELISpot were used to measure cellular immune responses in the spleen \( (\text{ Fig. 3}) \). The results that best correlated with protection were IFN-γ production by splenocytes from DNA alone- or DNA prime protein boost-immunized animals, when
Fig. 2. Protection against the experimental HIV-1/MuLV challenge. The animals were sacrificed 10 days after intraperitoneal inoculations of syngeneic HIV-1/MuLV-infected splenocytes. Ascites cells were collected and cultured in vitro together with hPBMCs or Jurkat-Tat cells for 21 days. (A) Differences between the groups of immunized animals. The cultures were monitored for HIV replication over time; an animal was regarded as culture positive (non-protected) at the time when HIV p24 antigen was detected in the cell supernatant (the numbers inside brackets represent the number of infected animals/total number of animals per group). The significant differences between protein primed DNA boosted mice and the other vaccine groups are highlighted in the figure. (B) Differences between the immunized animals and control animals. Table of p-values comparing vaccine groups to control groups, either separately (MockDNA alone or MF59 alone) or to control groups combined.

Fig. 3. Splenocyte IFN-γ and IL-2 responses after viral challenge. Splenocytes from individual spleens were isolated and cells were pooled group-wise at the time of sacrifice. The ELISpot analysis was performed using the same antigens for ex vivo re-stimulation as for the PBMC analyses in Fig. 4. No Tat-specific responses above cut-off were detected in any of the groups (data not shown). The HIV-1 Env-specific responses were measured by detecting IFN-γ (A) and IL-2 (B) producing cells. In addition, HIV-1 Gag-specific spleen cell responses were quantified by measuring IFN-γ (C) and IL-2 (D) SFC. Error bars show the standard deviation between triplicate wells.
cells were stimulated with Gag and Env antigens. In fact, mice in these two groups displayed high levels of IFN-γ production by lymphocytes in response to both antigens (Fig. 3A, C and 2A).

Notable is the clear-cut difference between the two prime–boost groups. In contrast to the highly protected DNA primed and protein boosted animals, the unprotected protein primed and DNA boosted mice displayed poor cellular responses in their spleens. There were no responses in MockDNA-immunized animals, whereas some challenge-induced reactivities against the Gag protein could be detected in the animals receiving MF59 alone (Fig. 3C and D), suggesting that the adjuvant itself had induced activation of the immune system allowing for a more potent reaction following the challenge. No background reactivity was observed in the IFN-γ assay when cells were stimulated with HBsAg, but the same control antigen gave rise to some unspecific IL-2 reactivity (Fig. 3D).

The peripheral cellular response was assessed group-wise by assaying pooled PBMCs for vaccine-specific induction of IFN-γ secretion prior to and after the experimental HIV-1 challenge (Fig. 4). The responses detected in PBMC after the experimental challenge were generally substantially lower against all three antigens when compared to pre-challenge responses (Fig. 4). Animals immunized with DNA alone displayed, as detected also in the spleen, responses against both Gag and Env (Fig. 4A and B). The largest difference in antigen-specific responses comparing splenocytes with PBMCs was seen in the prime boost groups. The DNA prime and protein boost group (well protected after challenge, Fig. 2), which displayed robust cellular responses in the spleen, also had very potent reactivities in the peripheral blood. The reverse order of immunogen delivery (unprotected, Fig. 2) resulted in robust peripheral blood cell cytokine production (Fig. 4), but as discussed above, only in low reactivity in splenocytes (Fig. 3).

Another interesting discrepancy was that the specificity of anti-Env responses of peripheral blood lymphocytes differed between the two prime boost groups. DNA followed by protein induced predominately IFN-γ responses towards the N-terminal part (aa 1–388) of the gp160, whereas the protein followed by DNA schedule induced IFN-γ production upon stimulation with peptides spanning the C-terminus (aa 389–849) of gp160 (Fig. 4A). Whether similar differences in epitope specificity also existed for Gag and Tat could not be determined since peptides from these antigens were not divided in distinct pools.

In control groups, PBMCs isolated both pre- and post-challenge did not respond when stimulated with media alone or with HBsAg (Fig. 4).

![Fig. 4. HIV-1-specific IFN-γ production by PBMCs. ELISpot assays were performed on group-wise pooled murine blood before (week 11) and after (week 13) the experimental HIV-1/MuLV challenge. (A) The results from envelope-specific ex vivo stimulations with a sequence matched gp160 peptide library (15-mers with 11 amino acid overlap) split into a N-terminal part (amino acid 1–388) and a C-terminal part (amino acid 378–849) or with a recombinant gp160 protein (rgp160). (B) IFN-γ production after re-stimulation with the HIV-1 Gag peptide library (amino acids 1–500), a recombinant Gag protein (rGag) or a recombinant Hepatitis B control antigen (rHBsAg). (C) Immunogen matched overlapping Tat peptides and a recombinant Tat protein were also used for ex vivo re-stimulation. The standard deviation shows the variation between ELISpot triplicates. The horizontal broken line shows the cut-off (50 SFC/million PBMCs).](image)
3.3. HIV-1-specific IgG production with detectable virus neutralization activity

Sera were collected after the final immunization as well as at the time of sacrifice, 10 days after the experimental HIV-1 challenge. No obvious correlation was found between antibody levels and the ability to clear the experimental challenge. First, we compared the pre-challenge HIV-1-specific IgG titers induced by different vaccine modalities (Fig. 5). All immunized groups responded with anti-Env-specific IgG (Fig. 5A); the highest titers were obtained in the protein-immunized animals (Fig. 5A). The geometric mean IgG titer (GMT) in the protein-immunized animals ($9 \times 10^5$) was almost 1000 times higher than in the DNA-immunized animals ($1.4 \times 10^3$) (Fig. 5A). Animals immunized twice with DNA followed by one protein boost had intermediate levels of anti-Env IgG (GMT $2 \times 10^5$) and the animals immunized with the same immunogens but in reverse order had even lower antibody titers (GMT $5 \times 10^4$). Statistical analyses were made within every group and between sera obtained prior to and following the challenge. The results show that after challenge, anti-Env IgG levels decreased in the protein-alone group ($p < 0.05$) but, interestingly, the same comparisons within the two prime-boost groups showed a significant increase in anti-Env and anti-Gag IgG in both these groups ($p < 0.05$ for both comparisons).

Further, for the Gag-specific IgG responses, DNA and protein followed by DNA induced low levels of anti-Gag IgG, whereas the levels induced by protein or DNA followed by protein were intermediate to high (Fig. 5B). A profile similar to that for the Gag-specific IgG levels was observed for the Tat binding IgG (Fig. 5C), although the anti-Tat responses were low and the differences between groups were not statistically significant (Fig. 5C).

The capacity of pooled sera, collected before and after challenge, to neutralize HIV-1 LAI was moderate (neutral-
ization titer of 40 or below). The neutralization capacity decreased after challenge in the group immunized with protein alone, whereas a slight increase was observed in the DNA followed by protein and the protein followed by DNA groups (Fig. 5D), analogous to what was seen for the titers of binding antibodies. Sera from DNA-immunized animals, which displayed low binding IgG levels in ELISA, still showed virus-neutralizing capacity after challenge, indicating that, although their levels were low, the antibodies induced by the DNA were of high affinity. No neutralizing effect was observed in sera obtained prior to immunization (data not shown), in sera from MockDNA-immunized animals or in sera from animals receiving MF59 alone (Fig. 5D).

4. Discussion

The aim of this study was to compare HIV-1 vaccine protocols. We found that a combination of our particular HIV-1 DNA plasmids expressing Env, Gag and Tat, either alone or combined with the corresponding protein immunogens formulated in the MF59 adjuvant, resulted in protection against an experimental HIV-1 infection. The only correlate of protection in this model was the appearance in the spleen of Gag-specific cellular immunity. Variables that did not correlate to protection were cellular reactivities in the periphery and antibody titers to Env, Gag or Tat.

Immunization with HIV-1 gp140, Gag and Tat DNA resulted in complete protection from the subsequent experimental HIV-1/MuLV challenge. This result confirms previous data on DNA immunization in this challenge model where a DNA plasmid cocktail encoding HIV-1 gp160, Gag, nef, rev and Tat mediated complete protection [2], while immunizations with the HIV-1 gp160 gene alone or the gag gene alone did not [1,5]. Similarly, in previously published experiments, mock-immunizations with equal amounts of non-encoding DNA plasmid did not confer any protection [2,26].

In this study, the animals that received DNA alone or were primed with DNA and boosted with protein were all completely protected after challenge. The immune parameter that best correlated with protection was the cellular response detected in the spleen of mice from these two groups. These animals displayed the most robust Gag- and envelope-specific cellular responses in the spleen. The clearest difference after challenge was detected in the Gag-specific response. The two protected groups displayed several-fold higher levels of IFN-γ producing cells in response to Gag antigens than the other groups. The heterologous DNA prime and protein or viral vector boost-mediated enhancement of cellular immunity has been previously described for HIV-1 immunogens in mice, macaques and man (reviewed in ref. [27]).

The poorly protected animals in the protein prime followed by DNA boost group displayed low levels of cellular responses in the spleen, but surprisingly, these animals had a potent pre-challenge cellular response in their peripheral blood, with high levels of reactivity to both Env and Gag peptides. This response, however, declined substantially after the challenge. The discrepancy observed between the different prime boost regimens suggests that the protein prime followed by DNA boost protocol does not induce a strong and long-lasting T cell response that could be augmented at the time of challenge and that the capacity of DNA to prime proper T cell responses is greater than that of protein. This can be due to several factors, including the known inherent effects of microbial DNA on the development of the adaptive immunity [28,29]. We believe that this difference in immune responses is linked to the inability to protect against the experimental HIV challenge.

Another explanation for the difference in protection between the two prime-boost groups is the epitope specificity of the envelope-specific cellular immunity. Cells from the group receiving DNA followed by protein recognized epitopes present preferentially in the N-terminal part of the gp160 antigen, which has been modified by a V2 deletion [30]. PBMC from the mice immunized with protein followed by DNA predominately recognized epitopes in the C-terminal part of the gp160 antigen. This indicates that the gp160 cellular epitope specificity is of importance for the protection and that the immunization modalities result in differences in the priming specificities of responses directed against certain epitopes.

The animals that received a cocktail of the recombinant HIV-1 gp140, Gag and Tat proteins formulated in the adjuvant emulsion MF59 were also protected from the experimental HIV-1 challenge. These animals responded with high levels of HIV-1-specific IgG but in contrast to animals receiving DNA, had only modest cellular responses against Env and Gag. The group of animals receiving the MF59 adjuvant alone was, however, partially protected (2/5 animals being negative for HIV-1 isolation) and it is therefore likely that the complete protection observed in the protein group is partly mediated by effects of the adjuvant in addition to the specific anti-HIV-1 responses. Since only low levels of post-challenge HIV-specific cellular immunity (directed against Gag) were detected in the adjuvant group alone, we assume that the observed protective effect in these animals is predominantly due to innate immune factors. The adjuvant MF59 have indeed been shown to induce influx of cells of the innate immune system when injected intramuscularly [31].

For the DNA followed by protein group, the dual DNA may have efficiently primed for the protein boost and with an extra effect from the MF59, this resulted in a protective response by a combination of innate and specific adaptive effector functions. The reasons why the protein followed by DNA was less effective were most likely that the effects of the adjuvant on the innate immunity had vanished by the time of challenge, which for these animals, occurred 12 weeks after the protein/MF59 immunization (group 4, see Fig. 1 for immunization protocol).

The serological data from mice receiving HIV-1 DNA (gp140, Gag and Tat) show low to intermediate levels of anti-Env IgG and no anti-Gag or anti-Tat-specific IgG. Ne-
tralization of HIV was detectable after challenge in serum from these animals, suggesting that low titer IgG induced by the DNA immunization were amplified by the infected cell challenge. Moreover, after the challenge, the levels of anti-Env- and Gag-specific IgG increased in the DNA followed by protein boost group, whereas the same responses decreased in the protein group. This can be interpreted in at least two ways: (i) the level of specific IgG (anti-Env GMT of $1 \times 10^6$) was already very high in the protein group and could not be further boosted or (ii) the DNA priming facilitated further enhancement of the level of humoral reactivity. Overall, no direct correlation could be established between protection and the level of antibody responses. This challenge model is based on inoculation with virus-infected cells and we believe that the primary protective immune response is the cell-mediated immune response and that neutralizing antibodies (targeting the mature HIV-1 envelope) probably have a limited inhibitory effect in this system. The results from the analysis of the humoral responses further strengthen the hypothesis that the most likely mediator of protection against this challenge is a highly specific T cell response, typically induced by a successful genetic immunization [32]. This might explain the relatively poor outcome of the protein immunizations, which predominately resulted in humoral responses. However, one cannot exclude that antibodies might elicit indirect protective effects, mediated for instance by mechanisms like antibody-dependent cell-mediated cytotoxicity (ADCC). In a cell-free virus challenge system (i.e. non-human primate SIV/SHIV models) antibodies might have greater impact on a cell-free virus challenge system (i.e. non-human primate antibody-dependent cell-mediated cytotoxicity (ADCC). In conclusion (Fig. 6), we have shown that a novel combination of multiple HIV-1 DNA and protein candidate immunogens can lead to protection against an experimental HIV challenge in mice. Genetic immunization alone induced complete protection, likely due primarily to induction of broad and strong cellular immunity. Heterologous prime-boost immunization resulted in enhanced cellular immunity and complete protection against challenge if the DNA and protein immunogens were delivered in the order of DNA followed by protein. Protein formulated in the MF59 adjuvant resulted on its own in robust protection in the challenge model; however, at least a part of this effect was most likely mediated by the adjuvant’s capacity to induce innate responses.

The correlates of protection in the HIV-1/MuLV challenge model are not fully characterized, but our data suggest that strong cellular responses both in the periphery (in PBMC) and, more importantly, centrally (in the spleen) are critical. Further, also the quality, epitope specificity and duration of these responses are probably highly important. Finally, it is likely that some, still unknown, innate immune mechanisms are involved in protection against the experimental HIV-1/MuLV challenge.

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