Use of alphavirus replicons expressing IL-12 as highly potent vaccine adjuvants

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ALPHAVAX
Vaccines Redefined

Introduction

Adjuvants are known to significantly enhance the effect of vaccines. Depending on composition and usage, the role of adjuvants can be i) dose-sparing, i.e. allowing for a lower dose of vaccine to be efficacious, ii) immune-enhancing, i.e. elevating the responses to poor antigens to levels found to be efficacious, iii) boost-sparing, i.e. allowing for fewer doses or even a single immunization to be required for efficacy. The precise mechanisms for protection differ between pathogens and are in general not fully understood, but in most cases neutralizing antibodies and/or pathogen-specific effector T cells are believed to be important.

We have developed an alphavirus replicon vector system for use as a platform vaccine technology primarily for the development of prophylactic and therapeutic vaccines for infectious diseases and cancer. Use of virus-like replicon particles (VRP) based on an attenuated strain of Venezuelan equine encephalitis (VEE) virus is especially attractive because VRP are propagation-defective, single cycle vectors that express heterologous proteins to high levels and target expression to dendritic cells. VRP vaccines have been shown to elicit both humoral and cellular immune responses to the vectored gene products that have conferred protection against challenge in many animal disease models.

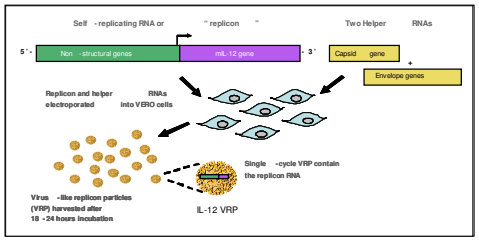
It was previously demonstrated by others (refs 1 and 2), as well as by ourselves (unpublished data), that VRP designed not to express any heterologous gene of interest (so-called Empty VRP) can serve as adjuvants to co-administered protein-based vaccines. While humoral responses, including mucosal, were enhanced, the effect on cell-mediated responses were more modest. The mechanism of action is not fully understood for Empty VRP as adjuvants but was shown to require type I interferon signaling. The alphavirus replicon initiate massive RNA replication activities that include the formation of dsRNA intermediate complexes. It is therefore tempting to hypothesize the role of signaling through TLR receptors. One study, however demonstrate that even non-infectious virions had adjuvant effects, suggesting several pathways to be involved.

In this poster, we present dramatic adjuvant activities of VRP expressing the well-characterized T_H1-inducing cytokine Interleukin 12.

Adjuvant Design

IL-12 VRP is an alphavirus replicon vector expressing murine interleuking 12 (mIL-12). The mIL-12 gene was made as a fusion construct where the subunits p40 and p35 were cloned as a single open reading frame separated with a short peptide linker (not shown).
The adjuvant contains a self-amplifying RNA (replicon) in which the structural protein genes of an attenuated strain of VEE virus, V3014, are replaced by the mIL-12 gene. The vaccine was produced by purification of VRP from Vero cells co-transfected with a self amplifying RNA (replicon) transcript containing the IL-12 gene and two additional RNA transcripts encoding the structural protein genes of V3014 (**Fig 1**). The use of two separate helper RNAs greatly reduces the chance of an intact genome being assembled, by RNA-RNA recombination events, which might generate replication competent virus (RCV). Each batch of VRP vaccine is tested to confirm the absence of detectable RCV. After VRP enter a cell, VEE virus non-structural proteins are synthesized, the replicon RNA is amplified, and the mIL-12 protein is expressed to a high level. Since the packaged replicon RNAs do not encode the VEE virus structural protein genes, the VRP are genetically restricted to a single round of replication and are not capable of producing progeny VRP in the infected cell. The titers of IL-12 VRP are determined by an immunofluorescence assay (IFA) where Vero cells are infected with VRP at serial dilutions and the concentrations of infectious units (I.U.) are scored using specific antiserum.

Figure 1. Production of IL-12 VRP Adjuvant



For immunizations, IL-12 VRP was mixed with soluble protein immunogens and the mixtures were stored on ice until vaccine administration. The stability of the IL-12 VRP in the combined vaccine solutions was monitored by IFA using Vero cells.

Humoral Responses

In order to evaluate the immune-enhancing activity of IL-12 VRP, BALB/c mice were immunized with recombinant Influenza A HA protein (Protein Sciences, CT, Fig 3 and 4) or with Trivalent Influenza Vaccine (TIV, Fig 5) alone or in combination with IL-12 VRP. The animals received two immunizations, three weeks apart. Humoral responses were measured in sera sampled prior to each immunization as well at one week post-boost. T-cell responses were measured in spleens harvested at one week post-boost (Fig 2). As seen in Figures 3-5, IL-12 VRP enchanced the humoral responses significantly, in a dose-sparing manner. Moreover, the adjuvant activity was significantly stronger than using VRP without any transgene (Empty VRP, green bars in figures 4-5).

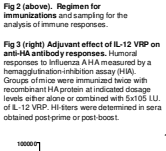


Fig 2 (above). Regimen for immunizations and sampling for the analysis of immune responses.

Fig 3 (right) Adjuvant effect of IL-12 VRP on anti-HA antibody responses. Humoral responses to Influenza A HA measured by a hemagglutination-inhibition assay (HIA). Groups of mice were immunized twice with recombinant HA protein at indicated dosage levels either alone or combined with 5x105 I.U. of IL-12 VRP. HI-titers were determined in sera obtained post-prime or post-boost.

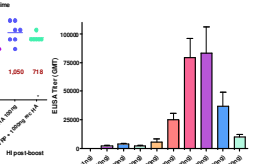


Fig 4 (above) Immune-enhancing role of IL-12 VRP. Humoral responses to Influenza A HA measured by ELISA. Groups of mice were immunized twice with recombinant HA protein alone or combined with IL-12 VRP at indicated dosage levels. One group received HA protein combined with VRP engineered not to over-express IL-12 or any other transgene (Empty VRP, green bar).

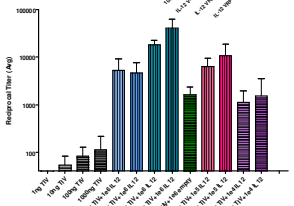


Fig 5 (left) Dose-sparing activity of IL-12 VRP on TIV. Humoral responses to Influenza A HA measured by ELISA. Groups of mice were immunized twice with TIV alone or combined with IL-12 VRP at indicated dosage levels. One group received HA protein combined with VRP engineered not to over-express IL-12 or any other transgene (Empty VRP, green bar). As seen, 10ng of TIV combined with 1x10^6 IU of IL-12 VRP elicited stronger responses than 1000ng of TIV alone indicating a 100-fold dose-sparing effect of IL-12 VRP at the dose of 1x10^6 IU. When the higher dose, 1x10^8 IU was used, the dose-sparing effect was at least 1000-fold.

Cell-mediated Responses

In order to assess the adjuvant activity of IL-12 VRP on the development of cell-mediated responses, Balb/c mice were immunized with recombinant Influenza A HA protein (Protein Sciences, CT, Fig 6) or with Trivalent Influenza Vaccine (TIV, Fig 7) alone or in combination with IL-12 VRP. As seen in figure 6, dose-escalation studies revealed that significant enhancement in the cellular responses to 100ng recombinant HA protein was achieved using 5x10^4 IU or higher doses of IL-12 VRP (Fig 6, orange bar). Recombinant protein or TIV (Fig 7) alone failed to induce cellular responses. Dose-escalation studies (Fig 7) demonstrated that IL-12 VRP displayed activity at a100-fold lower dose than Empty VRP.

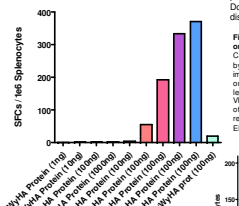


Fig 6 (left) Immune-enhancing role of IL-12 VRP on cellular responses to a influenza HA vaccine. Cell-mediated responses to Influenza A HA measured by peptide-specific ELISPOT. Groups of mice were immunized twice with recombinant HA protein alone or combined with IL-12 VRP at indicated dosage levels. One group received HA protein combined with VRP engineered not to over-express IL-12 or any other transgene (Empty VRP, green bar). Bars represent the number of Interferon gamma-secreting Elispot-forming units per million splenocytes.

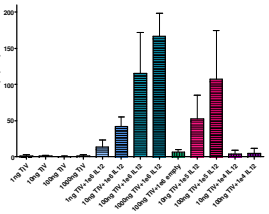


Fig 7 (right) Dose-sparing activity of IL-12 VRP cellular immune responses to TIV. Cell-mediated responses to Influenza A HA measured by peptide-specific ELISPOT. Groups of mice were immunized twice with TIV alone or combined with IL-12 VRP at indicated dosage levels. A control group received TIV protein combined with VRP engineered not to over-express IL-12 or any other transgene (Empty VRP, green bar).

Mechanisms

Characterization of in vivo IL-12 expression from IL-12 VRP. Mice were immunized with a recombinant Influenza HA vaccine combined with varying doses of IL-12 VRP, Empty VRP, or recombinant IL-12 protein. Plasma samples were obtained before the immunizations and at 1, 2, or 4 days post infection (p.i.). Plasma samples were analyzed using a quantitative cytometric bead array (CBA, BD Biosystems) for Interleukin 12. As seen in figure 8A below (left panel), a dose of 5x10^6 IU of IL-12 VRP resulted in systemic plasma IL-12 levels comparable to an administration of 50ng recombinant IL-12.

Assessment of the biological activity of IL-12 VRP. The plasma samples were also analyzed for levels of Interleron gamma, expected elevate as a result of in vivo stimulation of immune cells by IL-12 (figure 8B). At 48 h.p.i., the pIFN-g levels were equivalent between mice administered with 0.5ug of recombinant IL-12 and 5x10^6 IU of IL-12 VRP. These results suggest a higher specific IFN-g inducing activity of IL-12 expressed from IL-12 VRP than IL-12 in the form of recombinant protein.

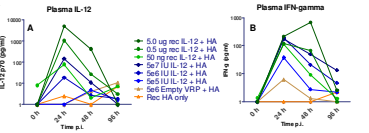


Fig 8 In vivo expression levels of IL-12 (A) and the resulting induction of IFN-gamma (B) levels following S.C. administration of IL-12 VRP into mice.

Quantitation of humoral and cellular immune responses enhanced by IL-12 VRP or recombinant soluble IL-12. Mice immunized as indicated above, were analyzed for cellular (spleen) anti-HA T-cell levels (Fig 9 A, B), as well as humoral (serum) anti-HA antibody titers (Fig 9 C). Responses were measure at 7 days after either one (A) or both (B) immunizations. Comparative analysis reveal drastically increased specific activity of IL-12 delivered by IL-12 VRP over recombinant soluble IL-12 (compare plasma levels of IL-12, Fig 8, and immune responses achieved, Fig 9).

Fig 9

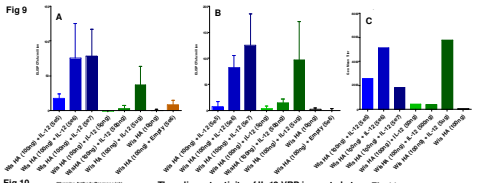
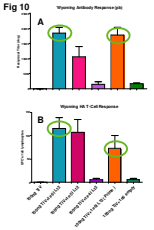


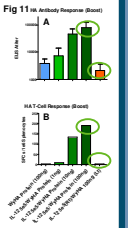
Fig 10



The adjuvant activity of IL-12 VRP is exerted at the time of priming (Left). Mice were immunized twice (see above), with TIV either alone or in combination with IL-12 VRP at indicated dosage levels. One group received TIV+IL-12 VRP at priming while the boost consisted of TIV alone (Fig 10 orange bar). Comparative analysis of immune responses, interestingly, failed to demonstrate any significant differences in the humoral (Fig 10 A) or the cellular (Fig 10 B) responses between the groups highlighted with green circles, indicating that the IL-12 VRP adjuvant is required at the priming only.

IL-12 VRP is only active as an adjuvant if administered locally together with the antigen component. Mice were immunized twice with HA protein alone or combined with IL-12 VRP, as indicated, by sc injection of into the right limb. One group, however, received the antigen component in the left limb. As seen in Fig 11, the results indicate a need for both components to be administered into the same site for an adjuvant effect to be observed.

Fig 11



Summary

- **Virus-like Replicon Particles carrying the gene for Interleukin 12 (IL-12 VRP) can act as a highly potent adjuvant to soluble protein-based vaccines.**
- **Immunization studies using Influenza A/HA vaccine preparations demonstrated a dose-sparing effect of IL-12 VRP that was at least 1000-fold compared to vaccination without IL-12 VRP adjuvant.**
- **In addition to enhancing humoral responses robust cell-mediated immune responses are induced. Effectively, by co-administering IL-12 VRP, soluble proteins could be made to serve as potent T-cell inducing antigens.**
- **The effects of IL-12 VRP are mediated locally as demonstrated by experiments where mice were immunized with antigen and adjuvant (Il-12 VRP) in separate flanks.**
- **Administration of IL-12 VRP at effective doses results in very low systemic levels of Interleukin 12, supporting the observations that IL-12 VRP acts locally, presumably at the site of the lymph node draining the site of injection.**

References:

1. Hidmark et al., Humoral Responses against Coimmunized Protein Antigen but Not against Alphavirus-Encoded Antigens Require Alpha/Beta Interferon Signaling, J. Virol., July 2006, p. 7100
2. Thompson et al., Mucosal and systemic adjuvant activity of alphavirus replicon particles, PNAS, Mar 2007, p. 3272