

Program #B190, Abstract #72



ABSTRACT An RNA replicon vector system derived from an attenuated strain of VEE virus was used to produce virus-like replicon particles (VRP) expressing SARS-CoV genes. We previously showed in mouse studies that complete protection was conferred by SARS S VRP and partial protection with SARS M VRP. In this study, two assays for cellular immunity were used to characterize the T cell phenotypes and cytokine profiles induced after SARS VRP vaccination. Following SARS S, N, M, or E VRP immunization of BALB/c mice, splenocytes were harvested for antigen-specific ICS and ELISPOT. The SARS S VRP induced both CD4+ and CD8+ responses with the latter being the dominant component as measured by ICS for IFN- γ or TNF- α . Polyfunctional CD8+ cells secreting both IFN- γ and TNF- α were also detected. Following SARS N VRP immunization, T cells secreting IFN- γ or TNF- α were readily detected with cells secreting both predominately identified as CD4+ cells. Only small amounts of Th1 cytokine secretion was detected in cells from SARS M VRP inoculated mice. SARS E VRP elicited a robust CD8+ response as shown by cytokine expression in cells stimulated with a 9-mer peptide. IL-4 was low or undetectable after any SARS VRP vaccination. The IFN- γ ELISPOT results paralleled those from ICS, indicating that the SARS VRP vaccines induced Th1-biased responses, although the cytokines elicited differed depending on the SARS gene expressed. This study was supported by NIH grant #UC1-A162582.

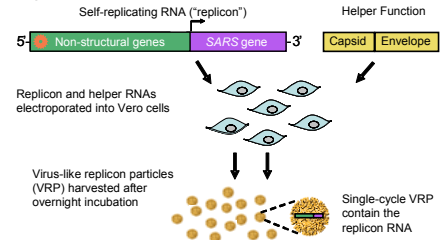
Evaluation of Cellular Immune Responses Induced in Mice by Alphavirus Replicon Particle Vaccines for SARS-CoV

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INTRODUCTION

VRP vaccines for SARS Coronavirus (SARS-CoV) were constructed by replacing the alphavirus structural protein genes with a gene of interest (e.g. SARS S, N, M, or E), resulting in a self-replicating RNA (replicon) that expressed proteins (e.g. SARS S, N, M, or E proteins) to high levels. The recombinant replicon was packaged into virus-like replicon particles (VRP) by transfecting Vero cells with replicon RNA and helper function nucleic acids that express the alphavirus structural proteins *in trans* (Figure 1). VRP have the same cell tropisms as parent alphavirus particles but are genetically restricted to a single round of infection and expression *in vitro* and *in vivo* due to the deletion of the alphavirus structural protein genes. Thus, VRP are single-cycle, replication-incompetent vectors that express proteins of interest upon cellular infection *in vivo* leading to the induction of robust cellular and humoral immune responses against the target disease proteins. This VRP vector system provides an attractive approach as a vaccine delivery system for the development of effective vaccines against SARS-CoV that may play a role in controlling future outbreaks of SARS.

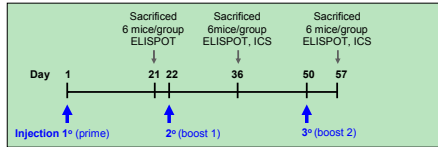
Figure 1. Production of SARS VRP Vaccines



We previously showed in mouse studies that complete protection against challenge was conferred by SARS S VRP and partial protection with SARS M VRP. To further evaluate the candidate SARS VRP vaccines under development, we have utilized two assays for cellular immunity to more fully characterize the T cell phenotypes and cytokine profiles induced in mice following SARS VRP vaccination (see Figure 2).

Figure 2. Study Design

Groups of 18, 6-8 week old BALB/c mice were vaccinated at Days 1, 22 and 50 with specific SARS vaccines (S, N, M, or E) at 1x10⁸ IU of VRP, sc in both rear footpads.



METHODS

Quantitative Assays for Cellular Immunity

Intracellular Cytokine Staining (ICS) and IFN- γ Enzyme-Linked ImmunosPOT (ELISPOT) assays were used to characterize the T cell phenotypes and their cytokine profiles induced after SARS VRP vaccine administration. Splenocytes were assayed for SARS S, N, M, or E specific cytokine secreting lymphocytes by standard ELISPOT and ICS assay methods with overlapping peptides.

Peptides used to stimulate antigen-specific T cells, SARS (Urban1 Strain)

S: 3 Pools covering the entire gene (18-mers overlapping by 11)

N: 1 Pool covering the entire gene (18-mers overlapping by 11)

M: 1 Pool covering the entire gene (18-mers overlapping by 11)

E: 1 immunodominant 9-mer peptide, amino acids 41-49 (AYCCNIVNV)

METHODS (Cont.)

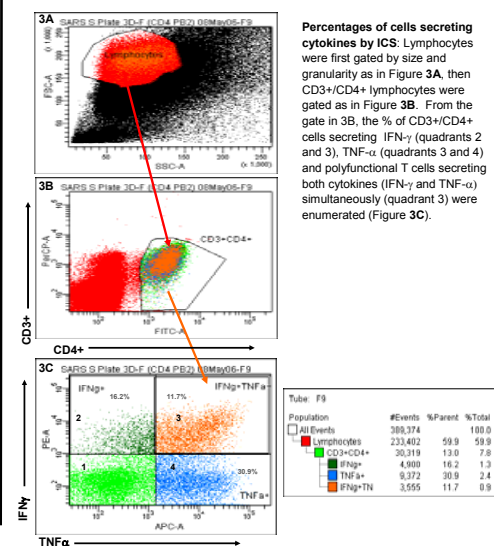
ELISPOT Assay (See Figure 4)

- Fresh splenic lymphocytes were tested at 1x10⁶ cells/well by stimulating overnight at 37°C in 5% CO₂ with peptide pools or a specific peptide.
- Wells were coated with anti-mouse IFN- γ antibody (Mab Tech) and were developed by sequential addition of biotin-labeled anti-mouse IFN- γ antibody (Mab Tech), avidin-HRP (Vector) and AEC substrate (Sigma).
- Spot forming cells (SFC) per well were counted by Zellnet, Inc.
- Data were expressed as SFC/10⁶ lymphocytes after subtraction of background (cells alone without peptide stimulant).
- A positive response was defined as mean SFC/10⁶ lymphocytes > 10 and mean SFC/well > 2X mean background SFC/well.

ICS Assay (See Figures 5-9)

- Spleens were harvested and cells cryopreserved from mice 14 days after boost 1 (Day 36) and 7 days after boost 2 (Day 57).
- Thawed lymphocytes were stimulated with SARS peptides or with no peptides (control) overnight in the presence of Brefeldin-A.
- Lymphocytes were surface stained with antibodies to CD4 (FITC) or CD8 (FITC) and CD3 (PerCP) and intracellular staining with antibodies to IFN- γ (PE), IL-4 (PE), TNF- α (APC) or IL-2 (APC) was performed following permeabilization with saponin.
- At least 300,000 total events per sample were collected on a LSR II flow cytometer.
- Data were expressed as the percentage of CD3+/CD4+ or CD3+/CD8+ cells secreting cytokines as shown in Figure 3.
- A response was considered positive if the difference between the percent of cytokine-secreting cells with and without peptides was > 0.01%.

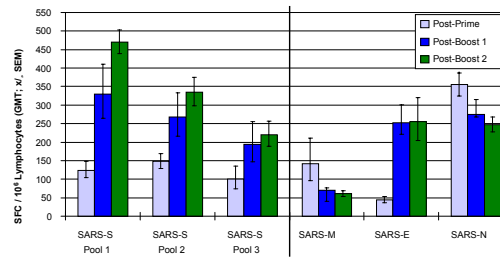
Figure 3. ICS Lymphocyte Gating Example: Cells Treated with Mitogen



RESULTS

- IFN- γ ELISPOT assay results demonstrated that VRP expressing SARS S, N, M, or E elicited robust cellular immune responses in BALB/c mice (Figure 4).
- As assessed by ICS, the pattern of secretion of other cytokines varied depending on the specific SARS protein that was expressed (Figures 5-9).
- In splenic lymphocytes from mice immunized with SARS S VRP and stimulated with SARS S peptides, high numbers of CD4+ T cells secreting IFN- γ , TNF- α , or IL-2 and low numbers of CD4+ T cells secreting IL-4, indicating a Th1 bias, were observed after boost 1. A similar profile was observed after boost 2 (Figure 5, left panel). High numbers of CD8+ T cells secreting IFN- γ and TNF- α were also observed after the first boost and the number of such cells increased after the second boost (Figure 5, right panel).
- In splenic lymphocytes from mice immunized with SARS N VRP and stimulated with SARS N peptides, high numbers of CD4+ T cells secreting IFN- γ , TNF- α , or IL-2 and low numbers of CD4+ T cells secreting IL-4, indicating a Th1 bias, were observed after the first boost, and the number of such cells was lower after the second boost (Figure 6, left panel). High numbers of CD8+ T cells secreting IFN- γ and TNF- α were also observed after the first boost although the number of such cells was low after the second boost (Figure 6, right panel).
- In splenic lymphocytes from mice immunized with SARS M VRP and stimulated with SARS M peptides, modest numbers of CD4+ T cells secreting IFN- γ , TNF- α , or IL-2 and low numbers of CD4+ T cells secreting IL-4, indicating a Th1 bias, were observed after the first or second boost (Figure 7, left panel) and low to undetectable numbers of CD8+ T cells secreting IFN- γ and TNF- α were observed after the first or second boost (Figure 7, right panel).
- In splenic lymphocytes from mice immunized with SARS E VRP and stimulated with a single, immunodominant 9 amino acid SARS E peptide, cells secreting IFN- γ and TNF- α were observed only in the CD8+ T cell population and not the CD4+ T cell population (Figure 8).
- Polyfunctional T cells secreting both IFN- γ and TNF- α were observed in the CD4+ and/or CD8+ T cell populations in mice immunized with each of the SARS VRP vaccines, although the specific pattern varied among the different vaccines (Figure 9).

Figure 4. IFN- γ ELISPOT Responses in Mice Immunized with SARS VRP



CONCLUSIONS

- VRP vaccines expressing any of four SARS-CoV proteins induced robust cellular immune responses in mice.
- VRP vaccines expressing SARS S, N, or M induced CD4+ T cell responses with a Th1 cytokine profile.
- VRP vaccines expressing SARS S, N, or E induced CD8+ T cell responses.
- Polyfunctional T cells have been associated with protective immunity in other models. Polyfunctional CD4+ T cell responses were induced by VRP vaccines expressing SARS S, N, or M, and polyfunctional CD8+ T cell responses were induced by VRP vaccines expressing SARS S or E.
- Because SARS S VRP induce robust and persistent, polyfunctional CD4+ and CD8+ T cell responses, and this vaccine was previously shown to be protective in a mouse challenge model, it is a potential candidate vaccine that warrants further development.

Figure 5. Cytokine Responses in Mice Immunized with SARS S VRP

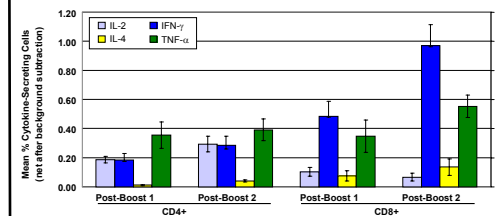


Figure 6. Cytokine Responses in Mice Immunized with SARS N VRP

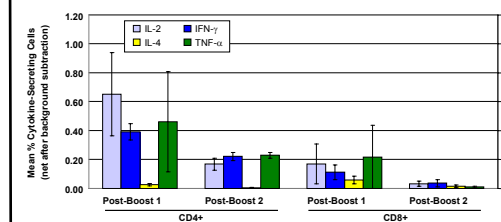


Figure 7. Cytokine Responses in Mice Immunized with SARS M VRP

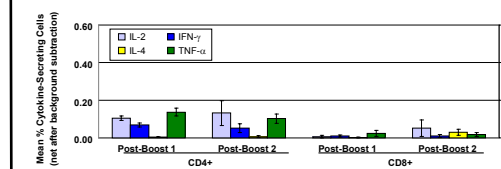


Figure 8. Cytokine Responses in Mice Immunized with SARS E VRP

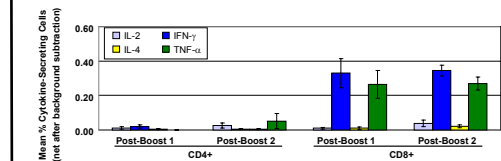


Figure 9. Polyfunctional Cytokine Responses in Mice Immunized with SARS VRP

