

Program #B191, Abstract #73



ABSTRACT

We previously evaluated the immunogenicity and efficacy of a virus-like replicon particle (VRP) vaccine expressing the SARS-CoV S glycoprotein in mice. SARS S VRP were highly immunogenic and conferred protection upon challenge. To extend that work we have evaluated the immune responses induced by SARS S VRP in macaques immunized at 0, 4 and 23 weeks. PBMC were assayed at two week intervals by ELISPOT and ICS. Post prime, both CD4+ and CD8+ T cells were found that secrete IFN- γ , IL-2 and TNF- α . The percentage of CD4+ cells secreting IFN- γ , IL-2 and TNF- α was substantially more than the percentage of CD4+ cells secreting IL-4 post prime indicating a Th1 bias. Post boost, the number of CD4+ cells secreting all measured cytokines were equivalent indicating that the SARS S VRP induced a balanced Th1/Th2 response after 2 inoculations. By week 8, a measured decline in the percentage of cytokine-secreting CD8+ cells was observed. There were no significant differences in the numbers of T cells secreting cytokines with 2 different VRP dosages given as measured by ICS, however a dose response was seen by ELISPOT. Neutralizing antibodies (NAb) to SARS CoV were measured in sera following post prime and boost inoculations. Three of 4 animals in the low dose group had measurable NAb post boost (GMT = 101) whereas all 4 animals in the high dose group had serum NAb after two doses (GMT = 190). These data provide support for further development of a SARS S VRP vaccine. This study was supported by NIH grant #UC1-AI62582

INTRODUCTION

The AlphaVax replicon vector system is based on an attenuated strain of Venezuelan equine encephalitis (VEE) virus and has been used as a vaccine vector delivery system for the development of a number of infectious disease and tumor vaccine candidates.

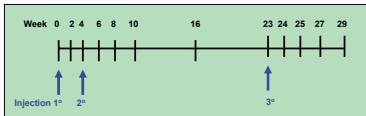
We have applied the VRP technology for the development of a SARS-CoV vaccine. In a previously published study, mice immunized with SARS S VRP developed high antibody titers and were completely protected against challenge (Kamrud et al. VIII International Symposium on Respiratory Viral Infections, V1-3). In another poster at this meeting (See Program #B190, Abstract #72) we describe the cellular immune responses, as assessed by ICS and ELISPOT assays, in mice immunized with VRP vaccines expressing any of four SARS proteins.

To extend that work, we have evaluated the cellular and humoral immune responses induced by SARS S VRP, at two dosage levels, in Chinese rhesus macaques.

METHODS

Two groups of 4 Chinese rhesus macaques were immunized with SARS S VRP, at either of two dosage levels (5×10^7 or 1×10^8 IU) by the i.m. route at 0, 4 and 23 weeks (Figure 1). Animals were vaccinated and housed at Southern Research Institute. At the time points indicated in Figure 1, whole blood was sent by courier to AlphaVax for same-day separation of peripheral blood mononuclear cells (PBMC). Separated PBMC were used immediately in IFN- γ ELISPOT and intracellular cytokine staining (ICS) assays.

Figure 1. Study Design



Micro-Neutralization Antibody Assay (See Table 1)

Heat-inactivated serum samples were diluted two-fold for titrating their neutralization potentials against 100 TCID₅₀ of SARS S-CoV in Vero E6 cells grown in 96-well plates. The neutralizing antibody titers were determined at 72-hrs and expressed as the reciprocal dilution of the serum showing a complete (100%) inhibition of virus-induced CPE.

Quantitative Assays for Cellular Immunity (See poster #72)

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 S VRP vaccine administration. PBMC were assayed for SARS S specific cytokine secreting lymphocytes by standard ELISPOT and ICS assay methods with one pool of SARS S overlapping peptides covering the entire gene.

For ICS assays, cells were surface stained with antibodies to CD3 (PerCP-Cy5.5), CD4 (FITC), and CD69 (PE). Following permeabilization cells were stained (APC) with antibodies to intracellular cytokines: IFN- γ , TNF- α , IL-2 and IL-4.

Immunogenicity of an Alphavirus Replicon Particle Vaccine Expressing SARS-CoV Spike (S) Glycoprotein in Non-human Primates

Elizabeth A Reap, Aubrey D Watson, Pamela K Norberg, Chad Cecil, Ronna E Dornsife, Kurt I Kamrud, and Jonathan Smith. AlphaVax Inc, 2 Triangle Drive, Durham, NC 27709

ICS

The number of cytokine-secreting CD4+ T cells was low at baseline (Week 0) and increased after the first dose (Week 4) for all cytokines tested (Figure 4, panels A and B). After the second dose (Week 8), the number of cytokine-secreting CD4+ T cells appeared to increase for IL-2 and IL-4 and was relatively unchanged for IFN- γ and TNF- α (Figure 4, panel C).

The number of cytokine-secreting CD4+ T cells had returned to baseline by Week 23 (Figure 5, panel A). At 4 weeks after the third dose (Week 27) the numbers of cytokine-secreting cells again increased similar to the balanced pattern at Week 8 (Figure 5, panel B).

The number of cytokine-secreting CD8+ T cells was low at baseline (Week 0) and increased after the first dose (Week 4) for IFN- γ and TNF- α (Figure 6, panels A and B). The number of cytokine-secreting CD8+ T cells remained low to undetectable for IL-2 and IL-4 (Figure 6, panels A and B). The number of cytokine-secreting CD8+ T cells returned to baseline by week 8 and remained at baseline at weeks 23 and 27 (data not shown).

Figure 4. Percentage of Cytokine-secreting CD4+ T Cells at Weeks 0, 4 and 8 in Chinese Rhesus Macaques Immunized with SARS S VRP

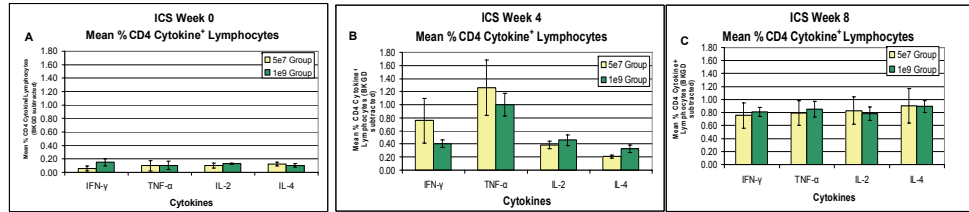


Figure 5. Percentage of Cytokine-secreting CD4+ T Cells at Weeks 23 and 27 in Chinese Rhesus Macaques Immunized with SARS S VRP

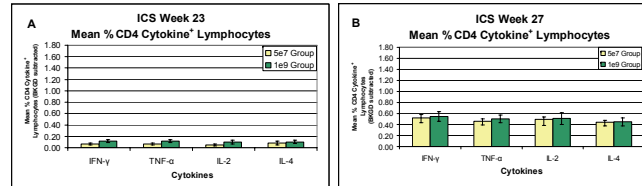
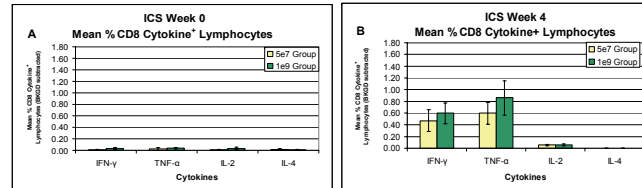


Figure 6. Percentage of Cytokine-secreting CD8+ T Cells at Weeks 0 and 4 in Chinese Rhesus Macaques Immunized with SARS S VRP



CONCLUSIONS

1. Non-human primates immunized with SARS S VRP developed SARS neutralizing antibodies at levels similar to those found in human SARS convalescent serum.
2. Non-human primates immunized with SARS S VRP developed potent cellular immune responses.
3. Substantially more CD4+ T cells secreted IFN- γ or TNF- α than IL-4 at 4 weeks after the prime, indicating a strong Th1-type response.
4. After the boost, the numbers of CD4+ T cells secreting each of the measured cytokines (IL-2, IFN- γ , IL-4, TNF- α) were equivalent, indicating that the SARS S VRP induced a balanced Th1/Th2 response after two inoculations.
5. Peak numbers of IFN- γ or TNF- α cytokine-secreting CD8+ T cells were seen 4 weeks after the prime.
6. Although the IFN- γ ELISPOT assay suggested there was a modest dose response, there were no significant differences in numbers of cytokine-secreting cells induced by the two different doses of SARS S vaccine as measured by ICS.
7. These findings support ongoing development of the AlphaVax SARS S VRP vaccine.

RESULTS

Neutralizing antibodies (NAb) to SARS-CoV were measured at baseline and after each inoculation. In the low-dose group, measurable NAb were detected in 0 (week 4), 3 (week 8) and 4 animals (week 24) after the first, second and third doses, respectively. In the high-dose group, measurable NAb were detected in 2 (week 4), 4 (week 8) and 4 animals (week 24) after the first, second and third doses, respectively (Table 1).

Table 1. Neutralizing Antibody Response to Immunization with SARS S VRP

SARS S, 5×10^7 IU	Week 0	Week 4	Week 8	Week 24
animal 4179	<20	<20	20	160
animal 4188	<20	<20	<20	160
animal 4193	<20	<20	160	1280
animal 4199	<20	<20	320	1280
GMT			101	453
SARS S, 1×10^8 IU	Week 0	Week 4	Week 8	Week 24
animal 4182	<20	<20	40	40
animal 4187	<20	<20	160	80
animal 4191	<20	<20	160	80
animal 4196	<20	<20	40	1280
GMT			190	113

IFN- γ ELISPOT

A dose-dependent antigen-specific response to SARS S peptides was seen in all animals, with similar responses in animals immunized with either the 5×10^7 or 1×10^8 IU dose. Responses for individual animals in the two dosage groups are shown in Figure 2 and the mean \pm SEM for each dose group are shown in Figure 3.

Figure 2. IFN- γ ELISPOT Responses in Individual Chinese Rhesus Macaques Immunized with SARS S VRP

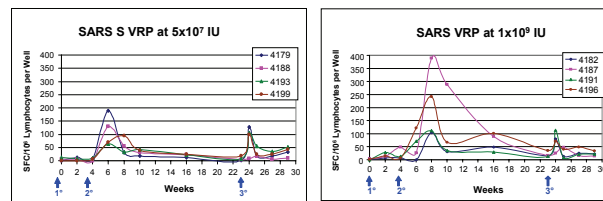


Figure 3. IFN- γ ELISPOT Responses (Mean \pm SEM) in Chinese Rhesus Macaques Immunized with SARS S VRP

