Novel live attenuated vector technology to elicit mucosal immunity against viral pathogens (SIV, HIV, Covid-19) and malaria

Tiffany Chen, Konstantin Virnik, Sanjai Kumar, and Ira Berkower, Center for Biologics, FDA, White Oak

Abstract

Background: The Berkower lab has been working on medical countermeasures for infectious diseases and pathogen reduction technologies. We have used live attenuated vaccine strains, such as RA27/3 from rubella, as a novel vector to express foreign antigens inserted between the structural genes of the vector. We have expressed a variety of viral antigens this way, including Gag and Env from HIV and SIV, S1 spike of Covid-19, and CSP protein of malaria. **Methodology:** Each vector is tested for stable expression after 5 to 10 passages in vitro, for stable growth in vivo by RT PCR of oral mucosa, and for immunogenicity. The best responders can be tested for protection against viral challenge. Advantages of this vector include safety and potency demonstrated in millions of children and adults and one or two doses protect for life. These antibodies can block virus entry at the mucosal surface, which is the frontline for aerosolized virus. The vectors are based on rubella vaccine strain. They replicate on mucosal surfaces, where they can elicit mucosal immunity, including nasal sinuses and upper airways. Current mRNA vaccines do not reach these sites well. For example, Mohamed and Gommerman (Mucosal Imm, 2022) have shown that only 20 to 40 % of subjects made detectable secretory IgA to RBD or S1 protein after immunization with mRNA vaccines. **Results:** Our RA27/3 based vectors replicate on the oral mucosa (see below). We have detected the vectors in mouth swabs from 10 out of 10 animals in the rhesus macaque model. These immunogens could elicit mucosal IgA antibodies where they are most needed, to reduce the challenge dose, prevent severe infection, and block viral transmission. By inhibiting different steps than conventional mRNA vaccines, these vectors have the potential for combined inhibition of viral binding and cell entry. **Conclusions:** Live viral vectors that express SARS CoV-2 antigens can grow in the oral mucosa, where they could elicit IgA antibodies. These antibodies could block viral binding, reduce cell entry, and prevent aerosol transmission. Mucosal immunity could enhance population immunity by targeting novel antigenic sites and viral functions that otherwise would be sensitive to escape mutations.

Introduction

We have used live attenuated vector technology to immunize against viral pathogens and malaria. The viruses include SIV, HIV, and Covid-19. The vaccine targets include: Gag protein, Env protein, and S1 spike protein. Additional targets include malaria circumsporozoite protein (CSP) with its Asn-Ala-Asn-Pro (NANP) tandem repeats. The vectors consist of the rubella vaccine strain, RA27/3, expressing the vaccine antigens as needed.

Materials and Methods

Rubella is a ss RNA virus 9.7 kB with the nonstructural proteins located at the 5' end and the structural proteins coding for C, E2 and E1 at the 3' end. The structural proteins are over expressed at the subgenomic promoter. Two insertion sites were identified. One is located between two Not I sites, where it is under control of the subgenomic promoter. The other is in the structural region, between env E2 and E1. As shown in **Figure 1**, antigen expression from both sites was shown by Western Blot, such as HIV MPER determinant expressed at the structural site.

Materials and Methods cont'd

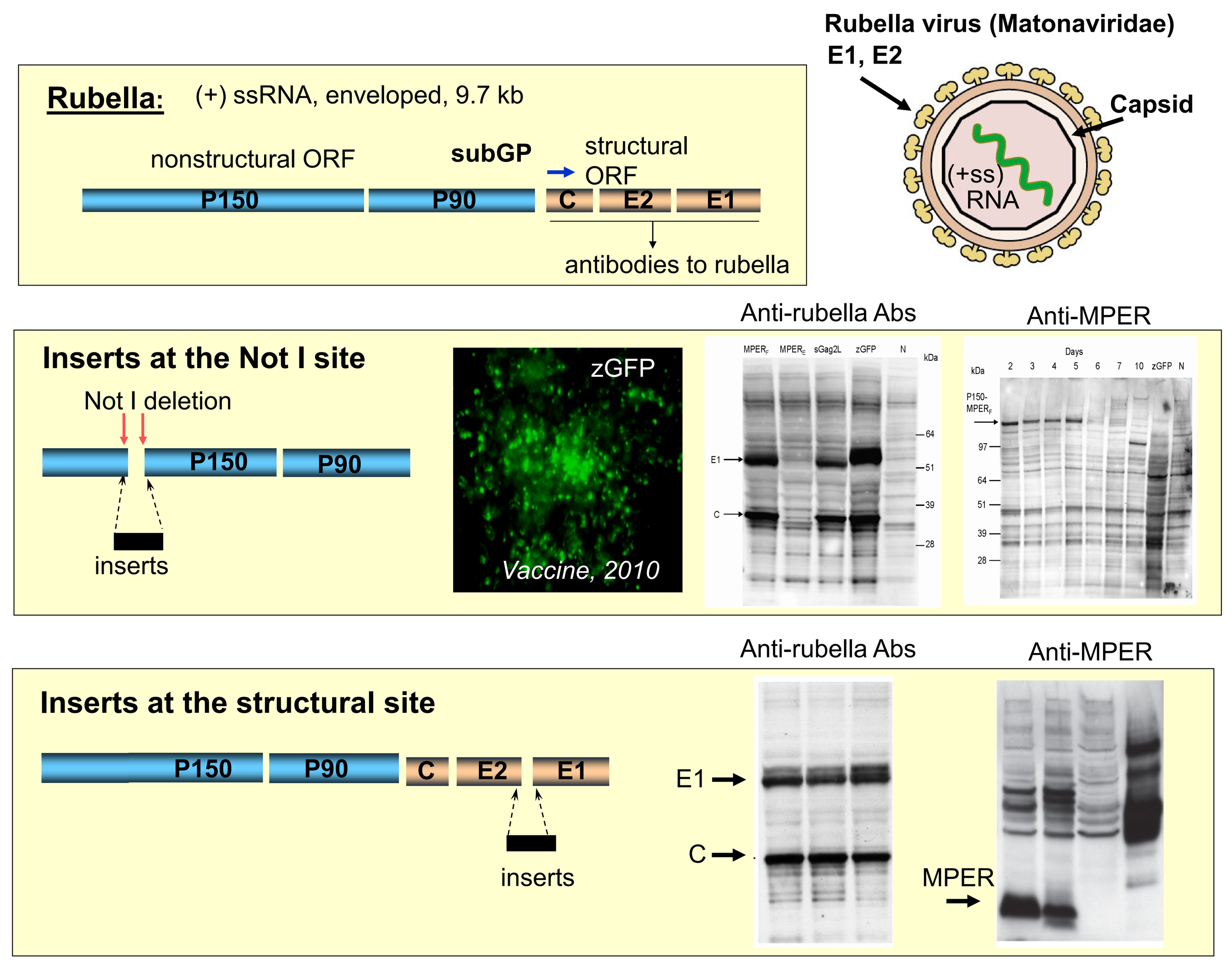

Figure 1. Insertion strategies for producing rubella vectors. Expression of the MPER antigen at the rubella structural insertion site.

For example, the insert for CSP protein of malaria was chosen for expression at the structural site (**Figure 2**). Over ¼ of the world's population are exposed to this pathogen. The CSP has an NANP repeat sequence that is an important target for immunity. The resulting NANP vectors serve as a ruler to determine if there are too many NANP repeats such that the vector doesn't replicate, or not enough NANP repeats for the epitope to form correctly. Constructs ranged from 9 to 12 to 18 repeats. All showed good vector replication, expression of NANP (right panel), and strong antigenicity for monoclonal anti-CSP.

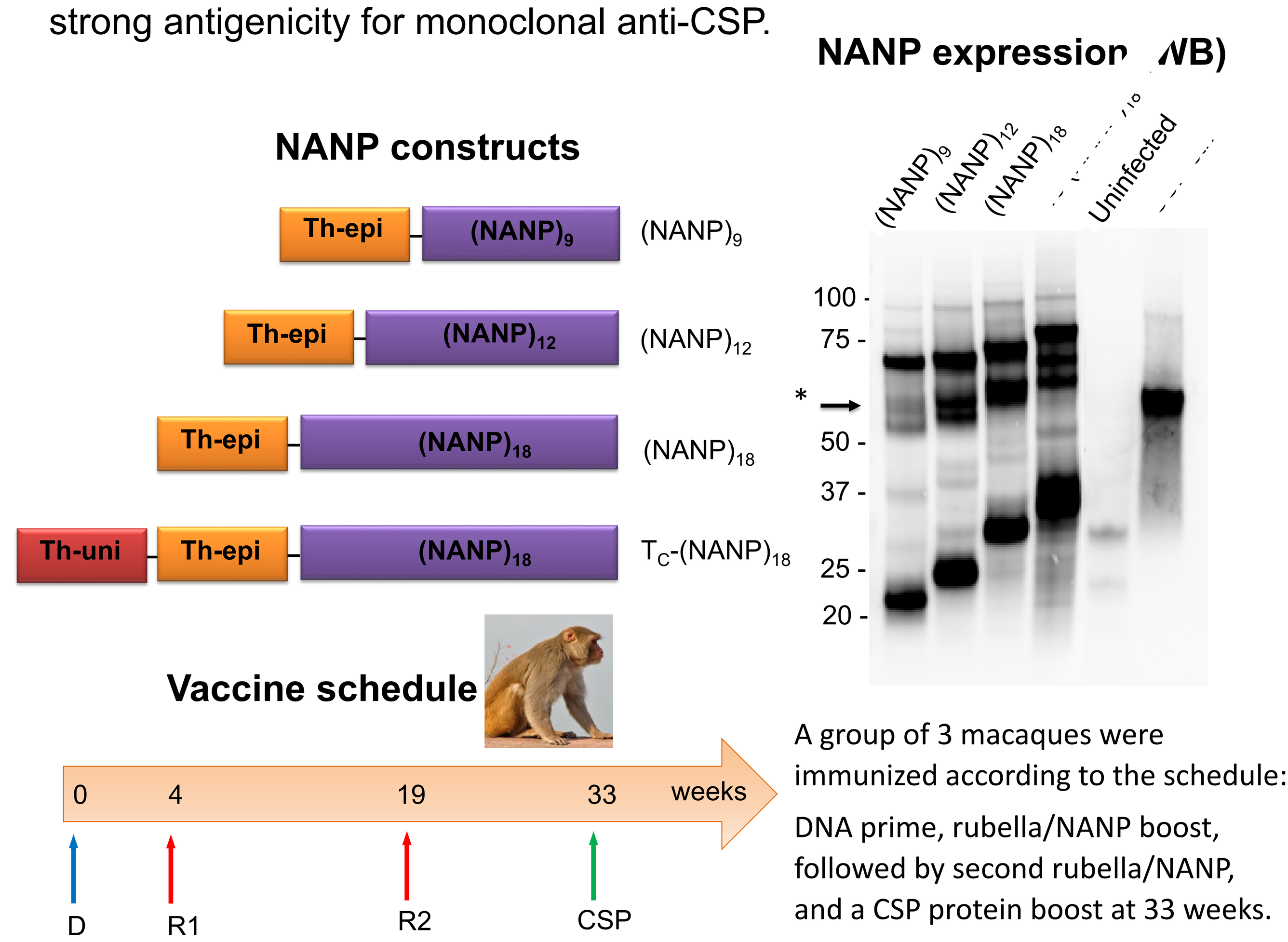

Figure 2. Immunization schedule in rhesus macaques, NANP tandem repeat construct design, and expression of NANP constructs (WB)

Results and Discussion

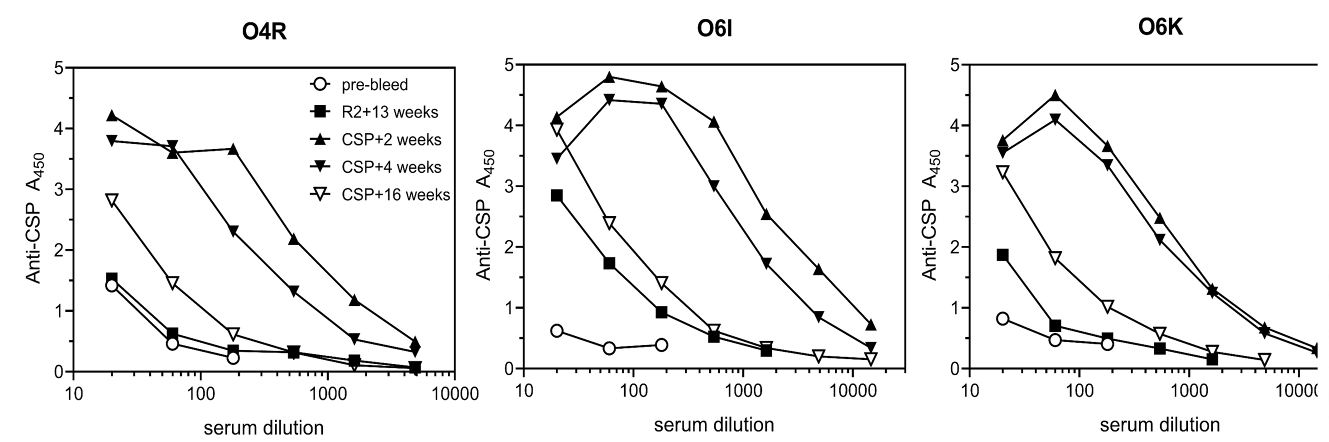

Figure 3. Rhesus antibodies to malaria CSP antigen by ELISA. The immune response was measured on CSP protein. It was strong in all animals, indicating a strong response to the rubella vector prime and CSP protein boost in all 3 animals. Durability will be critical for the intended use in children who are at risk for 5 to 9 years. It may be that more effective priming would allow for periodic boosting through natural exposure to infected mosquitoes.

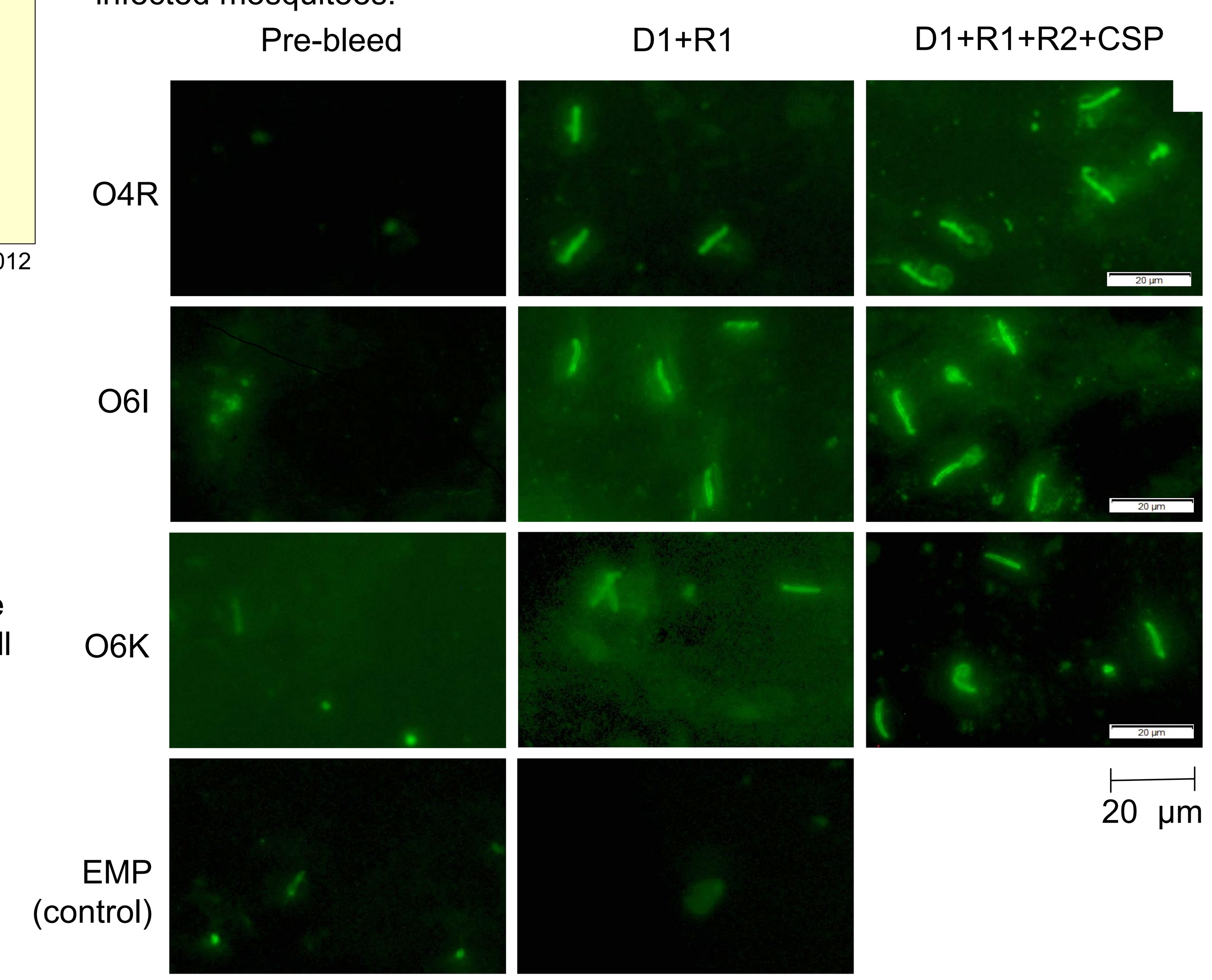

Figure 4. Rhesus antibody binding to malaria sporozoites. Based on the ELISA results, we tested antibodies at 1:400 to 1:800 dilution for the first rubella boost and at 1:3200 to 6400 for the rubella boost. They showed good sensitivity and specificity for sporozoites. Further testing for blocking entry into hepatocytes is underway in collaboration with the Navy Research Center, Silver Spring.

We followed the spread of rubella/CSP vaccine from immunization to response. Normal rubella infection travels from the mucosal surface to sites of proliferation, then to the oral mucosa, where it can spread by aerosol droplets to the next child. In the case of vaccination, the vaccine strain travels from the IM injection site to the oral mucosa, where it can be detected by mouth swabs followed by RT PCR. As shown in **Figure 6**, there was no rubella/MPER signal in the pre samples, but 2 out of 3 were positive 1 week after immunization, and the third macaque became positive at week 2.

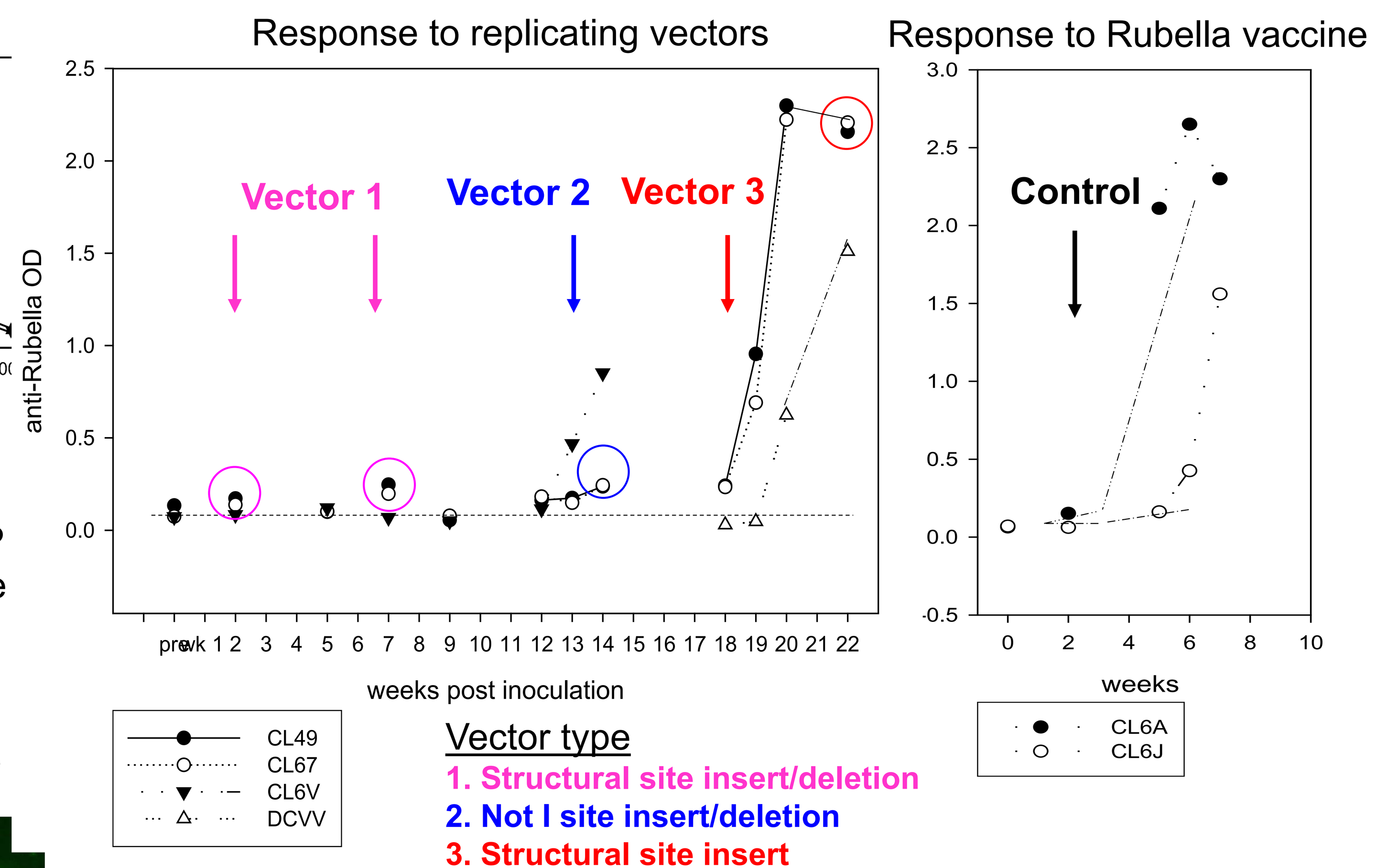

Figure 5. Rubella vectors with inserts at the structural site infected NHP efficiently (3/3 tested)

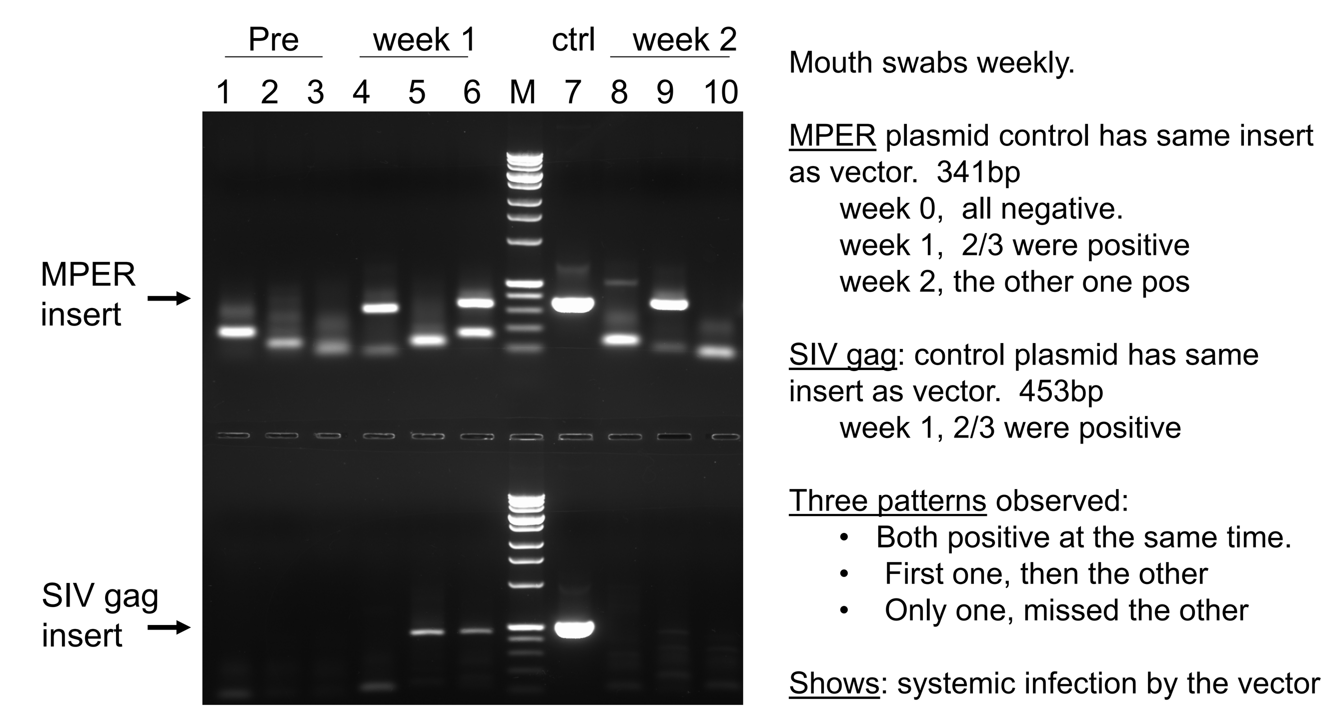

Figure 6. Rubella vectors detected in NHP mouth swabs by RT-PCR

Conclusion

We have used a novel live attenuated vector technology to produce more potent and versatile vaccines that are based on the safety and potency of classic rubella vaccine strain RA27/3. These new vectors will provide insight into the processes of infection and immunity. In the case of malaria, simply by reducing parasite burden, they may protect against severe disease.

Questions include:

a. Does vector immunization create new epitopes or a stronger response to existing epitopes?
b. Does mucosal immunization produce mucosal antibodies and mucosal immunity?
c. Can they increase duration of protection?
d. Can they reduce spread of aerosols and reduce mucosal transmission?
e. Can they add one more tool to the immunologist's tool kit for protection?