

Development of a DNA vaccine against SARS-CoV

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In November 2002, a new form of infectious pneumonia, known as severe acute respiratory syndrome (SARS), emerged in China. The virus causing SARS belongs to the family of Coronavirus and displays a large, positive stranded RNA genome of more than 29kb. In order to develop a potential vaccine against SARS-CoV, we examine a naked DNA immunization. Using viral genomic RNA as template, we have cloned the entire coding region of the Spike (S) glycoprotein by RT-PCR. Sequence analysis proved sequence identity with the Frankfurt 1 isolate and the S protein was cloned into a mammalian expression vector pVR1012. Correct expression of the protein was verified in mammalian cells by western blot analysis.

Different constructs encoding the S protein were generated in order to improve the immune response to this weak antigen. The modified construct carries a GPI-anchor sequence at the C-terminus of the S protein, missing the transmembrane and cytoplasmic region. GPI-anchor proteins are characterized to arrange in lipid rafts, which we can show with lipid raft extraction. Several features of GPI-anchored proteins have been described to optimize the presentation to immune responsive cells. Furthermore, we substituted the wt signal sequence of the S protein with the haemagglutinin leader (HA) of influenza. The HA leader sequence is expected to transport the protein more efficiently to the cell surface. This design may be useful for other DNA vaccines. The constructs were injected three times as naked DNA. ELISA and immunofluorescence showed diverse specific interaction for all constructs.

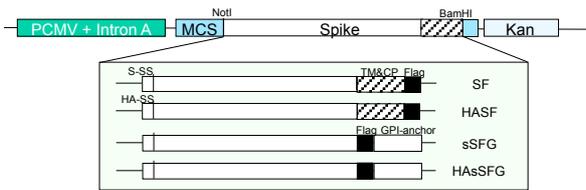


Fig. 1: Cloning strategy of the SARS-CoV DNA vaccine. The Spike protein contains 3768bp. Constructs with different localization signals are designed for an enhanced immune response of the Spike protein. The wt Spike protein contains an extracellular domain with a signal sequence as well as a transmembrane and an intracellular domain, recombinant constructs have an alternate signal sequence from influenza A/PR/8/34 haemagglutinin at the N-terminal part of the protein and/or a GPI-anchor sequence at the C-terminal end.

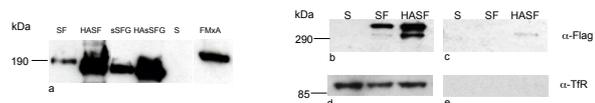


Fig. 2: Characterization of the S protein and plasma membrane extraction: HEK293T cells were transfected, proteins were extracted and equal amounts of total protein were applied on 7.5% SDS-gels. Blot a: expression control of all different constructs with anti-Flag antibody. Blot b: Plasma membrane extraction was conducted and the S proteins were detected with the mouse anti-Flag antibody. Blot c: proteins from the cytoplasmic debris and vesicular fragments were detected with the mouse anti-Flag antibody. Blot d: transferrin receptor, a marker for the plasma membrane, detected in the plasma membrane extraction with the mouse anti-TR antibody. Blot e: transferrin receptor detection of the cytoplasmic extraction.

Fig. 3: Lipid raft extraction: HEK 293T cells were transfected, lipid rafts were extracted and equal amounts of protein were loaded on each lane of the 7.5%/12.5% SDS-gels. Blot a: detection of sSFG and HASsFG in lipid rafts (left side) and of soluble proteins (right side) by anti-Flag antibody. Lipid raft extraction was done with a sucrose gradient (45-5%) and ultracentrifugation for 17h at 36000rpm. The lower band at 190kDa represents the Spike monomer, whereas the upper band represents the oligomeric form. Blot b: Detection of caveolin-1, a lipid raft marker, in the lipid raft extracts and very little in the fractions with soluble proteins (caveolin-1 under process). Detection is done with an anti-caveolin-1 antibody. Blot c: Detection of transferrin receptor as negative control.

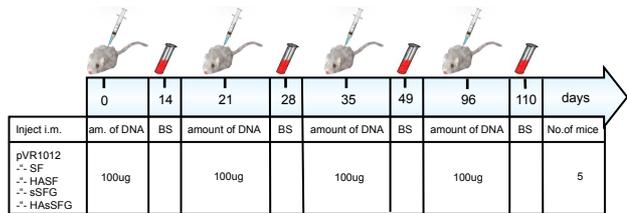
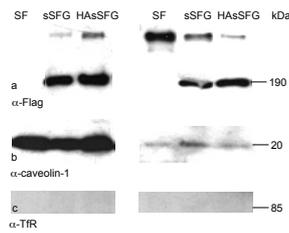


Fig. 4: C57BL/6 mice immunization protocol. Mice were immunized four times at intervals of three weeks. Blood samples were taken two weeks after each immunization. Five different groups of mice are immunized, each group containing five mice. pVR1012 as the negative control, pVR1012SF, pVR1012HASF, pVR1012sSFG and pVR1012HASsFG.

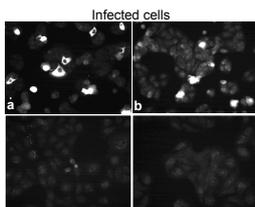


Fig. 5: SARS-CoV indirect immunofluorescence test (Euroimmun AG). Serum (1:50 dilution) was applied to the BIOCHIP with infected and non-infected cells. Specific binding of anti-SARS-CoV antibodies and infected cells is detected with anti-mouse or anti-human antibody coated with TRITC. a: serum of SARS-CoV infected patient (positive control); b: serum of healthy patient (negative control); c-g: sera from mice immunized with: c: empty vector; d: SF; e: HASF; f: sSFG; g: HASsFG.

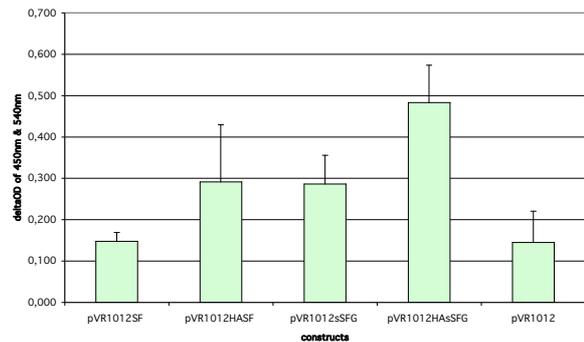
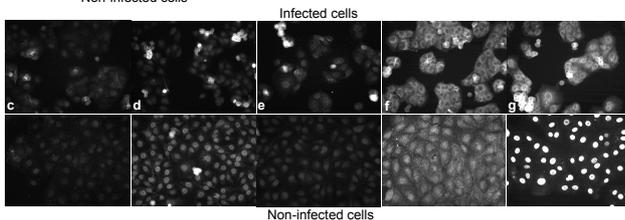


Fig. 6: ELISA analysis after the 4th immunization of the sera of immunized C57BL/6 mice (immunization protocol depicted in Fig. 4). The coating material, sSF was produced in HEK 293T cells and extracted to coat a 96 well plate at 4°C o/n (1:100 dilution). The mice sera (1:100 diluted) were applied in triplicates and incubated for 1h. Anti-mouse antibody coated with HRPO was added in a 1:10'000 dilution. The setup was incubated for 20min with fresh TBM and the reaction was stopped with 1M H₂SO₄. Absorbance was measured at 450nm and 540nm. The relative absorbance of 4 mice was subtracted from the absorbance of the serum of non-immunized mice.

Discussion and Conclusion

We have shown that the Spike protein carrying the HA signal sequence at its N-terminus, appears at a higher level at the plasma membrane than the wild type Spike protein. This effect is followed by an enhanced antibody production against the HASF construct, compared to the SF construct. Moreover we could show the localization of the Spike proteins provided with a GPI-anchor in lipid rafts, compared to the wild type Spike protein. The appearance of the originally weak antigen in lipid rafts contributes to its elevated antibody formation. Together, these two sequence modifications of the Spike protein show a better immune response in the SARS-CoV-IIFT and in the ELISA were mice sera were tested for SARS-CoV specific antibodies.

These results lead to the conclusion, that the Spike protein can be strengthened as an antigen by use of a strong signal sequence as well as a GPI-anchor. The HA signal sequence transports the protein to the cell surface more efficiently, whereas the GPI-anchor is leading to an enhanced antigen density on the surface of the cell and a longer half life of the protein. These two modifications can serve as a technology platform for other weak antigens, viral or cancer origin.