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 examined a naked DNA immunization. Using viral genomic RNA as template, we 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.

Discussion and Conclusion

We have shown that the Spike protein carrying the HA signal sequence at it’s N-terminus, appears at a higher level at the plasma membrane than the wt 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 it’s elevated antibody formation. Together, these two sequence modifications of the Spike protein show a better immune response in the SARS-CoV-IFT 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.