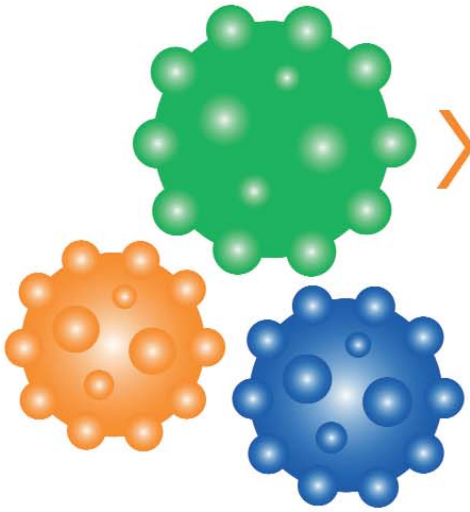

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cient protection against ASFV is probably dependent on both humoral as well as cellular immunological defence mechanisms, with especially the latter being most crucial for protection. In this project we addressed the question if it is possible to use current techniques of computational prediction of T-cell epitopes to develop a vaccine that is protective against ASFV. The ultimate goal is a broadly protective vaccine that is independent of the ASFV strain and is widely applicable for all pigs.

Methods: On the basis of the complete genome sequences of known ASFV isolates and the different swine leukocyte antigen (SLA) gene classes, the epitope prediction program NetMHCpan was used to predict a list of T-cell epitopes that are independent of virus strain or pig SLA class. Based on the prediction of the best twenty T-cell epitopes, a poly-epitope DNA vaccine was generated. The DNA sequence encoded 20 nonapeptide epitopes, separated by spacers of 2 to 5 amino acids that contain signals for proteasome cleavage and further processing by the intracellular antigen transport (TAP) machinery. In addition a universal PADRE B-cell epitope and an immunostimulatory CpG DNA motif were included to improve presentation of the epitopes to host T-cells. Groups of 6 pigs were vaccinated either three times with DNA vaccine (group 1), or two times with DNA vaccine followed by a combination of DNA vaccine and a peptide booster consisting of a cocktail of the twenty nonapeptides (group 2). A control group of 6 pigs was vaccinated three times with empty plasmid (group 3). Pigs were vaccinated with intervals of 2 weeks. The vaccine was applied intramuscularly followed by immuno-electroporationto improve the uptake of DNA by surrounding tissue. The peptides were applied by intramuscular vaccination with Stimune as an adjuvant. Two weeks after the final vaccination, pigs were challenged with the Netherlands ’86 strain and followed for 2 more weeks for clinical symptoms, virus levels in blood, antibody responses and levels of IFN-y secreting cells after in vitro stimulation with virus or the cocktail of twenty nonapeptides.

Results: ASFV infection of the non-vaccinated animals in control group 3 resulted in 40% survival, whereas infection of vaccinated animals in both groups 1 and 2 resulted in 83% survival. Pigs in group 2 had significant lower total clinical scores than pigs in the other groups. Pigs in this group also had a significant IFN-y secreting cell response against the cocktail of nonapeptides from day 42 post first vaccination (p.v.) (day 0 of challenge [p.c.]) until the end of the experiment. Pigs in group 1 had a significant IFN-y secreting cell response against the cocktail of nonapeptides from day 49 p.v.(day 7 p.c.). No IFN-y secreting cell response against the cocktail of nonapeptides was observed in the control group. All groups showed a significant IFN-y secreting cell response against the virus on day 56 p.v. (day 14 p.c.) There were no significant differences in levels of viral DNA in blood between groups. No significant differences were observed in levels of blocking percentages of the ASFV ELISA. The levels of infectious virus in blood and antibody levels detected by IPMA (immunoperoxidase monolayer assay) will be presented at the EPIZONE conference.

Conclusion: Promising results were obtained in this study by using the T-cell epitope prediction program NetMHCpan for development of an ASFV vaccine. Expression of predicted T-cell epitopes using a DNA vaccine provided at least partial protection against mortality and clinical disease. The immunological response could be further increased by using a peptide cocktail as booster. Based on these results, different vaccination strategies (e.g. using viral vectors) will be tested in future experiments.

Generation of a new Newcastle Disease vaccine by reverse genetics based on a recently genotype XI virus

Haijin Liu, Patricia Gil, Cécile Minet, Renata Servan de Almeida, Emmanuel Albina

CIRAD UMR CMAEE - 34398 Montpellie -, France

Objective: Newcastle disease (ND) is the major viral infection of poultry inducing high morbidity, mortality, and significant economic impacts on the poultry industry. ND is caused by virulent strains of avian paramyxovirus serotype 1 (aPMV-1) which have the capacity to spread over long distances by animal and human movements. All strains of aPMV-1 belong to a single serotype and current vaccines have been used worldwide for a long time and demonstrated their efficacy in terms of clinical protection. Up to date vaccines have been made of old genotypes that emerged several decades ago. However, recent studies have shown that the virus has undergone significant evolution which has led to the progressive emergence of new genotypes with potential antigenic drifts. The recently described genotype XI in Madagascar contains original amino acid substitutions on F and HN

proteins, some of them clustered in the head of the proteins, presumably exposed to the host immune system, suggesting that these substitutions may account for antigenic drifts. Indeed, we showed in vivo, under controlled conditions, that current vaccines conferred clinical protection against both the homologous genotype and genotype XI, but were unable to prevent virus shedding of heterologous genotype. Our objective was to produce genotype adapted vaccines, using reverse genetics to replace the F and HN of a live attenuated old-genotype virus (the genotype II Lasota strain) by the corresponding proteins of the more recent Madagascar genotype XI. This chimeric genotype XI-II will be characterized in vivo and evaluated in immunization/challenge trials.

Methods: NDV minigenomes expressing eGFP under two promoters (pT7 polymerase and pCMV) have been constructed and compared in vitro. Subsequently, segments of the full genome of the genotype XI MG-725 strain and LaSota strain have been generated by reverse transcription, cloned and assembled under the control of CMV promoter. Rescue of the virus is done by co-transfection of the full length genome and plasmids expressing NP, P and L proteins in BHK21 cells.

Results: The expression of the NP, P and L proteins of NDV MG725-08 strain has been confirmed on BHK 21 and by conventional RT- PCR on mRNA with specific NP, P and L primers.

Then, the NDV rescue system has been validated using eGFP minigenomes in antisens position to demonstrate the function of the NDV ribonucleoprotein. These results have been confirmed by extraction of mRNA and RT-qPCR or RT-PCR with specific eGFP primers. The NDV rescue system is now available to rescue the virus from an assembled full genome of NDV. Two genomes have been assembled from eight cloned segments and will be shortly rescued.

Conclusion: Full length genome of NDV MG725_08 strain is now available and the NDV rescue system is available in vitro. The rescue of the LaSota strain will be achieved soon and the corresponding infectious clones characterized in vitro and in vivo on embryonnated SPF chicken eggs. The next step will be to replace the F and HN protein of the LaSota strain (live attenuated vaccine strain) by the corresponding proteins of the MG725-08 strain (the more recent genotype XI with a modification of the F protein to introduce an avirulent cleavage site) and to evaluate the protection and viral shedding in chicken experiments.

A domain located at the amino terminus of the envelope glycoprotein (Gc) of an orthobunyavirus is targeted by neutralizing antibodies

Gleyder Roman-Sosa, Emiliana Brocchi, Horst Schirrmeier, Kerstin Wernike, Christian Schelp, Martin Beer

Institute of Diagnostic Virology, Friedrich-Loeffler-Institut - Südufer 10 17493 Greifswald - Insel Riems - Germany

Objective: The Schmallenberg virus (SBV) is an arthropod-borne virus of the genus *Orthobunyavirus* of the *Bunyaviridae* family. SBV is enveloped and possesses a tripartite genome composed of three single-stranded RNA molecules of negative sense of different sizes, designated as small (S), medium (M) and large (L) respectively. The single ORF of the M segment encodes for the surface proteins, which according to their order in the precursor are referred to as Gn and Gc, and in between the NSm.

The humoral immune response against the orthobunyaviral glycoproteins in the course of a natural infection has not been analyzed so far. Therefore, the present study aimed at identifying the domains of the SBV-glycoproteins targeted by antibodies from naturally and experimentally infected animals as well as monoclonal antibodies (mAbs).

Methods: The fragments encoding for the ectodomains of the glycoproteins Gn and Gc were cloned into an eukaryotic expression vector with a twin-strep tag at the carboxyl terminus. The Gc protein was either expressed as full-length fusion protein with the Gn (*Gn-L-Gc*) or in two truncated forms, including its amino terminal third (*Gc Amino*) or its two carboxyl terminal thirds (*Gc Amino* Δ). The reactivity pattern of bovine and mouse sera from negative or infected animals was analyzed with each antigen in an indirect ELISA. The reactivity of anti-SBV Gc murine monoclonal antibodies was investigated with the three antigens, and the effect of disulfide bond-reduction on the antibody-antigen interaction was tested.