Part Five

DISCUSSION

CHAPTER VIII

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The continued need for new and improved rabies vaccines is fuelled by several factors. From a point of practicality, the development of more cost effective vaccines, and other rabies biologics, is of great importance. The expense of purified cell culture vaccines are exorbitant and largely influence the general availability and use thereof in poorer countries. Reported vaccine failures are in many cases ascribed to incomplete regimens, which may be attributed to a lack of availability of a biologic and/or the expense of the treatment. It is also appreciated that strategies that target the reservoirs of the virus, rather than the treatment of dead end hosts, are a far more effective approach. Effective oral vaccination campaigns in several countries around the world, involving different wildlife species, have shown this. This type of strategy would alleviate the need for PEP, which holds substantial financial implications. Oral vaccination also presents the possibility of immunizing animals, such as bats, that would not be approachable by convential means. Therefore, the requirement for safe and effective oral rabies vaccines also remains an urgent point of investigation. Despite the successful use of oral vaccines such as V-RG and SAG2, in oral vaccination programs, there remains room for improvement of these vaccines. For example, the use of V-RG is hampered by the recognition of potential safety issues. On the other hand, despite the efficacy of SAG2, it lacks stability and requires multiple doses of the vaccine in order to induce long lasting protective immune responses through the oral route. Another valid objective is the development of vaccines with improved immunogenicity. Currently, multiple doses of vaccine are required to attain and maintain a protective level of immune response. Vaccines that may provide protective responses upon single administrations would be ideal. Some studies are now also focusing on expanding the immune repertoire elicited by vaccines in an attempt to enhance the immunogenicity thereof. Together with this point the lack of cross- protection of current rabies biologics with all the members of the lyssavirus group is also considered. Although it is not the intention to inflate the public

health burden of the non-rabies lyssaviruses, Mokola, Lagos Bat and West Caucasian bat viruses, it should nevertheless not be underestimated.

Attempts have been made to address these points in numerous vaccine studies. The development of recombinant subunit vaccines investigates the validity of various vaccine delivery systems for the appropriate presentation of rabies virus antigens to the immune system. Most prominently viral vaccine vectors, such as adeno- and different pox viruses (particularly vaccinia viruses), have been investigated as carriers for rabies antigens. Recombinant adenoviruses have proved safe and efficacious in various models and through various routes including the oral route. Possible stumbling blocks in the use of adenoviruses as vaccine vectors are the possibility of preexisting immunity against the vector, which is a highly immunogenic entity. Such a phenomenon could adversely affect the efficacy of such a vaccine. On the account of recombinant vaccinia viruses which has proven efficacy through its commercial use in several countries, safety issues offset its further implementation. A major movement in the development of not only rabies vaccines, but also vaccines for many other diseases, is the use of reverse genetics technology. This technology allows for the development of modified and attenuated vaccine viruses with novel properties. These are often developed through the generation of deletion mutants of rabies viruses. The antigenicity of strains has also been improved by the expression of multiple glycoprotein genes. With this technology the use of attenuated rabies viruses as vaccine carriers for other disease antigens, possibly for the development of dual vaccines, are also explored.

In this study the use of poxvirus based vectors are used to investigate several aspects of rabies vaccinology. Firstly the use of severely attenuated poxviruses, particulary in aspects of host range and replication efficiency, was explored as carriers for the major rabies antigen, the glycoprotein. Two candidate replication deficient poxvirus vectors, LSDV-SA and MVA, as recombinant rabies vaccines and particularly as oral vaccines, were examined here. These vectors are considered safer options, than for example the replication competent vaccinia viruses, since infection of non-permissive hosts are characteristically not productive, and infectious progeny is not produced after the initial infection cycle. Both these vectors have been shown to support the expression of foreign genes, even in non-permissive hosts. A second point of investigation involved

a study of the cross-protection and cross-reactivity of different recombinant vaccinia virus vaccines against rabies virus and lyssaviruses belonging to the so-called phylogroup II. Vaccinia viruses have time and again been shown to be effective carriers of rabies virus antigens. This feature is used in a proof-of-concept experiment to investigate the value of recombinant vaccines expressing different full-length lyssavirus glycoprotein genes.

To facilitate the development of vaccines based on the LSDV-SA strain of LSDV, a study was made towards the construction and convenient isolation of recombinant LSDV-SA viruses. The efficacy of different protocols and treatments for the isolation of homogenous recombinant vaccine viruses were investigated. It was suggested that a PCR screening step be included in the purification protocol of such viruses. Such a step would enable the worker to choose more homogenous virus samples that could be used to isolate pure samples through simple plaque purification protocols more readily. The efficacy of recombinant LSDV-SA expressing a rabies virus glycoprotein was also evaluated in a mouse model. This work was considered of importance for two reasons. Firstly the development of a small animal model that could be used in the testing of such recombinants would provide a practical and essential tool in the development of these vaccines. Secondly, the potential of using such a construct as a replication deficient vector was investigated. The lack of sufficient humoral responses elicited by these constructs are likely attributed to the lack of circulating antigens, which skews the immune responses to that of a Th1 type. Therefore little evidence is available to support the idea for the development of LSDV-SA as a non-replicative vaccine vehicle at this time. The value of using LSDV-SA as a vaccine carrier for the development of dual vaccines are however apparent and should remain a focus of investigation.

Recombinant MVA expressing a rabies virus glycoprotein was generated and tested in different animal models. The recombinant vaccine proved efficacious through the intramuscular route in mice. The efficacy of the vaccine was dosage dependant though, and higher doses of recombinant MVA was required to elicit responses equivalent to those obtained with recombinant Western Reserve strain and Copenhagen viruses. In addition, recombinant MVA elicited measurable anamnestic responses in previously vaccinated dogs and raccoons. This experiment once again highlighted the

capacity of recombinant MVA to elicit exceptional booster responses in prime-boost regimens. The vaccine, however, failed to induce humoral responses in mice upon oral administration, and also failed to boost immune responses in dogs and raccoons when administered *per os*. Since it is known that these animals are susceptible to oral inoculation with recombinant vaccinia virus, this observation can most likely be ascribed to the severe attenuation of this virus opposed to its replication competent counterparts. The further investigation of recombinant MVA as rabies vaccine may be validated in scenarios of preexisting immunity to vaccinia vectors and in booster regimens used in conjunction with other vaccines.

In summary, the study addressed valid questions on the immunogenicity of candidate recombinant rabies vaccines based on a newer generation of highly attenuated poxviruses. Although the usefulness of recombinant LSDV-SA as a replication deficient vaccine is not supported by the evidence produced here; the application of this system for the development of dual vaccines for ruminants is still appreciated. In theory the characteristics of the severely attenuated MVA seem to ideally fit the requirements of a recombinant rabies vaccine carrier. This may still ring true in some instances, particularly if applied in prime-boost regimens or considered as a booster vaccine in the presence of preexisting immunity to V-RG. The use of recombinant MVA as an oral vaccine is however not substantiated by the study done here. The balance between vaccine efficacy and the potency and attenuation of the vaccine carrier appears to be a fine one. The investigation of attenuated poxviruses as vaccine carriers is however not deterred. The potential advantages of such a vaccine still warrant the investigation of other poxviruses for this use. As an example, the effective use of a recombinant raccoonpoxvirus expressing rabies glycoprotein have been shown before and the use of such vaccines could be further explored. The comparison of such vaccines with the modified rabies virus strains produced through reverse genetics should however be investigated. The potential advantages of including a whole virus in a vaccine, opposed to only using particular antigens in subunit vaccines are recognized.

The enhancement of cross-protection capacity of rabies vaccines remains a relevant but less-studied topic in the field of rabies vaccinology. As a second facet of the study, the cross-protective and cross-reactive responses elicited by recombinant vaccinia

viruses expressing rabies, Mokola or West Caucasian bat virus glycoprotein genes either in single or in dual combinations were investigated. The efficacy of rabies glycoprotein vaccinia recombinants were once again illustrated, and highlighted the valuable use of recombinant vaccinia viruses in proof-of-concept studies. The lack of cross-reaction between rabies and the two non-rabies lyssaviruses involved were once again apparent. The divergent nature of these viruses is reiterated. Most notably there was no cross-reactions between Mokola and West Caucasian bat virus vaccines, even though these two viruses appear to group together in the two phylogroup division of lyssaviruses. Mokola vaccines induced responses that neutralized Lagos Bat viruses. The evidence therefore suggest that a recombinant vaccine expressing rabies and Mokola virus glycoprotein is most likely to protect against the spectrum of lyssaviruses, but excluding the highly divergent West Caucasian bat virus. This study once again motivates the necessity of continued and improved surveillance of these viruses. The importance of a more comprehensive account of the epidemiology and public health burden of these viruses are appreciated.