MOKOLA VIRUS:
A BRIEF REVIEW OF THE STATUS QUO.

L.H. Nel

1 INTRODUCTION.

Mokola virus is the genotype 3 member of the Genus Lyssavirus within the Family Rhabdoviridae of which all members, whether fish, animal or plant virus, share the same very characteristic enveloped bullet-shape morphology. Like rabies virus, the prototype lyssavirus, Mokola virus causes an acute encephalomyelitic disease. Whereas rabies is probably the most important and definitive viral zoonosis worldwide, Mokola virus infection has to date been reported from the African continent only (Nel et al., 2000). Two other lyssaviruses, Duvenhage and Lagos Bat virus (genotypes 4 and 2, respectively) also seems to be unique to the African continent (Swanepoel et al., 1993). The remaining lyssavirus genotypes appear to be exclusive to Eurasia (genotypes 5, 6; European Bat Lyssaviruses) and to Australia (genotype 7; Australian Bat Lyssavirus, Gould et al., 1998).

2 HISTORY OF MOKOLA VIRUS ISOLATIONS.

Very little is known about the epidemiology of the African rabies-related viruses. The isolation of Mokola virus has been reported rather haphazardly from only a small number of African countries where appropriate investigations have been undertaken (Figure 1). Mokola is the only lyssavirus never isolated from bats, but it has been found in a surprisingly diverse host range, considering the small number of virus isolates. The first isolation of the virus was already made in 1968, from organ pools of shrews in Nigeria (Kemp et al., 1972). Thereafter the virus was isolated from man (Nigeria – 1969/71 – Familusi and Moore, 1972; Familusi et al., 1972), again from shrews (Cameroon, 1974 – Le Gonidec et al., 1978), from a rodent (Central African Republic, 1983 – Saluzzo et al., 1984), from domestic cats and a dog (Zimbabwe, 1981/82/93 – Foggin, 1983; Bingham et al., 2001) and again in 1989 from a cat in Ethiopia (Mebatsion et al., 1992). All isolations of Mokola in South Africa were made from domestic cats; first in 1970 (described in Schneider et al., 1985), with the next isolates only made many years later, from 1995 to 1998 (Von Teichman et al., 1998; Nel et al., 2000).

3 MANIFESTATION OF DISEASE.

Disease caused by Mokola virus-infection is rabies-like in clinical manifestation. In fact, it is likely to be the good rabies surveillance programme (and improved lyssavirus diagnostics) that led to the recent isolations of Mokola in South Africa, which were all made from domestic cats with suspicious rabies-like symptoms. In the two human cases reported, both young girls, the symptoms were fever and convulsion (with full recovery) in one case and drowsiness, paralysis and terminal coma in the second case (Familusi and Moore, 1972; Familusi et al., 1972). One distinction between typical rabies infection and Mokola infection in domestic cats, seem to be the lack of unprovoked aggression in the latter. However, as with rabies, unusual behaviour, neurological disturbance, hypersensitivity, dehydration and salivation have been most commonly reported in the cases of Mokola infection of domestic animals (von Teichman et al., 1998).

1 Department of Microbiology - University of Pretoria - 0001 Pretoria - SOUTH AFRICA
4 Epidemiology.

Of all the lyssavirus genotypes, Mokola is genetically most distant from rabies, as demonstrated with serological studies (King and Crick, 1988) and through analyses of specific genomic nucleotide sequences (Bourhy et al., 1993). When Mokola virus isolates from southern Africa were analysed genetically, they were found to display a phylogenetic clustering arrangement which were in perfect agreement with their geographical sites of isolation, with one of the clusters composed of viruses which were isolated over a time period of 28 years (Nel et al., 2000; Figures 2 and 3). This striking geographical influence over Mokola virus evolution is in agreement with observations on the molecular epidemiology of classical rabies viruses associated with wildlife in southern Africa and elsewhere (Unpublished data; Nadin-Davis et al., 1999).

A phylogenetic analysis of the full-length glycoprotein sequences of Mokola virus and comparison with the glycoproteins of a wide range of rabies virus isolates indicates a comparable degree of variation within the two Genotypes (not shown). The significance of the finding lies in the fact that the genetic variation among only four isolates of Mokola (3 from southern Africa and one from Ethiopia) more or less equals the variation found among the most diverse classical rabies virus isolates from various host species throughout the world.
Figure 2: Geographical locations and numbers of Mokola virus isolates from southern Africa.

Mokola virus isolation sites in southern Africa

Zimbabwe
Harare, Zim (7 Isolates)

South Africa
Kwazulu/Natal, SA (4 isolates)
East London, SA (3 isolates)

Figure 3: Phylogenetic reconstruction indicating the relationship of rabies viruses, a Mokola isolate from Zimbabwe (Zim 82), seven South African isolates of the Mokola virus and an isolate of Lagos bat virus (see Figure 2 for geographical locations). The rabies virus genotype is represented by a group of viruses isolated from Herpestid species in South Africa. The inferred phylogeny is based on sequence alignment of the N1-N2 nucleoprotein gene sequences as previously described (Nel et al., 2000).

Phylogram based on partial nucleoprotein sequence

Viverrid rabies virus isolates
For lyssaviruses, the trimeric transmembrane glycoprotein (G) induces neutralizing antibodies and a large variety of rabies vaccines based on the delivery of this antigen confer protection against challenge with rabies virus (e.g. Perrin et al., 1985). In a comparison of the most important known antigenic domains of the G proteins of six Mokola viruses with rabies, several important dissimilarities were found. One of these was found in the antigenic domain III (Figure 4). It is well known that the context of this domain and the Arg333 specifically, determines the integrity of this antigenic site and the ability of rabies virus to produce lethal infection in mice (Dietzschold et al., 1983). Variations within this domain thus affect antigenicity as well as pathogenicity. In the case of rabies virus the important sites around Arg333 are all occupied by neutral amino acids (Figure 4). However, for all five Mokola viruses analysed, this domain differs significantly with that of rabies – with aspartic acid occupying position 333 and with two basic amino acids and one aliphatic amino acid in the important surrounding sites (highlighted in Figure 4). It is thus no surprise that rabies vaccines will not protect against Mokola infection in studies with mice and it has also been shown that rabies hyper-immune human sera very poorly cross-neutralizes Mokola virus (CDC, unpublished; Bahloul et al., 1998; Nel et al., submitted).

Failure of rabies vaccination to protect against Mokola has also been demonstrated with a dog in Zimbabwe (Foggin, 1983) and by the most recent cases of the disease in cats in South Africa - most of these animals were in fact vaccinated against rabies, as required by law for domestic animals in this part of South Africa (Von Teichman et al., 1998).

Figure 4: Dissimilarities in the glycoprotein antigenic domain III of Mokola virus isolates and rabies virus. Details are described in the text.

<table>
<thead>
<tr>
<th>Major antigenic site III</th>
<th>aa 330-338</th>
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<tbody>
<tr>
<td>mokola SA1</td>
<td>KRVDRWAD</td>
</tr>
<tr>
<td>mokola SA3</td>
<td>KRVDKWAD</td>
</tr>
<tr>
<td>mokola SA7</td>
<td>KRVDRWAD</td>
</tr>
<tr>
<td>mokola Zimbabwe</td>
<td>KRVDKWAD</td>
</tr>
<tr>
<td>mokola Ethiopia</td>
<td>KRVDRWAD</td>
</tr>
<tr>
<td>rabies (SAD)</td>
<td>KSVRTWNE</td>
</tr>
</tbody>
</table>

A second antigen that may also contribute to immunity against lyssaviruses, is the nucleoprotein (N), which tightly enfolds the viral RNA in a nucleocapsid structure. Although rabies N does not elicit neutralizing antibodies, it has been found to induce protective immunity under circumstances where vaccinated animals are challenged peripherally (Dietzschold et al., 1988; Dietzschold et al., 1989).

Several individual research groups have experimented with the use of DNA vaccines for protection against rabies (eg. Xiang et al., 1994; Bahloul et al., 1998; Osorio et al., 1999). Experimentation towards cross-reactive DNA vaccines, including the generation of chimeric lyssavirus glycoproteins (G) for rabies, Mokola and European bat lyssaviruses have also been described (Bahloul et al., 1998; Jallet et al., 1999). We have cloned the glycoprotein and nucleoprotein (N) genes from South African
Mokola viruses, and used these in the construction of different DNA vaccines for immunization against Mokola virus (Nel et. al, submitted).

Current thinking is that DNA vaccines may be most useful in combination with other vaccines (Williamson et al., 2000). Priming with a DNA vaccine prior to boosting with a live recombinant vector may significantly enhance the cellular immune responses to the recombinant protein; as reported for diseases such as malaria (DNA vaccination followed by boosting with modified Vaccinia Ankara (MVA)-recombinant, Schneider et al., 1998) and with Human Immunodeficiency Virus (HIV)-infection (Kent et al., 1998). Expressing only the gene of interest may serve to focus the immune response on these specific proteins. As a model for the utility of DNA vaccine/live recombinant vaccine prime/boost strategy, we have investigated whether enhanced cross-protection may be achieved with DNA/recombinant lyssavirus vaccine combinations. To date we have been unable to demonstrate any stimulation of a cross-reactive immune response using a variety of such combinations (unpublished), but cross-reactive protection in mice have been achieved with vectors which express chimeric lyssavirus genes (Bahloul et al., 1998; Jallet et al., 1999).

6 POSTEXPOSURE TREATMENT.

For rabies, the establishment of an effective post exposure vaccination regimen has been immensely useful in protecting against the disease. It may be assumed that the same would be true for Mokola virus, provided that dedicated biologicals are developed for such application. Conjectured from our experience with pre-exposure vaccination studies, it is likely that rabies-specific post exposure biologicals will not be effective against Mokola infection. Unfortunately, the DNA vaccines for Mokola so far developed may be at a disadvantage in post exposure application, due to the relatively slow humoral antibody responses elicited by these types of vaccines under conventional administration.

7 CONCLUSION.

Mokola virus has twice been isolated from humans and once from a dog, but most isolations to date have been from rabid cats (15 isolates) or small mammals (shrews – 5 isolates, rodent – 1 isolate). The central role of domestic cats along with small mammal species in the epidemiological evidence to our disposal allures speculation that small mammals, likely prey species for cats, serve as reservoir species for Mokola virus. Strengthening an argument for the likelihood of a bat reservoir for Mokola, is the fact that bats play an important or exclusive role in the epidemiological cycles of all the other lyssaviruses. Nevertheless, a recent survey (using the fluorescent antibody assay with a broad-spectrum conjugate) of 315 bats and 133 small mammals collected throughout South Africa, failed to indicate the presence of lyssavirus-specific antigens (W Hechter, unpublished results).

Mokola virus is a potentially dangerous agent. Our poor understanding of the reservoir of Mokola, its apparently underestimated incidence, its likely wide host range, its proven zoonotic potential (Familusi et al., 1972), the failure of protection by rabies vaccines and the absence of any post-exposure treatment regimen are discomforting. At risk of contracting encephalomyelitis from Mokola infection would be veterinarians, laboratory personnel (particularly those associated with rabies diagnostics) and others in regular contact with animals or samples which may expose them to Mokola virus.

However, to date the African rabies-related viruses like Mokola have mostly received attention because of scientific curiosity and interest in peculiar aspects of their epidemiology and pathogenicity (Von Teichman et al., 1998; Nel, et al., 2000; Foggin, 1988; CDC unpublished) or as models in molecular virology, for example in chimeric virus construction and study of defective interfering particles (Mebatsion et al., 1995) or in studies of immunogenicity and in studying vaccine development (Nel et al., submitted; Bahloul et al., 1998). Among the lyssaviruses of Africa, with consideration of the abundance of rabies virus and the very serious problems associated with classical rabies in wildlife, domestic animals and man throughout our continent, the rabies-related viruses will probably have to remain, for the time being, mostly “of interest”.

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ACKNOWLEDGEMENTS.

I would like to acknowledge and thank the following individuals and organizations whose crucial contributions are reflected in the information presented here:

APHL, John Bingham, George Bishop, CDC rabies section, Willem Hechter, Arthur King, Courtney Meredith, Chuck Rupprecht, Beate von Teichman and Alex Wandeler.

REFERENCES.


