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Monoclonal antibody studies on rabies-related viruses

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ABSTRACT

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Rabies and rabies-related viruses are divided into four serotypes, although it has been suggested that the inclusion of European bat lyssaviruses results in six genotypes. Sixty-four rabies-related viruses were tested against a panel of 36 anti-nucleocapsid monoclonal antibodies prepared from the immunization of Balb/$_c$ mice with five prototypic rabies-related viruses. Reaction patterns obtained confirmed the original distinction between serotype 1–4 viruses and revealed multiple variants of Lagos bat and Mokola viruses. In addition, two biotypes of European bat lyssavirus were identified and a clear distinction was shown between these biotypes and Duvenhage virus of Africa. The origins and importance of the rabies-related viruses are discussed.

INTRODUCTION

Taxonomically, rabies viruses belong to the order Mononegavirales, family Rhabdoviridae (Pringle 1991). The Rhabdoviridae are characterized by a negative-sense genome of single stranded RNA and the family is divided into two genera, *Vesiculovirus*, (which includes the viruses causing vesicular stomatitis and antigenically related viruses) and *Lyssavirus*, which includes rabies and rabies-related viruses (Calisher, Karabatsos, Zeller, Digoutte, Tesh, Shope, Travassos & St George 1989). The term "rabies-related viruses" was first used by Shope and his colleagues (Shope, Murphy, Harrison, Causey, Kemp, Simpson & Moore 1970) when they demonstrated that Lagos bat virus was related to rabies virus and that the degree of cross-reactivity among rabies, Lagos bat and Mokola viruses was sufficient to substantiate a distinctive sero-grouping within the Rhabdoviridae.

The genomic RNA of the rabies infectious particle contains five genes, each of which codes for a structural protein of the virion. The G (glycoprotein) and N (nucleoprotein) proteins are the most extensively studied of these proteins. The ectodomain G protein region binds neutralizing antibody and thus allows serotype definition. Currently, four serotypes are recognized on the basis of cross-neutralization tests in animals and the European bat lyssaviruses (EBL1 and EBL2) are ungrouped, but it is suggested that the *Lyssavirus* genus is composed of six genotypes (see Bourhy, Kissi & Tordo 1993). The amino acid sequences of the N proteins display a high degree of homology (Wunner 1991) and thus differences that are observed between anti-nucleoprotein Mab (Mab-N) reaction patterns may facilitate epidemiological studies.

Mab-N reaction pattern analyses of serotype 1 terrestrial animal isolates from an endemic area confirm surveillance observations of "compartmentation" (Bisseru 1972) of the disease in one major host species with occasional "spill-over" to other species within the same area. However, with the exception of the EBL viruses, there are too few rabies-related virus isolates to be able to determine species compartmentation, although "spill-over" of these viruses into domesticated animals and/or man is known to occur.

Most of the information regarding epitopic variation has been gathered from the use of Mabs prepared from fixed rabies viruses. More recently Mab-N

panels have been constructed from the immunization of Balb/$_c$ mice with rabies-related viruses (Bussereau, Vincent, Coudrier & Sureau 1988; Bussereau, Vincent & Sureau 1989; King 1991). These panels demonstrate that there is considerable variation amongst rabies-related viruses, including the disparity between the Duvenhage viruses of Africa and the EBL 1 and 2 viruses of European bats. Mab-N reaction pattern analyses provide a stepping-stone to precise typing by the determination of nucleotide sequences.

MATERIALS AND METHODS

A panel of Mab-Ns was prepared by using five rabies-related viruses as immunogens (King 1991). Briefly, the prototype strains of Mokola, Lagos bat, Duvenhage, Denmark bat and "Finman", an isolate made from a biologist who died in Finland of rabies believed to be of bat origin, were used to immunize Balb/$_c$ mice. Thereafter the usual fusion, cloning and RIPA techniques were employed to prepare and classify the Mab-Ns. Although no attempt was made to determine the antigenic site with which each Mab reacted, a panel of 36 Mab-Ns was constructed by testing them against a wide variety of viruses and selecting those Mab-Ns which were shown to react differently from the others.

The origins of 64 serotype 2–4 and EBL viruses are summarized in Table 1. Most of the known rabies-related viruses of Africa and isolates from European bats were obtained with the generous help of many researchers. One of two techniques was used to obtain the virus isolate Mab-N reaction patterns. Either the viruses were passaged in BHK-21 cells until such time as a sufficient number of positive cells was obtained to seed Greiner 84-well "Terasaki"-type plates, or the viruses were passaged in 3-week-old mice and their infected brains were harvested and used to prepare smears on 12-well Teflon-coated 3" x 1" glass slides. Prior experiments had shown that the two techniques were fully interchangeable.

TABLE 1 Origins of 64 serotype 2–4 and EBL viruses

Laboratory reference	Geographical location	Year isolated	Species	No. tested	Sender's reference
Serotype 2, Lagos bat viruses					
1	Nigeria	1956	*Eidolon helvum*	1	Boulger
3	RSA	1983	Bat	1	Pinetown
143	RSA	1982	Cat	1	839/82
190	RSA	1980	*Ep. wahlbergi*	1	687/80
133	Zimbabwe	1986	Cat	1	16302
43	CAR	1974	*Micropteropus pusilis*	1	Sureau
41	Senegal	1985	*E. helvum*	1	Dakar bat
Serotype 3, Mokola viruses					
4	Nigeria	1986	*Crocidura* sp.	1	Shope
5	RSA	1970	Cat	1	Umhlanga
39	Cameroun	1971	*Crocidura* sp.	1	Le Gonidec
40	CAR	1981	*Lophuromys sikapusi*	1	Saluzzo
174–177	Zimbabwe	1981	Cat	4	Foggin
Serotype 4, Duvenhage (Africa) viruses					
6	RSA	1970	Human	1	Meredith
131	Zimbabwe	1986	Bat	1	RS16
139	RSA	1981	Bat	1	1486/81
European viruses, EBL 1					
154	USSR	1985	Human	1	Yuli
Various	Europe	1968–1989	Bat[a]	41	Various
European viruses, EBL 2					
8	Finland	1985	Human	1	Lumio
29	Holland	1987	*Myotis dasycneme*	1	47072
30	Holland	1987	*M. dasycneme*	1	47129
228	Holland	1989	*M. dasycneme*	1	92666

[a] Most bats were *Eptesicus serotinus* (serotines) from western Europe but three were unidentified species and two bats (UB1 and UB2) were from *Vespertillio murinus* (particoloured) and *Nyctalus notula* (noctule) bats from the Ukraine. EBL = European bat lyssavirus

RESULTS

The distinctions between rabies (CVS 11) and the rabies-related viruses of Africa, recognized by cross-immunization tests, were maintained in the Mab-N analyses (Table 2). In addition the panel recognized multiple variants within two of the African virus groups (Table 3). For example, although isolates of Lagos bat virus were clearly more closely related to each other than they were to isolates of Mokola virus and vice versa, five reaction patterns in the N protein of these viruses were recognized by differential reactivity with Mab-Ns M6, M7, M11, D1, DB1, L18, L1 and L17. Similarly, three reaction patterns of Mokola virus were observed by use of Mabs L15, F2, F5 and F4. In comparison, the three Duvenhage viruses examined were defined by a single reaction pattern with 15 Mab-Ns.

A clear distinction was shown between the reaction patterns of Duvenhage (Africa) and the EBL I and EBL 2 viruses of Europe. Indeed, one (D1) of only three Mab-Ns prepared against Duvenhage virus did not react with the European isolates and conversely only five of twelve Mab-Ns prepared against viruses of European bat origin reacted with Duvenhage virus. In addition, at least two virus biotypes in European bats was demonstrated—only five of seven Mab-Ns prepared against the EBL 1 (Denmark bat) virus reacted with the EBL 2 (Finman) virus and conversely none of five Mab-Ns prepared against this latter virus reacted with the EBL 1 viruses.

Eight Mab-Ns (M11, DB3, DB4, F1, F2, F5, F4 and L25) which recognized epitopes in the EBL 2 prototype virus (Finman, RV 8) also recognized epitopes in the prototype Mokola virus (RV 4). Indeed, four of the Mab-Ns (F1, F2, F5 and F4) defined three reaction patterns within the Mokola virus serotype.

DISCUSSION

But for an upsurge in the number of rabid bats found in Europe, it is possible that the rabies-related viruses would have remained as scientific curios. Whilst it is true that they appear to have little public health significance, it is also true that little or no attempt is made to determine the extent of their existence. For example, although there have been more than 450 rabies positive European bats, in each of the countries affected by bat-rabies the disease came to light when a member of the public was bitten by an infected bat. European bats are considered as protected species and therefore only sick or dead bats are examined; in many countries no survey of sick or dead bats is carried out and some countries no longer examine bats for the presence of rabies unless a member of the public has been bitten.

TABLE 2 Reaction patterns of Mab-N panel with prototype viruses

Serotype virus	Mab-N reference number[a]																		
	M					D			DB							F			
	2	5	6	7	11	1	3	9	1	3	4	9	10	11	14	1	2	3	4	5
1. CVS 11	+								+	+	+			+	+					
2. Lagos bat	+	+	+	+					+	+	+									
3. Mokola	+	+	+	+	+					+	+					+	+		+	+
4. DUV[b] Africa	+					+	+	+	+	+	+			+	+					
EBL 1	+						+	+	+	+	+	+	+	+	+					
EBL 2					+		+	+	+	+	+					+	+	+	+	+

Serotype virus	Mab-N reference number[a]															
	L															
	1	2	3	4	8	11	15	17	18	19	20	23	25	26	27	28
1. CVS 11			+	+								+	+	+		+
2. Lagos bat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3. Mokola		+				+	+				+	+	+	+		+
4. DUV[b] Africa		+	+					+			+			+	+	
EBL 1											+					
EBL 2								+					+			

[a] M2 – 11 = 5 anti-Mokola Mab-Ns; D1 – 9 = 3 anti-Duvenhage Mab-Ns; DB1 – 14 = 7 anti-Denmark bat Mabs-Ns; F1 – 5 = 5 anti-Finman Mab-Ns; L1 – 28 = 16 anti-Lagos bat Mab-Ns

[b] DUV = Duvenhage

+ = positive reaction; o = weak positive reaction; v = variable, weak positive or no reaction; no symbol = no reaction

TABLE 3 Summary of tests with 36 Mab-Ns on 64 rabies-related viruses

Laboratory reference (RV)	Mab-N reference no.[a]																			Virus group
	M					D			DB							F				
	2	5	6	7	11	1	3	9	1	11	14	9	10	3	4	1	2	5	4	3	
1 3, 134, 190 133 43 41	+ + + + +	+ + + + +	+ + + + +	+		+ o			+ + o					+ + + + +	+ + + + +						Lagos bat
4 5, 39 40, 174–177	+ + +	+ + +	+ + +	+ + +	+ + +									+ + +	+ + +	+ + +	+ + +	+ +	+		Mokola
6, 131, 139	+					+	+	+	+	+	+			+	+						Duven-hage
154, various (39) UB1, UB2 8, 29, 20, 228	+ +			v +	 +		+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	 	 	 +	 +	 +	EBL 1 EBL 2

Laboratory reference (RV)	Mab-N reference no.[a]															Virus group
	L															
	18	1	17	3	27	8	19	4	15	25	11	23	28	2	26	20	
1 3, 134, 190 133 43 41	+ + +	+ + +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	Lagos bat
4 5, 39 40, 174–177									+ o	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	Mokola
6, 131, 139			+	+	+									+	+	+	Duven-hage
154, various (39) UB1, UB2 8, 29, 20, 228				 +						 +						+ +	EBL 1 EBL 2

[a] M2 – 11 = 5 anti-Mokola Mab-Ns; D1 – 9 = 3 anti-Duvenhage Mab-Ns; DB1 – 14 = 7 anti-Denmark bat Mabs-Ns: F1 – 5 = 5 anti-Finman Mab-Ns: L1 – 28 = 16 anti-Lagos bat Mab-Ns

[b] DUV = Duvenhage

+ = positive reaction; o = weak positive reaction; v = variable, weak positive or no reaction; no symbol = no reaction

Early reports suggested that the origin of European bat rabies was Duvenhage virus of Africa (although the index case in Europe occurred 16 years before the first isolation of Duvenhage virus). Duvenhage and EBL 1 viruses have both been isolated from insectivorous bats but the disparity between the isolates suggests that their relationship is somewhat more distant than was formerly supposed. Fewer EBL 2 viruses (from the index case and from *Myotis* spp. bats) have been isolated but relatively few of these bats have been examined for rabies. European bat rabies has been reported over a geographical

area ranging from the Ukraine to Spain and it is thus most likely that rabies in European bats occurs far more frequently than is reported.

Lagos bat virus appears to be the cause of rabies in fruit bats, although spill-over into cats and a dog has been reported; it remains the only rabies-related virus not to be associated with death in humans. The reservoir species of Mokola virus, assuming that there is one, has not been determined. The virus has not been isolated from a bat but the results above show that there is an antigenic relationship between the rabies viruses isolated from a man who died of rabies in Finland following a bat bite, bats in Holland (and Switzerland, results not shown) and shrews, cats and a rodent in Africa. Both Lagos bat and Mokola viruses have been isolated over a wide geographical range of Africa—from Senegal in the west to Ethiopia in the east and to South Africa in the south. There are too few isolates from these two virus groups, however, to permit the association of a particular reaction pattern with a particular species. During routine rabies diagnosis in Ethiopia, of 115 isolates Lagos bat virus was recovered from a dog and Mokola virus from a cat (Mebatsion, Cox & Frost 1992). The geographical spread of the rabies-related viruses in Africa suggests that, as in European bats, their occurrence is severely under-reported.

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