Monoclonal Antibodies to Mokola Virus for Identification of Rabies and Rabies-Related Viruses

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Rabies and rabies-related virus strains were studied by using a panel of monoclonal antibodies directed against either nucleocapsid proteins or cell surface antigens of Mokola virus (Mok-3). Each strain was used in parallel to infect cultured cells and mice. Then, the patterns of reactivity of the different monoclonal antibodies were determined by the immunofluorescent-antibody staining procedure. On cells, the monoclonal antibodies differentiated fixed rabies virus strains (serotype 1) from rabies-related virus strains. The seven fixed strains (CVS, PV₄, PM, Flury LEP and HEP, ERA, and SAD) reacted identically. The previous serotype groupings (serotype 2, Lagos-bat virus; serotype 3, Mokola virus; serotype 4, Duvenhage virus) established with anti-rabies monoclonal antibodies were confirmed, except for that of Lagos-bat Kindia, which appeared to be related to the African subtype of the Duvenhage serotype (Duv-2). Within the Mokola (Mok-1, -2, -3, and -5 and Umhlanga) and the Lagos-bat (Lag-1 and -2, Zimbabwe, Pinetown, and Dakar) serotypes, each strain appeared to be distinct. The African subtype of the Duvenhage serotype reacted differently from the European subtype. Within the Duvenhage serotype, subtypes Duv-4, -5, and -6 and Denmark reacted identically, while subtypes Duv-1, -2, and -3 and German Democratic Republic appeared to be distinct. The monoclonal antibodies specific for the cell surface antigens were also used in neutralization tests with all the strains. Two of them neutralized the infectivity of Mokola virus.

Viruses related to rabies were first isolated in Africa. Lagos-bat virus was discovered in 1956 and was isolated from a bat (1). Mokola virus was isolated from insectivores in 1968 (20). Duvenhage virus was isolated in 1971 from a human (11). Other isolates of these three viruses have been obtained since then (Table 1). Duvenhage virus was recently identified in bats from northern Europe (17, 18) and was considered to be responsible for cases of rabies-like disease in humans. In humans contaminated with Mokola and Duvenhage viruses through animal bites, symptoms identical to those of rabies are produced; this is followed by death (5, 6, 11, 19). Intracerebral inoculation of suckling mice with these lyssaviruses is followed by rabieslike encephalitic symptoms and death (14, 26).

In the lyssaviruses (subgroup of rhabdoviruses) (4), four serotypes correspond to rabies and rabies-related viruses; serotype 1 corresponds to rabies virus, serotype 2 corresponds to Lagos-bat virus, serotype 3 corresponds to Mokola virus, and serotype 4 corresponds to Duvenhage virus. Distinction of these serotypes was first obtained by seroneutralization with polyclonal antibodies, complement fixation, and cross-neutralization tests (30). Because of antigenic communities at the level of nucleocapsid proteins, positive immunofluorescent staining of cells infected with rabies-related viruses has been obtained with rabies polyclonal fluorescein-labeled antibodies. Since 1980, a panel of monoclonal antibodies (MAbs) directed against rabies virus nucleocapsid was used by Wiktor and Hattwick (30). This panel allowed the distinction of the four serotypes, and since then each new isolate has been identified from the results that were obtained with this panel of MAbs. Crossed immunization tests in mice revealed that only some antirabies vaccines can induce an effective protection against Duvenhage virus (8) and, to a lesser extent, against Lagos-bat virus

(27). No protection can be obtained, however, against Mokola virus, whatever vaccine is used (27).

Recently, we obtained a panel of MAbs against a Mokola virus strain (29). In the present study, the differentiation of strains in cultured cells was extended to all the recent isolates of these viruses. These strains were either from different species or from different origins.

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MATERIALS AND METHODS

Virus strains. Isolates of the three rabies-related viruses were collected in Africa or Europe (Table 1). Seven fixed strains with three different geographic origins were used (Table 2).

Cells. Cells were maintained in either Eagle modified essential medium (CER and BHK cells) or Dulbecco medium (Vero, Neuro-2a, and CH cells) supplemented with 5% fetal bovine serum (29). Sodium bicarbonate was added to allow cell multiplication in a 5 to 8% CO₂ atmosphere.

Virus multiplication in mice. Suckling OF1 mice were infected with each virus isolate by intracerebral inoculation. Brains were harvested at the terminal stage of the paralytic phase and were homogenized to obtain viral suspensions. A 20% (wt/vol) brain suspension was made in complete culture medium. After centrifugation for 30 min at 1,000 × *g*, the supernatant containing the virus was recovered and used immediately.

Virus multiplication in cell culture. (i) **Cell culture adaptation.** Neuroblastoma cells, which are usually the most sensitive cells for tissue culture propagation of street rabies viruses, were used for the first attempt at cell culture adaptation of the different virus isolates to the cell cultures. Alternatively, whenever growth could not be detected in Neuro-2a cells, the cell lines mentioned above were also used.

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TABLE 1. Rabies-related virus isolates

Virus ^a	Source of virus isolate			
	Country	Genus and species	Yr reported	Reference or source
Lagos-bat virus (serotype 2)				
Lag-1	Nigeria	<i>Eidolon helvum</i>	1956	1
Lag-2	Central African Republic	<i>Micropteropus pusillus</i>	1974	20, 22, 25
Lag-Pin	Pinetown, Republic of South Africa	<i>Epomophorus wahlbergi</i>	1980	3, 12
Lag-Dak	Dakar, Senegal	<i>Eidolon helvum</i>	1985	Lafon and Sureau, in press ^b
Lag-Kin	Kindia, Guinea	<i>Nycteris gambiensis</i>	1985	J. P. Digoutte
Lag-ZIM	Zimbabwe	Domestic cat	1986	C. M. Foggin
Mokola virus (serotype 3)				
Mok-1	Nigeria	<i>Crocidura</i> sp.	1968	20
Mok-2	Cameroon	<i>Crocidura occidentalis</i>	1974	9
Mok-3	Central African Republic	<i>Lophuromys sikapusi</i>	1981	16
Mok-5	Zimbabwe	Domestic dog	1981	7, 31
Mok-Umh	Republic of South Africa	Domestic cat	1980	19
Duvenhage virus (serotype 4)				
Duv-1	Republic of South Africa	Human brain	1971	11
Duv-2	Republic of South Africa	<i>Miniopterus</i> spp.	1981	28
Duv-3	Hamburg, Stade, and	Bats of unidentified species	1968	19
Duv-4	Bremerhaven, Federal Republic	Bats of unidentified species	1970	19
Duv-5	of Germany	Bats of unidentified species	1982	19
Duv-6	Poland	<i>Eptesicus serotinus</i>	1985	Lafon and Sureau, in press
Duv-DEN	Denmark	<i>Eptesicus serotinus</i>	1985	13
Duv-DDR	German Democratic Republic	<i>Eptesicus serotinus</i>	1986	H. Sinnecker

^a The virus strains belonged to the rabies unit virus collection, except for three of them. Lag-Pin corresponded to the original isolate 640/80; the virus was passaged on BHK-21; RV2 (Onderstepoort) and RV3 (NIV) were used after the fourth passage. Mok-Umh had two origins: the original isolate was 700/70; the virus was passaged on BHK-21 cells; RV5 was used after the third passage. Duv-DDR was received as a second passage in mouse brain.

^b M. Lafon and P. Sureau, in E. Kurstak and P. Thongcharoen, ed., *Impact of Viral Diseases on the Development of Asian Countries*, in press.

A 20% (wt/vol) suspension of virus-infected mouse brain in medium supplemented with fetal bovine serum (10%) was allowed to infect cells in suspension (10^6 cells per ml). After 1 h of contact at 37°C, the cells were dispensed in parallel in one 25-cm² flask and in one 60-well Terasaki plate ($10 \mu\text{l}$ per well, 10^5 cells per ml). The acetone-fixed Terasaki plate was stained with a polyclonal antinucleocapsid-antirabies conjugate (Diagnostics Pasteur) after 2, 3, or 5 days postinfection and served as the control for viral growth. When most of the cells in each well were found to be infected, the supernatant in the 25-cm² flask was harvested and stored at -80°C. When, after 3 to 5 days of incubation, only a small proportion of the cells in the Terasaki plate were found to be infected, the cells in the 25-cm² flask were trypsinized, mixed with uninfected freshly dispersed cells (1:1), and dispensed again in one 25-cm² flask and one Terasaki plate. The supernatant of the 25-cm² flask was harvested only when 100%, or nearly 100%, infected cells were obtained in the corresponding Terasaki plate after sequential passages.

(ii) **Virus passages.** Subsequent multiplications of virus were made and controlled as described above on CER, Vero, CH, and BHK cells.

(iii) **Preparation of Terasaki plates.** As the reactivity of the different MAbs was tested on acetone-fixed Terasaki plates with a given cell line and a given passage of each virus, homogeneous batches of plates were prepared. The cell concentration was adjusted so that a nonconfluent monolayer of well-spread infected cells could be obtained. Depending on the virus, the plates were incubated for 2 to 5 days at 37°C. Then, the cells were acetone fixed and used immediately or stored at -20°C (29).

MAbs. A panel of MAbs directed against Mokola (Mok-3) nucleocapsid proteins (nucleocapsid antigens [NCA]) and cell surface antigens (CSA) was used according to published procedures (29).

Immunofluorescent-antibody staining. Anti-rabies nucleocapsid polyclonal fluorescein isothiocyanate-labeled rabbit immunoglobulins (Diagnostics Pasteur) were used to control the presence of virus.

On cells in culture, an indirect immunofluorescence procedure was performed as described previously (29).

Virus neutralization. The protocol described previously (29) was used for neutralization tests.

RESULTS

Virus multiplication in cell culture. Rabies-related viruses could be adapted to one or more of the following cell lines that were used: mouse neuroblastoma cells (Neuro-2a), hamster cells (CH, BHK, and CER), and monkey cells (Vero) (Table 2). Most of the rabies-related virus strains were not cytopathogenic, and infected cells were still viable 15 days postinfection (29). These infected cells were easily trypsinized and then multiplied as monolayers. Five strains (Mok-5, Mok-Umh, Lag-Pin, Lag-Dak, and Lag-Kin) induced a cytopathic effect; at 3 days postinfection detached cells were found in the supernatant. The cells that were still attached to the substrate were not able to multiply.

All the cells that were infected with the fixed strains of rabies virus showed the same cytopathic effect, as described previously for the challenge virus strain (CVS). At 3 days postinfection detached cells were found in the supernatant. Cells attached on the substrate were not able to multiply (2).

TABLE 2. Reaction patterns of lyssavirus strains with NCA MAbs

Reaction group ^a	Reaction pattern ^b														Rabies ^c
	Mokola					Lagos-bat					Duvenhage				
	1	2	3	5	Umh	1	2, Pin	ZIM	Dak	Kin	1	2	3, 4, 5, 6, DEN	DDR	
1A	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
1B	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—
1C	—	—	+	+	+	—	—	—	—	—	—	—	—	—	—
2	—	+	+	+	+	—	—	—	—	—	—	—	—	—	—
3	—	—	+	—	—	—	+	+	—	—	—	—	—	—	—
4A	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
4B	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—
4C	+	+	+	+	+	—	—	—	+	—	—	—	—	—	—
5	+	+	+	+	+	—	—	—	—	—	—	—	+	+	—
6	+	+	+	+	+	—	+	+	+	—	—	—	—	—	—
7	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—
9	+	+	+	+	+	+	+	+	—	—	—	—	—	—	+
10A	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+
10B	+	+	+	+	+	+	+	—	+	+	—	+	+	—	+
10C	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

^a The MAbs were previously included in different groups (29). As different strains were characterized, the following subgroups were obtained: 1A, M18, 4-4, 24-3, 68-10, and 80-4; 1B, 153-8 and 153-12; 1C, 18-4; 2, M15 and 69-9; 3, 4-7; 4A, 131-8; 4B, 43-7, 43-8, and 183-14; 4C, 17-3; 5, 111-14; 6, M2, M7, and 71-5; 7, 51-2, 51-9, 62-1, 98-1, and 190-3; 8, 26-9; 9, 11-1, 44-1, 76-6, 121-6, and 184-14; 10A, 23-10, 28-4, and 28-5; 10B, M27; 10C, M28 and M30; and 11, 9-4, 22-3, 31-6, 59-1, 105-20, 128-19, 181-6, and 185-10.

^b Continuous cell lines from the following different origins were used: monkey (African green monkey kidney; Vero), mouse (Neuro-2a), and hamster (CH, BHK, and chicken embryo related [CER]). Fluorescent-antibody staining was observed on BHK cells for Mok-Umh and Lag-Pin; on Vero cells for Mok-1, Mok-2, Mok-3, Lag-ZIM, Duv-1, Duv-2, Duv-3, Duv-4, Duv-5, and Duv-DEN; on Vero and Neuro-2a cells for Duv-DDR; on Vero and CH cells for Lag-1, Lag-2, and Lag-Dak; on Vero and CER cells for Lag-Kin and Duv-6; on Vero, CER, and BHK cells for the fixed rabies virus strains; and on CER cells for Mok-5. The positive and negative immunoreactivities of hybridoma cell supernatants and ascites fluids are indicated.

^c The fixed strains of rabies virus correspond to the challenge virus strain (CVS) (2), the Pasteur strain (PV₄), the Pitman Moore strain (PM), two Flury strains (LEP and HEP), ERA, and SAD.

Reactivity pattern of anti-Mok-3 MAbs on infected cells. (i) Homologous virus: Mokola. All the immunofluorescence tests were performed at least 10 times at different dilutions. The same pattern of reactivity was obtained each time. As described above, all 60 MAbs gave a bright fluorescence; 45 of them produced only an intracytoplasmic fluorescence corresponding to an NCA (Fig. 1A), whereas 15 gave an intracellular plus a cell surface fluorescence pattern corresponding to those of the CSA (Fig. 1B). The same results were obtained with either the hybridoma cell supernatant or the corresponding ascites fluid (29).

(ii) Serotype 1: rabies. The fixed strains (CVS, PV₄, PM [Pitman Moore strain], Flury LEP and HEP, ERA, and SAD) gave a positive reaction with 19 NCA MAbs (Table 2) and 3 CSA MAbs (Table 3), but they reacted identically. These MAbs also cross-reacted with the rabies-related virus strains. The rabies virus fixed strains were distinguished from rabies-related viruses by comparison with NCA MAbs from groups 8 and 11. It must be noted that when an MAb was positive in tissue culture with any rabies or rabies-related virus strain, it always reacted at the same titer as it did with the homologous strain (29). No neutralizing capacity was found among the CSA MAbs.

(iii) Serotype 2: Lagos-bat. Thirty NCA MAbs recognized the Lagos-bat strains (Lag-Kin excluded), but all of them cross-reacted with Mokola virus. Among them, 15 and 19 MAbs also cross-reacted with Duvenhage and rabies virus strains, respectively (Table 2). NCA MAbs from group 4C cross-reacted only with Lag-Dak virus. A comparison between group 4C and groups 3 and 6 allowed us to differentiate Lag-1 from Lag-2 and Lag-Pin. Lag-2 and Lag-Pin reacted identically. A comparison between groups 3 and 10B allowed us to differentiate Lag-ZIM.

The four strains could be identified by 12 CSA MAbs, which also recognized all the strains, including rabies virus (Table 3), but none of the CSA MAbs had a neutralizing capacity. The strains were distinguishable by comparison between groups 4 and 5A for Lag-1 and Lag-Dak and between groups 5B and 5C for Lag-Pin. Strains Lag-2 and Lag-ZIM reacted identically.

Comparison between the two types of MAbs allowed use to identify all five strains. Strain Lag-Kin, however, showed different reactivity patterns with NCA MAbs compared with the other strains and was more related to the Duvenhage serotype (Duv-2).

(iv) Serotype 3: Mokola. Five NCA MAbs (group 1A) were specific for Mok-3 (Table 2). The other strains were distinguished by comparison between groups 1A and 1B for Mok-Umh, group 1C for Mok-5, group 2 for Mok-2, and group 4A for Mok-1. Thirty-three NCA MAbs (group 4B to group 11) cross-reacted with all five strains of Mokola virus. Thirty-one NCA MAbs that recognized Mokola virus also gave a positive staining reaction with the other lyssaviruses.

As a possible confirmation test, CSA MAb groups 1, 2, and 3 were used to identify Mok-1, -2, and -3 (Table 3). The other CSA MAbs gave positive fluorescent-antibody staining with the other lyssaviruses. In a previous report (29) it was shown that only CSA MAb group 2 (at a 1/40,000 dilution) was able to neutralize Mok-1, -2, and -3. In the present study, Mok-5 was neutralized by CSA MAb group 1 (1/20,000 dilution) and Mok-Umh was neutralized by CSA MAbs of group 2 (1/320 dilution) and group 1 (1/1,280 dilution).

(v) Serotype 4: Duvenhage. The Duvenhage strains were identified by 16 NCA MAbs (Table 2). These 16 MAbs also cross-reacted with Mokola virus, 15 of them cross-reacted

FIG. 1. Localization of viral antigens. Immunofluorescence staining of acetone-fixed cells (A and B) or mouse brain impressions (C and D) infected with a rabies-related virus by MAb directed against the NCA (A, C, and D) or the CSA (B). The same type of fluorescence was obtained, regardless of virus: Mokola, Lagos-bat, Duvenhage, or rabies. Magnifications: $\times 50$ (panels A, C, and D); $\times 150$ (panel B).

with Lagos-bat virus, and 14 cross-reacted with rabies virus. Group 5 or 10A allowed differentiation between African (Duv-1 and -2) and European (Duv-3 to Duv-DDR) strains, compared with group 8 or 11. Group 10B differentiated the two African strains and differentiated Duv-DDR from the other European strains.

No CSA MAb had any neutralizing activity on Duvenhage virus, but all the strains could be identified by five CSA MAbs that also recognized all the lyssavirus strains, including rabies virus (Table 3). A comparison between groups 6 and 7 differentiated Duv-3 from the other European strains.

DISCUSSION

Different groups of investigators have used rabies virus MAbs against rabies-related viruses. Differences in reactivity patterns were observed when antinucleocapsid antibodies were used. The results of all the studies have led to the following classification of the rabies group of the *Rhabdoviridae* family, genus *Lyssavirus*. Serotype 1 includes field and fixed strains of rabies virus; serotypes 2, 3, and 4 are commonly referred to as rabies-related viruses and correspond to Lagos-bat, Mokola, and Duvenhage viruses, re-

TABLE 3. Reaction patterns of lyssavirus strains with CSA MAbs

Reaction group ^a	Reaction pattern ^b													Rabies ^c
	Mokola					Lagos-bat					Duvenhage			
	1	2	3	5	Umh	1	2, ZIM	Pin	Dak	Kin	1, 2	3	4, 5, 6, DEN, DDR	
1	—	—	+	—	—	—	—	—	—	—	—	—	—	—
2	—	+	+	—	—	—	—	—	—	—	—	—	—	—
3	+	+	+	—	—	—	—	—	—	—	—	—	—	—
4	+	+	+	—	—	—	+	—	+	—	—	—	—	—
5A	+	+	+	—	—	+	+	—	—	—	—	—	—	—
5B	+	+	+	—	—	+	+	—	+	—	—	—	—	—
5C	+	+	+	—	—	+	+	+	+	—	—	—	—	—
5D	+	+	+	+	—	+	+	—	+	—	—	—	—	—
6	+	+	+	—	—	—	+	—	—	—	—	+	—	—
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+

^a The following MAbs were included in the following group or subgroup: 1, 244-11; 2, M24; 3, M17; 4, 116-10; 5A, 7-4 and 7-10; 5B, 53-21 and 189-12; 5C, 91-6; 5D, 62-47; 6, 135-10 and 135-18; 7, 29-1, 186-20, and 186-24.

^b See footnote b to Table 2.

^c See footnote c to Table 2.

spectively. At the same time, one MAb was produced by using the Mok-1 strain as the immunogen. This MAb, 422-5, is well known, as it reacts positively with only the rabies-related viruses (31). Because since 1980 increasing numbers of isolates of the rabies-related viruses have been obtained, it was decided at the Institut Pasteur to produce specific MAbs against one of the rabies-related viruses. The choice of Mokola virus was made, and the strain Mok-3 isolated recently by P. Sureau was used as the immunogen. A panel of MAbs that can identify antigenic differences among rabies and rabies-related virus strains was recently obtained. The MAbs could detect either NCA or CSA (29). In the present study, we tested all the rabies and rabies-related virus strains available; these strains originated from different countries and were tested by indirect immunofluorescence on infected cells in culture (Table 2). Considering that cell adaptation and the subsequent passage of a viral population (otherwise maintained by passage on mouse brains) correspond to a selection process that can lead to different populations according to the cell lines used, our first aim was to limit the adaptation process to the mouse neuroblastoma cell, the cell line that is most sensitive to rabies virus, and to limit the number of passages on the same type of cells. Because of the poor growth of some isolates on Neuro-2a cells, however, we had to turn to other cell lines from different origins. These included monkey Vero cells or hamster CH, BHK, and CER cells. These cells have the advantage (compared with Neuro-2a) that they spread on their growth substrate, always giving unambiguous fluorescent-antibody staining. With some isolates that could be adapted to more than one cell line, such as Lag-1, Lag-2, Lag-Dak, Lag-Kin, Duv-6, Duv-DDR, and all the fixed rabies strains, no difference could be noted in the reactivity patterns (Table 2). For this reason, we considered our results in cells in culture as valid. On the basis of their specificities, our NCA and CSA MAbs could be divided into groups 11 and 7, respectively, as summarized in Tables 2 and 3. NCA MAbs from group 11 and CSA MAbs from group 7 gave fluorescent-antibody staining with all the strains. Such a positive reaction would allow a new rhabdovirus to be considered as a lyssavirus. NCA MAb from group 8 reacted exclusively with rabies-related virus strains, allowing their discrimination from rabies virus strains. Each rabies-related virus strain had a unique pattern of reactivity. Such a reaction allowed us to distinguish the strains, and diagnosis panels could be made by using a few MAbs. Only a few strains reacted identically; these corresponded mostly to serotype 4. Strains Duv-4, Duv-5, Duv-6, and Duv-DEN reacted identically, as did strains Duv-2 and Lag-Kin. Study of additional Mokola MAbs should be helpful in the identification of Duvenhage virus strains. Production of MAbs against a Duvenhage virus strain used as an immunogen, however, would be a better choice. At the nucleocapsid protein level, a previous grouping with rabies virus antibodies allowed us to distinguish serotype 2 (Lagos-bat virus) from serotype 3 (Mokola virus) and serotype 4 (Duvenhage virus). The Mokola MAbs that belong to a given group react with different sets of strains, indicating that these strains share common epitopes. When the reference strain Mok-3 is considered, as all the MAbs cross-reacted with this strain, we could reconsider the relationships between all the strains. The Umhlanga strain (Mok-Umh), which was screened by J. Crick as being Mokola-related (personal communication), was in this group. Strains Mok-5 and Mok-Umh were related to Mok-3, while strains Mok-2 and Mok-1 were only distantly related. Lagos-bat and Duvenhage viruses were different from Mo-

kola virus, as groups of MAbs which failed to recognize the strains were identified. Lagos-bat virus was more closely related to Mokola virus than to Duvenhage virus. Among Lagos-bat virus strains, three types of reactions were found. (i) Lag-2, Lag-Pin, and Lag-ZIM shared a common epitope with Mok-3 (group 3); (ii) Lag-Dak and Lag-1 were closely related; and (iii) a common reaction pattern was observed for Lag-Kin and Duv-2. Among Duvenhage virus strains, two geographic subgroups were found. The European subgroup strains (Duv-3, Duv-4, Duv-5, Duv-6, and Duv-DEN) shared a common epitope with all the Mokola virus strains, while the African subgroup strains (Duv-1 and Duv-2) were more distantly related. It is well known that during evolution, numerous modifications in the rhabdovirus cell surface antigens were selected. Considering the results obtained with CSA MAbs inside each virus, the relationships between strains could also be reexamined. Mok-5 and Mok-Umh were recently found to have very few epitopes in common compared with Mok-3, Mok-2, and Mok-1. The same relationships were found from neutralization test results. Two neutralizing epitopes could be revealed: one that was common for Mok-1, Mok-2, Mok-3, and Mok-Umh and another that was different but common for Mok-5 and Mok-Umh. As determined by fluorescent-antibody staining, one MAb recognized Mok-2 and Mok-3 and the other recognized only Mok-3; a difference was noted between these two tests. Such a disparity was noted previously with rabies MAbs, but no explanation was proposed (10). This difference between neutralization and fluorescent-antibody staining might be due to a differential expression or conformation of the neutralizing epitope on the cell membranes and on the surface of the virion. We showed previously with one of these CSA MAbs and three Mokola strains that this lack of correlation was not related to the number of cell passages (29). Lag-2, Lag-ZIM, Lag-1, and Lag-Dak had common epitopes compared with Lag-Pin. Because few MAbs cross-reacted with Duvenhage virus, the relationships between the strains from different geographic regions were impossible to establish.

Different groups of investigators have selected rabies MAbs, including Wiktor et al. (31), Schneider et al. (19), Libeau et al. (10), and Smith et al. (21). This large collection was tested against different fixed strains and street isolates (15, 24). Most of the fixed strains were differentiated by the rabies MAbs (23). In the present study, 19 NCA MAbs (Table 2) and 3 CSA MAbs (Table 3) cross-reacted identically with the seven fixed rabies virus strains: CVS, PV₄, PM, Flury HEP and LEP, ERA, and SAD. Therefore, the Mokola MAbs are unable to distinguish antigenic differences among these strains. This finding suggests that rabies virus has evolved separately from rabies-related viruses. It is also clear from these results that a common epitope on the nucleocapsid and on the envelope proteins is shared by all the rabies and rabies-related viruses.

The possible use of these MAbs for the diagnosis of rabies-related viruses (possibly to replace polyclonal serum) in brain impressions is now under investigation, and all the new field isolates of rabies-related viruses will be examined. The reactivities of these MAbs will be further extended to new isolates of lyssaviruses.

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