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NEW STRAINS OF RABIES-RELATED VIRUSES ISOLATED FROM BATS IN THE UKRAINE

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Summary. – Two strains (UB-1 and UB-2) of rabies-related viruses were isolated from the brain of *Nyctalus noctula* and *Vespertilio murinus* captured from the hollows of tall trees on the left bank of Pripyat river in the Volynsky region of Ukrainian S.S.R. The viruses were isolated by means of intracerebral inoculation to white mice. The isolates were identified as rabies-related viruses of Duvenhage type in an indirect test of fluorescent antibodies with the panels of nucleocapsid monoclonal antibodies (NC Mab) provided by Wistar Institute (Philadelphia) and by Central Veterinary Laboratory (CVL, Weybridge). During the typing with the Wistar panel of NC Mab complete antigenic similarity was established between the newly isolated strain and Yuli virus. The reaction with CVL NC Mab revealed group-specific antigenic similarity between Yuli virus on one hand, Duvenhage-6 and Duvenhage-66 on the other hand, as well as between UB-1 and UB-2 and Duvenhage-26. The reaction with antibodies to clones DB-3,4,6,9, and 10 detected antigenic similarity between the viruses of chiropteric origin isolated in the U.S.S.R., North-West Europe as well in Africa, although some differences were discovered. Yuli, UB-1, and UB-2 viruses isolated in the U.S.S.R. were proved to belong to Duvenhage group of viruses (serotype 4).

Key words: Duvenhage virus; rabies-related virus; bats; monoclonal antibodies

Introduction

Latest communications report of unusually frequent isolations of rabies-related viruses of Duvenhage serotype 4 from insectivorous bats in some European countries. In 1986–1987 together 277 strains of this virus were isolated

from *Eptesicus serotinus* (93.1 %), mainly in the shore regions of the Baltic and North seas, i.e. 163 strains in Denmark, 86 in Netherlands, 25 in F.R.G., one in Poland, and 2 in Spain (Müller, 1988). In Jutland (Denmark) 163 virus strains were isolated from 1189 chiroptera examined (13.7 %), although no case of rabies infection has been registered among ground animals in that country since 1982 (Gaede, 1988). In Netherlands 86 viruses of Duvenhage type were isolated from 1225 bats tested (7.8 %) (Nieuwenhuige, 1988). In Finland Duvenhage virus was isolated from a man who died of encephalomyelitis (Lumino *et al.*, 1986). In the U.S.S.R. isolation of 7 rabies-like viruses of chiropteric origin (serotypes 1 and 4) has been reported, 2 of them from humans with lethal encephalomyelitis and 5 from insectivorous bats (Selimov *et al.*, 1987).

Materials and Methods

Bats. Carcasses of *Nyctalus noctula* and *Vespertilio murinus* were found in the hollows of tall trees along the left bank of Pripyat river (Volynsky region of the Ukrainian S.S.R.) near to Poland's border. Bodies of *Myotis bluthi, Myotis dasycneme* and *Myotis mystacinus* (one specimen each) were obtained from the caves of Shatsky lakes in the same region. The brain of each bat was placed into 50 % glycerol and brought to L. V. Gromashevsky Institute of Epidemiology, Microbiology, and Infectious Diseases (IEMID) Kiev, where it was stored at 4°C. Specimens as 20 % suspension of bat brain were examined by means of intracerebral (i.c.) inoculation of white mice weighing 7–8 g. To confirm the diagnosis of rabies, bat brain smears were examined by direct fluorescent antibody (FA) staining with commercial diagnostic antirabies γ-globulin.

Viruses. Yuli, rabies-related Duvenhage-6 virus (African virus), Duvenhage-26, and Duvenhage-66 isolated from bats in Denmark and Poland as well as strains of street rabies virus LOZ and PRO (isolated from humans with fatal hydrophobia following dog and cat bites, respectively) were used.

Virus identification. The viruses were titrated by means of i.c. inoculation into mice (weighing 6–7 g); the titres were calculated according to Reed and Muench (1938). The viruses were identified by indirect FA method with panels of NC Mab of Wistar Institute (U.S.A., Wiktor and Koprowski, 1980) and CVL (Weybridge, Great Britain). Mab-secreting selected and cloned hybridomas (CVL set) were obtained by means of hyperimmunization of BALB/C mice with inactivated murine brain suspension of Duvenhage-6 virus according to the conventional technique (EMBO Course, SKMB, 1980). Brain impression smears on glass slides obtained from mice infected with given viruses were stained with corresponding Mab (mouse ascitic fluids) and thereafter with labelled antimouse IgG according to the conventional technique. The specimens were viewed in luminescent microscope as described (Selimov *et al.*, 1964).

Results

On day 15 after i.c. inoculation of 20 % brain suspension of *Nystalus noctula,* 1 white mouse out of 7 inoculated fell ill with signs of general excitability, movement coordination disorders and paralysis. By 26 hr after inoculation, the animal died. Out of 7 mice inoculated by i.c. route with 20 % brain suspension of *Vespertilio murinus,* 2 fell ill on day 16 showing manifest signs of rabies and died 20 and 22 hr later. Luminescent microscopy of brain smears of dead white

mice revealed a faint positive staining. The isolated viruses were designated as UB-1 and UB-2 (Ukrainian bats 1 and 2).

Table 1 summarizes the results of comparative identification of UB-1 and UB-2 strains with Mab (from Wistar Institute). It is evident that UB-1 and UB-2 strains reacted with 502-2 clone and consequently, they are rabies viruses. Yuli, UB-1 and UB-2 viruses do not show bright staining (±) with the 422-5 Mab clone (specific for rabies-related viruses), whereas Duvenhage-6 and Duvenhage-66 strains show an obviously positive reaction (viruses from Africa and Poland). In our experiments, Duvenhage-26 virus from Denmark did not react

Table 1. Identification of UB-1, UB-2, Yuli and other strains with the use of Mabs (panel of Wistar Institute)

Mabs	Viral strains							
	Yuli	UB-1	UB-2	D-6	D-26	D-66	LOZ	PRO
502-2	+	+	+	+	+	+	+	+
422-5	±	±	±	+		+		
222-9							+	+
817-5							+	+
807-5							+	+
239-10							+	+
237-3							+	+
703-8							+	+
377-7							+	+
515-3	+	+	+		+	+	+	+
209-1							+	+
229-1							+	+
590-2							+	+
120-2							+	+
103-7							+	+
714-3	+	+	+		+	+	+	+
818-5							+	+
701-9							+	+
715-3							+	+
721-2							+	+
806-4							+	+
364-11							+	+
206-1							+	+
102-27							+	+
801-3*							+	+

* Footnote. „+" All viruses reacted with the remaining clones 111-14, 802-2, 804-9, 805-3, 111-2, 389-1, 822-7, 816-1, 808-3, 104-4, 803-6.
„±" faint fluorescence

with this antibody clone, although as it is known from the literature, all European clones of Duvenhage type exhibit a weak reaction with this clone (King and Crick, 1988). It should be noted that all rabies-related and classical viruses used in our experiments react with 515-3 and 714-3 Mab with the only exception of Duvenhage-6 (isolated in Africa).

Table 2 summarizes the antigenic reactivity of viruses under study with a panel of CVL Mab. It is evident from the Table that only LOZ and PRO strains of street rabies virus bind with M-2 Mab clone. Yuli, Duvenhage-6 and -66 viruses react with D-2,3,9,10 Mab whereas UB-1 and 2, Duvenhage-66 and LOZ and PRO strains of street rabies virus do not bind with these antibodies. Yuli virus does not bind with D-10 clone. The majority of viruses used in the

Table 2. Identification of viruses under study with NC Mab (CVL panel)

Mab		Virus							
		Yuli	UB-1	UB-2	D-6	D-26	D-66	LOZ	PRO
M	2							+	+
D	2	+	+		+		+		
	3	+			+		+		
	9	+			+		+		
	10				+		+		
DB	1	+	+	+	+	+	+	+	+
	2			+	+		+		
	3	+	+	+	+	+	+	+	
	4	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+		+
	9	+	+	+	+	+	+		
	10	+	+	+	+	+	+		
	11	+	+	+	+				
L	3								+
	16	+					+		
	20	+							

Footnote: None of viruses reacted with the following Mab: M-5, 6, 7, and 11; L-1, 4, 7, 8, 9, 10, 11, 12, 15, 17, 22, 23, 25, 26, 27 and 28.

experiment react with DB Mab. DB-1 clone binds with all rabies-related viruses and classic rabies viruses, DB-2 reacts only with UB-2 and Duvenhage-6 and -66 viruses. Mab to DB-3,4,6,9, and 10 bind with all viruses of chiropteric origin isolated in the European part of the U.S.S.R. as well as in Denmark, Poland, and Africa. According to our preliminary results, DB-11 antibody clone reacted with Yuli, UB-1 and UB-2 and Duvenhage-6 viruses whereas it did not bind with Duvenhage-26 and -66 viruses.

The viruses under study bound only with individual Mab clones L; this reaction apparently accounts for antigenic differences between Lagos (serotype 2), Mokola (serotype 3), and Duvenhage (serotype 4) viruses. However, an insufficient stability of Mab (CVL panel) cannot be ruled out. In addition, one should take into account the insufficient reproducibility of indirect IF when used for examination of brain smears used here as antigen.

Discussion

Using the panel of Mabs from Wistar Institute (Table 1) the antigenic profiles of Yuli, UB-1, and UB-2 viruses proved to be identical. Insignificant differences in the nucleocapsid antigen were found between rabies-related viruses isolated in the South of Russia and Ukraine as well as in Africa, Denmark, and Poland. Danish Duvenhage-26 virus did not react with Mab clone 422-5 and Duvenhage-6 did not react with 515-3 and 714-3 clones. As known, similar deviations noted in other laboratories were accounted for by possible low antibody concentration (Report of WHO Consultation, 1985). The reaction with Mabs D-2, 3, 9, and 10 revealed certain similarity between Yuli, Duvenhage-6 and -66 viruses on one hand, and UB-1 and UB-2 and Duvenhage-26 viruses on the other hand. The reaction with Mabs DB-3,4,6,9, and 10 showed antigenic similarity between all chiropteric viruses under study isolated in the U.S.S.R., North-West Europe, and Africa (Table 2).

Consequently, the results of our experiments indicate that UB-1 and UB-2 viruses isolated from bats in the Ukraine as well as Yuli virus belong to Duvenhage group of lyssa viruses (serotype 4). As known, in Europe up to the mid 80-s single report on lyssa virus isolations from *Chiroptera* were interpreted as occasional importation from the other continents. The latest communications from Denmark, Netherlands, F.R.G., and other European countries demonstrate that at least in northern and western Europe the natural cycle of rabies-related Duvenhage virus is maintained in the population of insectivorous bats, in particular in *Eptesicus serotinus species.*

Presumably, the peculiarities of chiropteric rabies-related viral infection in the U.S.S.R. are as follows. Rabies-related viruses of Duvenhage type were isolated in the South of the European part of our country, and in Siberia - lyssavirus of serotype 1 (Selimov *et al.*, 1987). Basing on our observations at present it is impossible to assume that *Eptesicus serotinus* is the main vector for rabies-

related viruses in the U.S.S.R. In addition, Yuli, UB-1, and UB-2 viruses of bat origin accumulate in the brain of inoculated white mice comparatively in low quantities (3.0 log LD_{50}/0.03 ml titre), the incubation period being 7–10 days.

The presented data on distribution of rabies-like virus infections in the U.S.S.R. should be regarded as incomplete: bat bites of man, sick bats or their bodies are infrequently available for laboratory investigations. Possible cases of biting a terrestrial animal by bats remain unnoticed.

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