ANTIGENIC CHARACTERISATION OF VIRUS ISOLATES FROM VACCINATED DOGS DYING OF RABIES

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SUMMARY

Four rabies virus isolates from dogs that succumbed to rabies infection in Nigeria within one year of anti-rabies vaccination were characterised by monoclonal antibodies (MAbs). The samples were screened for rabies and rabies-related viral antigens by the indirect fluorescent antibody test, performed with MAb 502–2, which recognises the nucleocapsid (NC) protein of all known Lyssaviruses and with MAb 422–5 which identifies African rabies-related viruses. All four canine virus isolates displayed positive fluorescence with MAb 502–2 and were negative with MAb 422–5. In the anti-NC MAb characterisation with a panel of 34 additional MAbs, all isolates displayed positive staining with 32 of the MAbs, were negative with MAb 102-27 and all displayed poor immunofluorescence with MAb 377–7. On the basis of reactivity with a panel of 40 anti-glycoprotein (G) MAbs the isolates were separated into four distinct viral subtypes. None of these canine isolates was identified as the common attenuated Flury LEP rabies strain used for domestic animal vaccination and none resembled other previously characterised rabies viruses from Nigeria.

INTRODUCTION

Rabies is endemic in Nigeria and is one of the most dreaded zoonotic diseases. Various communities have suffered sporadic outbreaks in both humans and dogs (Umoh and Belino, 1979; Fagbam, Anosa and Ezebuiro, 1981), but proper records of these outbreaks have not been kept (Nuru, 1973); neither has the yearly incidence on a national level been documented (Fagbam and et al., 1981; Ogunkoya, Will and Ezeokoli, 1984). Some human patients with rabies infection may not even receive medical attention because of the traditionally hopeless prognosis ascribed to the infection by the local population.

Despite major efforts to control the disease, rabies continues to be a major scourge of dogs and cats. Thousands of dogs are vaccinated annually with the Flury LEP vaccine strain produced in Vom, Nigeria (Boulger and Hardy, 1960; Nawathe, Banerjee, Okeke and Tiyagnet, 1981; Okoh, Umoh, Ezeokoli and Addo, 1988) but the prevalence of the disease in domestic animals has not declined (Durojaiye, 1984). Various workers have reported the death of dogs from rabies within one year of vaccination (Bobade, Aghomo, Akinyemi and Akpavie, 1983), perhaps due to a number of factors as suggested by Okoh et al. (1988). The present study used a panel of monoclonal antibodies (MAbs) to characterise viruses isolated from four dogs which died of rabies within one year of anti-rabies vaccination.

MATERIALS AND METHODS

Out of 2,500 dogs routinely examined and vaccinated in Ibadan, Nigeria within a period of two years with the Flury LEP vaccine produced in Vom, Nigeria, at least four dogs died of canine rabies at three, five, five and a half and
eight months after rabies vaccination. One of these dogs was a German shepherd and the other three were local breeds. All the dogs were house pets.

On clean glass microscope slides impressions were made in triplicate from the brains of mice used for the initial isolation of the viruses from the brain of each dog which died in spite of vaccination. The mouse brain impressions were air dried before and after fixation in 80% cold acetone at 4°C for 30 minutes. One set of impressions was examined using the fluorescent antibody test (FAT) by staining with anti-rabies FITC-labeled conjugate containing 1:2,000 dilution Evan’s blue (Wiktor, Flamand and Koprowski, 1980). Slides were incubated at 37°C for 30 min, then rinsed with distilled water and viewed while moist under fluorescent microscopy (Leitz Dialux) at ×200 magnification.

The two other sets of brain impressions were used in indirect staining with anti-nucleocapsid (NC) MAb 502-2 and 422-5 after fixation in 80% cold acetone at 4°C for 30 minutes. Slides were stained with either 502-2 or 422-5 and incubated in a moist chamber for 30 min at 37°C, after which they were washed twice with phosphate-buffered saline (PBS) (pH 7-0) and counterstained with fluorescein isothiocyanate (FITC)-labeled goat antimouse gamma globulin conjugate (Wiktor et al., 1980) for another 30 min incubation as above. Slides were rinsed in distilled water and viewed while moist under fluorescent microscopy at ×200 magnification.

**Virus isolation**

Virus isolation was made in both baby hamster kidney (BHK) and murine neuroblastoma (NA) cells. Ten per cent brain suspensions were made in Eagle's minimal essential medium (MEM) containing 10% foetal calf serum (MEM-10), 200 i.u. penicillin, 200 µg streptomycin, 200 µg/ml L-glutamine and 29-23 mg/ml fungizone, and were clarified by centrifugation at 2,000 rpm for 10 minutes. Approximately 0.25 ml of the virus-containing supernatant was mixed with either 2 ml of BHK or NA cells (1.5 × 10⁶/ml) in a T25 flask (Falcon, Oxnard, CA) and left to stand for 5 min before dilution to a final volume of 5-0 ml with MEM-10. From this 0.3 ml was transferred to a 96-well plate (Falcon, Denmark) from which 10 µl was placed in six wells of a Terasaki plate (NuncLon) to monitor percentage of cell infectivity. The T25 flask, 96-well plate and Terasaki plates were incubated for four days at 37°C in a 5.5% CO₂ incubator. Thereafter the Terasaki plate was washed twice with PBS and drained before fixation in 80% cold acetone for 30 minutes. After fixation and air drying each well was stained with 5 µl FITC-labeled anti-rabies gamma-globulin conjugate and incubated for 30 minutes. The plate was then rinsed in distilled water and observed while moist by fluorescent microscopy at ×200 magnification. The supernatant from the virus-infected cells was collected, clarified by centrifugation and stored at −85°C. The infected cells were passaged following the addition of trypsin. Fifty per cent of each cell passage was discarded and fresh MEM-10 was added to the T25 flask. Five such cell passages were carried out on three of the samples and seven on the fourth sample.

**Virus titration**

At 100% cell infection the virus-containing supernatant was titrated. Serial five-fold dilutions were done in MEM-10 (1:5–1:98415). Thirty µl of indicator NA or BHK cells (1·0 × 10⁶/ml) were added to each well and thoroughly mixed. Ten µl from each well was put into duplicate wells in a Terasaki plate and