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Rabies-Related Viruses

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Five viruses related to rabies occur in Africa. Two of these, Obodhiang from Sudan and kotonkan from Nigeria, were found in insects and are only distantly related to rabies virus. The other three are antigenically more closely related to rabies. Mokola virus was isolated from shrews in Nigeria, Lagos bat virus from fruit bats in Nigeria, and Duvenhage virus from brain of a man bitten by a bat in South Africa. The public health significance of the rabies-related viruses was emphasized in Zimbabwe where in 1981 a rabies-related virus became epizootic in the dog and cat population. It is postulated that the ancestral origin of rabies virus was Africa where the greatest antigenic diversity occurs and that the ancestor may have been an insect virus. Questions are raised why rabies has not evolved more rapidly in the New World, given the frequency and ease with which antigenic changes can be induced in the laboratory, and how the virus became so extensively established in New World bats.

Rabies, like poliomyelitis, at one time was believed to have a single causal virus. The discovery of multiple serotypes related to rabies virus parallels the early studies in which it was found that there were multiple poliomyelitis serotypes. In those early struggles with poliomyelitis, Dorothy Horstmann was an active participant to understand the significance of multiple serotypes [1]—studies which demonstrated the theoretical and practical virtues of serological classification. Her influence and encouragement have been significant factors in our study of rabies-related viruses. It is with sincere thanks that I dedicate to her this review of the rabies-related viruses and their evolutionary significance.

The viruses from Africa which were to be linked to rabies were not initially recognized as rabies-related. It was only through a many-faceted collaboration that the finding was established. The Rockefeller Foundation and several governmental agencies in the early 1950s embarked on programs to find viruses which caused encephalitis and tropical fevers. They established and supported field laboratories, fostering the concept of broad-based surveys of arthropods, wildlife, domestic animals, and patients. In those times it was quite fashionable to grind up bats, lizards, midges, mosquitoes, and almost any other of the earth's creatures to search for a viral pathogen by inoculation into mice. Boulger and Porterfield isolated an agent from *Eidolon helvum* fruit bats captured at Lagos Island, Nigeria, in 1956 [2]. They called the agent Lagos bat virus, registered it as a possible arbovirus, and distributed it widely to viral taxonomists in the hope of finding out more about its relationships.

In the following decade, several other seemingly remote events led to the isolation of other viruses which were later to be shown to be related to rabies. In 1963 Jack Schmidt working at the Naval Medical Research Unit in Cairo isolated a virus on

two occasions from *Mansonia uniformis* mosquitoes captured in the Sudan [3]. He called this agent Obodhiang virus after the site where it was found along the Nile River. He also sent this virus to Yale University, hoping to have it identified.

Then in 1967 Vernon Lee isolated kotonkan and in 1968 Graham Kemp isolated Mokola virus. These isolates were a direct result of The Rockefeller Foundation and Nigerian government's search for arboviruses in Ibadan, Nigeria. Vernon Lee, an entomologist, was fascinated by *Culicoides* midges, the vectors of the viruses causing ephemeral fever of cattle and bluetongue of sheep. He isolated kotonkan virus [4] from midges captured on cattle at the University of Ibadan farm. Kemp was a veterinarian equally fascinated by Africa's exotic creatures. Organs of trapped or dead *Crocidura* spp. shrews were ground up to recover Mokola, a mouse pathogen [5]. Later two strains of Mokola virus were isolated from cases of central nervous system (CNS) disease in Nigerian children [5,6]. The two Nigerian agents were also sent to Yale University for taxonomic study.

In 1969, we at the Yale Arbovirus Research Unit were studying Lagos bat, Mokola, and Obodhiang viruses. Lagos bat virus cross-reacted with Mokola by complement-fixation test. We were also collaborating with Fred Murphy, CDC, Atlanta, to classify the vertebrate animal rhabdoviruses. David Simpson from England independently sent Lagos bat virus to Fred Murphy in order that its morphology might be determined by electron microscopy. Murphy found Lagos bat virus to be a rhabdovirus, and phoned me with the suggestion that it should be tested serologically with rabies virus because of the remarkable similarity of its particles and intracytoplasmic inclusions to rabies. With this clue, we were immediately able to show that Lagos bat and Mokola viruses were related serologically to rabies [7]. The rabies serogroup was born.

The next observation was one which took some time to gain acceptance. Obodhiang virus, from mosquitoes, reacted serologically with Mokola virus. It seemed radical to believe a mosquito-borne or insect virus could be related to rabies. It would become more credible to us if we could demonstrate that Obodhiang virus was a rhabdovirus. Efforts by Dean Percy and Joseph Craft to visualize the virus were successful and it was indeed a rhabdovirus. Fred Murphy confirmed this observation and showed in addition that some Obodhiang particles had the cone shape which is characteristic of bovine ephemeral fever virus [4].

Dorothy Moore independently at the Virus Research Laboratory, University of Ibadan, found that kotontan virus was related to Mokola virus and kotonkan was also subsequently found to have morphology similar to Obodhiang virus [4].

Finally, in South Africa, Courtney Meredith read about the rabies-related viruses and thought of them when an agent he isolated from the brain of a man bitten on the lip by a bat did not react in the rabies immunofluorescence test, although the case appeared to be typically rabies [8]. This virus was named Duvenhage and was another new rabies-like virus [9].

Much of the more recent study of these viruses has dealt with their serological relatedness and differences. The two virus serotypes from insects are antigenically very different from each other and from the other members of the rabies serogroup. The relatedness was shown by complement-fixation and immunofluorescence with a potent antiserum. Mokola, Lagos bat, and Duvenhage viruses are more closely related to each other and to rabies virus by complement-fixation, immunofluorescence, and neutralization tests [7,9]. These four viruses now comprise the genus *Lyssavirus*. Surprisingly, however, rabies-immunized mice whose sera neutralized a

heterologous member of the serogroup were usually susceptible to challenge by the heterologous virus and died with encephalitis [10]. This observation indicates that probably rabies vaccine would not protect man or domestic animals against the disease caused by Mokola and Duvenhage viruses.

Minor antigenic differences are recognized among rabies strains [11] although a potent rabies vaccine will protect against challenge with these minor variants. Studies were initiated by Flamand, Wiktor, and Koprowski to determine the reactive sites on each of the antigens involved in the serologic reactions with the viruses related to rabies. They used monoclonal antibodies to show that there were shared epitopes on the surface glycoprotein as well as on the nucleocapsid protein [12,13]. Mokola, Lagos bat, and Duvenhage viruses from Africa shared many fewer reactive sites, i.e., reacted with fewer of the monoclones in the battery, than did strains of rabies from other parts of the world.

In a parallel study with monoclonal antibodies, Schneider and Meyer [14] identified two strains of Duvenhage virus from bats in northern Germany. These were the first rabies-related viruses isolated outside of Africa and their discovery led the authors to postulate that the bats had been carried there by ships from Africa.

The known distribution of Lagos bat virus, while limited to Africa, is much more extensive than originally thought. The agent was recovered a second time from bats in the Central African Republic [15]. Most recently Lagos bat virus was found in a sick bat [16], in the midst of a rabies outbreak among dogs in Natal, South Africa. It was first thought to be one of the prevalent rabies strains in the region, but was later shown to be a strain of Lagos bat virus [17], quite different from rabies. It was probably infecting bats in a cycle completely coincidental to the canine rabies epizootic in Natal.

What may be a highly significant event occurred in 1981 in Zimbabwe. From April to September, rabies-related viruses (not yet fully identified) were isolated from the brains of three rabid cats and a rabid dog. The dog had received rabies vaccine six months before. The isolates were distinct from rabies virus by neutralization and immunization-challenge tests in mice [18]. This is the first clear demonstration of epizootic spread of rabies-related viruses in domestic animals and may pose a new, serious public health problem in Africa, since rabies vaccine apparently did not protect.

The rabies-related viruses raise interesting questions of viral evolution. The most cohesive view holds that rabies and its related viruses evolved in Africa, since Africa is the site of the greatest antigenic diversity. In parts of the world other than Africa, rabies strains vary only in minor antigenic determinants [14]. Rabies virus, itself, may have moved with man from Africa in the form of infected domestic animals. The long incubation period of rabies makes this a plausible event despite the lengthy ship voyages to the New World. Those who doubt this theory point out that rabies virus is extensively and well-established in the New World bat populations and not just in domestic animals. The bat cycles are apparently compartmentalized from the domestic animal and other wildlife cycles; how this happened remains a mystery.

A second mystery is the slow natural evolution of lyssaviruses with little evidence of divergent evolution in the New World. In the laboratory, Mokola and Lagos bat viruses were antigenically stable. Multiple clones selected in cell culture with and without antibody pressure did not demonstrate antigenic differences [19]. By contrast, when rabies virus was pressured by monoclonal antibody, new non-neutralized viruses appeared readily in a frequency of 1:10,000 [20]. Although this might be a

dangerous experiment to carry much further (existing rabies vaccine might not protect if the strains should escape from the laboratory), the results indicated the ease with which a new rabies-related virus might evolve. Since rabies has been enzootic in North America since at least 1768 [21], why have not new serotypes evolved in the New World? One must assume that the predicted naturally occurring antigenically changed viruses are selected against and do not survive.

A third mystery is the role of insects as reservoirs for rabies and related viruses. There is no direct evidence that rabies virus is or can be transmitted by insects. A laboratory study by Thomas Aitken showed that Obodhiang virus replicated in *Aedes aegypti* mosquitoes following intrathoracic inoculation, appeared in the mosquito salivary glands after seven days' extrinsic incubation, and was maintained for at least 146 days in serial passage [unpublished data, 1973]. Obodhiang and kotonkan viruses were originally found in insects; neither kill adult mice nor do they cause rabies-like illness in dogs as do Mokola, Lagos bat, and rabies viruses. One can speculate that they are relics of an ancestor insect virus from which rabies, Mokola, Lagos bat, and Duvenhage viruses evolved. Perhaps rabies, with its high degree of vertebrate nerve cell dependence, is the most specialized of the lyssaviruses. Mokola, which also shares the most antigenic relationships with kotonkan and Obodhiang [22], may be the linking intermediate. If this were true, Mokola, unlike rabies, might also have retained its ability to replicate in insects.

The ability to infect insect cells and insects was examined experimentally. Sonja Buckley readily adapted Mokola virus to *Aedes albopictus* (Singh) cell culture [23] and maintained persistently infected cultures for four and one-half years. Thomas Aitken then inoculated *Aedes aegypti* mosquitoes with the mosquito cell-adapted strain and maintained Mokola virus in serial passage of infected mosquito bodies in *Aedes aegypti* for another 74 days [unpublished data, 1979].

We are not likely to prove or disprove these theories of viral evolution. The experiments do, however, offer insight into likely mechanisms and plausible explanations for the observed natural phenomena of rabies and rabies-related viruses. The studies have also alerted us to the real possibility that rabies-related viruses may become epizootic in domestic animals in Africa, thus posing a public health problem.

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