

ANTIGENIC RELATIONSHIPS AMONG TURLOCK SEROGROUP BUNYAVIRUSES AS DETERMINED BY NEUTRALIZATION TESTS

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Summary. — Antigenic relationships between the five recognized Turlock serogroup viruses (family Bunyaviridae, genus *Bunyavirus*) were determined by serum dilution-plaque reduction neutralization tests. Results indicated that Turlock, Umbre, M'Poko and Lednice viruses are distinct from each other and that Yaba-1 virus is a subtype of M'Poko virus.

Key words: Turlock serogroup; antigenic relationships; Bunyavirus; neutralization

Introduction

Five viruses constitute the Turlock (TUR) serogroup (family Bunyaviridae, genus *Bunyavirus* (Calisher *et al.*, in preparation). They have been isolated from culicine mosquitoes and birds in North and South America (Turlock virus), Europe (Lednice virus), Africa (M'Poko and Yaba-1 viruses), and Asia and Australia (Umbre virus). While none of these viruses has been associated with disease in humans or other animals, their widespread geographic distribution and prevalence causes them to be of interest. A review of the distribution of these viruses has been published (Bishop and Shope, 1979).

There is considerable confusion as to the antigenic relationships among TUR serogroup viruses. In preliminary studies, we confirmed the findings of Digoutte *et al.* (1970), demonstrating that M'Poko (Berge, 1975) and Yaba-1 (Simpson and Williams, 1965) viruses were essentially indistinguishable by complement-fixation tests but distinguishable by neutralization tests. Since members of the family Bunyaviridae often are found to share complement-fixing antigens, ostensibly attributed to possession of a common G₁ glycoprotein, we felt that application of the sensitive serum dilution-plaque reduction neutralization (N) test might be of use in separating the viruses of this group. This paper presents the results of cross-tests of recognized TUR serogroup viruses by N tests and demonstrates that antigenic differences among these viruses parallel their geographic separation.

Materials and Methods

Prototype viruses were available from the collection of this Branch or were obtained from Dr. Robert E. Shope, Yale Arbovirus Research Unit, New Haven, Connecticut (M'Poko and Yaba-1 viruses), or from Dr. D. Málková, Czechoslovak Academy of Sciences, Institute of Parasitology, Prague (Lednice virus). All viruses were passed in suckling mice (SM) by intracranial inoculation, and 10% clarified suspensions were prepared for seed virus pools. Each preparation was titrated by plaque assay in primary Pekin duck embryo serially propagated Vero, LLCMK₂, PS, and CER cell cultures. After selecting the most appropriate culture, based upon titer and size and clarity of plaques, viruses were cross-tested by N tests using the method published for California serogroup bunyaviruses by Lindsey *et al.* (1976). Briefly, equal 0.1 ml volumes of virus, containing 200 plaque-forming units, and serum dilutions of hyperimmune mouse ascitic fluids (Tikasingh *et al.*, 1966) were mixed, incubated at 4 °C for 18 hr, and 0.1 ml of the mixture was dropped onto cell cultures propagated in 6-well plastic plates. After 45 min of incubation at 37 °C to allow for adsorption, the cells were overlaid with nutrient agar and reincubated at 37 °C. Duck embryo cells were overlaid with agar containing 1 : 25,000 neutral red; other cell lines were overlaid first with agar not containing neutral red and on the third day after inoculation a second overlay containing neutral red. Each day after inoculation, cultures were examined for appearance of plaques, and when inoculated control cultures contained approximately 100 plaques, titrations were terminated. Titers were recorded as the highest dilution of antibody preparation inhibiting 90% or more of the plaques as compared with control titrations.

Results

Strains of Turlock virus from the United States (prototype strain 847-32 and Len 781-19), Ecuador (75V-2416), northern Brazil (BeAn 32260) and southern Brazil (SpAn 35260 and R-10667) were titrated in duck embryo and Vero cells for morphologic and biologic comparisons. In each culture, all viruses were similar in time of appearance of plaques, plaque size and clarity, and titer. Also, these six strains were identical in cross-neutralization tests.

When the 5 prototype TUR serogroup viruses were cross-tested by N, Turlock, M'Poko, Umbre and Lednice viruses were clearly distinguishable from each other, and Yaba-1 was shown to be a variant of M'Poko virus (Table 1). These results confirmed and extended previous complement-fixation test results which suggested that Turlock and Umbre viruses were distinct from each other and from viruses belonging to an antigenic complex,

Table 1. Results of serum dilution-plaque reduction neutralization (N) tests with five Turlock serogroup bunyaviruses

Virus	Strain	Titer of N antibody to:				
		TUR	MPO	Yaba-1	UMB	LED
Turlock	847-32	≥2560	160	— ^{a)}	—	40
M'Poko	BA-365	320	40,960	5120	—	20
	Yaba-1	40	2560	1280	—	160
Umbre	IG-1424	160	160	80	320	20
Lednice	6118	320	640	80	20	1280

a) — = <20

which includes M'Poko, Yaba-1 and Lednice viruses (D. J. Muth, in preparation).

Discussion

Turlock virus has been isolated primarily from culicine mosquitoes and passerine birds throughout the Americas (Berge, 1975). It is the only virus of the TUR serogroup in this region, and because no antigenic differences between strains have been detected, it is reasonable to assume that such homogeneity is due to continuous movement of the virus through wild birds.

A similar situation exists with Umbre virus. This member of the TUR serogroup has been isolated from culicine mosquitoes and birds in southern India and Queensland, Australia. As with Turlock virus, it is likely that Umbre virus is transported throughout its habitat in Asia and Australia by migrant birds and transmitted in epizootic cycles by culicine vectors.

The close antigenic relationships among M'Poko, Yaba-1, and Lednice viruses present a different picture. Although these viruses are also isolated from culicine mosquitoes and wild birds in Africa (M'Poko and Yaba-1) or Europe (Lednice), they are distinguishable from each other. Complement-fixation tests indicate that they share common antigens, but N tests (Table 1) demonstrate significant differences. For many years, it has been accepted that M'Poko and Yaba-1 are identical or very closely related to each other. Digoutte *et al.* (1970) using a virus dilution neutralization test, showed that Yaba-1 is a subtype of M'Poko virus. Extensive biological, biochemical and ecological studies of Lednice virus by Málková and others were performed with strains of this virus considered as closely related, if not identical, to Yaba-1 virus (Málková *et al.* 1972). The identifications, however, were done using complement-fixation and virus neutralization tests.

While Lednice virus of Europe is closely related to M'Poko virus, it is ecologically and antigenically distinct from it; whereas Yaba-1 virus, which is closely related to M'Poko virus, shares both antigens and ecologic setting.

From the studies reported here and in the literature, these viruses may be classified within the TUR serogroup as:

Complex	Virus	Subtype
Turlock	Turlock	
Umbre	Umbre	
M'Poko	M'Poko	M'Poko
	Lednice	Yaba-1

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