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PATHOGENICITY OF IFE VIRUS FOR SWISS ALBINO MICE

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Summary. — Pathogenicity of Ife virus was studied in Swiss albino mice following four inoculation routes. Mice of all ages survived oral infection without seroconversion; subcutaneous (i.c.) and intraperitoneal (i.p.) infections lead to low titre antibody production. Only suckling mice (1—5 days old) succumbed to intracerebral (i.c.) inoculation with infectivity titres which decreased by age and average survival time (AST) increasing with age. Following i.c. inoculation to suckling mice, the brain infectivity titres increased progressively by days post-infection (p.i.). Virus was not recovered from the lungs and kidney but in low titre it was obtained from the liver, spleen, heart and bloodat different days p.i. All organs examined showed evidence of complement fixing and immunofluorescent Ife virus antigen. No gross lesions were observed. The histopathological lesions were limited to the brain.

Key words: *pathogenicity*; *organ distribution*; *Ife virus*

Introduction

Ife virus, isolated first from bats (*Eidolon helvum*) at Ife, Nigeria in April, 1971 was subsequently isolated from the same bat species at Abuja (Nigeria) and Saa (Cameroun) in the same year (Virus Research Laboratory, Ibadan, 1971; Karabatsos, 1985). The virus was classified by complement fixation test as an ungrouped Orbivirus unrelated to any other known orbiviruses; based on physico-chemical characteristics it is considered as possible arbovirus (Karabatsos, 1985). Information on the epidemiology, properties and significance of this virus is very scanty. The pathogenicity and organ distribution of Ife virus in Swiss albino mice is described in this report.

Materials and Methods

Virus. Ife virus strain IbAN57928 obtained from Professor O. Tomori (Department of Virology, College of Medicine, University of Ibadan, Ibadan, Nigeria) was passaged i.c. in 2-day-old suckling Swiss albino mice and used in its 6th and 7th passages. Virus titres were determined by the method of Reed and Muench (1938).

Mouse infectivity assay. Swiss albino mice colony was established with parent stock obtained from the National Trypanosomiasis Research Institute, Vom, Nigeria and fed on standard mouse pellets (Pfizer Livestock feeds Ltd, Lagos, Nigeria). Litters of one- to five-day-old suckling mice (6—8 mice/litter), six weaning and six adult mice were infected with various doses of $10^{4.5}$ to $10^{5.5}$ MLD_{50} (mouse lethal dose fifty) per 0.02 ml of Ife virus. Inocula were given by i.c., s.c., i.p. and oral routes. Infected mice were observed daily for signs of illness and survivors were bled after 30 days (suckling mice) or 21 days (weaning and adult mice) and the development of complement fixing antibody was determined (Casals, 1967).

Organ distribution. Five litters of 2-day-old suckling mice (6 mice/litter) were inoculated by i.c. route with 0.02 ml of mouse brain suspension containing $10^{5.5}$ MLD_{50} of Ife virus. Five mice were sacrificed daily and brain, heart, lungs, liver, kidney, blood and spleen were assayed for virus content. Tissue samples were washed in sterile phosphate buffered saline (pH 7.2), ground in chilled mortars and prepared as 10 % suspensions in phosphate buffered saline (pH 7.2) containing 0.75 % bovine serum albumin, 100 μg/ml penicillin, 100 μg/ml streptomycin. The suspensions were titrated by i.c. inoculation of 0.02 ml vol. in 2-day-old suckling mice. Daily harvested organ samples were also tested for the presence of Ife virus antigen by indirect immunofluorescence (Riggs, 1979) and complement fixation (Casals, 1967) tests. Organs of mice that died on the fourth day p.i. were examined grossly, formalin-fixed, paraffin embedded, sectioned, and stained with haematoxylin and eosin. Three other litters of mice were inoculated similarly with 0.02 ml of normal mouse brain suspension as controls.

Table 1. Age-related brain infectivity titres, average survival time (AST) and response of Swiss albino mice inoculated intracerebrally or orally with $10^{4.5}$ MLD_{50}/0.02 ml Ife virus at the 6th mouse brain passage

Age (days)	Inoculation	Dose (ml) of inoculum	Average survival time (days)	Evidence of infection	Infectivity titres MLD_{50}/0.02 ml	Complement fixing antibody titre
1	Intracerebral	0.02	3.5	Paralysis, death	$10^{6.4}$	
2	Intracerebral	0.02	4.0	Paralysis, death	$10^{6.4}$	
3	Intracerebral	0.02	4.0	Paralysis, death	$10^{6.2}$	
4	Intracerebral	0.02	4.5	Paralysis, death	$10^{6.0}$	
Weaning mice	Intracerebral	0.03	> 21	Antibody, asymptomatic survival		1 : 20
1	Oral	0.03	> 30	No evidence, survived infection		No antibody
2	Intracerebral	0.04	> 30	No evidence, survived infection		No antibody
4	Intracerebral	0.04	> 30	Antibody, survived infection		No antibody
Weaning mice	Intracerebral	0.1	> 21	No evidence, survived infection		

Results

All suckling mice (1—5-day-old) succumbed to i.c. inoculation of $10^{4.5}$ MLD_{50} of Ife virus, the average survival time being 3.6 days and the infectivity titre between $10^{6.0}$—$10^{6.4}$ LD_{50}/0.02 ml; in contrast, the mice survived oral infection (Table 1). Weaning mice survived both i.c. and oral infections with and without antibody production. Both i.p. and s.c. inoculations did not kill mice, but mice of all ages seroconverted with low antibody titres (Table 2).

Table 2. Age-related response of Swiss albino mice inoculated intraperitoneally and subcutaneously with various doses of $10^{5.5}$ MLD_{50}/0.02 ml of Ife virus at the 7th mouse brain passage

Age of mice (days)	Route of inoculation	Dose (ml) inoculated	Average survival time (days)	Evidence of infection	Complement fixing antibody titre
1	intraperitoneal	0.02	> 30	antibody, asymptomatic survival	1 : 10
2	intraperitoneal	0.02	> 30	antibody, asymptomatic survival	1 : 10
2	intraperitoneal	0.02	> 30	antibody, asymptomatic survival	1 : 10
4	intraperitoneal	0.03	> 30	antibody, asymptomatic survival	1 : 20
Weaning mice	intraperitoneal	0.1	> 21	antibody, asymptomatic survival	1 : 40
Adult mice	intraperitoneal	0.2	> 21	antibody, asymptomatic survival	1 : 40
1	subcutaneous	0.02	> 30	antibody, asymtpmatic survival	1 : 5
3	subcutaneous	0.03	> 30	antibody, asymptomatic survival	1 : 5
5	subcutaneous	0.03	> 30	antibody, asymptomatic survival	1 : 10
Weaning mice	subcutaneous	0.1	> 21	antibody, asymptomatic survival	1 : 20
Adult mice	subcutaneous	0.2	> 21	antibody, asymptomatic survival	1 : 20

Table 3. Distribution of Ife virus infectivity titre in various organs of Swiss albino white mice inoculated intracerebrally with $10^{5.5}$ MLD_{50} of Ife virus

Days p.i.	Virus infectivity titres in various organs MLD_{50}/0.2 ml						
	Brain	Lungs	Liver	Kidney	Spleen	Heart	Blood
Day 1	$10^{2.4}$	N	$10^{1.2}$	N	$10^{1.0}$	N	N
Day 2	$10^{3.6}$	N	$10^{1.0}$	N	N	N	$10^{1.0}$
Day 3	$10^{5.2}$	N	N	N	N	$10^{1.0}$	$10^{1.0}$
Day 4	$10^{6.3}$	N	N	N	N	N	NT

N = No virus isolated
NT = Not tested

Virus titre in the mouse brain increased progressively by days p.i. from $10^{2.4}$ LD_{50}/0.02 ml on the first day to $10^{6.3}$ LD_{50}/0.02 ml on the fourth day (Table 3). Virus was not isolated from the lungs and kidney. In a low titre ($10^{1.0}$—$10^{1.2}$ MLD_{50}/0.02 ml) it was found in the liver, spleen, heart and blood at various intervals p.i. On the first day it was present in the spleen, on the first two days in the liver, on the third day in the heart muscle, and on the second and third days in the blood. No virus was isolated from controls. Immunofluorescent staining of the organs showed widespread foci of Ife virus antigens in all organs throughout the infection period. Except for kidney and myocardium which did not yield complement fixing (CF) antigen on the first day p.i., low titre of CF antigens were detected in the spleen, kidney, lungs, heart and liver throughout the observation period. CF antigen in high titre which increased with days p.i. was observed in the brain (Table 4). No gross lesions were seen in the organs of infected mice. Histopathological changes were confined to the brain, where areas of lymphocytic infiltration, neuronal degeneration and perivascular cuffing were detected (Figs. 1—2).

Table 4. Distribution of complement fixing antigen titres in various organs of Swiss albino white mice inoculated intracerebrally with $10^{5.5}$ MLD_{50} of Ife virus

Days p.i.	Reciprocal of antigen titres against 1 : 5 dilution of MIAF					
	Brain	Liver	Spleen	Lungs	Kidney	Heart
Day 1	10	5	10	20	N	N
Day 2	40	20	40	10	20	10
Day 3	160	5	20	40	40	20
Day 4	160	5	10	20	20	10

N = Negative for antigen
MIAF = Mouse immune ascitic fluid

Discussion

The suckling mouse is considered for a universal host system to detect arboviruses and it has been used most frequently for successful arbovirus isolations (Shope and Sather, 1979). In addition, it is of immense value in the third world countries, where facilities for the maintenance of tissue cultures may not be adequate. Ife virus strain used in this study can be easily propagated in suckling mice. However, the virus titre tended to decrease with increasing age. The i.c. route is the most efficient route for propagating Ife virus in suckling mice. Weaning and adult mice as well as oral, subcutaneous and intraperitoneal routes of inoculation are not useful for the propagation of this virus.

Arboviruses usually manifest some degree of tropism to certain organs when laboratory animals are experimentally infected. In this study, Ife virus, a possible arbovirus is more neurotropic than viscerotropic. Although there was minimal distribution of the virus to various organs, progressive multiplication of the virus occurred only in the brain.

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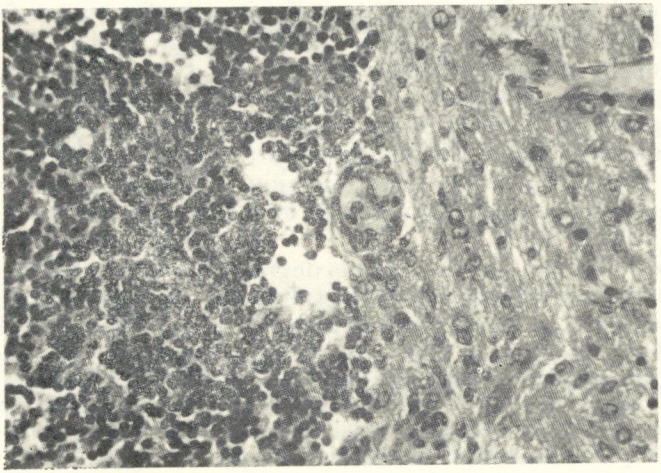

Fig. 1

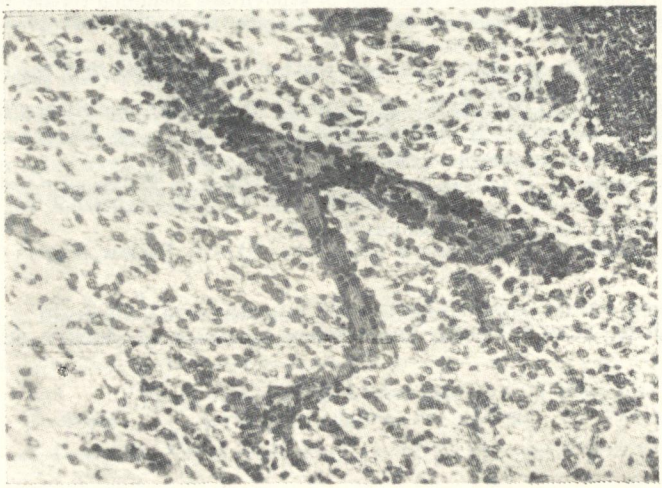

Fig. 2

Fig. 1. Ife virus-infected Swiss albino mouse cerebellum showing neuronal degeneration, HE staing (×250).
Fig. 2. Ife virus-infected Swiss albino mouse brain showing perivascular cuffing. HE staining (×250).