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SUSCEPTIBILITY OF MOUSE PERITONEAL MACROPHAGES TO INFECTION WITH ARBOVIRUSES

MACROPHAGE cultures from normal mice support the growth of mouse hepatitis viruses[2-4] and peritoneal macrophages (PM) from ectromelia-immune[1] mice support the growth of ectromelia viruses. Two arboviruses, *viz.*, West Nile (WN) and Yellow Fever (YF) have been shown to grow in mouse PM[5]. We report the results of susceptibility studies of mouse PM to thirteen different arboviruses isolated in India.

In the preliminary experiments, two ml of 2% starch[5] (in normal saline) was injected into mice by the intraperitoneal (IP) route (on the previous day) to activate the macrophages. In further experiments no such activator was used, because the yield of PM was adequate without its use. Three ml of sterile Hanks' balanced salt solution[6] was injected IP into 8–10 week old male Swiss albino mice and after kneading the abdomen gently, the IP fluid from each mouse was collected in a culture tube. After two hours of incubation at 37° C, saline was removed and the tubes fed with medium M 199 (Earle's), supplemented with 20% goat serum, loosely stoppered and incubated at 37° C in 5% CO_2 atmosphere[6]. After 24 hours, tubes with well-spread macrophages were selected and washed with the medium, tightly stoppered and incubated at 37° C for 48 hours. For the experiment, twenty tubes were selected and taken for inoculation of one virus. The viruses employed (Table I) were diluted in M 199 to contain approximately 3–4 dex[7] of infant mouse LD_{50} or $TCID_{50}$ virus and the tubes were inoculated with 0·1 ml of virus suspension per tube.

TABLE I

Antigenic group	Name of the virus, abbreviation strain number and passage history
Group A	Sindbis (SIN) (AR 339) M_5V_2; Chikungunya (CHIK) (634029) $M_{11}V_1$
Group B	Japanese encephalitis (JE) (P 20778) M_9; Kyasanur Forest disease virus (KFD) (P 9605) M_{15}; West Nile (WN)* (E 101) M_5V_2
VSV	Chandipura (CHP) (653514) $M_{22}V_1$
Kaisodi	Kaisodi (KSO) (G 14132) M_{43}
Bunyamwera	Batai (BAT) (G 20217) $M_{12}V_1$
Nairobi Sheep Disease	Ganjam (GAN)+ (G 619) M_5BH_1
Simbu	Ingwavuma (ING) (633970) M_7BH_1; Sathuperi (SAT) (G 11155) M_{13}
Ungrouped	Wanowrie (WAN) (G 700) M_{19}; Bhanja (BHA) (G 690) M_5BH_1

* Not isolated at Virus Research Centre.
+Renamed Nairobi Sheep Disease (NSD) since 1975.
M = Suckling mouse brain passage.
V = Vero passage.
BH = Baby hamster kidney (BHK–21) passage.

After adsorbing the virus for one hour at 37° C, the tubes were washed five times with neutralized Hanks' BSS and fed with M 199 with 10% goat serum and incubated at 37° C.

The tubes were observed daily for cytopathic effect (CPE) upto ten days. On the zero, third, seventh and tenth days of post-inoculation (PI) about 4–5 tubes were taken out and stored at — 50° C for a maximum period of one week. The tubes were subjected to three cycles of freezing and thawing and centrifuged at 250 g for 10 mins. The supernatant fluid was assayed in 2–3 day old infant mice by the intracerebral (IC) route. The sick mice were harvested and 10% brain suspension prepared in normal saline was tested by the quick complement fixation test[8] for identification of the viruses.

Out of the thirteen arboviruses tested, only Ingwavuma (ING) (633970) virus showed evidence of CPE and multiplication. The CPE was in the form of rounding of 75% cells on the second PI day and 100% on the next. Although the titre of the ING virus was not very high, the second passage tissue culture fluid, when titrated in infant mice, showed evidence of multiplication. The WN virus multiplied without any CPE; Kyasanur Forest Disease (KFD) and CHP viruses persisted in traces upto the 10th PI day, but could not be detected on subsequent passage. The remaining viruses did not show any evidence of multiplication, when the infected tissue culture fluids of the zero, 3rd, 7th and 10th PI days were assayed in infant mice.

It appeared that mouse PM supported the growth of only WN and ING viruses from among the arboviruses tested. This was further confirmed by carrying out three serial passages of these viruses in PM.

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ANTIVIRAL ACTIVITY OF 6MFA, GROWTH PRODUCT OF FUNGUS *A. OCHRACEUS*, AGAINST FOOT AND MOUTH DISEASE VIRUS

Foot and Mouth Disease (FMD) is an acute viral disease of the cloven footed animals. The virus comes in seven major types, namely, 'O', 'A', 'C', 'Asia-1', 'SAT-1', 'SAT-2' and 'SAT-3 and has over 62 subtypes.

Use of interferon (IF) and interferon inducers to control and manage FMD (picornavirus) virus infection in animals has been considered seriously in recent years. Particular mention may be made of yeast *RNA*, a product which, if injected continuously into infected mice, guinea pigs or cattle, is claimed to cause delayed clinical manifestations of FMD[1] presumably caused by interferon. Because virulent strains of FMD seem ot produce less of IF in host animals[2] and the avirulent strains more of it, there is a suggestion that FMD virus multiplication in mice can be restricted if adequate concentrations of IF in serum or body tissues is maintained through endogenous production or exogenous augmentation.

We recently reported isolation of an inducer of interferon trivially designated as 6–MFA[3-5] obtained from the fungus *Aspergillus flavus* Du/KR/162 *b* (Syn. *A. ochraceus* ATCC 28706), which inhibits Semliki Forest Virus (SFV) infections in experimentally inoculated Swiss Albino mice. We now report the results of tests of antiviral activity of 6–MFA against FMD virus.

6–MFA, a polysaccharide nucleoprotein complex, was prepared as per the technique described by Maheshwari and Gupta[3]. FMD virus 'O' and 'A' were obtained from IVRI in the form of 10% (w/v) muscle homogenates of the infected mice. Both strains are well adapted to multiply in 2–3 weeks old mouse[6]. For 'O' strain the MST values were 4·8 and 5·1 days, and for 'A' they were 4·5 and 4·5 days (*see* Table I). To assess anti-FMD virus activity, mice were first treated with 6–MFA through intraperitoneal route at the rate of 2 ml and 1 ml doses per mouse. This quantity of 6–MFA, chosen to treat mice against FMD, corresponds to that which usually gives 80% protection against SFV infection in similar mice[3]. Treated animals were challenged 24 hrs. later with the viruses ('O' and 'A') given by intramuscular route at the rate of 0·25 ml of $10^{2.6}$ and $10^{1.6}$ of type 'O', and $10^{2.2}$ and $10^{1.2}$ of type 'A' respectively (100 and 1000 LD_{50}) per mouse. The LD_{50} contained in the inoculum was determined by Reed and Muench[7] method. Mice were observed twice daily morning and evening for the development of specific paralytic symptoms and death upto 14 days after which the experiments were terminated.

In general, the proportion of animals protected against type 'O' was more than in type 'A' indicating that FMD type 'O' virus appears to be *more sensitive* to the antiviral action of 6–MFA than the type 'A'. Even then, the MST of treated mice (type 'A' infected animals) has been found to vary directly with the quantity of the inducer (Table I). Furthermore, the MST here was seen to increase with the size (100 and 1000 LD_{50}) of the challenge inoculum. This type of response can be explained on the basis of the participation of a specific type of immune (antibody) response increasing with