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Sero-epidemiological survey on Yaba and 1211 virus infections among several species of monkeys

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SUMMARY

The distribution of neutralizing antibody to Yaba virus and 1211 agent in the sera of three Asian and one African monkey species was examined.

Cynomolgus (*Macaca irus*), bonnet (*M. radiata*) and rhesus (*M. mulatta*) monkeys possessed antibody to Yaba virus at incidences of 19·9, 8·4 and 0%, respectively. In African green monkeys (*Cercopithecus aethiops*) the incidence was as high as 76·4%.

As for 1211 agent, no Asian monkeys had neutralizing antibody and 5·5% of African green monkey sera neutralized the virus.

INTRODUCTION

Since the discovery by Bearcroft & Jamieson (1958) of a contagious dermal disease in rhesus monkeys kept in open pens at a medical institute in Yaba, Lagos, Nigeria, no outbreak of the same disease in monkeys has been reported despite the increasing number of monkeys that has been used for various scientific studies and viral vaccine production. The causative agent, Yaba virus, has properties common to members of the poxvirus group (Andrewes *et al.* 1959; Niven *et al.* 1961; Woodroofe & Fenner, 1962) and, when experimentally inoculated, caused histiocytic tumour* in Asiatic macaques such as *M. mulatta, M. irus, M. nemistrinus* and *M. fuscata* (Ambrus, Feltz, Grace & Owens, 1963; Niven *et al.* 1961; Kato, Tsuru & Miyamoto, 1965) and man (Grace & Mirand, 1963). African monkeys are, in general, resistant to the virus (Ambrus *et al.* 1963).

In the meantime, the occurrence of dermal tumours similar to Yaba tumours in rhesus monkeys was reported and the causative agent was identified also as a poxvirus (Hall & McNulty, 1967; Casey, Woodruff & Butcher, 1967). This virus was tentatively named as 1211 agent (Nicholas & McNulty, 1968), Yaba-related virus (Crandell, Casey & Brumlow, 1969) or Yaba-like disease virus (Hull, 1968). The histological appearances of this tumour and Yaba tumour were quite different from each other in that the former consisted of epidermal cells with vacuolated nuclei while the latter consisted of histiocytes without nuclear vacuolation (Casey *et al.* 1967; Hall & McNulty, 1967). The causative agents of both types of tumour,

* We use the word 'tumour' in the widest sense as suggested by Bearcroft & Jamieson (1958), Andrewes *et al.* (1959) and Niven *et al.* (1961).

however, shared common antigens in complement-fixation and neutralization tests (Nicholas & McNulty, 1968; Crandell *et al.* 1969).

The present experiments were carried out to determine the natural hosts and geographical distribution of both viruses among monkeys. The results presented revealed that at least the African green monkey is a natural host of Yaba virus and Asian monkeys also acquire natural infection with Yaba virus or a closely related virus. It was also demonstrated that at least the Asian monkeys examined were not natural hosts of 1211 agent. Sufficient evidence to support the view that the African green monkey is a natural host of 1211 agent could not be obtained.

MATERIALS AND METHODS

Cell cultures and media

A cynomolgus monkey kidney cell line JINET was used. The properties of JINET cells and media used for the growth and maintenance of these cells have been described (Tsuchiya, Takayama & Tagaya, 1969).

Viruses

Yaba virus was propagated and titrated in JINET cells as previously described (Tsuchiya *et al.* 1969). The 1211 virus was a generous gift from Dr W. P. McNulty of the Oregon Regional Primate Research Centre, U.S.A., and was propagated and titrated in JINET cells. The latter virus was assayed by the plaque method.

Monkey sera

Cynomolgus monkey (*M. irus*) sera were collected in our laboratory by heart puncture at the time of nephrectomy. Some of the sera were provided by the Department of Veterinary Science of our institute. Indian bonnet (*M. radiata*) and rhesus (*M. mulatta*) monkey sera were obtained through the courtesy of Dr R. A. Feldman of the Christian Medical College and Hospital, Vellore, South India (present address: N.C.D.C., Atlanta, Ga., U.S.A.). African green monkey (*Cercopithecus aethiops*) sera were donated by the Chemo-serotherapy Institute, Kumamoto. All the sera were heated at 56° C. for 30 min.

Antisera

Yaba virus antisera were collected from cynomolgus monkeys bearing multiple tumours at the time of bleeding. The 1211 virus antisera were obtained by immunizing rabbits. The rabbits were inoculated subcutaneously and intravenously with 0·5 ml. each of fluorocarbon-treated virus 4 times at intervals of 2–3 days and booster injections were given by both routes 1 month after the last injections. The sera were collected by heart puncture under anaesthesia 8 days after the booster injections. All the sera were inactivated at 56° C. for 30 min.

Neutralization test

Neutralization was carried out by mixing equal amounts of appropriately diluted virus and serum. The virus–serum mixtures were incubated at 36° C. for 1 hr. and then 4° C. overnight. In the case of Yaba virus neutralization tests, 0·2 ml. amounts of virus–serum mixtures were inoculated into replicate tube cultures whose growth medium was replaced with 1 ml. amounts of fresh maintenance medium just before inoculation. In 1211 virus neutralization tests, 0·2 ml. amounts of inoculum were allowed to adsorb onto drained monolayers grown in 2 oz. prescription bottles for 3 hr. at 36° C., after which nutrient agar medium was added in 5 ml. amounts. The titres of the sera were expressed as the reciprocals of the highest dilution which caused more than 50 % reduction of the cellular foci of Yaba virus or plaques of 1211 virus.

Plaque assay of 1211 *virus*

The plaque method used in these experiments was the same as that reported previously for the variola–vaccinia subgroup of poxviruses (Tsuchiya & Tagaya, 1970). As plaque formation by 1211 agent was also found to be enhanced by the addition of DEAE-dextran and $MgCl_2$ in the agar overlay medium (Table 1) as in the case of other poxviruses (Tsuchiya & Tagaya, 1970), both drugs were incorporated in the agar medium at concentrations of 100 μg./ml. and 25 mM respectively. The plaques were counted 8–14 days after inoculation.

Table 1. *Effects of DEAE-dextran and* $MgCl_2$ *on plaque formation by* 1211 *agent*

Additive		Plaque titre*
None		$4{\cdot}8 \times 10^4$
DEAE-dextran	100 μg/ml	$7{\cdot}0 \times 10^4$
$MgCl_2$	25 mM	$1{\cdot}2 \times 10^5$
	35 mM	$1{\cdot}4 \times 10^5$
	45 mM	$8{\cdot}8 \times 10^4$
DEAE-dextran Plus $MgCl_2$	100 μg/ml 25 mM	$1{\cdot}4 \times 10^5$

* Plaque titre is expressed in p.f.u./ml. Additives were contained in agar overlay medium.

RESULTS

Yaba virus-neutralizing antibody in various monkey species

Screening tests for Yaba virus-neutralizing antibody were carried out on 265 Asian monkey and 55 African green monkey sera at a serum dilution of 1/4. The geographical origins of the monkeys from which the sera were collected and the results of the screening tests are summarized in Table 2. As shown in the Table, the incidences of neutralizing antibody in cynomolgus monkey sera varied according to the places where the monkeys were captured. It is interesting that the cynomolgus monkeys from the Asian Continent had a rather high incidence of antibody, while those from island countries had a rather low (Indonesia, 4·5 %)

or no (Philippines) incidence. The monkeys captured in Cambodia, Vietnam and Malaya possessed neutralizing antibody in 40·5, 20·0 and 25·0% respectively. In total, cynomolgus monkeys had antibody in 19·9% (33/166).

As to Indian monkeys, 7 out of 83 bonnet monkey sera (8·4%) possessed antibody, while none of 14 rhesus monkey sera had the antibody.

The frequency of antibody reached as high as 76·4% in cercopithecus monkeys.

Table 2. *Yaba virus-neutralizing antibody in monkey sera*

(Screening test at 1/4)

Monkey species	Place captured	No. tested	No. positive	% positive
Macaca irus	Cambodia	37	15	40·5
	Vietnam	35	7	20·0
	Malaya	40	10	25·0
	Indonesia	22	1	4·5
	Philippines	32	0	0
M. radiata	India	83	7	8·4
M. rhesus	India	14	0	0
Cercopithecus aethiops	Uganda	55	42	76·4

The focus numbers in control cultures ranged from 47·8 to 185·0.

Table 3. 1211 *agent-neutralizing antibody in monkey sera*

(Screening test at 1/4)

Monkey species	Place captured	No. tested	No. positive	% positive
Macaca irus	Cambodia	15	0	0
	Vietnam	34	0	0
	Malaya	40	0	0
	Indonesia	29	0	0
	Philippines	23	0	0
M. radiata	India	72	0	0
M. rhesus	India	14	0	0
Cercopithecus aethiops	Uganda	55	3	5·5

The plaque numbers in control cultures ranged from 49·5 to 137·3.

The 1211 *agent neutralizing antibody screening*

Two hundred and twenty-seven cynomolgus, bonnet and rhesus monkey sera were tested for neutralizing antibody to 1211 agent. All the sera failed to neutralize the agent at a serum dilution of 1/4 (Table 3). Only 3 out of 55 African green monkey sera (5·5%) showed neutralization at that dilution (Table 3).

Cross neutralization test

Yaba virus monkey and 1211 agent rabbit antisera and normal cynomolgus and African green monkey sera which had neutralizing antibody to Yaba virus were assayed for both Yaba and 1211 virus antibodies. The titres of the sera against both viruses are summarized in Table 4. As shown in the table, normal and anti-Yaba monkey sera did not neutralize 1211 virus at a serum dilution of 1/4 while

their titres to homologous virus ranged from 16 to 4096. In a separate experiment 5 out of 11 Yaba virus monkey antisera, including two sera shown in Table 4, neutralized 1211 agent when tested at a dilution of 1/4. In contrast, 1211 antisera prepared in rabbits neutralized both viruses at almost identical titres.

Table 4. *Cross-neutralization test*

Serum	Code	Virus: Yaba	Virus: 1211
Yaba antisera (monkey)	8300	4096	<4
	8292	1024	<4
1211 antisera (rabbit)	A	4096	1024
	B	4096	4096
Normal cynomolgus monkey sera	6823	16	<4
	6842	16	<4
Normal African green monkey sera	G–52	4096	<4
	G–62	1024	<4

DISCUSSION

The circumstances under which Yaba virus infection was first recognized were rather interesting. In 1957, Asian monkeys developed tumours while they were kept in captivity at an African institution (Bearcroft & Jamieson, 1958) and this is the only reported case of a natural outbreak of the disease in the literature. Although Ambrus, Strandstrom & Kawinski (1969) reported a 'spontaneous' outbreak of Yaba virus infection in their rhesus monkey colony, their report implied that the monkeys acquired infection through insect vectors in their laboratory, where Yaba virus studies using monkeys had been extensively carried out. In spite of the innumerable number of monkeys used all over the world in the past decade, no Yaba virus tumour has been recognized in freshly captured Asian monkeys even though tumours induced in these animals are so conspicuous that they can hardly escape recognition during quarantine period. The monkeys which are native to Africa, where the only Yaba virus outbreak was recognized, were in general resistant to the virus (Ambrus *et al.* 1963). These peculiarities in Yaba virus epidemiology and ecology stimulated us to carry out a sero-epidemiological survey on this virus together with 1211 agent which shares common antigens with Yaba virus (Nicholas & McNulty, 1968; Crandell *et al.* 1969).

The present experiments showed that both Asian and African monkeys had neutralizing antibody to Yaba virus. The incidence of the antibody to Yaba virus in African green monkeys reached as high as 76·4 % (Table 2), and the neutralizing titre was also very high. These facts suggest that at least the African green monkey may be a natural host of the virus. Despite the high incidence of neutralizing antibody to Yaba virus, spontaneous occurrence of Yaba tumour has not been reported in this monkey species. This may partly be due to the inherent resistance of this monkey species to the virus in regard to tumourigenesis.

It is a rather surprising finding that cynomolgus monkeys had an antibody incidence of 19·9 % because no outbreak of Yaba virus infection in this monkey

species has ever been reported although the virus causes tumours in cynomolgus monkeys which can hardly be overlooked. There are three possible explanations for this. The first is that another poxvirus indigenous among Asian monkeys which has antigens common to Yaba virus may exist and this hypothetical virus causes silent infection in these monkeys. The significantly low neutralizing-antibody titre to Yaba virus in cynomolgus monkeys compared to that in cercopithecus monkeys lends support to this suggestion. The second possibility is that Yaba virus may by nature be a non-tumour producing virus even in Asian monkeys and the Yaba virus isolated in the African outbreak is a mutant virus which produces tumours efficiently in Asian monkeys. The third possibility is that Yaba virus may be spread by air among monkeys in natural conditions and the lesions caused by the virus may be restricted to the respiratory organs and escape recognition. Experimental infection of monkeys with Yaba virus by aerosol has been reported (Wolfe, Griesemer & Farrell, 1968). Tumours in their experiments, however, appeared also on the body surface of the inoculated monkeys. Either of these possibilities may explain the rare recognition of Yaba tumours in monkeys. Although we could not demonstrate neutralizing antibody in rhesus monkeys, this may be due to the small number of sera tested.

No Asian monkeys had neutralizing antibody to 1211 agent and only 5·5% of African green monkeys had the antibody. This finding suggests that at least the Asian monkey species examined are not natural hosts of 1211 agent. It could not be concluded from the results of the present experiments whether African green monkeys are, or are not, natural hosts of 1211 agent because 1211 agent shares common antigens with Yaba virus. The African green monkey sera positive for 1211 agent were also positive for Yaba virus.

Some of the Yaba virus monkey antisera had neutralizing antibody against 1211 agent while others did not. On the contrary, hyperimmune rabbit antisera against 1211 agent neutralized both homologous and heterologous viruses at almost the same efficiency. This difference of immune sera in ability to cross-react with heterologous viruses may be due to the difference of the immunized animals. A similar phenomenon was also reported in herpes simplex virus (HSV) and *Herpesvirus simiae* (B virus) (Ueda, Tagaya & Shiroki, 1968). Monkeys produced neutralizing antibodies to HSV as well as B virus upon immunization with B virus or HSV but guinea-pigs produced antibodies which specifically neutralized homologous virus when they were immunized with either HSV or B virus. The Yaba virus monkey antisera which neutralize only homologous virus may be useful for differentiation of these viruses by immunological methods.

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