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Serological evidence of infection with Tana and Yaba pox viruses among several species of monkey

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SUMMARY

Sera from cynomolgus monkeys from Malaysia, from Indian rhesus monkeys, from various species of monkeys from Africa and from South America have been examined for neutralizing antibody to Tanapox and Yaba viruses. No antibody was found to either virus in the sera of rhesus monkeys or South American monkeys. A certain proportion of sera from cynomolgus monkeys and various species of African monkey showed antibody to one or other of the viruses, but few of the positive sera showed antibody to both. The results would seem to suggest that infection with the two viruses is endemic in African and Malaysian monkeys but does not occur or is very rare in Indian rhesus and New World monkeys.

INTRODUCTION

Yabapox and Tanapox viruses readily infect macaque monkeys, although Tanapox virus was first isolated from the lesions of a patient who acquired infection during an outbreak in 1963 among Africans living in the Tana River valley in Kenya (Downie *et al.* 1971). Yaba virus has also been shown to be pathogenic for man on experimental infection and one accidental infection in a laboratory technician has been reported (Grace & Mirand, 1963). On epidemiological grounds it has been suggested that African monkeys are the natural hosts of both viruses (Niven *et al.* 1961; Schmidt, 1970; Downie *et al.* 1971). The two viruses, although similar in morphology, produce lesions in monkeys which are clinically and histologically different, the lesions of Yaba viruses being large tumour-like masses involving mainly mesodermal tissues while Tanapox virus produces superficial hyperplastic nodules affecting almost entirely epithelial cells. Although there is some serological overlap (España, 1971; Nicholas, 1970) the viruses are immunologically distinct (Kupper, Casey & Johnson, 1970; Downie & España, 1973).

Tsuchiya & Tagaya (1971) have recently reported on the presence of antibodies to Yaba virus and '1211 agent' in the sera of two species of Asian monkeys (*Macaca iris*, *M. radiata*) and of African green monkeys (*Cercopithecus aethiops*). This '1211 agent' isolated from an outbreak of disease in macaque monkeys in the Oregon primate centre (Hall & McNulty, 1967; Nicholas & McNulty, 1968) is the same virus as the 'Yaba-like' virus (YLD) isolated by España (1966) from a similar outbreak in the California primate centre and was later shown to be identical with Tanapox virus (Downie & España, 1972). Tsuchiya & Tagaya found

antibodies to Yaba virus in 19·9% of cynomolgus (*M. iris*) sera, 8·4% of bonnet (*M. radiata*) sera but none in rhesus (*M. mulatta*) sera, while in the sera of African green monkeys (*C. aethiops*) the incidence of Yaba antibodies was as high as 76·4%. Antibodies to Tanapox virus (1211 agent) were not found in the sera of any Asian monkeys and only in 5·5% of African green monkeys. A similar survey of antibodies to Yaba and Tanapox virus in monkey sera has now been made in Liverpool and is reported in this paper. Sera from cynomolgus and rhesus monkeys have been tested as well as sera from several species of African and South American monkeys. As shown below the results have differed in certain respects from those obtained by Tsuchiya and Tagaya (1971).

MATERIALS AND METHODS

Cell cultures

All neutralization tests were made in cultures of BSC1, a continuous line of grivet monkey kidney cells, grown in Leighton tubes. The culture medium was Eagle's medium with 10·0% foetal calf serum added; but the concentration of foetal calf serum was reduced to 1·0% when the tubes were inoculated with serum-virus mixtures.

Viruses

The Yaba virus stock used was prepared from sonically-disrupted BSC1 cells which had been infected with the 5th subculture of Yaba virus in BSC1 cells 12 days previously. The original virus had been supplied by Dr Allison in the form of Yaba tumour tissue from an experimentally infected rhesus monkey. The Tanapox virus stock was similarly prepared from virus grown in bottle cultures of BSC1 cells as described by Downie & España (1972).

Monkey sera

The sera from cynomolgus, rhesus and patas monkeys were kindly supplied by Dr Frank Perkins of the Division of Immunological Products Control of the Medical Research Council. The animals had been bled 5 or 6 weeks after their arrival in this country. The cynomolgus monkeys came from Malaysia, the rhesus monkeys from India and the patas monkeys from Kenya. The sera from most of the African green monkeys were sent by Dr P. B. Stones and Dr McHugh of Pfizer Laboratories. The animals had been collected in the Lake Awasa region of Ethiopia and had been bled 1 week or 5 weeks after they arrived in Sandwich, Kent. A few sera from African green monkeys were from animals caught in Kenya. Seventeen sera from baboons caught in Kenya were generously given by Professor Nelson of the London School of Hygiene and eight sera from baboons caught in Senegal were supplied by Mr Hackett of Shamrock Farms, Essex. The seventeen sera from cercopithecus monkeys in Cameroon were sent by Dr Jan of Institut Pasteur, Yaounde (5 *C. cephus*, 8 *C. nictitans* and 4 *C. pogonias*). Sera from other West African primates were kindly supplied by Dr Nakano of the Communicable Diseases Centre, Atlanta, Georgia. These were 26 chimpanzee sera from the Ivory Coast and 32 cercopithecus and colobus monkeys from Liberia (14 *C. campbelli*,

6 *C. petaurista*, 3 *C. diana*, 1 *C. atys*, 2 *C. nictitans* and 6 *Colobus badius*). Most of the South American sera were sent by Dr Melendez of the Massachusetts primate centre but 13, given by Dr Marguerite Pereira, came from animals caught and bled in the Amazon valley. The 104 South American sera came from 5 species of monkey, namely, spider monkeys (*Atelas geofroyii*) 8, squirrel monkeys (*Saimiri sciureus*) 29, owl monkeys (*Aotus trivirgatus*) 32, marmosets (*saquinus oedipus*) 22 and capuchin monkeys (*cebus albifrons*) 13.

Antisera for the two viruses were prepared in rhesus or vervet monkeys as described by Downie & España (1972) and were used as positive controls along with known negative control monkey sera in each batch of tests.

Neutralization tests

These were carried out as previously described (Downie & España, 1972). All sera were inactivated at 58° C. for 15 min. before test. The test viruses were used in a dilution calculated to give 50–100 lesions in each control culture tube with negative sera. All sera were first screened at a dilution of 1/10. These diluted sera were mixed with virus suspension and the tubes containing the mixtures were rocked in a water bath at 37° C. for 2 hr. before the mixtures were tested. Of each of these, 0·2 ml. was inoculated into each of three or four Leighton culture tubes and 1·2 ml. of culture medium added to each tube before incubation. Tanapox virus lesions were counted after incubation of the tubes for 6–10 days. Yaba lesions, which developed more slowly, were counted after 10–12 days. At first lesion counts were made on the unstained sheets with a binocular microscope at a magnification of × 10. Latterly it was found more convenient to stain the cell sheets in the tubes with 1% Crystal violet and then to count the lesions with a hand lens. Both methods gave essentially the same counts. All sera which showed definite or doubtful neutralization in a dilution of 1 in 10 were retested in three-fold dilutions from 1/10 upwards.

RESULTS

The results of tests for neutralizing antibody to Tanapox virus in the various monkey sera are shown in Table 1 and for antibody to Yaba virus in Table 2. The numbers of sera tested against the two viruses are different because a few sera which were examined for antibody to Tanapox virus were not available when the technique for estimating antibody to Yaba virus was developed. However, all the sera tested for Yaba antibodies were also tested for antibody to Tanapox virus. It will be seen from the results in Tables 1 and 2 that antibodies to both viruses were present in a proportion of the sera from various species of monkeys in East and West Africa and in the sera from cynomolgus monkeys from Malaysia. Neutralizing antibody was not found in the sera of rhesus monkeys from India nor in the sera of various species of monkey from South America. Although the percentage of positive results from the two viruses is roughly the same for the various species of monkeys tested, few individual sera were positive for both viruses (Table 3). Only nine sera showed antibody to both viruses; two were from African green monkeys, three were from cynomolgus monkeys, one from a chimpanzee

Table 1. *Tanapox-neutralizing antibody in monkey sera (screening test at 1/10 serum dilution)*

Monkey species	Place of origin	No. tested	No. positive	% positive
Cercopithecus aethiops	Ethiopia and Kenya	78	13	16·6
Erythrocebus patas	Kenya	50	1	2·0
Papio anubis	Kenya	29	5	17·2
P. anubis	Senegal	8	1	12·5
Cercopithecus (see text)	Fr. Cameroon	17	2	11·8
Chimpanzees	Ivory Coast	26	2	7·7
Cercopithecus and *Colobus* (see text)	Liberia	32	8	25·0
Macaca mulatta	India	61	0	0
M. iris	Malaysia	51	10	19·6
Various (see text)	S. America	104	0	0

Table 2. *Yaba neutralizing antibody in monkey sera (screening test at 1/10 serum dilution)*

Monkey species	Place of origin	No. tested	No. positive	% positive
Cercopithecus aethiops	Ethiopia and Kenya	63	10	15·9
Erythrocebus patas	Kenya	49	6	12·2
Papio anubis	Senegal	8	1	12·5
Cercopithecus (see text)	Fr. Cameroon	17	2	11·8
Chimpanzees	Ivory Coast	26	4	15·4
Cercopithecus and Colobus (see text)	Liberia	32	4	12·5
Macaca mulatta	India	50	0	0
M. iris	Malaysia	51	10	19·6
Various (see text)	S. America	100	0	0

Table 3. *Analysis of results of antibody to Tanapox and Yaba viruses in those monkey species showing antibody*

		Antibody to Yaba virus		
		+	−	Total
Antibody to Tana virus	+	9	24	33
	−	27	184	211
Totals		36	208	244

and three from Campbell's monkeys. The analysis of the results shown in Table 3 indicates that antibody to either virus may be induced independently of the other. It may be of interest to note that of 40 sera from natives in the Tana River area, 20 of which showed antibody to Tanapox virus, none had neutralizing antibody to Yaba virus (Manson-Bahr & Downie, 1973). As mentioned above, all sera showing definite or doubtful neutralization of virus at a serum dilution of 1/10 were retested in dilutions from 1/10 upwards. The titres from the positive samples varied from 1/10 to 1/100, this latter titre being attained by a few sera. The titres found in the present study were similar to those seen in monkeys and humans suffering from clinical infection with Tanapox or Yaba viruses (España, 1971; Downie & España, 1973).

Twenty monkey sera that showed neutralizing antibody to Tanapox virus were tested for antibody by the complement-fixation technique. None was positive in a dilution of 1/5 or higher. The sera were not examined by the complement-fixation inhibition test which Hall, Olsen, Pakes & Yohn (1973) have recently found to give positive results with the sera of monkeys recovered from Yaba and Tanapox infection after the ordinary complement-fixation test has become negative.

DISCUSSION

The results of the serological survey reported here differ in some particulars from those recorded by Tsuchiya & Tagaya (1971). The percentage of cynomolgus monkeys from Malaysia showing neutralizing antibody in their sera to Yaba was similar in both series; but antibody to Tanapox was not found in the Japanese survey, whereas in our survey 20% of the sera showed antibody. The absence of antibody to both viruses from 14 rhesus sera examined by Tsuchiya & Tagaya was confirmed in our experience. However, the high proportion of sera from green monkeys in Uganda showing Yaba antibodies (76%) was not observed in our tests on green monkeys from Ethiopia and Kenya. It may be that the 1/4 serum dilution used in the screening test of the Japanese workers was responsible for their greater proportion of positive results, although there was not a corresponding difference in the results with cynomolgus sera. Furthermore, the very high titres of Yaba antibody found by the Japanese workers in some African cercopithecus sera were not obtained in our tests on similar sera. Another difference in the results concerns the antibody to Tanapox virus in the sera of African monkeys. Whereas only three of 55 cercopithecus sera were found to contain antibody in the Japanese tests, the proportion of positive sera found by us was considerably higher (Table 1).

Clinical infection with Yaba virus was first observed in rhesus monkeys housed in captivity in West Africa, whereas clinical infection with Tanapox was first reported in natives living in Eastern Kenya. Nevertheless, the results of our tests on monkey sera suggest that infection of monkeys with the two viruses is equally prevalent in both East and West Africa.

Our results suggest that various species of monkey in Africa and cynomolgus monkeys in Malaysia suffer from infection, probably latent, with Tanapox or Yaba viruses. Indian rhesus monkeys, which appear to be highly susceptible to clinical infection with both viruses, apparently rarely if ever acquire infection in the wild state. Our tests with sera of several species of South American monkeys failed to provide evidence of infection with either virus in the New World.

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