THE PICORNAVIRUSES OF ACUTE HAEMORRHAGIC CONJUNCTIVITIS : A COMPARATIVE STUDY

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INTRODUCTION

Epidemic outbreaks of acute haemorrhagic conjunctivitis (the "Apollo 11 disease") in 1969 were reported by Charterjee *et al.* (1970) in Ghana, Akinsete (1970) and Parrott (1971) in Nigeria. Attempts to isolate the causative agent were unsuccessful. A pandemic outbreak of a clinically similar eye condition which involved Southeast Asia made its appearance in Singapore around September 1970 (Lim and Yin-Murphy, 1971 a,b). The etiologic agent has been identified as an ether and acid (pH 3.0) resistant picornavirus (Lim and Yin-Murphy, 1972, and Yin-Murphy 1972). Following our outbreak reports of similar attacks were received from Malaysia, Thailand, Indonesia, Hong Kong, Taiwan, Philippines and Japan between the end of 1970 and 1971. A second epidemic of acute conjunctivitis appeared in Singapore between June and December 1971. The picornavirus responsible for the second epidemic differed from that isolated in 1970 in its cytopathogenicity for primary monkey kidney cell cultures and neutralising-antigen. The S.E.C./1971 virus however, was antigenically very similar to the Japanese acute haemorrhagic conjunctivitis (A.H.C.) virus isolated in Japan in year 1971 (Yin-Murphy and Lim, 1972, and Yin-Murphy, 1973). Presented here is a comparative study to establish, the relationship of picornavirus isolated from epidemics in Singapore in 1970 and 1971, Japan in 1971 (Kono, 1972) and Hong Kong in 1971 (Chang, pers. comm.).

MATERIALS AND METHODS

Viruses: Two strains of Japanese acute haemorrhagic conjunctivitis virus (Jap.A.H.C. 648/1971 and Jap.A.H.C. 670/1971) were obtained from Dr. R. Kono of Central Virus Diagnostic Laboratory, National Institute of Health, Tokyo, Japan and two strains of Hong Kong epidemic conjunctivitis virus (HK 3454/1971 and HK 3751/1971) were obtained from Dr. W.K. Chang, Department of Microbiology, University of Hong Kong. Two strains of Singapore epidemic conjunctivitis virus (S.E.C. 24/1970 and S.E.C. 146/1971) were included in the study.

Properties of Viruses: The size of these viruses was determined by filtration through 50 nm and 25 nm diameter pore size "Millipore" filters. They were tested for ether and acid resistance (Andrewes and Horstmann, 1949, and Tyrrell and Chanock, 1963). The nature of their nucleic acid was determined by the acridine orange staining method (Mayor, 1964). Viruses established in Hela cell cultures was tested for their cytopathogenicity for *Cynomolgus* monkey kidney (MK) cell cultures, pathogenicity for one-day old suckling mice and against serum-pools containing 42 enterovirus antisera by methods described (Yin-Murphy, 1972).

The antigenic relationship between these viruses was determined by neutralisation tests in Hela cell cultures against monkey immune sera to these viruses. A 10^2 to 10^3 $TCID_{50}$ virus suspension was tested against series of 1/5 to 1/640 serum dilutions. Nine paired (acute and convalescent) sera collected during year 1970 and 9 paired sera collected during

year 1971 outbreaks in Singapore were tested for neutralising antibody to the above viruses.

RESULTS

Virus isolates S.E.C. 24/1970, S.E.C. 146/1971, HK 3454/1971, HK 3751/1971, Jap. A.H.C.648/1971 and Jap.A.H.C. 670/1971 passed through "Millipore" filters of 50 nm and 25 nm diameter pore size. They were resistant to ether and acid pH 3.0. Infected Hela cell cultures stained an orange-red colour with acridine orange showing them to be RNA viruses. The S.E.C. 24/1970 and HK 3751/1971 viruses were not cytopathogenic for MK cell cultures whilst the remaining viruses produce marked CPE within 5 to 8 days of inoculation. With the exception of HK 3751/1971 which killed suckling mice within 5-8 days of inoculation by the intracerebral route, and within 7-10 days of inoculation by the subcutaneous route, the remaining viruses were non-pathogenic for suckling mice. None of the viruses was neutralised by antisera to poliovirus types 1, 2, 3, echovirus types 1 to 7, 9, 11 to 27 and 29 to 33, coxsackievirus types A7, A9, A16 and B1 to B6.

As shown in Table 1, S.E.C. 24/1970 virus showed a minor two-way neutralising-antigenic crossing with HK 3751/1971 virus and a one-way crossing with Jap.A.H.C. 670/1971. No antigenic crossing was found between S.E.C. 24/1970 and the remaining viruses. The HK 3751/1971 virus, apart from showing minor neutralising-antigenic crossing with S.E.C. 24/1970, showed no crossing with the remaining year 1971 isolates from Singapore, Hong Kong and Japan. On the other hand, S.E.C. 146/1971, HK 3454/1971, Jap. A.H.C. 648/1971 and Jap.A.H.C. 670/1971 were antigenically related to each other. Of the 9 paired sera from year 1970 outbreak which had a four-fold or greater neutralising-antibody rise ranging from 1/40 to $\geq$ 1/160 serum dilutions to S.E.C. 24/1970 virus, 4 had neutralising-antibody rises ranging from 1/40 to 1/80 to HK 3751/1971 and 2 low neutralising-antibody (1/20) to Jap. A.H.C. 670/1971. No neutralising-antibody to the remaining year 1971 isolates was found at

Table 1

Antigenic relationship of acute haemorrhagic conjunctivitis viruses (N.T. in Hela cell cultures).

Virus	Monkey immune Sera				
	SEC24/1970	SEC146/1971	HK3751/1971+	HK3454/1971+	JapAHC648/1971
EC24/1970	320*	< 5	20	< 5	< 5
SEC146/1971	< 5	320	< 5	160	320
HK3751/1971	20	< 5	20	< 5	< 5
HK3454/1971	< 5	320	< 5	1280	$\geq$ 640
JapAHC648/1971	< 5	320	< 5	320	320
JapAHC670/1971	20	320	< 5	$\geq$ 640	160

* Reciprocal of serum dilution.
+ MK immune serum from Dr. W.K. Chang.
SEC = Singapore epidemic conjunctivitis.
HK = Hong Kong.
JapAHC = Japanese acute haemorrhagic conjunctivitis.

Table 2

Neutralising-antibody in sera collected in year 1970.

Patient's Serum No.	Viruses					
	SEC24/70	SEC146/71	HK3751/71	HK3454/71	JapAHC648/71	JapAHC670/71
1a	< 20*	< 20	< 20	< 20	< 20	< 20
1b	40	< 20	40	< 20	< 20	< 20
11a	20	< 20	< 20	< 20	< 20	< 20
11b	80	< 20	80	< 20	< 20	< 20
13a	20	< 20	< 20	< 20	< 20	< 20
13b	80	< 20	< 20	< 20	< 20	< 20
17a	< 20	< 20	< 20	< 20	< 20	< 20
17b	40	< 20	< 20	< 20	< 20	< 20
18a	40	< 20	< 20	< 20	< 20	< 20
18b	160	< 20	< 20	< 20	< 20	< 20
21a	20	< 20	< 20	< 20	> 20	< 20
21b	⩾160	< 20	< 20	< 20	> 20	< 20
25a	20	< 20	< 20	< 20	< 20	< 20
25b	80	< 20	< 20	< 20	< 20	< 20
29a	20*	< 20	< 20	< 20	< 20	< 20
29b	⩾160	< 20	40	< 20	< 20	< 20
51a	20	< 20	< 20	< 20	< 20	< 20
51b	40	< 20	40	< 20	< 20	20

a = first serum sample.
b = second serum sample.
* = reciprocal of serum dilution.
SEC = Singapore epidemic conjunctivitis.
HK = Hong Kong.
JapAHC = Japanese acute haemorrhagic conjunctivitis.

1/20 serum dilution (Table 2). Of 9 paired sera from year 1971, no neutralising-antibody to S.E.C. 24/1970 and HK 3751/1971 was detected whilst a fourfold or greater neutralising-antibody rise ranging from 1/40 to ⩾ 1/160 were found to the remaining year 1971 isolates, namely S.E.C. 146/1971, HK 3454/1971, Jap.A.H.C. 648/1971 and Jap.A.H.C. 670/1971 (Table 3). The antibody titres to these viruses were identical or approximately the same.

DISCUSSION

In citing the outbreak of "Apollo 11 Disease" in Africa in 1969 we cannot conclude that the pandemic outbreak of acute haemorrhagic conjunctivitis in Southeast Asian originated from Africa. Although accounts of similarities in clinical symptoms were recorded, laboratory confirmations are necessary to relate outbreaks in the continent and the Southeast Asian region.

Table 3

Neutralising-antibody in sera collected in year 1971.

Patient's Serum No.	Viruses					
	SEC24/70	SEC146/71	HK3751/71	HK3454/71	JapAHC648/71	JapAHC670/71
16a	< 20	< 20	< 20	< 20	< 20	< 20
16b	< 20	40	< 20	40	80	40
68a	< 20	< 20	< 20	< 20	< 20	< 20
68b	< 20	⩾160	< 20	80	⩾160	⩾160
71a	< 20	< 20	< 20	< 20	< 20	< 20
71b	< 20	80	< 20	80	80	40
76a	< 20	< 20	< 20	< 20	< 20	< 20
76b	< 20	80	< 20	80	80	40
90a	< 20	< 20	< 20	< 20	< 20	< 20
90b	< 20	⩾160	< 20	80	⩾160	80
133a	< 20	< 20	< 20	< 20	< 20	< 20
133b	< 20	80	< 20	80	⩾160	80
136a	< 20	< 20	< 20	< 20	< 20	< 20
136b	< 20	80	< 20	80	80	40
157a	< 20	< 20	< 20	< 20	< 20	< 20
157b	< 20	80	< 20	80	⩾160	80
159a	< 20	< 20	< 20	< 20	< 20	< 20
159b	< 20	⩾160	< 20	⩾160	⩾160	80

a = first serum sample.
b = second serum sample.
SEC = Singapore epidemic conjunctivitis.
HK = Hong Kong.
JapAHC = Japanese acute haemorrhagic conjunctivitis.

Although the identity of the picornavirus of epidemic conjunctivitis isolated in Singapore in 1970 and the picornaviruses subsequently isolated from Hong Kong, Japan and Singapore in 1971 remain unestablished, their etiologic significance have been confirmed by serological findings. Furthermore, comparative study carried out on viruses isolated from Hong Kong, Japan and Singapore points to their being related to each other.

The Singapore epidemic conjunctivitis (1970 and 1971), Hong Kong epidemic conjunctivitis (1971) and Japan acute haemorrhagic conjunctivitis (1971) viruses were identical in their resistance to ether, acid pH 3.0 and filtrability through "Millipore" filters of 25 nm diameter pore size. Infected Hela cells manifested the colour of RNA viruses when stained with acridine orange. In these properties they resemble the enteroviruses rather than the rhinoviruses. But, none of these viruses was neutralised by the 42 enterovirus antisera tested. These picornaviruses differ in their pathogenicity for monkey kidney cell cultures and pathogenicity for one-day old suckling mice. Whereas the

S.E.C. 24/1970 and HK 3751/1971 do not produce CPE in MK cell cultures, the S.E.C. 146/1971, HK 3454/1971, Jap.A.H.C. 648/1971 and Jap.A.H.C. 670/1971 produce CPE in this cell line. The HK 3751/1971 virus alone was pathogenic for suckling mice.

Some antigenic differences were noted amongst the strains studied. The S.E.C. 24/1970 virus showed minor neutralising-antigenic crossing with HK 3751/1971 and Jap.A.H.C. 670/1971 but no crossing with the remaining year 1971 viruses. Of the two year 1971 strains which showed minor neutralising antigenic crossing with S.E.C. 24/1970 virus, HK 3751/1971 showed no crossing with S.E.C. 146/1971, HK 3454/1971 and Jap.A.H.C. 648/1971 viruses whereas Jap.A.H.C. 670/1971 was antigenically similar to these viruses. The S.E.C. 146/1971, HK 3454/1971 and Jap.A.H.C. 648/1971 were antigenically very similar to each other. These relationships were substantiated by serological findings performed with sera collected during the year 1970 and 1971 outbreaks in Singapore. Furthermore, 9 patients'sera supplied by Dr. Dora Tan, Institute of Medical Research, Kuala Lumpur, Malaysia, from the 1970 outbreak in Malaysia showed good neutralising-antibody to our S.E.C. 24/1970 virus.

From the above data it appears that the pandemic outbreak of acute haemorrhagic conjunctivitis was caused by a group of antigenically related picornaviruses. These viruses of acute haemorrhagic conjunctivitis may represent new variants of an established enterovirus or a new enterovirus-type. Alternatively, they may be classified as a subgroup of the picornaviruses distinct from the subgroups enteroviruses and rhinoviruses on basis of the distinctly different type of clinical syndrome produced by these viruses. The first picornavirus of epidemic conjunctivitis isolated in Singapore in 1970 could have undergone antigenic changes giving rise to subtypes as exemplified by isolates from subsequent outbreaks in the following year. However, although antigenically related more than one type of picornavirus could have been involved in the pandemic outbreak in the Southeast Asian region during 1970 and 1971.

SUMMARY

A comparative study of picornavirus isolated from Singapore (1970, 1971) Japan (1971) and Hong Kong (1971) was made to establish their antigenic relationship.

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