

Isolation of a virus responsible for an outbreak of acute haemorrhagic conjunctivitis in Morocco

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SUMMARY

An epidemic of acute haemorrhagic conjunctivitis occurred in Morocco in 1970-1. It was caused by an enterovirus which appeared to be a new antigenic type similar to a virus isolated in South East Asia during the same period.

INTRODUCTION

In December 1970 and January 1971 an epidemic of acute haemorrhagic conjunctivitis (A.H.C.) occurred in several towns in Morocco (Bourdieu, 1972). Because no bacteria could be isolated from conjunctival secretions, these secretions were stored and sent frozen at -20°C . from Rabat to the WHO Regional Centre for Enteroviruses in Lyon, France. The study of this material led to the isolation of a virus in the conditions described below.

MATERIALS AND METHODS

Clinical material

We obtained 100 conjunctival scrapings and swabs from patients with A.H.C. in Morocco within a few days of the onset of illness.

Cell cultures

Primary cultures of cynomolgus monkey kidney cells were prepared in tubes and incubated without rolling at 36°C . Eagle's minimum essential medium and lactalbumin-Earle's medium, each with 10% calf serum, were used as growth media. The maintenance medium was Parker's 199.

Human embryo fibroblasts (WI 38) and HeLa cells were grown at 36°C . without rolling in Eagle's basal essential medium with 10% calf serum. The maintenance medium was Parker's 199 with 2% foetal calf serum.

RESULTS

Ten strains, R5, R6, R7, R11, R20, R39, R73, R86, R87 and R98, were isolated on HeLa cells. The CPE was comparable with that caused by agents of the Picornavirus group. Attempts to isolate viruses on other cells were all unsuccessful.

The ten strains belonged to the same antigenic type, as shown by neutralization tests using an antiserum prepared in a monkey (*M. irus*) by inoculation with strain R6.

Further tests on the strains revealed the following characters:

(i) Infected cells showed an eosinophilic intracytoplasmic inclusion similar to the typical Picornavirus inclusion.

(ii) In the acid stability test infectivity was not diminished at pH 3.0.

(iii) Electron microscopic examination showed a characteristic Picornavirus structure with a diameter of 30 nm. and no envelope.

(iv) The virus was not neutralized by antisera against any of the following viruses: poliovirus types 1-3, Coxsackie A types 1-24, Coxsackie B types 1-6, echovirus types 1-33.

(v) One of the strains (R39), when inoculated into baby mice subcutaneously, provoked a slight temporary flaccid paralysis at the first passage but no symptoms at the second passage. All the other nine strains failed to produce any flaccid paralysis after two passages.

(vi) Monkey antiserum prepared against strain R6 has been tested against prototype enterovirus strains. Up to the present time this serum has failed to neutralize poliovirus types 1-3, Coxsackie A types 1-24, Coxsackie B types 1-6 and echovirus types 1-33.

(vii) Cross-neutralization tests between the 'A' Japanese strain (Kono *et al.* 1972) and the Moroccan R6 virus, using sera prepared in monkeys against the Japanese strain in Tokyo and the Moroccan strain in Lyon, show that these two viruses are of the same antigenic type.

DISCUSSION

Outbreaks of epidemic haemorrhagic conjunctivitis occurred in 1969 in Ghana (Chatterjee, Quarcoopome & Apenteng, 1970*a, b*) and in Nigeria (Parrott, 1971). No virus was grown in these cases, but the disease, which was called 'Apollo 11 disease', was clinically and epidemiologically similar to that later described in South East Asia and Japan.

A cytopathic agent was isolated from cases of epidemic haemorrhagic conjunctivitis in Singapore (Lim & Yin-Murphy, 1971). Preliminary evidence from the laboratory investigations of the virus strains grown from conjunctival swabs suggested that the agent was not an adenovirus. Later (Yin-Murphy, 1972) the laboratory findings suggested that the virus was a Picornavirus, not neutralized by 42 available enterovirus antisera, and it was referred to as 'Singapore Epidemic Conjunctivitis (1970) Virus'.

At a WHO Regional Seminar in Manila in December 1971, it appeared that

Singapore, Indonesia, Malaysia, Cambodia, Thailand, Hong Kong, the Philippines and Taiwan had been experiencing extensive outbreaks of a new type of conjunctivitis.

In Japan, a virus was isolated in 1971 (Kono *et al.* 1972) and it is suggested that this agent is an enterovirus of a new serotype. No neutralization was observed with antisera against poliovirus 1-3, echovirus 1-33 (except types 10 and 28), reovirus 1, Coxsackie B1-6, Coxsackie A7, 9 and 16. Electron microscopy showed virions with cubic symmetry, and a diameter of 29 nm.

An outbreak of acute conjunctivitis was observed in Singapore in 1970, but the virus isolated appeared to be antigenically different from Singapore Epidemic Conjunctivitis 1970 virus (Yin-Murphy & Lim, 1972).

In 1971, North Africa experienced an outbreak of the new epidemic conjunctivitis and 10 strains of an enterovirus were isolated in Morocco. The results reported here suggest that these strains are similar to those obtained in Singapore and Japan.

Cross neutralization between the Japanese strain and the antiserum prepared against it in Tokyo and the Moroccan strain R6 and the antiserum prepared against it in Lyon suggests that the Japanese and North African strains are identical.

The monkey antiserum prepared against the Moroccan strain did not neutralize any of the existing prototype enteroviruses and, with the Japanese and Singapore results (Kono *et al.* 1972; Lim & Yin-Murphy, 1971; Yin-Murphy, 1972; Yin-Murphy & Lim, 1972) this reinforces the suggestion that the enterovirus isolated in each place is a new serotype.

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