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The investigation of a recurrence of an AHC virus epidemic at Lucknow: a serosurvey for AHC virus antibodies before and after the epidemic

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SUMMARY

An epidemic of acute haemorrhagic conjunctivitis (AHC) recurred at Lucknow during July to September 1975, after a gap of 4 years. Out of the 35 cases investigated thoroughly, 20 Entero-70-like cytopathogenic agents were isolated from the conjunctiva which were neutralized by antisera against AHC virus J670/71 of Japan. Seroconversion was seen in 7 out of 11 paired sera from patients. Serological study was also done on 100 sera collected before the AHC epidemic of 1971, 100 sera after 1971 and 100 sera after 1975 epidemic. There were no neutralizing antibodies in the pre-epidemic period, while 18 % of sera after the first epidemic and 32 % after the second epidemic showed antibodies. The incidence of antibodies was highest (43 %) in children aged below 10 years. Of the children born after the first epidemic, 44 % had antibodies. Thus our findings show that the AHC virus appeared for the first time at Lucknow in 1971 and the almost complete absence of disease in children, and its mildness during second epidemic, may be due to immunity.

INTRODUCTION

The pandemic of acute haemorrhagic conjunctivitis (AHC), which originated at two widely separated foci at Ghana in West Africa in 1969, and Java and Indonesia in 1970, reached India in 1971. The epidemic in India was first reported from Bombay in March 1971 (Wadia, Irani & Katrak, 1972, 1973; Bharucha & Mondkar, 1972), a period when 'Huj Pilgrims' return in large number from Mecca, and, it was believed that Huj Pilgrims brought the disease into India. The next place to be affected was Calcutta during April 1971 which coincided with the influx of refugees from Bangladesh into West Bengal and Calcutta (Pramanik, 1971; Roy, Roy & Ahmed, 1972). Later, the disease spread all over India. At the same time the disease had been spreading along the coastal areas of Asia and West Africa but probably India is the only country where inland areas far away from sea coast were also affected. The cases first appeared at Lucknow in May 1971 and were found to be due to mixed infection with adenovirus type 2 and the AHC (Entero-70) virus (Saxena, Bhatia & Chaturvedi, 1972; Chaturvedi *et al.* 1975). A similar epidemic occurred at Lucknow during July–September 1975. The present paper reports our findings on the virological investigation of the cases of this

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epidemic and also of the serological survey carried out on sera collected before and after the epidemic for the presence of antibodies against AHC virus.

METHOD AND MATERIALS

Study of patients

The present study was carried out on 35 typical cases of acute haemorrhagic conjunctivitis. These cases were selected out of a large number of patients who attended the ophthalmology outpatient department or came directly to us.

For isolation of virus conjunctival swabs were collected from these cases and were immediately put in Melnick and Earle's solution kept on wet ice. Acute and convalescent phase sera were collected from 11 of the above patients for serological study. Conjunctival scrapings obtained from all the patients were stained by Giemsa technique and screened for the presence of inclusion bodies.

Virus isolation

Conjunctival swabs were centrifuged and inoculated on HeLa cell culture the same day or as early as possible. Each sample was inoculated into four tissue culture tubes and incubated at 35 °C. The tubes were examined daily for 10 days for cytopathogenic effect. After third pass the viruses were identified by the neutralization test using antiserum against AHC virus (J670/71 M.S. No. 11859) which was kindly provided by Dr R. Kono, Japan.

Study of paired sera

The paired sera collected from 11 patients were studied for antibodies against AHC virus using one of the local strains No. 75518, which was neutralized by the antiserum provided by Dr Kono. The third passage stock having an infectivity titre of $10^{6.5}$ TCID 50 per 0·1 ml was used for serological study. The sera were inactivated at 56 °C for 30 min. Doubling dilutions of the sera were mixed with 70–100 TCID 50 of the virus in equal volumes and incubated at 37 °C for 1 h. Each serum–virus mixture was inoculated in four HeLa cell culture tubes. After incubation for 5 days at 35 °C the tubes were read and findings were recorded. Adequate controls were also put with each test.

Study of human population

Three hundred sera collected from the healthy general population of Lucknow were studied for the presence of antibodies against AHC virus. Out of these 100 sera were collected during 1969–70, before the first AHC epidemic of 1971, 100 sera during 1972–4 after the first AHC epidemic and another 100 sera were collected during 1975–6 after the second AHC epidemic of 1975. The sera were collected from both sexes, and from age groups varying from 1 to 75 years. Care was also taken to include sera collected from children born after the first AHC epidemic to see that antibodies had been acquired after the 1971 epidemic only.

All the above sera were inactivated at 56 °C for 30 min and tested for the presence of neutralizing antibodies in a fixed dilution of 1/8 of the serum against 70–100 TCID 50 of the AHC virus 75518 as described above.

Table 1. *Results of neutralization test with paired sera from eleven patients*

Patient no.	Age	First serum		Second serum	
		Day*	Titre	Day*	Titre
1	35	3	1/32	25	1/128
2	20	2	1/16	23	1/64
3	22	1	1/8	27	1/128
4	20	1	1/8	28	1/8
5	21	3	1/16	28	1/64
6	23	2	1/8	24	1/64
7	18	2	1/8	24	1/8
8	25	2	1/8	20	1/16
9	27	3	1/8	24	1/16
10	22	3	1/16	29	1/64
11	19	1	1/8	24	1/64

* Day of illness on which the blood was collected.

RESULTS

Cytopathogenic agents were isolated from 20 (57·1 %) of 35 conjunctival swabs. The CPE appeared by day 4–6 in first passage which stabilized to the third day by third passage. The CPE was similar to that described by Kono *et al.* (1972) for AHC virus. All the 20 strains isolated were neutralized by AHC virus antiserum (J670/71).

Adenovirus-like agents were not isolated from any of the patients, nor were adenovirus-like inclusion bodies seen in any conjunctival scrapings.

Eleven paired sera were tested for neutralizing antibodies against one of the strains isolated in this outbreak (75518). Antibody titres in the first sera varied from 1/8 to 1/32. Seven of the 11 sera (64 %) showed a fourfold or greater rise of titre in the second serum (Table 1).

The results of the neutralization test on the three groups of sera collected from the normal population, 100 before the first epidemic, 100 between the epidemics and 100 after the second epidemic, are shown in Table 2. In the first group all were negative at a titre of 1/8. In the second group 18 %, and in the third group 32 % showed neutralization titres of $\geqslant$ 1/8.

The distribution of antibodies between different age groups of the normal population is summarized in Table 3, where it is seen that the highest percentage of positives is found in early life, falling to a minimum between the ages of 20 and 40, and rising slightly after the age of 40. The numbers in the groups are too small for much significance to be attached to this last rise.

Table 4 shows the distribution of neutralizing antibodies in sera collected from children born after the first epidemic and after the second epidemic. Only those children were tested who were old enough for maternal antibodies to have disappeared. Of the four children tested in the first group only one showed a titre of $\geqslant$ 1/8. *Among the 50 sera tested in the second group, 23, or just under 50 %, had* antibodies.

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Table 2. *Neutralizing antibodies in the population at different periods*

Period	Year	Total sera	Positive
Pre-epidemic	1969–70	100	0
Post 1st epidemic	1972–74	100	18
Post 2nd epidemic	1975–76	100	32

Table 3. *Distribution of antibodies in different age groups*

Age (years)	Total sera	Total positive	Positive (%)
< 5	54	24	44
5–10	18	7	39
11–20	19	5	28
21–30	41	5	13
31–40	30	3	10
41–50	26	4	16
> 50	12	2	17

Table 4. *Antibodies in children born after the first AHC epidemic*

Period	Year	Total no.	Positive	Positive (%)
Post 1st epidemic	1972–74	4	1	25
Post 2nd epidemic	1975–76	50	23	46
Total	1972–76	54	24	44

DISCUSSION

During the outbreak of AHC which recurred at Lucknow during 1975, 20 cytopathogenic agents were isolated on HeLa cells. They were neutralized by the antisera against the Japanese strain of AHC virus J670/71. This shows that the AHC virus prevalent in India is antigenically closely related to the virus J670/71 of Japan. During the 1971 epidemic at Lucknow a more than fourfold rise of neutralizing antibodies against AHC virus strain EC2/71 of Singapore was observed in paired sera (Chaturvedi *et al.* 1975). Antigenic similarity between the strains of AHC viruses isolated in Japan (1971), Taiwan (1971), Hong Kong (1971), Thailand (1972), Indonesia (1972), Singapore (1972), Morocco (1971) and England (1971) was demonstrated by Kono, Sasagawa, Miyamura & Etsuko (1975). In a comparative study of the different strains isolated from the cases of AHC at Singapore, Japan and Morocco antigenic similarity was also observed by Mirkovic *et al.* (1973). Thus both the epidemics of Lucknow were due to similar strains which were closely related to the above strains of virus. Unlike the epidemic of 1971 (Chaturvedi *et al.* 1975), no adenovirus could be isolated nor could adeno-like inclusion bodies be demonstrated in 1975.

In the present epidemic fourfold or greater rise of neutralizing antibody was observed in 63·7 % of the paired sera against J670/71-like agent isolated locally. This shows that AHC virus only is responsible for the conjunctivitis. Similar seroconversion was observed in 77·3 % paired sera in Japan and 66·7 % in Tunisia against J670/71 virus (Kono *et al.* 1975). Yin-Murphy (1973) also reported antibody rise against Japanese AHC virus in paired sera in Singapore in the 1971 outbreak.

Seroepidemiological studies have been carried out in different countries for the presence of antibodies in sera collected before and after the AHC epidemic. It was noted that 2·4 % of 244 sera in Japan, 10·3 % out of 39 sera in Ghana and 6·3 % of 111 sera in Indonesia had virus neutralizing substance in a dilution of 1/8 before the epidemic (Kono *et al.* 1975). Some authors have considered these antibody rises to be due to exposure to an antigenically related virus and not to AHC virus. Hierholzer, Hilliard & Esposito (1975) have also shown presence of antibodies in three out of 1014 (0·3 %) sera of the population of eastern or south-eastern U.S.A. where epidemics of AHC have not occurred so far. Out of 100 sera collected before the epidemic at Lucknow none showed presence of antibodies in a dilution of 1/8. It appears therefore that AHC virus was completely new for Lucknow. This was probably the reason for the involvement of very large numbers of persons during the 1971 epidemic. It is likely that the disease came here from Calcutta as the two cities are well connected by road, rail and air with much traffic.

It was further noted that after the first epidemic 18 % of the sera became positive while after the second epidemic 32 % were positive. In the post-epidemic studies 39·7 % of sera in Ghana and 45·2 % in Indonesia were positive while in Japan only 8·2 % were positive (Kono *et al.* 1975). That these antibodies have been acquired after the AHC epidemic is further supported by the fact that a significant number of children (44 %) born after the AHC epidemic had antibodies.

Another interesting feature which has come out from the present study is the fact that the incidence of antibodies was highest (43 %) in children aged below 10 years. It may be noted that the attack rate of AHC was highest in the second and third decades and lowest in the first decade (Saxena *et al.* 1972; Chaturvedi *et al.* 1975; Kono, 1975). It was also noted that even in those families where most of the members were involved the children were spared or least affected (personal observations; Salami, 1971; Lin & Yin-Murphy, 1973). The findings of higher antibody incidence in children in the present study and in Indonesia (Kono *et al.* 1975) denote a high rate of subclinical infection and immunity in children which may be one of the factors protecting them from clinical disease.

A door-to-door epidemiological study has not been done, but on the basis of the impressions gained from the patients, the clinicians of this hospital and the private practitioners of the city, it appears that the severity of the illness was much less during the second epidemic with fewer infected patients than in 1971. During the 1971 epidemic a number of cases of neurological involvement, especially paralysis of the soft palate, were seen (Saxena *et al.* 1972). During the second epidemic, in spite of a close watch, no such patient was seen which may indicate mildness of infection. The lesser severity of the illness may be due to herd immunity developed as a result of the 1971 epidemic which showed rising antibody titres in paired sera against EC2/71 virus which is antigenically similar to that isolated in the present epidemic.

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