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Immunofluorescence test for the epidemiological monitoring of acute haemorrhagic conjunctivitis cases

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An epidemic of acute haemorrhagic conjunctivitis (AHC) occurred in and around Chandigarh, north India, during June, July and August 1981. Considering the difficulty of virus isolation, the indirect immunofluorescence test was used for the demonstration of virus-specific antigen in the cytoplasm of exfoliated conjunctival cells, using reference antisera. The epidemic appeared to be primarily due to enterovirus type 70. The method was found suitable for the rapid diagnosis of AHC cases and for detecting subclinical infection among healthy subjects and convalescent carriers. During follow-up, it was observed that some of the AHC cases were shedding virus-infected conjunctival cells for a prolonged period, even after clinical recovery and during convalescence, before they became free from virus. In the absence of virus isolation, this method can be considered as suitable for the epidemiological monitoring of AHC.

Epidemics of acute haemorrhagic conjunctivitis (AHC) have been occurring in various parts of the world since the first report from Ghana in 1969 (*1*). India and countries in south-east Asia, for example, were affected in 1970–71 (*5*, *7*), and there was an epidemic in India in 1981 which followed a small epidemic outbreak in 1975 (*2*). The virus involved in the majority of cases has been found to be enterovirus type 70 (E70) (*5*), and occasionally coxsackievirus A 24 variant EH 24/70 (*2*, *4*).

Since virus isolation by tissue culture is not feasible in all laboratories, it was thought that the demonstration of virus-specific antigen in infected conjunctival cells by immunofluorescence (IF) could be a means for the rapid diagnosis of AHC cases during an epidemic. The practical importance of this procedure for the detection of enterovirus-infected cells in spinal fluid has been documented (*8*). The specificity of the IF test for the detection of E70 antigen in infected conjunctival cells and MRC-5 tissue culture cells (human embryonic lung) has been evaluated (*10*). The present paper describes the use of this test for the epidemiological monitoring and follow-up of AHC cases and apparently healthy subjects.

MATERIALS AND METHODS

AHC cases

The 1981 epidemic occurred in India from June to August in and around Chandigarh, a city in the Himalayan foothills at about 180 metres above sea-level. The distribution of AHC cases (by sex, age and month) is shown in Table 1.

The conjunctival cells collected with swabs from AHC cases during the epidemic period (22 July to 25 August 1981) were processed for the IF test after acetone fixation at −20 °C, as described previously (*10*). Repeated samples were collected from 10 AHC cases during the epidemic; 3 of them were followed up (not shown in Fig. 2) till 5 days post-infection (p.i.) and 7 (No. 1–7 in Fig. 2) to a maximum of 76 days p.i. during their late convalescence.

Eye swab samples from 13 apparently healthy subjects (without any past history of conjunctivitis) were collected about 4 weeks after the end of the epidemic for use as controls. Two of them were followed up for

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Table 1. Distribution of 590 AHC cases during the 1981 epidemic in and around Chandigarh, by month, sex and age

	Month			Sex		Age groups (years)							
	June	July	August	M	F	0–10	11–20	21–30	31–40	41–50	51–60	61–70	> 70
No. of cases	35	401	154	408	182	44	89	225	121	62	33	12	4

a further period of about 22 days. A total of 81 samples were collected from 51 clinical cases and 13 control subjects.

About 4 months after the end of the epidemic, 2 more AHC cases who reported at the end of December 1981 and early in January 1982 were also investigated. Three eye swab samples were taken from them, including one repeat sample (from one of them) on day 29 p.i.

Some 3–7 replicate slides were prepared from each sample. Some of these were sent to the laboratory in Houston (TX, USA) for testing.

Indirect fluorescent antibody technique for detection of virus-specific antigen

The indirect IF test was carried out by the standard method (*6*). All samples were tested with E70 monkey immune serum in Houston, USA. Some of the samples were also tested with E70 (J 670/71) monkey immune serum obtained from Dr R. Kono in Japan. FITC-tagged antihuman IgG (γ-chain specific) at a dilution of 1:10 was used as the second antibody in the IF test in Chandigarh, since monkey immunoglobulins are known to cross-react with human immunoglobulins. In the Houston laboratory, the replicate slides were tested with E70 monkey immune serum (1:64 dilution during the initial stage and 1:5–1:10 dilution later on) and with FITC-tagged anti-rhesus monkey IgG serum (diluted 1:15) as a second antibody in IF tests.

RESULTS

Antigen positivity and follow-up

The infected conjunctival cells of 51 out of 53 AHC cases during the epidemic and postepidemic period in 1981–82 showed cytoplasmic fluorescence with E70 antiserum (Fig. 1). During the clinical stage, 5–50% of the exfoliated conjunctival epithelial cells showed diffuse cytoplasmic fluorescence. E70 antigen could not be detected in the conjunctival cells of two AHC cases. One of the 3 antigen-positive cases in the early follow-up group was found to be shedding virus-infected conjunctival cells 5 days p.i., just after clinical recovery. The antigen positivity among clinical cases and apparently healthy subjects is shown in Fig. 2. Most of the cases had clinically recovered by 5–7 days p.i. Four of the 7 clinical cases were shedding virus-infected cells long after their clinical recovery, for a period varying from 36 to 61 days p.i., even after the end of the epidemic. A localized cytoplasmic fluorescence, like an inclusion, instead of a diffuse fluorescence was noted in the conjunctival cells of case No. 2 on day 61 p.i., late in the period of convalescence. These cases were followed up till antigen-containing cells could not be demonstrated in the smears. However, the first sample from one AHC case (No. 7) was not suitable for testing because of secondary infection on day 3 p.i. Later he was shown to be shedding virus-infected conjunctival cells on day 36 p.i. (thirty-fourth post-epidemic day). Other cases (No. 1, 3 and 6) were found to be negative for E70 antigen in the second follow-up samples, collected on days 51, 50, and 37 p.i. respectively.

Among the 13 healthy subjects, only 2 (No. 8 and 10) were found to be shedding conjunctival cells containing E70 antigen during the post-epidemic period, and became virus-negative about 3 weeks after the first sampling (Fig. 2).

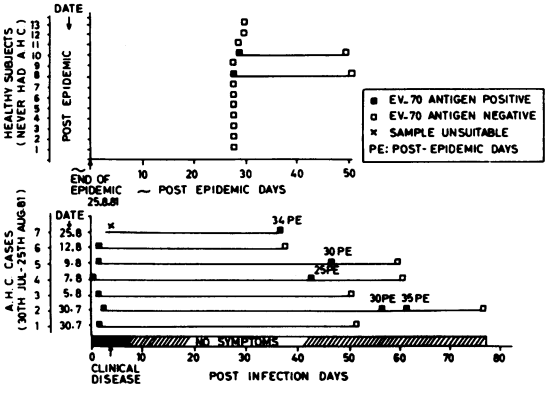

Fig. 2. Detection of E70 antigen, by the indirect immunofluorescence test, in the conjunctival cells from 7 AHC cases and from 2 out of 13 apparently healthy subjects; in the course of the post-infection period, all samples became antigen-negative.

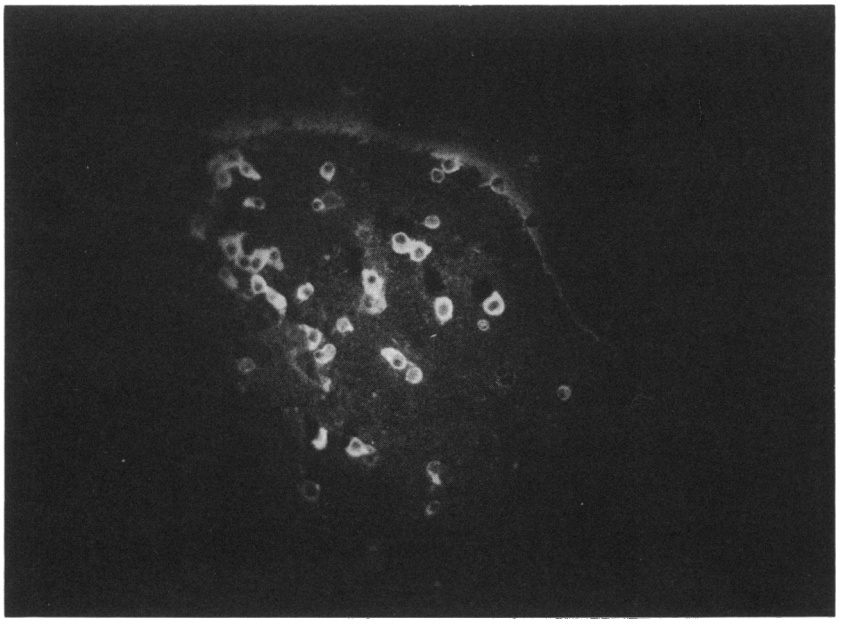

Fig. 1. Indirect immunofluorescence staining of conjunctival cells from an AHC case using E70 monkey immune serum to show the diffuse cytoplasmic fluorescence. (79×)

About 4 months after the end of the epidemic (in late December 1981), a 9-month-old child, with a history of contact with another child who had had conjunctivitis some 2 weeks earlier, was noticed to be suffering from mild conjunctivitis. The exfoliated conjunctival cells of this child revealed E70 antigen in the cytoplasm. On follow-up, the conjunctival cells collected on day 29 p.i. did not show virus-specific antigen. During early January 1982, a 21-year-old male with severe bilateral haemorrhagic conjunctivitis was found to be shedding E70-infected conjunctival cells on day 3 p.i. but he was not available for follow-up.

DISCUSSION

The immunofluorescence test has been used for rapid diagnosis of rabies, influenza, herpetic keratitis, and infections due to the respiratory syncytial virus and other viruses (*6*). It has also been used successfully for the demonstration of virus-specific antigen in the leukocytes of the cerebrospinal fluid from patients with enteroviral meningitis (*8*). Now for the first time it has been shown that the IF technique can be utilized for the rapid diagnosis of AHC cases due to infection with enterovirus 70. This test is most useful in cases where virus isolation is difficult, which was confirmed during the 1981 epidemic in India (*9*). The localized enterovirus antigen within the affected cells was demonstrated in the present study as a cytoplasmic fluorescence; and the specificity of the IF test was indicated by the gradual disappearance of these antigen-containing cells from previously positive subjects during follow-up. The specificity of this test for the detection of E70 antigen in virus-infected conjunctival and tissue culture cells has also been demonstrated (*10*).

Our results show that 51 out of the 53 AHC cases and 2 out of the 13 apparently healthy subjects were suffering from, respectively, clinical and subclinical infection with enterovirus 70. The IF test appeared to be very sensitive not only for the detection of clinical cases, but also for routine monitoring of subclinical cases and follow-up of the virus-carrier state during convalescence. In the present study, it was observed that virus replication in the conjunctival cells continued for as long as 61 days p.i. in one of the AHC cases, even after clinical recovery. The occasional emergence of clinical cases and occurrence of subclinical infection long after the end of the epidemic were also observed, which suggests that the virus persisted in nature during the post-epidemic period. Replication of the virus in the intestines of some cases with a post-conjunctivitis polio-like illness has also been noted (*3*). During the epidemic, the incubation period of this disease appeared to be 24 hours among family contacts, with probably direct spread of the virus because of the close contact. However, the exact time of entry of the enterovirus within families during the epidemic is not known. In the present study, one of the two sporadic cases (a 9-month-old child with mild conjunctivitis), which occurred about 4 months after the end of the epidemic, had a history of contact with a similar case in the neighbourhood about 2 weeks previously. Though the index case could not be studied, this enterovirus might have replicated in the intestine and produced mild conjunctivitis after an incubation period of 2 weeks, which is similar to the incubation periods for other enteroviruses. Another possibility is that the patient might have had contact with an inapparent carrier shortly before the onset. Thus, it appears that prolonged replication of the virus can occur not only in the intestine of some cases, as Kono et al. (*3*) have shown, but also in the conjunctiva long after clinical recovery, as shown in the present study.

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RÉSUMÉ

ÉPREUVE D'IMMUNOFLUORESCENCE DANS LA SURVEILLANCE ÉPIDÉMIOLOGIQUE DES CAS DE CONJONCTIVITE AIGUË HÉMORRAGIQUE

Une épidémie de conjonctivite aiguë hémorragique (CAH) s'est produite dans le nord de l'Inde, à Chandigarh et aux environs, en juin, juillet et août 1981. Des cas sporadiques ont été observés jusqu'au début de janvier 1982. Pour le diagnostic rapide de la CAH, on a mis au point une épreuve d'immunofluorescence indirecte destinée à détecter

l'antigène spécifique du virus dans le cytoplasme de cellules conjonctivales fixées à l'acétone, et qui avaient été prélevées à partir de malades et de sujets en bonne santé. Un immunsérum de référence, préparé sur le singe à l'égard de l'entérovirus 70 (E70) (dilué à 1:64), et une globuline anti-IgG humaine conjuguée à l'isothiocyanate de fluorescéine (diluée à 1:10) ont été utilisés à Chandigarh car on sait que les immunoglobulines de singe et les immunoglobulines humaines donnent des réactions croisées. A Houston (Etats-Unis d'Amérique), la spécificité de l'épreuve a été déterminée au moyen d'un immunsérum de référence dirigé contre E70 (dilution initiale à 1:64, ensuite de 1:5 à 1:10) et une globuline anti-IgG de singe rhésus conjuguée à l'isothiocyanate de fluorescéine (diluée à 1:15), qui ont été appliqués sur les lames fixées à l'acétone provenant de Chandigarh.

Cette épidémie a semblé principalement due à une infection par l'entérovirus 70. Les cellules conjonctivales provenant de 51 des 53 cas de CAH et de 2 des 13 sujets en bonne santé ont présenté une fluorescence cytoplasmique en présence de l'immunsérum anti-E70.

La conjonctivite aiguë hémorragique a été suivie pendant une période de 76 jours après infection chez 7 adultes (représentés à la figure 2) et un enfant âgé de 9 mois (non représenté dans la figure 2). Quatre de ces sujets ont éliminé des cellules conjonctivales infectées longtemps après leur guérison clinique, à savoir pendant une période allant de 36 à 61 jours après infection, avant de devenir virus-négatifs, à l'exception d'un cas qu'il n'a pas été possible de suivre au-delà. Les deuxièmes échantillons provenant des quatre cas restants, recueillis entre le 29ème et le 51ème jour après infection, se sont révélés négatifs. Deux porteurs en bonne santé sont devenus virus-négatifs au bout d'environ 3 semaines au cours de l'observation.

L'épreuve d'immunofluorescence semble être très sensible non seulement pour un diagnostic rapide des cas de CAH, mais aussi pour la détection de porteurs en bonne santé ou convalescents.

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