Conjunctivitis Due to Adenovirus Type 19

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An outbreak of unusual conjunctivitis occurred in Seattle in the summer of 1974. We found evidence of adenovirus type 19 (Ad19) infection in 28 of 42 (67%) referred cases from whom specimens for virus isolation and/or serology were obtained. Virus was isolated from conjunctiva, throats, and/or stools, often in week 2 of illness. Ad19-related cases more frequently had severe ocular pathology, transient visual impairment, and active disease for longer than a week. Secondary illnesses were more frequent in relation to Ad19 infection (26%) than to conjunctivitis of other etiology (5%, $P < 0.05$). Persons with Ad19 infection commonly experienced sore throats without coughs or fevers. No common source of infection was identified in the outbreak, and illness was unrelated to occupation, residence, or family characteristics. A 3- to 8-day incubation period was suggested by two related cases.

First cultured in 1955 from a Saudi Arabian child with trachoma (2), adenovirus type 19 (Ad19) has been isolated in Europe and North America since 1973. Related eye disease has included conjunctivitis, keratoconjunctivitis, and uveitis, in some cases clinically identical to that due to Ad8. (2-4, 7-9, 12-14, 18)

Ad19 has been implicated in common source outbreaks of keratoconjunctivitis (12, 14), but the mechanism of transmission in community outbreaks is still poorly understood.

In July, 1974, a Seattle ophthalmologist reported two cases of an acute "atypical" conjunctivitis with marked subconjunctival hemorrhage. These were the first Ad19 infections recognized in Seattle and were part of a small outbreak of conjunctivitis that lasted until early autumn. This report describes the occurrence of Ad19-related illness in Seattle from July, 1974, to July, 1976.

METHODS

Case finding. From June, 1974, through July, 1976, viral surveillance was conducted in the University of Washington Affiliated Eye Clinics, and conjunctival swabs for virus isolation were taken from any patients with acute conjunctivitis or keratoconjunctivitis, regardless of presumed etiology. In July, 1974, we learned of unusual eye illness in the community and contacted all 76 private ophthalmologists in the Seattle area and all University of Washington ophthalmology residents, asking for referrals. Patients referred between 1 July and 15 October 1974, were studied in detail. We interviewed cases as soon as possible, usually in the home, and obtained blood and specimens

for virus isolation as available. Eye examination results were collected from the referring physicians' records.

Virus isolation. Cotton swabs or scrapings from conjunctiva, throat swabs, and/or stool specimens were obtained during active conjunctivitis, usually in week 2 of illness. Specimens were processed and stored as described previously (5).

Each specimen was inoculated onto two different types of cultured cells. Diploid human fetal tonsil (FT) cells were inoculated with 0.1 ml of specimen per tube and incubated on a roller drum at 33°C for 2 weeks, with microscopic examination every 2 to 3 days. In the absence of cytopathic effect after 2 weeks, cells and culture fluid were passaged to fresh tubes and observed for another 2 weeks. If cytopathic effect was not evident at this time, results were reported as negative.

HeLa cell cultures were inoculated with 0.1 ml of specimen and incubated at 37°C in stationary racks. Cultures not showing cytopathic effects were passaged after 1 week and reported as negative after 1 more week. Confirmatory passages were made on cultures showing suspicious cytopathic effects.

Isolates were typed by neutralization with type-specific Ad hyperimmune sera (Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health).

Serology. Blood specimens were obtained from cases and contacts as early as possible in the index illness and from 2 to 5 weeks after onset, occasionally later. Sera were tested for neutralizing antibody to Ad3, Ad8, and Ad19, by the microtiter neutralization technique previously described (6). Sera were also tested for complement-fixing antibody to Ad group antigen. Control serum specimens drawn between 15 July and 30 September 1974 from 180 patients treated for acute pneumonia at a local outpatient clinic were tested for neutralizing antibody to Ad3 and Ad19. Acute and convalescent serum pairs, drawn from eight persons in relation to a previous outbreak of pharyn-

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goconjunctival fever due to Ad3 (10), were also tested for Ad19 neutralizing antibody.

RESULTS

Case finding. Between July and October, 1974, private ophthalmologists referred patients with hemorrhagic conjunctivitis. Surveillance in the University of Washington eye clinics found 11 Ad19-related conjunctivitis cases during the same period, but only 6 more over the next 22 months, despite an increasing number of specimens examined.

Clinical features of the conjunctivitis cases studied are shown in Tables 1 and 2, by etiology. This analysis includes only those cases referred because of hemorrhagic conjunctivitis. Nine cases without adequate isolation specimens and/or convalescent sera are excluded. Patients in the Ad19 group all had laboratory evidence of

Ad19 infection (isolation and/or serology). Those listed as "Other" had presumed viral conjunctivitis but negative specimens. The two categories were comparable with respect to source of referral and to quality of specimens obtained. The percentages shown in Tables 1 and 2 were calculated by assuming that features not specified were absent (percentage of total), and by assuming that only those features specified were evaluated (percentage of specified). Ad19 conjunctivitis, as compared with similarly referred cases of other etiology, more frequently involved photophobia, transient visual impairment, keratitis (usually epithelial), and preauricular adenopathy. Ad19 conjunctivitis began abruptly, worsening over several days. In contrast to conjunctivitis of other etiology, Ad19 conjunctivitis usually lasted more than a week ($P < 0.005$, the Fisher exact test). Recovery was complete in all cases.

TABLE 1. Symptoms of patients with conjunctivitis, by etiology

Symptom	No. specified ^a	Ad19 (N = 28)		Other (N = 14)	
		% Positive of total	% Positive of specified	% Positive of total	% Positive of specified
Fever	25/14	14	16	29	29
Malaise	25/14	36	40	43	43
Headache	26/12	32	35	7	8
Nausea/vomiting	26/14	21	23	29	29
Diarrhea	26/14	11	12	14	14
Sore throat	25/13	50	56	21	23
Cough	25/14	14	16	7	7
Coryza	26/14	39	42	21	21
Foreign body sensation	25/14	82	92	64	64
Eye pain	25/13	71	80	64	69
Photophobia	24/12	79 ^b	92 ^c	36	42
Discharge	27/14	86	89	93	93
Impaired vision	27/12	71 ^c	74 ^c	14	17

^a Number of Ad19/other patients with symptom specified in interview or on clinic record.

^b $P < 0.05$, the Fisher exact test, two tailed (Ad19 versus other).

^c $P < 0.01$, the Fisher exact test, two tailed (Ad19 versus other).

TABLE 2. Signs in patients with conjunctivitis, by etiology

Sign	No. specified ^a	Ad19 (N = 28)		Other (N = 14)	
		% of total	% of specified	% of total	% of specified
Lid edema	26/12	82	88	79	92
Follicles	20/5	43	60	14	40
Preauricular adenopathy	17/10	43 ^b	71 ^b	0	0
Keratitis	24/10	57 ^b	67 ^c	7	10
Epithelial only		36	42	7	10
Subepithelial only		4	4	0	0
Both epithelial and subepithelial		4	4	0	0
Ambiguous or unspecified		14	17	0	0

^a Number of Ad19/other patients with sign specified on clinic record.

^b $P < 0.01$, the Fisher exact test, two tailed (Ad19 versus other).

^c $P < 0.05$, the Fisher exact test, two tailed (Ad19 versus other).

Transmission of infection. Ad19 conjunctivitis was seen most frequently in adult males. Both sexes were affected, and cases ranged in age from 2 to 63 years. Differences in age, sex, family size, and presence of small children at home between Ad19 and other conjunctivitis cases were not statistically significant. Ad19 conjunctivitis was not related to residence, occupation, place of employment, recent eye examination, use of swimming pools, or use of public washrooms. No link was found with the cases seen concurrently in Portland, Ore. (4).

Illness in household contacts, occurring within 10 days of the index illness, is summarized in Table 3. Conjunctivitis occurred significantly more often in contacts of Ad19 conjunctivitis cases. Upper respiratory illness was also more frequent (not significant) in Ad19-related contacts. The highest Ad19 neutralizing antibody titer seen was in an Ad19-related contact with coryza, sore throat, and general malaise for several days, but without conjunctivitis.

Of 21 Ad19-related contacts from whom serum was obtained, 13 (62%) had Ad19 neutralizing antibody, including 4 (19%) who were asymptomatic.

Ad19 neutralizing antibody was detected in serum from 2 of 180 controls. Both were females in their late twenties, with no history of conjunctivitis in the previous 2 months. Neutralizing antibody to Ad3 was not found in any of these 180 specimens.

One case cluster was suitable for approximating the incubation period of Ad19 conjunctivitis. A family physician developed Ad19 conjunctivitis after treating a child with Ad19 infection. His only contact with the child occurred 3 and 8 days before his illness began. Another link suggested a 7-day incubation period, but multiple exposure could not be ruled out.

Virus isolation and antibody studies. Ad19 was recovered from eye, throat, and/or stool specimens, even in week 2 or 3 of active illness (Table 4). We were unable to isolate any viruses except Ad19 from those patients referred with hemorrhagic conjunctivitis. Other viruses

found by surveillance included herpes simplex, Ad3, and Ad8.

Acute (≤ 7 days after onset of illness) and convalescent (≥ 14 days after onset) serum pairs were obtained from only five referred patients. One of these patients, from whom Ad19 was isolated, had a fourfold or greater rise in Ad19 neutralizing antibody. The four patients who showed no rise in antibody titer included one who yielded Ad19 from a conjunctival specimen. Four other patients had fourfold or greater rises in Ad19 neutralizing antibody, even though the first specimen was obtained 10 to 12 days after eye symptoms began. From 13 Ad19 conjunctivitis cases, we obtained both a conjunctival swab during active illness and a convalescent phase serum specimen. Ten (77%) were isolation positive, and 12 (92%) had neutralizing antibody to Ad19.

Two of the five persons with a fourfold or greater rise in neutralizing antibody to Ad19 also had a fourfold rise to Ad3. We obtained at least one serum specimen from 59 persons—either referred cases or their contacts. In these specimens, presence of neutralizing antibody to Ad19 was strongly associated with antibody to Ad3 (chi-square test with continuity correction, one degree of freedom = 16.5, $P < 0.001$ [Table 5]). None of the eight serum pairs tested from a previous common-source outbreak of Ad3-related illness had neutralizing antibody to Ad19, although five of the eight had a fourfold or greater rise to Ad3 (10).

DISCUSSION

Epidemiology. Transmission of Ad19 infection, as indicated by prevalence of type-specific

TABLE 4. Ad19 isolation, by timing of specimen^a

Days after onset	Eye ^b	Throat ^b	Stool ^b
0-5	No specimens	No specimens	No specimens
6-10	4/7	2/4	0/1
11-15	2/5	2/3	2/2
16+	2/3	1/2	

^a Ad19-associated cases only.

^b Number positive/number of specimens.

TABLE 3. Illness in household contacts, by etiology

Contact	Ad19		Other	
	No.	%	No.	%
Total	42	100	38	100
Ill	13	31	2	5 ($P < 0.01$)
Conjunctivitis	11	26	0	0 ($P < 0.01$)
Upper respiratory illness	4	10	1	3
Conjunctivitis and upper respiratory illness	2	5	0	0
Other	0	0	1	3

TABLE 5. Neutralizing antibody to Ad3 and Ad19 among 59 referred cases and contacts

Reciprocal titer to Ad19	Reciprocal titer to Ad3						
	<4	4	8	16	32	64	128
128			1				
64			1				
32	1		1			1	
16	1	2	3	1	1		
8		2	5	4	2		
4			1	1			
<4	19	5	6	1			

antibody, has not been measured in open American populations, but is generally presumed to be low. Our finding of Ad19 neutralizing antibody in 2 of 180 persons, sampled without regard to Ad19 exposure, supports this impression, but is not conclusive. In contrast, Jung and Wigand (15) found Ad19 antibody by hemagglutination inhibition in 110 of 698 sera (15.8%) examined in West Germany before 1967. Ad19 neutralizing antibody was detected in 34 of 40 (85%) sera each, taken from healthy adults in Zaire (1970) and Mali (1972) (9). These results suggest recent arrival in the United States of an agent capable of substantial spread.

Recent occurrence of Ad19 conjunctivitis has been sporadic in some instances and related to common-source infection in others (12, 14). A Schiotz tonometer (14) and a dirty linen roll towel in a factory washroom (12) have been implicated in transmission. In late 1973, proven Ad19 conjunctivitis appeared concurrently in a common-source industrial outbreak (10 cases) and, independently, in the surrounding Nashville community (11 cases) (12). The Seattle cases that we report represent a widely scattered community outbreak, with no apparent common infectious source. We found no relation to known risk factors or to a variety of demographic variables. Although many of our cases were male adults, this may merely reflect treatment-seeking behavior and be unrelated to risk of disease.

Presumably, missing links in the chain of transmission involve infected persons either with less-severe eye symptoms, with some other anatomic site affected, or with no symptoms of any kind. We observed antibody-positive household contacts of Ad19 conjunctivitis in each of these categories. The importance of respiratory spread of the virus remains to be determined. We and others (16) have isolated Ad19 from throat cultures. Ad19 conjunctivitis patients commonly complained of sore throat, but only 10% reported cough. Our finding of Ad19 in stool specimens suggests that fecal/oral spread may be important, as with other Ad's, especially in children (11). Virus shedding in feces may predispose an infected person to eye contamination and thus to conjunctivitis.

Recording milder disease in contacts, we found household secondary attack rates higher than those reported previously (12), but the difference was not significant statistically.

Laboratory. Cross-neutralization between Ad19 and Ad3 has not been described (17). The association that we observed between presence of neutralizing antibody to Ad19 and Ad3 is not likely due to chance, even though the association is weaker when antibody titers are compared.

These findings might suggest that some persons are at high risk of adenoviral and/or eye infections. More likely is the possibility of a minor antigenic relationship between Ad19 and Ad3. Ad19 infection may frequently induce an anamnestic response to Ad3 in a person previously infected with Ad3. Although we were unable to detect neutralizing antibody to Ad19 in sera from patients infected with Ad3, these persons probably had no prior experience with Ad19.

Clinical. Physical signs, recorded usually several weeks before laboratory results were available, showed a consistent relation to etiology, even though compiled from the charts of 18 examining physicians. Compared with other conjunctivitis cases detected by identical criteria, those related to Ad19 were different in a number of respects. Ad19 conjunctivitis was more prolonged and more likely to show severe ocular pathology and disturbance of function. Nonetheless, these differences are not significant by the more conservative criteria appropriate for exploratory studies of this kind (1) and need to be confirmed by further research. Comparing Ad19 conjunctivitis with cases of other but unknown etiology may help to identify clinical features characteristic of severe Ad19 illness. Signs and symptoms observed with similar frequency in both groups may in part represent pathology likely to cause an affected person to seek care by an ophthalmologist.

Follicles were described in 60% of our Ad19 conjunctivitis cases. We found only two patients with subepithelial keratitis, although epithelial keratitis was recorded in 40%. These results differ from those reported in other studies (14, 16). It seems unlikely that such important signs would be present, yet selectively unrecorded by most referring physicians. The clinical spectrum of Ad19-related eye disease and its overlap with that due to other viruses remain controversial (3). Epidemiological studies of Ad19, therefore, should be based on laboratory rather than clinical diagnoses.

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LITERATURE CITED

1. Armitage, P. 1971. Statistical methods in medical research, p. 202-207. Blackwell Scientific Publications, London.
2. Bell, S. D., T. R. Rota, and D. E. McComb. 1960. Adenoviruses isolated from Saudi Arabia. III. Six new serotypes. *Am. J. Trop. Hyg.* 9:523-526.

3. Burns, R. P., and M. H. Potter. 1975. Epidemic keratoconjunctivitis due to adenovirus type 19. *Trans. Am. Ophthalmol. Soc.* 73:55-63.
4. Burns, R. P., and M. H. Potter. 1976. Epidemic keratoconjunctivitis due to adenovirus type 19. *Am. J. Ophthalmol.* 81:27-29.
5. Cooney, M. K., C. E. Hall, and J. P. Fox. The Seattle virus watch. III. Evaluation of isolation methods and summary of infections detected by virus isolations. *Am. J. Epidemiol.* 96:286-305.
6. Cooney, M. K., G. E. Kenny, R. Tam, and J. P. Fox. 1973. Cross relationships among 37 rhinoviruses demonstrated by virus neutralization with potent monotypic rabbit antisera. *Infect. Immun.* 7:335-340.
7. Darougar, S., M. P. Quinlan, J. A. Gibson, B. R. Jones, and D. A. McSwiggan. 1977. Epidemic keratoconjunctivitis and chronic papillary conjunctivitis in London due to adenovirus type 19. *Br. J. Ophthalmol.* 61:76-85.
8. Dawson, C. R., D. O'Day, and D. Vastine. 1975. Adenovirus 19, a cause of epidemic keratoconjunctivitis, not acute hemorrhagic conjunctivitis. *N. Engl. J. Med.* 293:46.
9. Desmyter, J., J. C. DeJong, K. W. Slaterus, and H. Verlaeckt. 1974. Keratoconjunctivitis caused by adenovirus type 19. *Br. Med. J.* 2:406.
10. Foy, H. M., M. K. Cooney, and J. B. Hatlen. 1968. Adenovirus type 3 epidemic associated with intermittent chlorination of a swimming pool. *Arch. Environ. Health* 17:795-802.
11. Foy, H. M., and J. T. Grayston. 1976. Adenoviruses, p. 53-69. In A. S. Evans (ed.), *Viral infections of humans: epidemiology and control*. Plenum Medical, New York.
12. Guyer, B., D. O'Day, J. C. Hierholzer, and W. Schaffner. 1975. Epidemic keratoconjunctivitis: a community outbreak of mixed adenovirus type 8 and type 19 infection. *J. Infect. Dis.* 132:142-150.
13. Hierholzer, J. C., B. Guyer, D. O'Day, and W. Schaffner. 1974. Adenovirus type 19 keratoconjunctivitis. *N. Engl. J. Med.* 290:1436.
14. Jackson, W. B., P. L. Davis, V. Groh, and R. Champlin. 1975. Adenovirus type 19 in Canada. *Can. J. Ophthalmol.* 10:326-333.
15. Jung, D., and R. Wigand. 1967. Epidemiology of group II adenoviruses. *Am. J. Epidemiol.* 85:311-319.
16. O'Day, D. M., B. Guyer, J. C. Hierholzer, K. J. Rosing, and W. Schaffner. 1976. Clinical and laboratory evaluation of epidemic keratoconjunctivitis due to adenovirus types 8 and 19. *Am. J. Ophthalmol.* 81:207-215.
17. Stevens, D. A., M. Schaeffer, J. P. Fox, C. D. Brandt, and M. Romano. 1967. Standardization and certification of reference antigens and antisera for 30 human adenovirus serotypes. *Am. J. Epidemiol.* 86:617-633.
18. Taylor, J. W., J. W. Chandler, and M. K. Cooney. 1975. Acute hemorrhagic conjunctivitis associated with adenovirus 19. *N. Engl. J. Med.* 292:978-979.