JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1995, p. 2728–2731 Vol. 33, No. 10
0095-1137/95/$04.00+0

Adenovirus Types 2, 8, and 37 Associated with Genital Infections in Patients Attending a Sexually Transmitted Disease Clinic

PAUL D. SWENSON,[1,2]* MICHAEL S. LOWENS,[1] CONNIE L. CELUM,[1,3] AND JOHN C. HIERHOLZER[4]

Seattle-King County Department of Public Health, Seattle, Washington 98104[1]; Department of Laboratory Medicine[2] and Department of Medicine,[3] University of Washington, Seattle, Washington 98195; and the Respiratory and Enterovirus Branch, Division of Viral and Rickettsial Diseases, Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[4]

Received 10 April 1995/Returned for modification 30 May 1995/Accepted 13 July 1995

Adenoviruses (Ads) are an important cause of respiratory illness, conjunctivitis, and gastroenteritis, but they are seldom recognized as a potential cause of sexually transmitted disease. We performed virus cultures on approximately 7,000 patients attending a sexually transmitted disease clinic or other health department clinics for the evaluation of genital ulcers, urethritis, or conjunctivitis. Ads were isolated from genital or conjunctival specimens obtained from 23 (0.33%) patients. Among the 20 Ad-positive men, 15 (75%) had urethritis, 12 (60%) had conjunctivitis, and 10 (50%) had both. All three Ad-positive women had vaginal discharge and genital ulcers or fissures. Ad isolates from 17 patients were available for serotyping. Ad type 37 was isolated from 14 patients, Ad type 8 was isolated from 2 patients, and Ad type 2 was isolated from 1 patient. In three of the Ad type 37 cases, Ad was recovered from both urethral and conjunctival specimens. One of the Ad type 8 cases had conjunctivitis, but the Ad type 2 case did not. Ads, particularly type 37, may be a sexually transmissible cause of genital ulcers, urethritis, and conjunctivitis.

The 47 currently recognized adenovirus (Ad) serotypes are divided into 6 subgroups (subgenera A to F) and are associated with a wide variety of clinical illnesses (7–9). Ad types 3 and 7 (subgenus B) and types 1, 2, and 5 (subgenus C) frequently cause respiratory illnesses in children; types 8, 19, and 37 (subgenus D) are commonly associated with epidemic keratoconjunctivitis (EKC); and types 40 and 41 (subgenus F) cause gastroenteritis in infants and young children. Subgenus A and E Ads and other Ad serotypes are less common causes of human disease or are nonpathogenic.

Although Ads are not widely recognized as a cause of genital infection, types 19 and 37 have been isolated from the genital tracts of patients attending sexually transmitted disease (STD) clinics in Australia (4, 5, 15, 16), The Netherlands (3, 17), Italy (2), and Sweden (3). Less frequently, types 1, 2, 8, 9, 10, 22, 26, and 32 have been isolated from urethral swabs (5), types 2 and 18 have been identified in endometrial tissues (14), and type 2 has been isolated from cervical swabs (3, 4). Genital Ad infections are associated with urethritis and penile ulcers in men (2–5, 16) and cervicitis and labial ulcers in women (3, 4, 15–17). Some patients with genital Ad infection also have conjunctivitis caused by the same serotype (2–5, 15–17).

In this study, we describe genital and oculogenital Ad infection and associated clinical findings in a series of patients attending an STD clinic in the United States.

MATERIALS AND METHODS

Patients. Patients attending the Seattle-King County Department of Public Health STD Clinic at Harborview Medical Center or other health department clinics who were cultured for herpes simplex virus (HSV) during the 4-year period from October 1989 to September 1993 were included in the study. Urethral or endocervical cultures for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were routinely performed on men and women, respectively. Syphilis serology was also routinely performed on all patients. HSV cultures were carried out for patients with undiagnosed genital ulcers and atypical or probable genital herpes lesions and on some patients with urethritis or conjunctivitis. Patients with at least two of the following were considered to have urethritis: a history of urethral discharge or dysuria, the presence of urethral discharge on examination, and a urethral Gram-stained smear showing five or more polymorphonuclear leukocytes per high-power field (1,000×). A clinical diagnosis of conjunctivitis was made if patients had conjunctival erythema and injection.

Collection of specimens. All specimens for virus culture were collected by experienced nurse-practitioners and physician assistants. Although the area around the collection site was not disinfected, genital specimens were carefully obtained to minimize local contaminants from urine or stool which may contain Ads. For urethral specimens, a Dacron swab on an aluminum shaft (Dacroswab, Spectrum Laboratories, Houston, Tex.; or Pur-Wraps, Hardwood Products, Guilford, Maine) was inserted approximately 1 cm, rotated, and removed. Genital lesion and conjunctival specimens were obtained by abrasion, as tolerated, with a Dacron swab on an aluminum or plastic shaft.

Virus cultures. Genital or conjunctival swab specimens were placed in 2 ml of viral transport medium containing Hanks' balanced salt solution, 0.5% gelatin, 1,000 U of penicillin per ml, 1,000 μg of streptomycin per ml, and 2.5 μg of amphotericin B per ml. Specimens were inoculated into two tubes of MRC-5 human embryonic lung fibroblasts (BioWhittaker, Walkersville, Md.) and one tube of A549 human lung carcinoma cells (ViroMed, Minnetonka, Minn.) and incubated at 36°C in stationary racks. Tubes of MRC-5 cells were received weekly and maintained in Eagle's minimum essential medium with 2% fetal bovine serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 0.25 μg of amphotericin B per ml. A549 cells were grown in-house each week in the same medium except with 10% fetal bovine serum, and were maintained as described above for MRC-5 cells. Inoculated cell cultures were examined daily for cytopathic effect (CPE) for 1 to 2 weeks before being discarded.

Virus identification. Ad isolates were initially identified by immunofluorescence staining of cell smears prepared from cultures with typical Ad CPE using a monoclonal antibody specific for Ad (Bartels Diagnostics, Issaquah, Wash.). When available, Ad isolates were sent to the Respiratory and Enterovirus Branch, Centers for Disease Control and Prevention, for confirmation by time-resolved fluoroimmunoassay (TR-FIA) (10) and electron microscopy and also subgrouping and serotyping by differential hemagglutination (HA), hemagglutination inhibition (HI), and serum neutralization (SN) assays (6, 7, 9, 11).

RESULTS

Patients with positive cultures. Ad was isolated from 23 (0.33%) patients out of approximately 7,000 who were cultured for HSV during the study period (Table 1). HSV was recovered from about 2,500 (36%) of these patients, none of whom had concurrent Ad infection. Among the 20 culture-positive men, Ad was isolated from the urethra in 10, the conjunctiva in 5,

* Corresponding author. Mailing address: Seattle-King County Department of Public Health Laboratory, 1303 Public Safety Building, Seattle, WA 98104. Phone: (206) 296-4740. Fax: (206) 296-4596.

TABLE 1. Characteristics of STD clinic patients with genital or conjunctival adenovirus infection

Patient no.	Age (yr)	Race	Sex	Sexual preference	No. of sex partners in the past 2 mo	New sex partner in the past 2 mo	HIV antibody result	Diagnosis/symptoms	Site(s) of virus isolation	Adenovirus serotype
1	30	White	Male	Homosexual	5	Yes	NA[a]	Urethritis, gonorrhea	Urethra	NA
2	43	White	Male	Heterosexual	1	No	Negative	Urethritis	Urethra	NA
3	52	White	Male	Homosexual	2	Yes	NA	Urethritis, conjunctivitis	Urethra, eye	NA
4	37	White	Male	Heterosexual	2	No	NA	Urethritis, conjunctivitis	Urethra	NA
5	28	Black	Male	Heterosexual	2	Yes	NA	Urethritis, conjunctivitis	Urethra	NA
6	27	White	Female	Heterosexual	1	No	Negative	Vaginal discharge, genital ulcers, mucopurulent cervicitis	Cervix	NA
7	26	White	Male	Heterosexual	3	Yes	NA	Dysuria, genital warts	Urethra	37
8	22	Black	Male	Heterosexual	1	Yes	NA	Urethritis, conjunctivitis	Urethra, eye	NA, 37 37
9	28	White	Male	Homosexual	NA	NA	Positive	Conjunctivitis	Eye	37
10	28	Hispanic	Male	Heterosexual	1	No	Negative	Urethritis, genital ulcers	Urethra	37
11	22	White	Male	Homosexual	6	Yes	Negative	Urethritis, conjunctivitis	Eye	37
12	36	NA	Male	Heterosexual	3	Yes	NA	Urethritis, conjunctivitis	Urethra	37
13	17	White	Female	Heterosexual	2	Yes	Negative	Vaginal discharge, cervical ulcers, pelvic inflammatory disease	Cervix	37
14	30	White	Male	Homosexual	1	Yes	Negative	Dysuria, genital warts, conjunctivitis	Urethra, eye	37
15	27	White	Female	NA	NA	NA	NA	Vulvar lesion, yeast vaginitis	Vulva	2
16	26	Black	Male	Homosexual	1	Yes	Negative	Urethritis, conjunctivitis	Eye	8
17	30	Black	Male	Heterosexual	1	Yes	Negative	Urethral erythema	Urethra	8
18	21	White	Male	Homosexual	3	Yes	Negative	Urethritis, genital warts	Urethra	37
19	20	Black	Male	Heterosexual	2	No	Negative	Urethritis, genital ulcers, conjunctivitis	Eye	37
20	42	White	Male	Heterosexual	2	Yes	NA	Urethritis, conjunctivitis	Eye	37
21	45	Black	Male	Heterosexual	1	No	Negative	Urethritis	Urethra	37
22	37	White	Male	Homosexual	2	Yes	NA	Proctitis, gonorrhea	Rectum	37
23	38	Black	Male	Heterosexual	1	Yes	Negative	Urethritis, conjunctivitis	Urethra, eye	37

[a] NA, not available.

both the urethra and conjunctiva in 4, and the rectum in 1. Among the women, Ad was isolated from the cervix in two and the vulva in one. These 23 Ad-positive patients had a mean age of 31.0 years (range, 17 to 52). Fourteen patients were white, seven were black, one was Hispanic, and race was unknown for one. Recent sexual activity was known for 21 patients, with a mean of 2.0 (range, 1 to 6) sexual partners during the previous 2 months. Fifteen of these 21 patients had a new sex partner during the previous 2 months. Sexual preference was known for 22 patients, of whom 8 were homosexual men, 12 were heterosexual men, and 2 were heterosexual women. Among the 13 patients with known human immunodeficiency virus (HIV) antibody status, 1 of 5 homosexual men was HIV antibody positive and all 8 heterosexual men and women were negative.

Clinical findings. Of the 20 Ad-positive men, 15 had dysuria, 13 had urethral discharge, 6 had urethral erythema or tenderness, 6 had inguinal lymphadenopathy, and 12 had eye pain. Fifteen (75%) of these 20 men were considered to have urethritis, 12 (60%) had conjunctivitis, and 10 (50%) had both. Of the five men without clinically defined urethritis, three had dysuria or urethral erythema alone, one had proctitis, and one had conjunctivitis. *N. gonorrhoeae* was also isolated from the urethra of a man with urethritis and from the rectum of the man with proctitis. Of the three Ad-positive women, all had vaginal discharge and genital ulcers or fissures, one had mucopurulent cervicitis, one had pelvic inflammatory disease, and one had yeast vaginitis. None of the Ad-positive women had conjunctivitis. No other sexually transmitted etiological agents were identified in these 23 patients. However, coinfection with other STD agents cannot be ruled out as the cause of symptoms in these patients.

Ad serotypes involved. Typical Ad CPE appeared a median of 6 (range, 3 to 13) days after inoculation for 20 isolates from 17 patients with detailed cell culture information available. Ad CPE was usually observed earlier and progressed more rapidly in A549 cells than in MRC-5 cells. Two isolates were only recovered in A549 cells. Nineteen Ad isolates from 17 patients were available for additional testing at the Centers for Disease Control and Prevention. All 19 of these isolates were positive for Ad antigen and negative for HSV antigen by TR-FIA. Electron microscopy on selected isolates also confirmed the presence of Ad and the absence of herpesvirus. The HA results were typical of HA subgroup 2A for 18 isolates, including 16 Ad isolates identified as type 37 by HI and SN assays and 2 isolates identified as type 8. The remaining Ad isolate had an HA pattern typical of HA subgroup 3A and was identified as type 2 by HI and SN assays. In two patients, Ad isolates from the urethra and eye were both identified as type 37. In a third patient, an Ad isolate from the eye was identified as type 37 but the Ad isolate from the urethra was not available for serotyping. Ad type 37 was isolated from 13 men and 1 woman, type 8 was recovered from 2 men, type 2 was isolated from 1 woman, and Ad isolates from 5 men and 1 woman were not available for serotyping. The 14 cases of Ad type 37 infection occurred sporadically during the study period with no obvious seasonal pattern.

DISCUSSION

In this study, we found that Ads were occasionally isolated from either the genital tract, the conjunctiva, or both sites in patients attending an STD clinic who were cultured for HSV in the evaluation of genital ulcers, urethritis, or conjunctivitis. Among the 17 patients whose Ad isolates were serotyped, all but 3 were infected with Ad type 37. Many of our male patients who were culture positive for Ad had urethritis, which frequently occurred together with conjunctivitis. These findings, together with those reported previously from outside the United States, suggest that Ad type 37 may be a significant oculogenital pathogen in sexually active immunocompetent adults.

Ad type 37 first appeared in 1976 and rapidly emerged as an important cause of EKC (7, 12). Ad infection of the genital tract was first observed in Australia in 1977 (4, 15), followed by The Netherlands (3, 17), Italy (2), and Sweden (3). Many of the Ads isolated in these studies were initially thought to be type 19, but most isolates were subsequently identified as type 37 (12, 16). In a more recent Australian study, Ads were isolated from the urethras of 0.36% of 35,800 men attending an STD clinic (5). Urethritis was present in 73% of 129 culture-positive men compared to only 23% of 129 culture-negative controls matched for age, sex, and date of specimen collection. These findings are very similar to our results. We recovered Ads from 0.33% of genital or conjunctival specimens collected from STD clinic patients. In addition, 75% of our 20 male patients with positive cultures for Ad had urethritis. Our Ad isolation rate probably would have been higher if we could have subpassaged all our cultures, but time and expense precluded our doing this on a routine basis. Unlike the Australian study in which only 14% of men with Ad-positive urethral cultures had conjunctivitis (5), our study found that 60% of our Ad-positive male patients had conjunctivitis. In addition to the single case of Ad type 2 isolated from a vulvar lesion in this study, Ad2 has been found in two cervical swabs in a Dutch study (3), in three cervical swabs in an Australian study (4), in 14 endometrial tissues in a Hungarian study (14), and in two urethral swabs from Australian men (5). Thus, of the Ads, the role of type 2 in genital disease is second only to that of type 37.

Although Ad type 37 was the predominant genital pathogen in our study and in those previously reported, other serotypes have been found occasionally in the same tissue sites. Most of the other types reported, Ads 1, 2, 9, 10, 18, 22, 26, and 32, were recovered from clearly genital sites and were not associated with conjunctivitis in these patients (3–5, 14). Ad type 8, however, was associated with oculogenital disease in one of two cases in this study. As with type 37, the occurrence of conjunctivitis in Ad type 8 cases would not be considered unusual because both serotypes are known major agents of EKC and follicular conjunctivitis (7).

What is so unusual about one of the Ad type 8 cases here is that the virus was recovered from the urethra, as in three previously reported Australian cases (5). Compared to the other Ads causing conjunctivitis, which cause multisystem disease and are usually isolated from multiple sites, Ad type 8 has only rarely been associated with respiratory or gastrointestinal symptoms and has been recovered almost exclusively from the eye. Type 8 was isolated from two sequential urine specimens from a 26-year old male with pre-AIDS lymphadenopathy and from throat, stool, and eye specimens from a 31-year old male AIDS patient with pharyngitis, pneumonia, gastroenteritis, and conjunctivitis (8). Ad type 8 was also recovered from throat swabs from one army recruit with acute respiratory disease, from throat and eye swabs from another recruit with acute respiratory disease and EKC, and from throat swabs of 10 of 43 patients with EKC but without any respiratory symptoms (19). Finally, in a worldwide survey based on World Health Organization compilations, Ad type 8 has been recovered, albeit infrequently, from respiratory and gastrointestinal tracts of children (0.18% of the virus associations reported) and of adults (1.39% of the virus associations reported) (18).

Two observations regarding Ad genital isolates remain unexplained. First, Ad type 37 and Ad type 8 have identical predilections for ocular tissues but not for genital tissues. Second, the serotypes associated with genital disease (types 2, 8, 19, and 37 and rarely others) have not been found in cases of acute hemorrhagic cystitis in children, and conversely, the viruses causing cystitis (types 11, rarely 21, 34, and 35) do not appear to cause genital lesions (7). Whether Ads 2, 8, 19, and 37 will prove to be important in neonatal Ad infections (such as those caused by types 3, 7, 21, 30, and others) remains to be seen. Neonatal Ad infections appear to result from respiratory or generalized illness in the mother around the time of vaginal delivery and are often fatal (1, 13, 20).

Ads, particularly type 37, may be a sexually transmissible cause of genital ulcers, urethritis, and conjunctivitis. The overall prevalence of isolation of Ads from samples collected in our STD clinic population was relatively low (0.33%), but all 23 Ad-infected patients had clinical manifestations of ocular or genital disease. Further studies are indicated to elucidate the association of sexual practices with Ad infection, to determine prevalence in HIV-positive and HIV-negative patients, and to determine the spectrum of clinical manifestations and natural history of oculogenital Ad infection.

REFERENCES

1. **Abzug, M. J., and M. J. Levin.** 1991. Neonatal adenovirus infection: four patients and review of the literature. Pediatrics **87:**890–896.
2. **Cevenini, R., M. Donati, M. P. Landini, and M. La Placa.** 1979. Adenovirus associated with an oculo-genital infection. Microbiologica **2:**425–427.
3. **de Jong, J. C., R. Wigand, G. Wadell, D. Keller, C. J. Muzerie, A. G. Wermenbol, and G. J. P. Schaap.** 1981. Adenovirus 37: identification and characterization of a medically important new adenovirus type of subgroup D. J. Med. Virol. **7:**105–118.
4. **Harnett, G. B., and W. A. Newnham.** 1981. Isolation of adenovirus type 19 from the male and female genital tracts. Br. J. Vener. Dis. **57:**55–57.
5. **Harnett, G. B., P. A. Phillips, and M. M. Gollow.** 1984. Association of genital adenovirus infection with urethritis in men. Med. J. Aust. **141:**337–338.
6. **Hierholzer, J. C.** 1973. Further subgrouping of the human adenoviruses by differential hemagglutination. J. Infect. Dis. **128:**541–550.
7. **Hierholzer, J. C.** 1989. Adenoviruses, p. 219–264. *In* N. J. Schmidt and R. W. Emmons (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections, 6th ed. American Public Health Association, Inc., Washington, D.C.
8. **Hierholzer, J. C.** 1992. Adenoviruses in the immunocompromised host. Clin. Microbiol. Rev. **5:**262–274.
9. **Hierholzer, J. C.** 1995. Adenoviruses, p. 947–955. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
10. **Hierholzer, J. C., K. H. Johansson, L. J. Anderson, C. J. Tsou, and P. E. Halonen.** 1987. Comparison of monoclonal time-resolved fluoroimmunoassay with monoclonal capture-biotinylated detector enzyme immunoassay for adenovirus antigen detection. J. Clin. Microbiol. **25:**1662–1667.
11. **Hierholzer, J. C., Y. O. Stone, and J. R. Broderson.** 1991. Antigenic relationships among the 47 human adenoviruses determined in reference horse antisera. Arch. Virol. **121:**179–197.
12. **Kemp, M. C., J. C. Hierholzer, C. P. Cabradilla, and J. F. Obijeski.** 1983. The changing etiology of epidemic keratoconjunctivitis: antigenic and restriction enzyme analyses of adenovirus types 19 and 37 isolated over a 10-year period. J. Infect. Dis. **148:**24–33.
13. **Kinney, J. S., J. C. Hierholzer, and D. W. Thibeault.** 1994. Neonatal pulmonary insufficiency caused by adenovirus infection successfully treated with extracorporeal membrane oxygenation. J. Pediatr. **125:**110–112.
14. **Kulcsar, G., J. Domotori, P. Dan, I. Nasz, S. Keskeny, J. Horvath, and P. Geck.** 1975. Virological studies on gynecological patients. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. A **231:**389–392.
15. **Laverty, C. R., P. Russell, J. Black, N. Kappagoda, R. A. Ben, and N. Booth.** 1977. Adenovirus infection of the cervix. Acta Cytol. **21:**114–117.

16. **Phillips, P. A., G. B. Harnett, and M. M. Gollow.** 1982. Adenovirus type 19 and a closely related new serotype in genital infections. Br. J. Vener. Dis. **58:**131–132.
17. **Schaap, G. J. P., J. C. de Jong, O. P. van Bijsterveld, and W. H. Beekhuis.** 1979. A new intermediate adenovirus type causing conjunctivitis. Arch. Ophthalmol. **97:**2336–2338.
18. **Schmitz, H., R. Wigand, and W. Heinrich.** 1983. Worldwide epidemiology of human adenovirus infections. Am. J. Epidemiol. **117:**455–466.
19. **Tai, F. H., J. T. Grayston, P. B. Johnson, and R. L. Woolridge.** 1960. Adenovirus infections in Chinese army recruits on Taiwan. J. Infect. Dis. **107:**160–164.
20. **Towbin, J. A., L. D. Griffin, A. B. Martin, S. Nelson, B. Siu, N. A. Ayres, G. Demmler, K. J. Moise, and Y. H. Zhang.** 1994. Intrauterine adenoviral myocarditis presenting as nonimmune hydrops fetalis: diagnosis by polymerase chain reaction. Pediatr. Infect. Dis. J. **13:**144–150.