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Rapid Diagnosis of Respiratory Adenovirus Infections in Young Adult Men

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Rapid viral diagnosis was attempted in 106 military conscripts with pneumonia and in 101 military conscripts with other types of respiratory infections. Nasopharyngeal suction specimens (NPS) were assayed for viral antigens by immunofluorescence and enzyme immunoassay (EIA). Sputum specimens from 97 pneumonia patients were assayed for viral antigens by EIA. Also, 71 NPS and 13 sputum specimens were examined for the presence of adenovirus DNA by a sandwich hybridization (HYB) method. The reference test was adenovirus isolation in cell culture from the NPS. Adenoviruses were isolated from 6 pneumonia patients and from 20 patients with other respiratory infections. Of these 26 NPS, rapid diagnosis was successful in 13, 16, and 14 cases by EIA, immunofluorescence, and HYB, respectively. Four antigen-positive specimens were found among the 181 specimens which were negative by virus isolation. Sputum was found to contain adenovirus antigen by EIA in 5 of 97 tested specimens. Of these 97 specimens, 13 were selectively tested in HYB, and a positive signal was observed in 4 cases. Serological testing of paired sera revealed 23 adenovirus infections in the pneumonia group and 42 in the group with other respiratory infections. Other viral infections were found only sporadically. All rapid virus detection methods showed excellent specificity but had a lower sensitivity (60%) than virus isolation. Our results show that rapid methods for diagnosing respiratory adenovirus infections can be successfully used in selected groups of adults.

Adenoviruses are important human pathogens which are responsible for several types of diseases, especially in children and in young adults (2, 7). In relatively closed communities, such as boarding schools and military camps, certain serotypes of adenoviruses are well-known causes of epidemic pneumonia and other types of respiratory tract infections (7). Serology and virus isolation from nasopharyngeal swabs are the conventional approaches for virological diagnosis of adenovirus infections. More recently, rapid methods for direct demonstration of viral antigens in the respiratory tract secretions have proved to be useful in speeding up the diagnosis (1, 15). Immunofluorescence (IF) is a widely used method (3) but requires fairly laborious specimen pretreatments. Solid-phase immunoassays, utilizing radioactive (radioimmunoassay [RIA]) or enzyme label (enzyme immunoassay [EIA]), are easier to carry out and have therefore extended the availability of rapid diagnostic methods. Demonstration of adenovirus DNA by nucleic acid sandwich hybridization (HYB) has also been shown to reveal the infection with sensitivity and reliability comparable to those of RIA (20). Most of the studies described above have been carried out with material collected from children by trained and experienced personnel. Less information is available on the suitability of these methods for demonstration of the virus in specimens taken from adults.

In this study on the etiology and differential diagnosis of acute pneumonia in military conscripts, adenovirus infection was demonstrated in a remarkable proportion of the pneumonia patients, as well as in the reference patients suffering from other types of acute respiratory tract infections. In this paper, we report observations on the relative power of three rapid diagnostic methods, IF, EIA, and HYB, for virus detection and compare their efficacy with that of the conventional methods, i.e., virus isolation and serology.

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MATERIALS AND METHODS

Patients. Patients were 207 men in compulsory military service (mean age, 20.5 years). None of them had any serious underlying diseases. All patients fell acutely ill with a febrile respiratory disease.

Of these patients, 106 had pneumonia, as diagnosed by the presence of pneumonic infiltrate in the chest X-ray examination, and 101 suffered from upper respiratory tract infection without pulmonic infiltrate. Most of the latter patients had an upper-respiratory-tract infection (common cold, 28 cases) or tonsillitis (61 cases) with or without symptoms of a common cold. Only a few patients had sinusitis (9 cases), otitis media (1 case), parotitis (1 case), or mononucleosis (1 case).

Specimens. Nasopharyngeal suction specimens (NPS) were collected through the nostrils of the patients with a mucus extractor. If the volume of the sample was less than 2 ml, sterile saline was added to yield an amount of 2 ml. Aliquots (1 ml each) of the NPS specimens were processed for demonstration of viral antigens by IF, and 0.5-ml aliquots were processed for virus isolation. A 0.5-ml portion was frozen to −20°C and later examined for the presence of viral antigens by EIA or for the presence of adenovirus DNA by HYB or for the presence of both by both methods. Sputum specimens (97 specimens) were also collected for bacterial cultivation from the pneumonia patients. A 0.5-ml amount was stored at −20°C and assayed by EIA for the different viral antigens and by HYB for adenovirus DNA.

Paired serum specimens for viral antibody determinations were collected on the day of admission to the hospital and 2 weeks later. Samples for bacterial culture and for antigen

TABLE 1. Sensitivity of rapid adenovirus-specific tests in patients with pneumonia (n = 106) or other respiratory infections (n = 101)

Adenovirus isolation result and illness (n)	No. of samples positive/no. of studied specimens by:		
	EIA[a]	IF[a]	HYB
Positive[b]			
Pneumonia (6)	3/6	4/6	4/6
Other respiratory infection (20)	9/19	9/13	10/17
Negative[c]			
Pneumonia (100)	0/100	1/77	0/17
Other respiratory infection (81)	1/80	2/61	0/31

[a] All three adenovirus isolation-negative, IF-positive cases were serologically confirmed; the adenovirus isolation-negative, EIA-positive case was not.
[b] A total of 23 cases showed diagnostic increase or positive IgM or both in adenovirus antibodies.
[c] A total of 42 cases showed diagnostic increase or positive IgM or both in adenovirus antibodies.

and bacterial antibody determinations were also obtained. These results will be presented in a separate report (K. Lehtomäki, M. Leinonen, A. Takala, and P. Makela, submitted for publication).

Detection of viral antigens. A standard IF method (3) was used for the detection of viral antigens. The direct IF test was used for influenza A antigens (fluorescein isothiocyanate-conjugated rabbit anti-influenza A antiserum; Flow Laboratories, Inc.). For the detection of the other antigens, indirect IF was used. Rabbit anti-adenovirus hexon and anti-parainfluenza type 1 virus antisera were obtained from Statens Bakteriologiska Laboratorium, Stockholm, Sweden, and rabbit anti-parainfluenza type 2 virus antiserum was obtained from J. Suni (Aurora Hospital, Helsinki, Finland). Bovine anti-parainfluenza type 3 and anti-respiratory syncytial virus antisera and chicken anti-influenza B virus antisera were provided by the Wellcome Research Laboratories (Kent, Bucks, United Kingdom). Fluorescein isothiocyanate-conjugated second antibodies (sheep anti-rabbit and anti-chicken and rabbit anti-bovine) were also obtained commercially (Wellcome). Specimens with an insufficient amount of epithelial cells were excluded from the comparisons.

EIA for adenovirus hexon antigen was done as previously described (15). EIAs for other respiratory viruses were done as described by Sarkkinen and co-workers (15–17). NPS or sputum specimens (400 μl) were further diluted in phosphate-buffered saline containing 20% inactivated fetal calf serum, 2% Tween, and 10 mM merthiolate (600 μl) and sonicated for 1 to 2 min (15).

Detection of adenovirus nucleic acids. HYB was carried out as described previously (13), by use of cloned reagents derived from the DNA of adenovirus type 3 (Ad3) (20). One of the reagents was chemically iodinated with ^{125}I (about 10^8 cpm/μg of DNA) to enable hybrid detection. This test detects both Ad3 and adenovirus type 7 (Ad7) with almost equal sensitivity but does not detect the adenoviruses of subgroup C (adenovirus type 2). Also, a test detecting viruses of subgroup C was used, but it remained negative with all specimens tested. The NPS and sputum specimens diluted in EIA dilution buffer (see above) were treated with sodium dodecyl sulfate and proteinase K and subjected to the assay after being boiled for 5 min (20). The limit of positivity of the HYB test was set based on the 97.5% confidence limit calculated with a series of negative specimens included in the test. The test detected 5×10^6 adenovirus DNA molecules. A total of 71 NPS and 13 sputum specimens were tested by HYB. They included most of the virus isolation-positive specimens and a comparable number of virus isolation-negative specimens.

Virus isolation. Virus isolations were carried out by inoculating 0.1-ml aliquots of the NPS into stationary-phase cultures of Vero, African green monkey kidney, and primary human amnion cells. Cultures were followed through one blind passage when necessary. Adenoviruses were typed by the hemagglutination inhibition test by use of type-specific antisera obtained from Statens Bakteriologiska Laboratorium. Cell lines used were not suitable for isolation of influenza or parainfluenza viruses.

Serological methods. The complement fixation tests for measurement of antibodies against 17 microbial antigens were carried out as described previously (19).

EIA for detection of antibodies against adenovirus hexon antigen (immunoglobulin G [IgG], IgA, and IgM classes) and influenza A and B and parainfluenza type 1, 2, and 3 viruses (IgG class) were done by using the principles previously described (10).

RESULTS

Rapid diagnosis of respiratory adenoviruses. The aim of the study was to evaluate the efficacy of rapid viral antigen detection methods in the diagnosis of pneumonia and other respiratory diseases in military recruits. Viruses other than adenovirus were found only occasionally. The comparisons of the different methods is restricted to the adenovirus-specific tests. The results obtained with the various antigen- and nucleic acid-detection methods are correlated to virus isolation in Table 1. Adenovirus was isolated from a total of 26 specimens (Ad3 from 11 cases, Ad7 from 14 cases, and untyped adenovirus from 1 case). Antigen detection methods were positive in 48% (EIA) and 68% (IF) of the isolation-positive specimens, and adenovirus subgroup B-specific nucleic acid was observed in 61% (HYB) of the isolation-positive specimens. Of the virus isolation-negative specimens (n = 181), one was positive by EIA (did not show diagnostic antibody rise in paired sera), and three were positive by IF (all were serologically confirmed cases). Diagnostic increase (or positive IgM in EIA) in serum adenovirus antibodies was observed in 23 pneumonia patients and in 42 patients with other respiratory infection.

Comparisons of the antigen or nucleic acid detection methods are shown in Table 2. Altogether, 22 specimens were positive with at least one test. There were no significant differences in the efficacies of the various rapid methods (chi-square test).

TABLE 2. Comparison of rapid adenovirus detection methods

No. of specimens (n = 22)	Reaction of specimen(s) by[a]:		
	EIA	IF	HYB
8	+	+	+
3	+	ND	+
1	+	–	–
1	+	ND	ND
2	–	+	+
4	–	+	–
1	–	+	ND
1	ND	+	ND
1	–	–	+

[a] +, Positive; –, negative; ND, test not done or insufficient specimen in IF. The total numbers of specimens positive by EIA, IF, and HYB were 13, 16, and 14, respectively.

TABLE 3. Detection of adenovirus antigens and nucleic acids in sputum specimens (n = 97) from pneumonia patients[a]

Specimen no.[b]	Reaction[c] of:					Virus isolation yielded (n = 99):
	Sputum specimens by:		Nasopharyngeal specimens by:			
	EIA (n = 97)	HYB (n = 17)	EIA (n = 99)	IF (n = 90)	HYB (n = 23)	
1	+	+	+	+	+	Ad7
2	+	+	+	+	+	Ad7
3	−	+	−	−	−	Ad3
4	−	+	−	−	−	
5	+	−	−	+	−	
6	+	ND	−	−	ND	
7	+	ND	+	+	+	Ad3
8–19	−	−	−	−	−	
20–97	−	ND	−	−	−	
98	ND	ND	−	+	+	Ad7
99	ND	ND	−	−	−	Ad7

[a] Pneumonia patients from Table 1.
[b] All antigen-, nucleic acid-, or isolation-positive cases were serologically confirmed.
[c] +, Positive; −, negative; ND, test not done.

Other viruses and viral antigens in NPS. Among pneumonia patients, four influenza B virus antigen-positive samples were found by IF, two parainfluenza type 2 antigen-positive samples were found (one by IF, one by EIA), and one parainfluenza type 3 antigen-positive sample was found by EIA. Virus isolation revealed one herpes simplex virus-positive specimen.

In patients with other respiratory infections, two influenza B (both by IF), two parainfluenza type 2 (one by IF, one by EIA), one parainfluenza type 3 (IF), and two respiratory syncytial (both by EIA) viral antigen-positive specimens were observed. By virus isolation, four herpes simplex virus-positive specimens and one enterovirus-positive specimen were detected.

Detection of adenoviral antigen and nucleic acids in sputum specimens. Sputum specimens were originally collected to identify putative bacterial agents in pneumonia patients. A total of 97 sputum specimens were studied for the presence of adenoviral and other viral antigens by EIA. A selected group of 17 specimens was also assayed by HYB for the presence of adenovirus nucleic acids. Five adenovirus-positive cases were detected by EIA, and four adenovirus-positive cases were detected by HYB (Table 3). These figures are higher than those obtained from NPS, since two additional positive cases were detected by both EIA and HYB. All sputum-positive cases showed a diagnostic increase in serum adenovirus antibodies or were positive by rapid tests on the corresponding NPS.

One parainfluenza type 2 virus antigen-positive specimen was also found by EIA.

DISCUSSION

In the present study, we have compared the diagnostic efficacy of modern rapid diagnostic methods in testing NPS and sputum specimens from young adult men.

IF was the first method used in the rapid diagnosis of respiratory infections caused by viruses (3). The method is sensitive and specific in the hands of an experienced microscopist. Single specimens can be studied as soon as they arrive at the laboratory, and the results are obtained within a few hours. IF has, however, proved to be less sensitive than virus isolation (3). Of great importance in IF is the collection and processing of the specimen; a sufficient number of infected respiratory epithelial cells should be obtained. In this study, IF was nearly as sensitive as virus isolation when samples with an insufficient amount of cells were excluded from the comparisons.

RIA and EIA methods have been applied for the detection of several viral antigens in nasopharynx, stools, and other body secretions (1, 11, 14, 15–17, 21). The advantages of the solid-phase assays are their large capacity, objectivity, and sensitivity. Sometimes, false-positive results may be observed, but with specific antisera (e.g., monoclonal antibodies) and a confirmatory test as described by Sarkkinen and co-workers (15–17), the specificity is improved. In studies concerning detection of rotavirus and adenovirus antigens in stools, RIA and EIA methods have been more sensitive than electron microscopy (5, 9). In NPS, their sensitivity is comparable to IF (4, 16–18), but they have been reported to be less sensitive than virus isolation (6). Our observations in the present study agree with previous ones in this regard. Virus isolation appeared to be more sensitive than adenovirus hexon antigen-specific EIA. This may be due to a number of insufficient specimens since some of the specimens contained little or no cells as judged by IF. The specimens were also stored for relatively long periods of time at −20°C and were often diluted for the EIA. This may also have impaired the observed sensitivity of the assays. Our study involved adult patients, who most likely experienced other types of adenovirus infections in childhood and thus had good hexon antibody levels. Consequently, a further explanation for negative EIA and IF results may be the possible presence of hexon antibodies in the nasopharynx interfering with the antigen detection, since excretion of IgA and IgG antibodies into secretions has been observed in respiratory infections (12). We did not, however, analyze secretory antibodies.

In recent years, methods for detecting microbial nucleic acids in clinical specimens have been developed for many viruses such as adenoviruses, enteroviruses, and herpesviruses (8, 13, 14, 18). In our method, the specimen is kept in solution. The hybrids are nevertheless collected onto a solid carrier with a capture probe reagent. This enables reliable testing of the specimens without background problems and with precise numerical values as results. The HYB method has shown sensitivity similar to that of adenovirus hexon antigen-specific RIA (20). In the present work, only a limited number of specimens were tested, and the sensitivity was again found to be comparable to that of IF and hexon antigen-specific EIA. Nucleic acid hybridization is highly specific in stringent conditions. Too high a specificity may be a problem with clinical specimens, but it can be overcome by choosing a probe that detects a wide range of various serotypes. Our Ad3-specific probe also detected Ad7 DNA.

In the present work, we have demonstrated that rapid diagnostic methods can successfully be used for detecting adenoviral antigens or DNA in respiratory infections of adults. Respiratory infections of adults characteristically lack mucus secretion, in which case it may be difficult to obtain a good sample for antigen detection. The HYB technique used here allowed exact quantitation of the viral DNA molecules in the specimens. It showed that the number of molecules varied between 5×10^7 and 5×10^9/ml of mucus, which is on an average 50 times less than that in the corresponding specimens taken from children (20). We also showed that sputum specimens originally taken for bacterial cultivation can well be used for detection of viral antigens

and nucleic acids. The specificity of the adenovirus detection methods was good, and their practical application would be in clarifying the etiology of outbreaks of respiratory infections in communities such as schools, hospitals, and military camps.

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