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Immunoassay Diagnosis of Adenovirus Infections in Children

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Direct detection of viral antigen in nasopharyngeal secretions and stool specimens by radioimmunoassay and the determination of serum antibody responses by complement fixation and immunoglobulin class-specific enzyme immunoassay against the hexon antigen were compared for diagnostic efficacy in 52 children with acute adenovirus infections. The highest diagnosis rate (85% of the cases) was obtained by antigen detection in nasopharyngeal secretions. Adenovirus antigen was also detected in stools of 72% of the 18 patients tested. The immunoglobulin G (IgG) antibody enzyme immunoassay detected 77% of the cases, being more sensitive than the complement fixation test with a 67% detection rate. The IgM antibody response was variable with no clear correlation with the age of the patient or severity of the clinical symptoms. IgM antibody response was detected in 48% of the patients and had the normal transient course, with a persistence of the IgM antibodies of approximately 2 months. Determination of IgA antibodies gave a diagnostic increase in titer in 37% of the cases and was found less suitable for serological diagnosis. Because of the clinical importance of rapid laboratory diagnosis, the direct detection of viral antigen in nasopharyngeal secretions or stool or both should be used as the primary diagnostic test in adenovirus infections.

During the last 5 years, modern immunological methods have been used increasingly in the laboratory diagnosis of viral infections. Virus isolation techniques have been challenged by methods capable of detecting virus antigens (14) or nucleic acids (19) directly in clinical specimens. In serology, classical methods such as complement fixation (CF) and hemagglutination inhibition have been substituted by radioimmunoassays (RIA) and enzyme immunoassays (EIA), tests with high sensitivities and the ability to separately assay antibodies belonging to different immunoglobulin classes (10).

In the present study, we compared the diagnostic efficacy of virus antigen detection in nasopharyngeal secretion (NPS) and in stool specimens as well as that of serology by the CF test and immunoglobulin class-specific EIA in the diagnosis of acute adenovirus infections in children.

MATERIALS AND METHODS

Patients. We studied 116 children admitted to the Departments of Pediatrics and Infectious Diseases, University of Turku, between 1 September 1981 and 31 December 1982 with a clinically suspected adenovirus infection. These children were tested by adenovirus antigen detection in NPS and by serology. Adenovirus infection was diagnosed by one or more of the methods in 52 children who formed the final study population. There were 33 males and 19 females with a mean age of 1 year 10 months (range, 4 months to 7 years 2 months). The main clinical symptoms of the patients are shown in Table 1. The study subjects were mostly patients with respiratory infections. Patients from whom adequate NPS specimens could not be obtained, e.g., most patients with solely gastrointestinal symptoms, were excluded. Four patients had fever as the only symptom except that two of them also had febrile convulsions.

Specimens. NPS specimens were collected by suction through the nostrils with a disposable mucus extractor (Vycon, Ecouen, France). One NPS specimen was obtained from each of 33 children, and 2, 3, 4, and 5 specimens were obtained from each of 12, 5, 1, and 1 children, respectively. The specimen volume obtained was usually 0.5 to 2.0 ml. One to three serial stool specimens were also collected from each of 18 children. Acute-phase serum specimens were taken at admission to the hospital, and the first convalescent-phase specimens were taken about 2 weeks later. Three to five additional specimens were obtained up to 90 days after onset of illness from 19 of the 52 children.

RIA for adenovirus antigen. The indirect RIAs used have been described in detail previously (8, 14). Briefly, adenovirus hexon antigens were captured from the specimens onto polystyrene beads coated with guinea pig antihexon antibody followed by sequential incubations with rabbit antihexon antibody and ^{125}I-labeled sheep anti-rabbit immunoglobulins. Borderline positive results were verified by a blocking-type confirmatory test where test specimens were incubated in parallel with preimmune and immune guinea pig serum

TABLE 1. Symptoms and signs in 52 hospitalized children with acute adenovirus infection

Symptom	% Showing symptom
Fever	92
Rhinitis	63
Cough	52
Tonsillitis	44
Otitis	27
Gastroenteritis	23
Conjunctivitis	15
Febrile convulsions	8
Pneumonia	4
Exanthema	4

before adding the rabbit anti-hexon antibody. In addition to adenovirus, the NPS specimens were also tested for respiratory syncytial virus, influenza A and B virus, and parainfluenza virus type 1, 2, and 3 antigens, and stool specimens were tested for rotavirus antigens by analogous RIAs.

EIA for adenovirus antibody. Flat-bottomed polystyrene microtiter plates (no. 76-201-05; Flow Laboratories, Irving, Scotland) were coated with purified hexon antigen from adenovirus type 2 (12) by incubating 0.5 μg of hexon antigen diluted in 75 μl of phosphate-buffered saline (PBS), pH 7.3, in each well overnight at room temperature. After incubation, the antigen suspensions were aspirated, and the wells were allowed to dry in air. The plates were stored at 4°C and washed with PBS before use.

Seventy-five-microliter volumes of test sera (fourfold serial dilutions starting at 1:40) were incubated in the antigen-coated wells for 2 h at 37°C. Positive and negative control sera were included in each test series. After washing with PBS containing 0.1% Tween 20, 75-μl volumes of peroxidase-conjugated heavy chain-specific antibodies to human immunoglobulin G (IgG), IgM, or IgA (Orion Diagnostica, Helsinki, Finland) at dilutions of 1:5,000, 1:1,000, and 1:100, respectively, were added, and the plates were incubated for 2 h at 37°C. PBS containing 5% normal porcine serum and 0.5% Tween 20 was used as diluent for both test sera and conjugated anti-human immunoglobulins. After washing as before, 75-μl volumes of freshly prepared substrate (1,2-phenylenediamine, 1 mg/ml, with 0.03% hydrogen peroxide in citrate phosphate buffer, pH 5.5) were added. The plates were incubated in the dark for 30 min at room temperature and 150 μl of 1 N hydrochloric acid was added to each well to stop the reaction. The absorbances in each well were measured directly on the plate with a vertically measuring photometer (Titertek Multiscan; Eflab, Finland) at 492 nm. The endpoint titer was regarded as the highest serum dilution in which the absorbance of the test serum was 2.1 times that of the negative control serum but at least 0.1. A significant (fourfold or greater) titer increase between paired serum specimens in IgG and IgA antibody assays and the detection of IgM antibodies were considered to indicate a recent infection.

CF tests. The CF tests were performed by a standardized microtechnique (17) which used crude cell extract type antigen from adenovirus type 5-infected Vero cells. In addition to adenovirus, antibodies to respiratory syncytial virus, influenza A and B viruses, parainfluenza virus types 1, 2 and 3, enteroviruses, and *Mycoplasma pneumoniae* were also assayed.

RESULTS

Antigen detection. Adenovirus antigen was detected in one or more NPS specimens of 44 of the 52 patients (Table 2). The diagnostic efficacy of antigen detection was dependent on the time of specimen collection (Fig. 1). From specimens taken during the first 5 days after onset of illness 26 of 31 (84%) were positive, but only 16 of 32 (50%) specimens taken 6 to 10 days after onset and 6 of 18 specimens taken 11 to 25 days after onset were positive. An NPS specimen taken during the first 5 days was available from three patients, and an NPS specimen taken during the first 10 days was available from all eight of the patients whose diagnosis could not be verified by antigen detection. In only one case was adenovirus antigen detected from subsequent NPS specimens after a negative specimen was obtained from the patient. A two-way comparison of the different tests is shown in Table 3.

Adenovirus antigen was also detected in stool specimens from 13 of the 18 patients from whom specimens were available. From the 11 stool specimens collected during the first 5 days, 10 (91%) were positive. Stool specimens were available from eight of the patients with gastrointestinal symptoms, and adenovirus antigen was detected in seven. In one case, a patient with tonsillitis, otitis, and gastroenteritis, adenovirus antigen was detected in a stool specimen but not in an NPS specimen, both taken 5 days after onset of illness.

CF test. A significant (fourfold or greater) increase in CF titer was observed in 35 cases (67%) (Table 2), and a stationary high antibody level was observed in 4 cases. The titer increases were in all cases detected between the first two serum specimens, which were taken about 2 weeks apart. The magnitude of the CF response was dependent on the age of the patient so that patients aged 3 years or more

TABLE 2. Diagnostic efficacy of virus antigen detection in NPS and serum in 52 children with an acute adenovirus infection

Diagnostic criterion	No. of cases diagnosed (%)
Viral antigen detection in NPS	44 (85)
Viral antigen detection in stool	13[a] (72)
CF titer increase	35 (67)
EIA IgG titer increase	40 (77)
EIA IgM positive	25 (48)
EIA IgA titer increase	19 (37)

[a] Specimens from 18 patients tested.

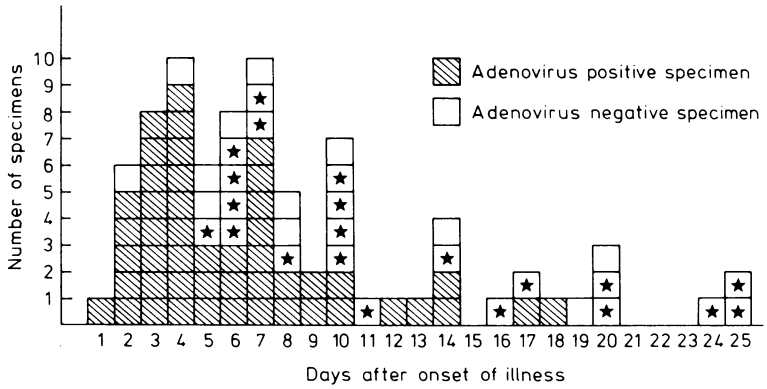

FIG. 1. Detection of adenovirus hexon antigen in NPS in relation to the time of specimen collection in 52 patients with acute adenovirus infection. Negative specimens from patients with a previous positive specimens are indicated by stars (★).

showed a much higher titer CF response than the younger children (Table 4).

IgG antibody EIA. The assay for EIA IgG antibody showed a diagnostic titer increase in 40 (77%) of the patients, slightly more sensitive than the CF test (Table 2). The remaining 12 patients already had high IgG antibody titers in acute-phase serum specimens. Eight patients had significant titer increases by the EIA IgG test but not by the CF test, whereas the opposite was demonstrated in three cases (Table 3). The IgG antibody response (Fig. 2) followed a rather uniform course in all patients but like the CF antibody response showed positive correlation with the age of the patient (Table 4).

IgM antibody EIA. An IgM antibody response to adenovirus was observed in 48 patients (Table 2), whereas no IgM antibodies, at least to hexon antigen, were produced in the remaining cases. When present, the IgM response followed the normal transient course, with maximum titers detected 10 to 20 days after the onset of illness and a persistence of approximately 2 months (Fig. 3). The presence of an IgM antibody response did not show any significant correlation with the age of the patient (Table 4). Of the patients with an IgG titer of 640 or below in the acute-phase serum specimen collected 0 to 7 days after onset of illness, 12 of 20 showed an IgM antibody response, whereas of 13 patients with an IgG titer of 1,280 or higher only 2 did so ($P = 0.05$; Fischer exact fourfold table test).

IgA antibody EIA. Great variations were observed in the IgA antibody responses. In 13 patients, IgA antibodies were not demonstrable in any of the serum specimens, whereas stable or decreasing IgA titers were observed in 20 patients. An increase of IgA antibody titer was shown in 19 patients (Table 2). All of these patients also had significant increases of IgG antibody titers (Table 3). In some patients the IgA titers declined to undetectable levels in 1 to

TABLE 3. Comparison of diagnostic efficacy of different tests in 52 children with an acute adenovirus infection

Test	Comparative result (no.)							
	CF		IgG		IgM		IgA	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
NPS								
Positive	29	15	32	12	22	22	15	29
Negative	6	2	8	0	3	5	4	4
CF								
Positive			32	3	17	18	17	18
Negative			8	9	8	9	2	15
IgG								
Positive					19	21	19	21
Negative					6	6	0	12
IgM								
Positive							12	13
Negative							7	20

TABLE 4. CF and immunoglobulin class-specific EIA antibody responses in relation to the age of the patients

Age group (yr)	No. of patients	Test	Geometric mean titer after onset of illness (days)			
			0–7	8–21	22–36	37–90
0–1	19	CF	<4	14	17	15
1–2	22	CF	<4	11	19	24
2–8	11	CF	10	23	71	102
0–1	19	IgG-EIA	209	299	1,452	1,280
1–2	22	IgG-EIA	214	672	1,859	2,560
2–8	11	IgG-EIA	349	2,874	5,653	10,240
0–1	19	IgM-EIA	50	149	110	<40
1–2	22	IgM-EIA	67	168	80	49
2–8	11	IgM-EIA	67	57	80	<40
0–1	19	IgA-EIA	67	80	141	54
1–2	22	IgA-EIA	53	186	113	131
2–8	11	IgA-EIA	135	453	390	254

2 months, but in others they persisted at stable levels up to the end of the follow-up. The magnitude of the IgA antibody response also showed positive correlation with the age of the patient. No correlation between the persistence of the IgA antibodies and the clinical state of the disease was found.

DISCUSSION

Direct detection of adenovirus antigens in NPS by immunofluorescence was introduced by Gardner and co-workers in 1972 (6). However, the sensitivity of the method for adenovirus was substantially lower than that for many other viral respiratory infections, since only 50 to 60% of the isolation-positive cases could be detected by immunofluorescence (7, 11). The experience gathered in our diagnostic unit during the last 5 years indicates that the RIA developed by Sarkkinen et al. (14) is a more sensitive and practical test than immunofluorescence. A probable explanation for the increased sensitivity is that the cytolytic type of infection caused by adenoviruses produces large quantities of extracellular virus and soluble antigenic proteins which can be detected by RIA but not by immunofluorescence.

RIA detection of adenovirus antigen in stool specimens has been shown to be a sensitive method for the diagnosis of adenoviral gastroenteritis (8, 18). However, virus excretion in feces also occurs in over 80% of predominantly respiratory adenovirus infections (4, 5). Although we only studied stool specimens from 18 of our patients, the results indicated that detection of adenovirus antigens in stool specimens can be used to increase the diagnostic efficacy in respiratory adenovirus infections.

Since adenoviruses can occasionally be isolated from human tonsils and can be intermittently excreted in the feces for a long time after the

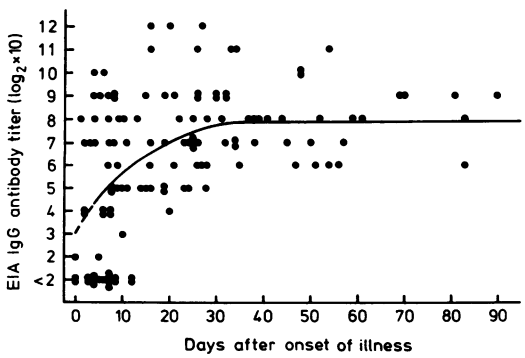

FIG. 2. Appearance and persistence of adenovirus IgG antibodies in serial serum specimens of 52 patients with acute adenovirus infection.

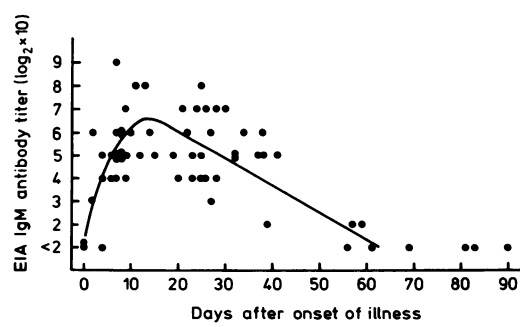

FIG. 3. Appearance and persistence of adenovirus IgM antibodies in serial serum specimens of 25 patients with acute adenovirus infection showing an IgM antibody response.

infection (4), it must be considered whether our finding of adenovirus antigen in NPS or in stool really indicates acute adenovirus infection. We have shown (O. Ruuskanen et al., submitted for publication) that adenovirus antigen can be found by RIA in stool specimens from 4% of children without symptoms. It has not been possible to study the carrier rate in NPS since adequate specimens cannot be obtained from children without nasal discharge. However, by using virus isolation, that apparently is at least as sensitive a method as RIA, Bronitki et al. (2) have shown adenoviruses in 2% of NPS from healthy children. These small background figures, together with the fact that 93% of our adenovirus infections diagnosed by antigen detection also had a serological response to adenovirus antigens, clearly indicate that our patients had true adenovirus infections.

The human adenoviruses share a common group-specific hexon antigen which can be used in group-specific serological tests, e.g., in the CF test (3, 15, 16), and in EIA (13). EIA is more sensitive than the CF test and therefore advantageous for diagnostic serology. However, it is evident that one single serological test is not capable of diagnosing all adenovirus infections.

Many viral infections are followed by a transient appearance of specific IgM antibodies, and the detection of these allow a rapid diagnosis to be made from a single serum specimen (10). Neutralizing serum IgM antibody responses after natural adenovirus infection (1) and after inoculation of soluble adenovirus antigens (9) have been studied previously. The responses observed were variable, including absent and prolonged IgM antibody responses as well as production of IgM antibodies against heterologous serotypes (9). In our material, the IgM antibody response followed the normal transient course in all cases. However, an IgM antibody response was only detected in 48% of the patients, and in many cases the titers were relatively low. Recently, Roggendorf et al. (13) also reported failure to detect IgM antibodies to the group-specific hexon antigen by EIA in a majority of the patients tested. Reinfections are often characterized by weak or absent IgM antibody responses (10), and in adenovirus infections the immunological memory to previously experienced serotypes might explain the suppressed production of IgM antibodies to the hexon antigen.

In conclusion, direct detection of viral antigen in NPS should be used as the primary method in the diagnosis of adenovirus infections with respiratory symptoms. The search for adenovirus antigen in stool specimens enhances the diagnostic efficacy in these infections and is of primary importance when respiratory symptoms are missing, e.g., when high fever is the only clinical manifestation or in adenoviral gastroenteritis. In serology, the CF test is a sufficiently reliable and sensitive method, but by a more sensitive EIA IgG antibody test a somewhat higher diagnostic efficacy can be obtained. Determination of specific IgM or IgA antibodies can be valuable in individual cases but is in general of low sensitivity.

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