Rapid Presumptive Recognition of Diarrhea-Associated Adenoviruses

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The quantity of adenoviruses in a diarrhea stool provided a strong presumptive indication of the presence or absence of an adenovirus from subgenus F or G (proposed species 40 or 41). These adenoviruses were found in the stools of 91% of 56 acutely ill diarrhea patients with one or more than one adenovirus particle per min of direct electron microscopic viewing, as compared with 40% of 20 acutely ill diarrhea patients with less than one detected adenovirus particle per min of viewing.

Adenoviruses in subgenera F and G (corresponding to proposed types or species 40 and 41) increasingly are being recognized as a cause of diarrhea, particularly in infants and young children (2, 5, 7–10, 13–20, 23–27). Accordingly, there now is clinical value in rapidly distinguishing these from other adenoviruses that are commonly found in the stools of children (1, 3, 4, 6, 11, 12, 21) but are not major causes of diarrhea.

Stools from pediatric inpatients and outpatients with diarrhea, vomiting, or both for 4 days or less were tested over a period of several years for viruses that could be visualized by direct electron microscopy (EM). For direct EM, freshly collected stools or stools that had been stored at 4°C for a few hours were prepared as 10% suspensions in deionized water. An equal volume of 1.5% phosphotungstic acid (brought to pH 7.1 with NaOH) was added as a negative stain. The mixtures were then dropped on Formvar-coated 400-mesh copper EM grids which were blotted dry after 2 min. The specimen grids were viewed at $\times 36,000$ to $\times 47,000$ magnification in an RCA EMU 4C or Hitachi HU 11E electron microscope.

Specimens in which no virus was visualized were retested by immune EM (3). Stools for immune EM preparations typically had been stored frozen at -60°C for a few days. All EM and immune EM preparations were read by the same experienced microscopist, who set a clock timer for 6 min and then scanned portions of each specimen grid for that time or until an adenovirus had been recognized and the quantity of that virus on the grid had been determined. The approximate number of viruses seen per minute was recorded for each preparation.

Attempts were also made to recover these adenoviruses in HEp-2 and 293 cell cultures (22, 23). Cultivated adenoviruses were then identified in 293 cell cultures by neutralization with antiserum (kindly supplied by S. E. Straus) prepared against one of our subgenus G adenoviruses. A 1:1,000 (homologous virus neutralization endpoint) dilution of this antiserum was used routinely; it completely neutralized diarrhea-associated ("enteral") adenoviruses (EAd) in subgenera F and G, including prototype adenoviruses 40 (Dugan) and 41 (Tak) without reacting with any other tested

adenovirus, including adenoviruses 1 through 7, 12, 18, and 31 (submitted for publication). Adenoviruses were also identified as belonging to subgenus F or G by restriction endonuclease electrophoresis of stool specimens or virus isolates, essentially by the method of Takiff et al. (23). Some non-EAd were identified by hemagglutination inhibition (4).

Adenoviruses were visualized in stools from 72 patients by direct EM and from four additional patients by immune EM. All of these viruses were then identified as EAd or non-EAd.

A total of 74 of the 76 viruses were tested by neutralization, and 57 (77%) were EAd. Of the 76 adenoviruses, 28 were selected at random and tested by restriction endonuclease electrophoresis; 25 of these (89%) were from EAd subgenus F or G. There was complete agreement as to EAd or non-EAd status whenever the same adenovirus was tested by both neutralization and restriction endonuclease electrophoresis. Overall, 81% of the visualized adenoviruses from patients hospitalized with acute gastroenteritis and 78% of those from the total group of tested inpatients and outpatients proved to be EAd (Table 1).

Of the 17 non-EAd, 4 were identified by hemagglutination inhibition after cultivation in HEp-2 cells. Three of these were type 1 adenoviruses, and one was a type 2 adenovirus. The remaining non-EAd have not been identified by serotype.

Interestingly, the mean concentration of adenoviruses in specimens containing EAd was about eight times greater than that in specimens containing non-EAd (7.5 adenovirus particles per min of direct EM viewing for EAd, as compared with 0.9 particles for non-EAd). Furthermore, only 8 (40%) of 20 patients with fewer than 1 adenovirus particle per min of direct EM viewing of an acute illness stool specimen were found to have an EAd, as compared with 51 (91%) of 56 patients with 1 to 100 or more such particles per min of direct EM viewing ($\chi^2 = 15.7$; $P < 0.001$). Findings restricted to patients who were hospitalized with acute gastroenteritis were essentially the same as those for the entire study group.

Clearly, the quantity of adenovirus found in an acute illness diarrhea stool can provide a strong presumptive indication of the presence or absence of an EAd. This determination can be made by EM within 15 min of specimen collection.

Of the several methods for the recognition or identification of EAd, which include cultivation in 293 cells (23), neutral-

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TABLE 1. Quantity of adenoviruses as an indicator of EAd in stools

No. of particles seen per min by EM	No. of patients with an EAd/no. tested (% positive) ^a	
	Hospitalized gastroenteritis patients	All patients ^b
<1	4/9 (44)	8/20 (40) ^c
≥1	34/38 (89)	51/56 (91) ^c
Total	38/47 (81)	59/76 (78)

^a Determined by EAd neutralization, restriction endonuclease electrophoresis, or both.

^b Includes inpatients and outpatients with diarrhea, vomiting, or both.

^c $\chi^2 = 15.7$; $P < 0.001$.

ization (9, 26), enzyme-linked immunosorbent assay (14, 27), restriction endonuclease electrophoresis (23), and dot blot hybridization (Takiff et al., submitted for publication), quantitative direct EM is by far the most rapid procedure and merely necessitates the recording of data that are obvious to the diagnostic microscopist.

About four of every five acutely ill pediatric gastroenteritis patients with any adenovirus visualized in their stools proved to have an EAd infection (infection by an adenovirus from subgenus F or G, or proposed species 40 or 41). When adenoviruses were visualized by direct EM in relative abundance (one or more virus particle per min of viewing), the ratio of EAd infections to total adenovirus infections increased to 9 of every 10.

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