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Identification by Immunofluorescence of Adenoviral Antigen in Exfoliated Bladder Epithelial Cells from Patients with Acute Hemorrhagic Cystitis[1,2] (38187)

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Adenovirus type 11 infection has been associated with a significant proportion of acute hemorrhagic cystitis (AHC) illnesses of children (1–5). AHC is a benign illness with no known sequelae and must be differentiated from the more serious genitourinary tract diseases that present with gross hematuria (2, 4). A rapid diagnostic method for identifying patients with adenoviruria could increase recognition of the infection, reduce the length of hospital stay, and decrease the number of diagnostic procedures now performed on patients suspected of having AHC.

Application of immunofluorescent procedures as a rapid method for the diagnosis of viral infections has been largely applied to respiratory tract material and infected tissue culture cells (6–13). Scrapings from vesicles of herpesvirus and smallpox infections have been used for the rapid diagnosis of these virus infections (14–16). Urinary sediment has been successfully employed to identify measles and coxsackie virus infections (17, 18). The purpose of this study was to examine urinary sediments by immunofluorescence for identification and localization of adenoviral antigen and to assess this procedure for the rapid diagnosis of adenovirus infection in patients with acute hemorrhagic cystitis.

Materials and Methods. Adenovirus type 11 antiserum. Antiserum to the Heard strain of adenovirus type 11 was produced in rabbits as previously described (19). Prior to use, the antiserum was adsorbed 3 times with HEp-2 cells by adding 1 ml of HEp-2 cell suspension (20% v/v packed cells in phosphate buffered saline pH 7.2) to 5 ml of heat inactivated (30 min at 56°) antiserum previously diluted 1:5 in phosphate buffered saline (PBS). After 1 hr adsorption at 4° with agitation of the mixture every 15 min the HEp-2 cells were separated by centrifugation. After 3 adsorptions the final dilution of adenovirus 11 antiserum was approximately 1:8 (at pH 7.2).

Fluorescein-Conjugated Anti-Rabbit Globulin. Commercial fluorescein-conjugated anti-rabbit globulin produced in sheep was obtained from Roboz Laboratories. Unconjugated fluorescein was removed by filtration through a G25 Sephadex column. The remaining nonspecific staining activity was removed by adsorbing the conjugate with mouse liver powder (0.2 gm mouse liver powder per ml of conjugate adsorbed for 1 hr at 4°). Finally the conjugated antiserum was adsorbed once with HEp-2 cells. The adsorbing procedures yielded fluorescein conjugated antiserum with highly specific staining properties when used

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at a 1:5 or a 1:10 dilution in PBS of pH 7.2.

Preparation and Staining of Smears of Urinary Sediment. A 25 ml aliquot of urine was centrifuged for 10 minutes at 1000 rpm and the sediment resuspended in ½ to 1 ml of residual urine. Smears were made of the resuspended urine sediments on FTA (fluorescent treponemal antibody) slides, air dried, and fixed for 2.5 hr in acetone at 4°, according to the method of Stevens and Watkins to stabilize adenovirus antigens and prevent their diffusion (13).

Smears were treated initially by layering 0.1 ml of 1:8 adsorbed adenovirus type 11 antiserum uniformly over the cells. Following incubation in 100% humidity chambers with adenovirus antiserum for 30 min at 35°, the smears were washed in PBS (pH 7.2) for 15 min, 0.1 ml of 1:5 fluorescein conjugated sheep anti-rabbit globulin was placed on the washed smears, and they were then incubated for 30 min at 35°. The smear was then washed for 15 min in PBS, air dried and mounted with buffered glycerol pH 9.0.

Control Slides. Slides of adenovirus type 11 infected and uninfected HEp-2 cells were prepared by trypsinizing tissue culture tubes to remove cells and fixing as above for urinary sediment. These slides were tested in parallel as controls to insure that the immunofluorescence was specific for adenovirus. Other control slides included infected HEp-2 smears treated with normal rabbit serum, horse adenovirus 11 antiserum, horse parainfluenza 2 antiserum and those treated with the fluorescein conjugate only.

Slides were coded and stained by one of us and read by the other without knowledge of the code. Smears were examined immediately after mounting the coverslip to minimize antigen diffusion in glycerol (13). All slides were examined using a high pressure mercury bulb, BG38 and BG3 exciter filters, 50 and 41 barrier filters, and a Ziess Standard Microscope. Photographs were taken using high speed Ektachrome film at 1250 magnification.

Virus Isolation. Results of virus isolation attempts in all patients, as well as bacteria and mycoplasma isolation, have been reported elsewhere (4). Evidence of adenoviruria was detected in approximately 21% of 69 AHC patients and bacteriuria occurred in 18% of cases; no mycoplasma isolates were identified. From these 69 patients, acetone fixed and frozen stored slides of urinary sediment were available from 4 AHC patients with adenoviruria, 1 control patient (no urinary tract disease) with adenoviruria, 17 AHC patients without adenoviruria, and 4 control patients without adenoviruria.

Results. Of 4 patients with AHC and adenoviruria, three had adenovirus antigen detectable by immunofluorescence in exfoliated bladder cells of their urinary sediment (Table I). Two of these adenoviruric positive AHC patients shed adenovirus type 11 in the urine, one adenovirus type 21, and one an adenovirus that was untyped. Multiple slides were examined from several urine specimens on each of the adenoviruric AHC patients. Of the 3–6 slides from each urine specimen tested for adenovirus antigen, usually less than one-half from each patient were positive. The one patient with AHC and adenoviruria from whom no positive slides were identified shed adenovirus 11 in the urine. All cells exhibiting fluorescent inclusions were characteristic transitional epithelial cells by morphologic criteria, possessing a central nucleus and being intermediate in shape between cuboidal and round. Fluorescence

TABLE I. Immunofluorescent Identification of Adenovirus in Urine Sediments from Patients with Acute Hemorrhagic Cystitis and Control Patients.

Category[a]	Adeno-viruria present	Number in group	Number with urine sediment positive by immuno-fluorescence
AHC	Yes	4	3
Control	Yes	1	0
AHC	No	17	0
Control	No	4	0

[a] AHC, Acute hemorrhagic cystitis; control, patients without urinary tract disease.

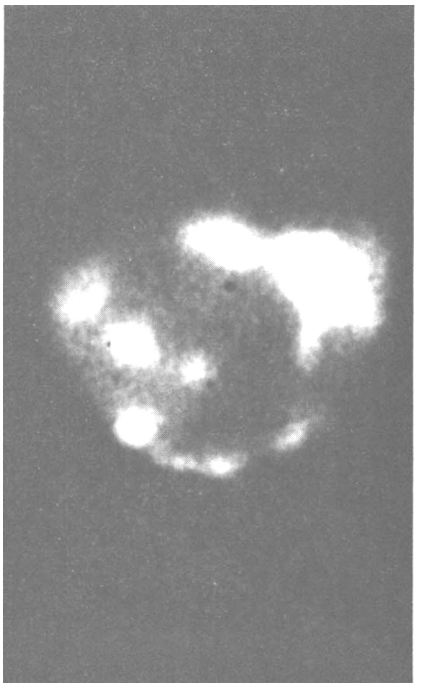

FIG. 1. Epithelial cell in acetone fixed urine sediment exhibiting fluorescent cytoplasmic granules after staining with adenovirus type 11 antiserum.

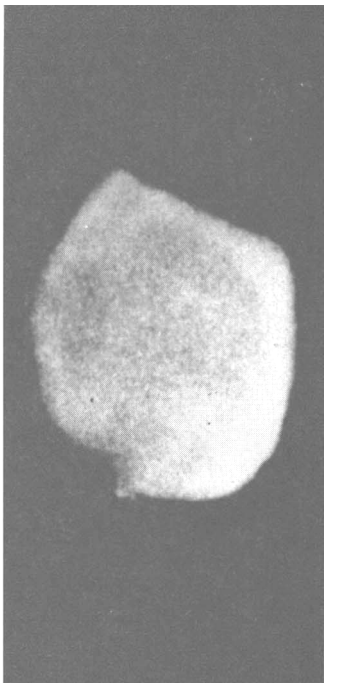

FIG. 2. Epithelial cell exhibiting diffuse cytoplasmic fluorescence. Treatment as in Fig. 1.

was observed mainly either as cytoplasmic granules (Fig. 1) or diffusely cytoplasmic (Fig. 2). Although, a few epithelial cells also exhibited fluorescent nuclear granules, cytoplasmic fluorescence was the predominant pattern. Unstained epithelial cells were detected both in negative urine specimens and among the cells of immunofluorescent positive urine sediment (Fig. 3).

Of 17 patients with AHC but no adenoviruria, none showed fluorescence in urinary sediment including one specimen from a patient with neutralizing antibody titer rise to adenovirus type 11. None of four urine sediments from control patients showed any specific fluorescence. One control patient without AHC but with adenoviruria had a negative urinary sediment by the immunofluorescent test.

Specific staining of leukocytes or casts was not observed in any urinary sediments tested. Large numbers of red blood cells were present in some urine specimens which made searching for epithelial cells tedious, but the erythrocytes did not take up stain. Specific cytoplasmic staining was evident in adenovirus infected HEp-2 cells employing adenovirus type 11 antiserum produced in rabbits, but horse adenovirus type 11 antiserum as well as parainfluenza 2 antiserum produced in horses and normal rabbit serum did not produce staining of adenovirus infected HEp-2 cells in this test system. Normal HEp-2 cells were not stained by rabbit adenovirus type 11 antiserum.

Discussion. The identification of adenovirus antigen in exfoliated bladder transitional epithelial cells from urinary sediment of patients with adenoviruric AHC provides further evidence of the association between adenovirus type 11 infection and AHC. The localization of adenovirus antigen in epithelial cells but not other formed elements in the sediment, especially casts, suggests that this virus infection occurs selectively in the bladder and not in the kidneys.

Both nuclear and cytoplasmic immunofluorescent staining have been reported in adenovirus infected HEp-2 cells as well as oropharyngeal scrapings from patients with

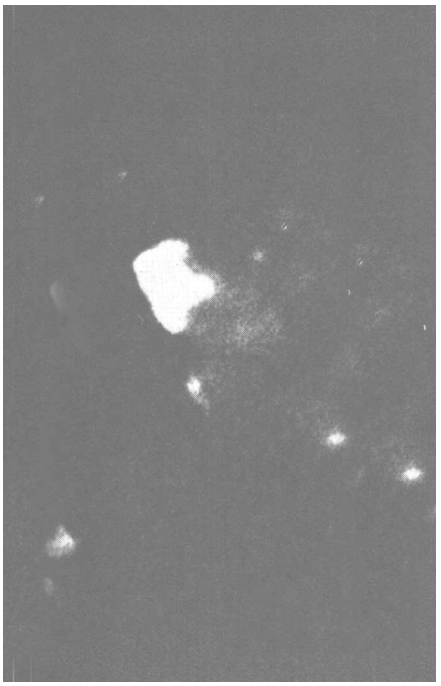

FIG. 3. Unstained epithelial cell. Treatment as in Fig. 1. The highlight is trapped dye in a particle of debris.

adenovirus upper respiratory infections (12). More cells with cytoplasmic staining than nuclear staining were detected in the urinary sediments from AHC patients. Preliminary evidence indicates that the pattern of staining depends on the duration of virus infection of the cells until tested and the conditions of cell growth in tissue culture (13).

One immunofluorescent positive urinary sediment was from a patient with AHC and type 21 adenoviruria, and another positive sediment was from a patient with AHC and adenoviruria that was untyped. This finding is consistent with the observation that detection of adenovirus antigen by indirect immunofluorescence is not type or subgroup specific. Using direct immunofluorescence, McCormick, Galapon, and Berling produced type specific conjugates for use in staining pharyngeal scraping (12). Homologous reactions between antiserum and virus infected cells may be relatively more intense than the heterologous reactions, and in the direct procedure these can be readily identified.

The diagnosis of adenoviruria using immunofluorescent techniques in patients with AHC circumvents the need for an extensive search for other causes of hematuria. The etiologic role of adenovirus infections in some cases of AHC can be established by immunofluorescent techniques quickly and thus facilitate case finding. The low yield of positive slides, however, limits the practicality of the test in general use. Thus the immunofluorescent test does not obviate the need for virus isolation procedures in the search for a viral etiology in cases of AHC.

Summary. Indirect immunofluorescent techniques were used to stain acetone fixed urine sediment smears from patients with acute hemorrhagic cystitis (AHC) and control without urinary tract diseases. Adenoviral antigen was identified in bladder epithelial cells of urine sediment from 3 of 4 patients with adenoviruric AHC. Urine sediment from seventeen virus negative AHC cases and four control cases failed to exhibit immunofluorescence. The finding of adenoviral antigen in epithelial cells of urine sediment from AHC patients supports the concept that adenovirus type 11 is etiologically related to this bladder infection.

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