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Viruses and interstitial cystitis: adenovirus genomes cannot be demonstrated in urinary bladder biopsies

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Abstract Microbes may be involved in the pathogenesis of interstitial cystitis (IC). Adenoviruses and BK virus (BKV) can infect epithelial cells in urinary bladder and they are causative agents for hemorrhagic cystitis. We therefore studied the presence of adenovirus and BKV genomes in urinary bladder tissue specimens of patients with IC using polymerase chain reaction (PCR) and in situ hybridization (ISH). Controls were specimens from cases with transitional cell carcinoma of the bladder. Nucleic acids were extracted from paraffin sections of the bladder tissue for PCR. Primers detecting all adenovirus types were used. In situ hybridization was carried out for the paraffin sections using digoxigenin-labeled DNA probes for adenovirus and BKV. The adenovirus DNA PCR was able to detect one to two infected cells/specimen. All the seven IC cases studied and six controls were negative for adenovirus DNA by PCR and ISH. The ISH test for BKV genomes was also considered negative in IC cases and controls. The specimens which were negative in PCR tests yielded a signal with β-globin primers, thus being amplifiable. We conclude that adenovirus and BKV do not play a major pathogenetic role in interstitial cystitis.

Key words Interstitial cystitis · Adenovirus · BK virus · Polymerase chain reaction · In situ hybridization

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Introduction

Microbes are probably involved in the pathogenesis of interstitial cystitis (IC). No known bacteria or viruses have been detected so far in diseased tissue. However, a recent polymerase chain reaction (PCR) study has demonstrated the presence of an unclassified microbe containing gram-negative bacterial DNA in bladder tissue of patients with IC [4]. In this study the bacterial DNA was present in 29% of the IC cases studied. It is possible that IC has a multifactorial etiology even if it is an infectious disease. Virological studies on IC have been carried out using virus isolation [5, 7] or serological examination [5] as methods. Herpes simplex virus, cytomegalovirus or varicella zoster virus have not been found to have roles as etiological agents for IC [5]. We studied the roles of adenovirus and BK virus (BKV) in IC, since these viruses are known to infect the cells in urinary bladder epithelium and they can cause hemorrhagic cystitis, especially in immunocompromised patients [2, 9, 13, 15]. Adenovirus types 11 and 21 are common etiological agents in acute hemorrhagic cystitis in children [13]. In the present study we used sensitive PCR and in situ hybridization methods for detection of adenovirus and BKV DNA in tissue specimens from urinary bladder biopsies of IC patients and control patients with bladder carcinoma.

Materials and methods

Patients and specimens

The IC patient group consisted of five men and two women, fulfilling the NIH and NIDDK criteria for IC [6]. Five patients had been subjected to bladder surgery (two resections and three cystectomies) due to intractable IC. The controls were six patients who had undergone total cystectomy due to carcinoma of the bladder. The study material was 5-μm-thick sections from paraffin-embedded tissue blocks. The sections for in situ hybridization (ISH) were taken on organosiliconized slides [12]. Sections for preparation of DNA

for PCR were cut using disposable knife blades. Sections of empty paraffin blocks were cut between the study specimens for PCR control purposes. The measures for prevention of PCR contamination are described below.

In situ hybridization

The probes for ISH were prepared by PCR with the primers shown below, using extract from adenovirus-infected A549 cells (for adenovirus probe) or dilutions of cloned BKV DNA (for BKV probe) as template. The preparative PCR reactions contained 50 μM digoxigenin(DIG)-11-dUTP (Boehringer, Germany) and 50 μM other deoxynucleotides (Pharmacia, Sweden). The PCR conditions were as described below. The DIG-labeled probes were recovered by ethanol precipitation.

The in situ hybridization and detection of DIG-labeled hybrids was carried out essentially as described by Hukkanen et al. [10] for DIG-labeled DNA probes. The modifications included use of 0.5 mg/ml denatured salmon sperm DNA and 0.5 mg/ml of transfer RNA (Sigma) as blocking agents in the hybridization mixture, use of a hot plate (95 °C) for 5 min for denaturation of target DNA in tissue slides before hybridization and use of a temperature of 42 °C for prehybridization and hybridization. The washes were done as described by Sandberg and Vuorio [14]. The detection of DIG-labeled hybrids was continued directly after washing. The procedure was as described previously [10]. Briefly, the sections were treated with 1% blocking reagent of the DIG DNA Labeling and Detection Kit (Boehringer, Germany) for 30 min at +20 °C, overlaid with the alkaline phosphatase-conjugated anti-digoxigenin antibody (750 mU/ml, Boehringer) and incubated in a humidified chamber for 40 min at +20 °C, washed and incubated with nitro-blue tetrazolium/X-phosphate color reagents in a dark, humidified dish for 18 h. The sections were briefly stained with eosin Y and the coverslips were mounted using Gurr's Aquamount. The controls for adenovirus in situ hybridization were sections from fixed adenovirus-infected and uninfected A549 cell pellets.

Polymerase chain reaction

For PCR studies the DNA was extracted from paraffin sections by two xylene extractions, two ethanol precipitations and a final wash with acetone [11]. In order to prevent PCR contamination, the working spaces for DNA extractions, construction of the PCR reactions, PCR run and gel analysis of the PCR products were kept separate from each other. The specimen handling was done in a designated laminar flow hood, which was treated with UV light for 20 min before and after the manipulations. This hood had not been used for cultivation of viruses. The specimens were pipetted using Finnpipette PCR positive displacement pipettors.

The primers for preparing the BKV probe by PCR bracketed the region at nucleotides #4388–4569 of the viral DNA (T antigen gene; *pyv.for*: 5′-TAGGTGCCAACCTATGGAACAGA and *pyv.rev*: 5′- GGAAAGTCTTTAGGGTCTTCTACC) [1]. The BKV PCR reaction contained 100 μM deoxynucleotides (Pharmacia), 250 nM primers and 1 U polymerase (DynaZyme, Finnzymes, Finland) with corresponding buffer, in a total volume of 50 μl/reaction. The DNA was boiled before PCR. The reaction temperatures were: 94 °C for 1 min/50 °C for 1 min/72 °C for 1 min, for 40 cycles, with a 3-min incubation at 94 °C before the first cycle and a 4-min extension after the last cycle.

The primers for adenovirus were from the hexon gene, representing the nucleotides #21589–21725 of adenovirus type 2 (5′- GCCGAGAAGGGCGTGCGCAGGTA and 5′-GACATGACTTTTGAGGTGGATCCCATGGA) [8]. These primers detect all adenovirus types. The DNA was boiled before PCR. The adenovirus PCR reaction contained 200 μM deoxynucleotides (Pharmacia), 200 nM primers, 1 U polymerase (DynaZyme, Finnzymes, Finland) and cor-responding buffer, in a reaction volume of 100 μl. The reaction temperatures were: 94 °C for 1 min/61 °C for 1 min/74 °C for 2 min, for 30 cycles, with a 10-min incubation at 94 °C before the first cycle. The positive control for adenovirus PCR was DNA extracted from sections of fixed adenovirus-infected A549 cells, corresponding to 200 infected cells/reaction. Negative controls were water and extracts of empty paraffin blocks. From each PCR product 30 vol% was run in a 2% agarose gel which was stained using ethidium bromide. Specimens which did not yield a signal in the virus PCR tests were subjected to PCR for human β-globin gene (268 bp fragment, primer sequences: 5′-CAACTTCATCCACGTTCACC and 5′-GAAGAGCCAAGGACAGGTAC).

Results

The sensitivity of the PCR test was evaluated using dilutions of extracted DNA from adenovirus-infected A549 cells. The adenovirus PCR was able to detect one to two infected cells. In PCR analysis all the IC specimens and controls were negative for adenovirus DNA (Fig. 1). The positive control, corresponding to 200 adenovirus-infected cells, yielded the positive product of the expected size of 137 base pairs (bp). The in situ hybridizations of IC specimens and controls for adenovirus DNA were also negative (Fig. 2). The background staining was heavy in parts of the tissue, especially in fibrous material. This reaction was also observed in control slides in which no probe was included in the hybridization mixture. The in situ hybridization for BK virus showed no clear-cut positive reactions with BKV probe (Fig. 3).

Discussion

Adenoviruses are frequent causative agents of hemorrhagic cystitis in humans [9, 13], and both adenoviruses and BKV are important pathogens causing

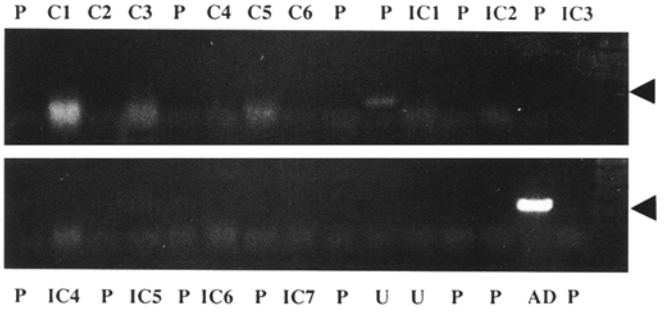

Fig. 1 Agarose gel electrophoresis of ethidium bromide-stained adenovirus PCR products. The type-common primers were derived from the hexon gene sequence. The location of the expected 137-bp PCR product is indicated by the *arrowheads* at the right margin. The positive control (*AD*) is located in the lower array of PCR products. The molecular size markers (*pEX3/Hinf*I digests) are in the *rightmost lane* of each gel. Specimens are: *P* empty paraffin sections, *C1–6* control cases with bladder carcinomas; *IC1–7* interstitial cystitis cases, *U* uninfected A549 cells, *AD* adenovirus-infected A549 cells, corresponding to 200 infected cells

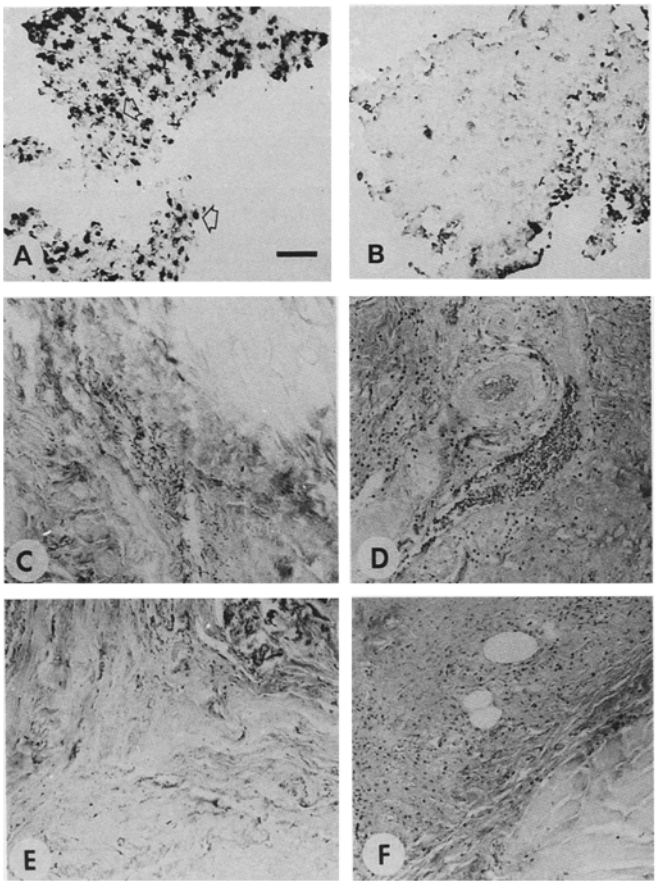

Fig. 2A–F In situ hybridization for adenovirus DNA using a digoxigenin-labeled DNA probe. Specimens were 5-μm paraffin sections of: adenovirus-infected A549 cells (**A**); uninfected A549 cells (**B**); bladder tissue of a case with transitional cell carcinoma (**C**); and bladder tissue from a case with interstitial cystitis (**D–F**). *Scale bar* in **A** represents 100 μm in all panels. *Arrows* in **A** indicate typical cells positive for the adenovirus DNA. The IC specimens and control cases were regarded as negative for adenovirus DNA

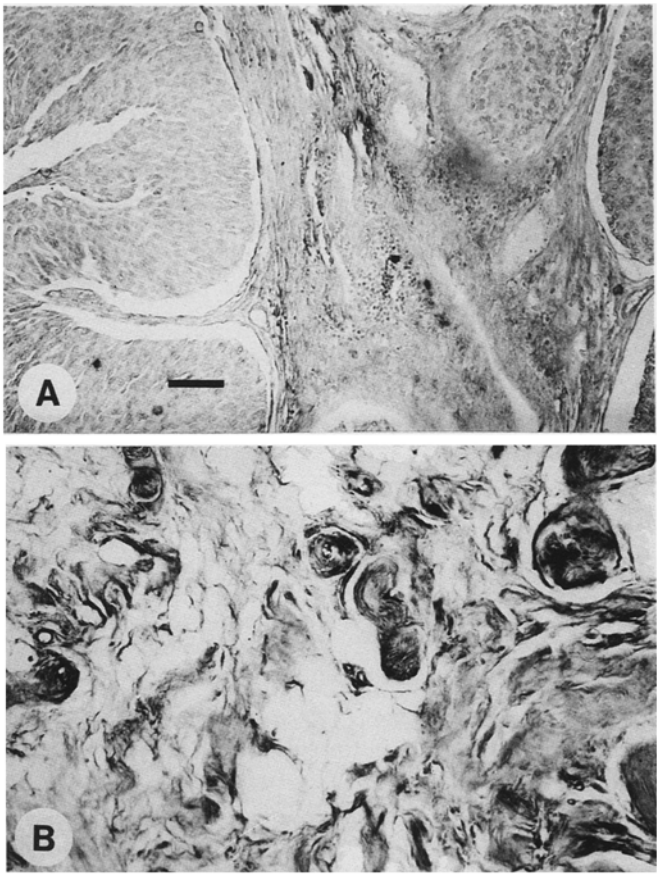

Fig. 3A, B In situ hybridization for BKV DNA using a digoxigenin-labeled DNA probe. Specimens were 5-μm paraffin sections of a control case with bladder carcinoma (**A**) and of a case with interstitial cystitis (**B**). *Scale bar* in **A** represents 100 μm. The hybridizations were considered negative for BKV DNA

cystitis in immunosuppressed patients, particularly in recipients of bone marrow or renal transplants [2, 9, 15]. A microbial etiology of IC has been suspected, since tissue-specific autoimmune phenomena have not been demonstrated in IC patients [4]. The causative roles of viruses, particularly herpesviruses, have been studied previously using the standard methods of virus cultivation and/or serology [5, 7]. In these studies no viruses were isolated from IC cases. We applied a more sensitive methodology in our approach. We studied the roles of adenoviruses and BKVs in IC using PCR and ISH techniques. Our application of PCR included use of paraffin sections of formalin-fixed bladder tissue as starting material for DNA extraction. A similar method has been used recently in the study of papillomavirus sequences in bladder tissue [3], indicating that the approach using paraffin sections is feasible. The suitability of the extracts for templates in PCR was controlled by amplification of human β-globin sequences from specimens which remained negative in virus PCR tests. We used nonradioactive ISH as a confirmatory test for our PCR findings, in order to investigate viral DNA, which would be present in only a few cells in the tissue.

Although the number of IC cases in our study is relatively limited (seven cases), any frequently associated viral pathogen should have been encountered among the cases. We used patients with bladder carcinoma as controls in order to assess the disease specificity of our findings. We were unable to demonstrate the presence of adenovirus sequences in any of the IC or control patients by PCR or ISH. The sensitivities of the techniques would have allowed detection of even individual scattered cells containing adenoviral DNA, since in experiments studying dilutions of adenovirus-infected cells in PCR we reached the detection limit of one to two infected cells/specimen (data not shown). The dilution of 200 infected cells/specimen, shown in Fig. 1, was regarded as a strong positive control. In the interpretation of ISH for adenovirus DNA we found that the detection and staining method itself yielded

background staining in fibrous material in the tissue. This was controlled by including such specimens in the ISH procedure which were left without the labeled probe but otherwise treated similarly to the study specimens. The presence of BKV in noteworthy amounts in the diseased tissue could not be demonstrated by the BKV ISH test (Fig. 3).

We conclude that adenoviruses and BK virus are not specifically associated with interstitial cystitis.

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