CHICKENPOX MONOARTHRITIS: DEMONSTRATION OF VARICELLA–ZOSTER VIRUS IN JOINT FLUID BY POLYMERASE CHAIN REACTION

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SUMMARY

A case of chickenpox monoarthritis is described. The presence of varicella–zoster virus (VZV) within the joint was demonstrated by the detection of viral DNA in synovial fluid at a time when peripheral blood cells were negative. This strongly suggests a direct role of VZV in causing monoarthritis complicating chickenpox. The use of the polymerase chain reaction allows more rapid (2 days) confirmation of the diagnosis. Early enough diagnosis would raise the question of using acyclovir to shorten the duration of arthritis.

KEY WORDS: Chickenpox, Monoarthritis, Varicella–zoster, PCR.

Arthritis is a rare, but well-recognized complication of acute varicella–zoster in children. Over the last 25 yr, there have been several isolated case reports, usually of a monoarthritis occurring at the time of onset of the exanthem or shortly afterwards. We describe a case in which the presence of varicella–zoster virus (VZV) was demonstrated by the detection of VZV DNA in joint fluid. This strongly suggests a direct role for VZV in causing this rare complication of chickenpox and the usefulness of a polymerase chain reaction (PCR)-based assay for rapid confirmation of the diagnosis.

CASE PRESENTATION

A 10-year-old girl presented to hospital with a 24 h history of acute swelling of the right knee which developed after apparently minor trauma. Over the preceding 10 days, she had had a sore throat and been subdued. Five days prior to presentation, she had developed a rash over her trunk which had persisted.

On examination, she was flushed and febrile (temperature 38°C). She had a maculopapular, vesicular eruption over her chest, back and abdomen. The right knee was extremely swollen, with a tense effusion. It was tender, and flexion was limited to 30°. She was unable to bear weight because of pain.

She was admitted to hospital and underwent arthroscopy and wash-out, in order to exclude a septic arthritis. A fibrinous exudate and a large quantity of straw-coloured fluid were removed. The synovium was noted to be boggy and hypertrophied. Synovial fluid showed 1650 white blood cells (WBC)/mm³: 95% mononuclear cells (mature lymphocytes and macrophages), 5% polymorphonuclear leucocytes (PMN). No organisms were noted on microscopy. Culture was negative for bacteria. Auromine-O stain for acid-fast bacilli was negative. Full blood count: haemoglobin 12.5 g/dl, white cell count 5.90, PMN 3.44, lymphocytes 1.74, monocytes 0.60, platelets 220, erythrocyte sedimentation rate (ESR) 8 mm/h, C-reactive protein (CRP) 1 mg/l. Blood cultures were negative. She was admitted for bed rest and splintage. A provisional diagnosis of chickenpox with monoarthritis was made.

Three days later, the knee had again developed a tense effusion. This was aspirated and 50 ml of amber-coloured fluid obtained and sent for viral studies. Swabs were also taken from the base of de-roofed crusted vesicles on her back. Over the next 3 days, her knee swelling did not return and she was discharged home. Assessment 2 months later showed her to be asymptomatic and functioning normally. However, there was mild persisting synovial thickening in the right knee joint and the presence of a small effusion was noted, consistent with mild persistent joint inflammation.

VIROLOGY TESTING

Samples submitted for virology analysis comprised washings from the right knee joint collected on 23 November 1996, aspirate from the right knee joint collected on 26 November 1996, swab from skin lesion collected on 26 November 1996, and peripheral white blood cells and serum obtained on 27 November 1996.

Methods

Samples were tested for the presence of VZV DNA by PCR amplification using primers targeting a sequence within VZV gene 31. The primer sequences were chosen from a region of VZV gene 31 displaying least homology to cellular or other viral sequences. Primer sequences are VZVK1-GCCCCGTC TCTATCTCCAAGAATT (upstream) and VZVK2-ACGACGGAGATACGTGCCAA (downstream).

Peripheral white blood cells were prepared from anticoagulated blood by red cell lysis, washing and concentration by centrifugation. Aliquots of 250 μl of sample, joint fluids or WBC suspensions were solubilized in SDS proteinase K lysis buffer and the DNA
extracted by phenol/chloroform extraction. DNA was recovered following overnight precipitation from 70% ethanol at −20°C. The DNA pellet was redissolved in 50 µl of water. An aliquot of this material was used for spectrophotometric determination of DNA concentration. The PCR reactions for knee washings and knee aspirate both contained the maximum volume of sample (10 µl), corresponding, respectively, to 30,000 and 100,000 cell equivalents of DNA. The PCR reaction for WBC contained 167,000 cell equivalents of DNA.

The reaction mixture contained 16.6 mM ammonium sulphate, 67 mM Tris–HCl (pH 8.8), 6.7 mM magnesium chloride, 10 mM β-mercaptoethanol, 6.7 µM EDTA, 1.5 mM each of dATP, dCTP, dGTP and dTTP, and 170 µg/ml of bovine serum albumin (Sigma). Two units of Taq polymerase (Life Technologies Inc.) were added to each 50 µl reaction mixture.

Two aliquots of lysis solution were passed through the DNA extraction procedure as controls for contamination; these were amplified (one result shown). DNA quality of the specimens was verified by parallel amplification of a 317 bp fragment of the β2 microglobulin gene (results not shown). DNA from peripheral WBC was controlled for inhibitors by spiking an aliquot with VZV DNA.

PCR reactions were denatured at 95°C for 3 min, followed by cycling at 60°C for 30 s, 65°C for 2 min, 95°C for 1 min. Forty cycles of amplification were performed. The 219 bp amplifier from the reaction was electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining.

Specific antibody to VZV was measured by indirect immunofluorescence. Cross-reacting antibodies were removed from the serum by prior absorption with a herpes simplex type I and II cell culture slurry. Slide preparations of VZV-infected human fibroblast cells were obtained from Gull (Cat #VZ211E; Gull Laboratories Inc., UT 84117, USA).

RESULTS

Knee washings, knee aspirate and skin swab were all positive for VZV DNA (Fig. 1). Peripheral WBC were negative for VZV DNA (Fig. 1). The identity of the amplified was confirmed by restriction endonuclease analysis (Fig. 2). Both negative control aliquots of lysis solution were negative for VZV DNA (one result shown). The peripheral WBC DNA control, spiked with VZV DNA, gave an amplified product of expected size, thus confirming there was no inhibition of VZV amplification (result not shown).

Antibody titres were as follows (Table I). Knee washings consisted of synovial fluid diluted to an unknown extent by saline wash-out solution. Knee aspirate was undiluted synovial fluid. Parallel mumps titres on these three samples (IgM, IgG, IgA) were all negative at 1:16 with the exception of the serum mumps titre of 1:128. Titres to mumps virus provided a marker antibody to control for passive contamination of synovial fluid with serum.

RESULTS

Non-bacterial arthritis occurring during the course of chickenpox in children has been recognized from at least 1931 [1]. More recently, there have been several individual case reports, involving children aged 2–10 yr. Most commonly a monoarthritis is described,
often affecting the knee [2–4]. Other joints have been involved and polyarticular cases have also been described [5, 6].

Although the clinical diagnosis of chickenpox is usually straightforward, by the time this patient presented with arthritis the skin rash had crusted and viral antigens were not demonstrable by direct immunofluorescence. It was, therefore, reassuring to confirm the diagnosis both by the presence of VZV-specific IgM and IgA in the serum, and by demonstrating VZV DNA in the swab from a skin lesion. Absence of VZV DNA in the peripheral WBC 9 days after cessation of new vesicle formation was consistent with clearance of viraemia.

VZV DNA was present at high titre in synovial fluid, both in the joint washings obtained at the acute phase of the arthritis and again 3 days later when the inflammation was less acute. Bacterial cultures of joint fluid were negative at all times. Although the presence of VZV DNA does not prove that viral replication was occurring at the site, the high titre of VZV DNA present in the joint fluid favours active local production. The absence of viral DNA in peripheral WBC at that time excludes passive contamination with viraemic blood. The DNA loading from peripheral WBC was five times that of the joint washings and twice that of the joint aspirate. The number of mononuclear cells amplified from peripheral WBC was, therefore, equivalent to or in excess of the number amplified from joint washings or aspirate. Antibody to VZV was present to excess in synovial fluid when compared to mumps antibody, selected as a comparative marker for passive entry of immunoglobulin into the joint. Excess of specific antibody suggests local antigenic stimulation.

There are rare reports in the literature describing isolation of infectious VZV from synovial fluid [7, 8] and, more recently, a report where VZV DNA was demonstrated in the joint effusion by PCR [9]. Our findings support these previous observations and reiterate the diagnostic utility of PCR-based technology.

Where there is fever and acute monoarthritis, a bacterial aetiology must be considered. Once negative bacterial cultures were obtained, the ability of PCR to provide a rapid positive diagnosis (2 days) was of great value to further clinical management. Classical viral culture methods require in excess of 10 days for the identification of VZV. Finding VZV within the joint raises the question of using acyclovir in an attempt to shorten the duration of arthritis.

REFERENCES