

# Fatal Adenovirus Type 7b Infection in a Child with Smith-Lemli-Opitz Syndrome

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Adenovirus type 7 causes worldwide respiratory tract infections, mainly in children. Severe systemic infections can occur, especially in immunocompromised patients and in patients with underlying chronic diseases. This report describes the first case of a fatal disseminated adenovirus type 7 infection in a child with Smith-Lemli-Opitz syndrome, a rare autosomal recessive disorder due to a primary enzymatic defect in cholesterol metabolism. Nasopharyngeal secretions and autopsy specimens including liver, lung, pleural fluid, and rectum were collected for viral culture. Adenovirus serotype 7 strains were obtained from all anatomic sites, except the liver. All these clinical isolates were analyzed using restriction endonuclease digestion of the genome, identifying them as genome type 7b, a virulent type. In this case, the fatal evolution could have been accelerated by the presence of an immunodeficiency although immunodeficiency is not included in the definition of Smith-Lemli-Opitz syndrome. The frequent recurrent banal infections in Smith-Lemli-Opitz syndrome could be prevented by a cholesterol supplementation regimen. Finally, this report emphasizes the need for efficient therapy for disseminated adenovirus infections, especially for virulent genome types. *J. Med. Virol.* 65:66–69, 2001. © 2001 Wiley-Liss, Inc.

**KEY WORDS:** adenovirus DNA; Smith-Lemli-Opitz syndrome; virulent genome type; restriction endonuclease digestion

## INTRODUCTION

The Smith-Lemli-Opitz syndrome is a rare autosomal recessive disorder due to a primary enzymatic defect in cholesterol metabolism [Honda et al., 1995], and characterized by multiple congenital anomalies [Smith et al., 1964]. The enzymatic defect is responsible for deficient plasma and tissue cholesterol levels, and accumulation of 7-dehydrocholesterol resulting in

growth restriction and mental retardation. The Smith-Lemli-Opitz syndrome has been subdivided into 2 types on the basis of clinical severity: type I is the classic one and type II the severe one. Recurrent banal infections are an important part of the medical problems associated with the Smith-Lemli-Opitz syndrome.

Adenovirus serotype 7 is frequently associated with severe clinical manifestations with residual damage and sometimes fatal outcome, mainly in children [Horwitz, 1996]. Adenovirus serotype 7 may cause localized diseases, such as pneumonia, acute respiratory disease, conjunctivitis, but also severe systemic infections with sepsis-like syndrome, high fever, and multiple-organ-system involvement, which can result in death [Horwitz, 1996; Munoz et al., 1998]. Host factors such as metabolic or genetic diseases, anatomic abnormalities, and immunologic deficiencies contribute to the severity of adenovirus infection [Munoz et al., 1998]. However, disseminated infections have also been reported in immunocompetent children and in children with underlying chronic illnesses [Munoz et al., 1998].

This report describes the first case of a fatal disseminated adenovirus type 7b infection in a child with a type II Smith-Lemli-Opitz syndrome.

## CASE REPORT

A 5-year-old girl had severe multiple morphologic anomalies including dysmorphic facies, cleft palate, polydactyly, and pyloric stenosis requiring gastrostomy. Since her birth, she had developed multiple ear and respiratory infections, and recurrent pyelonephritis although she had no renal tract abnormalities. The child had been living in a disabled patient center for two months before hospitalization. On 1 January, she was admitted to the Department of Pediatrics of Poitiers University Hospital Center for severe pneumonia and otitis due to respiratory syncytial virus (RSV). She was discharged a week later with anti-

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biotherapy. Nine days later, she developed fever and diarrhea. Despite symptomatic treatment, she was referred to the pediatric intensive care unit on 20 January for septic shock with acute pneumonia and diarrhea. On admission, she appeared hypotrophic (weight: 13,000 g), cyanotic and lethargic, with neurologic hypotonic signs. Physical examination showed high fever, important signs of acute respiratory infection, major hypotension (mean arterial pressure: 25 torr), tachycardia, hepatomegaly, and massive diarrhea. Chest X-ray showed bilateral reticulonodular infiltrates in the upper and lower lung lobes, with pleural effusion.

### Biological Investigations

Arterial blood gas values with a  $\text{FiO}_2$  of 1.0 were  $\text{pCO}_2$  6.3 kPa,  $\text{pO}_2$  26.2 kPa, oxygen saturation 99%, bicarbonates 21 mmol/l and pH 7.25. Laboratory data showed a leukocyte count of  $11.4 \times 10^9/\text{l}$  with 67% polynuclear leukocytes and 24% lymphocytes, hemoglobin at 10.8 g/dl, hematocrit at 31.3%, fibrinogen at 4.9 g/l, and C-reactive protein at 125 mg/l. Lactic acid was 5.51 mmol/l. Lumbar puncture showed clear cerebrospinal fluid (CSF), the erythrocyte count was  $5.1 \times 10^9/\text{l}$ , and the leukocyte count was  $0.075 \times 10^9/\text{l}$  with 100% mononuclears.

### Treatment and Evolution

Mechanical ventilation, fluid expansion, and vaso-pressive drugs were started immediately due to excessive respiratory workload and hypotension. The course worsened to episodes of cardiac arrests with refractory shock and acute respiratory distress syndrome. Despite intensive treatment including inotropic support and antibiotics, cardiovascular and respiratory functions deteriorated rapidly and the child died a few hours later.

### VIROLOGICAL INVESTIGATIONS

Nasopharyngeal secretions were processed for direct examination using immunofluorescence assay with monoclonal antibodies (Pasteur Diagnostics, Marnes-la-Coquette, France) for adenovirus, RSV, coronavirus, influenza viruses types A and B, and parainfluenza viruses types 1, 2, and 3.

Serology for adenovirus, influenza viruses type A and B, parainfluenza viruses types 1, 2, and 3, and enteroviruses was performed using the complement fixation test. A group-reactive antigen (Virion, Würzburg, Germany) was used for adenovirus complement fixation testing.

Nasopharyngeal secretions and autopsy specimens including liver, lung, pleural fluid, and rectum were inoculated onto HeLa-229 and MRC-5 cells. Characteristic cytopathic effect was confirmed by immunofluorescence assay using monoclonal antibodies against an adenovirus group-reactive epitope (Pasteur Diagnostics). Serotype determination was then performed by

neutralization with type-specific rabbit antisera (NIH, Bethesda, MD).

The adenovirus isolates were examined using restriction endonuclease digestion. Viral suspensions were harvested when an extensive cytopathic effect was present, and adenovirus strains were kept at  $-80^\circ\text{C}$  until restriction endonuclease analysis was carried out. Viral DNA was extracted and purified from infected cells as previously described by Shinagawa et al. [1983]. Restriction endonuclease analysis was carried out with *Bam*HI and *Bst*EII according to a well-known pathway used for genome type identification [Li et al., 1996; Wadell et al., 1985]. Briefly, aliquots containing 1–2  $\mu\text{g}$  of viral DNA were digested with 10–15 U/ $\mu\text{l}$  of each endonuclease according to the manufacturer's instructions (Boehringer Mannheim, Germany). After digestion, nucleic acid fragments were separated by electrophoresis at 38 V/cm for 16 hr on a 0.8% agarose gel and visualized by UV illumination following bromide staining. Adenovirus genotype 7c (no. 37300) and adenovirus genotype 7b (KCH4) strains circulating in Europe since the 1970s were used as reference.

### RESULTS

Direct examination by immunofluorescence assay on nasopharyngeal secretions was positive for adenovirus. Serology for adenovirus was negative, as well as serologies for influenza viruses types A and B, parainfluenza viruses types 1, 2, and 3, cytomegalovirus, and enterovirus. Only specific RSV antibodies were found positive at 1:128 by complement fixation testing.

Five days after inoculation, typical adenovirus cytopathic effects on heteroploid cells showing rounding and swelling of cells, refractile appearance, and clumping in grapelike clusters were observed in all the clinical specimens, except the liver. This preliminary identification was confirmed by immunofluorescence assay, showing adenovirus serotype 7 in all anatomic sites. It should be noted that specific neutralizing antibody titer was lower than 1:4 using the adenovirus serotype 7 isolates as antigen.

Each isolate obtained from the anatomic sites yielded cleavage patterns similar to the adenovirus genotype 7b reference strain. Restriction patterns with *Bam*HI and *Bst*EII showed a genome type 7b (Fig. 1). The use of *Bst*EII allowed discriminating between genome types 7b and 7b1 [Li et al., 1996].

Bacteriologic investigations including mycobacteria research in blood, CSF, pleural fluid, lung, and liver were negative as well as serologic tests for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Coxiella burnetii*.

### DISCUSSION

In the case reported, a child with a severe genetic defect presented a fatal adenovirus infection. The main question arising is the reason why this patient was particularly vulnerable to disseminated adenovirus infection. The presence of an immunodeficiency is the

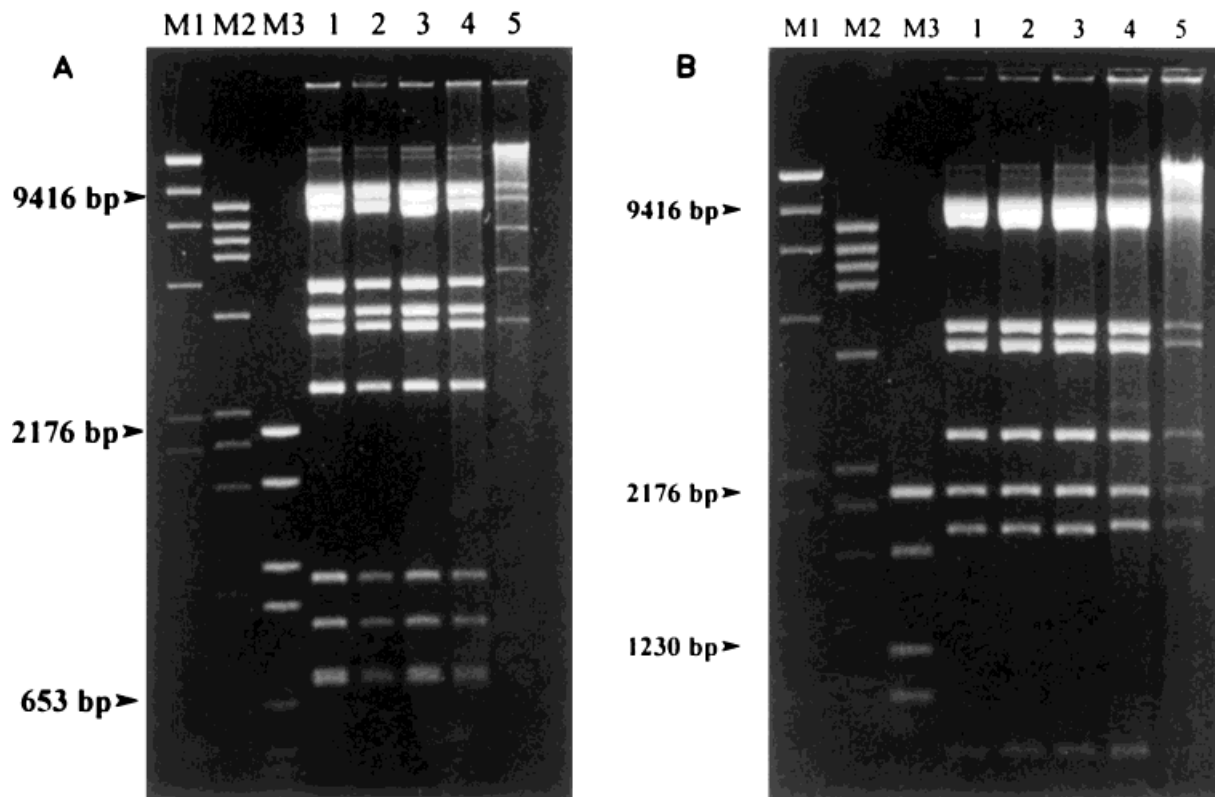


Fig. 1. Agarose gel electrophoresis showing *Bam*HI (A) and *Bst*EII (B) restriction patterns of adenovirus serotype 7 isolates. **Lanes M1, M2, M3:** size markers (bacteriophage DNA digested with *Hind*III, adenovirus serotype 2 digested with *Kpn*I and VI marker, respec-

tively); **lanes 1–3:** isolates from nasopharyngeal secretions, lung, and rectum, respectively; **lanes 4,5:** adenovirus genotype 7b (KCH4) and adenovirus genotype 7c (no. 37300) reference strains, respectively. Numbers to the left of the gels are molecular sizes in base pairs.

major hypothesis although immunodeficiency is not recognized as part of the Smith-Lemli-Opitz syndrome. Ostergaard et al. [1992] showed a defective monocyte oxidative metabolism in a child with Smith-Lemli-Opitz syndrome suffering from repeated febrile infections, but monocyte functions including chemotaxis, phagocytosis, and IL-1 production were normal. However, other unknown immune mechanisms might lead to a fatal evolution.

Viral infections induce a temporary suppression of T-cell-mediated immunity, or impair respiratory tissue and may increase the risk of developing severe secondary infections, mainly by adenovirus [Munoz et al., 1998]. In our patient, RSV detected at first admission might have contributed to the severity of adenovirus serotype 7 subsequent infection.

A decrease in the number and severity of intercurrent infections as well as improvement in developmental progress have been evidenced by a cholesterol supplementation regimen [Elias et al., 1997]. In the case reported, the patient was not supplemented with cholesterol.

Some adenovirus infections have been suggested to result from reactivation of latent viruses from an endogenous source [Horwitz, 1996; Munoz et al., 1998]. In this study, failure to detect adenovirus antibodies is more consistent with an acute infection than with a reactivation or a persistent infection.

However, some adenovirus infections that may not be detected by complement fixation testing can yield up to 50% false-negative results in children [Schmidt and Lennette, 1971]. In addition, nosocomial transmission might be suspected, since the interval between the two hospitalizations was consistent with the incubation period for adenovirus infection [Horwitz, 1996].

Disseminated adenovirus infections have been characterized by severe pulmonary disease, multiple-organ-system involvement including the liver, and frequent fatal outcome [Munoz et al., 1998]. We demonstrated nasopharynx, lung and gastrointestinal tract impairments but failed to find hepatic involvement although hepatitis has been commonly reported [Horwitz, 1996; Munoz et al., 1998]. The mortality rate is usually high, due to the type of underlying conditions and the severity of the disease. In addition, there is no specific therapy for adenovirus infections (ganciclovir and ribavirin have been used with no significant results [McCarthy et al., 1995]). Although no convincing link was established between a particular type and enhanced virulence, genome type 7b is the most common and virulent serotype 7 strain in Europe [Munoz et al., 1998]. However, numerous genome types and subtypes circulate and cause outbreaks with various degrees of severity [Horwitz, 1996].

Finally, this report emphasizes that adenovirus genotype 7b remains a virulent genome type, and that

the severity of the clinical outcome calls for an efficient therapy for adenoviral infection, especially in patients susceptible to threatening viral infections such as in the Smith-Lemli-Opitz syndrome.

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