Severe Pneumonia Due to Adenovirus Serotype 14: A New Respiratory Threat?

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Background. Adenoviruses are associated with sporadic infection and community and institutional outbreaks; they can cause especially severe disease in infants, young children, immunocompromised persons, and transplant recipients. Fifty-two adenovirus serotypes have been recognized and classified within 7 subgroups or species (A–G), with limited data available on associated clinical syndromes and disease severity in more than one-half of the known serotypes.

Methods. We describe the clinical presentation and virologic characterization of 1 adult and 2 pediatric patients admitted to 2 separate hospitals during April–May 2006 with severe acute respiratory tract infection. All patients had underlying chronic pulmonary disease; none were severely immunocompromised. All 3 experienced serious chronic sequelae or died.

Results. Adenovirus was isolated from all 3 case patients. Adenovirus serotype 14, a subspecies B2 serotype not previously associated with severe clinical illness, was confirmed by neutralization assay and sequencing of the hexon gene. Restriction enzyme analysis with BamHI, BglII, HindIII, and SmaI showed all 3 viruses to be identical and to belong to a new genome type that we have designated “Ad14a.”

Conclusions. Our identification of severe respiratory illness due to a previously rarely reported adenovirus serotype may signify the emergence in the United States of a new genomic variant that has the potential to spread globally and cause epidemics. These case reports highlight the need for rapid diagnosis and improved surveillance, with serotyping and molecular characterization, to identify emerging variants of adenovirus, which may assist with targeted development of antiviral agents or type-specific vaccines.

Human adenoviruses (Ads) are ubiquitous. The clinical spectrum of disease in humans can vary substantially, depending on the infecting serotype, and can include asymptomatic infection, pharyngoconjunctival fever, pneumonia, epidemic keratoconjunctivitis, acute hemorrhagic cystitis, gastroenteritis, meningocerebralitis, hepatitis, myocarditis, and life-threatening disseminated disease [1]. Ads have been associated with sporadic infection, as well as with community and institutional outbreaks. Common affected populations include new military recruits, healthy older children and adults infected in community outbreak settings, very young children, and immunocompromised persons (especially transplant recipients), with the latter group having the highest incidence of severe disease.

To date, ≥52 Ad serotypes have been recognized and classified within 7 subgroups or species (A–G) on the basis of distinctive characteristics, including sequence homology of their genomic DNA, genomic G+C content, tissue tropism, electrophoretic mobility of structural proteins, and other biological properties [2, 3]. Certain species, including B, C, D, and E, circulate globally and have been well characterized. However, more than one-half of known Ad serotypes are infrequently detected and have an unclear role with regard to human disease. For example, although subspecies B1 (Ad3, Ad7, and Ad21), species C (Ad1, Ad2, Ad5, and Ad6), and species E (Ad4) are well-recognized causes of acute respiratory tract infection, the subspecies B2
Ads have been only sporadically associated with clinical illness, most notably Ad11 with hemorrhagic cystitis in children [1,3,4]. In this report, we describe the clinical presentation and virologic characterization of 3 critically ill case patients with acute respiratory tract infection, including 1 patient who died of Ad14, a subspecies B2 serotype rarely identified and not previously associated with severe clinical illness [4].

CASE PATIENTS

Patient 1, a 49-year-old man with underlying chronic obstructive pulmonary disease, obstructive sleep apnea, obesity, and congestive heart failure, was hospitalized in May 2006 with a 4-month history of dry cough, pleuritic chest pain, shortness of breath, and wheezing. The patient had received treatment with multiple antibiotic courses and a 6-week course of oral prednisone (20 mg per day). The findings of a physical examination were unremarkable except for mild dyspnea, and admission laboratory test values were within normal ranges. Chest radiograph and CT showed bilateral “tree-in-bud” pattern of nodular opacities. Within 1 week, the patient had clinically deteriorated, developing acute respiratory distress syndrome, hypotension, and acute renal failure requiring intubation and pressor support. Results were negative for a battery of microbiological tests—including blood, sputum, and urine cultures; urinary antigen testing for Legionella pneumophila serotype 1; serologic testing for HIV, Chlamydia pneumoniae, Chlamydia psittaci, Mycoplasma pneumoniae, influenza A and B, respiratory syncytial virus, aspergillosis, coccidiomycosis, and cryptococcosis—and bacterial cultures and acid fast bacilli smears of bronchoalveolar lavage fluid. The patient died 2 weeks after hospital admission. Gross autopsy findings revealed severe acute interstitial pneumonitis. Subsequent viral culture from the bronchoalveolar lavage grew Ad.

Patient 2, a 17-month-old male infant, born prematurely after a 33-week gestation, with a history of multiple hospitalizations for lower respiratory tract infections, was hospitalized in April 2006 with a 2-day history of fever, cough, and decreased energy and oral intake. At admission, the patient was febrile (temperature, 41.2°C) and hypoxic (oxygen saturation on room air, 80%). Abnormal laboratory results included a slightly elevated WBC count, at 11.7 x 10^9 cells/L and a chest radiography demonstrating multilobar infiltrates. Despite treatment with broad-spectrum antibiotics, the child’s condition deteriorated, requiring intubation, with high-frequency oscillatory ventilation and extra corporeal membrane oxygenation. He developed recurrent pneumonias and a right upper lobe abscess that required surgical excision. After 350 days of hospitalization, the patient was discharged home but required long-term mechanical ventilation via a tracheostomy, aggressive pulmonary toilet, and G-tube feedings.

METHODS

An Ad isolate from patient 1 and nasopharyngeal and bronchoalveolar lavage specimens from patients 2 and 3, respectively, were forwarded to the California Department of Public Health Viral and Rickettsial Disease Laboratory (Richmond, CA) for serotyping. Isolates were also shipped on dry ice to the Lovelace Respiratory Research Institute (Albuquerque, NM) for further virologic characterization.

Virus culture and serotyping. At the Viral and Rickettsial Disease Laboratory, Ads were grown in culture with use of in-house prepared human fetal diploid cells and A-549 cells. Hyperimmune rabbit antisera to Ad types 1–49 were prepared and standardized. Sequential serotyping was performed, first with use of specific microneutralization assays for Ad types 1–5, 7, and 21 [5]. Testing was then performed with 12 intersecting
pools of immune sera that identify 35 different Ad types; antisera to each serotype is contained in 2 of the pools, and particular serotypes are identified when neutralization occurs in 2 pools that intersect on a grid [6].

**Viral DNA purification.** At Lovelace Respiratory Research Institute, the 3 isolates were passed once in A549 cells in 25 cm² flasks and were subsequently amplified in monolayers of A549 cells in 75 cm² flasks, for viral DNA extraction. Intracellular viral DNA was extracted from infected cells by the method developed by Shinagawa et al. [7], with modifications as described elsewhere [8].

**Sequence analysis.** Because Ad genomes are stable and recombinations are rare, sequence data can be used to identify species and serotype. The hypervariable region 7 (HVR7) of the hexon gene, shown to be useful for serotype determination by Sarantis et al. [9], was amplified using the primer pair and conditions reported by Metzgar et al. [4, 10]. Sequencing was performed by the DNA Research Services at the University of New Mexico Health Sciences Center (Albuquerque). Sequence analysis was performed using Seqman software for contig assembly and Megalign software (both programs are components of the Lasergene suite; DNASTAR) for sequence alignments.

**DNA restriction enzyme analysis.** For genome type identification, 1 μg of viral DNA was digested with restrictionendonucleases BamHI, BglII, HincII, and Smal, following the manufacturer’s recommendations (Promega). DNA fragments were separated by horizontal gel electrophoresis in 0.8% or 1.2% agarose gels run in 1× TBE buffer (Tris-borate, 0.09 M; EDTA, 0.002 M; pH, 8). Restriction profiles were visualized by UV transillumination at 303 nm, after staining with ethidium bromide, and were photographed in a Gel Doc imager (Biorad). Digests of DNA from the prototype strain of Ad14 (de Wit) were run in parallel, for comparison [11].

**RESULTS**

**Identification of serotype.** At Viral and Rickettsial Disease Laboratory, testing by microneutralization assays and intersecting pools identified all 3 isolates to be Ad14. The serotype was confirmed by specific neutralization with type-specific Ad14 rabbit antiserum (50–320 TCID₅₀ by ≥20 antibody units). At Lovelace Respiratory Research Institute, the sequence for HVR-7 for the 3 isolates was determined and was found to be identical. Alignment of the consensus sequence for the HVR-7 of the 3 isolates with the reference prototype strain Ad14 (de Wit) (GenBank accession number DQ149612) revealed a 99% sequence identity with 2 point mutations consistent with the serological identification of Ad14. Sequence data was deposited in GenBank under accession numbers EF653225, EF653226, and EF653227.

**DNA restriction enzyme analysis.** Restriction enzyme analysis of viral DNA with BamHI, BglII, HincII, and Smal revealed novel profiles for 3 of these endonucleases, easily distinguishable from the ones yielded by the reference prototype strain of Ad14 (de Wit) (figure 1). On the basis of the novel BamHI, the new genome type was named 14a, following the denomination system proposed by Li and Wadell [12].

**DISCUSSION**

In this article, we provide detailed clinical descriptions of 2 cases of severe illness and 1 death attributable to Ad14 infections, a previously recognized species B2 serotype that had been rarely associated with clinical illness in humans [4]. Certain Ad serotypes are well-described agents of acute respiratory tract infection. Ad1, Ad2, Ad5, and Ad6 are some of the more common causes of upper respiratory tract infections and pharyngogonjunctival fever in young children; up to 40%–60% of this population has serologic evidence of prior infection [13]. Ad4 and Ad7 are associated with upper and lower respiratory tract infection in young adults and are an important etiology of respiratory outbreaks in new military recruits, with up to 80% infected and 20%–40% hospitalized in some settings [13]. In immunocompromised patients, particularly bone marrow transplant or solid organ recipients, fulminant pneumonia and disseminated disease have been attributed to infections with Ad5, Ad31, Ad34, Ad35, and Ad39 [1]. Certain serotypes, especially 2, 3, 7, and 21, have been associated with serious chronic sequelae after acute respiratory tract infection, including irreversible atelectasis, bronchiectasis, bronchiolitis obliterans, and unilateral hyperlucent lung, with an estimated 14%–60% of children experiencing some degree of permanent lung damage [14].

Historically, the subspecies B2 Ads, including Ad14, have been infrequently recognized as a cause of acute respiratory illness. Until recently, most reports of Ad14 infection originated from outside the United States. After its original identification in acute respiratory tract infections among Dutch military recruits in 1955, Ad14 was associated with respiratory outbreaks in Great Britain in the same year, Uzbekistan in 1962, and Czechoslovakia in 1963 [11, 15–17]. More recently, a study of children hospitalized in Taiwan during 2001–2002 with acute respiratory illness identified Ad14 in 2%–11% of isolates; most of the case patients presented clinically with pharyngitis, tonsillitis, and bronchitis [18]. In 2007, there were new reports of the emergence of Ad14 in the United States. The virus has been detected in recent outbreaks of mild febrile respiratory illness in several military-recruit training centers in the United States, with HVR7 hexon sequences identical to the viruses we describe [4].

In contrast to these previous reports, the 1 adult and 2 pediatric patients with Ad14 infection we describe all presented with severe acute respiratory disease requiring intensive care, mechanical ventilation, and prolonged hospitalization. All 3
Figure 1. DNA restriction enzyme analysis of adenovirus 14 isolates ("Ad14p de Wit") from 3 cases of severe pneumonia. *Bam*H1, *Bgl*II, *Hin*dIII, and *Sma*I restriction enzyme patterns of adenovirus isolates from the reference prototype strain Ad14p (de Wit), a 49-year-old man who died after developing rapid, severe acute interstitial pneumonitis (case 1); a 17-month-old boy with multilobar pneumonia, bronchiolitis obliterans, and bronchiectasis (case 2); and a 3-year-old boy with multilobar pneumonia and severe bronchiectasis that required surgical excision of the lung and long-term ventilation via tracheostomy (case 3). Resolution was done by means of gel electrophoresis with ethidium bromide staining. Three endonucleases had novel patterns; on the basis of the novel *Bam*H1 and following the denomination system proposed by Li and Wadell [12], these 3 viruses have been designated to be a new genome type, Ad14a. M, molecular weight marker (1 kilobase + 100–base pair ladders; Bio-Rad).

patients had underlying chronic pulmonary disease; although 1 patient was given short-term corticosteroid treatment before onset of Ad infection, none were severely immunocompromised. All experienced serious sequelae, including bronchiolitis obliterans; severe, chronic lung disease requiring ventilator dependence; or death. Although little is known about mechanisms of pathogenicity of Ad, serotype, age of the patient at the time of infection, and other host factors (e.g., presence of immunosuppression or chronic lung disease) likely play key roles [1]. Mutations or recombinations may result in biological or antigenic changes in the type-specific epitopes in the major viral capsid protein, hexon, that can lead to increased virulence [3, 10]. Studies of Ad infection in children have identified increased production of cytokines, particularly TNF-α, IL-6, and IL-8 [19]. A low level of population immunity may also play a key role in susceptibility, clinical severity, and enhanced transmission of newly introduced Ad serotypes. For example, studies of new military recruits have documented Ad seroconversion rates of 34%–97% over a 6-week period, with associated high attack rates; in 1 study, 25% of enrolled recruits developed a febrile respiratory illness in that time frame, with 100% of recruits who had an initial titer of 1:4 becoming ill [20].

The 2 reported pediatric cases were admitted on the same day to adjacent beds in the pediatric intensive care unit. Although both Ad14 infections may have been community acquired, it is possible that 1 patient acquired infection from the other sometime during hospitalization. Although each child was cared for by separate medical teams and nurses using rigorous infection control precautions, their close proximity and the frequent aerosolized treatments required by 1 patient could have facilitated droplet transmission. Nosocomial outbreaks of Ad infection among institutionalized children in similar settings have been reported frequently [21, 22]. The commonly recognized route of transmission is respiratory droplet, although the contribution of contact transmission via fomites to the efficient spread of the virus in crowded, closed settings—such as military barracks, long-term care institutions, or day care centers—is increasingly recognized [20]. The rapid evolution of Ad outbreaks in institutionalized and hospital settings argues for improved awareness, better early detection, and strict adherence to infection control recommendations. Once a diagnosis is made, aggressive environmental decontamination and rapid cohorting and isolation of infected patients should be implemented.

Our Ad isolates were serotyped using serum neutralization assay and sequencing of the hexon gene. Further restriction enzyme analysis identified a new genomic variant, designated Ad14a. In the past 2 decades, advances in molecular testing capabilities have facilitated development of a new Ad classification system, better understanding of the emergence and shift in predominance of circulating Ad genomic variants, and correlation of specific variants with enhanced pathogenicity. For
example, between 1966 and 2000, Ad7 accounted for nearly 20% of Ads distributed worldwide [23]. The use of restriction enzyme analysis enabled the identification of the emergence and global spread of Ad7b in the 1970s, followed by Ad7d2 in the 1980s and 1990s [23, 24]; both viruses were associated with epidemics of severe respiratory illness and deaths [22, 24, 25].

Similar to Ad7, the confirmation of Ad14 in our 3 cases, as well as its recent association with acute respiratory tract infection in military recruits, may signify the emergence of a new genomic variant of Ad that has the potential to spread throughout the United States and globally. Although the advent of molecular techniques now allows for the quick, sensitive, and specific detection of Ad, few hospital laboratories have the capability to perform these tests on site. These findings highlight the need for improved access to rapid PCR assays to detect Ad in the clinical setting, as well as better public health surveillance, including serotyping and molecular characterization. Both may assist with the development of antiviral agents or type-specific vaccines that can substantially decrease Ad-associated morbidity and mortality in vulnerable populations.

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