

BRIEF COMMUNICATION

ADENOVIRUSES ISOLATED FROM CIVILIAN AND MILITARY PERSONNEL IN THE CITY OF RIO DE JANEIRO, BRAZIL

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SUMMARY

Adenovirus are important pathogen primarily associated to respiratory infections of children and military personnel, even though it is also associated to cases of conjunctivitis and keratoconjunctivitis. We analyzed respiratory secretion collected from subjects with and without respiratory infection symptoms, being 181 civilians and 221 military subjects. The samples were inoculated in HEp-2 and/or A549 tissue cultures for viral isolation. Samples presenting cytopathogenic effect (CPE) in any tissue culture were tested by a polymerase chain reaction (PCR) assay to confirm adenovirus isolation. The isolates confirmed as adenovirus were further analyzed by restriction endonuclease assay for determination of viral species. Three isolates were identified as specie A (two from civilian and one from military), one isolate from military was identified as specie C, and one isolate from civilian was identified as specie D. For two isolates the specie could not be identified.

KEYWORDS: Adenovirus; Respiratory infections; Epidemiology.

Human adenovirus comprises 51 serotypes classified into the genera *Mastadenovirus* of the *Adenoviridae* family, and are divided in six species (formerly subgenera) designated from A to F (BENKÖ *et al.*, 1999; DE JONG *et al.*, 1999, HORWITZ, 2001). The adenoviruses were first isolated and characterized as viral agent by two groups who were searching for the etiologic agents of acute respiratory infections (ROWE *et al.*, 1953; HILLEMANN & WERNER, 1954). Besides respiratory diseases, which includes acute respiratory disease of the military recruits, acute febrile pharyngitis, pharyngoconjunctival fever, and pneumonia, the adenoviruses have been associated to a variety of clinical syndromes, such as epidemic keratoconjunctivitis, acute hemorrhagic cystitis, hepatitis and gastroenteritis (HORWITZ, 2001). Recently, the detection of adenovirus genome was described in cases of myocarditis (PAUSCHINGER *et al.*, 1999), sudden infant death associated to myocarditis and pericarditis (SHIMIZU *et al.*, 1995), toxic shock-like syndrome (PRICE *et al.*, 2001), and unexplained deaths (PERKINS *et al.*, 1996). Isolation of adenovirus from patients with central nervous system manifestations such of fatal acute flaccid paralysis (CARDOSA *et al.*, 1999) and encephalitis with cerebral edema have also been described (CHATTERJEE *et al.*, 2000).

Epidemiologic studies initiated by the Commission of Acute Respiratory Disease of the U.S. Armed Forces confirmed that the adenoviruses were the cause of a large number of respiratory syndromes

among military recruits (GINSBERG *et al.*, 1955; DINGLE & LANGMUIR, 1968). Because of crowded living conditions, stressful working environment, and exposure to respiratory pathogens in disease-endemic areas, military trainees and newly mobilized troops are at particularly high risk for respiratory disease epidemics (GRAY *et al.*, 1994; GAYDOS & GAYDOS, 1995). During the 1950s and 1960s, up to 10% of recruits were infected with adenovirus, and these pathogens were responsible for approximately 90% of pneumonia hospitalizations (HILLEMANN, 1958; MILLER *et al.*, 1963). During the fall and winter seasons, specially in basic training camps, adenovirus caused widespread morbidity that overwhelmed medical resources, disrupted military training, and caused significant economic loss (COLLIS *et al.*, 1973). This scenario changed dramatically after 1971 with the introduction of an oral attenuated adenovirus vaccine against serotypes 4 (specie E) and 7 (specie B) (GAYDOS & GAYDOS, 1995). However, in 1996, the sole manufacture discontinues production of this vaccine and soon thereafter, as the supplies dwindled, new epidemics of respiratory infections among military subjects reemerged in the military settings. Since 1999, approximately, 10 - 20% of all recruits have become ill with adenovirus infection in basic training, similar to the prevaccine era (BARRAZA *et al.*, 1999; GRAY *et al.*, 1999; McNEILL *et al.*, 1999; MMWR, 2001; RYAN *et al.*, 2002).

In Brazil, epidemiological data to ascertain the impact of adenovirus respiratory infections in the military population are scarce (maybe

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nonexistent), and vaccination against this infection was never practiced in the country. Indeed, we are aware of one single study conducted among military recruits from an Army headquarters (Batalhão Oswaldo Cruz) located at Magalhães Bastos in the city of Rio de Janeiro, during an outbreak of respiratory disease (MACHADO, 1971). In the study, adenoviruses were isolated from 14 out of 161 samples. Eight isolates were demonstrated to belong to serotype 8, five to serotype 7 and one serotype 1. Information on the impact of adenovirus infections on the civilian adult population are rare as well, specially because the majority of epidemiologic studies that have been done in the country to evaluate the frequency of such infections were primarily targeted to the infantile population. In those studies the serotypes most common described were 1, 2, 3, 5 and 7 (TAKIMOTO *et al.*, 1969; SUTMOLLER *et al.*, 1983; NASCIMENTO *et al.*, 1991; MORAES *et al.*, 1997; KAJON *et al.*, 1999).

In this study, pharyngeal swabs were obtained from adults subjects: military recruits (221 specimens) from an army headquarter in Rio de Janeiro city, collected from April to November 2000, and civilian personnel (181 specimens), collected between March 2000 and November 2002, also in Rio de Janeiro city. The samples were collected from subjects with or without clinical symptoms. The symptoms included fever, sore throat, cough and rhinorrhea. The swabs were placed into virus transport medium and sent immediately to the laboratory where were stored at 4 °C. Prototype strains of adenovirus serotypes 31 (specie A), 3 (specie B), 1 (specie C), 10 (specie D), and 4 (specie E) provided by Dr. Dean Erdman (Centers for Disease Control and Prevention, USA) were cultivated in HEp-2 cells and used as positive controls. HEp-2 cells were provided by Dr. M.D. Wigg (Laboratório Experimental de Drogas Antivirais e Citotóxicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil). A549 cells were obtained from the Rio de Janeiro Cell Bank. Cell cultures were maintained in Dulbecco's Modified Eagle Medium (D-MEM) (Life Technologies, Rockville, MD) containing 50 µg/mL of gentamicin, 0.5 µg/mL of fungizone and 5 - 10% fetal calf serum. Specimens were inoculated into two tubes of HEp-2 and/or A549 cells and incubated at 37 °C in stationary racks. The cultures were daily examined for cytopathic effect (CPE) for one week and cultures homogenates were submitted to up to four blind passages. Samples that did not present CPE were considered negative. Supernatants from CPE positive cell cultures were tested for adenovirus by PCR. For adenovirus DNA extraction was achieved by using DNAzol (Invitrogen - Carlsbad, CA) according to the manufacturer's instructions. PCR amplification of adenovirus DNA extracted from positive tissue culture supernatant was performed in 50 µL volumes containing 45 µL of reaction mixtures, as described previously (ALLARD *et al.*, 2001). The primers used for PCR reaction amplify a region of the hexon gene of all adenovirus serotypes. Amplifications were carried out in a GeneAmp PCR system 2400 thermal cycler (Applied Biosystems, Foster City, CA). The PCR products were analyzed by 1.2% agarose gel electrophoresis and visualized by staining with ethidium bromide. Aliquots of 5 to 10 µL of the PCR product were digested for 3 h with 5 U of different endonucleases, *Ava* II, *Hae* II, *Csp* I (Promega, Madison, WI), and *Sma* I [Invitrogen], under conditions specified by the manufacturers. The endonuclease *Ava* II do not cleave adenovirus from species A or B but cleavage other adenovirus species. The enzyme *Hae* II cleave adenovirus from specie B and do not cleave specie A. The enzyme *Sma* I separate adenovirus from specie E from species C and D, and *Csp* I separate specie C from D (ALLARD *et al.*, 2001). Enzyme digests were analyzed on 3% NuSieve - 1% SeaKem ME agarose gels (FMC Bioproducts, Rockland, MN).

Five (2.26%; 5/221) samples from military recruits presented CPE in at least one of the cell cultures used. Among adults civilian subjects 10 (5.52%; 10/181) samples yielded CPE in one of the cells used. The culture supernatant of CPE positive samples was processed for adenovirus detection by PCR assay. Seven samples were positive confirming the isolation of adenovirus, being four samples from civilian (three symptomatic and one asymptomatic subjects) and three samples from military recruits (one symptomatic and two asymptomatic subjects) (Table 1). Three isolates were identified as specie A (two from civilian and one from military), one isolate from military was identified as specie C, and one isolate from civilian was identified as specie D. For two isolates (one from civilian and one from military) the specie could not be identified (Table 2).

Table 1

Frequency of adenovirus isolated from military and civilians with or without respiratory symptoms

	Clinical features				Total
	With symptoms		Without symptoms		
	Positive	Negative	Positive	Negative	
Civilians	3	86	1	91	181
Military	1	66	2	152	221
Total	4	152	3	243	402

Table 2

Adenovirus species determined by PCR-RFLP among the viruses isolated

Origin of sample	Specie	Month/Year
Civilian	A(2)	April/2000 June/2001
	D(1)	March/2001
Military	A(1)	August/2000
	C(1)	July/2000

Although adenoviruses are most frequently associated to respiratory infection in children some serotypes are often associated to acute respiratory disease among military recruits (GINSBERG *et al.*, 1955; DINGLE & LANGMUIR, 1968). Outbreaks of adenovirus disease among adults in the civilian population are rare and often associated to fatal cases (PERKINS *et al.*, 1996; SÁNCHEZ *et al.*, 1997; MMWR, 1998; PAUSCHINGER *et al.*, 1999; CARDOSA *et al.*, 1999; PRICE *et al.*, 2001).

To our knowledge no longitudinal study have ever been performed in Brazil to access the occurrence of adenovirus infections among military recruits, with the exception of one study in an army facility in Rio de Janeiro in 1971 (MACHADO, 1971). Nevertheless, this was not a longitudinal analysis, instead, it was a study of a solely outbreak of respiratory disease in a short period of time. Therefore, this would be the first time that such investigation has been conducted in the country. We found a very low incidence of adenovirus infection among the recruits of an army corporation located in the city of Rio de Janeiro as we detected only three positive samples out of 221 samples that have been analyzed.

The specie of the adenovirus isolates was identified for two of these samples: one sample recovered from a symptomatic subject belonged to specie A and the other one recovered from an asymptomatic subject belonged to specie C. These results are quite different from those described in the U.S.A. Perhaps it happened because the recruits in the corporation studied, differently from the American recruits, do not remain recluse in the unit during the full training period, where the living crowded conditions would certainly have a crucial role on respiratory infections spread. The data obtained in this study, although of little statistic significance, reveal a very important information, which is the low rates of adenovirus respiratory infection among military personnel, therefore precluding the implementation of an expensive vaccination program in military headquarters, at least in the city of Rio de Janeiro.

Although acute respiratory infections are important cause of morbidity and mortality in Brazil, few and sparse information are available on the viral etiology of those infection in the infantile and adult population as well. The few longitudinal studies that have been conducted demonstrated that adenoviruses are the second most important etiologic agent of acute respiratory disease among children (NASCIMENTO *et al.*, 1991; STRALIOTTO *et al.*, 2002). On the other hand, data on viral respiratory infections in the adult population in the country are still unknown. We found low rates of adenovirus infections among symptomatic and asymptomatic adults, as only four out of 181 analyzed samples gave positive results. The adenovirus specie involved was demonstrated for three isolates being two from specie A and one from specie C, all of them from symptomatic subjects. The specie of the fourth sample, recovered from an asymptomatic subject was not identified.

RESUMO

Adenovírus isolados de civis e militares da cidade do Rio de Janeiro, Brasil

Os adenovírus são importantes patógenos associados em geral, a infecções respiratórias em crianças e militares, embora, também estejam associados a surtos de conjuntivite e ceratoconjuntivite epidêmica. Neste estudo, foram analisadas 402 amostras de secreção respiratória colhidas de indivíduos com e sem sintomas de infecção respiratória, sendo 181 de civis e 221 de militares. As amostras foram inoculadas em cultura de células HEp-2 e/ou A549 para o isolamento viral; aquelas que apresentaram efeito citopatogênico (CPE) em alguma das culturas de células foram submetidas a reação da polimerase em cadeia (PCR) para confirmar o isolamento de adenovírus. Os isolados confirmados como sendo adenovírus foram posteriormente submetidos a digestão com endonucleases de restrição para a identificação da espécie viral. Três isolados foram identificados como espécie A (sendo dois isolados de civis e um de militar), um isolado de militar foi identificado como espécie C, e um isolado de civil foi identificado como espécie D. Para dois isolados a espécie não pode ser identificada.

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