Outbreak of Febrile Respiratory Illness Associated with Adenovirus 11a Infection in a Singapore Military Training Camp

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Outbreak cases of acute respiratory disease (ARD) associated with subspecies B2 human adenovirus 11a (HAdV-11a) infection were detected during 2005 in a military basic training camp in Singapore. The Singapore HAdV-11a strain is highly similar to other Asian strains of HAdV-11, including strain QS-DLL, which is responsible for the recently described 2006 outbreak of ARD in China.

Due to unique risk factors that include crowding and increased physical and psychological stress, military recruits in training are highly susceptible to outbreaks of acute respiratory disease (ARD), which are most often caused by viruses (26, 29). Human adenovirus (HAdV) infections have been recognized for decades as being important causes of ARD among military trainees in North America, Europe, and Asia (5, 8, 10, 15, 25, 27, 30, 32). The HAdVs serotypes most frequently associated with ARD in both military and civilian communities include subspecies B1 HAdV-3, HAdV-7, and HAdV-21 and species E HAdV-4. The association of subspecies B2 HAdV infection with ARD has historically been rarely reported and restricted mostly to closed community outbreaks, some of which were documented among military trainees (1, 2, 8, 24, 36).

From 1 January 2005 to 14 October 2005, a total of 226 male participants aged 18 to 24 years were enrolled in a study designed to detect and characterize viral agents responsible for ARD in the Singapore military recruit population. Analysis of laboratory data collected during this period identified influenza virus as the primary causative viral agent of respiratory illness among Singapore recruits in training (51 influenza virus-positive isolates out of 226 tested cases [22.5%]). HAdV-associated ARD cases were detected sporadically between January and October 2005. Thirty symptomatic trainees (13.3%) tested positive for HAdV during this period by a PCR assay described previously by Echavarria and colleagues (6). The temporal distribution of confirmed HAdV-associated cases of ARD is shown in Fig. 1. Cases of adenovirus-associated ARD were detected between February and June 2005 and in October 2005.

One case of coinfection with influenza A virus was detected among these patients. Recruits testing positive for HAdV were between 19 and 21 years old. The examination of clinical characteristics of the HAdV-positive cases of ARD showed that 23.3% of the recruits reported shortness of breath, 50% reported nasal congestion, 80% reported a headache, 80% reported body ache, and 13.3% reported signs of nausea or vomiting. The identified influenza A virus coinfection did not increase the severity of the respiratory symptoms. One patient presented with additional symptoms of conjunctivitis, but no eye swab samples were collected. Adenovirus isolation was accomplished for 27 of the 30 positive clinical specimens. All virus isolates were initially typed as species B HAdVs by PCR as described previously by Metzgar and colleagues (22). Isolates were further characterized by restriction enzyme analysis and sequencing of the hexon and fiber genes as described previously by Kajon and Erdman (13). Digestion with BamHI identified 26 of 27 isolates as belonging to subspecies B2 HAdVs and one isolate as being HAdV-3. Digestion of viral DNAs with BclI, BglII, BstEII, DraI, HindIII, PstI, SmaI, and XbaI determined that the 26 subspecies B2 isolates were identical and identified them as corresponding to genome type 11a (Fig. 2A) (17). The HAdV-3 isolate was identified as belonging to genome type 3a2 (Fig. 2B) (16).

By using the primer sets described in Table 1, identical hexon and fiber sequences were obtained for three randomly selected HAdV-11a isolates (GenBank accession no. FJ607010 and FJ603103 for isolate SNG1218, accession no. FJ607011 and FJ603104 for isolate SNG1222, and accession no. FJ607012 and FJ603105 for isolate SNG1223). Alignment of the sequences of the hexon gene corresponding to hypervariable regions 1 to 7 using the Basic Local Alignment Search Tool (BLAST) optimized for highly similar sequences (megablast) against the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) showed the three examined viruses to correspond to serotype 11 (19, 28). By using ClustalW implemented in Lasergene (DNASTAR, Inc., Madison, WI), the sequences...
for both the hexon and the fiber genes of the Singapore HAdV-11a strain were found to be highly similar to those reported for Asian and Middle Eastern HAdV-11 strains circulating over the last 2 decades in association with respiratory disease (Table 2). In addition, the complete genomic sequence obtained at the Walter Reed Army Institute of Research for isolate SNG1222 (GenBank accession no. FJ597732) was found to be 99.9% identical to that reported previously for strain QS-DLL, isolated in China in 2006 during an outbreak of ARD (33) (GenBank accession no. FJ643676). The identified differences between these two genomes are listed in Table 2. The results of this study suggest that, in contrast to other geographic locations where HAdV-3, -4, -7, and -21 rank among the most prevalent serotypes, HAdV-11 may be a relatively more important causative agent of ARD in military training facilities in Singapore. The circulation of HAdV-11 in South East Asia has been documented since the early 1960s in association with conjunctivitis, pharyngoconjuctival fever, ARD, and hemorrhagic cystitis among immunocompromised individuals (7, 9, 14, 17, 24, 31, 34–36). The work of Wadell and colleagues demonstrated the existence of two main clusters of relative homology for HAdV-11 genome types: the cluster of prototype-like genomic variants with a tropism for the renal epithelium, and the a-like cluster, with a distinct tropism for the respiratory tract. In addition to their unique restriction site maps, the p-like and a-like genomes differ in the sequences of the fiber gene involving the receptor binding domains (17, 20, 21). As noted by others previously (33), the fiber of the 11a-like genomes is more closely related to the fiber of the prototype strain of HAdV-14, de Wit (99.5% identity), than to the fiber of the prototype strain of HAdV-11, Slobitski (94.4% identity), suggesting that this genomic variant of HAdV-11 is an intertypic recombinant 11-14.

Except for strain BC34, isolated in Beijing, China, between 1965 and 1985 (17), none of the HAdV-11 strains with which the Singapore HAdV-11 isolates share high sequence similarities have been genotyped. However, the available sequence data indicate that all the Asian and Middle Eastern respiratory HAdV-11 strains used for comparison in this study represent closely related viruses likely belonging to genome type 11a, as described previously by Li and colleagues (17).

Our data confirm the long-lasting prevalence of genome type HAdV-11a in South East Asia and, together with data from recent studies from China (33, 36), support the observation that HAdV-11a is an important respiratory pathogen in the region and the hypothesis that this HAdV-11 genomic variant is a recombinant between HAdV-11 and HAdV-14 ancestral strains.

At present, the circulation of HAdV-11 has not been detected in association with respiratory illness outbreaks among U.S. military trainees, but HAdV-11 was confirmed to be the causative agent of a large outbreak of ARD in a job-training
facility in South Dakota in 1997, documenting the circulation of this serotype in association with respiratory disease in the United States (1). The detection of HAdV-11 in some collections of respiratory HAdVs gathered over the last 2 decades in South and North America, Asia, and the Middle East (3, 4, 11, 12; A. Kajon, unpublished data) and the recently reported emergence of HAdV-14 in North America (2, 18, 23) suggest an increasing role for these subspecies B2 serotypes in the etiology of ARD in several areas of the globe. Continuous surveillance and molecular characterization of respiratory adenovirus isolates worldwide will contribute to the elucidation of the natural history and pathogenesis of species B2 HAdV respiratory infections.

The protocol for this study was approved by the Joint Medical Committee for Research, Singapore Armed Forces, and participating institutions in the United States as IRB exempt.

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### REFERENCES


### TABLE 2. Comparative sequence analysis of Singapore HAdV-11a and other Asian strains of HAdV-11

<table>
<thead>
<tr>
<th>Strain or isolate</th>
<th>Origin</th>
<th>Reference</th>
<th>GenBank accession no.</th>
<th>% nt sequence identity</th>
<th>Identified difference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexon HVR1-7</td>
<td>China, 2006/ARD</td>
<td>36</td>
<td>DQ874353</td>
<td>99.9</td>
<td>1 synonymous point mutation at nt position 1647 of the hexon gene</td>
</tr>
<tr>
<td>QS-DDL</td>
<td>China, 2006/ARD</td>
<td>36</td>
<td>DQ874353</td>
<td>99.9</td>
<td>nt deletions at positions 1583, 1590, and 1597; one nonsynonymous point mutation at position 1617 of the hexon gene</td>
</tr>
<tr>
<td>91-038T</td>
<td>Japan, 1991/throat swab</td>
<td>Unpublished</td>
<td>AB162772</td>
<td>99.9</td>
<td>None</td>
</tr>
<tr>
<td>R1332</td>
<td>Kuwait, 2007</td>
<td>Unpublished</td>
<td>EU755357</td>
<td>100</td>
<td>1 nonsynonymous point mutation at nt position 1075 of the hexon gene</td>
</tr>
<tr>
<td>RKI-2797/04</td>
<td>Turkey, 2004</td>
<td>4</td>
<td>AY972815</td>
<td>99.9</td>
<td>1 nonsynonymous point mutation at nt position 127 of the fiber gene</td>
</tr>
<tr>
<td>Fiber BC34</td>
<td>China, 1965-1985/ARD</td>
<td>20</td>
<td>L08233</td>
<td>99.9</td>
<td>1 nonsynonymous point mutation at nt position 127 of the fiber gene</td>
</tr>
<tr>
<td>91-038T</td>
<td>Japan, 1991/throat swab</td>
<td>Unpublished</td>
<td>AB162822</td>
<td>99.9</td>
<td>1 nonsynonymous point mutation at nt position 127 of the fiber gene</td>
</tr>
<tr>
<td>RKI-2797/04</td>
<td>Turkey, 2004/ARD</td>
<td>4</td>
<td>AY972816</td>
<td>99.8</td>
<td>1 synonymous point mutation at nt position 138 of the fiber gene</td>
</tr>
<tr>
<td>QS-DDL</td>
<td>China, 2006/ARD</td>
<td>33</td>
<td>FJ643676</td>
<td>99.9</td>
<td>1 nonsynonymous point mutation at nt position 127 of the fiber gene</td>
</tr>
<tr>
<td>HAdV-11p Slobitsky</td>
<td>United States, 1957</td>
<td>20</td>
<td>AY163756</td>
<td>94.4</td>
<td>Multiple, plus one nonsynonymous point mutation at nt position 127 of the fiber gene</td>
</tr>
<tr>
<td>HAdV-14p de Wit</td>
<td>Netherlands, 1957/ARD</td>
<td>42</td>
<td>AB065116</td>
<td>99.5</td>
<td>Non-synonymous point mutations at nt positions 250, 346, and 412 of the fiber gene; 1 synonymous point mutation at nt position 774</td>
</tr>
<tr>
<td>Whole genome</td>
<td>China, 2006/ARD</td>
<td>33</td>
<td>FJ643676</td>
<td>99.9</td>
<td>Point mutations at positions 134, 202, 1721, 1885, 2170, 2823, 8351, 9253, 11677, 11293, 13328, 18072, 19849, 22846, 24348, 25509, 26060, 27198, 28745, 28827, 29204, 29429, 30259, 30378, 30501, 30901, and 34617; insertion of sequence TCCATATCCGTT downstream of the stop codon for E1A at nt position 1463; length of poly(A) tracts starting at positions 581, 3908, 29462c, 33984; length of poly(A) signals for L1, L2</td>
</tr>
</tbody>
</table>

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a Sequence data available for the hexon gene of strain 91-038T comprise nucleotide (nt) 346 to nt 1635.
b Sequence data available for the hexon gene of strain R1332 comprise nt 991 to nt 1604.
c Sequence data available for the hexon gene of strain RKI-2797/04 comprise nt 107 to nt 1541.
d Sequence data available for the fiber gene of strain RKI-2797/04 comprise nt 95 to nt 675.
e Nucleotide positions based on the genome of HAdV-11 strain QS-DLL (GenBank accession no. FJ643676).
f c, complementary strand.
g As described previously by Mei and Wadell (20) and Yang et al. (33)