Detection of viruses in human adenoid tissues using multiplex-PCR.

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Abstract

We detected a high frequency of viruses by polymerase chain reaction (PCR) in adenoids obtained from children without acute respiratory symptoms. Our results suggest that persistent/latent viral infection in the respiratory tract confounds interpretation of the association of PCR pathogen detection in these sources with acute respiratory infection.
Many aerobic and anaerobic bacteria form a well recognized normal bacterial flora in the upper respiratory tract and other bacteria though potentially pathogenic are often isolated from not only diseased but also healthy individuals (2).

Prompted by the observation that primary human adenoid epithelial cell cultures used as a model system for studying respiratory viruses (23) sometimes show viral cytopathic effects we investigated the presence of a wide variety of viruses in their adenoid tissue. Herpes viruses, in particular Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpes virus (HHV)-6 and HHV-7, have been detected in adenoid and tonsil tissue (1, 3, 5) and other studies have suggested that adenoids and/or tonsils can harbor these viruses latently (15, 16). In addition, adenoviruses (ADV) have been suggested to be harbored in latent form (7, 13). These viruses seem to form “a normal viral flora” but little is known whether any of other viruses associated with an acute respiratory infection may become latent or persist for a long time in the respiratory tract after an acute infection. To clarify this matter we performed PCR to detect viruses using adenoid tissue removed by adenoidectomy.

Thirty five adenoids were obtained from children with a median age of 4 years after adenoidectomy was performed at Vanderbilt Children’s Hospital between
May-December 2007, under a protocol approved by the Vanderbilt Institutional Review Board. The indications for adenoidectomy were determined by their primary care physician in conjunction with the otolaryngologist performing the surgery. The most common indication was respiratory obstruction secondary to hypertrophy. The patients had no acute respiratory symptoms at the time of surgery.

Approximately 25 mg of each adenoid tissue specimen was used for DNA and RNA extraction by using a QIAmp DNeasy Tissue Kit and a QIAmp RNesay Mini Kit (Qiagen, Valencia, CA), respectively. Eight monoplex PCR-EIA assays were used to detect HSV, CMV, EBV, varicella-zoster virus (VZV), HHV-6, HHV-7, HHV-8, and ADV, and a mutiplex RT-PCR to amplify influenza (Flu)-A, Flu-B, parainfluenza virus (PIV)-1, PIV-2, PIV-3, PIV-4, respiratory syncytial virus (RSV)-A, RSV-B, human metapneumovirus (hMPV), rhinoviruses (RhV), enterovirus (EnV) in a single reaction were performed as previously described (12, 18). RNA was also tested by real-time RT-PCR and conventional RT-PCR to detect human coronaviruses (hCoV) and reovirus (ReoV), respectively, as previously described (6, 10, 19). Positive and negative controls were included with each run.

At least one viral pathogen was detected from each of 35 adenoid tissues (Table 1). The viruses detected in greater than 15% of samples included ADV, HHV-7, EBV, EnV.
and RhV. Of 35 species 28 adenoids had multiple virus positive results (Table 2). In contrast, viruses not or rarely detected included: VZV, HHV-8, HHV-6, CMV, HSV, EnV hMPV, PIV-1, PIV-2, PIV-3, PIV-4, Flu-A, Flu-B, RSV A and B, hCoV and ReoV.

This study represents the broadest panels of viruses examined in a large number of adenoids. Review of the published data shows some variation in recovery of individual viruses (4, 12) but presents a consistent picture of identification of viruses from adenoidal tissue.

DNA and RNA viruses detected in this study may be in lymphoid cells or in the complex epithelial layer overlying the organized adenoid lymphoid tissue. Of the DNA viruses ADV, EBV and HHV-7 stood out as being commonly found. EBV and ADV are recognized as causes of viral tonsillitis and these viruses were detected frequently from our adenoids. EBV, in particular, is associated with more chronic disease in the respiratory lymphoid tissue (5, 15). The positive PCR results for EBV in 15 (43%) adenoids in present study support latent infection of EBV in the adenoid tissue though the high frequency of EBV in this mostly younger pediatric population is of interest as EBV infection in the United States is most typically associated with young adults (14).

HHV-6 and HHV-7 are members of the β-herpesviridie subfamily. In the present study HHV-7 was detected in 18 (51.4%) adenoids, but HHV-6 was detected in only 1
adenoid. This and other previous studies suggest a different latency between HHV-6 and HHV-7 in adenoid tissue as well as that in saliva in spite of the fact that HHV-7 is thought to occur later in life than HHV-6 (8, 9, 21, 22). Other DNA viruses that might have been expected in greater numbers were rarely seen, e.g. HSV and CMV.

All of the classic RNA viruses associated with acute respiratory illness, Flu A and B, RSV-A and RSV-B, PIV1-4, hCoV and hMPV were rarely found suggesting little long term carriage though it is noted that samples were not obtained in the peak of the winter respiratory season. In contrast, the picorna viruses - EnV and RhV - were seen in 31% and 17% respectively. We and others have recently demonstrated that RhV are frequently identified by PCR in well children and adults (12, 20). The high frequency of EnV was unexpected as they are most commonly associated with enteric disease. Our results demonstrate that picorna viruses may be shed virus for long term or cause a latent infection, and/or suggest that EnV may have a larger role in respiratory illness than previously appreciated. Little is known about ReoV as a cause of human disease. This study suggests it is unlikely to cause a chronic infection.

A caveat of using specimens from adenoidectomy or tonsillectomy is that they may be abnormal pathologic specimens not representative of the general population. Elective surgery is usually postponed in the face of illness and the majority of surgery is for
obstructive sleep apnea not recurrent infections (4).

Our results imply that viruses can be identified in adenoids and tonsil with regularity as “a normal viral flora” and suggest that some of respiratory viruses have more chronicity and hence a much less clear association with acute respiratory illness. We should consider the possibility of persistent/latent infection by many viral pathogens in the respiratory tract is confounding PCR diagnosis in a clinical setting and lends caution to interpretation of findings in primary epithelial cells derived from these sources.
References


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Herpesvirus DNA is consistently detected in lungs of patients with idiopathic


Table 1 Virus detection from 35 adenoid tissues

<table>
<thead>
<tr>
<th>Virus</th>
<th>No. of Positive specimens (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADV</td>
<td>28 (80.0)</td>
</tr>
<tr>
<td>HHV-7</td>
<td>18 (51.4)</td>
</tr>
<tr>
<td>EBV</td>
<td>15 (42.9)</td>
</tr>
<tr>
<td>EnV</td>
<td>11 (31.4)</td>
</tr>
<tr>
<td>RhV</td>
<td>6 (17.1)</td>
</tr>
<tr>
<td>HSV</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>CMV</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>HHV-6</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>PIV-1</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>VZV</td>
<td>0 (0)</td>
</tr>
<tr>
<td>HHV-8</td>
<td>0 (0)</td>
</tr>
<tr>
<td>PIV-2, 3, 4</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Flu-A, B</td>
<td>0 (0)</td>
</tr>
<tr>
<td>RSV-A, B</td>
<td>0 (0)</td>
</tr>
<tr>
<td>hMPV</td>
<td>0 (0)</td>
</tr>
<tr>
<td>hCoV</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ReoV</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
Table 2 Detection of multi viruses from adenoid tissue

<table>
<thead>
<tr>
<th>Double (n=14)</th>
<th>Triple (n=10)</th>
<th>Quadruple (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHV-7 + ADV</td>
<td>EBV + HHV-7 + ADV</td>
<td>EBV + HHV-7 + ADV + EnV</td>
</tr>
<tr>
<td>ADV + EnV</td>
<td>HHV-7 + ADV + EnV</td>
<td>EBV + HHV-7 + ADV + RhV</td>
</tr>
<tr>
<td>EBV + HHV-7</td>
<td>EBV + HHV-6 + RhV</td>
<td>EBV + HHV-7 + ADV + PIV</td>
</tr>
<tr>
<td>EBV + EnV</td>
<td>HHV-7 + ADV + RhV</td>
<td>CMV + EBV + HHV-7 + ADV</td>
</tr>
<tr>
<td>EBV + ADV</td>
<td>ADV + EnV + RhV</td>
<td></td>
</tr>
<tr>
<td>ADV + RhV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EnV + RhV</td>
<td></td>
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</tbody>
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