Epstein-Barr virus is an orally transmitted human herpesvirus that infects epithelial cells and establishes latency in memory B lymphocytes. Movement of virus between the two cell types is facilitated by changes in amounts of an envelope glycoprotein, gp42, which are effected by interaction of gp42 with HLA class II in a B cell. Here we used the differential ability of virus to bind to CD21-positive B cells and CD21-negative epithelial cells, which is also influenced by levels of gp42, to determine that the majority of virus shed in saliva is derived from an HLA class II-negative cell.

Epstein-Barr virus (EBV) is an orally transmitted human herpesvirus that infects more than 90% of the human population. Although the majority of primary infections are asymptomatic the virus can cause infectious mononucleosis, and long-term carriage can be associated with B-lymphocyte and epithelial-cell malignancies (16). These malignancies are reflective of the predominant tropisms of the virus. Current models of infection propose that productive replication in epithelial cells of the oropharynx facilitates access to B cells in Waldeyer’s ring (15, 18) where the virus can establish latency in long-lived memory B cells. Sporadic terminal differentiation of these B cells into plasmablasts triggers onset of productive replication to initiate another cycle of infection in the same or in a new host (8).

During this cycle of infection, ease of access of virus to a B cell and an epithelial cell can be driven by the cell type of origin because of differences in the proteins used for entry into each (1). Entry of virus into a B cell is initiated by attachment of virus glycoprotein gp350/220 to the complement receptor type 2, CD21 (13, 17). Attachment to a CD21-negative epithelial cell can be initiated by binding of a complex of two glycoproteins, gHgL, to an as-yet-unidentified receptor, gHgL (12, 14). Penetration of virus into a B cell requires glycoprotein gB and a complex of three proteins, gHgL and gp42 (5, 19). It is triggered by an interaction between gp42 and HLA class II (4, 10). Penetration of an HLA class II-negative epithelial cell requires gB and a two-part gHgL complex lacking gp42 (5, 20). It is triggered by a direct interaction between gHgL and a receptor which may be the same gHgL that mediates attachment (2). To accommodate these differences, the EBV virion contains both two-part gHgL complexes and three-part gHgLgp42 complexes with mutually exclusive functions. Only three-part gHgLgp42 complexes mediate B cell infection, and only two-part complexes lacking gp42 mediate epithelial infection (20). The presence of gp42 blocks the interaction with gHgL (2).

In an HLA class II-positive B cell, some three-part gHgLgp42 complexes are lost to an interaction with HLA class II which targets them to a degradative pathway. The ratio of gHgLgp42 to gHgL is thereby reduced. In an HLA class II-negative epithelial cell, this does not happen, and the ratio of gHgLgp42 to gHgL is higher (1). This results in a switch of tropism, with B-cell-derived virus being slightly more infectious for an epithelial cell and epithelial-cell-derived virus being as much as 2 logs more infectious for a B cell. It suggests a model in which virus replicating in epithelial cells in the oropharynx is strongly and rapidly targeted to the B-cell compartment at the beginning of the cycle of infection, whereas virus made in a differentiating B cell has a small increase in its ability to infect epithelial cells either for amplification in the existing host or, if shed directly from differentiating cells into saliva, for infection of epithelial cells in a new host.

We recently found that levels of gp42 in virus impact not only penetration but also attachment of virus to a gHgLR-expressing epithelial cell (2). Virus derived from HLA class II-positive B cells bound to gHgLR almost as well as it bound to CD21. In contrast, virus derived from an HLA class II-negative epithelial cell bound to CD21 as well as did the B-cell-derived virus but, because of a higher load of gp42, bound poorly to gHgLR. This difference suggested that the relative ability of virus to bind to CD21 or gHgLR might be used to determine whether virus in saliva originates from an HLA class II-positive or -negative cell.

Glycoprotein gp42 binds many but not all HLA class II alleles (6). Individuals expressing different alleles then presumably lose three-part gHgLgp42 complexes at levels consistent with the alleles that they express. To control for this, lymphoblastoid cell lines (LCL) were derived from six healthy donors by transforming their T-cell-depleted blood lymphocytes (11) with virus in their own saliva, which was obtained by use of a Salivette (Starstedt, Fisher Scientific International), centrifuged to remove cells, and clarified by filtration through a 0.8-μm filter. Virus was harvested from each individual LCL after induction with phorbol esters and sodium butyrate (9) and concentrated by centrifugation (12). LCL-derived virus and virus from saliva of the same donor were bound for 1 h on ice to EBV-negative CD21-positive Akata B cells or CD21-negative gHgLR-positive AGS epithelial cells (2). DNA was isolated with a QIAamp DNA blood minikit (QIAGEN Sciences) for real-time quantitative PCR (RQ-PCR) as described.
TABLE 1. Average ratio of binding of LCL- and saliva-derived virus to CD21− gHgLR− EBV− Akata B cells and CD21− gHgLR− EBV− AGS epithelial cells

<table>
<thead>
<tr>
<th>Donor</th>
<th>Binding ratio (no. of expts)</th>
<th>t test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LCL-derived virus</td>
<td>Saliva-derived virus</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.42 ± 0.22 (4)</td>
<td>5.72 ± 1.73 (6)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2</td>
<td>1.44 ± 0.18 (4)</td>
<td>18.98 ± 13.20 (5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3</td>
<td>1.69 ± 0.03 (3)</td>
<td>10.19 ± 6.40 (15)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4</td>
<td>1.58 ± 0.29 (4)</td>
<td>3.66 ± 0.59 (5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>2.27 ± 0.61 (3)</td>
<td>6.71 ± 0.44 (3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6</td>
<td>2.31 ± 0.55 (3)</td>
<td>18.09 ± 12.37 (21)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a Virus binding was measured by RQ-PCR as in Fig. 1.
b Fresh saliva was collected for each experiment.
c P values represent differences between LCL-derived and saliva-derived viruses for each donor.

determined by flow cytometry of virus bound to B cells and stained with a monoclonal antibody to gp42 or gH. Ratios are calculated from the percent of cells binding virus that with each antibody, to gp42 or gH, had a fluorescent signal above that of the isotype control.

Fresh saliva was collected for each experiment.

elsewhere (3) with primers that amplified the BamHI K region of EBV (5′-GGATGCGATTAAGGACCTTTGTTT-3′ and 5′-GGTCAAGCTGCACACAGTCA-3′; base coordinates 109677 and 109753, respectively; GenBank accession no. VO1555) and/or the cellular C-reactive protein (CRP) (5′-CTTGACCA GCCTCTCTCATGC-3′ and 5′-TGAGCTTCTAGACCCCA CCC-3′; base coordinates 132705 and 132605, respectively; GenBank accession no. AL445528). The BamHI K probe (5′-CAAAGCCGCTCTACTGCAATTATA-3′) was labeled with 6-carboxyfluorescein, and the CRP probe (5′-TTTGCC AGACAGTTAAGGCCACC-3′) was labeled with VIC (PE Applied Biosystems). Serial dilutions of DNA from IB4, a B-cell line containing five copies of EBV per cell (7), served as a standard, and EBV copy number per sample was normalized by amplification of virus in an HLA class II-negative cell. This is consistent with recent observations of EBV in tonsil epithelium of healthy carriers (15) and with a model in which amplification of virus in an HLA class II-negative epithelial cell is part of the normal cycle of persistence of EBV.

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