The Epstein–Barr Virus Receptor is Distinct from the C3 Receptor

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SUMMARY

Polyvalent serum directed against C3 receptors was employed in an attempt to block Epstein–Barr virus (EBV) binding to virus receptor-containing cell lines. The serum eliminated 90% of virus binding to Daudi and BJAB, lines which express only the C3d receptor. Raji and Ramos cells, which express the C3d, C3b and C3bi receptors, still adsorbed 70% of their virus capacity in the presence of excess antiserum. These effects were independent of the virus strain. In the light of previous reports, these data imply that, although the two receptors, EBV and C3d, are closely associated, the binding sites of EBV and complement are distinct. Additionally, an unusual EBV substrain-specific receptor found on U698 and P3HR-1/ASNP lines was shown to be independent of complement receptors.

An association between the receptor for the Epstein–Barr virus (EBV) and the C3d receptor has been demonstrated previously (Jondal et al., 1976; Yefenof et al., 1976; Yefenof & Klein, 1977). Several lines of evidence support this relationship. Initially it was seen that the two receptors were co-ordinately expressed on a number of cell lines and fresh lymphocyte fractions (Jondal et al., 1976). Various sublines of the same original Burkitt line, Jijoye, that vary in EBV receptor expression, presented an opportunity to investigate co-expression quantitatively; the results confirmed the association (Klein et al., 1978). At the membrane level, it was observed that EBV receptors and C3 receptors co-cap with each other but not with a variety of other cell surface structures (Yefenof et al., 1976). Membrane stripping results demonstrated that by removing either receptor, the binding potential of the other was eliminated (Yefenof & Klein, 1977). Lastly, cells pre-incubated with C3 and then with anti-C3 antibodies are incapable of binding virus (Yefenof et al., 1977, and unpublished observations).

Recently there has been a report of an unusual EBV receptor which binds only the lytic P3HR-1 virus substrain (Wells et al., 1981). This receptor appears on cells which are incapable of forming EAC-rosettes or binding C3. These receptors may represent C3-receptor-independent EBV receptors, or the cells may contain C3 components which are defective in C3 binding but contribute to EBV adsorption. Such a situation has been observed with a number of B-cell lymphoma biopsies (A. Wells et al., unpublished results). Using monospecific antisera against C3 receptors (C3b, C3bi, and C3d) (Gerdes & Stein, 1981; Gerdes et al., 1982; Stein et al., 1981), we analysed the nature of this unusual receptor. Additionally, the properties of the dual-virus-specific EBV receptor (Wells et al., 1981) were investigated in terms of C3 receptors. The connection between these receptors was found to be quite complex, being influenced by a number of factors, including tropism of the virus receptor and presence of complement receptors for C3b and C3bi and/or C3d.

Cell lines, their origins, and EBV and C3 receptor profiles are listed in Table 1. The methods employed do not distinguish between the C3b and C3bi receptors, the latter being the predominant complement receptor on Raji cells (Lambris et al., 1981). However, this presents no difficulties as the EBV receptor segregates independently of both these receptors (Jonssson et al., 1982). All cells were grown as suspension cultures at 37 °C under 60% humidity and 5% CO2 in RPMI 1640 culture medium supplemented with 10% foetal calf serum, penicillin (200 units/ml), and streptomycin (100 µg/ml). EBV producer B95-8 and P3HR-1 cells served as the source of B and P virus, respectively. The cells were grown in suspension to saturation for 1 week before harvesting. The virus was then purified and iodinated with 125I as described previously (Wells et al., 1981; Koide et al., 1981).
**Table 1. Cell lines and their characteristics**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Reference</th>
<th>Origin</th>
<th>Presence of EBV genome</th>
<th>Level of EBV receptors*</th>
<th>Complement receptors†</th>
<th>EBV binding inhibition†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raji</td>
<td>Epstein et al. (1966)</td>
<td>BL§</td>
<td>+</td>
<td>100 100</td>
<td>C3b 31 C3d 26</td>
<td></td>
</tr>
<tr>
<td>Ramos</td>
<td>Fresen &amp; zur Hausen (1976)</td>
<td>BL</td>
<td>-</td>
<td>78 78</td>
<td>B 105 P 30</td>
<td></td>
</tr>
<tr>
<td>Daudi</td>
<td>Klein et al. (1978)</td>
<td>BL</td>
<td>+</td>
<td>80 80</td>
<td>C3b 90 C3d 85</td>
<td></td>
</tr>
<tr>
<td>BJAB</td>
<td>Fresen &amp; zur Hausen (1976)</td>
<td>BL</td>
<td>-</td>
<td>100 100</td>
<td>C3b 90 C3d ND</td>
<td></td>
</tr>
<tr>
<td>U698</td>
<td>Nilsson &amp; Sundström (1974)</td>
<td>B-cell</td>
<td>-</td>
<td>0 100</td>
<td>C3b 0 C3d 0</td>
<td></td>
</tr>
<tr>
<td>P3HR-1/ ASNP</td>
<td>Klein et al. (1978)</td>
<td>P3HR-1</td>
<td>+</td>
<td>0 45</td>
<td>C3b 0 C3d 0</td>
<td></td>
</tr>
<tr>
<td>K562</td>
<td>Abu Sinna et al. (1980)</td>
<td>Erythroleukaemia</td>
<td>-</td>
<td>0 0</td>
<td>C3b NA C3d NA</td>
<td></td>
</tr>
</tbody>
</table>

* Arbitrary units; the binding level of 8 × 10^6 Raji cells is set at 100, and the negative controls, K562 and YAC, are 0 (Koide et al., 1981; Wells et al., 1981). The values shown are each the median value of 6 or more assays.
† Relative values at 3/4 subagglutination titre with EAC-rosettes (see Jonsén et al., 1982).
‡ Maximum level; see Fig. 1.
§ Burkitt lymphoma.
∥ ND, Not done; NA, not applicable.

A direct binding assay for radio-labelled virus was employed to detect the presence of viable EBV receptors (Koide et al., 1981). Briefly, aliquots of 125I-EBV were incubated with a known number of target cells for 30 min at 20 °C. The cells were pelleted and washed, then the radioactivity of the pellet and supernatants was measured independently. Various amounts of anti-C3 receptor serum (Gerdes & Stein, 1981; Stein et al., 1981) were incubated with the target cells for 30 min at 37 °C before performing a virus-binding assay. The effects of the anti-C3 receptor serum on EBV binding were directly quantifiable.

EBV receptor-positive cells incubated with C3 and then with anti-C3 antibodies are incapable of binding virus. As antigen–antibody complexes can be expansive, it was possible that the observed blockage of virus binding resulted from steric hindrance between two closely related, but distinct, binding sites. Directly blocking C3 receptors with receptor-specific antibodies would minimize the steric factor. Polyvalent sera which recognizes all C3 receptors, C3b, C3bi, and C3d (Gerdes & Stein, 1981; Gerdes et al., 1982; Stein et al., 1981), were employed to eliminate virus binding. One μl serum/10^6 Raji cells reduced the binding of FITC-conjugated C3 and EAC-rosettes by more than 90% (data not shown). A similar situation was observed with Daudi cells. The antisera, however, exhibited a heterogeneity in its ability to inhibit virus binding (Fig. 1a). Binding to Daudi, an EBV-carrying African Burkitt lymphoma (AfBL)-derived line, was completely inhibited by the antisera. This was also true for BJAB, an unusual, EBV-negative AfBL. The EBV-binding levels of Raji, an EBV-carrying AfBL, and Ramos, an EBV-negative American BL line, were only partially inhibited by the antisera. Maximum inhibition of virus binding was approached at antisera amounts comparable to those necessary to eliminate C3 adsorption in the case of Raji and Ramos. For Daudi and BJAB, however, excess antisera was required to eliminate virus binding.

Inhibition of EBV binding by the anti-C3 receptor serum was similar irrespective of the virus substrain employed (compare Fig. 1a, b). Binding of P3HR-1-derived virus to U698 and P3HR-1/ASNP, two lines which express an unusual P-virus-specific EBV receptor (Wells et al., 1981), was unaffected by preincubation with the antisera (Fig. 1b). This was expected, because the antisera was not seen to recognize U698 (data not shown). Thus the P-virus specific receptor is considered to be C3-receptor-independent.

The host range of the Epstein–Barr virus is extremely limited; by and large, only B-lymphocytes of human and certain primate origins and their derivatives can be infected (for review, see Epstein & Achong, 1979). The distribution of EBV receptors on these cells coincides with that of the C3d receptor. For this reason, it was postulated that the two receptors are related (Jondal et al., 1976). Many investigations have supported the initial association. The question...
Short communications

remains, nevertheless, of whether the two receptors are identical. We have presented evidence which suggests that, though the receptors are closely associated, the binding sites are distinct.

Polyvalent anti-C3 receptor serum (Gerdes & Stein, 1981) was employed to inhibit EBV binding. At levels greater than those necessary to completely eliminate C3 binding, the sera blocked virus binding to Daudi and BJAB. These two lines express only the C3d receptor. Two lines which possess the C3b, C3bi and C3d receptors, Raji and Ramos, still demonstrated a 70% EBV binding level in the presence of excess antiserum. Neither antiserum was effectively pre-
adsorbed on Daudi cells to remove the anti-C3d receptor components, nor did monoclonal anti-C3b receptor antibodies block C3 binding or EBV binding to lines expressing C3d receptor (data not shown). Thus, it is inferred that the anti-C3d receptor specificity is responsible for inhibition of the binding of EBV. Furthermore, the unusual P-virus-tropic receptor present on U698 and P3HR-1/ASNP was seen to be independent from the C3 receptors.

The data offer two possible explanations for the heterogeneity of virus binding inhibition. It is possible that Raji and Ramos contain two distinct EBV receptors, one associated with the C3d receptor and the other independent. In this case, Daudi and BJAB would express only the former type. Alternatively, the EBV receptors may be similar, being related to the C3d receptor with the intimacy of the association being determined, in part, by the presence of the C3b and/or C3bi receptor. All available evidence points to the second explanation. Results from membrane stripping (Yefenof & Klein, 1977) and antigen–antibody complexes situated on the C3 receptors (Yefenof et al., 1977) strongly imply that all EBV receptors present on Raji are associated with C3d receptor. The comparative levels of the EBV receptor on a large number of cell lines and their derived somatic cell hybrids segregate in parallel with the levels of the C3d receptor (Jönsson et al., 1982). Additionally, the kinetics of virus binding suggests that the virus receptors on a cell line are of one type (Wells et al., 1981; Koide et al., 1981).

If the virus receptors are considered to be similar and excess antiserum only partially inhibits virus binding to Raji and Ramos, then it is considered that the virus and C3d receptors are distinct sites. If this is so, binding of EBV is inhibited by spatial interference of C3d receptor-bound antibodies with the closely related virus-binding site. Preliminary studies using purified complement components demonstrate enhanced EBV binding in the presence of C3, further suggesting the distinctness and connection between the two receptors. These experiments elucidate the nature of the association between the EBV receptor and the C3d receptor as one of two closely related but functionally distinct receptors.

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REFERENCES


Short communications


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