Cell Surface Markers on Epithelial-Burkitt Hybrid Cells Superinfected with Epstein-Barr Virus

Ronald Glaser,2 Gilbert Lenoir, Soldano Ferrane,3 Michele A. Pellegrino, and Guy de-Thé

Department of Microbiology and Specialized Cancer Research Center, The Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033 [R. G.]; International Agency for Research on Cancer, 150 Cours Albert Thomas, 69008 Lyon, France [G. L., D. G.]; and Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037 [F. S., M. A. P.]

SUMMARY

Attempts were made to superinfect two epithelial-Burkitt hybrid cell lines, designated D98/HR-1 and D98/Raji, with Epstein-Barr virus (EBV) and to investigate the expression of some cell surface markers, including histocompatibility antigens, and the presence of B-cell markers, such as receptors for the third complement component and for monkey red blood cells. Successful superinfection of D98/HR-1 cells with EBV was made evident by the expression of early antigen and, to a lesser extent, virus capsid antigen. Only a rare D98/Raji cell was found to be positive for early antigen. The histocompatibility antigens of the parental cell lines D98, HR-1, and Raji were expressed on the surfaces of the hybrid cells. Receptors for third complement component b and d were not detected on the hybrid cells or on the D98 or HR-1 cell lines; they were found, however, on the Raji cells, indicating that EBV receptors and complement receptors can be separated. The significance of the infection of the hybrid cells with EBV and the expression of cell surface markers is described.

INTRODUCTION

We have studied the regulation and expression of the EBV4 genome in nonlymphoblastoid cell types, i.e., somatic cell hybrids of Burkitt tumor cells (9). With this procedure we have circumvented the problem of the failure of EBV to infect various cell types. We have maintained EBV in a repressed state similar to that observed in “nonproducer” Raji cells for long periods of time and have induced EBV to replicate and synthesize “complete” virus particles in epithelial hybrid cells after treatment with iododeoxyuridine (6, 8, 10).

The detection and characterization of antigenic markers and receptors expressed on the membranes of cells that are infectable with EBV may enable us to clarify the events in the infection process and to predict the permissiveness of other cell types. In this study we investigated cell surface markers expressed on the surfaces of epithelial-Burkitt hybrid cells that are infectable with EBV. Specifically, these markers are HLA and receptors for C3 and monkey RBC, receptors that are specific for B-cells (14).

MATERIALS AND METHODS

Cells. Burkitt’s lymphoma cell lines Raji and P3J-HR-1 (HR-1) were maintained in RPMI Medium 1640 supplemented with 10% FCS, penicillin (100 units/ml), streptomycin (100 µg/ml), and Mycostatin (10 units/ml). Epithelial-Burkitt hybrid cell lines D98/HR-1, clones 1 and 8, and D98/Raji, clones 4 and 16, (7, 9) and the parental D98 cells, which are variants of HeLa cells (5), were maintained in Eagle’s medium supplemented with 10% FCS, penicillin (100 units/ml), streptomycin (100 µg/ml), and Mycostatin (10 units/ml). Cells were grown in plastic tissue culture flasks (Falcon Plastics, Oxnard, Calif.) at 37°.

Virus. The procedure for preparing EBV concentrates has been described (1). Briefly, HR-1 cells were grown in RPMI Medium 1640 supplemented with 10% FCS at 35° for 11 days without feeding. The cell suspensions were centrifuged at 3000 rpm for 20 min, and the supernatants were filtered through a 1.2-µm Millipore filter. Virus was precipitated with polyethylene glycol and resuspended in 1% of the original volume of RPMI Medium 1640.

Antisera. HLA alloantisera were obtained from the serum bank at the National Institute of Allergy and Infectious Diseases. Antisera to C3 receptors were produced by injecting 1 rabbit with 106 Raji cells and reinoculating the animal 14 days later. The rabbit was then bled at weekly intervals. The presence of antibody to C3 receptors in this serum was demonstrated by the ability to inhibit the functional activity of C3 receptors after extensive adsorption with lymphoid cells that do not carry the C3 receptor. Detailed properties of the rabbit anti-C3 receptor xenoantisera are described elsewhere.6

6 S. Ferrone and M. A. Pellegrino. Characterization of an Anti-C3 Receptor Xenoantisera; submitted for publication.

Received January 26, 1977; accepted April 15, 1977.

JULY 1977

2291
Immunofluorescence. Indirect immunofluorescence was used to detect EBV early antigen or VCA. Glass coverslips of cell monolayers were washed in PBS, air dried, and fixed in acetone for 10 min at room temperature. Antisera previously characterized for activity against early antigen and VCA were adsorbed to the cells for 30 min. After being washed with PBS, the cells were reabsorbed with goat anti-human IgG conjugated to fluorescein isothiocyanate for an additional 30 min. The cells were washed again with PBS, counter-stained with 0.05% Evans blue, mounted in glycerol on glass slides, and examined with a Leitz microscope with UV.

Superinfection of Burkitt Hybrid Cells with EBV. Cloned D88/HR-1 and D98/Raji cells were grown as monolayers on glass coverslips. Approximately 24 hr after culturing had begun, 0.2 ml of a 10^5 early antigen-inducing units per ml suspension EBV (titrated in Raji cells) was added to each cell culture, and incubation was continued for 1 hr at 37°. After 5 ml of complete Eagle's medium were added to the culture, the cells were incubated for 6 days at 37° and were then fixed in acetone and examined for EBV early antigen and VCA.

Quantitation of HLA. The amount of HLA on Raji, HR-1, and D98 parent cells and on D98/HR-1 and D98/Raji hybrid cells was measured by a microquantitative adsorption technique (13). Briefly, HLA alloantisera at a dilution twice the minimal amount required to lyse 95% of the selected target cells were incubated with cell suspensions at varying concentrations for 60 min at room temperature. The cells were centrifuged, and the supernatant was examined for residual antibody adjusted to a concentration of 10^9 cells/ml in Veronal-buffered 0.9% NaCl solution (4). Rabbit serum deficient in C6 is immune adherence positive, and EAC1-3" is immune adherence negative (4); therefore, they were used to detect receptors for C3b and C3d, respectively.

Detection of Receptors for Monkey RBC and C3. Sheep erythrocytes were sensitized with rabbit anti-erythrocyte antibody adjusted to a concentration of 10^8 cells/ml in Veronal-buffered 0.9% NaCl solution and incubated with an equal volume of either rabbit serum deficient in C6 or EAC1-3" diluted 1:2 in Veronal-buffered 0.9% NaCl solution (4). Rabbit serum deficient in C6 is immune adherence positive, and EAC1-3" is immune adherence negative (4); therefore, they were used to detect receptors for C3b and C3d, respectively.

RESULTS

Superinfection of Burkitt Hybrid Cells with EBV. D98/HR-1 and D98/Raji cells in culture were infected with EBV prepared from HR-1 cells. Results of these experiments (Table 1) represent the averages from 6 independent experiments. Some early antigen-positive cells could be detected by 3 days postinfection; approximately 1% of D98/HR-1 cells were positive for early antigen 6 days after EBV infection (Fig. 1). A very small percentage (0.01%) were VCA positive. Only a rare D98/Raji cell was found to be early antigen positive, and no VCA-positive cells were observed. Under normal conditions the only antigen that can be detected in D98/HR-1 cells is the EBV-associated nuclear antigen; early antigen and VCA are repressed. No expression of EBV-specific antigens was detected in the D98 parent or in mouse fibroblast (CL1D) cells infected with the same virus stocks. In addition, early antigen was observed in a dividing D98/HR-1 cell in late telophase (Fig. 2), thus confirming previously published data that an early antigen-positive cell can divide at least 1 time (11).

Expression of HLA on Burkitt Hybrid Cells. D98/HR-1 clone 1 and 8 and D98/Raji clone 4 and 16 cells expressed HLA from both parental cell lines (Table 2). It could be determined that the number of antigenic sites present on the hybrid cells was similar to that detected on parental lines since similar numbers of both adsorbed equal amounts of HLA alloantisera. D98/HR-1 cells lost the ability to react with anti-HLA-B12 antiserum, which cross-reacted with HLA-B5 expressed on the parental line HR-1. In addition, D98/Raji cells acquired the ability to adsorb anti-HLA Victor alloantiserum directed to HLA-B5. Both clones of D98/Raji cells (including various passage levels) demonstrated.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Expression of early antigen and VCA in D98 and D98 hybrid cells after EBV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of EBV-specific markers (%)</td>
<td></td>
</tr>
<tr>
<td>Cell line</td>
<td>Early antigen</td>
</tr>
<tr>
<td>D98</td>
<td>0</td>
</tr>
<tr>
<td>CL1D</td>
<td>0</td>
</tr>
<tr>
<td>D98/HR-1</td>
<td>1</td>
</tr>
<tr>
<td>D98/Raji</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

R. Glaser et al.
strated variability in capacity to adsorb the Victor alloantiserum, as indicated by the ADₜₐ values between 1,500 and 30,000 cells/μl of antiserum. Analysis of the karyotype of these hybrids indicated that Chromosome 6, which has been linked to the HLA and C3 receptor phenotype (2, 16), is present in D98/HR-1 (Fig. 3) and D98/Raji (data not shown) cells.

Detection of Receptors for Monkey RBC and C3 on the Surfaces of Burkitt Hybrid Cells. Receptors for C3b and C3d were not detected on D98/HR-1 or D98/Raji cells or on the respective parental cells, with the exception of Raji cells (Table 3). D98/HR-1 and D98/Raji cells were also unable to adsorb a rabbit xenoantiserum directed to C3 receptors or closely associated membrane components at the ratio of 0.250 × 10⁶ cells/μl of antiserum (Chart 1). This antiserum was adsorbed by Raji cells at the ratio of 30,000 cells/μl of serum and was also adsorbed by other C3 receptor-positive cell lines, e.g., RPMI 6410. Thus, D98/HR-1 cells not expressing detectable levels of C3 receptors were still able to be superinfected with EBV.

Since the number of early antigen-positive D98/HR-1 cells was only about 1%, it was possible that the assay used to detect C3 receptors was not sensitive enough to detect this low level of cells. Therefore, we tested the sensitivity of the assay. Lymphoid cells were mixed with HeLa cells at decreasing concentrations. The percentage of rosette formation was determined (as described in "Materials and Methods") in mixtures in which the lymphoid cells were at 75, 25, 10, 5, 1, and 0% of the cell suspension. Tests were run in triplicate (blind coded) in 2 independent experiments (Chart 2). An average of 4.3% rosettes could be detected in which the lymphoid cells were 1% of the total cell suspension. No rosettes were observed when HeLa cells were assayed alone, confirming the results obtained with D98 cells. Therefore, the assay used to detect C3 receptors is sensitive enough to detect 1% early antigen-positive D98/HR-1 cells if they also express C3 receptors.

Assays were also performed to determine whether receptors for monkey RBC (associated with B-lymphocytes) were expressed on the hybrid cells. It was found that receptors for monkey RBC were expressed on the lymphoid cells but not on the epithelial-Burkitt hybrids or on D98 cells (Table 3).

**Table 2**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>HLA-A</th>
<th>HLA-B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADₜₐ</td>
<td></td>
</tr>
<tr>
<td>HR-1</td>
<td>—</td>
<td>2.0</td>
</tr>
<tr>
<td>D98</td>
<td>1.5</td>
<td>—</td>
</tr>
<tr>
<td>D98/HR-1</td>
<td>1.0</td>
<td>ND</td>
</tr>
<tr>
<td>Raji</td>
<td>—</td>
<td>1.5</td>
</tr>
<tr>
<td>D98/Raji</td>
<td>1.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* ADₜₐ value greater than 50,000 cells/ml.

**DISCUSSION**

We have shown that epithelial-Burkitt hybrid cells (D98/HR-1) can be superinfected with EBV, demonstrated by the synthesis of EBV early antigen and VCA. The positivity of these cells was clear, but the percentages were lower than those previously reported when epithelial-Burkitt hybrid cells were exposed to iododeoxyuridine and EBV was in-

---

**Table 3**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>EAC1-3</th>
<th>EAC1-5*</th>
<th>Monkey RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>D98</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HR-1</td>
<td>0</td>
<td>0</td>
<td>91</td>
</tr>
<tr>
<td>D98/HR-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Raji</td>
<td>87</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>D98/Raji</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* EAC1-5, rabbit serum deficient in C6.
Chart 2. Determination of the sensitivity of the rosette formation assay used to detect C3 receptors. Lymphoid cells (C3 receptor positive) were added to 2 x 10⁶ HeLa cells (C3 receptor negative) to make final concentrations of 75, 25, 10, 5, and 1% of the cell mixture. The rosette formation assay (described in "Materials and Methods") was used to detect C3 receptors.

produced to replicate (8, 10). In D98/Raji cells, EBV-specific early antigen could be detected at a very low level. There are several EBV genome-positive lymphoblastoid cell lines that, when infected with EBV, demonstrate low levels of early antigen that are comparable to those found in D98/HR-1 cells (3). The D98 cell line was not superinfected under the same conditions and with the same EBV preparation used for the susceptible hybrid cells.

Expression of early antigen was recently demonstrated in a Burkitt lymphoblastoid cell in metaphase, suggesting that an early antigen-positive cell can undergo cell division (11). Four days after EBV infected the culture, we found an early antigen-positive D98/HR-1 cell in late telophase. Thus, the sequence of cell division in which early antigen can be expressed can now be extended to telophase. These data confirm the work of Hampar et al. (11). It would seem then that a cell that synthesizes at least some component(s) of early antigen can divide at least once. What this means in terms of the function of early antigen is not clear.

The data obtained in this study suggest that the portion of the phenotype of the Burkitt tumor cell that is related to the receptor for EBV attachment is expressed in the hybrid cells. We have also shown that epithelial-Burkitt hybrids express HLA present on both parental cell lines. The D98/HR-1 hybrids lose the ability to adsorb cross-reacting antibodies to HLA-B12, which is expressed on both parental cells. In contrast, D98/Raji cells can adsorb an anti-HLA-B5 alloantisemun although neither of the parental cells can react with it. Adsorption of Victor serum is likely to reflect a cross-reaction between HLA-B5 and HLA-W35; the latter has reportedly been expressed on D98 cells (12). Changes of reactivity of hybrid cells with antisera to cross-reacting HLA specificities may reflect conformational changes of antigenic moieties inserted in the cell membrane and/or exposure of concealed membrane determinants as a result of alterations in membrane biosynthesis.

Two points regarding the expression of C3 receptors on hybrid cells deserve comment. Firstly, D98/HR-1 hybrid cells could be infected with EBV although C3 receptors were not detected. It has recently been suggested that C3 receptors may act as or be associated with EBV receptors (17). Our findings indicate that infection with EBV can occur without detectable C3 receptors. We have also shown that the procedure used to measure C3 receptors is sensitive enough to detect 1% of cells with C3 receptors, discounting the possibility that we would not detect the 1% early antigen-positive cells if they possessed the C3 receptor.

Secondly, the D98/Raji hybrids do not express C3 receptors although the Raji parental line carries C3 receptors. The lack of expression of C3 receptors on D98/Raji cells is reminiscent of the lack of secretion of α-fetoprotein and albumin from hybrids of mouse hepatoma cells (which secrete these proteins) and mouse or rat fibroblasts (which do not secrete them) (15). Recently, a synteny between HLA, C3 receptors, and Chromosome 6 has been shown (2, 16). Since the hybrid cells carry HLA antigens and contain Chromosome 6, their lack of C3 receptors cannot result from a loss of the chromosome carrying the genetic information for C3. The following possibilities can be envisioned for our experimental finding: (a) the C3 receptor is present on the cell membrane but cannot be detected because it is coated by a "masking" substance; or (b) the expression of C3 receptors is blocked at the level of synthesis, reflecting some interaction between the 2 genomes present in the hybrid nucleus, and/or at insertion in cell membranes. The latter possibility seems most probable since cells such as D98 may carry HLA although not expressing C3 receptor activity. This explanation would also explain the results obtained with the receptors for monkey RBC. These receptors were expressed on the surface of both Burkitt lymphoblastoid parental cell lines but not on either D98/HR-1 or D98/Raji cell lines.

We have shown that certain epithelial-Burkitt hybrid cells can be infected with EBV and that these cells express the HLA of both parental cell lines but not of C3 receptors. The availability of hybrid cells that lack cell surface markers expressed on 1 parental line is useful to define the genetic control and the regulatory mechanisms involved in their expression and to investigate their possible function in virus infection.

ACKNOWLEDGMENTS

The authors acknowledge the excellent technical assistance of M. F. Lavoue, M. C. Favre, C. Piccoli, and G. Baughman. We also thank M. Vuilloume for electron microscopy data, R. Ladda and Y. Dobelle for helpful discussion and chromosome analysis, and F. Rapp for constructive criticism of the manuscript.

REFERENCES

Cell Surface Markers on EBV-infected Burkitt Hybrid Cells


Fig. 1. Immunofluorescence photomicrograph of D98/HR-1 clone 1 cells infected with EBV derived from HR-1 cells 6 days postinfection, showing early antigen. Note cytoplasmic fluorescence. Approximately × 1125.

Fig. 2. Immunofluorescence photomicrograph of a D98/HR-1 clone 1 cell infected with EBV derived from HR-1 cells 4 days postinfection. Note that the early antigen-positive cell is in late telophase. Approximately × 1700.
Fig. 3. Photograph of trypsin-banded metaphase chromosomes of D98/HR-1 cells. Note 2 No. 6 chromosomes (arrows). D98/Raji cells also contain No. 6 chromosomes (not shown). Approximately x 6000.
Cell Surface Markers on Epithelial-Burkitt Hybrid Cells Superinfected with Epstein-Barr Virus


Updated version Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/37/7_Part_1/2291](http://cancerres.aacrjournals.org/content/37/7_Part_1/2291)

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.