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Identification and Characterization of the Epstein-Barr Virus Receptor on Human B Lymphocytes and Its Relationship to the C3d Complement Receptor (CR2)†

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In pursuing studies on the early events in the infection of human B cells by Epstein-Barr virus (EBV), we examined the host cell attachment phase with a panel of B-cell-specific monoclonal antibodies. One of the monoclonal antibodies, OKB7, directly blocked the attachment of purified EBV to B lymphocytes in the absence of a second anti-immunoglobulin antibody and thereby prevented EBV infection of tonsil and peripheral blood B cells. Although earlier studies have shown a close association of the EBV and complement receptor (CR2), an anti-CR2 monoclonal antibody, anti-B2, did not directly block the binding of EBV to B cells. A comparison of the structures recognized by these monoclonal antibodies on various cell types and their functional and physiochemical properties was undertaken. Flow cytometric analysis revealed that the molecules detected by OKB7 and anti-B2 were coexpressed to the same extent on B cells but were not expressed on T-cell lines. OKB7 and anti-B2 both immunoprecipitated a 145,000-molecular-weight membrane protein with an isoelectric point of 8.2 from membrane extracts of Raji lymphoblastoid cells. OKB7 and, to a lesser extent, anti-B2 directly blocked the attachment of C3d,g-coated fluorescent microspheres and sheep erythrocytes bearing C3d to B cells, indicating that these antibodies also react with CR2. These studies indicate that the EBV-CR2 receptor is a single membrane glycoprotein which possesses multiple antigenic and functional epitopes.

Epstein-Barr virus (EBV), an oncogenic herpesvirus of humans, selectively infects B lymphocytes (7, 12, 25) and probably nasoepithelial cells (31), suggesting that discrete membrane receptors are utilized by this virus to enter cells. Such receptors undoubtedly participate in the endocytosis of EBV in normal B cells (25, 26). Earlier studies demonstrated that the EBV receptor was closely associated with the complement C3d receptor (CR2), a 140,000- to 145,000-molecular-weight membrane glycoprotein (13, 18, 28, 34, 35). Recently, evidence for the identity of the EBV receptor with CR2 was obtained by using an anti-CR2 monoclonal antibody, HB-5, which does not directly block EBV binding (5). In the present studies, we examined the membrane structure recognized by OKB7, a B-lymphocyte-specific monoclonal antibody which directly blocks EBV attachment and infection, and the relationship of this structure to CR2.

MATERIALS AND METHODS

Cell lines, lymphocytes, and EBV isolation. EBV-transformed B-cell lines Raji and B95-8 were maintained in RPMI 1640 and 10% fetal bovine serum at 37°C. Peripheral blood B lymphocytes were isolated from normal adult donors by negative selection with neuraminidase-treated sheep erythrocytes as previously described (8). Tonsils were obtained at Children's Hospital, San Diego, Calif., from normal children of 2 to 11 years of age undergoing tonsillectomy.

EBV was grown in phorbol-myristate-acetate-stimulated B95-8 cells and purified as previously described (24). Virus was intrinsically labeled with [^{35}S]methionine by the method of Edson and Thorley-Lawson (2). Virus purity was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography and by negative-staining transmission electron microscopy (24).

Monoclonal and polyvalent antibodies and CR2 ligands. Monoclonal antibodies OKB2 and OKB7 were obtained from Ortho Diagnostics, Inc., Raritan, N.J.; anti-B1, anti-B2, and anti-B4 antibodies were from Coulter Immunology, Hialeah, Fla.; the monocyte- and macrophage-specific monoclonal antibody anti-MO2 was purchased from Coulter. Affinity-purified anti-mouse immunoglobulin M and anti-immunoglobulin G and M antibodies were purchased from Cappel Laboratories, Cochranville, Pa. The latter antibodies were cross-linked to CNBr-activated Sepharose 6B (Pharmacia Fine Chemicals, Piscataway, N.J. [Div. Pharmacia, Inc.]) as recommended by the manufacturer. Highly purified C3d,g, the physiologic fragment of C3 which binds to CR2 (1, 29), was generously provided by Hans Muller-Eberhard and Michael J. Pangburn. Coumarin (green) fluorescent microspheres (1.0 μm) (Covalent Technology Corp., Redwood City, Calif.) were coated with purified C3d,g or bovine serum albumin (BSA) exactly as recommended by the manufacturer. Antibody-sensitized sheep erythrocytes bearing the complement components C2 and C4 (EAC4,2) were coated with 10,000 to 20,000 molecules of C3d by sequential incubation with purified C3, factors H and I, and 5 μg of trypsin per ml as previously described (30).

Radiolabeling, SDS-PAGE, and IEF analysis of CR2. Raji lymphoblastoid cells were surface labeled with ^{125}I by using Iodo-Beads (Pierce Chemical Co., Rockford, Ill.). The cells were lysed in 1% Nonidet P-40, and the lysates were precleared by absorption with an equal volume of packed, fixed *Staphylococcus aureus* (Pansorbin; Calbiochem-Behring, La Jolla, Calif.) for 3 h at 4°C. The precleared extracts were reacted with 2 μg of anti-B2 antibody coupled to anti-mouse immunoglobulin-M-Sepharose or with an equivalent amount of OKB7 antibody coupled to anti-mouse

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TABLE 1. Effect of monoclonal antibodies on binding of EBV to B cells[a]

Monoclonal antibody	Immunoglobulin isotype	^{35}-labeled EBV bound (cpm ± SD)	% Inhibition of binding
Anti-MO2	IgM	421 ± 28	−3.0
Anti-B1	IgG2a	398 ± 32	2.5
Anti-B2	IgM	483 ± 80	−18.0
Anti-B4	IgG1	399 ± 12	2.3
OKB2	IgG1	488 ± 33	−19.6
OKB7	IgG2a	155 ± 26	62.0
Control		408 ± 18	

[a] Experiment was carried out with 10^6 peripheral blood B cells and 1 μg of each monoclonal antibody.

immunoglobulin G and M-Sepharose. The immunoprecipitates were washed and then eluted from the Sepharose beads with 2% SDS for SDS-PAGE (16) or with 8 M urea for isoelectric focusing (IEF) (4).

EBV binding to and infection of B cells in the presence of monoclonal antibodies. In preliminary studies, the binding of ^{35}S-labeled EBV to untreated Raji lymphoblastoid cells was examined first. Increasing amounts of ^{35}S-labeled EBV was added to 10^7 Raji cells in 1 ml of phosphate-buffered saline–BSA with constant mixing at 4°C for 60 min. The cells were then washed twice in phosphate-buffered saline–BSA, and the pellets were lysed and counted. For virus binding-inhibition assays, peripheral blood B cells (10^6) or tonsil or Raji cells (10^7) were pretreated with various amounts of the different monoclonal antibodies in phosphate-buffered saline–BSA or with buffer alone for 60 min at 4°C. The cells were washed and reacted with 4,000 cpm (approximately 10^7 virions) of purified ^{35}S-labeled EBV and incubated for an additional 60 min at 4°C. The cells were again washed, lysed with scintillation fluid (Cytoscint; Westchem, San Diego, Calif.), and counted. Control cells were pretreated with an irrelevant monoclonal antibody (anti-MO2) or with buffer alone. For viral infectivity assays, 6×10^5 peripheral blood B cells were incubated with the monoclonal antibodies for 60 min at 4°C. The monoclonal antibodies were diluted in RPMI–10% fetal calf serum and dialyzed against the same medium to remove azide before exposure to B cells. The cells were then washed with RPMI medium, plated into 96-well sterile trays at 2×10^5 cells per well, and then exposed to unlabeled EBV at a multiplicity of infection of 1.0, as determined by DNA content (27) and electron microscopy (24), for 14 days. Cell transformation was assessed by incorporation of [^{3}H]thymidine and by formation of cell colonies as previously described (24).

Binding of C3d,g and C3d to B cells in the presence of monoclonal antibodies. Binding of C3d or C3d,g to B cells was examined by two separate assays. In the first assay, the ability of monoclonal antibodies to influence C3d,g binding to B cells was studied by using C3d,g-coated fluorescent microspheres. B cells which had been reacted with various amounts of the monoclonal antibodies at 4°C for 60 min in 200 μl of phosphate-buffered saline–BSA were subsequently incubated for 45 min at 37°C with 10 μl of a 10% suspension of C3d,g-fluorescent microspheres or with microspheres which had been coated with BSA as a control. Cells were examined by combination phase-contrast and fluorescent microscopy. Cells with four or more microspheres were considered positive for C3d,g binding. In the second CR2 assay, B cells were pretreated with monoclonal antibodies at 4°C, washed, and then reacted at a ratio of 1:50 with EAC3d cells for 45 min at 37°C. Formation of rosettes was examined by light microscopy, and B cells with four or more EAC3d cells were considered positive. Controls included B cells incubated with EA or EAC4,2 cells.

RESULTS

Effect of B-cell-specific monoclonal antibodies on EBV attachment and infection. The specificity of ^{35}S-labeled EBV binding to B cells was first examined by incubating increasing amounts of ^{35}S-labeled EBV with Raji cells and determining binding. An uptake curve indicative of saturable binding was observed; at saturable binding, 85 to 90% binding of labeled virus was achieved. Such binding was inhibited by a 10-fold excess of cold EBV (data not shown), indicating the specificity of EBV binding. A panel of monoclonal antibodies specific for B cells was then screened for the ability to directly inhibit the binding of radiolabeled EBV to isolated B lymphocytes in the absence of a second antibody. In these experiments, an amount of each monoclonal antibody (1 to 2 μg) which gave maximal fluorescent staining of the cells was used together with an amount of ^{35}S-labeled EBV which resulted in the saturation of 50% of the EBV receptors on 10^7 untreated tonsil or Raji B cells. Only OKB7 inhibited the binding of labeled EBV to peripheral blood B cells (Table 1). There was no correlation of virus inhibition with the antibody isotype (Table 1) or with the percentage of B cells recognized by antibodies as determined by fluorescence-activated cell sorter analysis. The percentage of peripheral blood B cells labeled by anti-B1, OKB7, and anti-B2 antibodies, as determined by FACS analysis, was similar to the previously reported values (21–23). In addition, antibodies anti-B2 and OKB7 stained similar numbers of tonsil B cells and isolated peripheral blood B cells and did so with similar intensities.

Dose-response studies revealed that 50% inhibition of the binding of approximately 10^7 particles of EBV (4,000 cpm) to 10^7 tonsil B cells occurred with a dose of 300 ng of antibody OKB7, whereas 800 ng was required to achieve 50% inhibition of binding to 10^7 Raji cells (Fig. 1A). The anti-CR2 monoclonal antibody, anti-B2 (10), failed to inhibit EBV binding at all doses. Consistent with the ability of antibody OKB7 to inhibit the binding of EBV to tonsil B lymphocytes and Raji lymphoblastoid cells directly, this antibody also directly blocked EBV infection of peripheral blood B lymphocytes in a dose-dependent manner (Fig. 1B) as measured either by colony outgrowth in soft agarose or by [^{3}H]thymidine incorporation at 14 days of culture. A 50% inhibition of infection occurred with a dose of 300 to 500 ng of antibody OKB7 per 6×10^6 peripheral blood B lymphocytes.

Reactivity of OKB7 and anti-B2 monoclonal antibodies with CR2. Studies were next carried out to determine the possible CR2 specificity of monoclonal antibody OKB7. Antibody OKB7 efficiently inhibited both EAC3d and C3d,g binding to tonsil B cells and Raji cells, respectively, in the absence of a second anti-immunoglobulin antibody, indicating that this antibody blocks CR2 function (Table 2). Anti-B2 antibody showed a limited ability to inhibit CR2 function as indicated by the blocking of EAC3d and C3d,g microsphere binding to B cells, a finding consistent with previous reports (10). Two other monoclonal antibody controls, anti-MO2 and anti-B1, failed to alter CR2 function significantly. Inhibition was observed at 37°C (Table 2) as well as at 4°C (data not shown). This indicates that modulation by antibody redistribution of receptors was not responsible for the observed inhibition.

SDS-PAGE and IEF analysis of the antigen recognized by

antibodies anti-B2 and OKB7. Biochemical studies were carried out to define the antigens recognized by the OKB7 and anti-B2 monoclonal antibodies. Antibodies OKB7 (Fig. 2A, lane 1) and anti-B2 (lane 2) both immunoprecipitated a 145,000-molecular-weight protein from detergent lysates of labeled Raji cells. An irrelevant monoclonal antibody, anti-MO2 (lane 3), failed to do so. Immunoprecipitates were also subjected to IEF. Both antibodies anti-B2 (Fig. 2B, lane 1) and OKB7 (lane 2) detected a protein with an isoelectric point of 8.2, a value consistent with the isoelectric point of CR2 reported by Iida et al. (10). The molecular weight estimate for the protein recognized by antibody OKB7 was somewhat different than that originally reported (21).

DISCUSSION

Infection of B lymphocytes is initiated by the specific binding of EBV to a receptor expressed on the surface of B lymphocytes and B-lymphoblastoid cell lines (12–14, 25). Several lines of evidence have suggested that the EBV receptor is either a C3 receptor or is closely associated with a C3 receptor (3, 9, 13, 15, 20, 28, 34, 35). Direct analysis of the EBV receptor and its relationship to CR2 has recently been possible through the use of monoclonal antibodies. The 140,000- to 145,000-molecular-weight B-cell membrane protein is recognized by monoclonal antibodies termed anti-B2 (10, 23) and HB-5 (5, 32, 33), which immunoprecipitate a polypeptide chain of this molecular weight from normal B lymphocytes and B-lymphoblastoid cell lines and, together with an anti-immunoglobulin second antibody, block rosette formation between particles bearing C3d and B-lymphoid cells bearing the receptor (10, 33). Antibody anti-B2 also has some ability to block such rosette function directly in the absence of a second antibody (10). Antibody HB-5, although unable to block EBV binding directly, possessed this ability in the presence of a second anti-immunoglobulin antibody (5). Recently, good evidence for the identity of the EBV receptor with CR2 was obtained by using these antibodies, since pretreatment of B-lymphoblastoid cells with antibody HB-5 followed by anti-immunoglobulin blocked virus binding, and HB-5–CR2 complexes obtained by immunoprecipi-

TABLE 2. Inhibition of CR2 function by monoclonal antibodies

Antibody	Amt (μg)	% Positive EAC3d rosettes[a,b]	% Positive C3d,g rosettes[b,c]
None		73 (5)	93 (4)
Anti-MO2	2.0	67 (3)	88 (5)
Anti-B1	2.0	74 (5)	90 (5)
Anti-B2	0.5	40 (2)	78 (6)
Anti-B2	1.0	43 (8)	57 (4)
Anti-B2	2.0	40 (3)	25 (3)
OKB7	0.2	55 (4)	50 (4)
OKB7	1.0	45 (6)	20 (4)
OKB7	2.0	30 (2)	4 (1)

[a] Positive rosettes were tonsil B cells having four or more bound EAC3d cells.
[b] Numbers in parentheses indicate standard deviation from two experiments. At least 100 cells were counted for each antibody concentration.
[c] Positive rosettes were Raji cells having four or more fluorescent C3d,g microspheres.

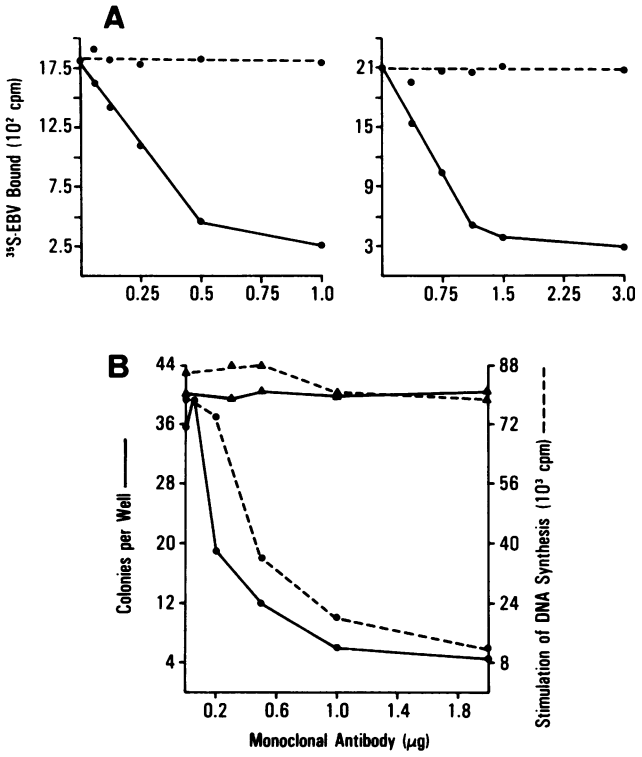

FIG. 1. Effect of monoclonal antibodies on ^{35}S-labeled EBV binding and infection. (A) Normal tonsil B (left) or Raji (right) cells (10^7) were reacted with various amounts of OKB7 (●——●) or anti-B2 (●---●) antibody before the addition of 4,000 cpm of ^{35}S-labeled EBV. *X* Axes, monoclonal antibody (μg). (B) B cells (6 × 10^5) were reacted with various amounts of OKB7 (●) or anti-B2 (▲) antibody, washed, and then cultured for 14 days in the presence of EBV. Infectivity was measured by the stimulation of DNA synthesis ([^{3}H]thymidine incorporation) and colony formation.

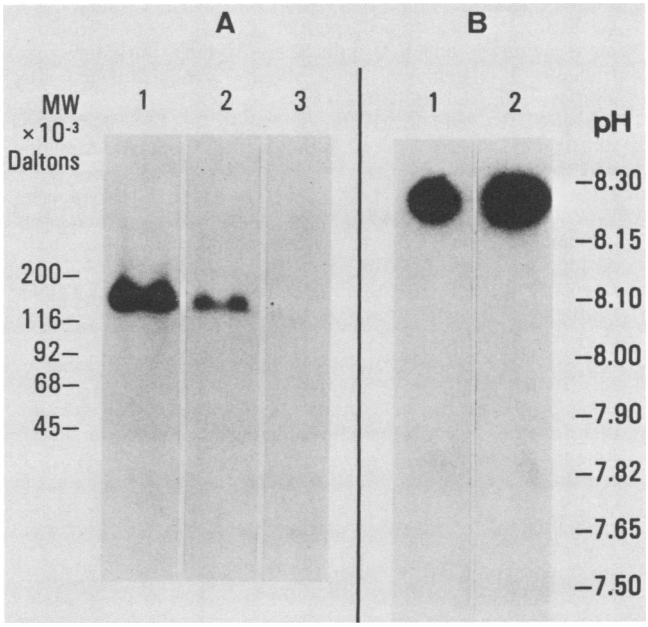

FIG. 2. Immunoprecipitation of Raji B cells with monoclonal antibodies. Surface-labeled Raji cells were lysed with 1% Nonidet P-40 and immunoprecipitated with OKB7 (lanes 1A and 2B), anti-B2 (lanes 2A and 1B) antibody, or MO2 (lane 3A) and analyzed by SDS-PAGE (A) or IEF (B). MW, Molecular weight.

tation from B-lymphoblastoid cell extracts and immobilized on *S. aureus* particles possessed the ability to bind EBV (5). The studies with OKB7 monoclonal antibody described here provide direct evidence that a single B-cell membrane protein functions as a dual receptor for EBV and C3d. This antibody reacts with a 145,000-molecular-weight protein with an isoelectric point of 8.2, values previously identified as characteristic of CR2, and directly blocks CR2 function as well as EBV binding and infection in the absence of a second antibody.

These studies thus indicate that EBV utilizes a normal cell constituent to achieve specific B-cell tropism. Other examples of viruses which use host proteins to infect cells are rabies virus, which may utilize the acetylcholine receptor on neural cells (18, 19), and lactate dehydrogenase virus, which may bind to Ia antigens (11) on macrophages.

These B-cell-specific monoclonal antibodies and corresponding ligands such as EBV and C3d,g, which all react with a common protein on lymphocytes but induce different biological functions, will provide very useful probes for dissecting the early stages of EBV infection as well as the biochemical events involved in B-cell activation and differentiation.

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LITERATURE CITED

1. **Davies, A. E., R. A. Harrison, and P. J. Lachmann.** 1984. Physiologic inactivation of fluid phase C3b: isolation and structural analysis of C3c, C3d,g (α2D) and C3g. J. Immunol. **132**:1960–1966.
2. **Edson, C. M., and D. A. Thorley-Lawson.** 1981. Epstein-Barr virus membrane antigen: characterization, distribution, and strain differences. J. Virol. **39**:172–184.
3. **Einhorn, L., M. Steinitz, E. Yefenof, I. Ernberg, T. Bakacs, and G. Klein.** 1978. Epstein-Barr virus (EBV) receptors, complement receptors, and EBV infectibility of different lymphocyte fractions of human peripheral blood. II. Epstein-Barr virus studies. Cell. Immunol. **35**:43–58.
4. **Ferreira, A., and D. Eichinger.** 1981. A simplified two-dimensional electrophoretic technique. J. Immunol. Methods **43**:291–299.
5. **Fingeroth, J. D., J. J. Weis, T. F. Tedder, J. L. Strominger, P. A. Biro, and D. T. Fearon.** 1984. Epstein-Barr virus receptor of human B lymphocytes is the C3d receptor CR2. Proc. Natl. Acad. Sci. U.S.A. **81**:4510–4514.
6. **Gerber, P., and B. H. Hoyer.** 1972. Induction of cellular DNA synthesis in human leukocytes by Epstein-Barr virus. Nature (London) **231**:46–47.
7. **Greaves, M. F., G. Brown, and A. B. Rickinson.** 1975. Epstein-Barr virus binding sites on lymphocyte subpopulations and the origin of lymphoblasts in cultured lymphoid cell lines and in the blood of patients with infectious mononucleosis. Clin. Immunol. Immunopathol. **3**:514–524.
8. **Huddlestone, J. R., and M. B. A. Oldstone.** 1978. T suppressor (T_G) lymphocytes fluctuate in parallel with changes in the clinical course of patients with multiple sclerosis. J. Immunol. **123**:1615–1618.
9. **Hutt-Fletcher, L. M., E. Fowler, J. D. Lambris, R. J. Feighny, J. G. Simmons, and G. D. Ross.** 1983. Studies of the Epstein-Barr virus receptor found on Raji cells. II. A comparison of lymphocyte binding sites for Epstein-Barr virus and C3d. J. Immunol. **130**:1309–1312.
10. **Iida, K., L. Nadler, and V. Nussenzweig.** 1983. Identification of the membrane receptor for the complement fragment C3d by means of a monoclonal antibody. J. Exp. Med. **158**:1021–1033.
11. **Inada, T., and C. A. Mims.** 1984. Mouse Ia antigens are receptors for lactate dehydrogenase virus. Nature (London) **309**:59–61.
12. **Jondal, M., and G. Klein.** 1973. Surface markers on human B and T lymphocytes. II. Presence of Epstein-Barr receptors on B lymphocytes. J. Exp. Med. **138**:1365–1378.
13. **Jondal, M., G. Klein, M. B. A. Oldstone, V. Bokisch, and E. Yefenof.** 1976. Surface markers on human B and T lymphocytes. VIII. Association between complement and Epstein-Barr receptors on human lymphoid cells. Scand. J. Immunol. **5**:401–410.
14. **Jonsson, V., A. Wells, and G. Klein.** 1982. Receptors for the complement C3d component and the Epstein-Barr virus are quantitatively co-expressed on a series of B cell lines and their derived somatic cell hybrids. Cell. Immunol. **72**:265–276.
15. **Klein, G., E. Yefenof, K. Falk, and A. Westman.** 1978. Relationship between Epstein-Barr virus (EBV) production and the loss of the EBV receptor/complement receptor complex in a series of sublines derived from the same original Burkitt's lymphoma. Int. J. Cancer **21**:552–560.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **Lambris, J. D., N. J. Dobson, and G. D. Ross.** 1981. Isolation of lymphocyte membrane complement receptor type two (the C3d receptor) and preparation of receptor-specific antibody. Proc. Natl. Acad. Sci. U.S.A. **78**:1828–1832.
18. **Lentz, T. L., T. G. Burrage, A. L. Smith, J. Crick, and G. H. Tignor.** 1982. Is the acetylcholine receptor a rabies virus receptor? Science **215**:182–184.
19. **Lentz, T. L., P. T. Wilson, E. Hawrot, and D. W. Speicher.** 1984. Amino acid sequence similarity between rabies virus glycoprotein and snake venom curaremimetic neurotoxins. Science **226**:847–848.
20. **Magrath, I., C. Freeman, M. Santaella, J. Gadek, M. Frank, R. Spiegel, and L. Novikovs.** 1981. Induction of complement receptor expression in cell lines derived from human undifferentiated lymphomas. II. Characterization of the induced complement receptors and demonstration of the simultaneous induction of EBV receptor. J. Immunol. **127**:1039–1043.
21. **Mittler, R. S., M. A. Talle, K. Carpenter, P. E. Rao, and G. Goldstein.** 1983. Generation and characterization of monoclonal antibodies reactive with human B lymphocytes. J. Immunol. **131**:1754–1761.
22. **Nadler, L. M., K. C. Anderson, G. Marti, M. Bates, E. Park, J. F. Daley, and S. F. Schlossman.** 1983. B4, a human lymphocyte associated antigen expressed on normal, mitogen-activated, and malignant B lymphocytes. J. Immunol. **131**:244–250.
23. **Nadler, L. M., P. Stashenko, R. Hardy, A. Van Agthoven, C. Terhorst, and S. F. Schlossman.** 1981. Characterization of a human B cell specific antigen (B2) distinct from B1. J. Immunol. **126**:1941–1947.
24. **Nemerow, G. R., and N. R. Cooper.** 1981. Isolation of Epstein-Barr virus and studies of its neutralization by human IgG and complement. J. Immunol. **127**:272–278.
25. **Nemerow, G. R., and N. R. Cooper.** 1984. Early events in the infection of human B lymphocytes by Epstein-Barr virus: the internalization process. Virology **132**:186–198.
26. **Nemerow, G. R., and N. R. Cooper.** 1984. Infection of B lymphocytes by a human herpesvirus, Epstein-Barr virus, is blocked by calmodulin antagonists. Proc. Natl. Acad. Sci. U.S.A. **81**:4955–4959.
27. **Nemerow, G. R., F. C. Jensen, and N. R. Cooper.** 1982. Neutralization of Epstein-Barr virus by nonimmune human serum. Role of cross-reacting antibody to herpes simplex virus and complement. J. Clin. Invest. **70**:1081–1091.
28. **Rosenthal, K. S., S. Yanovich, M. Inbar, and J. L. Strominger.** 1978. Translocation of a hydrocarbon fluorescent probe between Epstein-Barr virus and lymphoid cells: an assay for early events in viral infection. Proc. Natl. Acad. Sci. U.S.A. **75**:5076–5080.
29. **Ross, G. D., S. L. Newman, J. D. Lambris, J. E. Devery-Pocius, J. A. Cain, and P. J. Lachmann.** 1983. Generation of three

different fragments of bound C3 with purified factor I or serum II. Location of binding sites in the C3 fragments for factors B and H, complement receptors and bovine conglutinin. J. Exp. Med. **158**:334–352.

30. **Schreiber, R. D., M. K. Pangburn, A. B. Bjornson, M. A. Brothers, and H. J. Muller-Eberhard.** 1982. The role of C3 fragments in endocytosis and extracellular cytotoxic reactions by polymorphonuclear leukocytes. Clin. Immunol. Immunopathol. **23**:335–357.
31. **Sixbey, J. W., E. H. Vesterinen, J. G. Nedrud, N. Raab-Traub, L. A. Walton, and J. S. Pagano.** 1983. Replication of Epstein-Barr virus in human epithelial cells infected in vitro. Nature (London) **306**:480–483.
32. **Tedder, T. F., L. T. Clement, and M. D. Cooper.** 1984. Expression of C3d receptors during human B cell differentiation: immunofluorescence analysis with the HB-5 monoclonal antibody. J. Immunol. **133**:678–683.
33. **Weis, J. J., T. F. Tedder, and D. T. Fearon.** 1984. Identification of a 145,000 M_r membrane protein as the C3d receptor (CR2) of human B lymphocytes. Proc. Natl. Acad. Sci. U.S.A. **81**:881–885.
34. **Yefenof, E., and G. Klein.** 1977. Membrane receptor stripping confirms the association between EBV receptors and complement receptors on the surface of human B lymphoma lines. Int. J. Cancer **20**:347–352.
35. **Yefenof, E., G. Klein, M. Jondal, and M. B. A. Oldstone.** 1976. Surface markers on human B and T lymphocytes. IX. Two color immunofluorescence studies on the association between EBV receptors and complement receptors on the surface of lymphoid cell lines. Int. J. Cancer **17**:693–700.