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Characterization of a T-Lymphocyte Epstein-Barr Virus/C3d Receptor (CD21)

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The Epstein-Barr virus/C3d receptor (EBVR-CR2) was detected on three T-lymphoblastoid cell lines. The apparent M_rs of purified EBVR-CR2 of T-cell and B-cell origin were identical. The N-terminal amino acid sequence from the T-cell EBVR-CR2 confirmed the placement of this receptor in a multigene family of complement regulatory proteins. All EBVR-CR2-positive T-cell lines were T6 and T4-T8 antigen positive.

Epstein-Barr virus (EBV), a human herpesvirus, causes infectious mononucleosis and polyclonal B-cell lymphoma (7). EBV is associated with African Burkitt's lymphoma and nasopharyngeal carcinoma (7). The human B-lymphocyte receptor for EBV is a 145,000-M_r glycoprotein (8, 9, 37) that is also the receptor for the d region of C3 (CR2) (17, 55). A role for this receptor in normal B-lymphocyte activation has been postulated (10, 15, 29, 36, 49, 57). Internal amino acid sequences derived from tryptic peptides and a partial cDNA clone demonstrates that EBV CR2 (EBVR-CR2) is homologous to complement receptor type 1 (CR1) (19, 54), which places it in a multigene family (38) that includes the complement regulatory proteins C4-binding protein (5), factor H (21, 41), and decay-accelerating factor (4, 28), all five of which are linked on chromosome 1 (27, 42, 53). Additional complement proteins which share a characteristic 60-amino-acid consensus repeat and concomitant serine protease activity include factor B (11, 32), C2 (11), and C1r-C1s (24, 45). The noncomplement proteins beta-2 glycoprotein I (26), the beta subunit of factor XIII (16), the alpha[1] chain of haptoglobin (22), and the interleukin-2 receptor (23) are also members of this extended multigene family (38).

EBVR-CR2 was originally found on B lymphocytes with the exception of the T-lymphoblastoid cell line Molt 4 (8, 30). Immunohistochemical staining procedures suggest that the receptor may also be expressed on follicular dendritic cells (40), histiocytosis X cells (1), and a subset of oropharyngeal epithelial cells (58). Identity of EBVR-CR2 with cross-reactive antigens on these distinct cell types has not been established by biochemical means or functional studies.

EBVR-CR2 (CD21) was purified from (13, 18) a B-lymphoblastoid cell line (JY) and from a T-lymphoblastoid cell line (HPB-ALL) by lectin (18) and immunoaffinity chromatography (44). To purify the receptor, membrane lysates (36, 37) in detergent were passed over three serial lectin columns (ricin, lentil, and wheat germ) which were eluted with appropriate sugars (37); the lysates were then pooled in the presence of sodium deoxycholate (final concentration, 0.5%), and this lysate pool was applied to an immunoaffinity column of anti-HB-5 MAb (40), which was washed as previously described (37). The column was preeluted with 1 column volume of 50 mM diethylamine (pH 11.5) (Fisher Scientific Co.)–0.1% sodium deoxycholate (which removed little of the EBVR-CR2 [<2%] but rapidly cleared the column of minor contaminants), washed with 2 column volumes of 10 mM Tris (pH 7.5, 0.1%), and finally eluted in 2 to 3 column volumes of 3 M KSCN–10 mM Tris (pH 7.5)–0.1% sodium deoxycholate. Separate columns were used for the respective cell lines throughout the purification. The eluted protein was dialyzed against 10 mM Tris–0.04 M NaCl–0.1% sodium deoxycholate, precipitated with 5 volumes of cold acetone, suspended in 500 μl of water, and analyzed for yield and purity. The molecular weights of highly purified proteins from JY and HPB-ALL appeared to be identical when assessed by silver stain (33) of the purified receptor run on sodium dodecyl sulfate-polyacrylamide gels under reducing (Fig. 1) and nonreducing conditions. A simple and rapid purification scheme yielded an average of 0.5 to 3 μg of pure protein per g of cells from 50- to 100-g batches.

Purified EBVR-CR2 was precipitated in ethanol, dried, suspended in 100 μl of water–0.01% recrystallized sodium dodecyl sulfate, and sequenced on a gas-phase sequenator (18). The N-terminal sequence from 1 to 2 nmol of purified HPB-ALL EBVR-CR2 was obtained on two occasions (Table 1). No N-terminal amino acid sequence was obtained from 1 nmol of reduced and alkylated JY receptor, suggesting qualitative or quantitative alterations in the protein preparation or amino acid substitution in the B-cell protein resulting in N-terminal cyclization or other biochemical modification that inhibited the sequencing reaction. The N-terminal amino acids of HPB-ALL EBVR-CR2 were homologous to internal peptides from the B-cell protein (Table 1). Identification of the consensus repeat at the extreme N terminus was consistent with the structure of several members of the 60-amino-acid-repeat family, including C4-binding protein, factor H, decay-accelerating factor, beta-2 glycoprotein I, the b subunit of factor XIII, and probably CR1 (19), whose members share a similar organization, with tandem repeats proceeding from or near the amino end of the molecule and terminating in a short C-terminal domain (38) (Table 1). The N-terminal portions of selected consensus sequences from more structurally diverse family members (Table 1), including factor B, C2, C1r-C1s, factor I, the alpha[1] chain of haptoglobin, and the interleukin-2 receptor, also demonstrate significant similarity at positions 8, 10, 12, and 13 beyond the absolute conservation at positions 4 and 7. Secondary structure analysis of the EBVR-CR2 N terminus by a modified Chou-Fasman pseudoprobabilities code (37a) reveals a beta turn originating close to or at the N terminus, followed by a small beta pleated sheet. Because the C3b-C4b binding domains of C4-binding protein, factor H, the C2b fragment of C2, and the Ba fragment of factor B have been localized to their

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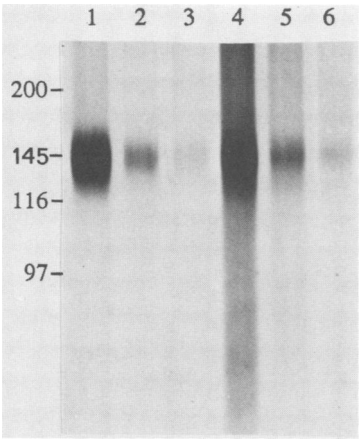

FIG. 1. Silver-stained 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lanes 1 to 3 contain serial dilutions of purified EBVR-CR2 from the T-lymphoblastoid cell line HPB-ALL. Lanes 4 to 6 contain serial dilutions of purified receptor protein from the JY cell line. Numbers on the left indicate molecular weights of standard proteins (the purified protein is at 145).

respective N termini (38), it will be interesting to determine whether EBVR-CR2, which is distinguished by binding C3d and EBV, also contains N-terminal sequences important for ligand attachment.

The observation that EBVR-CR2 was expressed on the T-cell line HPB-ALL as well as on Molt 4 (8, 30) (Fig. 2) prompted us to examine several additional T lymphocytes. Two monoclonal antibodies (MAbs), HB-5 (50) and anti-B2 (34), with specificity for distinct epitopes on EBVR-CR2 were employed. HB-5 was prepared by 45% ammonium sulfate precipitation of murine ascites obtained following intraperitoneal injection of 10^7 HB-135 hybridoma cells (American Type Culture Collection). Anti-B2 was purchased from Coulter Immunology, as was the control MAb anti-B1 (34). The MAb EBVCS (48) was a gift of Bill Sugden. The 7F.10 MAb directed to T6 (CD1) was provided by Nancy Jones. The anti-CR1 MAb anti-C3bR was purchased from Dako, and the irrelevant MAb P3 was provided by Martin Hemler. Fluorescein isothiocyanate (FITC)-coupled goat $F(ab')_2$ anti-mouse immunoglobulins G, A, and M were purchased from Organon Teknika. EBV was purified from the B-958 cell line and fluoresceinated as described previously (8). Established cell lines were maintained as described previously (8). For staining, samples containing 5×10^5 cells each were washed twice with RPMI–5% heat-inactivated fetal calf serum, incubated with antibody 1 for 30 min on ice, washed twice, reincubated with FITC-labeled antibody 2 for 30 min on ice, washed twice more, and either fixed with 1% paraformaldehyde in phosphate-buffered saline or analyzed immediately following the addition of 10 μl of propidium iodide at 0.5 mg/ml. Duplicate samples were stained with FITC-EBV as described elsewhere (8). Flow cytometry was performed on an EPICS V (Coulter) dual laser cell sorter with an MDADS data acquisition package.

Analysis of cells stained with HB-5, anti-B2, and FITC-EBV indicated that the T-cell line Jurkat also expressed EBVR-CR2, while the T-cell line T-ALL-1 did not (Fig. 2). Incubation with HB-5 and anti-mouse immunoglobulin blocked binding of FITC-EBV on all receptor-bearing lines

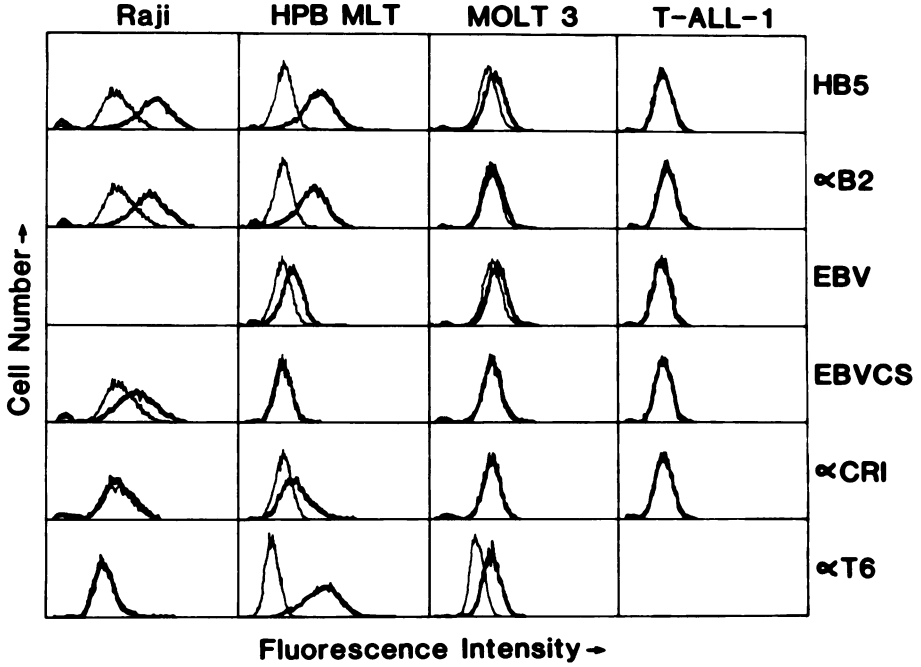

FIG. 2. Flow cytometry of T- and B-cell lines. The mean channel fluorescence for the EBVR-CR2 was for the B-cell lines Raji 111 and JY 17 (not shown) and for the T-cell lines HPB-MLT 26, Jurkat 5 (not shown), Molt 3 5, T-ALL-1 0, and HSB-2 0 (not shown). To obtain these values, the mean channel fluorescence in arbitrary logarithmic units was calculated from data obtained with MAb HB-5 and FITC-labeled goat $F(ab')_2$ anti-mouse immunoglobulins G, A, and M (Organon Teknika) and converted to a linear scale, and the value of the control antibody, P3, was subtracted. Fluorescence was achieved with 500 mW of 488-nm light from an argon ion laser. A 525-nm band pass–560-nm short-pass filter combination was used for detection.

TABLE 1. N-terminal amino acid sequence of EBVR-CR2 compared with sequences of internal peptides from CR2 and with best-fit consensus repeat sequences from members of the 60-amino-acid consensus repeat family[a]

N-terminal sequence EBVR/CR2:	Ile-Ser-Xaa[1]-Gly-Ser-Pro-Pro[2]-Xaa[3]-Ile-Leu-Asn-Gly-Arg-Ile-Glu-Tyr-Gly-Ser-Thr-Ser-Ile-Lys-Xaa[4]-Lys-Xaa[4]-Ser/Glu
Internal peptide CR2(54):	Val-Lys-Glu-Pro-Pro-Xaa-Ile-Leu
Internal peptide CR2 (54)	Cys-Gln-Pro-Pro-Pro-Glu-Ile-Leu-
CR1, 367-392(19):	Ile-Phe-Cys-Pro-Ser-Pro-Pro-Val-Ile-Pro-Asn-Gly-Arg-His-Thr-Gly-Lys-Pro-Leu-Glu-Val-Phe-Pro-Phe-Gly-Lys
H, cHt.49.14, 10-35(21)	Leu-Pro-Cys-Lys-Ser-Pro-Pro-Glu-Ile-Ser-His-Gly-Val-Val-Ala-His-Met-Ser-Asp-Ser-Tyr-Gln-Tyr-Gly-Gln-Gln
C4 bp, 124-149(5):	Val-Lys-Cys-Lys-Pro-Pro-Pro-Asp-Ile- -Asn-Gly-Arg-His-Ser-Gly-Glu-Glu-Asn-Phe-Tyr-Ala-Tyr-Gly-Phe-Ser
DAF, 1-25(4):	Asp-Cys-Gly-Leu-Pro-Pro-Asp-Val-Pro-Asn-Ala-Gln-Pro-Ala-Leu-Glu-Gly-Arg-Thr-Ser-Phe-Pro-Glu-Asp-Thr
B2 gpl, 184-208(26):	Val-Lys-Cys-Pro-Phe-Pro-Ser-Arg-Pro-Asp-Asn-Gly-Phe-Val-Asn-Tyr-Pro-Ala-Lys-Pro-Thr-Leu-Tyr-Tyr-Lys-Asp
B subunit F XIII, 314-339(16):	Val-Ala-Cys-Glu-Glu-Pro-Pro-Phe-Ile-Glu-Asn-Gly-Ala-Ala-Asn-Leu-His-Ser-Lys-Ile-Tyr-Tyr-Asn-Gly-Asp-Lys
alpha Hptgn, 13-37(22):	Asp-Gly-Cys-Pro-Lys-Pro-Pro-Glu-Ile-Ala-His-Gly-Tyr-Val-Glu-His- -Ser-Val-Arg-Tyr-Gln-Cys-Lys-Asn-Tyr
B, 138-161(11):	Gly-Tyr-Cys-Ser-Asn-Pro-Gly- -Ile-Pro-Ile-Gly-Thr-Arg-Lys-Val-Gly-Ser- -Gln-Tyr-Arg-Leu-Glu-Asp-Ser
C2, 138-162(11):	Gly-Xaa-Cys-Pro-Asn-Pro-Gly- -Ile-Ser-Leu-Gly-Ala-Val-Xaa-Thr-Gly-Phe-Arg-Phe-Gly-His-Gly-Asp-Leu-Val
C1r, 374-399(24):	Arg-Cys-Cys-Gly-Gln-Pro-Arg-Asn-Leu-Pro-Asn-Gly-Asp-Phe-Arg-Tyr-Thr-Thr-Thr-Met-Gly-Val-Asn-Thr-Tyr-Lys
IL-2R, 102-127(23):	Gly-His-Cys-Arg-Glu-Pro-Pro-Pro-Trp-Glu-Asn-Glu-Ala-Thr-Glu-Arg-Ile-Tyr-His-Phe-Val-Val-Gly-Gln-Met-Val
Consensus sequence (38):	- - 4 - - 7 -
Similarities:	- - - - - - 8 - 10 - 12 13 - - - - - - - - - - - - - -

[a] Superscripts are as follows. [1], No signal obtained on this cycle was consistent with a cysteine, tryptophan, or glycosylation site. Of note is the marked conservation of cysteine at this position among family members. [2], High probability. However, proline does not always cleave fully; therefore, an unambiguous assignment could not be made. [3], No signal obtained. [4], Unknown amino acid assignment. [5], Personal observation based on nucleic acid sequence from a JY cDNA library. Underscoring is as follows. ___, Consensus sequence. Positions 4 and 7 as defined by the original consensus sequence of approximately 60 amino acids found in most repeating homology units of family members (38). _____, Strongly conserved amino acids not in the defined consensus sequence but found in 50% or more of the proteins enumerated above. ....., Positions with some identity to the EBVR-CR2 N terminus. Numbers after abbreviated proteins indicate numerical amino acid assignments or clone designations indicated by the original authors. Abbreviations: H, factor H; bp, binding protein; DAF, decay-accelerating factor; B2 gpl, beta-2 glycoprotein I; F XIII, factor XIII; Hptgn, haptoglobin; B, factor B; IL-2R, interleukin-2 receptor.

examined, as previously demonstrated (8). The rank order of mean channel fluorescence for these T-lymphoblastoid cell lines was HPB-ALL (also called HPB-MLT) > Jurkat (also called JM) > Molt 3 (from the same patient as Molt 4) (see the legend to Fig. 2). The mean fluorescence intensity of the HPB-ALL cell line was comparable to that of the B-cell line JY but was less intense than that of Raji (see the legend to Fig. 2), suggesting approximately equivalent receptor densities among receptor-positive T cells compared with B-cells, i.e. 8,000 to<70,000 receptors per cell (8). EBVR-CR2-positive T-cell lines HPB-ALL and Jurkat also expressed CR1, while the cell line Molt 3, which expressed low levels of EBVR-CR2, did not have CR1 detectable by flow cytometry. The B-cell-specific antigens B1 and EBVCS (CD23) (Blast-2) (Fig. 2), the latter an antigen present on many B-lymphoblastoid cell lines and associated with EBV transformation (48, 51, 52), were absent on all T-lymphoblastoid cell lines. Phenotypic analysis of three EBVR-CR2-bearing T-lymphoblastoid cell lines revealed that they all expressed T6 (CD1) (3) (Fig. 2) as well as both T4 and T8 cell surface antigens and were HLA-DR negative (31). No EBVR-CR2 was detected on cloned T4- and T8-positive cells from peripheral blood. Southern blot analysis of EBVR-CR2-positive T-cell DNA, with the EBV *Bam*HI W genomic fragment used as a probe, indicated that the cells did not harbor endogenous virus.

Previous identification at low density of an EBVR-CR2 (8, 30) ostensibly incapable of internalizing virus (30) on the T-cell line Molt 4 was regarded as an unusual event related to the transformed state of this tumor. Several T-cell tumor lines previously examined, including HSB-2, Hut-78, CEM, and T-ALL-1, were EBVR-CR2 negative. However, the T-cell line HPB-ALL expressed receptors at a much higher density than that observed on several B-lymphoblastoid cells, while the T-cell line Jurkat expressed low levels of EBVR-CR2. No other B-cell-specific markers were found on these T cells. However, all the cell lines identified were T6 and also T4-T8 antigen positive. A fresh T-lymphoid neoplasm of thymic phenotype stained with the MAb OKB7 also directed to EBVR-CR2 was previously described (20). Although it was reported that T6-positive cells from normal thymus are EBVR-CR2 negative (50), the recently defined complexity of the CD1 antigen family of proteins including T6 (3) suggests that reexamination of receptor expression during thymic ontogeny could prove interesting. T6-positive T lymphocytes have been reported to circulate transiently in the peripheral blood of burn patients (J. Wood, J. B. O'Mahoney, S. B. Palder, M. L. Rodrick, P. O'Eon, and J. D. Mannick, Letter, J. Invest. Dermatol. **82**:387–388, 1984) and patients treated for Wiskott-Aldrich syndrome (39). Very rare T6-positive lymphocytes have also been identified in normal cord blood and peripheral blood (6, 14). Of interest in this regard is a recent report of an EBV-genome-positive thymoepithelioma (25) and also of an EBV-transformed T-cell line from cord blood (47). These reports support the notion that rare T lymphocytes expressing EBVR-CR2 may exist in vivo and may be susceptible to transformation by EBV. Increased rates of spontaneous recombination (2) and trisomy associated with myeloid malignancy (43) have been ascribed to the complement regulatory protein locus, raising the possibility that a direct genetic alteration accounts for EBVR-CR2 expression in the T-cell neoplasms identified. Interestingly, the CD1 multigene family also maps to chromosome 1 (3); however, its location in relation to the complement regulatory protein locus is unknown. The significance of concurrent expression of T6 and T4-T8 antigens on EBVR-CR2-positive T-cell neoplasms remains to be determined.

B cells which harbor virus but continue to express receptors can bind exogenous EBV, and genome-negative receptor-bearing B-lymphoblastoid lines can be converted to latent genome-positive cell lines by superinfection with active virus (46). The tropism of EBV for the human B lymphocyte has been explained on the basis of receptor specificity. However, the process of virus internalization and establishment of latency is more complex. Infected B-lymphoblastoid cell lines do not internalize virus by the same pathway as virgin B lymphocytes from peripheral blood (12, 35, 49). Possible explanations for this observation include interferon effects on the cell membrane such as those described for other viruses (56), specific EBV-mediated cell membrane alterations secondary to latent viral proteins or to activated cellular proteins, or, in the case of EBV-negative tumor lines, membrane alterations otherwise associated with the transformed state. Identification of the virus receptor on several EBV-negative T-lymphoblastoid cell lines allows one to approach the question of whether a functional receptor is both necessary and sufficient for the establishment of latent EBV infection or whether the B-cell milieu itself is required for internalization-fusion or the establishment of latent versus lytic infection. Comparison of different pathways of infection should prove informative.

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LITERATURE CITED

1. **Bieber, T., D. Hanau, E. Heid, and M. D. Kazatchkine.** 1985. Histiocytosis-X cells express C3b, C3d, and C3bi receptor (CR1, CR2 and CR3) antigens. Arch. Dermatol. Res. **277**: 496–498.
2. **Brito-Babapulle, V., and N. B. Atkin.** 1981. Breakpoints in chromosome 1 abnormalities of 218 human neoplasms. Cancer Genet. Cytogenet. **4**:215–225.
3. **Calabi, F., and C. Milstein.** 1986. A novel family of human major histocompatibility complex-related genes not mapping to chromosome 6. Nature (London) **323**:540–543.
4. **Caras, I. W., M. A. Davitz, L. Rhee, G. Weddell, D. W. Martin, Jr., and V. Nussenzweig.** 1987. Cloning of decay-accelerating factor suggests novel use of splicing to generate two proteins. Nature (London) **325**:545–549.
5. **Chung, L. P., D. R. Bentley, and K. B. M. Reid.** 1985. Molecular cloning and characterization of the cDNA coding for C4b-binding protein, a regulatory protein of the classical pathway of the human complement system. Biochem. J. **230**:133–141.
6. **Dezutter-Dambuyant, C., D. Schmitt, M. Faure, M. Cordier, and J. Thivolet.** 1984. Detection of OKT6-positive cells (without visible Birbeck granules) in normal peripheral blood. Immunol. Lett. **8**:121–126.
7. **Epstein, M. A., and B. G. Achong.** 1986. Introductory considerations, p. 1–11. *In* M. A. Epstein and B. G. Achong (ed.), The Epstein-Barr virus: recent advances. John Wiley & Sons, Inc., New York.
8. **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. USA **81**:4510–4516.
9. **Frade, R., M. Barel, B. Ehlin-Henriksson, and G. Klein.** 1985. gp140, the C3d receptor of human B lymphocytes, is also the Epstein-Barr virus receptor. Proc. Natl. Acad. Sci. USA **82**: 1490–1493.
10. **Frade, R., M. C. Crevon, M. Barel, A. Vazquez, L. Krikorian, C. Charriaut, and P. Galanaud.** 1985. Enhancement of human B

cell proliferation by an antibody to the C3d receptor, the gp 140 molecule. Eur. J. Immunol. **15**:73–76.
11. **Gagnon, J.** 1984. Structure and activation of complement components C2 and factor B. Philos. Trans. R. Soc. Lond. B. Biol. Sci. **306**:23–31.
12. **Goldmacher, T., J. Lambert, and S. Schlossman.** 1986. EBV binding induces internalization of the C3d receptor: a novel immunotoxin delivery system. J. Immunol. **137**:1387–1391.
13. **Gorga, J. C., J. Foran, S. J. Burakoff, and J. L. Strominger.** 1984. Use of the HLA-DR antigens incorporated into liposomes to generate HLA-DR specific cytotoxic lymphocytes. Methods Enzymol. **108**:607–613.
14. **Gothelf, Y., N. Sharon, and E. Gazit.** 1986. A subset of human cord blood mononuclear cells is similar to Langerhans cells of the skin: a study of peanut agglutinin and monoclonal antibodies. Hum. Immunol. **15**:164–174.
15. **Hutt-Fletcher, L. M.** 1987. Synergistic activation of cells by Epstein-Barr virus and B-cell growth factor. J. Virol. **61**: 774–781.
16. **Ichinose, A., B. A. McMullen, K. Fujikawa, and E. W. Davie.** 1986. Amino acid sequence of the b subunit of factor XIII, a protein composed of ten repetitive segments. Biochemistry **25**:4633–4638.
17. **Iida, K., L. Nadler, and V. Nussenzweig.** 1983. Identification of the membrane fragment for the complement fragment C3d by means of a monoclonal antibody. J. Exp. Med. **158**:1021–1033.
18. **Jones, N. H., M. Clabby, D. P. Dialynas, H.-J. S. Huang, L. Herzenberg, and J. Strominger.** 1986. Isolation of complementary DNA clones encoding the human lymphocyte glycoprotein T1/Leu-1. Nature (London) **323**:346–349.
19. **Klickstein, L. B., W. W. Wong, J. A. Smith, J. A. Weis, J. G. Wilson, and D. T. Fearon.** 1987. Human C3b/4b receptor(CR1): demonstration of long homologous repeating domains that are composed of short consensus repeats characteristic of C3/C4 binding proteins. J. Exp. Med. **165**:1095–1112.
20. **Knowles, D. M., II, L. D. Dodson, R. Raab, R. S. Mittler, M. A. Talle, and G. Goldstein.** 1983. The application of monoclonal antibodies to the characterization and diagnosis of lymphoid neoplasms: a review of recent studies. Diagn. Immunol. **1**:142–149.
21. **Kristensen, T., R. A. Wetsel, and B. F. Tack.** 1986. Structural analysis of human complement protein H: homology with C4b binding protein, $beta_2$-glycoprotein I and the Ba fragment of B. J. Immunol. **136**:3407–3411.
22. **Kurosky, A., D. R. Barnett, T. H. Lee, B. Touchstone, R. E. Hay, M. S. Arnott, B. H. Bowman, and W. Fitch.** 1980. Covalent structure of human haptoglobin: a serine protease homolog. Proc. Natl. Acad. Sci. USA **78**:3388–3392.
23. **Leonard, W. J., J. M. Depper, M. Kanehisa, M. Kronke, N. Peffer, P. B. Svetlik, M. Sullivan, and W. C. Greene.** 1985. Structure of the human interleukin-2 receptor gene. Science **230**:633–639.
24. **Leytus, S. P., K. Kurachi, K. S. Sakariassen, and E. W. Davie.** 1986. Nucleotide sequence of the cDna coding for human complement C1r. Biochemistry **25**:4855–4863.
25. **Leyvraz, S., W. Henle, A. P. Chahinian, C. Perlmann, G. Klein, R. E. Gordon, M. Rosenblum, and J. F. Holland.** 1985. Association of Epstein-Barr virus with thymic carcinoma. N. Engl. J. Med. **312**:1296–1299.
26. **Lozier, J., N. Takahashi, and F. W. Putnam.** 1984. Complete amino acid sequence of human plasma $beta_2$-glycoprotein I. Proc. Natl. Acad. Sci. USA **81**:3640–3644.
27. **Lublin, D. M., R. S. Lemons, M. M. Le Beau, V. M. Holers, M. L. Tykocinski, M. E. Medof, and J. P. Atkinson.** 1987. The gene encoding decay-accelerating factor (DAF) is located in the complement regulatory locus on the long arm of chromosome 1. J. Exp. Med. **165**:1731–1736.
28. **Medof, M. E., D. M. Lublin, V. M. Holers, D. Ayers, R. R. Getty, J. F. Leykam, J. P. Atkinson, and M. L. Tykocinski.** 1987. Cloning and characterization of cDNAs encoding the complete sequence of decay-accelerating factor of human complement. Proc. Natl. Acad. Sci. USA **84**:2007–2011.
29. **Melchers, F., A. Erdei, T. Schultz, and M. P. Dierich.** 1985. Growth control of activated, synchronized murine B cells by the C3d fragment of human complement. Nature (London) **317**:264–267.
30. **Menezes, J., J. M. Seigneurin, P. Patel, A. Bourkas, and G. Lenoir.** 1977. Presence of Epstein-Barr virus receptors, but absence of virus penetration, in cells of an Epstein-Barr virus genome-negative human lymphoblastoid T line (Molt 4). J. Virol. **22**:816–821.
31. **Minowada, J., H. G. Drexler, M. Menon, H. Dubinstein, H. Messmore, S. Krasnow, J. Takeuchi, and A. A. Sandberg.** 1985. A model scheme of hematopoietic cell differentiation based on multiple marker analysis of leukemia-lymphomas: T cell lineage. Hamatol-Bluttransfus. **29**:426–429.
32. **Morley, B. J., and R. D. Campbell.** 1984. Internal homologies of the Ba fragment from human complement component factor B, a class III MHC antigen. EMBO J. **3**:153–157.
33. **Morrissey, J. H.** 1981. Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced sensitivity. Anal. Biochem. **117**:307–310.
34. **Nadler, L. M., P. Stashenko, R. Hardy, A. von Agthoven, C. Terhorst, and S. F. Schlossman.** 1981. Characterization of a human B cell specific antigen (B2) distinct form B1. J. Immunol. **126**:1941–1947.
35. **Nemerow, G. R., and N. R. Cooper.** 1984. Early events in the infection of human B lymphocytes by Epstein-Barr virus DNA: internalization process. Virology **132**:186–196.
36. **Nemerow, G. R., M. E. McNaughton, and N. R. Cooper.** 1985. Binding of monoclonal antibody to the Epstein-Barr virus (EBV)/CR2 receptor induces activation and differentiation of human B lymphocytes. J. Immunol. **135**:3068–3073.
37. **Nemerow, G. R., R. Wolfert, M. E. McNaughton, and N. R. Cooper.** 1985. Identification and characterization of the Epstein-Barr virus receptor on human B lymphocytes and its relationship to the C3d complement receptor (CR2). J. Virol. **55**:347–351.
37a. **Ralph, W. W., T. Webster, and T. F. Smith.** 1987. A modified Chou and Fasman protein structure algorithm. CABIOS **3**: 211–216.
38. Reid, K. B. M., D. R. Bentley, R. D. Campbell, L. P. Chung, R. B. Sim, T. Kristensen, and B. F. Tack. 1986. Complement system proteins which interact with C3b or C4b. Immunol. Today **7**:230–234.
39. **Reinherz, E. L., M. D. Cooper, S. F. Schlossman, and F. S. Rosen.** 1981. Abnormalities of T cell maturation and regulation in human beings with immunodeficiency disorders. J. Clin. Invest. **63**:699–705.
40. **Reynes, M., J. P. Aubert, J. H. Cohen, J. Audouin, V. Tricottet, J. Diebold, and M. D. Kazatchkine.** 1985. Human follicular dendritic cells express CR1, CR2 and CR3 complement receptor antigens. J. Immunol. **135**:2687–2694.
41. **Ripoche, J., A. J. Day, A. C. Willis, K. T. Belt, R. D. Campbell, and R. B. Sim.** 1986. Partial characterization of human complement factor H by protein and cDNA sequencing: homology with other complement and noncomplement proteins. Biosci. Rep. **6**:65–72.
42. **Rodriquez de Cordoba, S., D. M. Lublin, P. Rubenstein, and J. P. Atkinson.** 1985. Human genes for the three complement components that regulate the activation of C3 are tightly linked. J. Exp. Med. **161**:1189–1195.
43. **Rowley, J. D.** 1978. Abnormalities of chromosome 1: significance in malignant transformation. B Cell Pathol. **29**:139–144.
44. **Schneider, C., R. A. Neuman, D. R. Sutherland, V. Asser, and M. F. Greaves.** 1982. A one-step purification of membrane proteins using a high efficiency immunomatrix. J. Biol. Chem. **257**:10766–10769.
45. **Spycher, S. E., H. Nick, and E. E. Rickli.** 1986. Human complement component C1s partial sequence determination of the heavy chain and identification of the peptide bond cleaved during activation. Eur. J. Biochem. **156**:49–57.
46. **Steinitz, M., and G. Klein.** 1975. Comparison between growth characteristics of an EBV-genome negative lymphoma line and its EBV-converted subline in vitro. Proc. Natl. Acad. Sci. USA **72**:3518–3520.
47. **Stevenson, M., B. Volsky, M. Hedenskog, and D. Volsky.** 1986.

Immortalization of human T lymphocytes after transfection of Epstein-Barr virus DNA. Science **233**:980–984.

48. **Sugden, B., and S. Metzenberg.** 1983. Characterization of an antigen whose cell surface expression is induced by infection with Epstein-Barr virus. J. Virol. **46**:800–807.
49. **Tanner, J., J. Weis, D. Fearon, Y. Whang, and E. Kieff.** 1987. Epstein-Barr virus gp350/220 binding to the B lymphocyte C3d receptor mediates adsorption, capping and endocytosis. Cell **50**:203–213.
50. **Tedder, T. F., L. T. Clement, and M. D. Cooper.** 1984. Expression of C3d receptors during human B cell differentiation: analysis with the HB-5 monoclonal antibody. J. Immunol. **133**:678–683.
51. **Thorley-Lawson, D. A., and K. P. Mann.** 1985. Early events in Epstein-Barr virus infection provide a model for B cell activation. J. Exp. Med. **162**:45–59.
52. **Wang, F., M. Gregory, M. Rowe, A. B. Rickinson, D. Wang, M. Birkenbach, H. Kikutani, T. Kishimoto, and E. Kieff.** 1987. Epstein-Barr virus nuclear antigen 2 specifically induces expression of the B-cell activation antigen CD23. Proc. Natl. Acad. Sci. USA **84**:3452–3456.
53. **Weis, J. H., C. C. Morton, G. A. P. Bruns, J. J. Weis, L. B. Klickstein, W. W. Wong, and D. T. Fearon.** 1987. A complement receptor locus: genes encoding C3b/C4b receptor and C3d/Epstein-Barr virus receptor map to 1q32. J. Immunol. **138**: 312–315.
54. **Weis, J. J., D. T. Fearon, L. B. Klickstein, W. W. Wong, S. A. Richards, A. de Bruyn Kops, J. A. Smith, and J. H. Weis.** 1986. Identification of a partial cDNA clone for the C3d/Epstein-Barr virus receptor of human B lymphocytes: homology with the receptor for the fragments C3b and C4b of the third and fourth components of complement. Proc. Natl. Acad. Sci. USA **83**:5639–5643.
55. **Weis, J. J., T. F. Tedder, and D. T. Fearon.** 1984. Identification of a 145,000 M_r membrane protein as the CR2 receptor (CR2) of human B lymphocytes. Proc. Natl. Acad. Sci. USA **81**:881–885.
56. **Whitaker-Dowling, P. A., D. K. Wilcox, C. C. Widnell, and J. S. Younger.** 1983. Interferon-mediated inhibition of virus penetration. Proc. Natl. Acad. Sci. USA **80**:1083–1086.
57. **Wilson, B. S., J. L. Platt, and N. Kay.** 1985. Monoclonal antibodies to the 140,000 mol wt glycoprotein of B lymphocyte membranes (CR2 receptor) initiates proliferation of B cells in vitro. Blood **66**:824–829.
58. **Young, L. S., D. Clark, J. W. Sixbey, and A. B. Rickinson.** 1986. Epstein-Barr virus receptors on human pharyngeal epithelia. Lancet **i**:240–242.