Epstein–Barr virus (EBV) induces expression of B-cell activation markers on *in vitro* infection of EBV-negative B-lymphoma cells

(herpesvirus/nuclear antigens/cell growth/transformation)

ALAIN CALENDER*, MARC BILLAUD*, JEAN-PIERRE AUBRY†, JACQUES BANCHEREAU†, MICHELE VUILLAUME*, AND GILBERT M. LENOIR*‡

*International Agency for Research on Cancer, 150, cours Albert Thomas, 69372 Lyon Cedex 08, France; and †Unicet, Centre de Recherche, 69572 Dardilly, France

Communicated by Werner Henle, July 6, 1987 (received for review March 24, 1987)

ABSTRACT A set of B-cell activation markers, including the EBV/C3d receptor [complement receptor type 2 (CR2) (CD21)], the 45-kDa lymphoblastoid cell-associated (Blast-2) antigen (CD23), and the B-cell restricted activation (Bac-1) antigen (which was recently identified as a potential B-cell growth factor receptor) can be turned on by infecting lymphoma cells that are genome negative for Epstein–Barr virus (EBV) with the B95-8 immortalizing strain of the virus. The nonimmortalizing EBV variant, strain P3HR-1, which possesses a deletion within the *Bam*HI WYH region of the genome containing the coding sequence for the EBV-determined nuclear antigen 2, does not induce expression of these markers. Other lymphoblastoid cell-associated antigen markers can be activated by infection with either immortalizing or nonimmortalizing viruses. These results suggest that the immortalizing potential of EBV is correlated with its ability to induce expression of B-cell activation markers, which are suspected to play a major role in the physiological pathway leading to lymphoid cell proliferation. The viral genomic region deleted in the nonimmortalizing strain of EBV seems to be required for activation of some of these markers. Human lymphoma cell lines, such as those used in this study, can thus help identify the specific EBV genes involved in lymphoid B-cell proliferation and the mechanism of action of these genes.

Epstein–Barr virus (EBV), a human lymphotropic herpesvirus, causes infectious mononucleosis and is associated with two human malignancies—Burkitt lymphoma (BL) and nasopharyngeal carcinoma (1). *In vitro* EBV can transform human B lymphocytes to a state of continuous proliferation, known as cell “immortalization,” generating permanent lymphoblastoid cell lines (LCL) that contain multiple copies of the viral genome in an episomal form (2). The process of cell immortalization has been suggested to occur via EBV-mediated stimulation of a physiological B-cell activation pathway (3). Thus, experiments by Gordon *et al.* (4) indicate that the process could in part be associated with induction of an autocrine secretion loop of B-cell growth factors (BCGFs) that act on specific receptors.

A phenotypic, biochemical, or molecular study of EBV-induced B-cell immortalization should not compare a heterogeneous population of primary lymphocytes with a homogeneous population of rapidly dividing EBV-infected cells: because of their heterogeneity, primary lymphocyte populations are not true controls for LCL. Therefore we have developed an *in vitro* model with which to study mechanisms of EBV-induced phenotypic alterations of B cells. Some EBV-negative lymphoma cell lines do not express the B-cell activation markers normally expressed at the surface of

EBV-immortalized cells (5); however, those lymphomatous cells are susceptible to EBV infection, leading to stable EBV-positive lines, the phenotypic characteristics of which can be easily studied and compared with those of parental cells.

We selected a panel of such cell lines and focused on the expression of three B-cell activation surface markers: C3d receptor (CR2 or CD21), 45-kDa lymphoblastoid cell-associated (Blast-2) antigen (CD23), and B-cell restricted activation (Bac-1) antigen. CR2 has recently been suggested both as being involved in the control of the cell cycle of activated B cells (6) and as being the EBV receptor expressed on the surface of B lymphocytes (7, 8). The Blast-2 antigen (CD23) is an early marker of B-cell activation (9) and was recently shown to be homologous to the low-affinity IgE/Fc receptor on B cells (10, 11). The third B-cell activation marker selected for this study is that recognized by the Bac-1 monoclonal antibody (mAb); it may correspond to one of the BCGF receptors (12). In this report, we demonstrate that infection of these cell lines *in vitro* with immortalizing strains of EBV leads to stable EBV-positive converted lines that express CR2/CD21, Blast-2/CD23, and Bac-1 activation markers. Expression of these markers is not seen after infection of the same lymphoma cell lines with the nonimmortalizing EBV strain P3HR-1, suggesting that the DNA region deleted from this virus codes for proteins required for activation of the corresponding cellular genes.

MATERIALS AND METHODS

Cell Lines, mAbs. The BL 02, BL 30, BL 31, BL 40, BL 41, and BL 70 cell lines were established at the International Agency for Research on Cancer (IARC) (Lyon, France). The BJAB cell line was a gift from G. Klein (Karolinska Institute, Stockholm), and the Louckes line was from A. B. Rickinson (C.R.C., University of Birmingham, U.K.). The phenotypes of the original lines analyzed within the first 30 passages *in vitro* and again during the experiments underwent no major modification. The IARC 211 line (LCL) was established by infection of peripheral blood lymphocytes with EBV *in vitro* (B95-8). Table 1 shows the panel of mAb used for cell-surface phenotyping. mAbs MHM6 and mAb25, initially used to study antigen Blast-2 and IgE/Fc receptor, respectively, were shown during the course of the study to recognize the same molecules, CD23 (10, 11). MHM6 and AC2 mAbs were used as ascitic fluids; Ki24 mAb was a tissue culture supernatant, and the others were used as purified immuno-

Abbreviations: EBV, Epstein–Barr virus; EBNA2, EBV nuclear antigen 2; mAb, monoclonal antibody; BCGF, B-cell growth factor; BL, Burkitt lymphoma; LCL, lymphoblastoid cell line; CR2, complement receptor type 2; CD23, Blast 2 antigen (45-kDa lymphoblastoid cell-associated differentiation antigen); Bac-1, B-cell restricted activation antigen.

‡To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Table 1. mAbs used for cell-surface phenotyping

mAb	Specificity and cluster (designation)	Source and reference
OKB7	140-kDa C3d/EBV receptor CR2 (CD21)	Ortho Diagnostics,* ref. 13
Bac-1	B-cell restricted activation antigen	T. Suzuki & M. D. Cooper, [†] ref. 12
MHM6	45-kDa Blast-2 lymphoblastoid cell-associated antigen (CD23)	A. B. Rickinson, [‡] ref. 14
mAb25	44-kDa IgE Fc receptor on human lymphocytes (CD23)	J. Y. Bonnefoy, [§] ref. 11
AC2	80-kDa lymphoblastoid cell-associated antigen (CD39)	A. B. Rickinson, [‡] ref. 14
Ki24	Sternberg-Reed cell-associated antigen	A. B. Rickinson, [‡] ref. 15
Anti-CALLA	100-kDa human common acute lymphoblastic leukemia antigen (CD10)	Becton Dickinson, refs. 16, 17
OK1 A	29 kDa and 34 kDa human HLA-DR antigen complex	Ortho Diagnostics,* ref. 18

*Raritan, NJ.

[†]Tumor Institute, University of Alabama, Tuscaloosa, AL.[‡]C.R.C., University of Birmingham, U.K.[§]Unicet, Centre de Recherche, Dardilly, France.^{||}Mountain View, CA.

globulin. Control immunoglobulin was a polyclonal mouse IgG plus IgM raised against γ interferon; this control immunoglobulin was produced and provided by the Unicet Laboratory (Dardilly, France).

Virus Preparation, Cell Infection, and Cloning. Three EBV isolates were used in this study. Two immortalizing strains, B95-8 (19) and AG876 (20), differ mainly at their EBV nuclear antigen 2 (EBNA2) locus (21, 22); the third isolate used was the nonimmortalizing strain P3HR-1, which is deleted within the *Bam*HI WYH region and consequently lacks the coding sequence for EBNA2 (23). Viral suspensions were concentrated 100-fold from supernatants of B95-8, P3HR-1, or AG876 cell cultures, according to the procedure of Adams (24). Infectivity of the viral preparations was tested by induction of EBNA on the EBV-negative BJAB cell line 3 days after infection (25). For the conversion experiments, the virus was used at a concentration that induced 50% EBNA positivity in BJAB cells. After infection, cells were cultivated at concentrations maintained between 1 and 2×10^6 cells per ml. EBNA was analyzed with the anticomplement immunofluorescence method described by Reedman and Klein (26). Independent EBNA-positive and EBNA-negative cell clones were obtained by single-cell cloning 2 and 4 weeks after infection.

Fluorescence Analysis. Cells were incubated with mAb at dilutions of 1:50 for the purified antibodies, 1:1000 for ascitic fluids, and 1:5 for the tissue culture supernatant. After being washed, the cells were further incubated with fluorescein-conjugated F(ab')₂ antibodies to mouse immunoglobulin (BioArt, Meudon, France). mAbs of the same isotype as test mAbs were included as routine negative controls for non-specific binding. Immunofluorescence staining was first assessed by fluorescence microscopy (Leitz) and further analyzed with a FACS 440 cell sorter (Becton Dickinson). The number of mAb binding sites per cell was estimated using the technique developed by Poncelet and Carayon (27).

Quantification of EBV Genome Copies. Cellular DNA extraction, nick translation of the *Bam*HI W fragment corresponding to the internal repeat unit of the EBV genome (a cloned fragment of the EBV genome, provided by G. W. Bornkamm, Freiburg University, F.R.G.), and quantifications by dot hybridization were done by standard procedures (28). Cellular DNA was digested with *Eco*RI before hybridization.

RESULTS

Original Cell Surface Phenotypes. A set of six EBV genome-negative lymphoma/leukemia cell lines were selected for their low expression level of such B-cell activation markers as CR2, Blast-2 (CD23), and Bac-1; BL 31 showed, however, a high level of constitutive expression of antigen Bac-1 (Fig. 1). Two EBV-negative lymphoma cell lines, Louckes and BJAB, which naturally express these activation markers, were selected as controls for some experiments. CR2, Blast-2 (CD23), and Bac-1 were expressed on 82, 26, and 48% of Louckes cells and on 39, 37, and 40% of BJAB cells.

Phenotypic Alterations Associated with EBV Conversion. Expression of the surface markers, the 100-kDa human common acute lymphoblastic leukemia antigen (CALLA), HLA-DR, and surface immunoglobulin (sIg) was similar after infection with immortalizing and nonimmortalizing EBV, as assessed by the percentage of positive cells and the intensity of fluorescence staining (data not shown) with one exception—there was a dramatic reduction in HLA-DR expression after conversion of BL 70 with P3HR-1 virus, confirmed by the analysis of six independent cell clones (data not shown).

Major differences in expression were noted with activation markers (Fig. 1). CR2, Bac-1, and the CD23-related molecules—Blast-2 and the IgE/Fc receptor—were strongly activated in B95-8- and AG876-converted cells with the two following exceptions: Bac-1 expression was not induced in B95-8-converted BL 02 cells, and CR2 was only moderately activated in BL 30 and BL 40 cells after B95-8 conversion. However, when the nonimmortalizing EBV strain P3HR-1 was used for the conversion, no significant change in the expression of these activation markers was seen in any of the six cell lines studied. These results were confirmed in independent EBV-converted cell clones, as shown for antigens CR2 and CD23 in the BL 02 cell line in Table 2.

As shown in Fig. 2, both immortalizing and nonimmortalizing viruses were capable, to some extent, of activating the expression of the 80-kDa lymphoblastoid cell-associated antigen AC2 (CD39) and the Sternberg-Reed cell-associated antigen Ki24. Whereas antigen Ki24 was equally activated or constantly expressed by P3HR-1 or B95-8 conversion, antigen AC2 was, in general, significantly more expressed in B95-8-converted cells than in P3HR-1 converts.

Number of Antibody Binding Sites per Cell. The number of CR2, Bac-1, and CD23 molecules per cell was estimated for five of the lymphoma cell lines and their P3HR-1 and B95-8 converts (Table 3) in the EBV-positive BL line Raji and on a LCL (IARC 211). In most cell lines analyzed, conversion with P3HR-1 virus did not affect the number of CR2, CD23, and Bac-1 molecules per cell, whereas conversion with B95-8 or AG876 dramatically increased expression of these markers to levels comparable to, or higher than, those observed in LCL and in Raji cells. The slight increase in CR2 molecules in B95-8-converted BL 30 and BL 40 cells, as well as the lack of induction of Bac-1 molecules in B95-8-converted BL 02 cells, are consistent with the results of the previous analysis shown in Fig. 1.

Effect of Culture Conditions on Expression of Markers. To eliminate the possibility that selective effect of culture procedures causes a nonspecific activation of B surface-activation antigens, mock infection experiments were done on BL 30 cells using a concentrated supernatant prepared

FIG. 1. Expression of four B-cell activation markers on six EBV-negative lymphoma cell lines and their EBV-positive converts. Original cell line was phenotypically analyzed as well as clones in which 100% of the cells expressed EBNA after conversion with the B95-8 or P3HR-1 strains of EBV. These results represent the analysis of a single-cell clone, but similar results always occurred with other independent clones and with the initial converted cell population before cloning (see also Table 2). Results for each cell line are grouped as follows: EBV-negative control (C), P3HR-1-converted (P3), B95-8-converted (B95), and AG876-converted (AG) cell lines.

from the EBV-negative BJAB cell line, thus containing no viral particles. No effect was seen on CR2, CD23, and Bac-1 expression between 8 and 60 days after mock infection (data not shown). Furthermore, expression of the three activation markers on independent EBV-negative cell clones obtained by cell cloning after B95-8 infection was similar to that of the parental EBV-negative cell line, as for two clones of the BL 70 cell line in Table 3.

No EBV-Induced Inhibition of Constitutionally Expressed CR2 and Bac-1. To rule out the possibility that the low level of CR2 expression in P3HR-1-converted lines results from inhibition or from difference in the cytopathogenicity of P3HR-1 and B95-8 viruses, the following experiment was done. Louckes and BJAB cells, which constitutionally express CR2, were converted with P3HR-1 and B95-8 viruses, and the level of CR2 expression was studied after 1 month on a 100% EBNA-expressing cell population. P3HR-1 virus conversion had no significant effect on CR2 expression, which occurred on 39.1, 36.1, and 62.6% of the BJAB control, P3HR-1-converted cells, and B95-8-converted cells. The Louckes EBV-negative cell line expressed CR2 on 82% of

cells, and this expression was maintained on 88% and 88.5% of their P3HR-1- and B95-8-converted counterparts. Similarly, the constitutional expression of Bac-1 in BL 31 cells was unaffected by infection with either virus (Fig. 1).

No Significant Variation in the Number of Viral Genome Copies in Converted Cells. A mean of 0.8–1.4 copies of the viral genome was found per cell in the P3HR-1 and B95-8 converts of the cell lines tested (data not shown).

DISCUSSION

Infection of EBV-negative lymphoma cells with immortalizing EBV can induce a dramatic increase in the expression of the B-cell activation markers CR2, Blast-2/CD23, and Bac-1, markers usually detectable in <5% of EBV-negative cells. More than 60% of B95-8-converted cells express these markers at induced levels up to 10^5 molecules per cell.

Some Activation Markers Are Induced only by Immortalizing EBV Isolates. The fact that markers CR2, Blast-2/CD23, and Bac-1 are activated by the two immortalizing strains B95-8 and AG876 and not by the nonimmortalizing P3HR-1 virus strongly suggests that the deleted portion of the P3HR-1 genome contains sequences required for turning on expression of these activation markers in the converted cells (Fig. 1). Similar observations were made by Klein *et al.* (29), demonstrating that EBV conversion of the EBV-negative Ramos lymphoma line by transforming B95-8 substrain regularly induced the expression of CR2, as detected by B2 (Coultronics) mAb, whereas the nontransforming P3HR-1 had no such effect. Surface phenotypic differences cannot be explained by quantitative variations in the number of viral genome copies because dot hybridization experiments give similar values for P3HR-1- and B95-8-converted cells.

Table 2. CR2 (CD21) and Blast-2 (CD23) expression in independent clones of EBV-converted BL 02 cell line

Surface marker	BL 02 control	% positive cells					
		BL 02/P3HR-1			BL 02/B95-8		
		1	2	3	1	2	3
CR2	10.7	7.2	8.0	14.9	54.4	84.3	67.9
CD23 (MHM6)	1.1	1.8	3.9	1.5	57.2	47.5	54.4

The numbers 1, 2, and 3 refer to clones 1, 2, and 3.

FIG. 2. Expression of the 80-kDa lymphoblastoid cell-associated antigen AC2 (CD39) and the Sternberg-Reed cell-associated antigen Ki24 on the panel of control (C), P3HR-1-converted (P3), and B95-8-converted (B95) cell lines.

Control experiments on Louckes and BJAB EBV-negative cell lines, which constitutively express CR2, indicate that

Table 3. Quantitative flow cytometry analysis of the number of CR2, CD23, and Bac-1 molecules per cell

Cell line	CR2 (CD21)	CD23 (MHM6)	Bac-1
BL 02	<100	<100	<100
BL 02 P3HR-1	<100	<100	500
BL 02 B95-8	18,000	400	100
BL 30	<100	<100	300
BL 30 P3HR-1	<100	<100	400
BL 30 B95-8	100	1,000	9,000
BL 40	<100	100	300
BL 40 P3HR-1	<100	<100	1,700
BL 40 B95-8	200	5,500	107,000
BL 41	100	100	700
BL 41 P3HR-1	<100	<100	<100
BL 41 B95-8	76,000	260,000	101,000
BL 41 AG876	44,000	57,000	15,000
BL 70	<100	<100	300
BL 70 P3HR-1	<100	<100	<100
BL 70 B95-8	18,000	1,200	2,600
BL 70(B95-8)*	<100	100	200
LCL IARC 211	1,600	47,000	45,000
Raji	16,000	100	5,000

*EBV-negative selected clone.

conversion with P3HR-1 is not associated with a selection of cells that express low levels of CR2, as suggested by the known cytopathic effect of the P3HR-1 virus (30). However, infection with EBV does not significantly affect the percentage of cells that express other surface markers, such as antigens [the 100-kDa human common acute lymphoblastic leukemia antigen (CALLA)], HLA-DR, and surface immunoglobulin. Three control experiments clearly suggest that expression of certain viral functions is necessary to trigger the B-cell activation pathway, and these experiments show the observed phenomena not to be the consequence of experimental selection bias on the following grounds: (i) mock infection of EBV-negative cells was not associated with activation; (ii) EBV genome-negative clones isolated by single-cell cloning after B95-8 infection express none of the selected activation markers (Table 3); and (iii) activation seen in the converted cells was confirmed by analysis of independent EBV genome-positive cell clones (Table 2).

Some Markers Are Activated by Both Immortalizing and Nonimmortalizing EBV Isolates. The finding that both P3HR-1 and B95-8 viruses can activate some markers, such as lymphoblastoid cell-associated antigens AC2 and Ki24, indicates that the viral functions expressed by both immortalizing and nonimmortalizing strains can also alter B-cell phenotype, and these functions might even be involved in the process of B-cell immortalization.

Implications of this Cellular System for Studying Viral Functions Involved in B-Cell Immortalization and Proliferation. Activation markers induced by EBV have been implicated at different stages of the B-cell activation and proliferation pathways. Blast-2/CD23 is synthesized as a 43- to 46-kDa precursor and is expressed at the cell surface after activation of B cells before being shed into the culture supernatant as a soluble 25- to 33-kDa derivative (31). Such behavior is consistent with a dual role for Blast-2/CD23 as a receptor and as a growth factor for the processed molecule, as recently suggested (32). Other studies have revealed that antigen CD23 is involved in triggering the progression of activated B lymphocytes through the G₁ phase of the cell cycle (33), and several authors have recently demonstrated that antigen CD23 is also the low affinity receptor for the Fc fragment of IgE (11, 34). This surface molecule is a B-cell-specific differentiation marker restricted to mature B cells before immunoglobulin isotype switching and is induced on human bone marrow B cells by purified B-cell stimulating factor 1 (IL-4) lymphokine (34). The same authors showed that the IgE/Fc receptor is not expressed on Daudi cells, which are known to carry an EBV strain with an EBNA2 gene deletion similar to that in the P3HR-1 virus, suggesting again a relationship between EBNA2 and CD23 expression. The Bac-1 mAb, which recognizes an antigen expressed on B95-8-converted Burkitt cells, was shown to inhibit the BCGF-induced enhancement of DNA synthesis in B cells activated with anti-IgM antibody (12), suggesting that Bac-1 is a receptor for a BCGF. Autocrine production of BCGF is believed to play an important role in EBV-induced proliferation (35, 36). CR2 is defined as the EBV/C3d receptor on lymphocytes and was characterized as a 140-kDa glycoprotein recognized by OKB7 mAb (7, 37). This receptor has recently been shown to play a role in B-cell proliferation (6, 38). The initial EBV-CR2 interaction is probably a critical step in triggering the B-cell activation pathway. Whether continuous expression of CR2 is required for EBV-induced cell immortalization remains to be assessed.

In summary, our experimental system provides a panel of converted cell lines, obtained with different strains of EBV characterized with varying biological properties, that might permit analysis of some viral functions involved in B-cell immortalization and proliferation. An obvious possibility is that of analyzing the effect of specific EBV genes on different segments of the B-cell activation pathway. This panel of converted cell lines would also enable the dissection of molecular level at which EBV affects the regulation of specific cellular genes, such as those that code for CR2 and CD23, or for oncogenic sequences, such as *c-fgr*, known also to be activated by EBV (39). Apart from analyzing the role of EBV in the immortalization process, the described experimental model might be most useful for studying the role of EBV in BL. In particular, it could be used to test whether HLA class I restricted T-cell killing is directed against EBNA2 or against antigens induced by the EBNA2-related activation pathway. The recent demonstration that EBV-positive BL cells have a nonlymphoblastoid phenotype *in vivo* (40) coupled with the fact that freshly established BL cells do not necessarily express EBNA2 points to the intriguing hypothesis that the activation pathway related to EBNA2 expression is not involved in the development of malignant BL cells. Should this hypothesis be confirmed, the P3HR-1-converted B-lymphoma cell lines may prove an ideal tool with which to study whether viral gene-encoded proteins provide a growth advantage to cells carrying the characteristic chromosomal translocation, thus cooperating with a deregulated *c-myc* gene.

We acknowledge M. F. Lavoue for her excellent technical assistance in immunofluorescence techniques. We are indebted to Prof.

G. W. Bornkamm, Dr. J. P. Lamelin, and Mrs. E. Heseltine for helpful suggestions and critical revision of the manuscript. We thank A. B. Rickinson, T. Suzuki, D. M. Cooper, and J. Y. Bonnefoy for their gift of mAbs. A.C. is supported by a Special Training Fellowship from the International Agency for Research on Cancer. M.B. is supported by a fellowship from the Association pour la Recherche sur le Cancer.

1. Epstein, M. A. & Achong, B. G., eds. (1979) *The Epstein-Barr Virus* (Springer, Berlin).
2. Henle, W., Diehl, V., Kohn, G., Zur Hausen, H. & Henle, G. (1967) *Science* **157**, 1064-1065.
3. Thorley-Lawson, D. A. & Mann, K. P. (1985) *J. Exp. Med.* **162**, 45-59.
4. Gordon, J., Ley, S. C., Melamed, M. D., English, L. S. & Hughes-Jones, N. C. (1984) *Nature (London)* **312**, 145-147.
5. Nilsson, K. & Klein, G. (1982) *Adv. Cancer Res.* **37**, 319-379.
6. Melchers, F., Erdei, A., Schulz, T. & Dierich, M. P. (1985) *Nature (London)* **317**, 264-267.
7. Fingerth, J. D., Weis, J. J., Tedder, T. F., Strominger, J. L., Biro, P. A. & Fearon, D. T. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4510-4514.
8. Frade, R., Barel, M., Ehlin-Eriksson, B. & Klein, G. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1490-1493.
9. Thorley-Lawson, D. A., Nadler, L. M., Bahn, A. K. & Schooley, R. T. (1985) *J. Immunol.* **134**, 3007-3012.
10. Yukawa, K., Kikutani, H., Owaki, H., Yamasaki, K., Yokota, A., Nakamura, H., Barsumian, E. L., Hardy, R. R., Suemura, M. & Kishimoto, T. (1987) *J. Immunol.* **138**, in press.
11. Bonnefoy, J. Y., Aubry, J. P., Peronne, C., Wijdenes, J. & Banchereau, J. (1987) *J. Immunol.*, in press.
12. Suzuki, T., Komiyama, K., Buttler, J. L. & Cooper, M. D. (1986) *J. Immunol.* **137**, 1208-1213.
13. Mittler, R. S., Talle, M. A., Carpenter, K., Rao, P. E. & Goldstein, G. (1983) *J. Immunol.* **131**, 1754-1761.
14. Rowe, M., Hildreth, J. E. K., Rickinson, A. B. & Epstein, M. A. (1982) *Int. J. Cancer* **29**, 373-381.
15. Stein, H., Gerdes, J., Schwab, U., Lemke, H., Diehl, V., Mason, D., Bartels, H. & Ziegler, A. (1983) *Haemat. Oncol.* **1**, 21-29.
16. Greaves, M. F. (1981) *Monoclonal Antibodies in Leukaemia Markers*, ed. Knapp, W. (Academic, New York), p. 19.
17. Ritz, J., Pesando, J. M., Norris-McConarty, J., Lazarus, H. & Schlossman, S. H. (1980) *Nature (London)* **283**, 583-586.
18. Lampson, L. A. & Levy, R. (1980) *J. Immunol.* **125**, 393-398.
19. Miller, G., Shope, T., Lisco, H., Stitt, D. & Lipman, M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 383-387.
20. Pizzo, P. A., Magrath, I. T., Chattopadhyay, S. K., Biggar, R. J. & Gerber, P. (1978) *Nature (London)* **272**, 629-631.
21. King, W., Dambaugh, T., Heller, M., Dowling, J. & Kieff, E. (1982) *J. Virol.* **43**, 979-989.
22. Dambaugh, T., Hennessy, K., Chamnankit, L. & Kieff, E. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7632-7636.
23. Bornkamm, G. W., Hudewentz, J., Freese, U. K. & Zimmer, U. (1982) *J. Virol.* **43**, 952-968.
24. Adams, A. (1973) *J. Gen. Virol.* **20**, 391-394.
25. Klein, G., Lindahl, T., Jondal, M., Leibold, W., Menezes, J., Nilsson, K. & Sundstrom, C. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3283-3287.
26. Reedman, B. M. & Klein, G. (1973) *Int. J. Cancer* **11**, 499-520.
27. Poncelet, P. & Carayon, P. (1985) *J. Immunol. Methods* **85**, 65-74.
28. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
29. Klein, G., Ehlin-Henriksson, B. & Schlossman, S. F. (1983) *J. Immunol.* **130**, 1985-1988.
30. Klein, G., Manneborg, A. & Steinitz, M. (1979) *Int. J. Cancer* **23**, 197-200.
31. Thorley-Lawson, D. A., Swendeman, S. L. & Edson, C. M. (1986) *J. Immunol.* **136**, 1745-1751.
32. Swendeman, S. & Thorley-Lawson, D. A. (1987) *EMBO J.* **6**, 1637-1642.
33. Gordon, J., Rowe, M., Walker, L. & Guy, G. (1986) *Eur. J. Immunol.* **16**, 1075-1080.
34. Kikutani, H., Suemura, M., Owaki, H., Nakamura, H., Sato, R., Yamasaki, K., Barsumian, L., Hardy, R. R. & Kishimoto, T. (1986) *J. Exp. Med.* **164**, 1455-1464.
35. Gordon, J., Aman, P., Rosen, A., Ernberg, J., Ehlin-Henriksson, B. & Klein, G. (1985) *Int. J. Cancer* **35**, 251-256.
36. Blazar, B. A., Sutton, L. M. & Strome, M. (1983) *Cancer Res.* **43**, 4562-4570.
37. Nemerow, G. R., Siaw, M. F. E. & Cooper, N. R. (1986) *J. Virol.* **58**, 709-712.
38. Frade, R., Crevon, M. C., Barel, M., Vasquez, M., Krikorian, L., Charriaud, C. & Galanaud, O. (1985) *Eur. J. Immunol.* **15**, 73-76.
39. Cheah, M. S. C., Ley, T. J., Tronick, S. R. & Robbins, K. C. (1986) *Nature (London)* **319**, 238-240.
40. Rowe, D. T., Rowe, M., Evan, G. I., Wallace, L. E., Farrel, P. J. & Rickinson, A. B. (1986) *EMBO J.* **5**, 2599-2607.