Immunology 1986 **58** 591–595

Control of human B-lymphocyte replication

II. TRANSFORMING EPSTEIN–BARR VIRUS EXPLOITS THREE DISTINCT VIRAL SIGNALS TO UNDERMINE THREE SEPARATE CONTROL POINTS IN B-CELL GROWTH

J. GORDON,* LEONIE WALKER,* G. GUY,† G. BROWN,* M. ROWE‡ & A. RICKINSON† *Departments of *Immunology, †Biochemistry and ‡Cancer Studies, University of Birmingham, Birmingham*

Accepted for publication 14 March 1986

SUMMARY

Highly purified resting (G_0) B lymphocytes were monitored for their response to transforming Epstein–Barr virus (B95-8 strain), to a non-transforming mutant (P3HR-1) containing a deletion in the EBNA-2 coding region, and to inactivated virus of either type. All preparations induced an early appearance of two activation antigens, which included the CD23,p45 ('Blast-2') antigen. Thus, virus binding was sufficient for an initial activation step. Further change required an active viral genome. Infection with the P3HR-1 strain prompted the exit of cells out of G_0 but led to an arrest in the early G_1 phase of the cycle. While initially showing sequels to activation indistinguishable from those observed with P3HR-1 virus, cells infected with B95-8 virus continued through G_1 to express late activation antigens, enter S-phase and complete the replicative cycle. The addition of the phorbol ester TPA was found to compensate for the abortive cell cycle entry achieved with the P3HR-1 mutant, but could not supplement the minimal activation observed with inactivated virus. These findings demonstrate that the Epstein–Barr virus undermines three separate control points in the growth cycle of human B lymphocytes, and exploits three distinct viral signals to achieve this end.

INTRODUCTION

The Epstein–Barr virus (EBV) is one of the very few potentially oncogenic viruses documented for man. Its presence is intimately associated with two tumours endemic in geographically restricted areas—Burkitt lymphoma in equatorial Africa/New Guinea and nasopharyngeal carcinoma in Southern China (Ernberg & Kallin, 1984). Burkitt lymphoma represents a malignant proliferation of B lymphocytes that are known to bind the virus through their receptor for the C3d component of complement (CR2) (Fingeroth *et al.*, 1984). This receptor has recently been implicated in the control of B-cell replication (Melchers *et al.*, 1985). Infection of B lymphocytes with EBV *in vitro* leads to the outgrowth of autonomous cell lines expressing a transformed phenotype (Gordon *et al.*, 1984a; Nilsson & Klein, 1982). One of the elements essential for transformation appears to be EBNA-2, since the non-transforming P3HR-1 mutant of EBV contains a major deletion in the coding region for this protein (Hennessy & Kieff, 1985). By following the infection of B cells with both the transforming and non-transforming strains of virus and, in addition, with inactivated virus, we have shown that the interaction of EBV with its host cell is a multi-step process, with each stage dependent on distinct elements of the virus and its genome.

Abbreviations: EBNA, Epstein–Barr nuclear antigen; EBV, Epstein–Barr virus; FCS, fetal calf serum; TdR, thymidine; TPA, 12-0-tetradecanoyl-phorbol-13-acetate.

Correspondence: Dr J. Gordon, Dept. of Immunology, The Medical School, Vincent Drive, Edgbaston, Birmingham B15 2TJ, U.K.

MATERIALS AND METHODS

Preparation of resting B lymphocytes

B cells were isolated from tonsils obtained at routine tonsillectomy by negative selections of cells binding sheep erythrocytes as described previously (Gordon, Guy & Walker, 1985). Cells banding below a 62·5% Percoll (Pharmacia, Uppsala, Sweden) gradient constituted the resting populations used in this study. These preparations were in a G_0 stage of the cell cycle, were of high purity with regard to contaminating monocytes (<0·2%) and T cells (<0·2%), and were >98% surface immunoglobulin-positive.

Preparation of virus

Virus was obtained from mycoplasma-free B95-8 and P3HR-1 cell lines by the following procedure. Cells were grown from a starting concentration of 2×10^5/ml for 7–10 days at 37° in an air-tight container. The cells were then pelleted and the supernatant spun at 100,000 ***g*** for 1 hr at 4°. The pellet was then resuspended at 1/200 volume in RPMI-1640 containing 10% fetal calf serum (FCS) and stored at −70°. Where indicated, aliquots of virus were exposed to a dose of UV irradiation five times greater than was required to abolish all cord blood B-cell transforming activity in the B95-8 virus preparation. UV-inactivation of the EBNA-1- and latent membrane protein-inducing functions of the B95-8 and P3HR-1 virus preparations used in these experiments was checked in each case by exposing the EB virus-negative IARC-BL2 cell line (kindly provided by Dr G. Lenoir, IARC, Lyon, France) to the relevant preparations, assessing viral antigen expression by immunofluorescence after 48 and 72 hr.

Infection and culture of B cells

Freshly isolated resting B cells were cultured at 5×10^5/ml in the presence of virus at an equivalent strength of four-fold original concentration, which was found to be optimal for the subsequent activations noted. All B-cell cultures were performed in flat-bottomed wells in RPMI-1640 containing 10% FCS, 2 mM L-glutamine, penicillin/streptomycin 50 μg/ml and 5×10^{-5}M 2-mercaptoethanol.

Measures of B-cell activation

Surface antigens were detected in an indirect rosetting assay using sheep erythrocytes coated with sheep antibodies to mouse immunoglobulins as indicator cells and the following monoclonal antibodies as the initial layer: BK19.9 recognizing a ubiquitous proliferation antigen structurally similar to, but serologically distinct from, the transferrin receptor (Gatter *et al.*, 1983); MHM6 identifying the CD23,p45 (Blast-2) antigen (Rowe *et al.*, 1982; Thorley-Lawson *et al.*, 1985); A2 (a gift of Dr A. Bernard, Institut Gustave-Roussy, Villejuif) describing the transferrin receptor; 11EF7, an antibody developed by Dr N. Ling in the Dept. of Immunology, Birmingham, which defines a new B-cell restricted activation antigen, and B2 (a gift of Dr L. Nadler, Dana-Farber Institute, Boston, MA) recognizing the gp140 CR2 receptor, which shares identity with the receptor for EBV (Nadler *et al.*, 1981). Jo5 recognizing human DR class II polymorphic determinants was used in indirect immunofluorescence with FITC-labelled sheep anti-mouse immunoglobulins comprising the second layer. Antibodies were in the form of ascitic fluid and used at dilutions between 1/20 and 1/50. RNA and DNA synthesis were determined by pulsing 200 μl cultures with 50 μl of [^{3}H]uridine and [^{3}H]thymidine, respectively, at 0·01 mCi/ml. RNA and DNA content were assessed on a FACS IV (Becton-Dickinson, Mountain View, CA) by determining the fluorescence emission from triton-permeabilized cells stained with acridine orange according to the method of Darzynkiewicz *et al.* (1980). Intercalation of dye with DNA emits maximally at 530 nm (green fluorescence), whereas RNA-bound acridine orange emits in the red spectrum with a maximum at 640 nm. The amount of light deflected at 90° was simultaneously collected with 5×10^4 cells analysed for each run. In some experiments the extent of forward light scatter was determined for viable (i.e. unfixed) cells.

RESULTS

Tables 1 and 2 detail the early sequels to infecting G_0 B cells with the virus preparations used in this study. Following an 18 hr exposure to all preparations, a greatly reduced number of cells were positive for the B2 antibody that recognizes the CR2 receptor. This indicates that efficient binding and internalization of EB virions had occurred. A major consequence of virus binding was the induction of two activation antigens (Table 1).

Table 1. Effect of EBV infection on early marker changes

	% cells rosette-positive*				
	B2	MHM6		BK19.9	
	18 hr	6 hr	18 hr	6 hr	18 hr
Control	92	4	6	4	3
P_3HR-1	17	58	59	32	35
B95-8	20	54	73	48	52
P_3HR-1+UV	14	37	40	27	31
B95-8+UV	24	35	44	30	39

* Cells were cultured for indicated times with different preparations of virus, then tested for their ability to form rosettes with the antibodies shown (B2 recognizes the EBV receptor).

Table 2. Effect of EBV infection on changes in size and class II expression

	Mean channel no.*				
	Exp. 1			Exp. 2	
	Fwd. sctr.		DR	Fwd. sctr.	DR
	18 hr	44 hr	18 hr	18 hr	18 hr
Control	75	71	54	80	54
P_3HR-1	81	94	59	79	61
B95-8	79	100	59	82	66
P_3HR-1+UV	72	76	ND†	80	ND
B95-8+UV	75	77	ND	82	ND

* As for Table 1, but the forward scatter (Fwd. sctr.) and level of Class II (DR) antigen expression were determined by FACS analysis.

† ND, not determined.

One of these is the B-lineage restricted CD23,p45 ('Blast-2') antigen recognized in this study by MHM6. The other is a novel lineage-unrestricted 'proliferation' antigen described by the monoclonal antibody BK19.9. Both antigens appeared remarkably early, being first detected 3–4 hr post-infection and present on a large number of cells by 6 hr. It should be noted, however, that while virus binding was sufficient to induce these antigens, the level of expression, particularly at later times, was higher for cells that had been exposed to active transforming virus.

Early sequels that have been described for the activation of murine B lymphocytes include changes in size and increased expression of MHC class II antigens (Mond *et al.*, 1981; Rabin, O'Hara & Paul, 1985). We examined these parameters using flow cytometry, both to measure forward scatter as an indicator of size, and to determine the level of DR antigens following fluorescent labelling of cells in an indirect technique. The results obtained are summarized in Table 2, and it is apparent that while small changes were observed, increased class II expression was not a reliable early marker for the activation of human B

Table 3. Effect of EBV infection on late marker changes

	% cells rosette-positive*					
	Exp. 1				Exp. 2	
	A2		11EF7		A2	11EF7
	18 hr	44 hr	18 hr	44 hr	40 hr	40 hr
Control	1	2	0	0	3	0
P_3HR-1	3	2	0	1	4	2
B95-8	3	35	0	28	42	30
P_3HR-1+UV	ND†	1	ND	0	2	1
B95-8+UV	ND	2	ND	0	3	0

* As for Table 1 (A2 recognizes the transferrin receptor).

† ND, not determined.

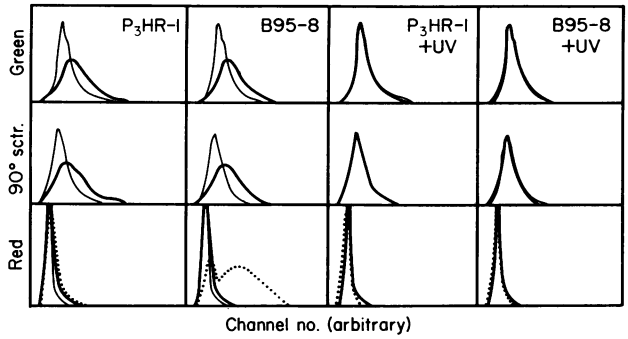

Figure 1. Flow cytometric analysis of B cells exposed to virus. Histograms constructed from the data of 5 × 10^4 cells infected with virus are shown with control values indicated (——). In addition to the changes occurring at 24 hr (——), the RNA content (red fluorescence 600–650 nm) at 68 hr is also shown (. . . .). Green fluorescence (515–575 nm) indicates emission from acridine orange bound to DNA.

cells exposed to EBV. Similarly, changes in cell size became significant only on the second day post-infection, and only with cells that had been infected with active forms of the virus (Table 2).

We next investigated the appearance of antigens associated with later stages of activation. The transferrin receptor (Tf-R) is required for the iron-dependent G_1 to S transition of haemopoietic cells (Larrick & Cresswell, 1979), while 11EF7 defines a new B-lineage restricted antigen that appears on activated cells with similar kinetics. The results in Table 3 show that only the full transforming strain of EBV was capable of inducing these two 'late' activation antigens on a significant number of cells. Neither simple virus binding nor the contribution of the P3HR-1 genome could drive G_0 B cells to a stage where the transferrin receptor or the 11EF7-defined antigen was expressed.

Recent studies have shown that the native DNA of high-density B lymphocytes has low-level accessibility for intercalating dyes, which is paralleled by the amount of light scattered at 90° (Walker *et al.*, 1986). We have found that activators capable of prompting B cells out of G_0 induce an increase in these parameters so that they achieve a level comensurate with cycling cells. These changes are independent of actual DNA synthesis and, as argued in the preceding paper (Walker *et al.*, 1986), appear to reflect the decondensation of gross chromatin structure prior to new gene transcription. In this study it was found that chromatin-related changes were induced by both the transforming and non-transforming active viruses but not by inactive virus (Fig. 1). Increased DNA stainability and 90° scatter in the absence of *de novo* DNA synthesis, first noted between 12 hr and 16 hr, was maximal by 24 hr. At the same time, a small but significant increase in RNA content was noted (Fig. 1), but again only in cells that had received an active virus. Whereas cells that had been infected with B95-8 transforming virus continued to increase their RNA content, those exposed to P3HR-1 maintained only a modest level above that of control cultures, indicating an arrest in the early G_1 phase of the cell cycle.

The above changes in RNA content were reflected by active RNA synthesis (Fig. 2), with P3HR-1 inducing a small but significant increase over the first 40 hr of infection while inactivated virus failed to provoke any measurable change. B95-

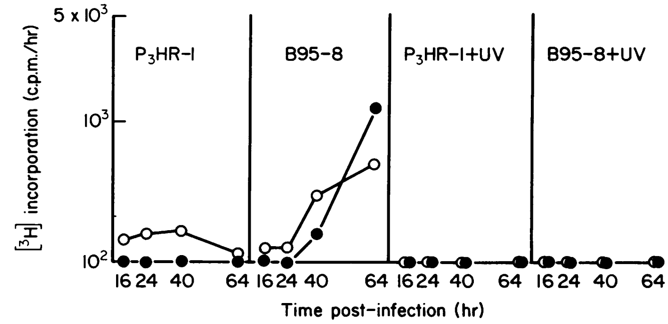

Figure 2. DNA and RNA synthesis in virus-infected B cells. The incorporation of [^{3}H]thymidine (●) and [^{3}H]uridine (○) is shown as change above controls at an hourly rate over the time-points indicated.

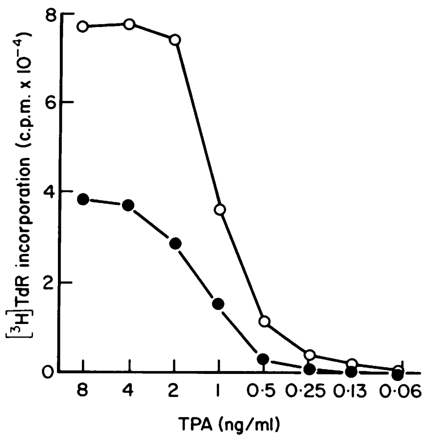

Figure 3. DNA synthesis in B cells exposed to P3HR-1 and TPA. B cells infected with P3HR-1 virus were cultured for 68 hr in the presence of TPA at indicated concentrations (○). Cultures were pulsed with 0·5 μCi of [^{3}H]TdR over the final 16 hr of culture and results represent means of triplicate determinations, which were always within 10% of each other. For comparison, cells infected with B95-8 virus incorporated 85,659 c.p.m. of [^{3}H]TdR over the same period. The data from uninfected cells cultured with TPA alone are also shown (●).

8-infected cells not only gave a continual increase in RNA synthesis over the whole 64 hr but, in addition, initiated DNA synthesis between 24 hr and 40 hr. No DNA synthesis above background levels was detected in cells exposed either to inactivated virus or to intact P3HR-1 virus over the whole period of observation.

Finally, we examined whether the early cell cycle entry observed with P3HR-1 virus could be completed by the addition of the phorbol ester TPA. We have recently shown that TPA through its ability to activate protein kinase-C can synergize with other signals to deliver a full growth message to G_0 B cells (Guy *et al.*, 1985). The results detailed in Fig. 3 indicate that TPA could indeed compensate, at least partially, for the defect present in the P3HR-1 virus for signalling the S-phase entry of resting B lymphocytes. When TPA was added to cells that had been exposed to inactivated virus, no augmentation of DNA synthesis was seen above that obtained with TPA alone (data not shown).

DISCUSSION

The unique host specificity of EBV for B lymphocytes is expressed partly at the level of virus binding. It has been shown that the structure exploited by the virus to obtain entry into B cells is the CR2 receptor that normally binds the C3d fragment of complement (Fingeroth *et al.*, 1984). The recent observation that C3d controls a growth restriction point for cycling murine B cells (Melchers *et al.*, 1985) opens the possibility that the interaction between CR2 and EBV contributes more to the transformation process than simple capturing and internalization of the virus. This was found to be the case in the present study as witnessed by the rapid appearance of two activation-related antigens at the surface of B cells that had bound EB virions. One of these antigens has recently been described as being the first lineage-specific marker to appear on the activation of human B cells, although the rapidity of its induction had not been fully appreciated (Thorley-Lawson & Mann, 1985). Our studies show that the appearance of the CD23,p45 antigen is independent of an intact viral genome. We have found that any minimal activator of B cells (e.g. anti-immunoglobulin, phorbol ester, calcium ionophores) is capable of triggering the appearance of the activation markers that were induced on virus binding (Walker *et al.*, 1986). In view of the minor changes observed in class II expression and cell size, our results suggest that the induction of CD23,p45 and the lineage-unrestricted antigen described by BK19.9 provides the most reliable marker for early B-cell activation in the human system that is currently available.

At the time of this study, a series of reports emerged to indicate a role for the CR2 receptor in the activation of human B cells. Several groups have now demonstrated that both monoclonal and polyclonal antibodies to CR2 will trigger T-cell dependent DNA synthesis in B cells (Wilson, Platt & Kay, 1985; Nemerow, McNaughton & Cooper, 1985; Frade *et al.*, 1985), while Aman *et al.* (manuscript submitted) have documented an early loss in surface IgD from cells exposed to inactivated virus. Our studies place some of the events that result from the triggering of CR2 as occurring within the G_0 compartment and prior to the entry of B cells into the growth cycle proper. Indeed, no increase in either RNA synthesis or content was observed for cells that had bound inactivated virus, even though efficient induction of early activation antigens had occurred.

An active viral genome was required for cells that had been primed through their receptor to enter the cell cycle. This step was heralded by changes consistent with a loosening of chromatin structure prior to increased RNA synthesis and content. The entire EBV genome has now been sequenced and several regions are known to be transcribed in latently infected cells (Baer *et al.*, 1984). Of the early viral gene products, the EBNA-2 protein cannot be responsible for bringing about this initial movement into cell cycle since the P3HR-1 mutant has a gene deletion that precludes expression of this protein (Hennessy & Kieff, 1985). Other viral gene products that may be responsible for this effect are the EBNA-1 protein, the latent membrane protein, or one of the other recently described but much less well-characterized nuclear antigens (Hennessy *et al.*, 1984; Hennessy, Fennewald and Kieff, 1985; Kallin *et al.*, 1986). Any one, or indeed any combination, of these proteins could bring about entry of infected cells into cycle.

Whatever coding region of the viral genome is responsible for the exit from G_0, infected cells become arrested in the early G_1 phase of the cycle in the absence of the EBNA-2 protein.

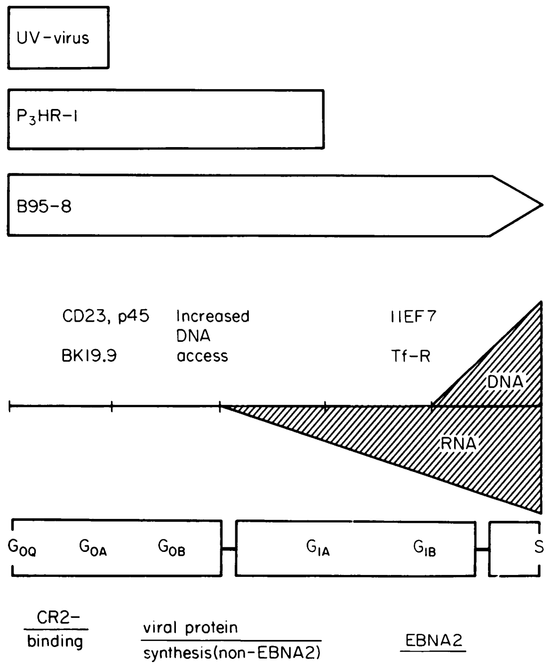

Figure 4. A model for the activation of G_0 B cells by EBV. The ability of inactive, deleted and transforming virus to drive G_0 B cells into cycle is shown with the associated phenotypes indicated. Note the heterogeneity of the G_0 stage, which is highlighted by the early induction of activation antigens (reached with all virus preparations) and an increased accessibility of the native DNA for intercalating acridine orange (not achieved with inactivated virus). These changes precede the entry of cells into cycle, which is defined by increased RNA content and accompanied by an increase in cell size. Only virus that contained an intact EBNA-2 coding region was capable of allowing B cells to complete the cycle as judged by the initiation of cellular DNA synthesis and eventual replication of transformed cells. The ability of B95-8 but not P3HR-1 virus to induce the expression of the 11EF7-defined antigen maps its appearance to the late G_1 stage of the cell cycle.

Thus, P3HR-1 virus-infected cells retained a low RNA content, did not express 'late' activation antigens and failed to synthesize DNA. The ability of cells that had been infected with B95-8 virus to continue through G_1 and enter the S-phase of the cycle maps this progression stage firmly to the EBNA-2 coding region. The ability of the phorbol ester TPA to fulfill a similar function raises the possibility that the EBNA-2 protein could contribute a C-kinase activity to the transformation process. It is interesting to speculate on the relationship between second messengers that are generated through an active viral genome, and the growth factors induced and required by EBV-transformed cells for their progression through the cell cycle (Gordon *et al.*, 1984a, b).

By preparing B lymphocytes in a resting state essentially free of contaminating monocytes and T cells, and by following the detailed sequels to their activation, we have been able to identify three distinct stages in the interaction of EBV with its host cell and, in addition, determine the contribution of separate components of the viral genome to these steps through the use of fully transforming, defective and inactive preparations of the virus. The scheme outlined in Fig. 4 summarizes these observations within the context of the B-cell activation cycle. The findings presented in this report not only provide valuable information on the action of EBV, but also reinforce a new model of human B-cell growth, which is highlighted by the recognition of phenotypic subcompartments and associated growth control points in what had been previously considered a homogenous G_0 compartment (Walker *et al.*, 1986). The model implies that multiple checks operate at a variety of levels in B-cell growth. It is an attractive notion that this reflects, at least in part, the establishment of safeguards to protect against uncontrolled proliferations. Our observation that a potentially oncogenic virus has exploited three separate constituents to usurp these controls supports this concept and offers new insight into the processes that can lead to malignant transformation.

ACKNOWLEDGMENTS

This work was supported by the Medical Research Council (U.K.). We wish to thank Ann Milner for her excellent assistance with running the FACS IV.

REFERENCES

Baer R., Bankier A.T., Biggin P.L., Deininger P.L., Farrell P.J. & Gibson T.J. (1984) DNA sequence and expression of the B95-8 Epstein–Barr virus genome. *Nature (Lond.)*, **310**, 207.

Darzynkiewicz Z., Sharpless T., Staiano-Coico L. & Melamed M.R. (1980) Subcompartments of the G1 phase of cell cycle detected by flow cytometry. *Proc. natl. Acad. Sci. U.S.A.* **77**, 6696.

Ernberg I. & Kallin B. (1984) Epstein–Barr and its association with human malignant diseases. *Can. Surveys*, **3**, 57.

Fingeroth J.D., Weis J.J., Tedder T.F., Strominger J.L., Biro P.A. & Fearon D.T. (1984) Epstein–Barr virus receptor of human B lymphocytes is the C3d receptor CR2. *Proc. natl. Acad. Sci. U.S.A.* **81**, 4510.

Frade R., Crevon M.C., Barel M., Vazquez A., Krikorian L., Charriaut C. & Galanaud P. (1985) Enhancement of human B cell proliferation by an antibody to the C3d receptor, the GP140 molecule. *Eur. J. Immunol.* **15**, 73.

Gatter K.C., Brown G., Trowbridge I.S., Woolston R.-E. & Mason D.Y. (1983) Transferrin receptors in human tissues: their distribution and possible clinical relevance. *J. clin. Pathol.* **36**, 539.

Gordon J., Guy G. & Walker L. (1985) Autocrine models of B-lymphocyte growth. I. Role of cell contact and soluble factors in T-independent B-cell responses. *Immunology*, **56**, 329.

Gordon J., Ley S.C., Melamed M., Aman P. & Hughes-Jones N.C. (1984a) Soluble factor requirements for the autostimulatory growth of B lymphocytes immortalized by the Epstein–Barr virus. *J. exp. Med.* **159**, 1554.

Gordon J., Ley S.C., Melamed M.D., English L.S. & Hughes-Jones N.C. (1984b) Immortalized B lymphocytes produce B-cell growth factor. *Nature (Lond.)*, **310**, 145.

Guy G.R., Bunce C.M., Gordon J., Michell R.H. & Brown G. (1985) A combination of calcium ionophore and TPA stimulates the growth of purified resting B cells. *Scand. J. Immunol.* **22**, 591.

Hennessy K., Fennewald S., Hummel M., Cole T. & Kieff E. (1984) A membrane protein encoded by Epstein–Barr virus in latent growth transforming infection. *Proc. natl. Acad. Sci. U.S.A.* **81**, 7207.

Hennessy K., Fennewald S. & Kieff E. (1985) A third viral nuclear protein in lymphoblasts immortalized by Epstein–Barr virus. *Proc. natl. Acad. Sci. U.S.A.* **82**, 5944.

Hennessy K. & Kieff E. (1985) A second nuclear protein is encoded by Epstein–Barr virus in latent infection. *Science*, **227**, 1238.

Kallin B., Dillner J., Ernberg I., Ehlin-Henriksson B., Rosen A., Henle W., Henle G. & Klein G. (1986) Four virally determined nuclear antigens are expressed in Epstein–Barr virus-transformed cells. *Proc. natl. Acad. Sci. U.S.A.* **83**, 1499.

Larrick J.W. & Cresswell P. (1979) Modulation of cell surface iron transferrin receptors by cellular density and state of activation. *J. Supramol. Struct.* **11**, 579.

Melchers F., Erdei A., Schulz T. & Dierich M.P. (1985) Growth control of activated, synchronized murine B cells by the C3d fragment of human complement. *Nature (Lond.)*, **317**, 264.

Mond J.J., Seghal E., Kung J. & Finkleman F.D. (1981) Increased expression of I-region associated antigen (Ia) on B cells after cross-linking of surface immunoglobulin. *J. Immunol.* **127**, 881.

Nadler L.M., Stashenko P., Hardy R., van Agthovan A., Terhorst C. & Schlossman S.F. (1981) Characterization of a human B-cell specific antigen (B2) distinct from B1. *J. Immunol.* **126**, 1941.

Nemerow G.R., McNaughton M.E. & Cooper N.R. (1985) Binding of monoclonal to Epstein–Barr virus (EBV)/CR2 receptor induces activation and differentiation of human B lymphocytes. *J. Immunol.* **135**, 3068.

Nilsson K. & Klein G. (1982) Phenotypic and cytogenetic characteristics of human B-lymphoid cell lines and their relevance for the etiology of Burkitt lymphoma. *Adv. Cancer Res.* **37**, 319.

Rabin E.M., O'Hara J. & Paul W.E. (1985) B-cell stimulatory factor 1 activates resting B cells. *Proc. natl. Acad. Sci. U.S.A.* **82**, 2935.

Rowe M., Hildreth J.E.K., Rickinson A.B. & Epstein M.A. (1982) Monoclonal antibodies to Epstein–Barr virus-induced, transformation-associated cell surface antigens: binding patterns and effect upon virus-specific T-cell cytotoxicity. *Int. J. Cancer*, **29**, 373.

Thorley-Lawson D.A. & Mann K.P. (1985) Early events in Epstein–Barr virus infection provide a model for B cell activation. *J. exp. Med.* **162**, 1985.

Thorley-Lawson D.A., Nadler L.M., Bhan A.K. & Schooley R.T. (1985) Blast-2 (EBVCS), an early cell surface marker of human B-cell activation, is superinduced by Epstein–Barr virus. *J. Immunol.* **134**, 3007.

Wilson B., Platt J.L. & Kay N.E. (1985) Monoclonal antibodies to the 140,000 mol. wt glycoprotein of B lymphocyte membranes (CR2 receptor) initiates proliferation of B cell *in vitro*. *Blood*, **66**, 824.

Walker L., Guy G., Brown G., Rowe M., Milner A.E. & Gordon J. (1986) Control of human B-lymphocyte replication. I. Characterization of novel activation states that precede the entry of G_0 B cells into cycle. *Immunology*, **58**, 583.