Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 10411–10416, September 1996
Medical Sciences

c-*myc* activation renders proliferation of Epstein–Barr virus (EBV)-transformed cells independent of EBV nuclear antigen 2 and latent membrane protein 1

[phenotype of Burkitt lymphoma/t(2;8) chromosomal translocation/c-*myc* function/TP1 promoter]

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Communicated by George Klein, Karolinska Institute, Stockholm, Sweden, June 21, 1996 (received for review March 28, 1996)

ABSTRACT **Two genetic events contribute to the development of endemic Burkitt lymphoma (BL) infection of B lymphocytes with Epstein–Barr virus (EBV) and the activation of the protooncogene c-*myc* through chromosomal translocation. The viral genes EBV nuclear antigen 2 (EBNA2) and latent membrane protein 1 (LMP1) are essential for transformation of primary human B cells by EBV *in vitro*; however, these genes are not expressed in BL cells *in vivo*. To address the question whether c-*myc* activation might abrogate the requirement of the EBNA2 and LMP1 function, we have introduced an activated c-*myc* gene into an EBV-transformed cell line in which EBNA2 was rendered estrogen-dependent through fusion with the hormone binding domain of the estrogen receptor. The c-*myc* gene was placed under the control of regulatory elements of the immunoglobulin κ locus composed of a matrix attachment region, the intron enhancer, and the 3′ enhancer. We show here that transfection of a c-*myc* expression plasmid followed by selection for high MYC expression is capable of inducing continuous proliferation of these cells in the absence of functional EBNA2 and LMP1. c-*myc*-induced hormone-independent proliferation was associated with a dramatic change in the growth behavior as well as cell surface marker expression of these cells. The typical lymphoblastoid morphology and phenotype of EBV-transformed cells completely changed into that of BL cells *in vivo*. We conclude that the phenotype of BL cells reflects the expression pattern of viral and cellular genes rather than its germinal center origin.**

Epstein–Barr virus (EBV) is involved in the development of human neoplasias like Burkitt lymphoma (BL), nasopharyngeal carcinoma, Hodgkin disease, and lymphomas occurring in immunodeficient individuals (1). Except for the last entity, these tumors are all of a monoclonal origin, indicating that apart from EBV other genetic events are involved. EBV transforms primary human B cells very efficiently, leading to rapidly growing lymphoblastoid cell lines (LCLs). At least four EBV nuclear antigens (EBNA1, EBNA2, EBNA3A, and EBNA3C) and one latent membrane protein antigen (LMP1) are required for the initiation of transformation (2–6). EBNA2 plays a crucial role for initiation as well as maintenance of transformation (7). Together with EBNA–LP, EBNA2 is the first viral gene expressed in primary B cells infected with EBV (8, 9). It acts as a transcriptional regulator and governs the expression of viral latent membrane and nuclear antigens (except EBNA1) as well as the expression of cellular genes involved in the control of cell proliferation (10–17). In BL cells *in vivo*, viral gene expression is restricted to the EBV-encoded, small nonpolyadenylylated RNAs (EBER I and II) and EBNA1, which is involved in maintaining the episomal state of the viral genome (18). Upon cultivation *in vitro*, BL cells tend to change their phenotype, the so-called group I phenotype, toward that of EBV-transformed lymphoblastoid cells (group III phenotype) (19) concomitantly with an up-regulation of the EBV latent gene expression program, i.e., EBNA-LP, EBNA-2-3A, -3B, -3C, LMP1, and LMP2. There is no evidence that EBNA1 is involved in growth control; therefore, proliferation of BL cells *in vivo* must be driven by other than viral genes. One candidate for this is the protooncogene c-*myc*. BL cells are invariably characterized by chromosomal translocations involving the c-*myc* locus on chromosome 8 and one of the immunoglobulin (Ig) loci on chromosome 2, 14, or 22 (20). The juxtaposition of regulatory elements of the different Ig loci into the vicinity of c-*myc* leads to constitutive high expression of this oncogene. For the Igκ locus, we were able to define the regulatory elements required for the BL specific c-*myc* activation (21–24). The concerted action of two enhancers identified 5′ and 3′ of the constant region of the Igκ gene locus together with the Igκ matrix attachment region is necessary to confer all features of c-*myc* activation in BL cells, i.e., (*i*) strong transcriptional activation of the gene, (*ii*) preferential usage of promoter P1, (*iii*) loss of regulation at the level of transcript elongation, and (*iv*) sensitivity to the action of sodium butyrate (21, 25).

Another important difference between EBV-transformed lymphoblastoid cells and BL cells concerns the morphology, growth behavior, and the phenotype of the cells. Lymphoblastoid cells are relatively large and irregularly shaped and grow in large clumps. BL cells, on the other hand, are uniformly small and round cells growing in single cell suspension. The different morphology and growth behavior are reflected in a substantial difference in cell surface antigen expression. Freshly isolated BL cells of group I phenotype express the differentiation markers CD10, CD38, and CD77 but are negative or almost completely negative for the expression of activation markers like CD21, CD23, and CD39 or adhesion molecules like CD44, CD54, and CD58. LCLs or BL group III

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Abbreviations: BL, Burkitt lymphoma; EBV, Epstein–Barr virus; EBNA2, EBV nuclear antigen 2; LMP1, latent membrane protein 1; LCL, lymphoblastoid cell lines.

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cells strongly express the activation markers and adhesion molecules but have switched off CD10, CD38, and CD77 (19).

In this study, we have asked whether c-*myc* activation can substitute for EBNA2 and the downstream target genes of EBNA2, including LMP1 in growth control. To address this question, we have introduced an activated c-*myc* gene into an LCL conditional for EBNA2. By complementing the EBNA2 defect of the transformation-deficient P3HR1 virus with an EBNA2-estrogen receptor fusion gene, a lymphoblastoid cell line was established that can proliferate only in the presence of estrogen. We show here that expression of an activated c-*myc* gene renders cell proliferation independent of EBNA2 and LMP1. These cells grow in single cell suspension and have adopted the group I phenotype of BL cells *in vivo*.

MATERIALS AND METHODS

Cell Lines and Cell Culture. All cells were grown in RPMI 1640 cell culture medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. ER/EB2-5 is a conditionally transformed lymphoblastoid cell line expressing a conditional mutant of EBNA2 (ER-EBNA2) that requires 1 μM β-estradiol for activation of the EBNA2 function (7). Photographs of the cells (Nikon model AFX-IIA) were digitized with a charge-coupled device camera (Cybertech, Berlin) and further processed with Adobe Systems (Mountain View, CA) PHOTOSHOP.

DNA Constructs, Transient and Stable Transfections, Reporter Gene Assay, and RNA Analysis. The c-*myc* expression construct pKH348 (see Fig. 1) is based on pKH199-7, which was described previously (23). It differs only in that the described tag in exon 2 is absent in pKH348, therefore allowing expression of MYC protein. Stable transfection and selection of hygromycin-resistant cells were done as described (21). Standard procedures were followed for preparation of RNA and Northern blotting (26). For the detection of LMP1, a probe spanning position 167,131 to 169,568 of the standard EBV genome was used. c-*myc* specific transcripts were detected with a probe representing the *Cla*I–*Eco*RI fragment of the germ line c-*myc* gene corresponding to exon 3 (22). Northern blots were quantified with a Fuji BAS1000 radioactivity scanner. Standard procedures were followed for transient expression of the TP1-luciferase (27) construct, the preparation of cellular extracts, and the measurement of luciferase activity (23).

Western Blot Analysis. Cellular extracts for Western blot analysis were prepared in lysis buffer [25 mM Tris·HCl, pH 7.5/25 mM EDTA/5% glycerin/1% (vol/vol) 2-mercaptoethanol/1% SDS/0.1% bromphenol blue] and sonicated. Proteins were separated on SDS/PAGE and blotted onto nitrocellulose membrane. Equal loading of the gels was verified by Ponceau red staining. Expression of protein was analyzed using the following antibodies: monoclonal antibodies specific for EBNA1 [EBNA1-1H4 (28)], EBNA2 [EBNA2-R3 (29)], LMP1 (CS1-4, Dako), and BCL-2 (124, Dako), and a polyclonal antibody specific for MYC (OPA 02/1, Medac, Hamburg, Germany). Immunoreactive proteins were detected by peroxidase-coupled secondary antibodies and Enhanced Chemiluminiscence (ECL, Amersham) as described (7).

Flow Cytofluorometry. The following monoclonal antibodies were used for cell surface phenotyping: anti-CD10 (ALB1; Immunotech, Luminy, France), anti-CD19 (BC3; Immunotech), anti-CD21 (BL13; Immunotech), anti-CD23 (9P25; Immunotech), anti-CD38 (T16, Immunotech), anti-CD39 (AC2; Immunotech), anti-CD40 (5C3, PharMingen), and anti-CD58 (AICD58-1; Immunotech). Cell-surface antigen expression was assessed by indirect immunofluorescence staining with a fluorescein isothiocyanate (FITC)-conjugated polyclonal goat anti-mouse antibody (Dako). ER/EB2-5 cells and the transfectants were analyzed for surface marker expression using a FACScan flow cytometer and the PC-LYSIS analysis program (Becton Dickinson).

RESULTS

Proliferation of ER/EB2-5 Cells Becomes Independent of Hormone in Cells Highly Expressing c-*myc*. Three elements in the Igκ locus drive c-*myc* expression in BL cells, which carry a chromosomal t(2;8) translocation: (*i*) the intron enhancer, (*ii*) the 3′ enhancer situated 12 kb downstream of the κ constant region, and (*iii*) a matrix attachment region located between the κ joining and the intron enhancer (21, 23). An episomal vector carrying these three elements downstream of c-*myc* (construct pKH348; Fig. 1) introduced into Raji cells can confer all features of c-*myc* deregulation observed in BL cells (23). The construct pKH348 was introduced into ER/EB2-5 cells by electroporation. Hygromycin-resistant cell clones were selected in cell culture medium supplemented with 1 μM β-estradiol and 12 hygromycin-resistant cell clones were obtained. An initial estrogen withdrawal experiment was performed immediately after hygromycin-resistant clones were established. All 12 clones were still dependent on hormone for proliferation. After 12 weeks of continuous culture in the presence of estrogen, hormone was again withdrawn. At this time, two of the clones continued to proliferate without lag phase and without any evidence of cell death after hormone deprivation.

Northern and Western blot analyses were performed to compare c-*myc* RNA and protein expression levels in cells sensitive or resistant to estrogen deprivation. The level of c-*myc* RNA expression was an order of magnitude higher in the two hormone-independent cell clones, A1 and B4, as compared with parental

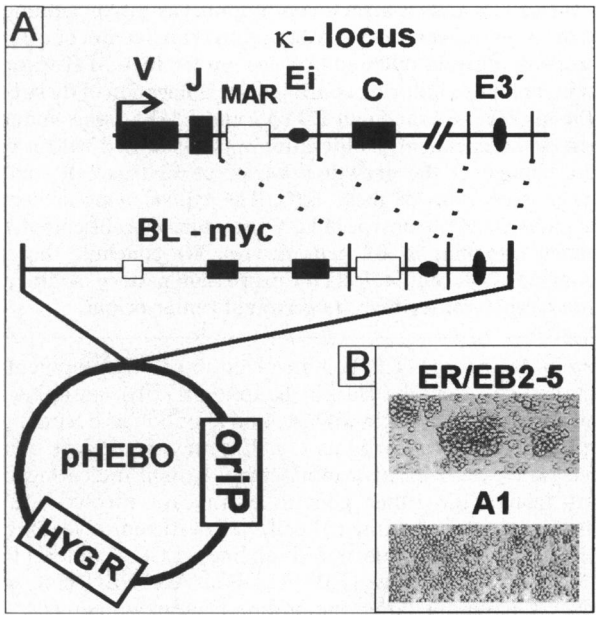

FIG. 1. An activated c-*myc* gene renders proliferation of conditionally EBV-transformed cells estrogen-independent and alters their growth behavior. (*A*) c-*myc* overexpression construct pKH348. The self-replicating vector pHEBO (30) was used to express c-*myc* under the control of three different regulatory elements derived from the Igκ locus: (*i*) matrix attachment region, (*ii*) intron enhancer (Ei), and (*iii*) 3′ enhancer (E3′). (*Upper*) A schematic representation of the Igκ locus. V, variable region; J, joining region; C, constant region of the Igκ gene. Details of the construction are given in ref. 23. pKH348 is based on pKH199-7 (9), differing only in that the described tag in exon 2 is absent in pKH348. (*B*) Morphology of parental ER/EB2-5 cells grown in the presence of estrogen (*Upper*) and the pKH348-transfected cell line A1 grown in the absence of hormone (*Lower*). ER/EB2-5 cells grow in large clumps, are large, and have an irregular shape, whereas A1 cells are round and small and grow in single cell suspension.

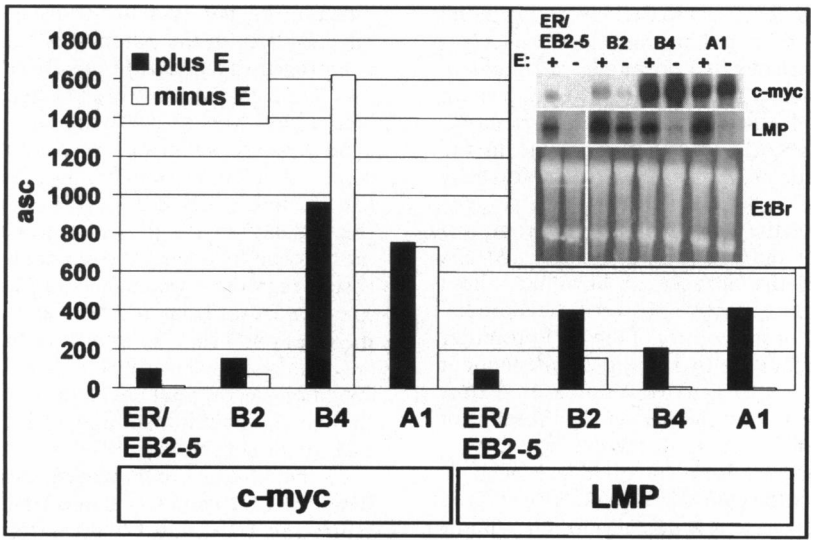

FIG. 2. c-*myc*-transfected hormone-independent cells express high c-*myc* RNA levels. Northern blot analysis (26) (*Inset*) of c-*myc* and LMP1 expression in ER/EB2-5 cells and cell lines A1, B2, and B4 obtained by stable transfection of ER/EB2-5 with pKH348. The cells were grown in the presence or absence of estrogen (E: +/−). B2 cells are still dependent on estrogen, whereas B4 and A1 proliferate also in the absence of estrogen. The ethidium bromide stain of the RNA gel is shown underneath. The blot was hybridized with an LMP1 and, after stripping, with a c-*myc* specific probe. Northern blots were quantified with a Fuji BAS1000 radioactivity scanner. asc, Arbitrary scanning units.

ER/EB2-5 cells (Fig. 2). A cell clone that had been transfected with pKH348 and selected for hygromycin resistance but that failed to survive upon withdrawal of estrogen (B2) exhibited an about 5-fold lower c-*myc* RNA expression level than the hormone-independent clones A1 and B4. Southern blot analysis revealed about 50 copies of pKH348 plasmid in A1 and B4 cells, and about 5 copies in cell clone B2 (data not shown).

These results suggested that estrogen-independent growth was correlated with a selection for fairly high c-*myc* expression levels. To verify this point, the c-*myc* transfection experiment was repeated and another 11 hygromycin-resistant cell clones were established. After a variable lag phase of 2 to 6 weeks, seven of these clones continued to proliferate also in the absence of hormone. Protein extracts were prepared from the hormone-dependent and -independent clones and analyzed by Western blot for the expression of MYC protein (Fig. 3*A*). In all cell clones growing in the absence of estrogen, the expression of c-*myc* had increased dramatically, indicating that an expression of c-*myc* at a high level is a prerequisite for EBNA2-independent growth.

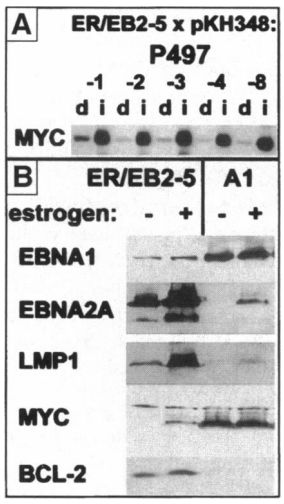

FIG. 3. Expression of MYC, BCL-2, and viral antigens in c-*myc*-transfected hormone-independent cell lines. (*A*) Myc expression is high in hormone-independent and low in hormone-dependent c-*myc*-transfected ER/EB2-5 cells. Extracts for Western blots were prepared of five pKH348-transfected hygromycin-resistant cell lines (P497-1 to P497-4 and 497-8) before (d) and after acquisition of hormone-independent (i) growth. (*B*) ER-EBNA2 and LMP1 expression is regulated by estrogen in c-*myc*-transfected hormone-independent A1 cells. Western blot analysis with antibodies directed against the indicated proteins is shown. A1 cells were continuously grown in the absence of hormone (A1). The cells were washed and reseeded in fresh cell culture medium without or with hormone (− or +) 4 days before the preparation of protein extracts. ER/EB2-5 cells treated in the same way served as controls.

EBNA2 Function and LMP1 Expression Are Regulated by Estrogen in Cell Clones Growing Independently of Hormone. It was important to exclude the possibility that hormone-independent proliferation was a consequence of a mutation in the chimeric EBNA2–estrogen receptor fusion protein, which could have rendered EBNA2 function hormone-independent. To rule this out, LMP1 expression was studied by Northern blot and Western blot analyses in cell clones A1 and B4 that had been grown in the presence or absence of estrogen. LMP1 is not only a direct target gene of EBNA2, it is also a transforming oncogene that by itself may perhaps be capable of inducing continuous proliferation if constitutively expressed (31). As shown by Northern blot (Fig. 2) and Western blot analyses (Fig. 3 for A1 cells), LMP1 expression was clearly regulatable by hormone in A1 and B4 cells. LMP1 expression was reduced to undetectable levels in the absence of hormone and was reinduced by the readdition of hormone, although to a lesser extent as compared with the parental ER/EB2-5 cells. Expression of the chimeric EBNA2 protein was similarly

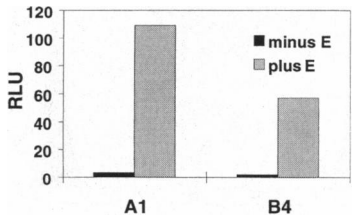

FIG. 4. Transactivation of the EBV-TP1 promoter by EBNA2 is dependent on estrogen in c-*myc*-transfected ER/EB2-5 cells. c-*myc*-transfected hormone-independent A1 and B4 cells were transfected with the (−804) TP1 promoter luciferase construct (29) and luciferase activity was measured in extracts of cells maintained in the absence (minus E) or presence of estrogen (plus E).

regulated by estrogen. Its level decreased after hormone deprivation concomitantly with an increase in the electrophoretic mobility and increased when hormone was readded. The change in the abundance of the chimeric EBNA2 protein reflects the autoregulatory feedback loop of the Cp promoter positively regulated by EBNA2 (15–17), the change in the electrophoretic mobility posttranscriptional regulation by phosphorylation (32).

EBNA2 strongly regulates the activity of the two promoters of the terminal protein, TP1 and TP2 (also designated LMP2A and LMP2B) (14, 29). In the absence of hormone, these promoters should therefore be inactive. To verify this prediction, the luciferase gene under the control of the TP1 promoter was transiently transfected into the two estrogen-independent cell lines A1 and B4 and luciferase activity was measured after keeping the cells for 48 hr either in the presence or absence of estrogen. In the absence of hormone, luciferase activity was almost undetectable but high in hormone-treated cells (Fig. 4). This supports the notion that the chimeric EBNA2 is inactive in c-*myc* transfectants growing in the absence of hormone and its function is still regulatable by addition and withdrawal of estrogen. We conclude that hormone-independent growth is not a consequence of either constitutive EBNA2 or LMP1 expression.

EBNA1 expression was not affected by withdrawal or readdition of hormone. Remarkably, EBNA1 expression was significantly higher in c-*myc* transfected hormone-independent A1 cells than in the parental ER/EB2-5 cells.

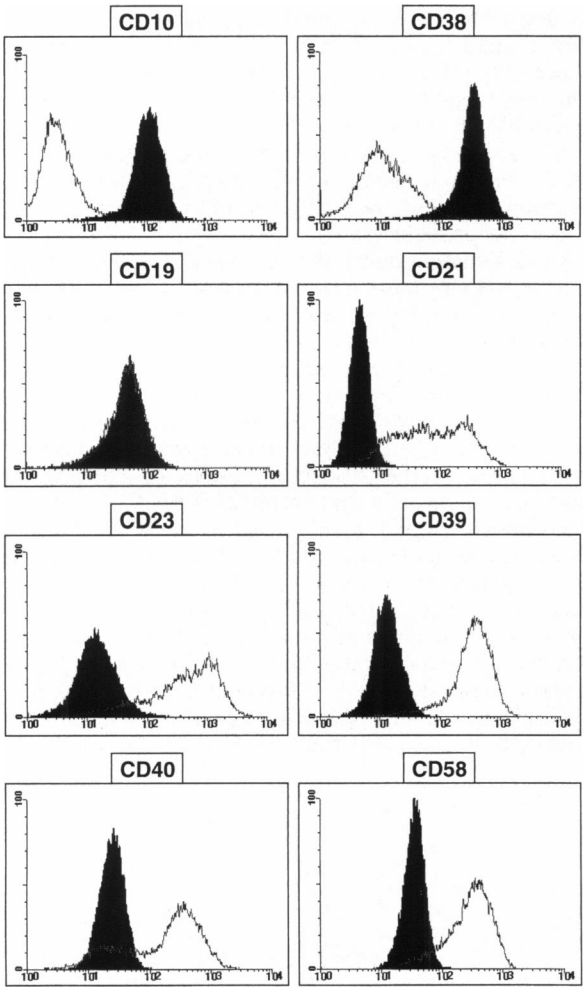

FIG. 5. CD10 and CD38 expression is up-regulated and expression of adhesion molecules and activation markers is down-regulated in c-*myc*-transfected hormone-independent A1 cells. FACScan analysis of A1 cells grown in the absence of hormone (solid profiles) and ER/EB2-5 cells grown in the presence of hormone (white profiles) with monoclonal antibodies specific for the indicated surface antigens.

Estrogen-Independent Cells Do Not Express BCL-2. In LCLs, the BCL-2 gene is actively expressed. BCL-2 was reported to be induced by LMP1 (33). Whether up-regulation of BCL-2 reflects induction or selection for expression of BCL-2-positive cells in the course of cell transformation by EBV is still a matter of debate (34, 35). In ER/EB2-5 cells, BCL-2 protein can easily be detected (Fig. 3) and remains present even upon removal of estrogen. In the estrogen-independent c-*myc* transfectants, BCL-2 protein was absent regardless whether estrogen was added or withdrawn. Therefore, expression of LMP1 and BCL-2 does not correlate in these cells and survival as well as proliferation have apparently become independent of BCL-2. At least under normal growth conditions (10% fetal calf serum), the A1 and B4 cells do not show an elevated level of apoptosis compared with the parental LCL (data not shown).

In the Absence of Estrogen, c-*myc*-Transfected Cells Up-Regulate CD10 and CD38 and Down-Regulate Adhesion Molecules and Activation Markers. High c-*myc* expression induced not only hormone-independent growth but also a dramatic shift in the growth behavior. The cells no longer grew in large clumps that is a characteristic of EBV-transformed cells, but rather began to grow in a single cell suspension reminiscent of BL cells with group I phenotype (Fig. 1) (19). This change was associated with a change in cell surface marker expression (Fig. 5). Activation markers such as CD21, CD23, and CD39 and adhesion molecules such as CD54 (data not shown) and CD58 were down-regulated, whereas CD10 and CD38, not expressed on parental ER/EB2-5 cells, became readily detectable. Expression of the B cell-specific marker molecule CD19 was not affected by the expression of c-*myc*. Addition of hormone to A1 cells increased CD21, CD23, CD39, and CD58 expression and decreased the level of CD10 and CD38, again indicating that the signal transduction cascade initiated by EBNA2 is intact (data not shown). Upon addition of hormone to A1 cells, however, the level of expression of these surface markers did not return to the expression level of ER/EB 2–5 cells, consistent with the observation that the expression levels of ER/EBNA2 and LMP1 are reduced in estrogen-treated A1 and B4 as compared with parental ER/EB 2–5 cells (Fig. 3). Similar observations were made when an activated c-*myc* gene was introduced into nonconditionally transformed LCLs resulting in an intermediate phenotype with down-regulation of adhesion molecules (36–38).

The correlation between c-*myc* expression and phenotypic shift was confirmed by studying the cell surface phenotype of the same series of c-*myc* transfectants already shown in Fig. 3*A* during establishment of hygromycin-resistance and hormone-independent growth (Fig. 6). In hygromycin-resistant hormone-dependent clones, the cell surface expression was still high for

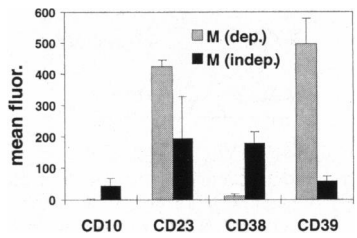

FIG. 6. Hormone-independent growth of c-*myc*-transfected cells is associated with the up-regulation of CD10 and CD38 and down-regulation of CD23 and CD39. FACScan analysis [compilation of mean (M) fluorescence values plus standard deviation] of five different pKH348-transfected hygromycin-resistant cell lines (P497-1 to P497-4 and 497-8) before (shaded bars) and after (solid bars) acquisition of hormone-independent growth. The cell lines are identical to those shown in Fig. 3*A*.

CD23 and CD39 and low for CD10 and CD38, whereas it shifted to the BL group I expression pattern concomitantly with the rise in c-*myc* expression and acquisition of hormone-independent growth.

DISCUSSION

Endemic BL is characterized by two features: (*i*) the presence of the EBV genome in the tumor cells and (*ii*) chromosomal translocations activating the c-*myc* gene. EBV can cause unlimited proliferation of primary human B cells. EBNA2 is a pleiotropic activator of viral and cellular genes involved in growth control and plays a decisive role in the induction of cell proliferation by EBV. EBNA2 induces c-*myc* expression at a moderate level as revealed by the conditional EBNA2 system (7). Overexpression of c-*myc* to high levels allowed these cells to proliferate continuously in the absence of estrogen. A highly active c-*myc* gene is thus able to substitute for EBNA2 and EBNA2-regulated genes such as LMP1 and to impose by itself a proliferation program onto these conditionally transformed cells. This is particularly important in light of the fact that EBV-positive BL cells *in vivo* only express EBNA1 but not the set of viral nuclear and membrane antigens required for driving proliferation of normal B cells. It cannot be excluded that EBNA1 expression in BL cells *in vivo* as well as the elevated EBNA1 expression in A1 cells are also involved in proliferation control. Remarkably, EBNA1 appears to be nonimmunogenic (39), whereas a strong cytotoxic T cell response is mounted against all other viral antigens expressed in transformed cells. Therefore, switching-off the viral gene expression program of transformed cells appears to be a prerequisite for the development of BL. Furthermore, our phenotypic data suggest that high c-*myc* expression not only confers a direct stimulus to proliferation but presumably also severely impairs immune recognition of BL cells by affecting the expression of molecules involved in T cell stimulation as well as cytotoxic T cell recognition. High c-*myc* expression is thus associated with down-regulation of the HLA class I and II molecules (40) and of activation markers and adhesion molecules.

An important implication of this finding is that the cell surface phenotype of a tumor reflects the activity of transcription factors that drive cell surface antigen expression rather than the origin from which a given tumor is derived. The phenotype of BL cells therefore does not imply that the tumor is derived from germinal center B cells. A cell with a c-*myc* translocation will rather adopt a phenotype similar to that of germinal center cells. The phenotype of BL cells does, however, not rule out the possibility that BL cells are derived from germinal center cells. In fact, the acquisition of hypermutations in the Ig genes is a valid argument in favor of a germinal center or postgerminal center origin of BL (41).

The phenotypic change observed in c-*myc* transfected conditionally EBV-transformed lymphocytes may provide an ideal system to define c-*myc* target genes and to dissect at a molecular level the mechanism by which the MYC protein alters the expression of cell surface antigens (36–38). It should be noted, however, that part of the effects that appear to be the result of c-*myc* overexpression may rather be the consequence of EBNA2 and LMP1 down-regulation.

Based on the experiments described here, we propose the following model for the pathogenesis of BL. EBV infects primary B cells and confers growth and survival advantages to the infected cells until an effective T cell response is raised. As an accident of normal B cell development or facilitated or provoked by EBV, chromosomal translocations involving the Ig loci will take place. An involvement of EBV in this process is suggested by the recent observation of Srinivas and Sixbey (42) that the recombinase activating genes RAG1 and RAG2 are induced by EBV. Only chromosomal translocations giving rise to high levels of c-*myc* expression will allow for selection of cells that have shut off the viral antigens expressed in transformed cells. Switching off the expression program of transformed cells is associated with a switch in the promoter usage from the C/W-promoter to a promoter located in the *Bam*HI–Q fragment (43, 44). Usage of this Q-promoter gives rise to a message that only encodes EBNA1. The transcription factors governing the activity of this Q-promoter are still unknown. MYC/MAX binding sites (CANGTG) (45) are located at positions −441, −958, and −1128 5′ of the Q-promoter transcription initiation site, suggesting that MYC might be directly involved in the promoter switch from C/W- to the Q-promoter. The observed increase of EBNA1 expression in A1 cells could therefore be a result of high c-*myc* expression. Experiments are in progress to test this hypothesis. On the other hand, the promoter switch may as well occur independently of c-*myc* as part of the viral strategy for latent persistence. Cells harboring the viral genome in the peripheral blood of normal healthy individuals were thus shown to express EBNA1- but not EBNA2- or LMP1-RNA (46, 47). Switching-off the viral expression program of transformed cells may therefore occur stochastically or may be induced by factors provided by the microenvironment. This form of latency guarantees the survival of virus-infected cells *in vivo* in face of a fully competent immune system.

The system described here is apparently not restricted to the study of MYC function and the pathogenesis of BL. A variety of additional chromosomal translocations have been described in B cell leukemias and lymphomas in which oncogenes other than c-*myc* are either transcriptionally activated or involved in the formation of fusion proteins (48). In cases in which two reciprocal fusion proteins are generated, the molecular analysis of the fusion products may not elucidate which of the two is the actual oncogene or whether both fusion proteins must cooperate to exert their oncogenic effect. The system described here allows one to screen for the most important phenotypes relevant for leukemogenesis and lymphomagenesis, i.e., survival and the induction of proliferation. Attempts are in progress to include other oncogenes involved in B cell leukemogenesis such as bcr-abl, E2A-PBX, and the MLL gene into the system described here (49–52).

We thank C. Rottenberger for excellent technical help and C. Hall for critically reading the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (PO325/1), the Deutsche Krebshilfe, and the Fonds der Chemischen Industrie.

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