Expression of Class I Major Histocompatibility Complex Antigens in Epstein-Barr Virus-carrying Lymphoblastoid Cell Lines and Burkitt Lymphoma Cells

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Expression of Class I Major Histocompatibility Complex Antigens in Epstein-Barr Virus-carrying Lymphoblastoid Cell Lines and Burkitt Lymphoma Cells[1]

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ABSTRACT

Epstein-Barr virus (EBV) carrying lymphoblastoid cell lines (LCLs) and EBV-positive Burkitt lymphoma (BL) cells were compared for their expression of class I antigens of the major histocompatibility complex. Five common BL lines, LCLs, pokeweed mitogen-stimulated blasts and resting B-cells from healthy donors, and eight pairs of BL cells and LCLs, each pair originating from one patient, were tested. Quantitative analysis was performed using a radioimmunoassay; qualitative aspects were studied by one- and two-dimensional gel electrophoresis. In general, LCLs expressed significantly higher amounts of class I antigens than BL cells, the latter showing class I densities similar to or lower than peripheral resting B-cells. From analysis of the expression of class I-specific RNA, there is some evidence that class I antigen expression is regulated on the transcriptional level. In two BL cells studied, class I expression could be enhanced by γ-interferon, whereas the corresponding LCLs seemed to be refractory to this treatment. One- and two-dimensional gel electrophoresis showed that in some BL lines, in addition to the generally lower class I expression, distinct class I specificities were down-regulated. None of these alterations in class I expression was EBV specific; however, they may well play a role in the recognition of BL cells and LCLs by cellular immune mechanisms. Thus, down-regulation of class I antigens may contribute to the resistance of BL cells to cytotoxic T-lymphocytes, whereas their enhanced expression may improve the recognition of EBV-infected LCLs.

INTRODUCTION

From EBV[3] infected B-lymphocytes LCLs with indefinite growth potential can be established *in vitro*. In the healthy EBV-positive individual, however, these cells are efficiently controlled by specific CTL restricted by class I antigens of the MHC (1). EBV-carrying BL cells, on the other hand, can escape this immune surveillance, although BL patients possess CTL reacting against their normal EBV-infected B-lymphocytes (2); *in vitro* experiments showed that most BL cells are indeed resistant to HLA-matched EBV-specific CTL (3). This raised the question as to how CTL recognize EBV-infected B-lymphocytes and how, on the other hand, BL cells can avoid being recognized. Virus-specific CTL usually recognize viral structures on the surface of infected cells. To date two EBV-coded proteins produced in latently infected cells are known as targets for CTL: the membrane protein encoded by the reading frame BNLF1 (BNLF1-MA), often denoted as latent membrane antigen (4, 5), and one component of the EBNA complex, EBNA 3 (6). In BL cells the BNLF1-MA is present only in reduced amounts in a truncated version (7, 8) and, therefore, probably does not function as a target for CTL; EBNA 3, however, is usually expressed in BL cells (9) and should be recognized. Recently, Torsteinsdottir *et al.* (10) demonstrated that certain BL cells were not only resistant to EBV-specific HLA-matched CTL but also to allospecific CTL. Thus, apart from the lack of viral antigens there must be another mechanism rendering BL cells resistant to specific CTL, possibly an alteration in the expression of class I antigens of the MHC which present processed viral antigen to CTL. Data in the literature about class I antigens at the surface of EBV-infected cells are in part contradictory (3, 10–14); we therefore decided to study the expression of these structures in more detail using EBV-infected LCLs and BL cells and, in comparison, EBV-negative BL cells, resting B-cells, and pokeweed mitogen-stimulated B-blasts.

MATERIALS AND METHODS

Cell Lines. BL lines were established by direct outgrowth from tumor biopsies; LCLs were generated from peripheral B-cells of BL patients or healthy donors, either by spontaneous outgrowth or by *in vitro* infection with the B95–8 strain of EBV. The BL lines studied were the EBV-positive lines Jijoye, P3HR1, Daudi, Nawalma, and Raji, the EBV-negative line BJAB, and its subline BJAB/B95–8 which was converted to the EBV-positive status. The eight pairs of BL lines and corresponding LCLs from the same patients were kindly provided by G. Lenoir, Lyon, France (BL 2/LCL 304, BL 29/LCL 167, BL 36/LCL 174, BL 64/LCL 549, BL 67/LCL 309) and A. Rickinson, Birmingham, United Kingdom (WEW BL/LCL, ELI BL/LCL, CHEP BL/LCL) and have been described in detail elsewhere (15, 16). Cells were maintained in RPMI 1640 supplemented with 2 mM glutamine and 10% fetal calf serum containing 100 IU/ml penicillin and 100 μg/ml streptomycin.

Antibodies. The monoclonal antibody W6/32, specific for a common framework determinant of all class I molecules (17), was used for radioimmunoassay and immunoprecipitation experiments. High titered antisera against EBV-positive BL cells and LCLs were raised in rabbits.

Radioimmunoassay for Quantitative Analysis of Class I Antigens. Cells (10^4/well in phosphate-buffered saline, pH 7.2, containing 0.5% bovine serum albumin and 10 mM sodium azide) were incubated for 2 h on ice in microtiter plates with monoclonal antibody W6/32 at saturating levels. After the cells were washed ^{125}I-labeled protein A (Amersham, Braunschweig, FRG; ~100 000 cpm in 10 μl) was added and after another 2 h incubation on ice the amount of bound radioactivity determined. In parallel experiments cells were incubated with a saturating concentration of a mixture of two rabbit antisera directed against EBV-positive BL cells and LCLs (anti-BL/LCL). In immunoprecipitation experiments this antiserum mixture recognized the vast majority of exposed membrane proteins on BL cells, LCLs, and peripheral B-cells. Radioactivity bound by anti-BL/LCL-treated cells was considered to be a measure for the area of the cell surface accessible to antibodies. The relative cell surface density of class I antigens was calculated by dividing cpm obtained with W6/32 by cpm obtained with anti-BL/LCL, times 100. This correction of the absolute number of cpm obtained with the class I-specific antibody seemed necessary because LCLs are often larger than BL cells; a calculation of the surface

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[3] The abbreviations used are: EBV, Epstein-Barr virus; LCL, lymphoblastoid cell line; CTL, cytotoxic T-lymphocytes; MHC, major histocompatibility complex; BL, Burkitt lymphoma; EBNA, Epstein-Barr virus nuclear antigen; poly(A)$^+$ RNA, polyadenylated RNA; SDS, sodium dodecyl sulfate; PBL, peripheral blood lymphocyte.

area from the diameter of the cells, however, is difficult because BL cells possess more surface villi than LCLs (18). All samples were tested in triplicate.

Analysis of Class I RNA. Total RNA was prepared from about 10^8 lymphocytes using the guanidinium rhodanid/cesium chloride method described by Maniatis *et al.* (19). Poly(A)$^+$ RNA was prepared from 100–500 μg of total RNA by affinity chromatography on polyuridylate paper (Hybond mAP; Amersham, Braunschweig, FRG) according to the manufacturers' instructions. For Northern blot experiments standard methods were used (19). Briefly, 10 μg of total or 0.5 μg of poly(A)$^+$ RNA was denatured in 50% formamide/2.2 M formaldehyde and electrophoresed on a 2.2 M formaldehyde-containing agarose gel for 4–5 h. RNA was transferred to nylon filters (Biodyne, Pall BioSupport division, New York) overnight. Slot blot analysis was performed using a Minifold II apparatus (Schleicher and Schüll, Dassel, FRG). Total (10 μg) or poly(A)$^+$ RNA (0.5 μg) was denatured in glyoxal and spotted on nitrocellulose. Filters were hybridized using standard conditions (19) with a human HLA complementary DNA probe (kindly provided by E. Weiss, Munich, FRG) labeled with ^{32}P by means of a commercially available nick-translation kit (Boehringer-Mannheim, Mannheim, FRG). For quantitation of RNA autoradiographs were scanned using an Elscript densitometer (Hirschmann, Munich, FRG) and the relative densities of specific RNA in BL cells and LCLs were compared directly.

Analysis of Class I Antigens by One- and Two-Dimensional Gel Electrophoresis. Iodination of cell surface proteins was done exactly as described by Hubbard and Cohn (20). Cells were metabolically labeled in the presence of 250 μCi [^{35}S]methionine for 12 h. Lysis of labeled cells and immunoprecipitation was performed as described earlier (21). Immunoprecipitates were either analyzed by SDS-polyacrylamide gel electrophoresis or subjected to two-dimensional gel electrophoresis according to the method of O'Farrell (22). Because LCLs usually expressed higher amounts of class I antigens than BL cells, for a qualitative comparison of the class I patterns of an LCL and the corresponding BL line, immunoprecipitates of the two cell lines were brought to equal specific radioactivity and equal amounts of radioactivity were applied on the gel.

Induction of Class I Antigen Expression by γ-Interferon. Cells (2×10^6) were incubated with 0, 20, 200, or 2000 IU/ml of recombinant γ-interferon (kindly provided by F. J. Brzoska, Bioferon, Laupheim, FRG). After 24, 48, and 72 h aliquots were removed and class I antigen expression was determined by radioimmunoassay (see above). Results were expressed as the ratio between the cpm values obtained for the treated cells and the cpm values of the untreated control measured simultaneously.

Miscellaneous. PBLs were isolated from heparinized blood by density gradient centrifugation over Ficoll-Hypaque. B-lymphocytes were prepared from PBLs by removing T-cells by rosetting with 2-aminoethylisothiouromium bromide hydrobromide-treated sheep erythrocytes according to the method of Beverly (23). For the generation of B-blasts PBLs were treated with pokeweed mitogen (10 μg/ml; Gibco) for 5–7 days.

RESULTS

Density of Class I Antigens on LCLs and BL Cells. In preliminary experiments expression of class I antigens in the eight pairs of BL lines and the corresponding LCLs from the same patient was studied by immunoprecipitation of ^{125}I-labeled class I proteins and analysis of the precipitated compounds by SDS-gel electrophoresis followed by autoradiography. In five of these pairs (BL 67/LCL 309, BL 2/LCL 304, ELI BL/LCL, CHEP BL/LCL, BL 29/LCL 167) class I antigens were expressed in clearly higher density on LCLs than on the corresponding BL lines, as shown for two such pairs in Fig. 1. For quantitative determination of class i antigen density a radioimmunoassay was used; in addition to the eight BL/LCL pairs five commonly used BL lines and five LCLs derived from healthy donors were studied (Table 1).

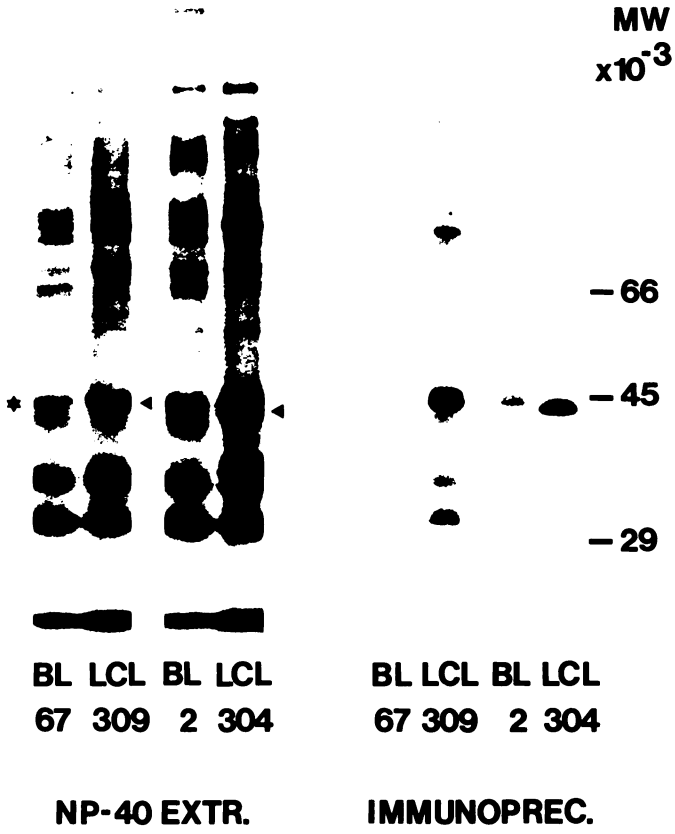

Fig. 1. Expression of MHC class I antigens on the surface of two pairs of BL cells and corresponding LCLs from the same patients. To allow a comparison of the density of class I antigens Nonidet P-40 (NP-40) extracts of labeled cells were adjusted to equal amounts of radioactivity and corresponding immunoprecipitates obtained with monoclonal antibody W6/32 were adjusted accordingly. Class I antigens can clearly be identified even in Nonidet P-40 extracts (*arrowheads*), although there is some interference with actin (*). The higher class I density in both LCLs is clearly demonstrated as well as a qualitative difference in class I antigens. MW, molecular weight.

The relative density varied considerably within the groups of BL cells and LCLs; In general, however, LCLs showed a significantly higher class I expression than BL cells (relative densities, 48.3 *versus* 13.7, respectively; $P < 0.001$, Student's t test).

A comparison of the eight BL/LCL pairs revealed an only slightly higher class I density on LCLs for three pairs (WEW, 64/549, 36/174; ratios, 1.2–1.4), whereas in the remaining pairs LCLs showed a 3.2- to 27.0-fold higher class I density than the corresponding BL lines.

Analysis of Class I RNA. Northern blot hybridization of class I RNA of six BL/LCL pairs with a class I-specific DNA probe revealed one RNA species of about 2000 base pairs in both BL and LCL cells (data not shown). A quantitative evaluation of the expression of class I RNA in these six BL/LCL pairs by slot blot hybridization showed in five pairs higher values of specific RNA in LCLs than in BL cells; this effect was more pronounced when poly(A)$^+$ RNA was used (Table 2). However, despite this general trend to higher class I RNA expression in LCLs, there was in three pairs (67/309, 64/549, ELI) a clear discrepancy between the LCL/BL ratio for RNA expression and the expression of class I antigen.

Influence of γ-Interferon on the Expression of Class I Antigens. BL/LCL pairs from two patients were incubated with varying amounts of γ-interferon and the expression of class I antigens was studied. A response was observed in both BL lines: after 48 h of treatment the expression of class I antigens was

Table 1 *Levels of class I antigens on the surface of BL cells and EBV-transformed LCLs as measured by radioimmunassay*
Each cell line was tested at least twice, one representative experiment is shown.

	BL cells				LCLs		
	Anti-class I (cpm)[a]	Anti-BL/LCL (cpm)[a]	Relative density[b]		Anti-class I (cpm)[a]	Anti-BL/LCL (cpm)[a]	Relative density[b]
P3HR1	1,882	10,159	18.5	Si-S	13,444	31,637	42.5
Daudi	374	21,993	1.7	Wo-S	14,321	22,308	64.2
Jijoye	2,898	23,793	12.2	Ma-S	11,060	23,960	46.2
Namalwa	2,838	21,377	13.3	Pr-L	16,518	34,838	47.4
Raji	2,230	22,888	9.7	Cd-L	12,058	36,155	33.4
BL 67[c]	3,793	28,636	13.2	LCL 309[c]	17,997	36,537	49.3
WEW-BL[d]	3,379	19,869	17.0	WEW-LCL[d]	4,076	19,938	20.4
BL 64[e]	13,507	28,157	48.0	LCL 549[e]	30,548	46,710	65.4
BL 2[f]	5,187	28,821	18.0	LCL 304[f]	15,815	35,953	44.0
ELI-BL[g]	464	20,109	2.3	ELI-LCL[g]	5,367	27,676	19.4
BL 36[h]	5,626	25,558	22.0	LCL 174[h]	6,340	22,784	27.8
CHEP-BL[i]	1,676	35,587	4.7	CHEP-LCL[i]	32,123	25,332	127.0
BL 29[j]	435	23,366	1.9	LCL 167[j]	23,539	57,455	41.0

[a] Mean of triplicate, after subtraction of background activity (no antibody added).

[b] $\frac{\text{(cpm obtained with anti-class I antibody)}}{\text{(cpm obtained with anti-BL/LCL serum)}} \times 100$.

[c–j] Pairs of corresponding BL cells and LCLs.

Table 2 *Relative amounts of class I-specific RNA in BL cells and corresponding LCLs*

Slot blot analysis of total (10 μg/slot) and poly(A)$^+$ (0.5 μg/slot) RNA; autoradiograms were measured by densitometry and values given in arbitrary units.

BL/LCL pairs	Total RNA			Poly(A)$^+$ RNA		
	BL	LCL	LCL/BL	BL	LCL	LCL/BL
67/309	16.1	17.0	1.06	9.5	11.9	1.25
WEW	49.2	41.1	0.84	35.7	39.6	1.11
64/549	60.0	86.9	1.45	28.6	88.7	3.10
2/304	20.0	28.5	1.43	9.5	46.5	4.90
ELI	40.8	52.3	1.28	13.7	19.0	1.39
36/174	72.3	100.0	1.38	36.9	56.6	1.53

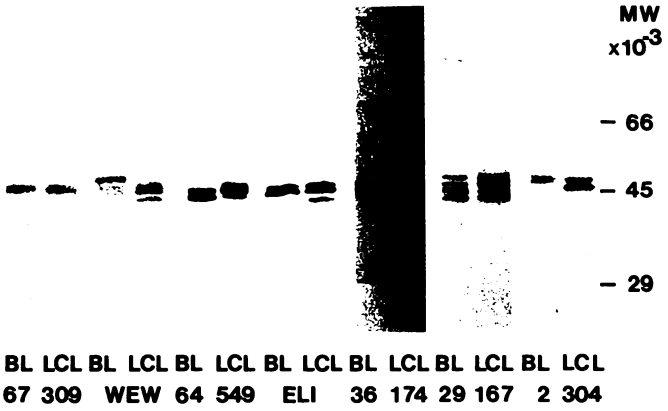

Fig. 3. SDS-gel electrophoresis of class I antigens of BL cells and corresponding LCLs. Class I antigens were precipitated from metabolically labeled cells using monoclonal antibody W6/32. As far as possible equal amounts of radioactivity were applied. MW, molecular weight.

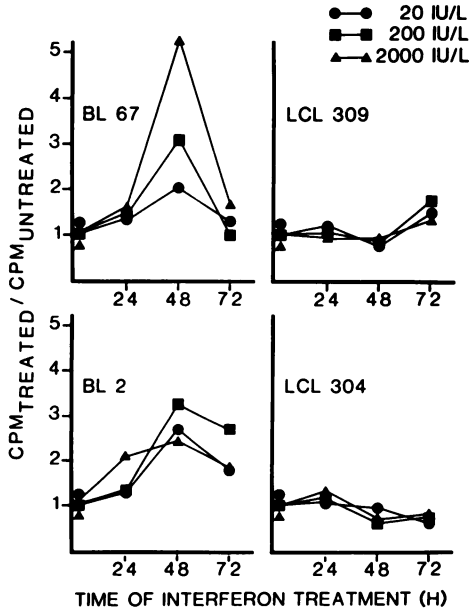

Fig. 2. Influence of γ-interferon on the level of expression of class I antigens on BL cells (*left*) and the corresponding LCLs (*right*). Class I antigen expression was measured by radioimmunoassay; results are expressed relative to the values obtained for the untreated cells (measured simultaneously) taken as a unit. Cpm values for the untreated cells ranged from 1035 cpm (BL2) to 5622 cpm (LCL 309).

enhanced 3- to 5-fold, thus approaching the levels regularly seen in corresponding LCLs. The treatment had no significant influence on the latter (Fig. 2). The response to γ-interferon was similar in both BL lines tested, irrespective of the presence (BL 67) or absence (BL 2) of EBV.

Analysis of Class I Antigens by One- and Two-Dimensional Gel Electrophoresis. Radioactively labeled class I antigens were immunoprecipitated and analyzed by gel electrophoresis. As already assumed from the experiments shown in Fig. 1 one-dimensional SDS-gel electrophoresis revealed clear differences in the class I antigen pattern between the BL-cells and the corresponding LCL in five of the seven cell pairs studied (67/309, WEW, 64/549, ELI, 2/304) (Fig. 3). When cells were labeled in the presence of tunicamycin to inhibit *N*-linked glycosylation, the two-dimemsiomal patterns changed but differences between BL cells and the corresponding LCL still persisted, indicating that these differences are not caused by altered glycosylation (data not shown).

The class I antigens of four cell pairs were further analyzed by two-dimensional gel electrophoresis (Fig. 4). The cells of pair 36/174 which did not show any class I antigen differences in the one-dimensional analysis also showed an identical two-dimensional pattern. In the BL cells of the three other pairs,

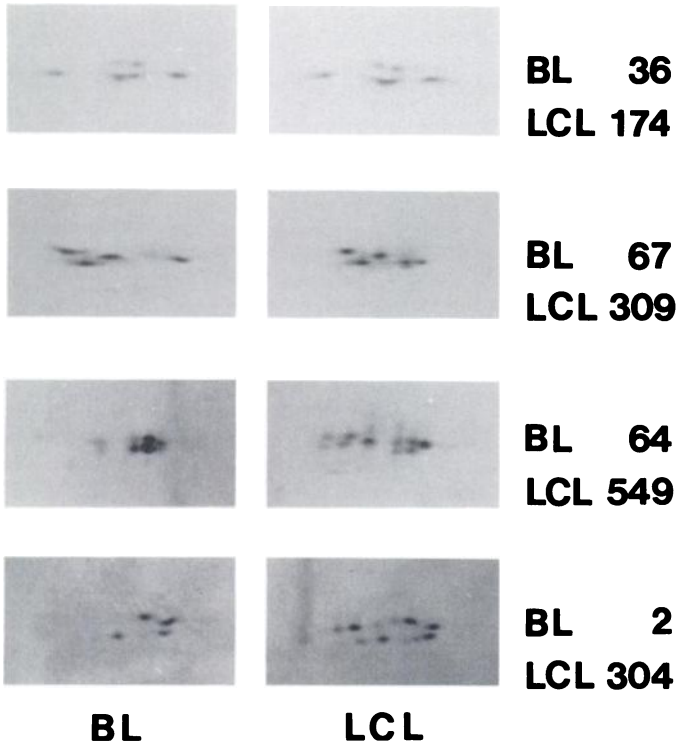

Fig. 4. Two-dimensional gel electrophoresis of class I antigens of BL cells and corresponding LCLs. Class I antigens were precipitated from metabolically labeled cells using monoclonal antibody W6/32. The acidic end of the isoelectric focusing gel is on the *right*. Equal amounts of radioactivity were applied.

Table 3 *Influence of EBV infection on expression of class I antigens*

	Anti-class I (cpm)[a]	Anti-BL/LCL (cpm)[a]	Relative density[b]
Donor S. J.			
B-cells	1,095	11,407	9.6
B-blasts	5,598	18,512	30.2
EBV LCLs	10,267	18,760	54.7
Donor R. W.			
B-cells	418	7,002	6.0
B-blasts	12,952	34,535	37.5
EBV LCLs	12,363	30,156	41.0
Donor W. J.			
B-cells	1,114	8,802	12.7
B-blasts	4,120	12,893	32.0
EBV LCLs	11,588	27,031	42.9

[a] Mean of triplicate, after subtraction of background activity (no antibody added).

[b] $\frac{\text{(cpm obtained with anti-class I antibody)}}{\text{(cpm obtained with anti-BL/LCL serum)}} \times 100.$

however, one (67/309) or several (64/549, 2/304) spots were not detected, which were clearly visible on the corresponding LCLs. Conventional HLA typing of these four pairs showed complete identity in the case of pair 36/174, whereas the BL line 67 lacked HLA B 16 which was present on the LCL (309) of this patient; BL 64 and BL 2 could not be typed because of strong multispecific reactions.

Influence of EBV Infection on the Expression of Class I Antigens. The influence of EBV infection on the expression of class I antigens was studied in peripheral B-cells and in BL cells which were originally EBV negative. Infection of isolated B-lymphocytes with EBV led to a 3.4- to 6.8-fold increase in class I expression; a similar effect was obtained by mitogenic stimulation, *i.e.*, B-blasts showed 2.5- to 6.3-fold higher values (Table 3). Neither in LCLs nor in the EBV-infected BL cells, however, did EBV infection change the pattern of class I specificities as shown by two-dimensional gel electrophoresis (Fig. 5).

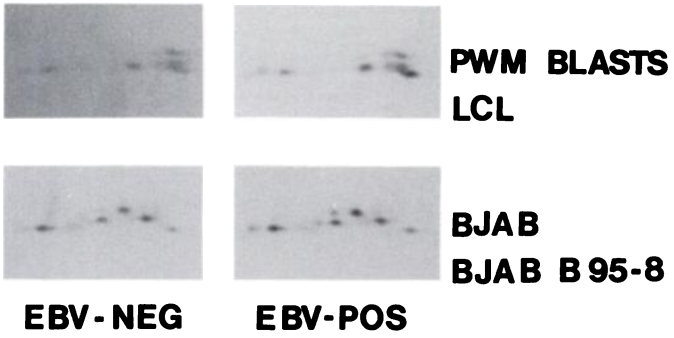

Fig. 5. Two-dimensional gel electrophoresis of class I antigens of pokeweed mitogen (PWM) stimulated B-blasts and EBV-transformed LCLs of the same individual (*above*) and of BJAB BL cells and their EBV-infected subline BJAB/B95-8. Class I antigens were precipitated from metabolically labeled cells using monoclonal antibody W6/32. The acidic end of the isoelectric focusing gel is on the *right*. Equal amounts of radioactivity were applied.

DISCUSSION

Several studies so far have dealt with the expression of class I antigens of the MHC on BL cells and LCLs with, however, somewhat conflicting results. Some authors described a high class I expression on LCLs (11) and a low or even absent class I expression on BL cells (12, 13, 24), whereas Rooney *et al.* (3) and Torsteinsdottir *et al.* (10) did not observe significant differences in class I expression in BL/LCL pairs. Down-regulation of single class I specificities in BL cells was first described by Masucci *et al.* (25), who found a selective loss of HLA A11 in two BL lines resistant to A11-restricted EBV-specific cytotoxic T-cells; in two recent reports this could be extended to other specificities as well (26, 27).

Our experiments demonstrate that the class I antigens of the MHC are expressed differently on BL cells and on EBV-transformed LCLs. Despite considerable individual variations, LCLs in generál revealed significantly higher class I levels than BL cells; the latter showed a class I expression similar to or, in the case of two lines (ELI BL, BL 29), considerably lower than peripheral B-lymphocytes. The lines ELI BL and BL 29, in fact, showed cpm values in the radioimmunoassay not much higher then the BL line Daudi which is known to lack class I antigens (13). These findings are in agreement with some earlier studies (11, 12, 24) but conflict with the results of Rooney *et al.* (3) and Torsteinsdottir *et al.* (10). The observed discrepancies might be caused by differences in the cell lines studied, but more probably they are due to the different techniques used [especially because four of the BL/LCL pairs of our study were also analyzed by Rooney *et al.* (3)].

Our radioimmunoassay applies saturating antibody concentrations (which was only possible by reducing the number of cells/well to 1×10^4) and uses a labeled second ligand of high affinity (^{125}I-labeled protein A). This technique allows the detection of even small differences in class I density between different cells, differences which might well remain undetectable under the conditions used by others (3, 10) who measured class I expression by means of the fluorescence-activated cell sorter.

In our study LCLs also showed, in general, a higher expression of class I RNA than BL cells. However, there were some

discrepancies between the class I RNA and the protein expression: two pairs showed low differences in RNA expression but high differences in antigen expression; the opposite was found in another pair. Thus, although one might assume that the differences in the expression of these antigens might be due to different expression on the transcriptional level, these data do not fully support this idea. On the other hand, such a mechanism would well correspond with our finding that γ-interferon increases class I antigen expression on both of the BL lines studied because γ-interferon is known to enhance transcription of MHC antigens in other cells (28). Thus, according to our results it is very likely that the down-regulation of class I expression occurs at the transcriptional level but this has to be definitely proven by further experiments. Interestingly, the treatment with γ-interferon did not alter class I expression in the two LCLs; therefore, in the latter class I antigens seem to be maximally expressed.

The qualitative variations in the pattern of class I specificities seen in the two-dimensional gel electrophoresis experiments point strongly to down-regulation of certain class I specificities in three of the four BL lines studied. This technique cannot rule out completely that in BL cells only modifications of class I antigens are missing, *e.g.*, molecules differing in their carbohydrate content; however, because differences between BL lines and LCLs were still present after treatment with tunicamycin, and because in two of the BL lines four or even five spots seen in the corresponding LCLs were missing, the most probable explanation would indeed be the lack of expression of one or, in the case of BL 2 and BL 64, even more class I specificities. These results are in accordance with the studies (25–27) which demonstrated down-regulation of HLA A11 in certain BL cells by means of specific monoclonal antibodies. In addition our study shows for two BL lines (ELI, BL 29) a complete down-regulation of all (or at least most) class I specificities far below the level seen in resting B-lymphocytes.

All of the observed differences in class I expression (the quantitative variations between LCLs and the corresponding BL lines or the normal resting B-lymphocytes, as well as the down-regulation of certain class I specificities in BL cells) turned out to be independent of infection with EBV. Mitogenic stimulation of peripheral B-cells led to a significant increase in class I expression similarly to the effect of EBV infection; the two-dimensional gel electrophoresis pattern of class I antigens of normal B-lymphocytes was not altered by EBV infection. Similarly, infection of the EBV-negative line BJAB did not influence the pattern of class I expression. On the other hand, down-regulation of some class I antigens was found also in the EBV-negative BL line BL 2. Thus, the alteration of class I expression in BL cells [down-regulation of some (BL 67, BL 64, BL 2) or obviously all (ELI BL, BL 29) class one specificities] seems to be linked mainly to the tumorigenic nature of these cells.

Altered MHC class I antigen expression is a well-known phenomenon in several tumors such as malignant melanoma (29), colorectal carcinoma (30), certain B-cell lymphomas (31), small cell lung cancer (32), and neuroblastoma (33). For the latter system Bernards *et al.* (34) recently showed that down-modulation of class I antigens is a consequence of overexpression of the cellular oncogene N-*myc*. Such a mechanism may prove true for BL cells as well in which the typical chromosomal translocations (35) are thought to lead to a deregulated expression of c-*myc*; overexpression of c-*myc* may cause down-regulation of class I antigens.

It is tempting to assume that reduced class I expression influences the recognition of BL cells by specific cytotoxic T-cells. In EBV-infected cells viral structures should play the definitive role in their recognition by specific CTL; the reduced expression or the complete lack of viral target structures as the BNLF1-MA (4, 7) is thought to be a main mechanism involved in BL cells escaping immune surveillance. Nevertheless, cellular factors may be important as well. Gregory *et al.* (36) recently showed that the down-regulation of cell adhesion molecules paralleled the ability of BL cells to escape the attack by CTL. On the other hand, Masucci *et al.* (25) could clearly demonstrate that the selective lack of HLA A11 in two EBV-positive BL lines prevented their killing by EBV-specific CTL, thus shedding light on the role of class I antigens in the immune recognition of these cells. It may well be that several factors, viral and cellular, contribute to the resistance of BL cells to cellular immune attacks. Our data show that down-regulation of all or single class I antigens in BL cells seems to be a rather frequent event. The overexpression of these structures observed in LCLs, on the other hand, may well render these cells more susceptible to specific lysis and, therefore, play a role in the effective control of EBV-transformed lymphocytes.

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