DOWNREGULATION OF CELL ADHESION MOLECULES LFA-3 AND ICAM-1 IN EPSTEIN-BARR VIRUS-POSITIVE BURKITT'S LYMPHOMA UNDERLIES TUMOR CELL ESCAPE FROM VIRUS-SPECIFIC T CELL SURVEILLANCE

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Cell surface adhesion molecules are thought to play an important part in establishing the intercellular contacts that are necessary for immunological reactions (1). One such adhesion pathway in man involves the lymphocyte function-associated antigen (LFA-1),[1] one of a family of leukocyte cell surface proteins (LFA-1, Mac-1, p150, 95) that are heterodimers with a common β chain and distinct though homologous α chains (1, 2). The principal ligand for LFA-1 seems to be the intercellular adhesion molecule ICAM-1, a protein expressed on many differentiated cell types (3). The LFA-1/ICAM-1 pathway mediates a variety of cell-cell adhesions by T and B lymphocytes, natural killer cells, granulocytes, and macrophages (1–5) and also appears to be responsible for the homotypic cell adhesions shown by certain leukocyte-derived cell lines in vitro (5).

Interactions between cytotoxic T lymphocytes (CTL) and their target cells involve an initial phase of effector/target adhesion, detectable by rapid conjugate formation in vitro, which is independent of antigen-specific recognition (4). mAb blocking studies indicate that this interaction involves two separate adhesion pathways (4, 6). One is the effector LFA-1/target ICAM-1 pathway described above, the other is mediated via the T cell-specific CD2 antigen (T11, LFA-2) interacting with a widely distributed adhesion protein, LFA-3 (7), on the target cell surface. Both pathways appear to be required for optimal effector/target conjugation, and therefore might be important accessories for CTL formation.

One of the best characterized CTL surveillance systems operative in man is that which is specifically directed against Epstein-Barr virus (EBV), an agent with oncogenic potential in vivo (8) and with cell growth transforming ability for human B cells in vitro (9). EBV-specific CTL, reactivated from memory T cells in the blood of virus-immune donors, recognize EBV-transformed B lymphoblastoid cell lines (LCL) in a HLA class I antigen-restricted manner (10).

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[1] *Abbreviations used in this paper:* BL, Burkitt's lymphoma; BLA, Burkitt associated glycolipid antigen; CALLA, common acute lymphoblastic leukemia antigen; ICAM, intercellular adhesion molecule; LCL, lymphoblastoid cell line; LFA, lymphocyte function-associated antigen.

In studying the possible importance of this surveillance system as a defense against EBV-associated tumors, we found that early passage cultures of virus-genome-positive Burkitt's lymphoma (BL) cell lines were not killed by HLA-matched EBV-specific CTL in assays where EBV-transformed LCL derived from the normal B cells of the same patients were clearly recognized (11). We postulated that this reflected the in vivo selection of a malignant BL cell clone that was no longer sensitive to virus-specific T cell surveillance.

The above result with BL cells could not be explained by any altered expression of HLA class I antigens on the tumor cell surface (11) but in several cases it did correlate with an altered pattern of virus gene expression. Thus, certain of the EBV latent gene products that are consistently found in all in vitro-transformed LCL were not detectably expressed in the early passage BL cells, suggesting a downregulation of those viral proteins providing the target antigens for virus-specific T cell recognition (12). However, we noted that not every case in which BL cells escaped recognition could be explained in this way. Thus, on serial passage a number of BL cell lines showed a broadening of virus latent gene expression (and an accompanying change in cell surface/growth phenotype) to that characteristic of LCL, yet certain of these lines still remained insensitive to EBV-specific CTL killing (12). This showed that the capacity of malignant BL cells to evade T cell surveillance was not based solely on altered virus gene expression and suggested that some other feature of the tumor cell phenotype was also important. Here we identify that second feature as altered adhesion molecule expression.

Materials and Methods

Cell Lines. BL lines were established from EBV genome-positive BL biopsy samples and grown in suspension culture in RPMI-1640 containing FCS as described (13). Initially all BL cells grew as single cell suspensions, but on serial passage some lines moved towards a more LCL-like growth pattern and cell surface phenotype. Lines could be grouped according to their growth morphology and their expression of the following leukocyte differentiation antigens: the BL-associated glycolipid antigen, BLA, the common acute lymphoblastic leukemia antigen, CALLA (CD10), and the LCL-associated antigens Ki24, CD23, CD30, and CD39. The phenotype grouping of BL cell lines, described in detail elsewhere (14), can be summarized thus: (group I) single cell growth, BLA^+ $CALLA^+$ $Ki24^-$ $CD23^-$ $CD30^-$ $CD39^-$; (group I/II) single cells/small clumps, BLA^+ $CALLA^+$ $Ki24^+$ $CD23^-$ $CD30^-$ $CD39^-$; (group II) medium/large clumps, BLA^+ $CALLA^+$ $Ki24^+$ $CD23^+$ $CD30^+$ $CD39^+$; (group III) large clumps, BLA^- $CALLA^-$ $Ki24^+$ $CD23^+$ $CD30^+$ $CD39^+$.

LCL were generated from BL patients by EBV-induced transformation of normal circulating B cells using the B95.8 or QIMR-WIL strain of virus. All LCL display the group III phenotype.

Surface Immunofluorescence and FACS Analysis. Adhesion molecule expression by BL lines and LCL was analyzed by surface immunofluorescence labeling and flow cytofluorometry. Cells were initially labeled with saturating concentrations of mouse IgG mAbs, washed, and further incubated with polyclonal FITC-conjugated goat anti-mouse IgG (Sigma, London, England). The mAbs used were MHM24 and MHM23 (15) specifying the α and β chains, respectively, of LFA-1 (both kindly provided by Professor A. J. McMichael, John Radcliffe Hospital, Oxford, England), RR1/1 (3) specifying ICAM-1 and TS2/9 (7) specifying LFA-3 (both kindly provided by Dr. T. Springer, Dana Faber Cancer Institute, Boston, MA). In addition, the mAbs W6.32 (16) and A21BC1 (kindly provided by Dr. N. Ling, University of Birmingham, England) specifying HLA class I and class II molecules, respectively, the CD19 mAb 8EB1 (from Dr. Ling) and the CD20 mAb B1 (obtained from Coulter Clone, Luton, England) were used as reference reagents in the same experiments. Labeled cells were analyzed using a FACS IV (Becton Dickinson & Co., Mountain View, CA) and results were

expressed as fluorescence/volume ratios, thus facilitating the comparison of cell lines with slightly different cell size distributions. Mean fluorescence/volume ratios were calculated from measurements of fluorescence intensity and of 180° light scatter (proportional to cell volume) from 20,000 individual cells as described (17). Background fluorescence was detected using cells that had only received FITC-conjugated antibody during the labeling procedure. For any one combination of cell line and mAb, repeated testing under the same conditions of labeling gave highly reproducible values of the mean fluorescence/volume ratio.

Qualitative Homotypic Aggregation Assay. Cultured cells were dispersed by repeated gentle pipetting, resuspended in RPMI-1640 containing 10% FCS and 20 mM Hepes (RPMI-10H) and reseeded at 5×10^5 cells in 0.25 ml in 2-ml wells (Nunc, Roskilde, Denmark). To each well was added 0.25 ml RPMI-10H either alone or containing the mAbs 8EB1, MHM23, MHM24, RR1/1 or TS2/9 at a saturating final concentration, generally 1:100 dilution of ascitic fluid. After 30 min, incubation at 37°C on a rocking platform operating at 80 cycles per minute, homotypic adhesion was assessed visually by observation of cell clumps using an inverted phase contrast microscope.

EBV-specific T Cells: Preparation and Use in Conjugate Formation and Cytotoxicity Assays. EBV-specific, HLA class I–restricted CTL were reactivated from the blood of virus-immune individuals and expanded in IL-2 as described (17). The capacity of CTL to bind BL or LCL targets was assessed in a FACS conjugate assay (4, 6). Briefly, 2.5×10^4 CTL effectors labeled with fluorescein diacetate (100 μg/ml) and 5×10^4 target cells labeled with hydroethidine (48 μg/ml) were cocentrifuged at 4°C in 50 μl RPMI-10H in 12 × 75 mm plastic tubes (Falcon Labware, Oxnard, CA). After a 6-min incubation in a 37°C water bath, the cell pellets were resuspended by vigorous vortexing in 0.8 ml cold (4°C) PBS containing 0.2% BSA, and the cell suspensions were held on ice and analyzed immediately in the FACS IV. Some assays were conducted in the presence of saturating concentrations of the mAbs 8EB1, MHM23, MHM24, RR1/1, and TS2/9 used either individually or in pairs. In other assays, target or effector cells were pretreated with single mAbs and then excess antibody was removed by washing before completion of the conjugate-forming assay in normal medium. Where EDTA was used to inhibit Mg^{2+}-dependent conjugate formation (4), effectors and targets were brought together in the presence of 5 mM EDTA. Conjugate formation was assayed by FACS analysis; 10,000 or 20,000 total fluorescent events were collected, and the proportion of total events emitting both red (from targets) and green (from effectors) fluorescence signals (i.e., CTL-target conjugates) was divided by the proportion of the total cells emitting green signals (i.e., the total effector population), thus indicating the percentage of effectors in a given CTL population that had formed conjugates. "Background" conjugate formation was estimated using effector/target cell mixtures that were not cocentrifuged, but rather dispersed immediately into 0.8 ml PBS/BSA. Background values of effector conjugate formation that ranged from <1% to 6% were always subtracted from the observed results.

The lytic capacity of EBV-specific CTL towards HLA class I antigen-matched BL and LCL targets was assessed by short-term ^{51}chromium-release assay (17).

Results

Expression of Adhesion Molecules in Relation to BL Cell Surface/Growth Phenotype. Levels of expression of LFA-1, ICAM-1, and LFA-3 were examined in BL cell lines that had retained the single cell/small clump growth pattern and biopsy-like phenotype (groups I, I/II) versus those BL lines that had changed with serial passage to grow in large clumps and to express a more "lymphoblastoid" cell surface phenotype while retaining the chromosomal translocation indicative of their malignant origin (groups II, III; see Materials and Methods). The FACS profiles obtained from two representative group I/II cell lines (ELI BL, WW2 BL) in Fig. 1 indicate their very low expression of LFA-1 and ICAM-1 and the virtual absence of LFA-3. In the same analysis, two representative group III lines (BL18, BL36) showed increased levels of LFA-1 and dramatically higher expression of ICAM-1 and LFA-3. Fig. 1 also shows the

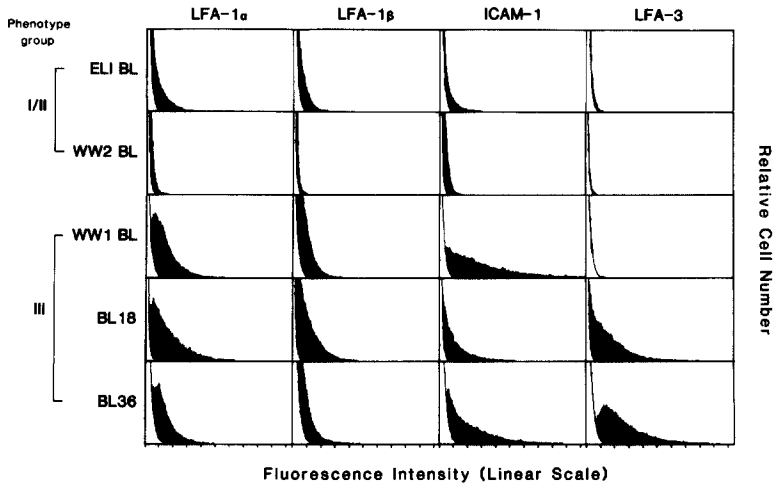

FIGURE 1. Expression of adhesion molecules by BL cell lines of group I/II (biopsy-like) and group III (LCL-like) phenotypes. Surface immunofluorescence labeling was performed using mAbs MHM24, MHM23, RR1/1, and TS2/9 to LFA-1α, LFA-1β, ICAM-1, and LFA-3, respectively. Samples were analyzed on a FACS IV using linear fluorescence intensity settings. The abscissa of each graph is graduated in arbitrary units from 0 to 200 in increments of 20.

unusual profile obtained with one particular group III line, WW1 BL, where high levels of LFA-1 and ICAM-1 were observed in the absence of detectable LFA-3. A total of 14 EBV^+ BL cell lines were analyzed in this way, each on at least two occasions, and the complete results are presented as fluorescence/volume ratios in Table I. Group I and I/II cell lines generally expressed low levels of LFA-1 and little or no ICAM-1 and LFA-3, even though these same cells were strongly labeled by mAbs to other membrane proteins, such as HLA class I or class II antigens, CALLA, and the pan-B cell markers CD19 and CD20 (Table I and data not shown). By contrast, group III BL cell lines generally expressed LFA-1, ICAM-1, and LFA-3 at the same high levels as are seen on EBV-transformed LCL, with the single exception of WW1 BL which, as already illustrated, lacked LFA-3. Table I also includes, as an additional reference, the fluorescence/volume ratios obtained with an LCL derived from an LFA-1-deficient patient (18); this line expressed ICAM-1 and LFA-3 but lacked LFA-1 and grew predominantly as single cells.

Functional evidence regarding the involvement of adhesion molecules in the altered growth pattern of group III BL cell lines was obtained using a qualitative cell aggregation assay. As shown in Fig. 2, lines such as BL18 and BL36 formed well-defined cell aggregates within 30 min of seeding into wells as a dispersed single cell suspension. Of several mAbs tested for their ability to affect aggregation when included in the culture medium, specific inhibition was observed both with the anti-LFA-1 reagents (MHM23 and 24) and with the anti-ICAM-1 reagent (RR1/1); on the other hand, mAbs to LFA-3, to HLA class II antigens, or to the pan-B cell marker CD19 had no such effect. Precisely similar results were obtained using LCL cells rather than group III BL cells in the same assay (data not shown).

Antigen-independent Adhesion of EBV-specific CTL to BL Cells. Combinations of BL

TABLE I

Adhesion Molecule Expression by BL Lines Showing Varying Degrees of Phenotypic Change In Vitro*

Line	Phenotypic group	Growth pattern	Fluorescence/volume ratio of cells expressing (mAb): LFA-1α (MHM24)	LFA-1β (MHM23)	ICAM-1 (RR1/1)	LFA-3 (TS2/9)	HLA I (W6.32)
ELI BL			5.3	5.9	4.5	0.4	78.7
CHEP BL			1.6	2.5	8.6	1.6	57.3
WW2 BL			1.9	0.7	2.4	0.1	71.2
BL60	I + I/II	Single cells/	1.4	1.6	4.1	0.4	93.6
BL29		small clumps	4.1	2.7	0.7	3.0	60.4
BL37			3.9	3.8	0.3	2.3	61.5
WAN BL			7.1	2.5	11.2	0.3	69.3
BL 74			14.6	16.2	0.3	1.6	69.5
OBA BL	II	Medium clumps	10.8	5.2	26.7	16.0	98.2
WW1 BL			21.9	13.4	49.5	0.2	61.0
BL16			31.1	19.2	47.1	57.8	54.5
BL18	III	Large clumps	30.7	19.3	12.0	27.4	64.3
BL36			17.1	11.1	32.5	42.8	48.3
MUTU BL			24.7	33.4	43.6	51.0	53.0
Control LCL‡	III	Large clumps	22.1	13.5	16.2	49.7	54.8
LFA-1-ve LCL§	III	Single cells	0.1	0.7	20.1	37.9	14.1

* All BL cell lines were assayed between passage 30 and 50 in vitro, by which time they had achieved a stable cell surface/growth phenotype.

‡ Mean of six normal LCL.

§ LCL derived from an LFA-1-deficient individual.

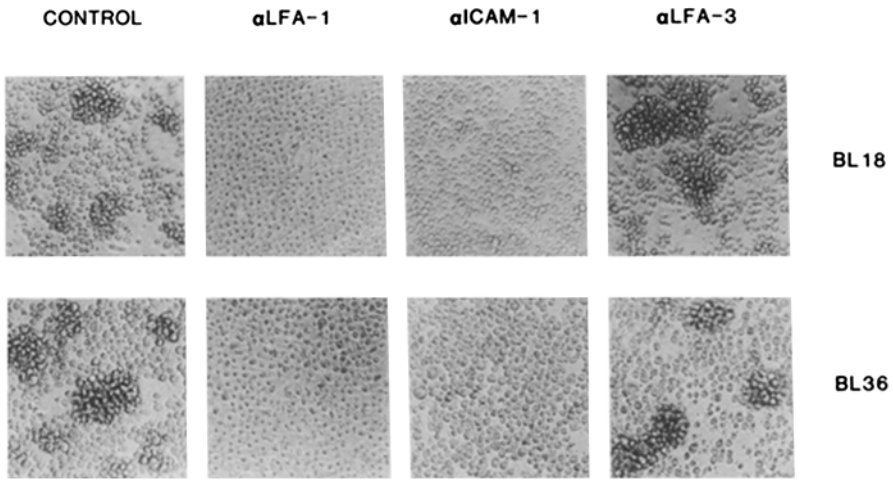

FIGURE 2. Homotypic adhesion by two BL-derived cell lines (BL18, BL36) of group III phenotype. Dissociated cells were allowed to aggregate for 30 min either in medium alone (CONTROL) or in medium containing mAbs MHM23 (αLFA-1), RR1/1 (αICAM-1), or TS2/9 (αLFA-3). Control cultures containing mAbs to CD19 or to HLA class II molecules did not affect aggregation. mAb MHM24 (anti-LFA-1α) produced the same effect as MHM23 (anti-LFA-1β).

target lines and EBV-specific CTL from HLA-mismatched individuals were used in short-term conjugate formation assays to measure the efficiency of BL-CTL adhesions that were independent of any antigen-specific (i.e., HLA-restricted) recognition. Fig. 3 shows the results of an experiment comparing group I/II and group III

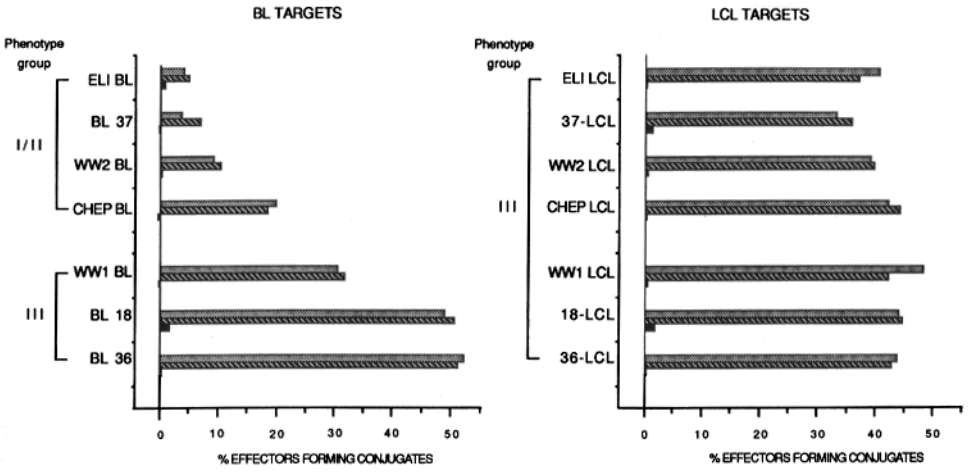

FIGURE 3. Conjugate formation between BL cell lines or corresponding LCL and HLA-mismatched EBV-specific CTL. Fluorochrome-labeled CTL and target cells were cocentrifuged in medium alone (▩), in the presence of the CD19 mAb 8EB1 (▨), or in a mixture of mAbs MHM23 and TS2/9 (anti-LFA-1 and anti-LFA-3, respectively; (■). The percent effectors showing the ability to form conjugates with target cells was estimated after incubation at 37°C for 6 min followed by FACS analysis. In the same assay these effectors showed 54% conjugate formation with their autologous target LCL.

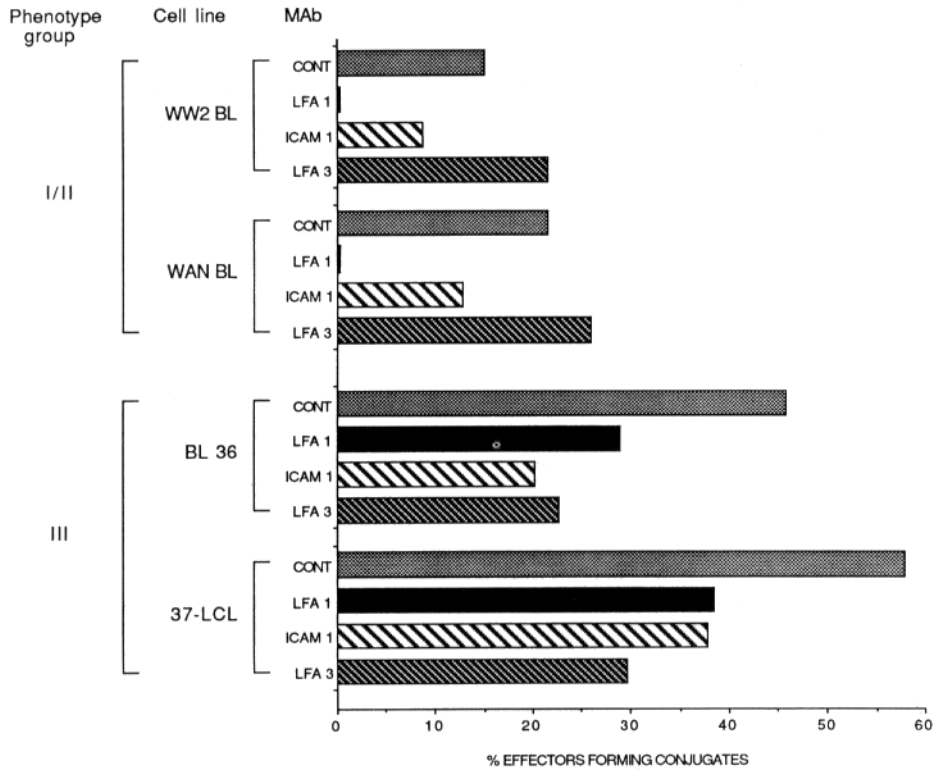

FIGURE 4. mAb blocking of effector/target conjugate formation. EBV-specific HLA-mismatched CTL were allowed to bind to the indicated BL and LCL targets in the presence of either medium alone (CONT) or of mAbs MHM23, RR1/1 or TS2/9 against LFA-1, ICAM-1, and LFA-3, respectively. In the same assay these effectors showed 50% conjugate formation with their autologous target LCL.

BL lines in combination with the same mismatched effectors; the corresponding LCL from these particular BL patients are included as control targets for comparison. The group I/II BL cell lines displayed significantly reduced conjugate forming ability when compared either with the group III BL lines or with their control LCL. This pattern of results was obtained on many occasions, with the level of conjugate formation by group I/II BL cell lines usually being <25% of that shown by the corresponding LCL. The conjugates being detected by FACS analysis in this assay were the result of cell–cell interactions mediated by adhesion molecules; since conjugate formation was consistently abolished in the presence of a combination of anti-LFA-1 and anti-LFA-3 mAbs but unaffected by the CD19 control mAb.

In further mAb blocking experiments, conjugate formation between LCL targets and EBV-specific CTL was inhibited by ~50% in the presence of either anti-LFA-1 or anti-ICAM-1 or anti-LFA-3 (37-LCL, Fig. 4; WW1 LCL, Fig. 5). Combinations of anti-LFA-1 and anti-ICAM-1 were no more effective than either mAb alone, whereas anti-LFA-1 and anti-LFA-3 gave complete inhibition; interestingly, combinations of anti-ICAM-1 and anti-LFA-3 also gave substantial inhibition, but this was never

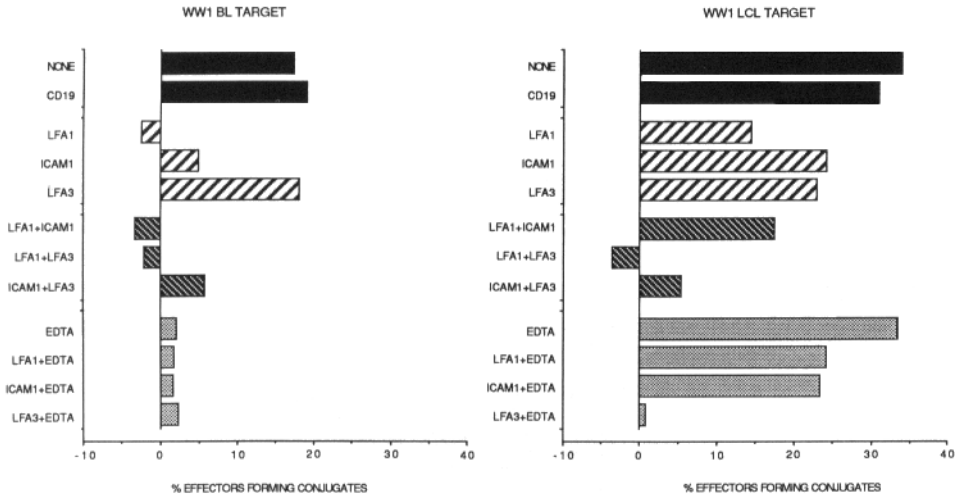

FIGURE 5. Conjugate formation of EBV-specific HLA-mismatched CTL with WW1 BL and WW1 LCL targets in the presence of individual anti-LFA-1, anti-ICAM-1, or anti-LFA-3 mAbs (MHM23, RR1/1, and TS2/9, respectively) or with dual mAb combinations. Control conjugate assays were performed either in the presence of medium alone (NONE) or with the CD19 mAb (8EB1). Conjugate formation was also performed in the presence of 5 mM EDTA either alone or in combination with individual mAbs against LFA-1, ICAM-1, or LFA-3. In the same assay these effectors showed 45% conjugate formation with their autologous target LCL.

complete (WW1 LCL, Fig. 5). Pretreatment of target or of effector cells, then removal of unbound mAbs before the assay, showed that anti-ICAM-1 and anti-LFA-3 mediated their inhibition through binding to the target cells, anti-LFA-1 through binding to the effectors (data not shown). Such results are consistent with there being two pathways of antigen-independent adhesion for LCL-effector conjugations, one involving LFA-1 and ICAM-1, the other involving LFA-3 (see also references 4, 19).

All group III BL cell lines (with the exception of WW1 BL, see below) showed the same sensitivity to mAb blocking of conjugate formation as did LCL (BL36, Fig. 4) and therefore appeared to interact with CTL through both LFA-1 and LFA-3 pathways. In contrast, the lower but still significant levels of conjugate formation that group I/II BL cell lines displayed was completely abrogated by anti-LFA-1, was partially inhibited by anti-ICAM-1, and was unaffected by anti-LFA-3 (WW2 BL and WAN BL, Fig. 4). This indicates that all effector/target conjugation involving group I/II BL cell lines occurs through an LFA-1 mediated pathway, and not through LFA-3. Analysis of conjugate formation by the unusual group III cell line, WW1 BL, showed that this followed the group I/II BL pattern, all conjugates being inhibited in the presence of anti-LFA-1 (Fig. 5). In this context, Fig. 5 also shows the results of assays conducted in the presence of 5 mM EDTA in an effort to impair selectively the Mg^{2+}-dependent LFA-1 pathway (4). Although conjugate formation by WW1-LCL cells was, at best, only slightly reduced by EDTA alone, a combination of EDTA and anti-LFA-3 was completely inhibitory; in contrast, EDTA alone was able to prevent all conjugate formation by WW1 BL cells.

Relationship between Adhesion Molecule Expression, Conjugate Formation, and Sensitivity

TABLE II

BL Lines and Corresponding LCLs: Adhesion Molecule Expression, Conjugate Formation, and Sensitivity to EBV-specific T Cell Cytotoxicity

Target cell line*	Adhesion molecules‡		Conjugate formation§	Lysis by EBV-specific T cells‖				
				CM-T cells		SW-T cells	KS-T cells	
	ICAM-1	LFA-3		10:1	5:1	5:1	10:1	5:1
			%	%		%	%	
p25	4.1	0.7	9.6	0	0	0	0	2
BL72 p80	13.0	12.9	22.8	7	4	0	15	10
p140	24.1	21.5	46.3	23	18	11	34	26
72-LCL	34.4	58.9	26.0	37	27	18	47	44
WW1 BL p60	48.0	0.9	30.0	5	3	1	8	4
WW1 LCL	21.1	40.2	48.0	42	29	31	40	40
Allo LCL	20.8	48.2	44.0	3	2	2	7	5

* BL-derived cell lines at indicated passage numbers in vitro together with the corresponding in vitro-transformed LCL from the same patients. Allo-LCL is an LCL from a normal donor that shows no HLA class I antigen matching with the EBV-specific effector T cells shown.

‡ Expression of adhesion molecules is indicated by fluorescence/volume ratios as measured by FACS. Cells were surface-labeled for ICAM-1 using mAb RR1/1 and for LFA-3 using TS2/9.

§ EBV-specific HLA-mismatched effectors binding in short-term assays to the target cells indicated. These same effectors showed 45% binding in the same assay to their autologous target LCL.

‖ Results of 5-h ^{51}Cr-release cytotoxicity assays using EBV-specific effector T cells from three HLA class I antigen-matched donors; CM, SW, and KS: E/T ratios of 10:1 and 5:1 are indicated.

of BL cells to EBV-specific CTL-mediated Lysis. Of several EBV$^+$ BL cell lines found to be insensitive to HLA-matched EBV-specific CTL (11), the BL72 and WW1 BL lines were known to be expressing the full spectrum of EBV-latent proteins and so their lack of recognition could not be ascribed to downregulation of the viral target antigens for CTL responses (12). In the present study, access to cryopreserved stocks of the BL72 line allowed cells at passages 25, 80, and 140 to be tested in the same experiment for adhesion molecule expression, for conjugate formation, and for susceptibility to lysis by three independent CTL preparations from the HLA-matched EBV-immune donors CM, SW, and KS. The corresponding LCL derived from the same patient (72-LCL) provides an important reference target in these same assays. As shown in Table II, the increasing expression of ICAM-1 and of LFA-3 that was observed on BL72 cells with increasing passage number was reflected both in the relative efficiency with which these cells formed conjugates and in their relative sensitivity to EBV-specific cytolysis. We noted in particular that, while BL72 cells in passage 25 did express some ICAM-1 and did form detectable numbers of conjugates, significant CTL-mediated lysis was not observed until later passages by which time LFA-3 was also expressed.

A critical role for LFA-3 in EBV-specific cytolysis was more strongly indicated by the results obtained in this same set of assays using WW1 BL target cells and the corresponding WW1 LCL control (Table II). Even at the high passage number used in this particular study, WW1 BL cells displaying the group III phenotype and

expressing the full spectrum of known EBV-latent proteins remained insensitive to EBV-specific CTL. The very low level of WW1 BL killing observed in these and in many other assays was never significantly different from that of HLA-mismatched LCL included as control targets, while the WW1 LCL itself was always strongly killed. Note that the WW1 BL target did form appreciable numbers of conjugates with EBV-specific CTL (Table II), even when tested with the same HLA-matched effector T cell preparations as were used in the cytotoxicity assays (data not shown), yet clearly these conjugates did not lead to EBV-specific cytolysis of the target cells.

Discussion

EBV^+ BL cells in very early passage display a homogeneous cell surface phenotype, like that of the original biopsy population, and a single cell pattern of growth. With serial passage some, but not all, lines move towards a more "lymphoblastoid" (i.e., LCL-like) pattern of cell surface markers and begin to grow in large clumps (13). Where such changes occur, they appear to be directly induced by a broadening of expression of the resident EBV genome; thus certain EBV-latent proteins that are downregulated in BL tumor cells in vivo may begin to be expressed in vitro (14). The present study shows that one important, and hitherto unrecognized feature of this EBV-induced phenotypic change is a dramatic increase in expression of the cell adhesion molecules LFA-1, ICAM-1, and LFA-3 from the very low levels present on early passage cells (Fig. 1, Table I). Indeed, our results suggest that the movement of BL cells towards a more aggregated pattern of growth in vitro simply reflects the increased availability of surface LFA-1, and ICAM-1, i.e., those molecules involved in homotypic cell adhesion (Fig. 2). In most of the phenotypically altered BL cell lines, LFA-1 ICAM-1, and LFA-3 appear to have been coordinately upregulated; however the WW1 BL cell line was an exception in that it displayed the classic group III (LCL-like) surface phenotype, grew in clumps, and expressed high levels of both LFA-1 and ICAM-1 in the absence of detectable LFA-3 (Fig. 1, Table I).

Several recent papers examining the antigen-independent phase of CTL/target interactions have identified effector LFA-1 and target ICAM-1 molecules as being involved in one adhesion pathway, and effector CD2 and target LFA-3 molecules in another (4, 6, 19). Here we show that group I and I/II BL cell lines retaining a biopsy-like phenotype and expressing low levels of adhesion molecules, formed conjugates with EBV-specific CTL much less efficiently than did group III BL cell lines or LCLs (Fig. 3). Moreover, the conjugates formed by group I/II lines were mediated entirely via an LFA-1 pathway, and not via LFA-3. In contrast, most group III BL cell lines showed a pattern of conjugate formation that was indistinguishable from that of LCL and that involved both the LFA-1 and the LFA-3 pathways. The exception among group III lines was again WW1 BL, which is phenotypically LCL-like in all respects other than LFA-3 expression, and which appeared to form conjugates by exclusive use of the LFA-1 pathway(s) operative for group I/II BL cell lines. In these LFA-3-independent adhesions, it was interesting that the anti-ICAM-1 mAb never achieved the complete inhibitory effect shown by the anti-LFA-1 mAbs (Figs. 4 and 5). Other studies have already indicated the possibility of a second target cell ligand for LFA-1 (19) and the present work suggests that such a molecule is as important as ICAM-1 in mediating conjugate formation by group I/II BL cell lines.

The final set of experiments (Table II) reveal how reduced adhesion molecule expression and reduced conjugate formation by BL cells are reflected in terms of tumor cell sensitivity to EBV-specific T cell cytolysis. In particular, the results resolve a long-standing paradox from earlier studies, namely that certain BL cell lines (BL72 and WW1 BL) that had begun to express the full spectrum of EBV-latent proteins in vitro nevertheless remained insensitive to lysis by HLA class I antigen-matched EBV-specific CTL (11, 12). Thus, BL72 cells at passage 25, though already showing an LCL-like pattern of virus gene expression, still expressed only low levels of ICAM-1 and were virtually negative for LFA-3; it was only when these molecules were upregulated on serial passage that EBV-specific lysis occurred. The results with the other unusual line, WW1 BL, were even more instructive because here the target cells' resistance to EBV-specific lysis was retained in late passage and correlated with a continuing downregulation of LFA-3. Conjugates formed with CTL effectors through the LFA-1 pathway(s) alone are therefore insufficient to achieve target cell lysis, even when the appropriate viral target/HLA class I antigen complex is available for specific interaction with the T cell receptor. This observation adds to the body of recent evidence that suggests that LFA-3 binding to CD2 on the T cell surface is not just providing adhesive stabilization of cell-cell contact but is also contributing to functional activation of the effector T cell (20, 21), possibly by facilitating an interaction between CD2 and the T cell receptor complex itself (22). We do not yet know whether the WW1 BL cell genome retains an intact LFA-3 coding sequence, but it may be possible to use these particular cells as recipients in LFA-3 gene transfection experiments to examine the functional role of LFA-3 in mediating T cell recognition and lysis.

Placing the present work in a wider context, we now consider that there are two features of EBV^+ BL tumor cells that guarantee their evasion of EBV-specific T cell surveillance in vivo. The first is a downregulation of several, but not all of those EBV-latent proteins that could provide target antigens for the virus-specific CTL response (14). The second is a downregulation of the cellular adhesion molecules that facilitate CTL/target interactions, and in particular of LFA-3 without which such interactions appear not to achieve target cell lysis. Our present findings on group I and I/II BL cell lines, which we consider close but not necessarily exact parallels of the BL biopsy phenotype in vivo, have led us to examine adhesion molecule expression directly on biopsy cells themselves. In our studies to date, we find that the tumor cells do express very low levels of LFA-1 (see also reference 23), but do not express detectable amounts either of ICAM-1 or of LFA-3 (Gregory, C. D., unpublished results). Such downregulation of ICAM-1 and LFA-3 expression on EBV^+ tumor cells emerging in the face of virus-specific T cell surveillance may reflect the in vivo selection of an immunoresistant malignant clone.

Summary

Some EBV^+ BL cell lines continue to grow as single cells on in vitro passage, show an unusually restricted expression of EBV-latent genes and retain a BL biopsy-like cell surface phenotype (group I/II lines); others change to growth in aggregates, show a broader pattern of virus latent gene expression, and develop a cell surface phenotype more characteristic of EBV-transformed LCL (group III lines). Here we show that the cell surface adhesion molecules LFA-1, ICAM-1, and LFA-3 are expressed at very low levels, if at all, on group I/II lines and are coordinately upregu-

lated as BL lines move towards group III. The change to growth in aggregates reflects the increasing availability of LFA-1 and ICAM-1, the two ligands whose mutual interaction underlies homotypic BL cell adhesion in vitro. The low levels of ICAM-1 and LFA-3 on group I/II BL cell lines are also associated with an impaired ability to interact with EBV-specific CTL in the antigen-independent phase of effector/target conjugation. mAb blocking studies show that the small number of conjugates that are formed with group I/II BL targets involve the LFA-1/ICAM-1 adhesion pathway but not the LFA-3 pathway; in contrast, both pathways contribute to the efficient conjugate formation shown by group III BL or LCL targets. Earlier work identified one group III line, WW1 BL, as unusual since it expressed the full spectrum of EBV-latent proteins yet remained insensitive to lysis by EBV-specific CTL. Here we show that this line has an anomalous pattern of adhesion molecule expression with high levels of LFA-1 and ICAM-1 in the absence of detectable LFA-3. The WW1 BL cells form conjugates with EBV-specific CTL through the LFA-1/ICAM-1 pathway, but in the absence of a target LFA-3/effector CD2 interaction these conjugates do not achieve target cell lysis. This may reflect an important role for target LFA-3 molecules in activating EBV-specific CTL function. From these in vitro studies, we postulate that downregulation of the adhesion molecules LFA-3 and ICAM-1 on EBV^+ BL underlies the ability of the malignant clone to evade EBV-specific T cell surveillance in vivo.

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