JOURNAL OF VIROLOGY, June 1983, p. 800–807
0022-538X/83/060800-08$02.00/0

Vol. 46, No. 3

Characterization of an Antigen Whose Cell Surface Expression Is Induced by Infection with Epstein-Barr Virus

BILL SUGDEN* AND STAN METZENBERG
McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706

Received 8 December 1982/Accepted 8 March 1983

Metabolically labeled monoclonal antibodies were used to measure the number of determinants per cell for an Epstein-Barr virus (EBV) cell surface antigen (EBVCS) (C. Kintner and B. Sugden, Nature [London] **294**:458–460, 1981) which is expressed on the surface of EBV-transformed cells. The antigenic determinants were present approximately 5×10^5 times per in vitro-transformed cell. Immunoprecipitation followed by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate indicated that four independent monoclonal antibodies to EBVCS recognized a protein of 47,000 daltons. The identification of EBVCS isolated from EBV-transformed cells grown in tunicamycin demonstrated that the antigen when isolated from cells grown without this drug was glycosylated. Finally, preclearing experiments with monoclonal antibodies to EBVCS or to HLA (class I products of the human major histocompatibility locus) and to β2-microglobulin indicated that EBVCS is not a major histocompatibility type 1 antigen.

Epstein-Barr virus (EBV) induces the expression of one or more antigens, termed EBV cell surface antigen (EBVCS) or B-last-1, on the surface of adult human B-lymphocytes soon after it infects them (8, 14, 20, 21). In preliminary experiments the antigens recognized by anti-EBVCS antibodies and the anti-B-last-1 antibody appear to be distinct (David Thorley-Lawson, personal communication). When established human lymphoid cell lines have been tested, the surface antigen(s) has been detected only on those which carry EBV (8, 14, 16, 19, 21). The antigen(s) is undetectable on primary human B-lymphocytes, detectable on a small percentage of pokeweed mitogen-induced blasts only after cell sorting (21), and detectable on some samples of peripheral leukocytes from patients with chronic lymphocytic leukemia (21; our unpublished data). This distribution indicates that EBVCS and B-last-1, although induced by EBV, are probably not encoded by the virus. This antigen(s) is expressed uniformly on EBV-transformed cells and therefore is not the membrane antigen, which is found only on productively infected cells (6). The EBVCS (B-last-1) antigen(s) is often expressed at diminished levels in Burkitt's lymphoma cell lines (8, 14, 20, 21) and is undetectable in some of them (8, 14). These latter observations are consistent with the antigen(s) being involved in specific T-cell-mediated cytotoxic responses directed against EBV-infected cells in vivo; that is, EBVCS or B-last-1 is a candidate for the lymphocyte-determined membrane antigen (19) found on EBV-transformed cells. Cells that express less of an antigen required for stimulation of or recognition by cytotoxic effector cells presumably would have an increased chance of proliferating in vivo. Compelling experiments which associate EBVCS (B-last-1) with or dissociate it from the lymphocyte-determined membrane antigen are difficult and have not yet been presented.

We have characterized the EBVCS antigen with five independent monoclonal antibodies. EBVCS is a prevalent, cell surface glycoprotein of approximately 47,000 daltons. It does not coprecipitate with β2-microglobulin and does not cross-react with an antibody to a framework determinant of HLA molecules.

MATERIALS AND METHODS

Cell lines. All cell lines were propagated in RPMI 1640 plus 10% fetal bovine serum in stationary-suspension cultures or in roller bottles.

Hybridomas. Four of the five hybridomas that we isolated have been described (8). The fifth was isolated as described previously (8), but we used SP2/0 as a fusion partner. This hybridoma secretes an immunoglobulin G (IgG) monoclonal antibody. We also used two additional hybridomas not isolated by us, BBM.1 and PA2.6, which secrete antibodies to β2-microglobulin and HLA-A,B,C (class I products of the human major histocompatibility locus), respectively (2). The hybridomas were propagated in vitro in roller bottles or in vivo as ascites tumors in syngeneic mice.

Metabolic labeling. Monoclonal antibodies were labeled metabolically (3) by incubating the hybridoma cells in RPMI 1640 medium lacking lysine or leucine and in the absence of serum. Hybridoma cells (1×10^7 to 2×10^7) were incubated in 10 ml of this medium supplemented with 1 mCi of [^{3}H]lysine or [^{3}H]leucine (50 to 100 Ci/mmol) for 24 h. The supernatant medium

was removed, and an equivalent number of fresh hybridoma cells was added to the supernatant medium for an additional 24 h. This medium was removed and served as a source for ^{3}H-labeled antibodies. The ^{3}H-labeled antibodies were isolated by clarifying the medium by centrifuging at 275 × *g* for 10 min, centrifuging at 180,000 × *g* for 30 min, and then precipitating the antibodies (at 4°C overnight) with ammonium sulfate at 50% of saturation. The pellet was dissolved in a small volume of phosphate-buffered saline (PBS) and separated from unincorporated label with Sephadex G-75. Between 100 and 500 μg of a ^{3}H-labeled monoclonal antibody with a specific activity of approximately 10^5 cpm/μg was recovered from each experiment.

Binding of metabolically labeled monoclonal antibodies. Cells were washed twice in PBS, and their numbers and viability were determined by eosin exclusion. If the percentage of cells that were viable was below 90%, the cells were additionally banded on Renografin-Ficoll for selection of viable cells. Between 5×10^5 and 1×10^7 cells were added to a 50-μl reaction mixture containing 0.15 M NaCl, 5% fetal calf serum, 0.4 mg of Human Gamma Globulins (Miles Laboratories, Inc.) per ml, 0.1% sodium azide, and 0.01 to 500 μg of a tritiated monoclonal antibody with a specific activity ranging from 1×10^2 to 3×10^5 cpm/μg. (The different specific activities were obtained by mixing labeled and unlabeled antibodies and used to permit detectable signals and a reasonable cost.) The reaction mixture was incubated at 0°C for 1 to 3 h and then washed twice in PBS containing 5% calf serum and 0.4 mg of Human Gamma Globulins per ml at 0 to 4°C. The cells were banded on a 1-ml layer of Renografin-Ficoll and then washed again as described above. A portion (95%) of the cells was then placed in a scintillation vial and solubilized with 5 ml of Aquassure. The remaining 5% were lysed with a drop of 10% sodium dodecyl sulfate (SDS) and analyzed for DNA content by the method of Labarca and Paigen (10). DNA content was used as a measure of cell recovery, and recovery was typically 80 to 90%.

Labeling of viable cells with ^{125}I. Cells (1×10^7 to 5×10^7) in 1 ml of PBS containing 100 μg of lactoperoxidase (Boehringer Mannheim Corp.) and 2 mCi of Na^{125}I (Amersham Corp.) per 10^7 cells were incubated at 0°C for 5 min with 50 μl of 0.06% H_2O_2. An additional 50 μl of 0.06% H_2O_2 was added for an additional 5 min, and the cells were separated from free ^{125}I by being washed four times in 10 m of PBS–5% calf serum–0.01 M KI. The labeled cells were lysed by occasional vortexing in PBS–0.05% Nonidet P-40–1% Trasylol at 0°C for 10 min. The nuclei were removed by centrifugation, and the supernatant served as the starting material for immunoprecipitations.

Immunoprecipitation and gel electrophoresis. The immunoprecipitation of antigens from labeled cell extracts and their resolution on SDS-polyacrylamide gels were performed generally as described previously (4). To remove labeled human immunoglobulin from the cell extracts, a preclearing step was performed. Extracts were incubated with 50 μg of affinity-purified rabbit anti-human $(Fab')_2$ antibodies per 2×10^7 cells for 60 min at 0°C and then with 250 μl of a 10% suspension of formaldehyde-fixed *Staphylococcus aureus* for 15 min at 0°C. This suspension was clarified by centrifugation at 30,000 rpm for 30 min. The

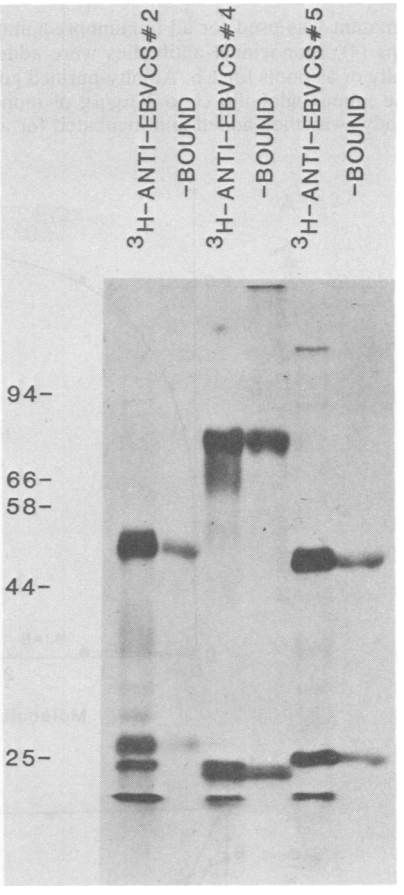

FIG. 1. Analysis of metabolically labeled anti-EBVCS antibodies before and after binding to cells. Labeled antibodies 2 (IgG), 4 (IgM), and 5 (IgG) were incubated in a binding mix with 10^7 cells (clone 11/17-1), the cells were washed three times, a portion was counted to determine the amount bound, the cells were lysed in PBS–0.5% Nonidet P-40, and the antibodies were immunoprecipitated. All of antibodies 2 and 4 and 50% of antibody 5 bound to the cells were recovered in the immunoprecipitates. The preparations of the antibodies used for the experiment (unbound) and those recovered in the immunoprecipitates (bound) were resolved on a 10% polyacrylamide gel containing SDS and were detected by fluorography. Molecular mass markers (numbers on left, $\times 10^3$) were phosphorylase α (94,000 daltons), bovine serum albumin (66,000 daltons), glutamate dehydrogenase (58,000 daltons), ovalbumin (44,000 daltons), and chymotrypsinogen (25,000 daltons). Only signals from IgG heavy chains (55,000 daltons), IgM heavy chains (78,000 daltons), and light chains (25,000 daltons) are found in the bound samples, indicating that the labeled species bound to cells are immunoglobulin molecules. The additional bands (near 25,000 daltons) present in the unbound antibody probably represent immunoglobulin light chains secreted by the fusion partner NS-1 used in the creation of the hybridomas anti-EBVCS 2 and 4. SP2/0, which does not express light chains, was used to create anti-EBVCS 5. The fastest-migrating bands in the unbound lanes are at the pH front of the gel.

supernatant was used for all immunoprecipitation reactions (4). Monoclonal antibodies were added individually or as pools for 1 h. Affinity-purified goat anti-mouse immunoglobulin (1 to 2 μg/μg of monoclonal antibody) was then added and incubated for an additional 1 h. Finally, fixed *S. aureus* (1 to 2 μl of a 10% suspension per μg of total antibody) was added for 15 min, pelleted, and washed four times as described previously (4). Immunoprecipitates were resolved on 10% linear or 10 to 15% gradient polyacrylamide gels

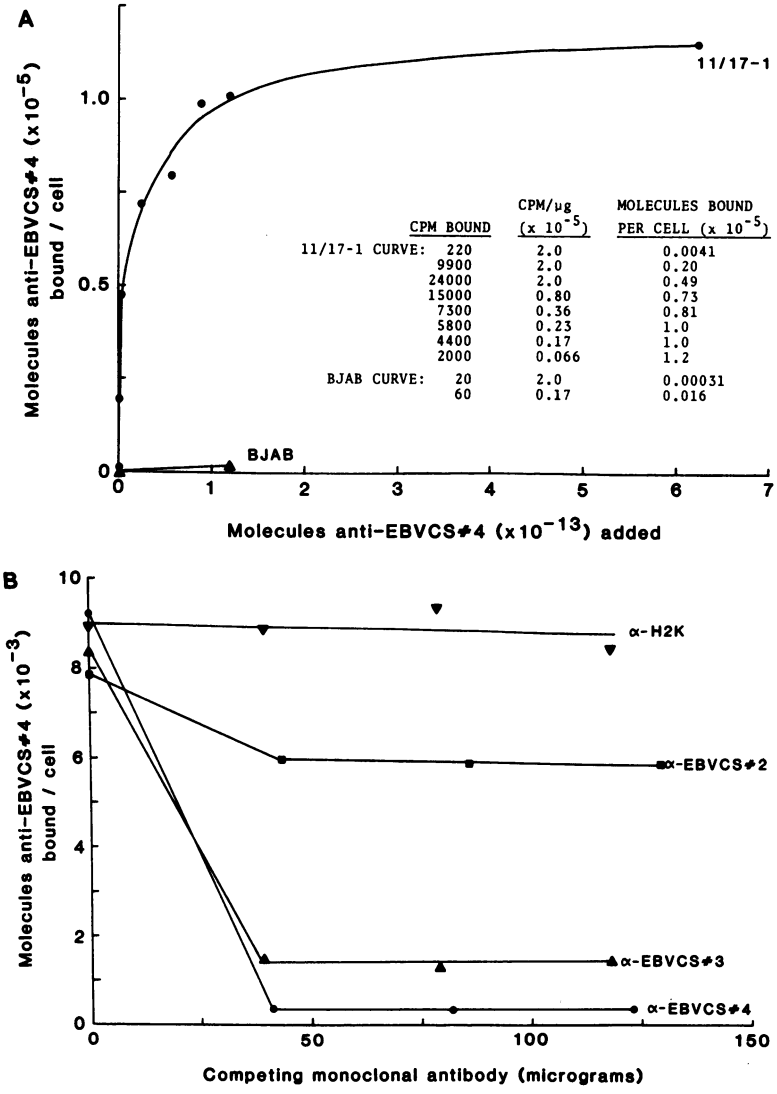

FIG. 2. Binding of antibodies to viable cells. (A) Titration of EBVCS 4 determinants with the ^{3}H-labeled monoclonal antibody. Specific binding of anti-EBVCS 4 (an IgM antibody) to EBV-transformed cells (clone 11/17-1) was measured as a function of total anti-EBVCS 4 added. Depicted is one representative experiment. The counts (CPM) bound above background (20 cpm) are given in the inset along with the specific activity of the antibody (counts per minute per microgram) and the calculated values for the number of molecules bound per cell. A total of 2×10^6 11/17-1 cells and 1.3×10^6 BJAB cells (an EBV-negative cell line) were used per point. There is a plateau in binding at 10^5 anti-EBVCS 4 molecules bound per 11/17-1 cell and negligible binding to BJAB cells. (B) Competition between unlabeled monoclonal antibodies and ^{3}H-labeled anti-EBVCS 4 for binding to EBV-transformed cells (clone 11/17-1). A total of 3.3×10^{11} molecules of ^{3}H-labeled anti-EBVCS 4 (0.41 μg at a specific activity of 1.82×10^5 cpm/μg) were tested for binding to 2×10^6 11/17-1 cells, along with increasing amounts of unlabeled monoclonal antibodies anti-H2-K^k, anti-EBVCS 2, anti-EBVCS 3, or anti-EBVCS 4. Binding to clone 11/17-1 produced signals ranging from 190 to 6,440 cpm above background (20 cpm). This level of ^{3}H-labeled antibody was chosen to permit both ready detection of competition and efficient use of the labeled reagent.

containing SDS (11), and the radioactive species were detected by fluorography and autoradiography (1).

RESULTS

The monoclonal antibodies to EBVCS antigen are the only available tools to define this antigen. To characterize both the antibodies and the molecules to which they bind, we measured how many of each of the antibodies bind at saturation to several cell lines and which of the antibodies compete with one another for binding. For simplicity of presentation, we shall term the antibodies anti-EBVCS 1 through 5 and term the antigen EBVCS.

Binding of anti-EBVCS to cells. To confirm that it is the labeled antibodies and not contaminants that bind to cells, three different preparations of labeled antibodies were incubated with viable cells, the cells were washed three times and lysed, and the antibodies were immunoprecipitated. Between 50 and 100% of the label bound to the cells was recovered in the immunoprecipitates. The immunoprecipitates and their respective antibody preparations were resolved on SDS-polyacrylamide gels (Fig. 1). The major radioactive species in the antibody preparations were the immunoglobulin heavy and light chains. The only obvious radioactive species bound to the cells were the immunoglobulin molecules, indicating that the measurement of ^3H counts per minute bound reflects the binding of the monoclonal antibodies themselves and not some labeled contaminant.

The binding of the five ^3H-labeled antibodies to viable cells was measured over as large a range of concentrations as practicable. A typical experiment showing both the value for binding at apparent saturation and competition for binding of one antibody by others is shown in Fig. 2. Binding was complete within 15 min at 0°C (data not shown). Not only did homologous antibodies compete for binding, but two pairs of antibodies (anti-EBVCS 2 and 5 [data not shown] and anti-EBVCS 3 and 4) also competed for binding (Fig. 2B). Saturation binding values for the different anti-EBVCS antibodies ranged from 1×10^5 to 3×10^6 for EBV-positive cells (Table 1).

Recognizing that EBVCS determinants constitute a major cell surface antigen on EBV-transformed cells, we used the antibodies to characterize the antigen. Surface proteins of EBV-transformed cells were isotopically labeled with ^{125}I, lactoperoxidase, and H_2O_2, solubilized, and immunoprecipitated, and those immunoprecipitated were resolved on polyacrylamide gels containing SDS.

EBVCS is a protein of 47,000 daltons. The five antibodies were used separately to immunoprecipitate labeled surface proteins of cells from a clone of EBV-transformed cells. Four of the five immunoprecipitates after resolution on gels and detection by autoradiography contained a major band with a mobility of approximately 47,000 daltons (Fig. 3). The fifth immunoprecipitate, that of anti-EBVCS 3, had no major signal, although there was competition between this antibody and anti-EBVCS 4 for binding to viable

TABLE 1. Enumeration of cell surface determinants with ^3H-labeled monoclonal antibodies

Cell lines[a]	No. of ^3H-antibody molecules per cell bound at saturation[b]						
	EBVCS:					HLA-A,B,C	β2-microglobulin
	1	2	3	4	5		
11/17-1	2×10^5	2×10^6	2×10^5	2×10^5	2×10^5	3×10^6	3×10^6
THL-B1	ND[c]	ND	ND	9×10^4	ND	1.5×10^6	2×10^6
721	ND	ND	ND	2×10^5	1×10^5	3×10^6	3×10^6
721-61A°B°	ND	3×10^6	ND	3×10^5	ND	1.5×10^5	2.5×10^5
BJAB	<6,000	<100	ND	<500	<600	4.5×10^5	4×10^5
RAMOS	<6,000	ND	<300	ND	<4,000	ND	ND

[a] Cell lines tested for levels of ^3H-labeled antibodies bound at saturation included four clones of cells transformed in vitro by EBV (clones 11/17-1, THL-B1, 721, and 721-61A°B°). These cell lines were derived from three donors (7, 18). Clone 721-61A°B° was selected from a mutagenized sample of clone 721 to be resistant to anti-HLA-A,B typing antisera plus complement (7). Two additional cell lines (BJAB and RAMOS) which are EBV negative and were established from Burkitt's lymphoma biopsies (9, 12) were tested.

[b] The determinants recognized by the ^3H-labeled monoclonal antibodies were on the EBVCS molecule (EBVCS 1 through 5), on the HLA framework (HLA-A,B,C), and on the β2-microglobulin molecules (β2-microglobulin). The number of molecules of each ^3H-labeled antibody bound at saturation was measured by using antibody preparations with specific activities of 1×10^3 to 5×10^5 cpm/μg. Saturation values were constant over 2- to 10-fold ranges of antibody concentrations and occurred usually when there was a 100- to 1,000-fold excess of unbound to bound antibody. Counts bound to the 10^6 cells tested ranged between 500 and 50,000 cpm above a background of 20 cpm. Anti-EBVCS antibodies bound to the BJAB and RAMOS cell lines gave counts of less than 200 cpm above background.

[c] ND, Not determined.

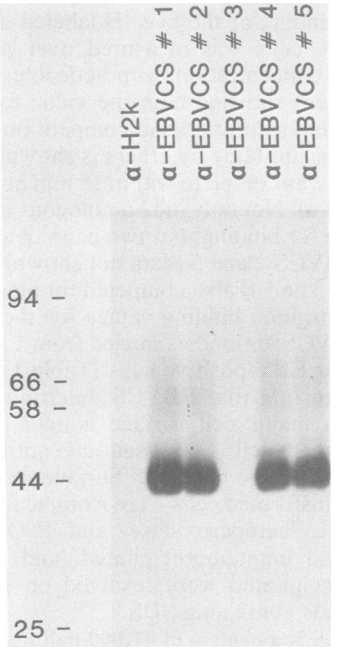

FIG. 3. Immunoprecipitation of EBVCS with anti-EBVCS monoclonal antibodies. Surface proteins of cells (clone 11/17-1) were labeled with $Na^{125}I$, solubilized, and immunoprecipitated with anti-EBVCS 1 through 5 and a monoclonal antibody to murine H2-K as a control. After preclearing, 0.7 to 0.8% of the incorporated label was precipitated by anti-EBVCS 1, 2, 4, and 5; 0.07 to 0.09% was precipitated by anti-EBVCS 3 and anti-H2-K antibodies. The immunoprecipitates were resolved on a 10% polyacrylamide gel containing SDS and visualized by autoradiography. The molecular mass markers included were the same as in Fig. 1. EBVCS in the gel has an apparent molecular mass of 47,000 daltons.

cells (Fig. 2 and 3; Table 1). That anti-EBVCS 3 fails to precipitate a solubilized antigen but does bind to living cells may indicate that the determinants to which it binds are lost upon solubilization of the EBVCS antigen.

EBVCS is a glycoprotein. A clone of EBV-transformed cells was propagated in tunicamycin (1 μg/ml) for increasing times. Surface proteins of these treated cells were labeled, and extracts were made, immunoprecipitated, resolved on gels, and detected by autoradiography. HLA, a prevalent cell surface glycoprotein (17), was analyzed in parallel in the same lysates. By 24 h of incubation in tunicamycin, a faster-migrating signal could be observed for both EBVCS and the heavy chain of HLA (Fig. 4). These signals most likely reflect the synthesis and insertion into the cell membrane of proteins which lack the high-mannose residues whose addition is blocked by tunicamycin (5). The fully glycosylated forms of EBVCS and HLA diminished, presumably because of turnover as the cells were maintained for as long as 48 h (Fig. 4). This experiment indicates that EBVCS is a glycoprotein synthesized by the cell and is not simply adsorbed onto the cell surface.

EBVCS is not associated with β2-microglobulin. EBVCS is a prevalent cell surface glycoprotein with a molecular weight of approximately 47,000 and is a candidate for being the lympho-

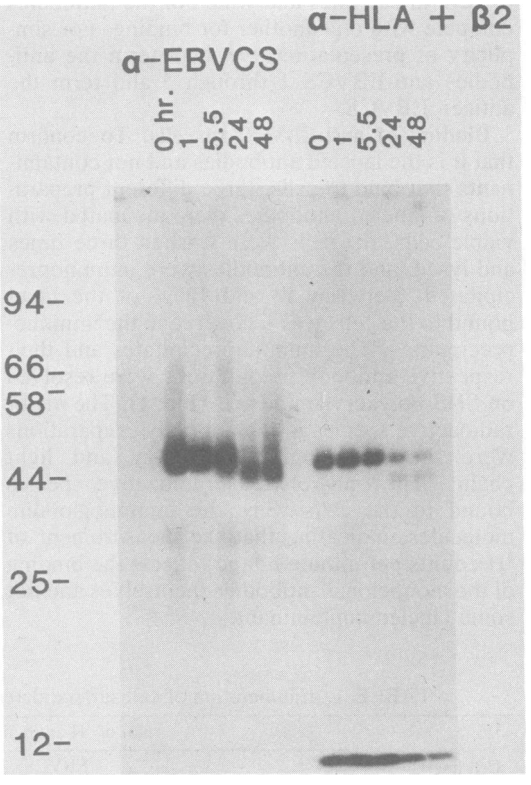

FIG. 4. Effect of tunicamycin on the expression and structure of EBVCS and HLA antigens. Cells (clone 11/17-1) were incubated in tunicamycin (1 μg/ml) for 0, 1, 5.5, 24, and 48 h. Surface proteins of each sample of cells were labeled with $Na^{125}I$, solubilized, and immunoprecipitated with a pool of anti-EBVCS 1, 4, and 5 antibodies. The supernatants of these immunoprecipitations were used as sources of HLA, which was immunoprecipitated with a pool of anti-HLA and anti-β2-microglobulin antibodies. The immunoprecipitates were resolved in a 10 to 15% gradient polyacrylamide gel containing SDS. The molecular weight markers were those used in Fig. 1 and cytochrome *c* (11,500 daltons). Apparent molecular weights (in daltons) are as follows: the fully glycosylated EBVCS, 47,000; the less-glycosylated form which appears in the 5.5- (visible on longer exposures), 24-, and 48-h samples, 44,000; the fully glycosylated heavy chain of HLA, 45,000; the less-glycosylated heavy chain which appears in the 5.5- (visible on longer exposures), 24-, and 48-h samples, 43,000; β2-microglobulin, 11,000.

cyte-determined membrane antigen. These characteristics indicate that it might be a type 1 major histocompatibility antigen; that is, it might be associated with β2-microglobulin and even be a modified form of HLA itself. One piece of evidence against this hypothesis is that anti-EBVCS antibodies bound as well to an HLA-A,B-negative variant cell line, 721-61A°B°, as they did to the HLA-A,B-positive parent, 721 (Table 1).

To test in a different manner for an association among EBVCS, HLA, and β2-microglobulin, we removed from one labeled-cell lysate either EBVCS or HLA-A,B,C plus β2-microglobulin. We used each of these precleared lysates as a source for the other antigen, which was detected by immunoprecipitation, gel electrophoresis, and autoradiography (Fig. 5). One portion of the lysate precleared of EBVCS (sup I of Fig. 5) contained no EBVCS but just as much HLA plus β2-microglobulin as found in the portion first precipitated with anti-HLA and anti-β2 microglobulin (II of Fig. 5). Conversely, when one portion of the lysate was precleared of HLA and β2-microglobulin, it was found to contain no HLA and no β2-microglobulin (sup II of Fig. 5) but just as much EBVCS as in the portion first precipitated with anti-EBVCS (I of Fig. 5). These data indicate that EBVCS is not coprecipitated with β2-microglobulin and, within the limits of the monoclonal antibodies used, does not cross-react with an antibody to an HLA framework determinant.

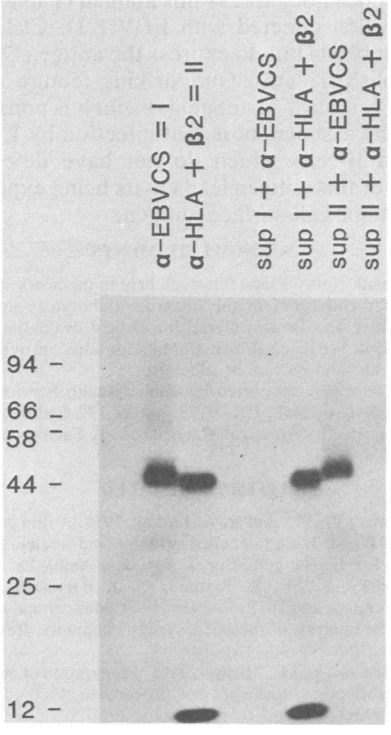

FIG. 5. Lack of association of EBVCS with β2-microglobulin. Surface proteins of one clone of cells (THL-B1) were labeled with $Na^{125}I$, solubilized, and divided into two portions. One portion (α-EBVCS = I) was immunoprecipitated with a pool of anti-EBVCS 1, 4, and 5 antibodies. A 500-μg portion of this pool of antibodies, which is 10 times that used in the experiments shown in Fig. 3 and 4, was used for an extract of 10^7 cells. A total of 0.6% of the incorporated label was precipitated by this pool. The second portion (α-HLA + α-β2 = II) was immunoprecipitated with a pool of anti-HLA and anti-β2-microglobulin antibodies. Again 500 μg of a pool of these antibodies was used, which is 10 times that used in the experiment shown in Fig. 4. A total of 1.1% of the incorporated label was precipitated by this pool. The supernatants from these two immunoprecipitations were each divided into two portions. One portion of the supernatant precleared of EBVCS was immunoprecipitated with 50 μg of a pool of anti-EBVCS antibodies (sup I + α-EBVCS); the other was immunoprecipitated with 50 μg of a pool of anti-HLA plus anti-β2-microglobulin antibodies (sup I + α-HLA + α-β2). One portion of the supernatant precleared of HLA and β2-microglobulin was immunoprecipitated with 50 μg of a pool of anti-HLA and anti-β2-microglobulin antibodies (sup II + α-HLA + α-β2); the other was immunoprecipitated with 50 μg of a pool of anti-EBVCS antibodies (sup II + α-EBVCS). All immunoprecipitates were resolved on a 10 to 15% gradient polyacrylamide gel containing SDS and were visualized by autoradiography. The molecular mass markers were those used in Fig. 4. Preclearing of a lysate of EBVCS (as evidenced by a second, negative immunoprecipitation with anti-EBVCS antibodies) does not remove HLA or β2-microglobulin. Conversely, preclearing of a lysate of HLA and β2-microglobulin (as evidenced by a second, negative immunoprecipitation with anti-HLA and anti-β2-microglobulin antibodies) does not remove EBVCS.

DISCUSSION

We have used five independent monoclonal antibodies to enumerate and characterize an antigen whose expression at the cell surface is induced in human B-lymphocytes by infection with EBV. Binding of the antibodies at high concentrations to EBV-transformed cells indicates that there are approximately 5×10^5 determinants for each of the antibodies per cell (Fig. 2 and Table 1). If we make the assumption that there is one determinant for each of the antibodies per antigen, then there are approximately 5×10^5 antigen molecules per cell. Similar experiments indicate that there are approximately 3×10^6 HLA-A,B,C heavy-chain molecules per cell (Table 1), which agrees with published values (2, 13). That there are roughly similar levels of EBVCS and HLA-A,B,C per cell is corroborated by the experiments in which cell surface proteins were labeled with ^{125}I and

exhaustively immunoprecipitated (Fig. 5). A total of 0.6% of the labeled proteins was immunoprecipitated by a pool of anti-EBVCS antibodies, whereas 1.1% of the labeled proteins was immunoprecipitated by the anti-HLA plus anti-β2 microglobulin antibodies. Recognizing that the number and accessibility of tyrosine residues in the different surface antigens probably differ, the closeness of the values for the percentage of ^{125}I-labeled protein precipitated indicates that the numbers of each antigen per cell are roughly similar.

The unlabeled homologous antibodies and, in two cases, heterologous antibodies can compete with the metabolically labeled antibodies for binding to EBVCS. The failure to compete among three of the antibodies indicates that an IgM molecule of 750,000 daltons on binding to an antigen of 47,000 daltons does not inhibit binding of an IgG molecule of 150,000 daltons. This conclusion does illustrate that recognition of a surface determinant by an antibody or even by a T-cell may be difficult to prevent by the binding of one monoclonal antibody to a site near that surface determinant. The failure of a monoclonal antibody to block T-cell killing, therefore, cannot easily be used to test whether the antigen to which the antibody binds is distinct from the structure recognized by the T-cell (14, 16).

Four of our five antibodies precipitate a surface protein of approximately 47,000 daltons, as do the other three reported antibodies with similar specificities (14, 16, 20, 21). In addition, the distribution of the antigens among cell lines, when measured both qualitatively and quantitatively, is similar (Table 1) (8, 14, 16, 20, 21). However, in preliminary experiments EBVCS and B-last-1 appear to be distinct antigens (David Thorley-Lawson, personal communication).

The EBVCS antigen is a glycoprotein (Fig. 4); in the presence of tunicamycin it turns over at the cell surface more than once per 24 h (Fig. 4). If it turns over at the same rate in the absence of the drug, then EBVCS turns over faster than the generation time of the cell, which is approximately 24 h. This turnover rate indicates that EBVCS is either shed from cells or internalized and catabolized.

The EBVCS antigen is not a type 1 major histocompatibility antigen; that is, it is not detectably associated with β2-microglobulin (Fig. 5). In addition, the antigen is not detectably associated with HLA-A,B,C (Fig. 5). Similar experiments with the adenovirus glycoprotein E3/19K indicate that it is associated with HLA-A,B,C antigens (15), but no other virus-associated antigens which are candidates for being recognized by cytotoxic T-lymphocytes have been shown to be physically associated with HLA antigens (15). The only reasons still to suspect that EBVCS may be the lymphocyte-determined membrane antigen are its conspicuous expression on EBV-transformed cells (Table 1) and its reduced expression on Burkitt's lymphoma cell lines (8, 14, 20, 21).

Recent findings indicate that B-last-1 and perhaps EBVCS, which behaves so similarly, is probably not encoded by EBV and may be specific for a differentiated B-lymphoblast (21). This assignment, although intriguing, does not yet easily mesh with all of the observations on this antigen(s). An EBV-negative Burkitt's lymphoma cell line, RAMOS, which is a B-cell blast, does not express this antigen (Table 1) but does when infected with EBV (21). CLL cells are not blasts but do express the antigen (21; our unpublished data). One striking feature of the EBVCS (B-last-1) antigen(s) which is consistent with the assignment is that infection by EBV of human B-cells which do not have detectable levels of this antigen leads to its being expressed as a major cell surface antigen.

ACKNOWLEDGMENTS

We thank Kathy Paape for much help in this work. We also thank our colleagues in the McArdle Laboratory and Aida Metzenberg and Donata Oertel for critical evaluation of the manuscript. We thank Robert DeMars for kindly providing the 721 cell line and its variant subclones.

This work was supported by Public Health Service grants CA-09135, CA-22443, CA-97175, and 5 T32 GM07215. B.S. was supported by American Cancer Society Faculty Research Award FRA-023.

LITERATURE CITED

1. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
2. **Brodsky, F. M., P. Parham, C. J. Barnstable, M. J. Crumpton, and W. F. Bodmer.** 1979. Monoclonal antibodies for analysis of the HLA system. Immunol. Rev. **47**:3–61.
3. **Galfre, G., and C. Milstein.** 1981. Preparation of monoclonal antibodies: strategies and procedures. Methods Enzymol. **73**:3–46.
4. **Grunwald, D. J., B. Dale, J. Dudley, W. Lamph, B. Sugden, B. Ozanne, and R. Risser.** 1982. Loss of viral gene expression and retention of tumorigenicity by Abelson lymphoma cells. J. Virol. **43**:92–103.
5. **Heifetz, A., R. W. Keenan, and A. D. Elbein.** 1979. Mechanism of action of tunicamycin on the UDP-GlcNAc:dolichyl-phosphate GlcNAc-1-phosphate transferase. Biochemistry **18**:2186–2192.
6. **Henle, W., and G. Henle.** 1979. Seroepidemiology of the virus, p. 61–78. *In* M. A. Epstein and B. G. Achong (ed.), The Epstein-Barr virus. Springer-Verlag, Berlin.
7. **Kavathas, P., F. H. Bach, and R. DeMars.** 1980. Gamma ray-induced loss of expression of HLA and glyoxalase I alleles in lymphoblastoid cells. Proc. Natl. Acad. Sci. U.S.A. **77**:4251–4255.
8. **Kintner, C., and B. Sugden.** 1981. Identification of antigenic determinants unique to the surfaces of cells transformed by Epstein-Barr virus. Nature (London) **294**:458–460.
9. **Klein, G., B. Giovanella, A. Westman, J. S. Stehlin, and D. Mumford.** 1975. An EBV-genome-negative cell line established from an American Burkitt lymphoma; receptor characteristics. EBV infectibility and permanent conver-

sion into EBV-positive sublines by *in vitro* infection. Intervirology 5:319–334.
10. **Labarca, C., and K. Paigen.** 1980. A simple, rapid, and sensitive DNA assay procedure. Anal. Biochem. 102:344–352.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680–685.
12. **Menezes, J., W. Leibold, G. Klein, and G. Clements.** 1975. Establishment and characterization of an Epstein-Barr virus (EBV)-negative lymphoblastoid B-cell line (BJA-B) from an exceptional EBV-genome-negative African Burkitt's lymphoma. Biomedicine 22:276–284.
13. **Parham, P., C. J. Barnstable, and W. F. Bodmer.** 1979. Use of a monoclonal antibody (W6/32) in structural studies of HLA-A,B,C antigens. J. Immunol. 123:342–349.
14. **Rowe, M., J. E. K. Hildreth, A. B. Rickinson, and M. A. Epstein.** 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 29:373–381.
15. **Signäs, C., M. G. Katze, H. Persson, and L. Philipson.** 1982. An adenovirus glycoprotein binds heavy chains of class I transplantation antigens from man and mouse. Nature (London) 299:175–178.
16. **Slovin, S. F., D. M. Frisman, C. D. Tsoukas, I. Royston, S. M. Baird, S. B. Wormsley, D. A. Carson, and J. H. Vaughan.** 1982. Membrane antigen on Epstein-Barr virus-infected human B cells recognized by a monoclonal antibody. Proc. Natl. Acad. Sci. U.S.A. 79:2649–2653.
17. **Strominger, J. L., D. L. Mann, P. Parham, R. Robb, T. Springer, and C. Terhorst.** 1977. Structure of HL-A A and B antigens isolated from cultured human lymphocytes. Cold Spring Harbor Symp. Quant. Biol. 41:323–329.
18. **Sugden, B., M. Phelps, and J. Domoradzki.** 1979. Epstein-Barr virus DNA is amplified in transformed lymphocytes. J. Virol. 31:590–595.
19. **Svedmyr, E., and M. Jondal.** 1975. Cytotoxic effector cells specific for B cell lines transformed by Epstein-Barr virus are present in patients with infectious mononucleosis. Proc. Natl. Acad. Sci. U.S.A. 72:1622–1626.
20. **Thorley-Lawson, D. A., C. M. Edson, and K. Geilinger.** 1982. Epstein-Barr virus antigens—a challenge to modern biochemistry. Adv. Cancer Res. 36:295–348.
21. **Thorley-Lawson, D. A., R. T. Schooley, A. K. Bhan, and L. M. Nadler.** 1982. Epstein-Barr virus superinduces a new human B cell differentiation antigen (B-last-1) expressed on transformed lymphoblasts. Cell 30:415–425.