SURFACE MARKERS ON HUMAN B AND T LYMPHOCYTES

II. Presence of Epstein-Barr Virus Receptors on B Lymphocytes*

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The association of the lymphotropic Epstein-Barr virus (EBV)[1] with certain human lymphoproliferative diseases and its ability to transform normal lymphocytes into established in vitro lines have prompted the present investigations. Primary EBV infection of certain age groups causes infectious mononucleosis, a self-limiting lymphoproliferative disease (1). Furthermore, the regular association of EBV with Burkitt's lymphoma cells in vivo has led to serious consideration of this virus, possibly some special subtype, as the etiological agent of the disease, alone or together with cofactors (2).

Three lymphotropic herpesviruses are known to cause neoplastic disease in animals: Marek's disease herpesvirus (MDHV) in chickens, and herpesvirus *Saimiri* (HVS) and herpesvirus *Ateles* (HVA) in certain new-world monkeys. Like EBV, the proliferative interaction between the genomes of these herpesviruses and their lymphoid target cells is dependent on a nonpermissive relationship (3–5). It is a so far unchallenged rule for all known herpesviruses that the synthesis of viral proteins, including early products made before viral DNA synthesis, leads to an irreversible damage and eventually death of the host cell. MDHV and HVS can induce a lytic cycle in epithelial cells and fibroblasts, and their proliferation-inducing effect is limited to lymphoid cells. For EBV, the site of the lytic viral replication is not yet known, whereas the nonpermissive, proliferative interaction with the lymphoid cells has been clearly demonstrated. It is important to define the human lymphoid cells that are the target for the proliferation-inducing effect of EBV in vitro and in vivo.

In the mouse and in man the lymphoid system is divided into two major compartments, thymus dependent (T) and bone marrow dependent (B), respectively. Surface markers and purification procedures now exist that allow the isolation and

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[1] *Abbreviations used in this paper:* BSS, balanced salt solution; E, sheep erythrocytes; EA, SRBC sensitized with 7S rabbit antibodies; EAC, SRBC sensitized with 19S rabbit antibodies and mouse complement; EBNA, Epstein-Barr virus-associated nuclear antigen; EBV, Epstein-Barr virus; F-Abwao, fluorescein isothiocyanate-conjugated MA-positive IgG; HVS, herpesvirus *Saimiri*; MA, EBV-induced membrane antigen; MDVH, Marek's disease herpesvirus; TRITC-Abwao, tetramethylrhodamine isothiocyanate-conjugated MA-positive IgG.

classification of these subpopulations in the human system (6–8). The most reliable marker for peripheral B lymphocytes seems to be the presence of easily detectable surface-bound immunoglobulin molecules that are believed to function as antigen-specific receptors. EBV-carrying in vitro lines are known to have surface-bound immunoglobulin (9) and it is therefore probable that they have originated from B lymphocytes. The issue has been somewhat confused, however, by the recent finding of surface-bound immunoglobulin on T lymphocytes by some investigators (10–12) although in much smaller quantities and possibly with another localization in the cell membrane. If this is correct, EBV transformation of T lymphocytes might conceivably induce some steric change that could make these molecules more easily detectable. This might lead to a misinterpretation of the actual origin of the transformed cells.

On the basis of these considerations, it seemed logical to combine an effort to classify EBV-carrying in vitro lines with regard to B- and T- cell markers, with studies of the presence of virus receptors on normal peripheral B and T lymphocytes. Since EBV-induced lymphocyte transformation can be only neutralized by antibodies against the viral envelope,[2] the target lymphocyte must carry specific receptors that combine with some envelope component(s). Such receptors can be demonstrated on the cell surface is most established, EBV-carrying nonproducer lines (13). We have previously studied virus adsorption to the cell surface in such lines by staining the viral envelope antigens with fluorescein-conjugated antibody immediately after infection. We tried to apply the same method to peripheral lymphocytes but found that they adsorbed much less virus than the established cell lines. The fluorescence readings became less clear and it was difficult to estimate the number of the receptor-positive cells. In the present study, we have therefore reversed the system. Virus receptor-positive normal lymphocytes were specifically adsorbed to the surface of virus-producing cells in the P3HR-1 line. This approach was based on the following considerations:

P3HR-1 is a high virus-producer clone, derived from the Jijoye line (14). Approximately 5% of the cells are in the productive cycle under ordinary culture conditions. These cells accumulate in rapid succession, early antigen, viral capsid antigen, and mature virus particles (15, 16). In parallel, viral envelope antigens appear in the cell membrane in large quantities (17). They share antigenic specificity(ies) as MA, the EBV-induced membrane antigen (18, 19), can can be demonstrated by staining live P3HR-1 cell suspensions with anti-MA-positive, fluorescein-conjugated human IgG (17). Unlike most other EBV producer lines where cells tend to accumulate in the early phases of the viral cycle, with an excess of MA + VCA − cells (17), P3HR-1 consists essentially of an MA + VCA + minority and an MA − VCA − majority, with little or no intermediate forms.

Our approach was based on the consideration that the viral envelope components incorporated into the membrane of the virus-producing cells may attach to the viral receptors on the surface of receptor-bearing cells, including normal lymphocytes. This was found to be the case, since all MA-positive cells and none of the MA-negative cells formed rosettes with normal lympho-

[2] de Schryver, A., G. Klein, D. J. Moss, J. H. Pope, G. Henle, W. Henle, J. Hewetson, and G. Rocchi. Manuscript under preparation.

cyte suspensions. In this paper, we have studied the question whether B or T lymphocytes are responsible for the binding and whether rosette formation could be specifically blocked by anti-MA-positive sera.

Materials and Methods

Preparation of E, EA, and EAC.—Sheep erythrocytes (E) were stored in Alsever's solution at 4°C. Before use they were washed three times in balanced salt solution ([BSS] pH 7.3) and adjusted to a 1% solution.

SRBC sensitized with 7S rabbit antibodies (EA) were prepared as follows: A rabbit anti-SRBC serum was fractionated on a Sephadex G-200 column into 19S and 7S fractions. Washed SRBC were shaken for 1 h at room temperature with the lowest dilution of 7S antibodies not giving hemagglutination and then incubated for another hour at 4°C. The sensitized SRBC were washed three times in BSS before use.

SRBC sensitized with 19S rabbit antibodies and mouse complement (EAC) were prepared as follows: Washed 5% SRBC were shaken for 1 h at room temperature with the lowest dilution of 19S antibodies that failed to hemagglutinate and were then washed three times. An equal volume of 5% 19S-treated SRBC was then mixed with an equal volume of fresh A-strain mouse serum diluted 1:2. The serum was adsorbed with SRBC once before use. The cells were shaken horizontally at 37°C in a water bath for 15 min and washed three times. EAC incubated with heat-inactivated mouse serum for 30 min at 56°C served as the control.

Tests for Surface Markers on Human Lymphoblastoid Lines.—

E binding: 100 μl of a 1% SRBC solution was mixed with 100 μl of a cell suspension containing 4×10^6 cells/ml. The cells were spun down in a small plastic tube. The pellet was incubated at 37°C for 15 min and then at 4°C for an additional hour, whereafter the cells were gently lifted onto a glass slide and examined under the microscope.

EA binding: The lymphoblastoid cells and the indicator cells were spun down to a pellet as when testing for E-binding capacity. The pellet was then incubated at 37°C for 30 min and shaken apart. EA-binding cells were counted in a Bürker chamber.

EAC binding: Same as for EA.

Surface immunoglobulin: A commercially available polyvalent goat antihuman immunoglobulin serum was used in a direct immunofluorescent assay (Hyland 072-202; Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.). The conjugate was used in a dilution of 1:10. This dilution failed to stain a control cell line (MOLT-4, received from Dr. Minowada, Roswell Park Memorial Institute, Buffalo, N. Y.) that was known to lack surface immunoglobulin (20). 5×10^5 cells in a small plastic tube were treated with 50 μl of antiserum for 30 min at 4°C. The cells were then washed three times in BSS and resuspended in 50 μl of a glycerol-BSS (1:1) solution and immediately examined in the ultraviolet (UV) microscope.

Purification of Lymphocytes.—7-ml portions of freshly drawn heparinized whole blood were layered on 3-ml Ficoll-Isopaque gradients in 15-ml glass centrifuge tubes. The gradients were spun for 12 min at 680 *g*. The lymphocyte-monocyte layer at the upper interphase was removed and washed twice in BSS and then further purified by the iron carbonyl technique. 0.2 g of iron powder was mixed with a 10 ml cell suspension prepared in BSS and 10% human AB+ serum. The tube was incubated in a 37°C water bath for 30 min with shaking every 5 min. The iron powder was removed by magnetism and the cells were washed three times.

EB Virus Receptor Assay.—10^5 P3HR-1 cells were labeled with 50 μl of a fluorescein- or tetramethylrhodamine isothiocyanate-conjugated MA-positive IgG (F-Abwao or TRITC-Abwao, footnote 3) for 30 min at 4°C and washed twice in cold BSS. The cells were then

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[3] Lamon, E. V., I. Ernberg, and G. Klein. Detection of antigens determined by the Epstein-Barr virus (EBV) in human lymphoblastoid cell culture lines by elution of specific radioiodine labeled antibody. Manuscript submitted for publication.

resuspended in 50 μl of BSS containing 2×10^6 purified lymphocytes and incubated for 1 h at 4°C. Thereafter the cells were shaken gently and examined in the UV microscope for rosette formation. Cells that had more than two lymphocytes attached to their surface were scored as rosettes.

Preparation of Anti-Immunoglobulin Column.—The columns were prepared as earlier described by Wigzell et al. (7). Degalan beads (V26; Degussa Wolfgang AG, Hanau-am-Main, Germany) were incubated for 30 min at 50°C with human gamma globulin (Beriglobin; Behringwerke AG, Marburg-Lahn, West Germany) at a concentration of 5 mg/ml in BSS. The plastic beads were incubated overnight at 4°C before being packed in the column and washed. A rabbit antihuman immunoglobulin serum diluted 1:2 was incubated in the column for 30 min at room temperature and then washed away. The column was then ready for use. The efficiency of every column passage was checked by determining the percentage of Ig-positive cells in the passed population. Samples containing more than 2% contaminants were discarded.

Blocking Experiments.—Two groups of African patient sera were used. One was known to contain high antibody levels against the EBV-determined membrane antigen, as measured by the blocking of direct membrane fluorescence (21), whereas the other had a low or negative antibody level. 50 μl of each serum was added to 10^5 P3HR-1 cells, pretreated with the F-Abwao conjugate and incubated for 30 min at 4°C to allow saturation of the viral determinants on the surface of the virus-producing cells. After one wash in cold BSS the cells were mixed with 2×10^6 lymphocytes in 50 μl of BSS as described above. It was previously shown by two color fluorescence (22) that conjugates are too dilute to block all MA-reactive sites on the cell surface, whereas undiluted sera with high anti-MA level can block more or less completely.

Maintenance of Lymphoblastoid Lines.—The cell lines were propagated as stationary suspension cultures in Eagle's basal medium with 20% fetal calf serum. They were fed every 4th day and adjusted to an initial cell concentration of 2×10^5 cells/ml each time.

Detection of EBV-Associated Nuclear Antigen, EBNA Test.—Smears were prepared in the cytocentrifuge, or by spreading a concentrated suspension of washed cells on clean slides and then air dried and fixed in chilled acetone. Human serum, which gave no reaction with Raji cells in the EBNA test (23) and which contained no detectable antibodies to EBV antigens by complement fixation or immunofluorescence, was used as a source of complement. All dilutions and washes were made in BSS.

After fixation, the smears were dipped in BSS and treated with test serum (usually diluted 1:8 and inactivated at 56°C for 30 min) containing complement (final dilution 1:10) at 37°C for 30 min in a humid chamber. They were washed with stirring for 30 min, stained with a suitable dilution of FITC-conjugated antihuman β_{1C}/β_{1A} globulin (Hyland Laboratories, Los Angeles, Calif.) at 37°C for 30 min or 4°C for 90 min, washed again, and mounted in BSS:glycerol 1:1. The slides were examined in a Leitz Ortholux microscope equipped with a vertical Ploem-type illuminator at oil immersion (54 × or 100 ×).

RESULTS

Surface Markers on Human Lymphoblastoid Lines.—Table I shows the surface characteristics of the human lymphoblastoid lines tested. All EBV genome-carrying lines had surface-bound immunoglobulin and none of these lines could bind SRBC. In contrast, the T-cell-derived line MOLT-4 (20), which does not carry the EBV genome according to the EBNA test (23), did not have surface-bound immunoglobulin but could bind SRBC. 11 of the 17 EBV-carrying lines had receptors for 7S Fc, ranging from 1 to 61% of the cells. All lines had receptors for C'3 ranging from 2 to 100%.

Surface Markers on Monkey Lymphoblastoid Lines.—The B and T charac-

TABLE I

Surface Characteristics of Human Lymphoblastoid Lines

Line		Presence of EBNA antigen (23)	T-lymphocyte characteristics	B-lymphocyte characteristics		
			Capacity to bind SRBC	EA-binding* cells	EAC-binding* cells	Surface-bound immunoglobulin
				%	%	
Daudi	BL‡	POS	NEG	61	100	POS
Raji	BL	"	"	1	100	"
Seraphine	BL	"	"	1	98	"
Namalwa	BL	"	"	12	23	"
Akuba	BL	"	"	57	7	"
Maku	BL	"	"	0	100	"
Sulubu	BL	"	"	0	6	"
Muniungi	BL	"	"	29	58	"
Rael	BL	"	"	0	2	"
LY-46	BL	"	"	48	62	"
NC-37	N	"	"	30	100	"
CB-B-1	CB	"		3	98	"
CB-B-3	CB	"	"	14	91	"
CB-B-9	CB	"	"	0	33	"
L4-28	NPC	"	"	0	100	"
SKL-1	L	"	"	40	48	"
JHTC-33	IM	"	"	0	19	"
MOLT-4§	ALL	NEG	POS	0	63	NEG

For the origin of the lines, see ref. 13 and 20.

* EA and NAC were tested with rat RBC covered with Fc and C'3 as described in Materials and Methods.

‡ *BL*, Burkitt's lymphoma-derived lines. *N*, lines derived from normal lymphocytes of EBV-positive donor. *NPC*, nasopharyngeal carcinoma-derived lymphoblastoid line. *IM*, infectious mononucleosis-derived line. *ALL*, line established from acute lymphatic leukemia. *CB*, cord blood lymphocytes transformed with EBV (received from Doctors José Ménézes and Wolfgang Leibold). *L*, leukemia-derived line.

§ In the MOLT-4 line about 50% of the cells formed rosettes with SRBC with the technique described.

teristics shown in Table II are defined in analogy with the human system. Experiments with peripheral lymphocytes from squirrel monkeys had earlier shown that there was no over-lapping between the two populations of lymphocytes that bind SRBC and carry surface immunoglobulin. All three EBV-transformed lines tested lacked receptors for SRBC. They seemed to carry immunoglobulin on their surface although the staining was very weak. In

contrast, the herpesvirus *Saimiri* (HVS)-carrying lines did bind SRBC and were clearly negative for surface immunoglobulin. The EBV-transformed marmoset line B-95-8 had receptors for C'3 that were not present on the other lines. No 7S Fc receptors were found on any of the simian lines tested.

EBV Receptors on Human Peripheral Lymphocytes.—Table III shows that all MA-positive P3HR-1 cells, labeled with the F-Abwao conjugate, were capable of binding lymphocytes to their surfaces. Virtually no MA-negative

TABLE II

Surface Characteristics of Monkey Lymphoblastoid Lines

Line		Viral genome	T-lymphocyte characteristics	B-lymphocyte characteristics		
			Capacity to bind SRBC	EA-binding cells	EAC-binding cells	Surface-bound immunoglobulin
				%	%	
KCSM-80*	SM‡	EBV	NEG	0	2	POS- ±?
KCSM-81*	SM‡	EBV	NEG	0	3	POS- ±?
B-95-8§	MM‡	EBV	NEG	0	86	POS- ±?
1670*	MM‡	HVS‖	POS	0	0	NEG
MLC¶	MM‡	HVS‖	POS	0	0	NEG
70-BM-2*		HVS‖	POS	2	0	NEG

* Received from Doctors Larry Falk and Friedrich Deinhardt.

‡ *MM*, line derived from marmoset monkeys; *SM*, line derived from squirrel monkeys. 60–80% of HVS-transformed cells formed SRBC rosettes. Surface-bound immunoglobulin was stained with the same conjugate as for the human lines. The surface Ig staining of the three EBV-carrying lines was very weakly positive, with distinctly stained spots on most cells.

§ Received from Dr. George Miller.

‖ Herpesvirus *Saimiri*.

¶ Received from Dr. Alan Rabson.

TABLE III

Binding of Human Peripheral Lymphocytes to Virus-Producing Cells in the P3HR-1 Line

	Rosette-forming cells*	MA-positive cells forming rosettes‡	MA-negative cells forming rosettes§
	%	%	%
Exp. I	4.2	98	0.1
Exp. II	2.3	93	0
Exp. III	3.6	95	0

* Binding was performed as described in Materials and Methods. 1,000 P3HR-1 cells were screened in a Bürker chamber and the percentage was calculated.

‡ 100 MA-positive P3HR-1 cells were screened in the UV microscope and the percentage was calculated.

§ 1,000 MA-negative P3HR-1 cells were screened in the UV microscope and the percentage was calculated.

cell had the capacity to form rosettes (Figs. 1–4). The percentage of rosette-forming cells varied from 2.3 to 4.2 in three repeat experiments.

Table IV shows that the number of rosette-forming cells was essentially the same, no matter whether P3HR-1 cells were pretreated with the MA-reactive conjugate or not. This rules out the possibility that binding was due to 7S Fc receptors on the lymphocytes.

Table V shows that the binding of lymphocytes to MA-positive P3HR-1 cells could be partially blocked by sera with high antibody titers against EBV antigens but not, or only to a more limited extent, by sera with low EBV antibody titers.

Table VI shows that passage of lymphocytes through a column that retained B lymphocytes, also retained the cells that could bind to virus-producing P3HR-1 cells almost completely (Figs. 5 and 6).

DISCUSSION

EBV is only known to infect human and some nonhuman primate cells of lymphoid origin. Since the virus can convert resting lymphoid cells with a limited life-span into permanent in vitro lines and can induce benign and perhaps even malignant lymphoproliferative disease in vivo, it is important to define the type of lymphocyte that is susceptible to the virus. We have attempted this in two different ways. Our first approach was to test established, EBV-carrying lines for three receptors normally associated with B lymphocytes, surface immunoglobulins, 7S Fc receptors, and C'3 receptors. We have also tested the lines for the capacity to form rosettes with SRBC, associated with normal T lymphocytes. A human lymphoblastoid line that lacks the EBV genome, MOLT-4 (20), was used for comparison. In addition, a number of simian lymphoblastoid lines, converted by EBV or by herpesvirus *Saimiri* (HVS), respectively, were also tested in the same way.

All EBV-carrying human lines tested had surface-associated immunoglobulin. The majority also carried receptors for C'3 and/or 7S Fc, although they expressed them in different degrees. This is in line with the earlier findings of Nishioka (24), although the distribution of the receptors were slightly different in our experiments. These discrepancies may be due to the difference in complement sources and in the techniques involved in rosette formation. Since none of the EBV-carrying lines could bind SRBC, all this evidence points towards a B-lymphocyte origin, although it cannot be regarded as conclusive. It must be remembered that the markers used have been defined with regard to peripheral B and T lymphocytes. These are relatively small and inactive, compared with the lymphoblastoid lines. Since T-cell blasts induced in a one-way mixed leukocyte culture or by concanavalin A stimulation retain their capacity to bind SRBC, however, this marker might be expected to appear on in vitro lines as well, provided they are T cell derived (Jondal, M., unpublished observation). Only one line showed this property, however–the EBNA antigen-

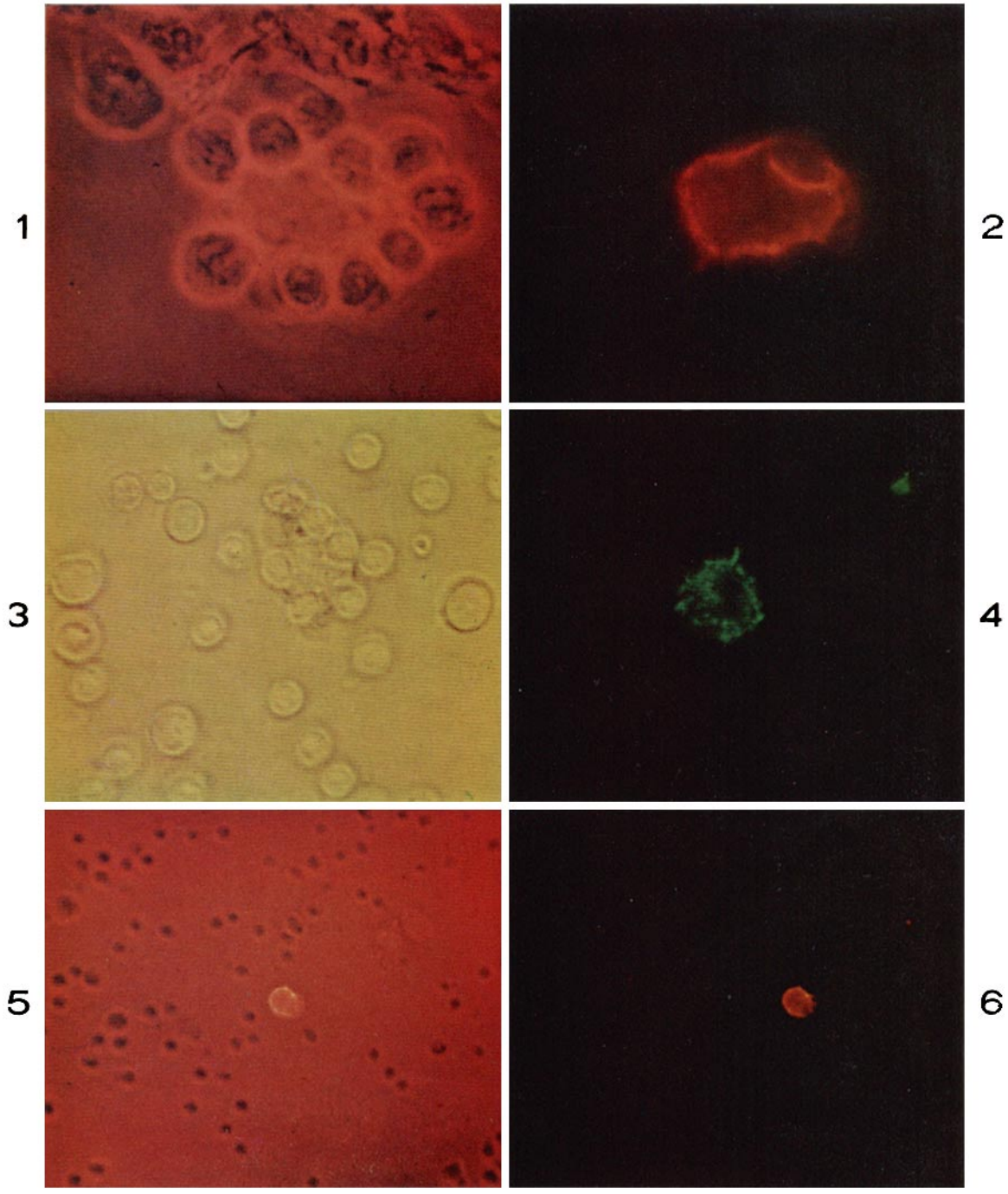
1
2
3
4
5
6

TABLE IV

Binding of Human Peripheral Lymphocytes to Virus-Producing Cells in the P3HR-1 Line in the Presence and Absence of the MA-Reactive Abwao Conjugate

	Rosette-forming cells	
	MA-stained P3HR-1 cells	Unstained P3HR-1 cells
	%	%
Exp. I	4.2	4.5
Exp. II	2.3	2.6
Exp. III	3.6	3.5

Binding assay was performed as described in Materials and Methods. 1,000 P3HR-1 cells were screened for rosette formation in a Bürker chamber and the percentage was calculated.

negative MOLT-4 that did not carry surface-bound immunoglobulin. This confirms the original suggestion that this line has been derived from T cells (20).

Our second approach was to look for EBV receptors on the surface of peripheral lymphocytes. Adsorption of the virus is obviously a prerequisite for virally induced transformation, and while the presence of viral receptors does not prove sensitivity to the virus, absence of such receptors minimizes the probability that a category of cells will be susceptible to viral infection and/or transformation. It has been shown that EBV-induced transformation of cord blood lymphocytes is due to the attachment of enveloped virus.[2]

An attempt to assess EBV adsorption to lymphocyte surfaces by immediate membrane fluorescence (13) was unsuccessful. Presumably, receptors on mature lymphocytes are relatively scarce, or the number of receptor-positive lymphocytes is not high enough. This difficulty could be circumvented by using the virus-producing P3HR-1 line as the source of viral envelopes with the appropriate combining specificity for virus receptors on the surface of

FIG. 1. Virus-producing P3HR-1 cell, prelabeled with TRITC-conjugated Abwao (MA-reactive reference reagent). The cell is surrounded with adherent lymphocytes. Phase contrast. × 3720.

FIG. 2. Same field and magnification as in Fig. 1, photographs for TRITC fluorescence, with 546 nm green excitation light, 4 mm BG38 + 2 mm BG36 + AL546 as primary filters, 580 nm interference plate and K590 barrier filter. × 3720.

FIG. 3. Low-power view of a virus-producing P3HR-1 cell, prelabeled with the FITC-conjugated Abwao reference immunoglobulin, surrounded by adherent lymphocytes. × 2025.

FIG. 4. Same field as in Fig. 3, photographs for FITC fluorescence. 470 nm blue excitation light, with 4 mm BG38 + 1.5 mm BG12 + AL470 as primary filters, 495 nm interference plate and K510 barrier filter. × 2025. Note that none of the free P3HR-1 cells, not covered with adherent lymphocytes in Fig. 3, show any MA staining.

FIG. 5. Low-power view, combined with contrast and TRITC fluorescence, of TRITC-Abwao-labeled P3HR-1 cells, admixed to column-purified T lymphocytes. Note the absence of rosettes. × 936.

FIG. 6. Same field as in Fig. 5, photographs for red emission, as in Fig. 2. × 936. Note that the MA-reactive cell is free of adherent lymphocytes.

TABLE V

Specific Inhibition of Rosette Formation by Sera with High EBV Titers

	Sera with high EBV antibody titers				Inhibition of rosette formation around MA-positive cells	
	BI*	BT*	VCA titer‡	Early antibody titer§	Exp. I	Exp. II
					%	%
S.M.	1.00	8	80	<10	26	18
W.F.	0.98	16	320–640	>320	88	76
G.K.	0.97	16	1,280	80	58	28
K.K.	0.96	n.t.	160	n.t.	78	88
M.M.	0.94	n.t.	640	2560	84	76
D.L.	0.88	n.t.	1,280	n.t.	62	58
O.J.	0.85	64	320–640	80	72	64
E.J.	0.85	4	320	n.t.	70	56
					Mean 67.3	Mean 58.0
Sera with low EBV antibody titers						
M.K.	0.16	<1	160	10	12	20
A.K.	0.18	<1	20	<10	2	8
P.H.	0.03	<1	20	10	6	2
N.M.	0.03	<1	<10		0	2
R.T.	0.05	<1	<10	<10	18	22
S.O.	0.06	<1	10	<10	22	16
R.K.	0.02	<1	10–20	<10	8	6
					Mean 9.7	Mean 10.9

Two representative experiments are shown in the table. 100 MA-positive P3HR-1 cells were screened in the UV microscope for rosette formation.

* Direct membrane fluorescence-blocking assay for EBV-determined membrane antigens (MA) was performed as previously described.[3] Blocking Index (*BI*) is defined as the capacity of the serum to inhibit staining of a fluorescein-conjugated reference serum (Mutua) by first treating the cells with the test serum and then with the reference serum (1:8) after washing.[3] Blocking Titer (*BT*) is defined as the concentration of test serum giving a 50% reduction of positive cells labeled by the reference conjugate.

‡ Antibody titer against EBV-determined viral capsid antigen (15).

§ Antibody titer against EBV-determined early antigen (16).

other cells. It has been shown previously that the virus cycle proceeds relatively rapidly to completion in the P3HR-1 line and that cells containing late viral envelope material with MA specificity in their plasma membranes (17). By prestaining the plasma membrane with MA-positive conjugates these cells could be easily identified. Almost all MA-positive P3HR-1 cells formed rosettes with unfractionated lymphocytes, whereas none of the MA-negative cells did so. It might be objected, however, that the lymphocytes attached to the MA-stained P3HR-1 cells by their 7S Fc receptors, rather than via EBV

TABLE VI

Binding of Column-Purified Human T Lymphocytes to Virus-Producing Cells in the P3HR-1 Line

	MA-positive cells forming rosettes	
	Unfractionated cells	Fractionated cells
Exp. I	98	5
Exp. II	93	7
Exp. III	95	8

100 MA-positive P3HR-1 cells were screened in the UV microscope and the percentage of rosette-forming cells was calculated. For details of column passage see Materials and Methods. EM, 4001, p 2.

receptors. When the experiments were repeated with unstained P3HR-1 cells, the proportion of rosette-forming cells was the same, however, as in the prestained population. Therefore, it could be concluded that the binding was not mediated through the 7S Fc receptors. The second alternative that lymphocytes attached by EBV receptors was confirmed by blocking experiments. Sera containing high antibody titers against the EBV-determined MA could inhibit rosette formation to a considerable extent, whereas sera with insignificant or low MA titers failed to do so. The fact that this blocking was partial rather than complete is either due to a large excess of viral envelope material on the surface of virus-producing cells, and/or to a steric difference between the anti-MA antibody-binding sites and the sites combining with the virus receptors on the lymphocyte surface.

The finding that unfractionated lymphocyte populations formed rosettes regularly with 100% of the MA-positive P3HR-1 cells whereas lymphocytes passed through an anti-immunoglobulin column were unable to make rosettes must be interpreted to mean that only B lymphocytes had EBV receptors. A more direct way to prove this would be to stain the adherent lymphocytes in the rosettes with a fluorescein-conjugated anti-immunoglobulin serum. This was attempted with a double-staining procedure, but most of the rosettes disintegrated during these manipulations. The few incomplete rosettes left consisted entirely of immunoglobulin-positive lymphocytes. This would further support the view that the relevant function of the column in the present experiments was the removal of immunoglobulin-positive lymphocytes. Care should otherwise be taken in interpreting the effect of such a column passage as the column consists of immune complexes and secondarily removes cells with 7S Fc receptors. Current knowledge suggests, however, that these receptors are also localized on B lymphocytes with the exception of educated T lymphocytes in the mouse system (25, 26).

The results obtained in this study thus suggest that EBV acts on the bone marrow-derived lymphocyte system in man. It remains an open question,

however, whether all B lymphocytes or only a minority are susceptible to the virus.

SUMMARY

Human peripheral lymphocytes were investigated for receptors binding Epstein-Barr virus (EBV) because of the regular association of this virus with infectious mononucleosis and Burkitt's lymphoma.

This was done by a cytoadherence technique where virus-producing cells, displaying fresh viral determinants in their cytoplasmatic membrane, were mixed with lymphocytes. Unfractionated lymphocytes were found to adhere to these cells in contrast to column-purified T lymphocytes. The specificity of the binding was confirmed by blocking experiments that showed that sera containing high titers of antibodies directed against the virus could partially inhibit the adherence in contrast to low-titer sera. It is concluded that B lymphocytes, in contrast to T lymphocytes, have receptors for EBV.

In a second line of experiments it was found that established human lymphoblastoid lines that carry the EBV genome had receptors characteristic for B lymphocytes and did not form T-lymphocyte rosettes. In contrast, a line of known T-lymphocyte origin that did not carry the EBV genome had receptors characteristic for T lymphocytes.

EBV-transformed simian lymphoblastoid lines had surface markers indicating a B-lymphocyte origin in contrast to HVS-transformed simian lines that lacked surface immunoglobulin but carried receptors for sheep red blood cells.

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