Epstein-Barr Virus-Lymphoid Cell Interactions

III. Effect of Concanavalin A and Saccharides on Epstein-Barr Virus Penetration

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Received 22 October 1981/Accepted 28 December 1981

To study some aspects of Epstein-Barr virus (EBV) penetration into target cells, the effect of concanavalin A (ConA) and various saccharides on virus infectivity and cell susceptibility to EBV infection was examined. ConA treatment of the target cells, EBV, or EBV-cell complexes was found to inhibit virus antigen expression. Several control experiments with α -D-methyl-mannoside elution of ConA, removal of nonfused EBV particles from the cell surface by trypsin treatment, and addition of ConA at different times postinfection were performed to define the site of ConA action on EBV infection. ConA appeared to have a dual action: (i) it inhibited EBV binding to virus receptors, and (ii) it blocked the penetration of receptor-bound virus into target cells at a trypsin-sensitive stage, thus indicating that ConA prevented the fusion of viral envelope with the target cell membrane. A high sucrose concentration (0.25 M), known to inhibit cell membrane movements, was also found to block EBV penetration at a trypsin-sensitive stage, thus suggesting the implication of cell membrane movements and underlying activities (or both) in viral envelope fusion. Lower concentrations of various monosaccharides (0.12 M) did not influence EBV infection. Under conditions of ConA treatment that did not influence EBV infectivity and target cells susceptibility, ConA was able to mediate virus binding to EBV receptor-negative cell lines, but no virus antigens were expressed in these cells. These observations reinforced the idea that the mere attachment of EBV to lymphoid cells is not sufficient to lead to infection. In light of the present and previously published data, we postulate the existence of a specific cellular mechanism that allows the penetration of EBV into the target (B) lymphocyte.

Epstein-Barr virus (EBV) is a human herpes-virus that causes infectious mononucleosis (11, 25), and its genome is associated with two human cancers: Burkitt's lymphoma (5) and nasopharyngeal carcinoma (7). The main target cell for EBV infection is the B lymphocyte which bears specific EBV receptors (14, 18); this virus is also found in nasopharyngeal carcinoma cells which are of epithelial nature, however, and it is still unknown whether EBV is able to infect epithelial cells directly or indirectly by fusion of an EBV genome-carrying B-lymphoid cell and these cells (7). The mode of entry of EBV into target cells seems to involve fusion of the viral envelope with the cell membrane, followed by release of the viral nucleocapsid into the cytoplasm (21, 32). The cellular and viral factors that govern these processes are still unknown; they may prove to contribute largely to the narrow host range restriction of EBV, as indicated by the ability of this virus to bind to Molt-4 cells, but not to release its nucleocapsid

into their cytoplasm (21). The function of the different EBV envelope glycoproteins regarding receptor binding and envelope fusion has not been studied, mainly because of the lack of viral mutants with abnormal envelope functions and the lack of a model system for the study of EBV penetration. Model systems have been successfully developed for paramyxoviruses such as Sendai virus (SV) and have contributed to a clearer definition of the functions of such glycoproteins as hemagglutinin-neuraminidase and fusion factor ($F_{1,2}$) of SV (13, 27, 37). The simplest of these model systems consisted of the use of SV receptor-negative horse or cat erythrocytes and concanavalin A (ConA)-mediated binding of SV to these cells which thus became susceptible to SV-induced hemolysis; this indicated that the specific binding of viral hemagglutinin-neuraminidase to virus receptors is not necessary for the $F_{1,2}$ protein to function in envelope fusion (37).

To initiate similar studies on the mechanism of

penetration of EBV into target cells we studied the effect of ConA on EBV infectivity and cell susceptibility to EBV infection and also determined conditions in which ConA-mediated binding of EBV to EBV receptor-negative cells might be used as a model system to study factors that govern virus penetration into target cells. Additionally, we investigated the effect of various saccharides on EBV penetration into target cells to assess the role of carbohydrate-mediated interactions and the role of cell membrane movements in virus penetration. Saccharides at relatively low concentrations can be used as competitive inhibitors of carbohydrate-mediated interactions such as lectin binding to polysaccharides (8). However, higher concentrations of saccharides are able to block non-specifically the movement of cellular membranes and related membrane fusion events as shown in the SV-Ehrlich ascites tumor cell system (17, 24). Interestingly, in this system high saccharide concentrations have no influence on SV envelope fusion with the cell membrane which is controlled by the viral $F_{1,2}$ protein, not by a cellular mechanism. Therefore, it seems possible to distinguish fusion events that depend on a cellular mechanism from those which do not by using high saccharide concentrations as a probe.

Our data indicate that: (i) EBV penetration into target cells is blocked by ConA and high sucrose concentration (0.25 M) at a trypsin-sensitive stage on the cell surface; (ii) lower concentrations of various monosaccharides (0.12 M) did not influence EBV infection; (iii) ConA-mediated binding of EBV to EBV receptor-negative cell lines did not lead to viral antigen expression under conditions in which ConA did not influence EBV infection of susceptible targets. Taken together, these data suggest that EBV penetration depends on a cellular mechanism involving membrane movements and emphasize the importance of other factors than virus binding to the cell surface in determining the host range of EBV.

(Preliminary results on this subject were presented at the International Workshop on Herpesviruses, Bologna, Italy, 27 through 31 July 1981, abstract, p. 268.)

MATERIALS AND METHODS

Cells. The cell lines used in these studies and their main characteristics are listed in Table 1. The cells were grown in culture medium RPMI-1640 (RPMI) supplemented with 10% heat-inactivated fetal bovine serum and antibiotics as described previously (18). This medium will hereafter be referred to as RPMI-10.

Virus preparation. EBV-producer P3HR-1 and B95-8 cell cultures were used as a source of the nontransforming P3HR-1 and lymphocyte-transforming B95-8 strains of EBV, respectively. Cell-free supernatants from these cultures were prepared as described previ-

TABLE 1. Detection of ConA receptors on lymphoid cell lines by using FITC-ConA

Cell line	Cell type ^a	Origin ^b (reference)	EBV genome status ^c	FITC-ConA binding cells ^d	
				%	Fluorescence intensity
Raji	B	BL (30)	+	100	+++
P3HR-1	B	BL (12)	+	100	+++
BJA-B	B	BL (20)	-	95	+
B95-8	B	M (22)	+	100	+++
Molt-4	T	ALL (23)	-	89	+
1301	N	ALL (15)	-	85	+

^a B, Thymus-independent B lymphocyte; T, thymus-processed lymphocyte; N, non-B, non-T lymphocyte.

^b BL, Burkitt's lymphoma; M, EBV-transformed monkey (Marmoset) lymphocytes; ALL, acute lymphoblastic leukemia.

^c +, EBV genome positive, as determined by the presence of EBNA and/or EBV DNA; -, EBV genome negative.

^d The percentages given here are mean values from two separate experiments. Relative fluorescence intensity is indicated by the + signs.

ously (19) and concentrated by centrifugation for 90 min at $45,000 \times g$ and 4°C. The resulting virus pellets were gently suspended in 0.0025 of the original supernatant volume of Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline (PBS). EBV was purified by centrifugation of concentrated EBV preparations through two consecutive dextran T-10 gradients, 5 to 30% and 10 to 30%, as described previously (6). The purified virus was appropriately concentrated by centrifugation and suspended in PBS. The purified EBV preparations thus obtained were kept at 4°C and used within a week.

Preparation of FITC-ConA and detection of ConA receptors. ConA (Pharmacia Fine Chemicals) was dissolved in PBS at 25 mg/ml and stored at -20°C in small working samples until use. Fluorescein isothiocyanate (FITC)-ConA was prepared by mixing 1 mg of FITC (Isomer I, Sigma) and 50 μ l of carbonate-bicarbonate buffer (0.5 M, pH 9.5) with 0.5 ml of ConA solution (25 mg/ml) with gentle shaking at room temperature (21°C) for 2 h. The mixture was incubated overnight at 4°C, and FITC-ConA was isolated by gel filtration and then stored at -20°C in small working samples until use. FITC-ConA was used at a final concentration of 100 μ g/ml as follows: a pellet of 5×10^5 test cells was suspended in 50 μ l of FITC-ConA solution and incubated for 30 min in ice with occasional shaking; the cells were then washed three times with cold PBS, suspended in 2 drops of glycerol-PBS (1:1), and observed with a UV microscope.

Virus-cell complexes. Virus-cell complexes were formed at 0 to 4°C by incubation of 5×10^5 BJA-B cells with 0.1 ml of a 1:8 dilution of B95-8 EBV preparation for 1 h in ice. Free virus was washed away with cold PBS and centrifugation at 4°C.

ConA treatment and MM elution. Unless otherwise stated, all ConA treatment of cells or virus or virus-

cell complexes were performed at 0 to 4°C to study the effect of ConA on these three targets under similar conditions. A total of 5×10^5 BJA-B cells or virus-cell complexes were pelleted by centrifugation at 4°C, suspended in 0.2 ml of cold PBS (control) or cold ConA solution at the desired concentration, incubated for 30 min in ice, and washed twice with cold PBS. When necessary, ConA was eluted from the cells or from the virus-cell complexes by washing five times with a cold 0.3 M solution of α -D-methyl-mannoside (MM) in PBS. The virus-cell complexes were then washed and suspended in RPMI-10 and cultured for 48 h. The ConA-treated cells were incubated with B95-8 EBV and washed with PBS as described for the preparation of virus-cell complexes. When MM elution after EBV binding was necessary, it was applied as indicated above, and the cells were incubated in RPMI-10 for 48 h under normal culture conditions.

EBV (B95-8 or P3HR-1 strain) was treated with ConA or sham treated as follows. One volume (10 μ l) of PBS or ConA solution at 11 times the desired final ConA concentration was mixed with 10 volumes (100 μ l) of undiluted EBV suspension. After incubation in ice for 30 min, cold PBS (0.69 ml) was added to the mixture to obtain a final EBV dilution of 1:8. In experiments in which the effect of ConA was studied at different EBV concentrations, the starting undiluted EBV preparation was first serially diluted; then, 100 μ l of each EBV dilution was treated with 10 μ l of ConA solution and diluted with 0.69 ml of PBS as above. ConA elution from the virus was accomplished immediately after incubation with ConA by dilution of the virus to 1:4 with a cold 0.5 M solution of MM (0.29 ml) and incubation of the diluted suspension for 30 min in ice. The virus was then further diluted 1:2 by the addition of cold PBS (0.4 ml). Samples (100- μ l) of the final (1:8) EBV dilution were added to 5×10^5 Raji or BJA-B cells, respectively. These cells were then processed as for the formation of virus-cell complexes, and, when desired, they were eluted with MM as described above; then, the cells were cultured in RPMI-10 for 48 h. After each 48 h of culture, the cells were examined for EBV-induced early antigens (EA) or nuclear antigens (EBNA) as described below.

Effect of ConA on EBV penetration into target cells. The effect of ConA on B95-8 EBV penetration into BJA-B targets was examined in two different ways. In the first approach, the ConA- or sham-treated virus-cell complexes were incubated for 2 h at 37°C in RPMI, and virus particles that did not fuse with or penetrate the cells were removed by trypsin treatment. Trypsin (Difco Laboratories) was applied at a final concentration of 0.25% for 15 min at 37°C. The cells were then washed with RPMI-10 and either immediately cultured or allowed to recover for 20 min at 37°C in RPMI-10, eluted with MM at 4°C, and cultured for 48 h. After culture, the cells were examined for EBNA as described below.

In the second approach, all operations were conducted at 37°C as follows. B95-8 EBV preparation was diluted 1:8 with RPMI at 37°C; 100 μ l of this EBV dilution was added per pellet of 5×10^5 BJA-B cells, either immediately or after mixing the virus with 10 μ l of ConA (2.2 mg/ml), and the cells were incubated at 37°C. Each sample with untreated virus was treated with 10 μ l of the above ConA solution at a predetermined moment between 5 and 60 min postinfection

(after EBV addition). The cells were washed twice with warm (37°C) RPMI 45 min after ConA addition and then cultured for 48 h and examined for the presence of EBNA as indicated below.

Effect of low ConA concentrations on EBV receptor-negative and receptor-positive cell lines. The effect of low ConA concentrations on the ability of cells to bind EBV and on their susceptibility to EBV infection was studied using various EBV receptor-positive and receptor-negative cell lines; 5×10^5 cells from each line were treated with 50 μ l of either 0, 1, 10, or 20 μ g of ConA per ml for 30 min at 4°C. The cells were then pelleted, suspended in 100 μ l of EBV preparation previously diluted 1:8, and then incubated in ice for 1 h. Subsequently, the cells were washed twice with either cold PBS or RPMI-10 at room temperature and, respectively, examined for EBV binding by using the membrane immunofluorescence assay as described earlier (21) or cultured for 48 h. After culture, the cells were examined for EBV-induced antigens as described below.

Detection of EBV antigens. After culture for 48 h under the usual conditions (as indicated above), the infected cells were harvested and washed in PBS by low-speed centrifugation. The cells were then smeared, air dried, and fixed with cold (-20°C) acetone-methanol (1:1). The cell smears were stained for EBNA by the two-step anticomplement immunofluorescence method (18) or stained for the EA by using anti-EA antibody-positive human serum and rabbit anti-human γ chain-specific conjugate as described previously (10).

Effect of various sugars and ovalbumin on EBV penetration into target cells. The effect of sugars [sucrose, *N*-acetyl-D-glucosamine, D-(+)-galactose, *N*-acetyl-D-galactosamine, L-(-)-fucose or MM] or ovalbumin on B95-8 EBV penetration into BJA-B cells was tested by including these compounds in the B95-8 EBV suspension. The final concentration of ovalbumin was 1.5 mg/ml. A 100- μ l sample of the virus suspension (diluted 1:8) containing the test compound was added to 5×10^5 BJA-B cells for 2 h at 37°C, and then 10 μ l of a 2.8% trypsin solution in PBS containing the corresponding test compound was added. The mixture was incubated for 15 min at 37°C with continuous shaking. The samples with sugars were then washed with RPMI-10 and cultured for 48 h, whereas the samples with ovalbumin were cooled to 0 to 4°C in ice, washed with cold PBS, and treated with 0.25% trypsin at 4°C for 30 min; they were then washed with RPMI-10 and cultured for 48 h. At the end of the culture period the cells were examined for the presence of EBNA as indicated above.

RESULTS

Effect of ConA on EBV infectivity. Before studying the outcome of ConA-mediated binding of EBV to receptor-negative cell lines, it was important to know the effect of various concentrations of ConA on the EBV replication cycle. Therefore, we first studied the effect of ConA on EBV infectivity by using the two EBV strains P3HR-1 and B95-8 and two different target cell lines Raji and BJA-B, respectively. The main properties of these two targets and the other cell

FIG. 1. Effect of ConA on EBV infectivity and cell susceptibility to EBV infection. Symbols: ●, EBNA induction by B95-8 EBV in ConA-treated BJA-B cells; ▲, EBNA induction by ConA-treated B95-8 EBV in BJA-B cells; ■, EA induction by ConA-treated P3HR-1 EBV in Raji cells.

lines used in these studies are shown in Table 1, along with their ability to bind FITC-labeled ConA (FITC-ConA). The infectivity of P3HR-1 EBV was assessed by the EA induction assay in Raji cells, and that of B95-8 EBV was assessed by the EBNA induction assay in BJA-B cells. Treatment of EBV with ConA was found to inhibit the expression of EA and EBNA in a concentration-dependent manner (Fig. 1). The extent of inhibition of EBV infectivity by ConA also depended on virus concentration (Fig. 2), thus indicating that the observed inhibition of antigen expression was mainly due to ConA binding to the virus and not to an effect of residual unbound ConA on target cell susceptibility to EBV infection. The effect of ConA on virus infectivity was reversed by the incubation of ConA-treated virus with MM, a competitive

FIG. 2. Effect of ConA on EBNA induction in BJA-B cells at different EBV concentrations. Symbols: ▲, B95-8 EBV treated with 50 µg of ConA per ml; ●, sham-treated (control) B95-8 EBV.

TABLE 2. Elution of ConA by MM: effect on inhibition of EBNA by ConA^a

Treatment of:		% EBNA-positive BJA-B cells ^b		
Cells	EBV	MM elution		PBS
		Before EBV binding	After EBV binding	
PBS	ConA (100 µg/ml)	22	8	3
PBS	ConA (200 µg/ml)	21	5	0.3
PBS	ConA (500 µg/ml)	18	1	0
ConA (500 µg/ml)	PBS	23	10	2
ConA (1 mg/ml)	PBS	24	5	0.4
ConA (4 mg/ml)	PBS	21	0.2	0
PBS	PBS	26	24	25

^a B95-8 EBV and BJA-B cells were used. ConA treatments and MM elutions were carried out as described in the text.

^b The percentages given here are mean values from three separate determinations.

inhibitor of ConA binding, before infection, thus showing that the virus was not irreversibly altered by ConA (Table 2). However, when the ConA-treated virus was first incubated with BJA-B cells, and the cells were subsequently eluted with MM, cultured at 37°C, and examined for the presence of EBNA, the effect of ConA was only partly reversed. The reversion rates obtained in this case were 36, 24, and 5% of those obtained by MM elution before infection in samples treated with 100, 200, and 500 µg of ConA per ml, respectively (Table 2). This indicates that only a fraction of the ConA-treated virus succeeded in binding to EBV receptors on the cell surface, and that the proportion of virus that bound to its receptors decreased with increasing ConA concentration.

Effect of ConA on target cell susceptibility to EBV infection. To know whether the treatment of target cells alone with ConA would affect their susceptibility to EBV, we pretreated BJA-B cells with ConA at various concentrations and then infected them with EBV and examined them for the presence of EBNA. It was found that ConA treatment inhibited EBNA expression in these cells in a concentration-dependent manner (Fig. 1). The ConA concentration needed to reach a given level of inhibition was reproducibly higher than that needed to produce a similar level of inhibition by pretreatment of the virus (Fig. 1). When the ConA-treated cells were eluted with MM before incubation with

EBV, the effect of ConA on virus antigen expression was reversed (Table 2). However, when the virus was allowed to adsorb to the ConA-treated cells and then MM elution was applied, only partial reversal of the inhibition of antigen expression was obtained; the extent of reversal in this case decreased with increasing ConA concentration (Table 2). Therefore, it appears that EBV binding to its receptors was inhibited by treatment of the cells with ConA.

The relatively high ConA concentration (4 mg/ml) necessary to completely inhibit EBV binding to its specific receptors (Table 2) may be related to the fact that this concentration of ConA is also required to saturate BJA-B target cells with this lectin, as found in competition experiments between unlabeled ConA and FITC-ConA (data not shown).

Effect of ConA on EBV penetration into target cells. To study the effect of ConA on EBV penetration into cells, we eliminated the effect of ConA on EBV binding by allowing the virus to bind to BJA-B cells in the cold (to prevent viral envelope-cell membrane fusion) before ConA treatment. The virus-cell complexes thus obtained were treated with various ConA concentrations in the cold and then warmed to 37°C and cultured under normal conditions. It was thus found that ConA inhibited EBNA expression in a concentration-dependent manner (Fig. 3). Elution of ConA from the virus-cell complexes restored the expression of EBNA (Table 3). This reversal of ConA-induced inhibition was obtained either when the cold, ConA-treated complexes were immediately eluted with MM or incubated for 2 h at 37°C and subsequently cooled down to 4°C and eluted with MM (Table 3). These data indicate that ConA is able to inhibit EBV interaction with its target cells beyond the receptor-binding stage.

To define the site of this inhibition, two types

FIG. 3. Effect of ConA on preformed virus-cell complexes.

TABLE 3. Induction of EBNA: effects of trypsinization of EBV-cell complexes or MM elution of ConA from complexes^a

ConA (500 µg/ml at 4°C)	Sequential treatment of EBV-cell complexes:			% EBNA- positive cells ^b
	2 h of incubation at 37°C	Trypsin (0.25%)	MM elution	
Yes	Yes	No	No	0
Yes	Yes	No	Yes	19
Yes	No	No	Yes	20
Yes	Yes	Yes	No	0
Yes	Yes	Yes	Yes	0
No	Yes	Yes	Yes	21
No	Yes	No	No	24

^a EBV-cell complexes were prepared and treated with ConA, trypsin or MM (or a combination) as described in the text. BJA-B cells and B95-8 EBV were used.

^b The percentages given in this table are mean values from two separate experiments.

of experiment were performed. In the first type, trypsin treatment was used to remove from the cell surface virus particles that did not undergo fusion with the cell membrane. In some samples, the ConA-treated virus-cell complexes were incubated for 2 h at 37°C and subsequently treated with trypsin, treated with trypsin and eluted with MM (to remove any ConA bound to trypsin-resistant molecules, such as glycolipids), or sham treated. In no case was EBNA detected. However, in similarly treated samples in which complexes had not been treated with ConA, EBNA was expressed normally (Table 3). These results indicate that ConA arrested EBV at a trypsin-sensitive stage on the cell surface and therefore prevented fusion of viral envelope with cell membrane.

In the second type of experiment, we wanted to see whether the above results could be confirmed by following a different approach and also to eliminate possible artifacts due to low temperatures or trypsin treatment (or both). ConA (at a final concentration of 200 µg/ml) was added to BJA-B cells simultaneously with EBV or at different moments after virus addition (postinfection) at 37°C; its effect on EBNA induction was then examined. It was found that when ConA and EBV were added simultaneously to the cells, EBNA expression was inhibited to the same extent as when EBV or EBV-cell complexes were treated with the same concentration of the lectin in the cold. By increasing the delay between virus addition and ConA addition to the cells, the level of inhibition of EBNA induction decreased; at 1 h postinfection, ConA addition produced no significant inhibition (Fig. 4). These data indicate that ConA acted on the

FIG. 4. Effect of ConA treatment at different times postinfection on EBNA induction by B95-8 EBV in BJA-B cells. Symbols: ●, ConA (200 µg/ml)-treated samples; —, sham-treated (control) samples.

early stages of virus infection and confirm the effect of this lectin on virus penetration.

Effect of low ConA concentrations on EBV receptor-negative and receptor-positive cell lines. As the above results showed that ConA binds to both the virus and the target cell and that low ConA concentrations do not affect EBV antigen expression, we decided to determine whether ConA at these concentrations would mediate EBV binding to virus receptor-negative cell lines. In the positive cases, we were also interested to know whether this ConA-mediated binding would be followed by EBV infection of cells that otherwise are not susceptible to EBV infection.

We therefore treated various EBV-susceptible and nonsusceptible cell lines with low concentrations of ConA (1, 10, and 20 µg/ml) and allowed the EBV to interact with these cells at

4°C. Subsequently, we examined virus binding by the membrane immunofluorescence assay or cultured the cells for 48 h and then examined EBV antigen expression in these cells. The results (Table 4) indicate that a ConA concentration of 1 µg/ml did not modify binding and antigen expression of EBV to any detectable extent; higher ConA concentrations (10 and 20 µg/ml) rendered the EBV receptor-negative cell lines (1301, P3HR-1, and B95-8) able to bind EBV to a degree that seems to be related to the ability of these cells to bind ConA (Table 1). EBV antigens were expressed normally in the infectable Raji and BJA-B cell lines, but no antigens were induced in the nonsusceptible Molt-4, 1301, and P3HR-1 cell lines.

These results indicate that the mere binding of EBV to the cell surface, either through EBV-specific receptors (in Molt-4 cells) or through ConA receptors (in 1301 and P3HR-1 cells), is not sufficient to lead to virus infection. Therefore, some unknown cellular functions seem to determine the fate of cell surface-bound EBV.

EBNA induction in the presence of ovalbumin and various saccharides. The inhibition of early EBV-target cell interactions by ConA described above could have resulted from the blocking of essential saccharide recognition markers by the lectin. Therefore, different saccharides [*N*-acetyl-D-glucosamine, D-(+)-galactose, *N*-acetyl-D-galactosamine, L-(+)-fucose, and MM] were assayed for their ability to inhibit EBV penetration at a final concentration of 0.12 M. Ovalbumin, which bears high mannose-type oligosaccharides that bind to ConA (2), was also assayed at a final concentration of 1.5 mg/ml. None of these competitive inhibitors of oligosaccharide-mediated interactions influenced EBNA expression (data not shown). In view of the nonspecific action of saccharides at high concentrations on

TABLE 4. Effect of ConA on EBV binding and antigen expression by receptor-positive and receptor-negative cells

Cell line	EBV strain ^a	% Positive cells ^b											
		Control			Treated at ConA concn:								
		MIF	EA	EBNA	1 µg/ml			10 µg/ml			20 µg/ml		
Raji	P	98	27	+	98	27	+	97	26	+	100	25	+
P3HR-1	P	0	2	+	ND	2	+	55	1	+	71	2	+
B95-8	P	0	ND	+	ND	ND	+	64	ND	+	83	ND	+
	B	0	ND	+	1	ND	+	60	ND	+	81	ND	+
BJA-B	B	72	ND	30	71	ND	28	72	ND	30	75	ND	27
Molt-4	P	41	0	0	40	0	0	45	0	0	51	0	0
1301	B	0	0	0	0	0	0	11	0	0	27	0	0

^a P, P3HR-1, non-transforming strain of EBV; B, B95-8, transforming strain of EBV.

^b The figures given here were obtained from three separate experiments, and each percentage represents the mean value of at least three separate determinations. MIF, Membrane immunofluorescence assay; +, as Raji, P3HR-1, and B95-8 cells are EBV genome carrying and EBNA positive, EBNA induction was not considered here; ND, not done.

TABLE 5. Effect of sucrose on EBNA induction by B95-8 EBV in BJA-B cells

Treatment ^a of cells and EBV with:		% EBNA-positive cells ^b
Sucrose (0.25 M)	Trypsin (before removal of sucrose)	
Yes	No	27
Yes	Yes	0
No	Yes	21
No	No	29

^a Treatments of cells and virus with sucrose or trypsin (or both) were conducted as described in the text.

^b The percentages given here are mean values from three separate experiments.

the movement of cell membranes (as stressed above), the above sugars were not assayed at higher concentrations.

Additionally, to learn about the role of cell membrane movements in EBV penetration, we examined the effect of high sucrose concentration (0.25 M) on this process. It was thus found that sucrose blocked EBV penetration at a trypsin-sensitive stage on the cell surface (Table 5). The action of sucrose was reversible as shown by the full expression of EBNA when trypsin treatment, before the removal of sucrose, was omitted (Table 5).

DISCUSSION

Previous studies in this laboratory have led to the identification of several lymphoid cell lines which bind EBV but do not allow expression of viral antigens (21, 28). One of these cell lines, Molt-4, was studied by both immunofluorescence and electron microscopy; it was then found that the virus bound to the surface of Molt-4 cells, but neither viral antigens nor nucleocapsids could be detected in them (21). These studies raised the possibility that, in these cells, EBV infection may be controlled at the level of virus penetration and prompted us to investigate the mechanism of penetration of this virus.

In the present studies, we wanted to evaluate the relative importance of cellular and viral functions that may contribute in determining the fate of cell surface-bound EBV: either penetration and antigen induction (in susceptible cells) or lack of penetration or infection or both (in Molt-4 and other resistant cells).

The results indicate that ConA inhibits EBV infectivity and protects the target cells against virus infection. This is in agreement with observations reported on other enveloped viruses (26, 34). Neither the virus nor the cells were irreversibly altered by ConA since they were released from its effect by elution with MM. The MM

elution data also indicate that EBV binding to its specific receptors was inhibited by the lectin. This inhibition may be a consequence of the ability of ConA to mediate EBV binding to ConA receptors on the cell surface (Table 4); it is thus conceivable that in the presence of high amounts of ConA on the virus or cell surface (Table 2), EBV binds predominantly through ConA receptors and is later eluted by MM from the cell surface.

The most interesting feature of the effect of ConA on EBV was the ability of this lectin to inhibit virus penetration, despite the fact that EBV was allowed to bind to its specific receptors before ConA treatment. This is indicated by the fact that when the virus-cell complexes, preformed and treated with ConA at low temperature, were incubated at 37°C for 2 h, the virus remained susceptible to the action of trypsin. A similar conclusion was also reached regarding the effect of ConA on virus penetration when ConA treatment was applied at different time intervals postinfection. It appears also from the latter experiments that ConA does not influence antigen induction by internalized virus; this, along with the ability of MM to reverse the effect of ConA on EBV penetration, indicates that the action of this lectin is exerted at the cell or virus surface.

In light of the known properties of ConA (8, 36), the effect of ConA on EBV penetration may be explained by one or both of the two following mechanisms. (i) ConA-blocked virus or cellular oligosaccharides (or both) might participate in virus penetration after the binding stage; interestingly, the importance of adequate glycosylation of viral glycoproteins for the production of infectious virus has been established (3, 29). (ii) ConA, by virtue of its tetravalence, could cross-link and restrict the mobility of virus and cell surface glycoconjugates (9, 36). This action of ConA, in turn, would inhibit envelope fusion which requires the mobility of membrane components (1, 16, 31).

We were not able to distinguish between these two possible mechanisms of ConA action by using a variety of inhibitors of oligosaccharide-mediated interactions (4, 8, 33); these inhibitors (sugars and ovalbumin) were intended to compete with viral or cellular carbohydrate markers that might contribute to virus penetration, but did not show any effect. Therefore, if oligosaccharide-mediated interactions are actually involved in EBV penetration, they would rely on sugar configurations different from those assayed here; alternatively, the lack of effect of these inhibitors might indicate that the involved oligosaccharide-mediated interactions are irreversible.

In agreement with the second possible mecha-

nism of ConA action, we found that high sucrose concentration (0.25 M) blocked EBV penetration, thus confirming the importance of the mobility of membrane components. Sucrose at high concentration is a strong inhibitor of cell membrane movements, as shown in the SV-Ehrlich ascites tumor cell system (17, 24). In this system, sucrose does not prevent SV envelope fusion which is controlled exclusively by the viral fusion factor $F_{1,2}$ whereas it prevents SV-induced cell-cell fusion which, in addition to the $F_{1,2}$ glycoprotein, requires specific movements of the cell membrane and some function(s) of the cytoskeleton.

Surprisingly, high sucrose concentration blocked EBV penetration at a trypsin-sensitive stage, indicating the absence of virus envelope fusion with the cell membrane. This suggests that EBV envelope fusion, unlike that of SV, depends on target cell membrane movements. However, our data do not show whether these membrane movements or the related mechanism of EBV penetration is induced upon EBV binding to specific virus receptors or represents a normal characteristic of EBV-susceptible cells. In any event, the need for specific virus-cell interactions to allow virus penetration is suggested by the inability of low ConA concentrations to render various resistant cell lines susceptible to EBV infection. At these concentrations, ConA was able to mediate EBV binding to receptor-negative cell lines and did not inhibit EBV infectivity in susceptible ones. It should be noted here that we had also chosen for these experiments EBV receptor-negative cell lines on the basis of their ability to support EBV antigen expression after the implantation into their membrane of EBV receptor-bearing Raji cell membrane fragments (35). Therefore, it seems likely that the absence of EBV antigen induction in these resistant cells is due to the lack of virus penetration. The failure of ConA-mediated binding of EBV to lead to EBV antigen expression is in line with the observation that several EBV receptor-positive cell lines are not susceptible to EBV infection (28); it further consolidates the concept that factors other than the mere presence of virus receptors are responsible for the unusually narrow host range of EBV.

In conclusion, our results emphasize both the specificity and complexity of the events leading to EBV penetration into target cells. They constitute the first experimental evidence that EBV envelope fusion follows a different and more intricate pathway than that of the well-studied fusion of the SV envelope. This new pathway is not yet known, but it may rely partly or entirely on a cellular mechanism involving membrane movements that may be specifically triggered upon EBV binding to its specific receptors. It is

also possible that these membrane movements or a related mechanism allowing EBV penetration represents, at least in part, membrane activities specific to the EBV-susceptible B-lymphoid cell. The understanding of the events governing EBV penetration may even lead to the identification of new properties of the B-lymphocyte membrane.

ACKNOWLEDGMENTS

We thank Cancer Research Society, Inc., Montreal, and The Canadian Medical Research Council for their support. R.K. has a graduate studentship from the Fondation du Conseil des Clubs Sociaux, Montreal, and J.M. is a senior Research Scholar from the Conseil de la Recherche en Santé du Québec.

We thank Sylvie Cardinal for typing the manuscript.

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