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Sendai Virus Envelopes Can Mediate Epstein-Barr Virus Binding to and Penetration into Epstein-Barr Virus Receptor-Negative Cells

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Epstein-Barr virus (EBV) receptor-negative cells were treated with UV-inactivated Sendai virus (SV) or with reconstituted SV envelopes having a low hemolytic activity and then assayed for EBV binding or for susceptibility to EBV infection. EBV binding was assessed by using both unlabeled and fluoresceinated EBV preparations. It was found that SV or SV envelope treatment renders these cells able to bind EBV. Various experiments were performed to clarify the mechanism of this SV-induced binding. The EBV receptor-negative 1301 cells were treated with SV either at 0°C or at both 0 and 37°C successively and then examined for EBV binding at 0°C. It was thus found that when SV treatment was performed exclusively at 0°C, the target cells showed higher fluorescence intensity after their incubation with fluoresceinated EBV. In addition, *Clostridium perfringens* neuraminidase treatment of 1301 cells did not induce any EBV binding to these cells. These data indicate that EBV binding is not due to the disturbance of the cell membrane by SV envelope fusion or to the uncovering of EBV binding sites on the cells after the enzymatic action of SV neuraminidase. Moreover, bound EBV was partly eluted from SV-treated 1301 cells at 37°C, and the treatment of EBV with *C. perfringens* neuraminidase inhibited its SV-mediated binding. These data indicate that EBV binds to the hemagglutinin-neuraminidase of SV on the target cell surface and that a fraction of the bound EBV becomes irreversibly associated with the SV-treated cell membrane. Our data also show that EBV can penetrate into 1301 cells which have incorporated SV envelopes into their membrane, as demonstrated by the induction of the EBV-determined nuclear antigen by B95-8 EBV in SV envelope-treated 1301 cells.

The host range of Epstein-Barr virus (EBV) is determined by specific interactions between the viral envelope and the host cell membrane. These specific interactions involve virus binding to EBV receptors on the cell surface (5, 8, 17), followed by fusion of the viral envelope with the target cell membrane (11, 20, 26). Although only human and some nonhuman primate B lymphocytes and B lymphoid cell lines can be infected by EBV in vitro, the virus appears to also transform epithelial cells in vivo. This is shown by the regular presence of EBV DNA and EBV-determined nuclear antigen (EBNA) in the epithelial cells of undifferentiated or poorly differentiated nasopharyngeal carcinoma (12, 22, 31). It is still unclear how EBV gets into epithelial cells of the nasopharynx, since attempts to infect such cells by direct exposure to EBV were unsuccessful (3), and the presence of EBV receptors has never been demonstrated on normal human epithelial cells. To answer the question as to how EBV enters the postnasal epithelial cell, it is very important to understand the basic mechanism of EBV penetration into target B lymphocytes and to perform model studies on alternative mechanisms which may allow EBV binding and penetration in the absence of specific EBV receptors.

In a previous paper (11) we reported initial studies on the mechanism of EBV penetration into target cells; we used a model system in which the tetravalent lectin concanavalin A (ConA) served to attach EBV to EBV receptor-negative cells, to define the important factors that lead to EBV penetration into target cells. The absence of EBV infection in this model system and in the EBV receptor-positive Molt-4 cells led us to the conclusion that the EBV envelope may not possess a fusion factor such as $F_{1,2}$ of paramyxoviruses. This conclusion is in

line with the data of other investigators (1) showing that EBV induces cell-cell fusion from within and not from without. The above and other observations led us to postulate the existence of a mechanism in the cell membrane of B lymphocytes, which allows EBV envelope fusion (11). Therefore, in subsequent studies with the above model system and UV-inactivated Sendai virus (SV) as a source of the fusion factor $F_{1,2}$, we wanted to see the effect of incorporating a fusion factor in the cell membrane on EBV infection. Thus, while examining the cells for EBV binding before and after the addition of SV or ConA to the EBV receptor-negative targets, we found that SV-treated cells were able to bind EBV in the absence of ConA (unpublished data). Since we did not know the mechanism of this SV-induced EBV binding, we also decided to investigate in parallel the binding of a well-characterized ligand, ConA, which is known to bind specifically to α-D-manno (or gluco)-pyranoside moieties on the SV envelope (SVE) (23).

The cells were grown in RPMI 1640 medium supplemented with heat-inactivated fetal bovine serum at a final concentration of 10% and antibiotics (RPMI-10) as described previously (17). EBV preparations of P3HR-1 and B95-8 strains were obtained from cell-free supernatants of the corresponding EBV producer cell cultures P3HR-1 and B95-8, respectively (18). EBV was concentrated, labeled with fluorescein isothiocyanate (FITC), and purified on Sepharose 4B columns (Pharmacia Fine Chemicals, Inc.) as described previously (10). SV was grown in 10-day-old embryonated eggs for 48 h. The SV-containing chorioallantoic fluid was clarified by two cycles of low-speed centrifugation (20 min at 1,000 × *g*), and the virus was pelleted by centrifugation at 45,000 × *g* for 60 min. SV pellets were suspended in calcium- and magnesium-free phosphate-buffered saline (PBS) at a suitable SV concentration.

SV concentration was determined as hemagglutinating units (HAU) by Salk's pattern method (25), using 50 μl of 0.5% chicken erythrocytes and 50 μl of serially diluted SV suspension. SV was UV inactivated before use in all experiments except for SVE reconstitution.

Table 1 shows that when various EBV receptor-positive and receptor-negative cell lines were treated with SV at 0 and 37°C, they bound FITC-EBV to the same extent regardless of their EBV receptor status. On the other hand, in sham-treated samples only EBV receptor-positive cell lines (Raji and BJA-B) bound FITC-EBV.

The data in Table 1 also show that FITC-labeled ConA (FITC-ConA) binding to the SV-treated EBV genome-negative cell lines was much stronger than its binding to sham-treated counterparts, whereas FITC-ConA binding to EBV genome-positive cells was not significantly increased by the SV treatment. However, in the absence of SV treatment, FITC-ConA binding to EBV genome-positive cells was much stronger than its binding to EBV genome-negative ones, as could be expected from previously published data (28). It is therefore possible that the density of ConA receptors on SVE is similar to that on the membrane of EBV genome-positive cells, but higher than that of EBV genome-negative cells.

Since the cell lines used here are of various origin and nature (Table 1), the effect of SV appears to be independent of the cell type used. Therefore, for the following experiments, we chose to use only one of the EBV receptor-negative, EBV genome-negative cell lines, 1301. To exclude the possibility that fluoresceinated material of cellular origin eventually contained in the FITC-EBV preparation may account for all membrane fluorescence observed, we performed the following control experiments. Samples of FITC-EBV preparation were adsorbed at 0°C either with SV-treated or sham-treated 1301 cells or with the EBV receptor-positive BJA-B cells, and the residual unbound FITC-EBV was assayed for binding to Raji cells. The data in Table 2 show that SV-treated 1301 cells (SV-1301) adsorbed FITC-EBV to a similar extent as the BJA-B cells, whereas sham-treated 1301 cells did not adsorb FITC-EBV at all. These data indicate that FITC-EBV binds to SV-1301 cells.

Although we have previously shown that EBV fluoresceination does not alter its main biological properties (10), we wanted to know whether the above-described FITC-EBV binding to SV-treated cells is a property of EBV or only of its fluorescein-labeled counterpart. Therefore, the above adsorption experiments were repeated with unlabeled EBV, and the residual unbound EBV was used to induce EBNA in BJA-B cells. The results (Table 2) show very clearly that infectious EBV particles of both P3HR-1 and B95-8 strains bound to SV-1301 cells to a similar extent as to BJA-B cells. This shows that FITC-EBV binding to SV-treated cells is not due to an eventual modification in EBV as a consequence of its labeling with FITC.

The possible mechanisms of the SV-induced EBV binding were investigated in light of the known properties of SV. The following possibilities were considered: (i) uncovering of hidden nonspecific sites able to bind EBV, ConA, and probably other ligands due to disturbance of the cell membrane by SV (14) or to the enzymatic action of the SV neuraminidase on cell surface glycoproteins; (ii) uncovering of hidden specific EBV and ConA receptors for the reasons indi-

TABLE 1. Effect of the treatment of various cell lines with SV on binding of FITC-EBV and FITC-ConA

Cell line	Origin (nature; reference)[a]	EBV genome status[b]	Treatment of cells with SV[c]	FITC-EBV binding[d]		FITC-ConA binding[d]	
				% Fluorescent cells	Fluorescence intensity[e]	% Fluorescent cells	Fluorescence intentsity[e]
Raji	BL (B; 24)	+	No	98	++	100	+++
			Yes	100	+++	100	+++
BJA-B	BL (B; 19)	−	No	70	+	93	+
			Yes	100	+++	100	+++
P3HR-1	BL (B; 6)	+	No	0	−	100	+++
			Yes	100	+++	100	+++
B95-8	M (B; 21)	+	No	0	−	100	+++
			Yes	100	+++	100	+++
1301	ALL (N; 9)	−	No	0	−	86	+
			Yes	100	+++	100	+++
K-562	CML (N; 13)	−	No	0	−	80	+
			Yes	100	+++	100	+++

[a] BL, Burkitt's lymphoma; M, EBV-transformed monkey (marmoset) lymphocytes; ALL, acute lymphoblastic leukemia; CML, chronic myelocytic leukemia; B, thymus-independent B lymphocyte; N, non-B, non-T lymphocyte.

[b] +, EBV genome positive, as determined by the presence of EBNA or EBV DNA or both; −, EBV genome negative.

[c] The cells were treated with SV by adding 750 HAU of SV in 25 μl of PBS to a pellet of 10^6 cells at 0°C (in ice). The cells were incubated for 20 min at 0°C with occasional shaking and, then washed twice with cold RPMI and resuspended in 2 ml of this medium; the cells were then warmed to 37°C and incubated for 30 min at this temperature with regular shaking (SV treatment at 0 and 37°C). At the end of incubation the cells were washed twice with RPMI and placed at 0°C for binding studies.

[d] To assay for FITC-EBV or FITC-ConA binding, 50-μl samples of FITC-EBV preparation or of a FITC-ConA solution at 100 μg/ml, prepared as described previously (11), were added to 10^6 test cells at 0°C. The cells were then incubated at 0°C for 20 min with occasional shaking; at the end of incubation the cells were washed twice with cold RPMI. The cells were then washed once with PBS at 4°C, suspended in 2 drops of cold glycerol-PBS (1:1), and observed for membrane fluorescence. FITC-EBV used here was of the B95-8 strain. The percentages given are rounded means from two separate experiments. Cells treated with uninfected (control) chorioallantoic fluid processed similarly as for the preparation and concentration of SV gave results identical to those in the absence of SV treatment shown above.

[e] Fluorescence intensity of preparations in this study was assessed as described earlier (10).

cated above; or (iii) that the SV hemagglutinin-neuraminidase molecule binds *N*-acetylneuraminic acid residues on the EBV envelope in the same way as it binds to SV receptors on cells, whereas ConA binds to specific ConA receptors of the cell membrane-associated SVE.

Several experiments were performed to test these possibilities or mechanisms.

Since it is known (14) that only SV binding occurs at low temperature with neither SV envelope nor cell membrane fluidity changes, and without detectable neuraminidase action, mechanisms i and ii were first investigated by treating 1301 cells with SV at 0°C exclusively.

The data in Table 3 show that 1301 cells treated with SV at 0°C bound FITC-EBV or FITC-ConA with a higher fluorescence intensity than 1301 cells treated with SV at 0 and 37°C. These data also show that SV agglutinated the cells as long as they were kept in the cold, indicating that SV neuraminidase did not function at low temperature. Furthermore, 1301 cells treated with *Clostridium perfringens* neuraminidase did not bind any FITC-EBV or FITC-ConA (Table 4). These data indicate that SVE components are directly involved in EBV and ConA binding to SV-treated cells. In addition, FITC-ConA could be eluted from the surface of both SV-treated and sham-treated cells with α-D-methylmannoside (a competitive inhibitor of ConA binding), regardless of whether SV treatment was done at 0°C only or at 0 and 37°C (data not shown). Therefore, FITC-ConA was specifically bound to ConA receptors on the cell membrane and on the SVE associated with it. As the above data argue against the occurrence of mechanisms i and ii, the possibility that the hemagglutinin-neuraminidase glycoprotein of SV mediated EBV binding (mechanism iii) was then tested as follows. First, the hemadsorption

TABLE 2. Adsorption of FITC-EBV and unlabeled EBV with SV-treated cells

EBV adsorption with:	Residual FITC-EBV[a] binding to Raji cells [% fluorescent cells (fluorescence intensity)]		EBNA induction[b] in BJA-B cells by residual EBV[a] (% EBNA positive cells)			
	Adsorption at 0°C	Adsorption at 0 and 37°C	Adsorption at 0°C		Adsorption at 0 and 37°C	
			P3HR-1 EBV	B95-8 EBV	P3HR-1 EBV	B95-8 EBV
None	98 (+++)	97 (+++)	31 ± 3	47 ± 5	34 ± 4	47 ± 4
1301	99 (+++)	98 (+++)	32 ± 3	48 ± 4	33 ± 4	49 ± 3
SV-1301[c]	55 (+)	81 (++)	3 ± 1	3 ± 1	22 ± 3	28 ± 5
BJA-B	60 (+)	50 (+)	6 ± 2	8 ± 2	2 ± 1	4 ± 2

[a] FITC EBV was of the P3HR-1 strain. Samples of 0.1 ml of EBV or FITC-EBV suspension in RPMI were added to pellets of 10^7 BJA-B cells or 1301 cells pretreated as described in the text with or without SV. The resulting virus-cell mixture was shaken and incubated either at 0°C for 80 min (adsorption at 0°C) or at 0°C for 20 min; then these preparations were transferred to 37°C and incubated for 60 min (adsorption at 0 and 37°C) with regular shaking. After incubation, the cells were pelleted and the supernatant containing residual EBV or FITC-EBV was harvested and centrifuged at 1,000 × *g* for 10 min to remove contaminating cells. A 50-μl amount of the final FITC-EBV or EBV-containing supernatant were used to assay for FITC-EBV binding to Raji cells or to induce EBNA in BJA-B cells, respectively.

[b] For EBNA induction, pellets of 5×10^5 BJA-B cells were suspended in 50 μl of either original or absorbed EBV preparation. The cells were then incubated at 37°C for 1 h with occasional shaking, washed twice with RPMI, resuspended in 1 ml of RPMI-10, and cultured for 48 h. The cells were harvested and processed for EBNA detection by anti complement immunofluorescence as described before (17).

[c] Pellets of 10^7 cells of the 1301 cell line were treated with 3,000 HAU of SV in 0.1 ml of PBS at 0 and 37°C, as described in Table 1, footnote *c*.

test was performed at 0°C on SV-1301 cells, using sheep erythrocytes; then the elution of sheep erythrocytes was followed at 37°C in parallel with the elution of FITC-EBV from the surface of SV-1301 cells. The data in Table 5 show that, whereas sheep erythrocytes were completely eluted from the hemadsorbing cells after 1 h of incubation at 37°C, FITC-EBV was only incompletely eluted during this time, since 84% of the cells retained a weak fluorescence. It is noteworthy that 55% of these cells were capped, and shedding of caps was frequently observed.

Next, adsorption of FITC-EBV or unlabeled EBV was performed at 0 and 37°C and compared with their adsorption at 0°C. The results (Table 2) show that both FITC-EBV and unlabeled EBV were eluted extensively but incompletely from the SV-1301 cells, thus indicating the involvement of hemagglutinin-neuraminidase in EBV binding. Further evidence of the involvement of hemagglutinin-neuraminidase in EBV

TABLE 3. Effect of temperature of treatment with SV or with SVE on FITC-EBV or FITC-ConA binding to 1301 cells and on cell agglutination

Treatment of 1301 cells[a]	Cell agglutination[b]	FITC-EBV binding[c]		FITC-ConA binding[c]	
		% Fluorescent cells	Fluorescence intensity	% Fluorescent cells	Fluorescence intensity
None (sham-treated control)	−	0	−	85	+
SV at 0°C	+++	100	++++	100	++++
SV at 0 and 37°C	±	100	+++	100	+++
SVE at 0°C	+++	100	++++	100	++++
SVE at 0 and 37°C	−	100	+++	NT	NT

[a] Cells were treated with SV at 0 and 37°C as indicated in Table 1, footnote *c*. SVE treatment was performed under identical conditions as for SV. In SV or SVE treatment exclusively at 0°C, the step of incubation of cells at 37°C was omitted. Reconstituted SVE having a low hemolytic activity were prepared as described previously (29, 30). Briefly, SV was extracted by Triton X-100 to solubilize envelope components, and the SVE solution thus obtained was dialyzed at 4°C for 40 h against 10 mM Tris-hydrochloride buffer (pH 7.5) containing 2 mM $CaCl_2$, 2 mM $MgCl_2$, 2 mM NaN_3, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mg of bovine serum albumin per ml. This SVE suspension was then layered on 10% sucrose, centrifuged at 100,000 × *g* for 1 h at 4°C, and the SVE pellet was suspended in cold (4°C) PBS and kept at 4°C until its use within 1 week.

[b] Cell agglutination was recorded before addition of FITC-EBV or FITC-ConA.

[c] The values given are rounded means from two separate experiments. NT, Not tested.

TABLE 4. Effect of neuraminidase[a] on FITC-EBV[b] and FITC-ConA binding to SV-treated and control cells

Cells and cell treatment	Treatment of FITC-EBV or FITC-ConA	FITC-EBV binding[c]		FITC-ConA binding[c]	
		% Fluorescent cells	Fluorescence intensity	% Fluorescent cells	Fluorescence intensity
1301	PBS	0	−	86	+
SV-1301	Heat-inactivated neuraminidase	100	+++	100	+++
SV-1301	Neuraminidase	87	+	100	+++
Neuraminidase-treated 1301	PBS	0	−	87	+
Raji	Neuraminidase	99	++	NT	NT
Raji	PBS	98	++	NT	NT

[a] The treatment of 1301 cells, FITC-EBV, or FITC-ConA with *C. perfringens* neuraminidase (Millipore Corp.) was at 1.3 U/ml for 1 h at 37°C. FITC-EBV and FITC-ConA were also sham treated with PBS or heat-inactivated neuraminidase (5 min at 100°C). At the end of incubation with neuraminidase, the cells were washed twice with PBS and kept at 0°C, whereas FITC-EBV or FITC-ConA was used without removal of the neuraminidase.

[b] FITC-EBV used was of the B95-8 strain.

[c] The values are means from two separate experiments. NT, Not tested.

binding to SV-1301 cells was given by the inhibitory effect of the treatment of FITC-EBV by *C. perfringens* neuraminidase on its binding to SV-1301 cells (Table 4). The specificity of neuraminidase treatment was shown by the lack of effect of neuraminidase treatment of FITC-ConA on its binding to SV-1301 cells and by the inability of heat-inactivated neuraminidase to affect either FITC-EBV or FITC-ConA binding to these cells. The observation that EBV elution from SV-1301 cells was incomplete may indicate that a fraction of the EBV particles have become irreversibly associated with the SV-1301 cell membrane, probably by action of $F_{1,2}$. The role of $F_{1,2}$ in this irreversible association of EBV with SV-1301 cells was therefore investigated by adsorbing EBV preparations on SV-1301 cells or SV-1301 cells treated with trypsin to selectively inactive $F_{1,2}$ without affecting hemagglutinin-neuraminidase (15). However, the effect of trypsin treatment was more complex than expected, since in addition to abrogating irreversible EBV binding at 37°C, it also decreased EBV binding at 0°C (data not shown).

To know whether some of the bound EBV particles underwent fusion of their envelope with the SV-1301 cell membrane and then succeeded in releasing their nucleocapsid into the cytoplasm, we examined the ability of B95-8 EBV to induce EBNA in SV-1301 cells.

In preliminary experiments we found that the treatment of cells from various lymphoid cell lines (1301, BJA-B, and Molt-4) with SV at concentrations in the range of those used for FITC-EBV and unlabeled EBV binding and adsorption studies (i.e., 300 to 750 HAU/10^6 cells) did not affect cell viability significantly even up to 4 h after SV treatment. However, when examined after 18 h of culture in RPMI-10, the cells were found to be degenerated completely (data not shown). To prevent this cell degeneration, it was necessary to treat the cells with an

TABLE 5. Elution of FITC-EBV and SRBC from SV-1301 cells at 37°C and capping of the bound FITC-EBV[a]

Incubation time (min) of cells at 37°C after SRBC or FITC-EBV binding at 0°C	FITC-EBV binding			% Hemadsorption-positive cells[b]
	% Fluorescent cells	Fluorescence intensity	% Capped cells	
0	100	+++	0	98
30	90	++	31	24
60	84	+	55	0

[a] FITC-EBV of the B95-8 strain was used. The cells were treated with FITC-EBV at 0°C as indicated in Table 1, footnote *d*, and then placed at 37°C with regular shaking for FITC-EBV elution studies. At the end of each incubation period, the cells were washed twice with cold PBS and processed for microscopic observation. SRBC, Sheep erythrocytes. Values given are rounded mean values from two separate experiments.

[b] The hemadsorption test (16) was performed by adding 0.1 ml of a cold (4°C) 0.1% suspension of sheep erythrocytes in RPMI to a pellet of 10^6 control 1301 or SV-1301 cells. The cells were mixed and incubated at 0°C for 30 min, with gentle shaking at 10-min intervals; then the cells were either immediately observed for hemadsorption or further incubated at 37°C for elution of the bound sheep erythrocytes. Control samples for elution were kept at 0°C. Untreated 1301 cells showed no hemadsorption.

SV concentration (5 HAU/10^6 cells) which mediated a barely detectable FITC-EBV binding. Therefore, we chos to use an intermediate SV concentration (50 HAU/10^6 cells), which rendered 1301 cells clearly positive for FITC-EBV binding while still causing extensive cell death (Table 6), and to remove dead cells from the various samples by centrifugation through Ficoll-Isopaque.

The data in Table 6 show, first, that no EBNA induction was detected in SV-1301 cells 48 h after incubation with EBV. Despite the high susceptibility of BJA-B cells to the cytotoxic effect of SV, EBNA induction in the surviving SV-treated BJA-B cells was not significantly inhibited by the SV treatment. This suggested that the absence of EBNA induction in the surviving SV-treated 1301 cells is due to the lack of EBV penetration into the cells, probably because the surviving cells were those which did not bind enough SV to allow EBV envelope fusion. To eliminate the cytotoxic effect of SV, we used nonhemolytic reconstituted SVEs which did not cause cell death (Table 6). SVEs were as efficient as SV in mediating FITC-EBV binding when the same number of HAU per 10^6 cells was used (Table 3). Moreover, the data in Table 6 show that when 1301 cells were treated with SVE they became infectible by EBV as shown by the induction of EBNA in a small fraction (0.2 to 0.6%) of these cells.

The present study shows that EBV binding and penetration take place in EBV receptor-negative cells which have integrated SVE in their membrane. In earlier studies, other investigators (27, 30) have used reconstituted SVEs as vehicles to deliver EBV or to transfer EBV receptors from Raji cells to various receptor-negative cell lines; these manipulations allowed EBV binding and penetration into EBV receptor-negative targets. Our findings indicate that the treatment of EBV receptor-negative cells with reconstituted SVEs alone is sufficient to allow EBV binding and penetration into these cells.

The low frequency of EBV-infected SVE-1301 cells is not surprising and may reflect one or a combination of the following situations. (i) The SVE-mediated binding of EBV is not EBV specific and the EBV preparations used may eventually contain cell membrane fragments and vesicles; therefore, competition between EBV and membrane material of cellular origin for binding would decrease the chances of EBV infection. (ii) Only a fraction of the bound EBV particles fuse with the cell membrane, the remainder being eluted by action of the SV neuraminidase. (iii) The SVEs used have a very low ability to mediate cell-cell fusion in suspension (29) and, by analogy, they may have a low EBV-cell fusion capacity. In addition, the possibility that only a fraction of the 1301 cells are able to express EBV antigens should not be disregarded.

TABLE 6. Effect of treatment of cells with SV or SVE on EBNA induction by B95-8 EBV in these cells[a]

Cell line and cell treatment	% Cell viability[b] at:		% EBNA-positive cells[b]	
	1 h[c]	18 h[c]	EBV-positive serum	EBV-negative serum
1301	98	98	0	0
1301 + SV	95	65	0	0
1301 + SVE	95	95	0	0
1301 + EBV	96	97	0	0
1301 + SV + EBV	94	62	0	0
1301 + SVE + EBV	98	95	0.2, 0.6	0, 0
BJA-B	94	97	0	0
BJA-B + SV	95	21	0	0
BJA-B + SVE	96	96	0	0
BJA-B + EBV	96	98	33	0
BJA-B + SV + EBV	93	20	29	0
BJA-B + SVE + EBV	91	95	32, 28	0

[a] In these experiments 5×10^6 cells were treated with 250 HAU of SV in 0.1 ml of RPMI, at 0 and 37°C as described in Table 1, footnote *c*. To assay for the effect of SVE on cell infectibility by EBV, 5×10^5 cells were treated with 1,500 HAU of SVE in 25 μl of PBS at 0 and 37°C as above. For samples in which SV-treated 1301 or BJA-B cells were used as targets for EBV infection, 5×10^6 target cells were infected with 0.5 ml of EBV preparation as described in Table 2, footnote *b*. In these experiments the target cells were passed, at 18 h after EBV infection, over Ficoll-Isopaque to remove dead cells; the surviving cells thus obtained were further cultured for 30 h at a concentration of 5×10^5 cells per ml. The cells were then harvested and processed for the detection of EBNA as above. SVE-treated cells were infected by EBV as described in Table 2, footnote *b*.

[b] All percentages given are mean values from two separate experiments. The sets of two values represent the data obtained with two different SVE preparations. The use of different EBV-positive and EBV-negative sera did not change the results to any significant degree, and not less than 2,000 cells per smear were counted to determine the percentage of EBNA-positive cells. Assays for the detection of infectious EBV in the supernatant of EBV-infected SVE-1301 cells gave negative results; the expression of EBV antigens other than EBNA was not investigated.

[c] Time after the end of incubation with EBV.

The ability of SVE to mediate EBV infection of EBV receptor-negative cells suggests to us a possible mechanism for the association of EBV with nasopharyngeal carcinoma. This mechanism would consist in the infection of normal epithelial cells of the human nasopharynx by a "helper" virus able to mediate EBV binding and

envelope fusion. Under appropriate conditions, the helper virus would be able to establish a special type of interaction with some of the epithelial cells so that they will express helper virus envelope components on their surface at an optimal density, without being lysed during the virus replication cycle. This natural helper virus-epithelial cell system may be equivalent to the SVE-1301 cell model system and, by analogy, may be infected by EBV under certain conditions which may lead to the development of nasopharyngeal carcinoma. This helper virus hypothesis may also explain the following observations, which otherwise seem to be contradictory. It was reported that one of three nasopharyngeal carcinoma tumors was superinfected by EBV (3). It was also found that normal human nasopharyngeal epithelial cells could be stimulated by EBV into rapid outgrowth suggestive of transformation (7), whereas recent attempts to infect such cells by direct exposure to EBV have failed (4). The infectibility of cells of one of the nasopharyngeal carcinoma tumors and probably of some normal epithelial cells by EBV may indicate that these cells were expressing envelope components of a helper virus on their surface. Furthermore, the U cell line, presumably of epithelioid type, was reported to be susceptible to EBV infection (2). It would be very interesting to see whether this cell line is persistently infected with a given virus which may play the helper role.

In conclusion, the present studies show for the first time that EBV binding and penetration into EBV receptor-negative cells may be mediated by SVE components inserted in the cell membrane. Moreover, our data also point to a new approach for investigating the etiological role of EBV in human nasopharyngeal carcinoma.

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