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Use of Fluoresceinated Epstein-Barr Virus to Study Epstein-Barr Virus-Lymphoid Cell Interactions

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As a direct approach to visualize Epstein-Barr virus (EBV) binding to its cellular receptors and to learn more on the nature of this binding, virus preparations were conjugated to fluorescein isothiocyanate and used to detect EBV receptors on lymphoid cells. Different enzymatic and chemical treatments were also applied either to the virus or to target cells or to both, and the effect of these treatments on virus binding was then examined. The results obtained show that: (i) EBV can be fluoresceinated without affecting its infectivity or cell binding ability, and the fluoresceinated virus represents an important tool to investigate the biology and nature of EBV interactions with its cellular receptors; (ii) the two virus strains (P3HR-1 and B95-8) share common receptors on Raji cells; (iii) protease treatment of EBV or target cells abrogates virus binding; (iv) EBV receptors regenerate after removal of the protease, and this regeneration is inhibited by cycloheximide or sucrose; (v) EBV particles bear concanavalin A receptors, and this lectin hinders the interaction of the virus with its cellular receptors; (vi) neuraminidase treatment, various monosaccharides, ovalbumin, and glycopeptides derived from EBV or cell surface do not inhibit virus binding. Taken together, the above data also demonstrate that some cellular and viral surface (glyco-) proteins are required for EBV binding to its targets.

Epstein-Barr virus (EBV) receptors are found on human B lymphocytes (4, 6, 13) and also on cells from some established cultures of T and "null" (non-B, non-T) lymphocyte lineage (7, 16, 17). These EBV receptors have not yet been sufficiently characterized, and therefore very little is known of their nature and biology. It has been suggested that EBV receptors are closely associated with receptors for the third component of complement (7, 10, 26) but are distinct from them (22). Various methods have been used to detect EBV receptors; the most frequently used of these methods are the membrane immunofluorescence (MIF) assay with fluorescein-labeled antibodies (9, 17) and the adsorption assay consisting of titration of residual, unbound EBV on different target cells (21). Other assays include rosette formation between EBV producer and target lymphocytes (6) or between tanned erythrocytes treated with EBV preparations and target cells (3). The use of in vivo [^{3}H]thymidine-labeled EBV has also been reported (24), but the procedure of virus labeling and its efficiency or reproducibility were not described; in our hands, in vivo labeling of EBV for receptor studies has yielded unreliable results (unpublished data). At least two efficient approaches of EBV labeling may be envisaged: (i) improving in vivo labeling, which may be achieved if a host cell fully permissive for EBV replication or a more specific and potent virus inducer or both are found; to date, a satisfactory system in which this approach is used has yet to be reported; and (ii) in vitro labeling of EB virions without, however, affecting virus binding ability and infectivity. In regard to the latter, the identification of a reagent that can bind covalently to EBV without altering virus binding properties and infectivity will permit the preparation of labeled virus, either radioactive or fluorescent, by using radioactive or fluorescent analogs of that reagent, respectively.

In this investigation, we have established the feasibility of using covalently labeled EBV for a direct virus receptor assay and the usefulness of this labeled virus in studies of the biochemical nature of EBV interactions with the target cell surface. EBV was covalently labeled through its amino groups with fluorescein isothiocyanate (FITC), and the fluoresceinated virus (FITC-EBV) was examined for its ability to bind and infect target cells; the effect of different enzymatic and chemical treatments of the virus or cells or both on EBV binding was also studied. The data obtained indicate that: (i) it is possible to obtain FITC-labeled EBV that has the same

binding and infectivity characteristics as unlabeled virus; (ii) the interaction of EBV with its cellular receptors appears to depend on the integrity of some viral and cellular surface (glyco-) proteins, and (iii) this interaction is hindered by concanavalin A (ConA), a lectin which binds specifically to α-D-mannopyranoside or α-D-glucopyranoside residues (1).

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MATERIALS AND METHODS

Cells. The cell lines used for EBV receptor studies and their main features are shown in Table 1. The cells were cultured in RPMI 1640 medium (RPMI) supplemented with 10% heat-inactivated fetal bovine serum and antibiotics as described previously (RPMI-10) (13).

Virus preparation. Cell-free supernatants from EBV-producing P3HR-1 (5) and B95-8 (18) cell cultures were prepared and used as a source of virus, as described previously (14). These EBV-containing supernatants were concentrated by centrifugation at 45,000 × *g* for 90 min at 4°C. The resulting pellets were gently suspended in cold (4°C) calcium- and magnesium-free phosphate-buffered saline, pH 7.2 (PBS), or RPMI culture medium to obtain the desired EBV concentration relative to that of the unconcentrated, cell-free supernatant.

Virus labeling with FITC. FITC labeling of EBV was performed as follows. A 1/9 volume of a 2-mg/ml solution of FITC (Isomer I; Sigma Chemical Co.) in 0.5 M carbonate-bicarbonate buffer at pH 9.5 was added, while mixing, to 1 volume of a ≥200×-concentrated EBV preparation in PBS. The mixture was then kept at room temperature (21 to 22°C) for 1 h in the dark. Subsequently, three different procedures were used to separate free FITC. The first procedure involved the purification of FITC-EBV on two consecutive dextran T-10 gradients as described for the purification of EBV particles (2). The second procedure was as follows. The mixture was diluted 20 times or more with cold PBS and centrifuged at 45,000 × *g* for 90 min at 4°C, and the resulting pellets were suspended in this buffer to obtain the original volume of virus preparation. The conjugated EBV preparation was then centrifuged at 1,000 × *g* for 20 min to discard large aggregates and stored at −85°C. For electron microscopic examination, some of the pellets thus obtained were washed two times with PBS and resuspended in this buffer, and a drop of the resulting aggregate suspension was placed on a Formvar-covered, carbon-coated grid; the preparation was then negatively stained with 2% phosphotungstic acid at pH 7.2 and examined with a Philips 201 transmission electron microscope. In the third procedure, FITC-EBV was separated from free FITC and purified by gel chromatography on a Sepharose 4B column (Pharmacia Fine Chemicals, Inc.).

Preparation of FITC-labeled ConA. ConA (Pharmacia Fine Chemicals) was dissolved in PBS at 25 mg/ml and stored at −20°C in small samples until use. For FITC labeling, 1 mg of FITC and 50 μl of carbonate-bicarbonate buffer were added to 0.5 ml of ConA solution (25 mg/ml); the mixture was then gently shaken at room temperature for 2 h and further incubated at 4°C overnight. Free FITC was removed by gel filtration on Sephadex G-25, and FITC-labeled ConA (FITC-ConA) was stored at −20°C.

EBV receptor assays. EBV receptors were detected by two methods: (i) indirect MIF with unlabeled EBV, anti-EBV antibody-positive human serum, and rabbit anti-human γ-chain-specific conjugate as described previously (17); and (b) FITC-EBV binding assay. The latter was carried out at 0 to 4°C as follows. Twenty-five microliters of a 200×-concentrated FITC-EBV preparation was added to a pellet of 5 × 10^5 target cells; the mixture was then gently shaken on a Vortex mixer and kept for 45 min in ice with occasional shaking. The cells were then washed three times with cold PBS, resuspended in a mixture of glycerol-PBS (1:1), mounted, and observed for membrane fluorescence with a UV microscope.

Blocking experiments with unlabeled EBV. Equal numbers of target cells (5 × 10^5) were pelleted, suspended in 25 μl of an unlabeled EBV preparation,

TABLE 1. Detection of EBV receptors on different human lymphoid cell lines, using both FITC-EBV and MIF assays

Cell line	Origin (nature; reference)[a]	EBV genome status[b]	% EBV receptor-positive cells for[c]:			
			P3HR-1 EBV		B95-8 EBV	
			FITC-EBV	EBV	FITC-EBV	EBV
Raji	BL (B; 20)	+	97 ± 1	97 ± 2	98 ± 1	95 ± 4
Daudi	BL (B; 8)	+	96 ± 3	ND	97 ± 3	ND
BJA-B	BL (B; 15)	−	83 ± 5	81 ± 7	72 ± 7	73 ± 8
K-562	CML (N; 11)	−	0	0	0	0
1301	ALL (N; 7)	−	0	0	0	0

[a] BL, Burkitt's lymphoma; ALL, acute lymphoblastic leukemia; CML, chronic myelocytic leukemia; B, thymus-independent B lymphocyte; T, thymus-processed 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 numbers represent rounded means of three separate determinations together with standard deviations. ND, Not done, because Daudi cells bear both surface immunoglobulins and Fc receptors, and the MIF assay as described above is therefore inappropriate for these cells.

and then incubated in ice. Concentrations of unlabeled EBV and incubation times are specified in Results. After incubation with unlabeled EBV, the cells were pelleted and resuspended in 25 μl of a 200×-concentrated FITC-EBV preparation and incubated for the time period as indicated below (Results). The operations were then continued as for the FITC-EBV binding assay.

EBNA induction assay. Pellets of 5×10^5 BJA-B cells were suspended in 0.1 ml of EBV suspension and allowed to adsorb virus for 1 h at 37°C; the cells were then washed two times with RPMI, resuspended in RPMI-10, and cultured at 37°C for 48 h. The cells were then harvested and processed for EBV nuclear antigen (EBNA) detection by anticomplement immunofluorescence as described before (13).

Protease treatments and EBV receptor regeneration assay. Cells or virus was treated at pH 7 with 0.25 mg of pronase (protease type VI, repurified; Sigma) per ml or with 1 mg of crystalline trypsin (Sigma) per ml for 10 min at 37°C and then washed with cold RPMI-10 by centrifugation at 4°C. For EBV receptor regeneration studies, 2×10^6 Raji cells were incubated for 10 min at 37°C with RPMI alone, RPMI containing 300 μg of cycloheximide per ml, or RPMI containing 0.3 M sucrose and then trypsin treated as described above. The trypsinized cells were washed three times with, and resuspended in, the corresponding medium (with or without cycloheximide or sucrose) supplemented with 10% fetal bovine serum, and incubated at 37°C under normal culture conditions. Control samples were treated similarly in the absence of trypsin, cycloheximide, and sucrose. Samples of 4×10^5 cells were withdrawn at different time intervals from 0 to 18 h after removal of trypsin, and the percentage of FITC-EBV binding cells was determined.

Crude glycopeptide preparations were obtained by exhaustive pronase digestion of cell surface glycoproteins or whole EBV preparations essentially as described (23).

Treatments directed towards carbohydrates of target cells or EBV or both. ConA treatment of cells (10^6 Raji or B95-8 cells) or EBV was for 45 min at 4°C. In all ConA experiments, 200×-concentrated, labeled or unlabeled EBV preparations of the B95-8 strain were used. Conditions for saturation of cells with ConA were determined by preincubating the cells with ConA at different concentrations and adding FITC-ConA at 100 μg/ml for 45 min; the percentage of FITC-ConA binding cells was then determined. Elution of ConA from the cell surface was achieved by 10 successive washings with cold 0.5 M solutions of α-D-methyl-mannoside (MM) in PBS. After the various treatments of cells or virus or both with ConA (see below), the treated cells were washed two times with PBS, and the treated virus was separated from free ConA by gel filtration through Sepharose 4B. The cells were then incubated with virus for 45 min and subsequently eluted with MM or washed with PBS. Neuraminidase, glycopeptide preparations, ovalbumin, and different sugars were assayed for inhibition of FITC-EBV binding to Raji cells (Table 2).

RESULTS

Effect of FITC on EBV infectivity. To determine whether FITC labeling of EBV affects its

TABLE 2. Summary of treatments that did not affect FITC-EBV binding to Raji cells

Treatment of cells or virus or both with:	Treatment conditions (concn, time, temp [°C])	Control for treatment efficacy
Neuraminidase from *Clostridium perfringens*[a]	0.07 to 1.3 U/ml, 1 h, 37°C	Inactivation of Sendai virus receptors on Raji cells
Glycopeptides from EBV or Raji cells	Equivalent to 100 μl of 400× EBV or to 10^7 Raji cells per 50 μl, 90 min, 4°C	Inhibition of FITC-ConA binding to Raji cells
Ovalbumin	4 mg/ml, 45 min, 4°C	Inhibition of FITC-ConA binding to Raji cells
α-D-Methyl-mannoside	0.5 M, 45 min, 4°C	Inhibition of FITC-ConA binding to Raji cells
D-(+)-Mannose	0.5 M, 45 min, 4°C	Inhibition of FITC-ConA binding to Raji cells
D-(+)-Galactose	0.5 M, 45 min, 4°C	None
L-(−)-Fucose	0.5 M, 45 min, 4°C	None
N-Acetyl-D-galactosamine	0.5 M, 45 min, 4°C	None
N-Acetyl-D-glucosamine	0.5 M, 45 min, 4°C	None
Equimolar mixture of the above-mentioned sugars	0.5 M, 45 min, 4°C	None

[a] Neuraminidase was obtained from Millipore Corp.

main biological properties, we studied first the ability of FITC-EBV to infect and induce EBNA in BJA-B cells. Therefore, samples of the same virus stock were either fluoresceinated or sham treated and then assayed for EBNA induction in BJA-B cells (Fig. 1). These results indicate that, when free FITC was not removed from the FITC-EBV preparation (Fig. 1A) or was removed by molecular sieving through a Sepharose 4B gel (Fig. 1C), no difference in the EBNA-inducing ability of labeled and unlabeled EBV was detected. However, when free FITC was washed away by centrifugation at 45,000 × *g* for 90 min at 4°C (Fig. 1B), infectivity of FITC-EBV was drastically reduced. This centrifugation procedure (see above) also resulted in the formation of large virus aggregates, observed by electron microscopy, which contained almost exclusively EBV nucleocapsids (data not shown). It is therefore possible that FITC conjugation enhanced aggregation of virus particles brought into close contact by centrifugation, since the resulting pellets required stronger and prolonged shaking for virus resuspension, thus resulting in a remarkable decrease in virus titer as compared with unlabeled, control virus preparations (Fig. 1B). Treatment of EBV with carbonate-bicarbonate buffer (pH 9.5) alone did not influence its infectivity. On the basis of the above results, we routinely used Sepharose 4B-purified infectious FITC-EBV preparations for all subsequent studies unless otherwise stated.

FITC-EBV binding assay. After establishing that we could obtain fluoresceinated EBV that was as infectious as its unlabeled counterpart (control), we investigated the use of FITC-EBV to detect virus receptors on different target cells. Examples of fluorescence observed after FITC-EBV binding to Raji cells are shown in Fig. 2. The FITC-EBV binding assay gave essentially the same results as the indirect MIF when both strains of EBV, P3HR-1 and B95-8, and different lymphoid cell lines were used (Table 1). Only EBV receptor-positive cells bound FITC-EBV. This binding was EBV specific, since preincubation of this labeled EBV with anti-EBV antibody-positive human serum completely abolished its binding ability, whereas its preincubation with anti-EBV antibody-negative serum was without effect (Table 3). Furthermore, blocking experiments showed that unlabeled and labeled EBV bind to the same recep-

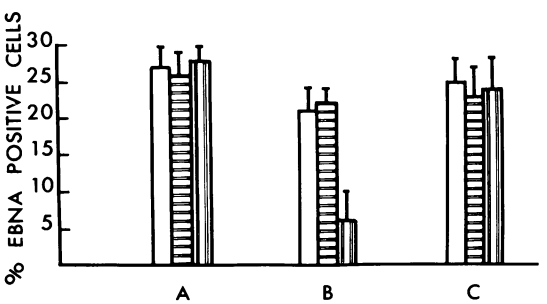

FIG. 1. Effect of fluoresceination on EBV infectivity. A 200×-concentrated B95-8 EBV preparation was reacted with FITC at pH 9.5 (▥) or sham treated at pH 7.2 (PBS) (□) or at pH 9.5 (carbonate-bicarbonate buffer) (▤). The reaction mixture was diluted 5 times with PBS and used in the EBNA induction assay (A) or subjected to the centrifugation procedure for removal of free FITC (B). The resulting EBV preparation was then diluted five times with PBS and used for EBNA induction. The Sepharose 4B gel filtration method for removal of free FITC was applied, and the obtained EBV preparation was brought to a final dilution of five times and assayed for EBNA induction in BJA-B cells (C).

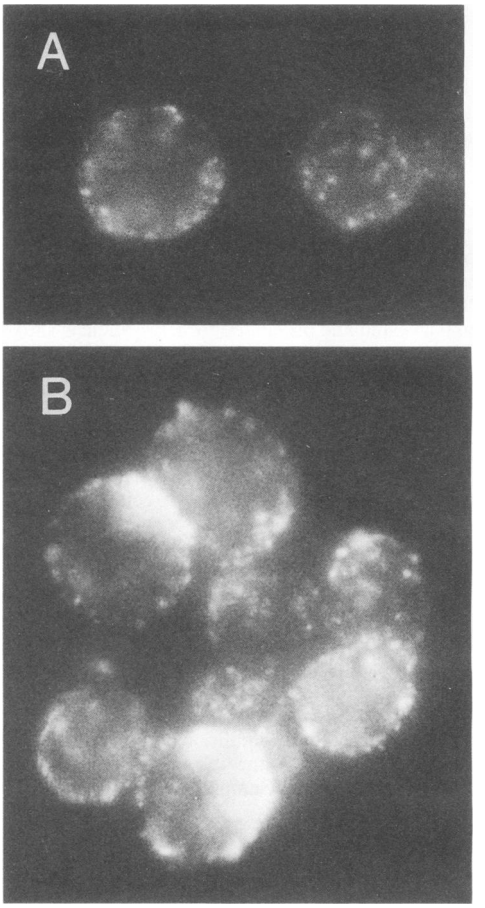

FIG. 2. Membrane fluorescence on Raji cells incubated with FITC-EBV of the B95-8 strain. Both isolated (A) and clumped (B) fluorescent cells are shown. Relative fluorescence intensity for (A) was assessed as + +, whereas that of B was assessed as + + + + (see legend to Fig. 3).

TABLE 3. Blocking of FITC-EBV binding to target cells with anti-EBV antibody-positive serum and with unlabeled EBV

Unlabeled EBV			FITC-EBV		% Membrane fluorescence[a] (positive cells)[c]	
Concn (×)	Strain	No. of incubation periods[b]	Strain	Treatment[c]	Raji	BJA-B
0 (PBS)	None	1	P3HR-1	None	93	82
0 (PBS)	None	1	P3HR-1	PBS	83	74
0 (PBS)	None	1	P3HR-1	− Serum	82	71
0 (PBS)	None	1	P3HR-1	+ Serum	0	0
25	P3HR-1	1	P3HR-1	None	71	44
50	P3HR-1	1	P3HR-1	None	50	27
100	P3HR-1	1	P3HR-1	None	20	18
200	P3HR-1	1	P3HR-1	None	3	2
200	P3HR-1	2	B95-8	None	0	ND
200	B95-8	2	P3HR-1	None	0	ND

[a] The percentages given are rounded mean values from two separate experiments. ND, Not done.
[b] Twenty-five microliters of unlabeled EBV was added for each 45-min incubation period.
[c] Treatment of FITC-EBV was with an equal volume of PBS, anti-EBV antibody-negative human serum (− serum), or anti-EBV antibody-positive human serum (+ serum) for 90 min at 4°C. Incubation of cells with FITC-EBV was for 45 min.

tors, since the preincubation of EBV receptor-positive cells with concentrated, unlabeled EBV preparations completely prevented the binding of FITC-EBV (Table 3). In addition, our results indicated that the two EBV strains bind to the same receptor on Raji cells, since the preincubation of these cells with unlabeled EBV of one strain prevented the binding of FITC-EBV of the second strain, and vice versa (Table 3). More evidence on the identity between binding of labeled and of unlabeled virus to Raji targets was obtained when their binding rates were compared (Fig. 3); the binding of unlabeled EBV was assessed by its ability to block FITC-EBV attachment. It was thus found that 80 to 90% of the cells bound labeled or unlabeled EBV within 5 to 10 min of incubation with virus; subsequently, the binding increased relatively slowly over a 35- to 40-min incubation period; this slow increase in virus binding was characterized by relative changes in fluorescence intensity rather than a change in the number of fluorescent cells (Fig. 3). Most of the above binding experiments were also performed by using fluoresceinated EBV preparations separated by centrifugation procedures (with or without the use of dextran gradient), and identical results were obtained (data not shown).

Effect of proteases on EBV binding. After establishing the FITC-EBV binding assay, we investigated the biochemical nature of the interactions between EBV and its cell surface receptors. We therefore applied different chemical and enzymic treatments to either Raji cells or EBV or to both and then studied the effect of these treatments on virus binding to Raji targets. It was thus found that mild protease treatment of unlabeled EBV preparations rendered them unable to block FITC-EBV binding to Raji cells (Table 4). On the other hand, protease treatment of Raji cells completely inactivated their EBV receptors (Table 4). However, these receptors regenerated after trypsin treatment (Fig. 4), and this regeneration was significantly inhibited by cycloheximide (an inhibitor of protein synthesis) and completely blocked by sucrose, a potent inhibitor of the movement and fusion of cell membranes (12). It should also be noted here that, during these different treatments, cell via-

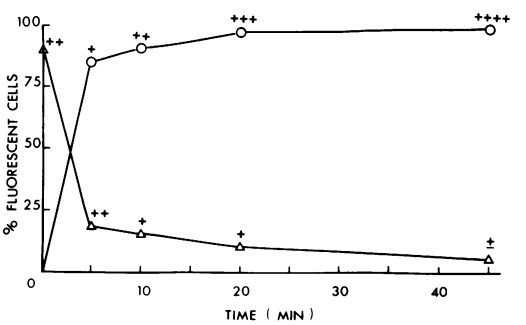

FIG. 3. Kinetics of labeled and unlabeled EBV binding. Raji cells were incubated with a 200×-concentrated FITC-EBV preparation for the indicated time (○) or preincubated with a 200×-concentrated, unlabeled EBV preparation for the indicated time, and then incubated with FITC-EBV for 10 min (△). Both labeled and unlabeled EBV preparations used in these experiments were of the P3HR-1 strain. +, Relative fluorescence intensity observed.

TABLE 4. Effect of proteases on EBV binding to Raji cells[a]

Raji cells treated or incubated with:[b]	% Membrane fluorescence (FITC-EBV)-positive cells[c]
Pronase	0
Trypsin	0
Control unlabeled EBV	0
Pronase-treated, unlabeled EBV	96
Trypsin-treated, unlabeled EBV	98
PBS	98

[a] Cells or virus were treated with proteases as described in the text. Labeled and unlabeled virus preparations used were the B95-8 strain of EBV.

[b] Cells were preincubated for 45 min with a 400×-concentrated, unlabeled EBV preparation which was treated with proteases or sham treated.

[c] FITC-EBV was added to the treated cells for 45 min. All numbers are rounded mean values from two separate experiments.

bility was regularly monitored by means of trypan blue dye exclusion and was found not to be affected by them.

Effect of ConA on EBV binding. To learn whether oligosaccharide chains on the cell membrane or on virus envelope may participate in EBV-cell surface interactions, we investigated the effect of the α-D-mannoside- and α-D-glucoside-specific lectin ConA on EBV binding. We had found in our preliminary experiments that the treatment of FITC-EBV or Raji cells or both with 0.1 to 1 mg of ConA per ml did not affect virus binding to these targets; it was unclear whether the fluorescence observed on Raji cells after ConA treatment was due to FITC-EBV binding to virus receptors or to ConA-mediated binding of fluoresceinated virus to ConA receptors on the cell surface. Therefore, more experiments were performed to assess the effect of ConA on EBV binding, using both Raji and EBV receptor-negative, ConA-receptor rich B95-8 cells.

The effect of ConA treatment on EBV binding was demonstrated as follows (Fig. 5). (i) When Raji or B95-8 cells were saturated with ConA, they were equally well stained when either FITC-EBV or unlabeled EBV and MIF were used, whereas control B95-8 cells did not bind FITC-EBV. MM (a specific inhibitor of ConA binding) eluted the bound virus to the same extent from ConA-treated Raji and B95-8 cells, whereas it was unable to elute FITC-EBV bound to virus receptors on control Raji cells. This observation indicates that FITC-EBV or unlabeled EBV bound to ConA on the cell surface and not to EBV receptors. (ii) When the FITC-EBV preparation alone was treated with ConA at a final concentration of 0.5 mg/ml, fluoresceinated material bound to the same extent to Raji and B95-8 cells and was equally well eluted from both cell surfaces by MM. Therefore, it seems clear that ConA-treated FITC-EBV did not bind to virus receptors, but to ConA receptors on the cell surface. (iii) When both cells and FITC-EBV preparation were treated with a saturating concentration of ConA (10 mg/ml), very little binding of fluoresceinated material to the cells occurred, and the bound material was again eluted by MM. This observation indicates that ConA not only diverted EBV binding towards ConA receptors, but hindered the interaction of ligand sites on EBV, with virus receptors on the cell surface.

Taken together, these data indicate that: (i) ConA mediated EBV binding to the cell surface through ConA receptors and (ii) ConA inhibited EBV binding to virus receptors.

Role of carbohydrates in EBV binding. The effect of ConA on EBV binding suggested to us that some carbohydrate residues at the cell-virus interface may be necessary for EBV binding to its receptors; therefore, we undertook a series of experiments to assess the role of carbohydrates in EBV-cell surface interactions (Table 2). Neuraminidase treatment did not affect EBV receptors, whereas it inactivated Sendai virus receptors, as was found from control experiments.

No further inactivation of Sendai virus receptors could be achieved by increasing the enzyme concentration from 0.07 to 1.3 U/ml, thus indicating that all the susceptible *N*-acetyl-neuraminic acid residues were removed from the cell surface at the lower neuraminidase concentra-

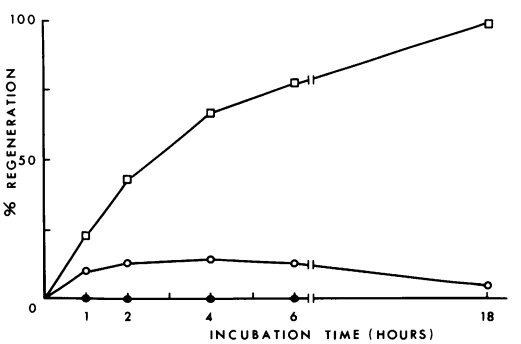

FIG. 4. Regeneration of EBV receptors on Raji cells after trypsin treatment and removal of trypsin. The percentage of regeneration was calculated relative to sham-trypsinized samples incubated for 18 h under the same conditions as the trypsin-treated samples. After trypsin treatment and removal of trypsin, the cells were incubated in RPMI-10 alone (□), in RPMI-10 plus cycloheximide (300 μg/ml) (○), or in RPMI-10 plus sucrose (0.3 M) (●). Each point represents rounded mean values from two separate experiments. FITC-EBV from strain B95-8 was used in these experiments.

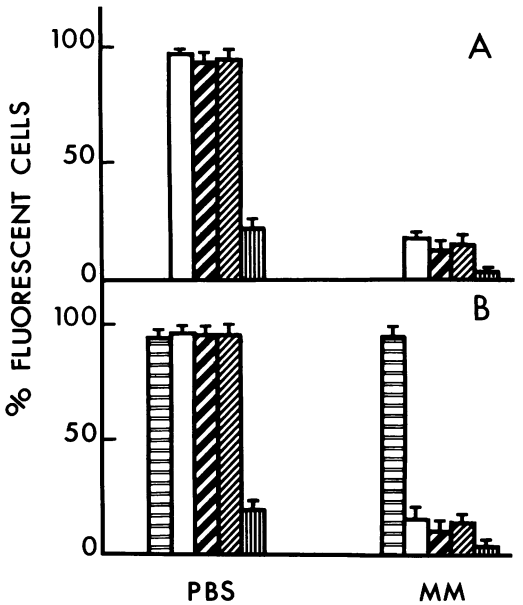

FIG. 5. Effect of ConA on EBV binding. (A) B95-8 cells; (B) Raji cells. A total of 95 to 100% of Raji and B95-8 cells bound FITC-ConA; a maximal inhibition of ConA binding occurred when cells were preincubated with 8 to 10 mg of unlabeled ConA per ml, and at this ConA concentration, the cell agglutination observed was minimal (data not shown). ▤, Sham-treated samples in the absence of ConA. All of the B95-8 cells examined were found to be negative for EBV receptors. □, Cells treated with 10 mg of ConA per ml, to which FITC-EBV was then added. ▨, Cells first treated with 10 mg of ConA per ml, to which unlabeled EBV was then added, and subsequently examined for MIF. ▨, FITC-EBV treated with 0.5 mg of ConA per ml. ▥, Cells and FITC-EBV treated with 10 mg of ConA per ml.

tion. Incubation of Raji cells or FITC-EBV or both with 0.5 M solutions of one of the monosaccharides listed in Table 2 or of an equimolar mixture did not influence FITC-EBV binding to Raji cells. Furthermore, ovalbumin, which is a glycoprotein with high mannose-type oligosaccharides, had no effect on virus binding, whereas it inhibited FITC-ConA binding to Raji cells. Since the interaction of saccharide-binding sites with oligosaccharides is considerably more complex than their interaction with simple monosaccharides (1), we continued our sugar inhibition experiments, using crude glycopeptide preparations obtained by exhaustive pronase digestion of EBV preparations or Raji cell surface glycoproteins. All glycopeptide preparations used blocked FITC-ConA binding as much as did MM or ovalbumin. However, none of these glycopeptide preparations influenced FITC-EBV binding to Raji cells.

DISCUSSION

The development of new techniques for the quantitation and analysis of EBV receptors will advance the understanding of EBV-cell surface interactions. In these studies, we have covalently labeled EBV with the amino group reagent FITC and demonstrated the usefulness of this approach in the investigation of several aspects of EBV-cell surface interactions. The principal advantage of covalent labeling is its adaptability to a wide range of radioactive or fluorescent markers for a variety of purposes; this procedure is also more controllable and efficient than in vivo labeling of EBV with radioactive precursors. Previously reported studies of EBV binding to target cells have not used covalently labeled virus (3, 6, 9, 17, 24); however, EBV covalently labeled with [^{14}C]formaldehyde has been used in an indirect radioimmunoassay (2) to precipitate purified virions, indicating that the labeled viral proteins retained their antigenicity. Furthermore, in biophysical studies of EBV binding to target cells, the modification of EBV with amino group-specific reagents apparently did not influence its binding ability and specificity (25). These findings are in line with our results, which show for the first time that FITC labeling of EBV does not affect its principal biological properties (i.e., specific binding to target cells and infectivity). FITC-EBV preparations certainly have a great potential for future applications, particularly since FITC-EBV binding may be analyzed in a fluorescence-activated cell sorter for qualitative and quantitative studies of virus-cell interactions (e.g., percent of EBV receptor-positive lymphocyte subpopulations as well as kinetic analysis of EBV binding to these cells, etc.). Such studies may also provide important new information on EBV infection of lymphocytes from different individuals. Moreover, covalent labeling of EBV with a fluorescent marker makes it possible to study the simultaneous binding of different EBV strains labeled with different such markers (e.g., fluoresceinated B95-8 EBV and rhodaminated P3HR-1 EBV). This kind of investigation is not possible with the use of labeled anti-EBV antibodies since, to our knowledge, there are no antibodies that can differentiate between EBV strains. It is noteworthy that differential binding of two EBV strains to some lymphoid cell lines due to strain-specific receptors on these cells has been recently reported (19); future studies on the interaction of EBV with these lines, using a fluorescence-activated cell sorter and fluorescent virus preparations, may provide important new information in this field.

With regard to EBV-cell surface interaction, the observed abrogation of virus binding by trypsin and pronase could be due to a general

disturbance of the fundamental organization of the cell and virus surface or may indicate that EBV receptors and the ligand molecules on EBV envelope are proteins or are closely associated with a protein structure essential for EBV-cell surface interactions. The ability of ConA to inhibit EBV binding suggests that the receptor or ligand structures are of glycoprotein nature.

In conclusion, the present studies demonstrate that EBV can successfully be covalently labeled with FITC and that fluoresceinated EBV preparations represent a unique tool for the detection of virus receptors on target cells and for the investigation of the nature and biology of EBV-cell surface interactions.

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