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A Study on Epstein Barr Virus Receptor Activity in Cell Free Extracts of Human Lymphoblastoid Cells

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With 3 Figures

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Summary

Epstein Barr Virus (EBV) receptor activity in cell free extracts is operationally defined as one which causes a reduction in the effective concentration of the early antigen inducing particles of an EBV preparation when the latter is preincubated with the extract before infection. Such activity was detected in the surface extract of Raji cells and to a lesser extent in that of BJAB cells, both of which are B lymphoblastoid cells that are susceptible to infection with EBV. Receptor activity was not detected in similar extracts of P_3HR-1 cells and human diploid fibroblasts neither of which are known to be susceptible to EBV infection.

Receptor activity in the Raji cell extract was found to be associated with membranous structures. This may have rendered the activity resistant to treatment with trypsin and sonication. The activity was however abrogated if the extract was exposed to neutral detergent.

Binding of receptor activity was observed when Raji cell extract was chromatographed on a column of immobilized EBV. Subsequent electrophoretic analysis showed however that this procedure did not result in an appreciable purification of the receptor activity. Neutral detergent treated extract was similarly chromatographed. The resulting eluates did not contain detectable receptor activity but were less heterogeneous in protein content as compared with that of the original extract. It is not certain at present if these EBV binding proteins are involved in the receptor activity of the extract.

Introduction

Nasopharyngeal carcinoma (NPC) cells have been shown to harbor Epstein-Barr Virus (EBV) genomes (3, 8, 13, 16) and they regularly display EBV nuclear antigens (3, 7, 8). NPC cells are of epithelial origin and the persistence of EBV in these tumor cells is the only known exception to the lymphotropism of EBV

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(9). There is now mounting evidence causally linking EBV with this malignancy (2). It seems possible that susceptibility to NPC might be related to susceptibility of certain epithelial cells to infection with EBV (6) which, in turn, may depend on the ability of these cells to adsorb the virus. To test this possibility, we undertook to isolate and purify EBV receptors. It is envisaged that antibodies against EBV receptors might allow a direct examination of epithelial cells in various biopsy specimens for their ability to adsorb EBV.

EBV receptors have been shown to occur on certain human B lymphoblastoid cell lines (17). In our earlier studies, we have subjected one of such cell lines, Raji (4), to treatments with buffers of low and high ionic strength containing glycerol (10). Chemically these extracts contain a heterogeneous mixture of proteins that were accessible to whole cell radioiodination catalysed by lactoperoxidase (10). Two types of antigenic activities have been demonstrated in these extracts; one elicits NPC-associated *in vitro* cellular immune responses (12) and the other, as defined by hyperimmune rabbit serum against the extracts, appears to be EBV-related and occurring on Raji cell surface (unpublished observations). We report in this paper the detection of EBV receptor activity in one of the Raji cell extracts. We also present results of our preliminary attempt at purification of the receptors from the extract using a column containing immobilized EBV components.

Materials and Methods

EBV

A batch of EBV preparation was obtained from Pfizer Co. through the Special Virus Cancer Program, National Cancer Institute, U.S.A. This preparation containing about 10^8 virus particles per ml of water that had been partially purified from the P_3HR-1 lymphoblastoid cell culture supernatant as described by Traul and Mayyasi (14). The virus suspension was made to 0.1 M $NaHCO_3$, pH 8.3 and 0.5 per cent sodium deoxycholate immediately before conjugation onto cyanogen bromide activated sepharose 4B (Pharmacia, U.S.), coupling 4.3 mg protein to 1.2 g of activated gel at room temperature for 2 hours initially and then overnight at 4° C. The gel was then thoroughly washed and it is referred to hereafter as the immobilized EBV.

EBV used for infection studies was prepared according to Adams (1) from supernatants of P_3HR-1 cultures that had been incubated for 21 days without feeding. The virus preparation was stored at −70° C in small aliquots until use.

EBV Titration

EBV was assayed by its ability to induce early viral antigens (EA) in the non-virus producing Raji cells according to the method described by Adams (1). 5×10^6 Raji cells were pelleted and resuspended in 0.1 ml of a serially diluted aliquot of an EBV preparation and 0.9 ml of culture medium (RPMI-1640 supplemented with 10 per cent fetal calf serum obtained from Grand Island Biologicals, U.S.A.). Virus adsorption was allowed for one hour at room temperature. Four ml of culture medium was then added and the culture was incubated at 37° C for 2 days in 10 per cent CO_2. The cells were washed with HBSS and smeared. Cell smears were fixed in chilled acetone. To determine the per cent of EA positive cells, the smears were reacted for one hour at 37° C against a 1:200 diluted human serum, Tu 1101, that has an anti-EA and anti-VCA titers of 1:640 and 1:1280 respectively. The serum was a generous gift from Dr. G. de Thé of the International Agency for Research on Cancer, Lyon, France. The smears were washed in HBSS and EA positive cells were developed by counterstaining with a 1:16 diluted fluorescein anti-human immunoglobulin (Hyland, U.S.A.) for one hour at 37° C. After washing with HBSS, the number of EA positive cells were enumerated from a total of not less than 500 cells.

Cell Cultures

Lymphoblastoid cells, i.e. Raji BJAB and P_3HR-1, were grown as stationary suspension cultures in RPMI-1640 supplemented with 10 per cent fetal calf serum. The cultures were fed with an equal volume of the medium every 4 days and the cells harvested on the third day after the last feeding when cell viability was better than 90 per cent as indicated by 0.1 per cent eosin exclusion.

Human fibroblasts (FS-4) were grown as monolayers in RPMI-1640 supplemented with 5 per cent fetal calf serum and harvested at confluency using a rubber policeman.

Protein Determination

The method of LOWRY *et al.* (11) was used. Protein was also estimated by absorbance at 280 nm.

Cell Extracts

The Raji cell extract TS was prepared by treating these cells with a Tris HCl buffer (Tris HCl 0.02 M; 10 per cent V/V glycerol, pH 7.2) followed by centrifugation at $12{,}000 \times g$ for 30 minutes as previously described (10). Extracts of the other lymphoblastoid cell lines were prepared similarly. Human diploid fibroblasts (FS-4) were harvested by scraping confluent layers of the cells with a rubber policeman. The cells were washed once with PBS and extracted with the Tris HCl buffer as the lymphoblastoid cells.

Chromatography

An immobilized EBV column, 0.9×5 cm, containing approximately 4.3 mg protein, was used throughout. Samples and the column were equilibrated with phosphate buffered saline (PBS) containing 0.1 per cent NP 40. After sample application, the column was washed with PBS and then eluted with PBS containing 0.25 M NaCl followed by PBS-0.5 M NaCl and PBS-1.0 M NaCl. Samples were applied at 7 ml per hour and the washing and elution at 18 ml per hour. Effluent from the column was collected as 1.4 ml fractions. All operations were performed at room temperature.

Polyacrylamide Gel Electrophoresis

Samples were heated at 100° C for 15 minutes in 2 per cent each of 2-mercaptoethanol and sodium dodecyl sulfate (SDS) prior to application to 4—24 per cent linear gradient polyacrylamide gel slabs, $0.3 \times 7 \times 8$ cm, cast in 0.1 per cent SDS and 0.05 M phosphate buffer pH 7.2. Electrophoresis was carried out in the same buffer for 38—40 hours at 20 volts.

Results

Receptor Assay and Calculations

Cell free extracts were tested for EBV receptor activity by their ability to interfere with EBV infection. Unless otherwise stated tests were performed by incubating a cell extract with a given amount of EBV at room temperature for one hour. A virus control was set up in parallel which contained the same amount of EBV and the buffer of the extract. Effective concentrations of EBV in the test and control mixtures are then determined by the standard EA induction assay as described by ADAMS (1) and compared.

Results of a typical experiment are shown in Figure 1. In the virus control, per cent of EA positive cells is inversely proportional to the reciprocal of virus dilution. A linear regression line of per cent EA positive cells on $\log_2$ reciprocal virus dilution was constructed using values obtained at the lower virus dilutions. It has a correlation coefficient (r) of 0.99 indicating a good correlation between the two parameters. Standard error (Sxy) is 0.03, reflecting the assay error. The slope of the linear regression line (−4.05) reflects the inherent susceptibility of

the target cells (Raji) to infection with the virus. Geometric mean virus titer is arbitarily chosen as the reciprocal of virus dilution corresponding to the intercept on the linear regression line at the level of 2 per cent EA positive cells (2 per cent titer) but it must be stated that intercepts at any other levels of EA positive cells may be chosen equally well to represent virus titers. The actual mean concentration of EA inducing particles in the infecting virus preparation is calculated as the product of the geometric mean virus titer, the number of EA positive cells (2 per cent of the total Raji cell infected) and the volume of the virus preparation used per infection assay (1 ml). The range of virus titer which provides a measure of assay error is obtained as the values of reciprocal dilution corresponding to the intercepts on lines drawn parallel to but at $+$Sxy and $-$Sxy from the linear regression line at the 2 per cent EA positive cell level. Table 1 shows the results of computation by linear regression from data obtained with different virus controls. It is noted that in all these instances, there was a good correlation between the observed per cent EA cells and $\log_2$ reciprocal virus dilution. The slopes of the linear regression lines however varied on different occasions, and while the basis for this variation is not understood, it is possible that this might reflect a difference in the state of the Raji cell cultures used for infection. The value of assay error of ± 30 per cent of the geometric mean titer observed in one of the assays is the maximum value thus far encountered. Changes in the geometric mean virus titer observed in the test preparation which exceeds 30 per cent of that of the corresponding virus control was therefore considered as a significant change in the effective concentration of EA inducing particles.

Table 1. *Determination of virus concentration*

Virus Preparations	Linear regression ($Y = a_0 x + a_1$)[a]				Virus concentration				
	Slope (a_0)	Y-intercept (a_1)	Correlation coefficient (r)	Standard error (Sxy)	GMT[b] at Y = 2% EA	Error[c] Lower titer (L)	Higher titer (H)	$\left(\frac{GMT}{L}\right)$	$\left(\frac{H}{GMT}\right)$
1	-4.05	20.78	0.99	0.03	24.93	24.76	24.93	$+1.01$	-1.00
2	-1.90	10.90	0.96	0.73	25.63	19.70	33.59	$+1.30$	-1.31
3	-2.00	8.43	0.99	0.09	9.32	9.00	9.58	$+1.04$	-1.03
4	-5.11	23.45	0.99	0.44	18.38	17.27	19.43	$+1.06$	-1.06

[a] Y = per cent EA; X = $\log_2$ (reciprocal virus dilution)

[b] Geometric mean titer at 2 per cent EA = $10^{0.30}\left(\frac{2-a_1}{a_0}\right)$

[c] Error (range) expressed *either* as lower (L) and higher (H) titers where

$$L = 10^{0.30}\left[\frac{(2-a_1)+Sxy}{a_0}\right]; \quad H = 10^{0.30}\left[\frac{(2-a_1)-Sxy}{a_0}\right]$$

or as folds reduction i.e. $\frac{GMT}{L}$ and $-\frac{H}{GMT}$

Note, titer $>$ GMT is assigned a negative sign ($-$) to denote relative increase

For the test virus preparations, lines of best fit having the same slope as the linear regression line were drawn. The mean 2 per cent titers thus obtained were compared with an expressed as ratios to that of the corresponding virus controls. When the control titer exceeds the test titer, the ratio $\frac{\text{Control titer}}{\text{Test titer}}$ represents folds reduction in effective virus concentration. In the instance when the test titer exceeds the titer of the virus control, a negative value is assigned to the ratio $\frac{\text{Test titer}}{\text{Control titer}}$ which represents folds increase in effective virus concentration.

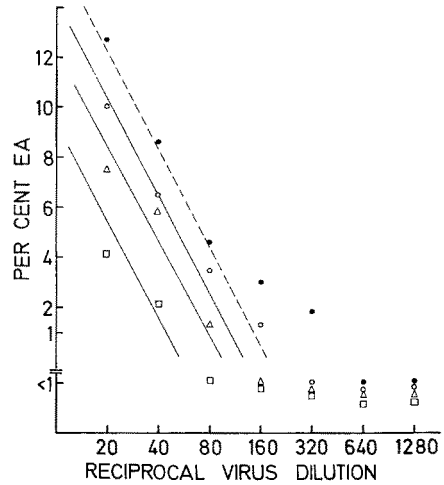

Fig. 1. Induction of EA synthesis in Raji cells by serially diluted aliquots of EBV that had been previously reacted for 1 hour at 37° C with 0 (•), 3.0 (○), 6.0 (△) and 18.0 (□) μg of TS protein. Broken line is the linear regression line of per cent EA positive cells on reciprocal dilution of the control virus preparation using values observed between virus dilution of 1:20 and 1:80 (dilution refers to that of the original virus stock). Solid lines are lines of best fit observed with the different test mixtures and have the same slope as that of the linear regression line. See also text and Tables 1 and 2

Detection of Receptor Activity in the Raji Cell Extract, TS

Table 2 summarizes results obtained from the experiments shown in Figure 1 and from similar experiments using different preparations of Raji cell TS. All extracts were freshly prepared before testing and they were found to cause consistently a substantial reduction in the effective virus concentration relative to those of the corresponding virus controls. The extent of reduction however varied among different batches of extracts. In two of the experiments in which different amounts of the same batches of extracts were tested, reduction in virus concentration was found to depend on the amounts of extracts present. It should be noted that, as in the results shown in Figure 1, lines of best fits parallel to the linear regression lines of the corresponding virus control may be drawn for the test preparations in all the instances. This suggested that the presence of TS did

not affect the inherent susceptibly of the target cells to infection with EBV. In other experiments, it was found that the addition of TS to Raji cell suspensions 4 hours after they had been infected with EBV did not affect the subsequent development of EA in these cells (results not shown). These results therefore indicate that the interference phenomenon might be related to an effect which is exerted on the virus and is therefore dependent on preincubation of EBV with these extracts.

Table 2. *Interference of EBV infection by different preparations of the Raji cell extract, TS*[a]

TS		Virus			
		Control			Reduction in titer (folds)
Batch	Protein (μg per reaction)	EA particles $\times 10^{-5}$	Titer	Test (Titer)	
8/31	3.5	54.6	27.3	16.2	1.7
8/31	7.0	54.6	27.3	10.4	2.6
8/31	21.0	54.6	27.3	5.5	5.0
8/23	3.0	50.2	25.1	17.2	1.5
8/23	6.0	50.2	25.1	13.4	1.9
8/23	18.0	50.2	25.1	7.1	3.5
12/11	21.5	21.4	10.7	5.9	1.8
1/13/H	36.8	18.6	9.3	4.0	2.3
1/13/T	36.8	18.6	9.3	3.4	2.7
1/13/E	36.8	18.6	9.3	2.5	3.7

[a] See text for experimental details

Table 3. *Interference of EBV infectivity by TS observed at different virus concentrations*[a]

TS		Virus			
		Control			Reduction in titer (folds)
Batch	Protein (μg per reaction)	EA particles $\times 10^{-5}$	Titer	Test (Titer)	
1/13/H	36.8	18.6	9.3	4.0	2.3
1/13/H	36.8	125.5	62.8	78.8	−1.3
1/13/T	36.8	18.6	9.3	3.4	2.7
1/13/T	36.8	125.5	62.8	52.0	1.2
1/13/E	36.8	18.6	9.3	2.5	3.7
1/13/E	36.8	125.5	62.8	52.0	1.2

[a] The same virus preparation was allowed to react at 2 different concentrations with 3 different batches of TS. See text for experimental details

The effect of virus concentration on EBV receptor activity was tested by reacting different preparations of TS with a higher (123.6×10^5 per ml) and a lower (18.6×10^5 per ml) concentration of the virus (Table 3). At the lower virus concentration, all three batches of TS caused an appreciable reduction in virus concentration relative to that of the corresponding virus controls but receptor activity could not be detected in any of these extracts if they were allowed to react with an excess of the same virus. In view of these, between 20 to 50×10^5 EA

particles per reaction mixture was used for subsequent studies; virus controls of this concentration range gave rise to about 10 per cent of EA positive cells in our assay system.

Characterization of Receptor Activity in Raji Cell Extract

The Raji cell extract, TS, was subjected to different treatments and then tested for receptor activity (Table 4). In one type of experiment, TS was centrifuged at 100,000 × *g* for 2 hours. The pellet was resuspended to the original volume and when tested concurrently with the supernatant, it was found to cause a substantially greater reduction in EBV titer than the supernatant although the protein concentration of the former was lower than that of the supernatant (Table 4, experiment 1).

Table 4. *Effects of different treatments of TS on its ability to interfere with EBV infection*[a]

		Virus				
	Extract	Control				
Treatments		Protein (μg per reaction)	EA particles $\times 10^{-5}$	Titer	Test (Titer)	Reduction in titer (folds)
100,000 × *g* for 2 hours	Control	31.2	28.8	14.4	4.6	3.1
	Supernatant	20.7	28.8	14.4	5.1	2.8
	Pellet	6.0	28.8	14.4	3.4	4.2
0.25% w/v trypsin for 1 hour at 37° C	TS + Tryp then TI[b]	31.2	25.6	12.8	4.4	2.9
	TS + TI then Tryp[b]	31.2	25.6	12.8	5.5	2.3
	TS untreated	31.2	25.6	12.8	4.3	3.0
Sonication 2 minutes	Unsonicated	31.2	28.8	14.4	4.6	3.1
	Sonicated	25.5	28.8	14.4	4.6	3.1
Reaction temperatures	37° C	30.0	23.4	11.7	4.4	2.7
	22° C	30.0	23.0	11.5	6.2	1.9
	4° C	30.0	19.0	9.5	5.7	1.7
0.1% NP-40	Treated	21.5	21.4	10.7	12.3	−1.2
	Untreated	21.5	21.4	10.7	5.9	1.8

[a] See text for details of experiments
[b] Trypsin (Tryp); Soya bean trypsin inhibitor, TI

TS was incubated in 0.25 per cent w/v of trypsin for 1 hour at 37° C. The mixture was then made to 0.5 per cent w/v with respect to soya bean trypsin inhibitor and tested for receptor activity. Control extracts included the untreated TS and one which was obtained by mixing the extract first with trypsin inhibitor and then, trypsin. These mixtures were allowed to react with EBV as before and titrated for residual EA inducing particles. The results were compared with a virus control made up by first mixing the virus with trypsin inhibitor and then with trypsin at the respective concentrations as used in the test mixtures. The results show that receptor activity is not sensitive to treatment with this proteolytic enzyme (Table 4, experiment 2).

TS was sonicated for 2 minutes at 10 seconds bursts with a Bronson sonifier at maximum output. The sonicated extract was shown to retain its capacity to interfere with EBV infection as the unsonicated preparation (Table 4, experiment 3).

TS was made to contain 0.1 per cent Nonidet P40 and dialysed against HBSS. Receptor activity in the neutral detergent-treated extract was assayed and the result compared with that of a control extract which had not been exposed to the detergent but otherwise similarly treated. It was found that exposure to NP40 abrogated the receptor activity originally present in TS resulting in an increase in concentration of the effective EA inducing particle relative to that of the virus control (Table 4, experiment 4).

TS was allowed to react with EBV at different temperatures and titrated for residual EBV. The results were compared with the virus controls similarly incubated with the buffer alone. A substantial receptor activity was detected in the mixture which had been incubated at 4° C although the reduction in effective virus concentration seemed to be greater in the mixture that had been incubated at 37° C (Table 4, experiment 5).

The above experiments had been repeated and similar results were obtained (not shown).

Detection of Receptor Activity in Extracts From Other Cell Lines

Extracts from 3 human lymphoid cell lines, human diploid fibroblasts (FS) were prepared in a similar manner as that used to prepare the Raji cell TS (see Materials and Methods). Receptor activity was detected in TS of Raji and BJA-B cells but not in that prepared from the P_3HR-1 cells (Table 5). The latter is a lymphoid cell line that is also known to be not susceptible to infection with EBV and these cells do not contain detectable EBV receptors on their cell surface (17), while BJA-B is a lymphoid cell line that is susceptible to infection with EBV (5). Receptor activity was not detected in extracts from the other nonlymphoid human cell line and it is of interest that with rare exceptions (6), EBV is a lympho-

Table 5. *Interference of EBV infection by extracts prepared from different cell lines*

Extracts			Virus			
			Control			
Cell line origin	Batch	Protein (μg per reaction)	EA particles $\times 10^{-5}$	Titer	Test (Titer)	Folds reduction in titer
Raji	1/18	31.3	38.0	19.0	1.8	10.4
	1/13/H	36.8	18.6	9.3	4.0	2.3
	1/13/T	36.8	18.6	9.3	3.4	2.7
BJA-B	(1) 1/13/B	24.8	18.6	9.3	6.6	1.4
P_3HR-1	(1) 1/13/P_1	30.0	18.6	9.3	9.7	−1.04
	1/13/P_2	30.0	18.6	9.3	8.9	1.04
FS	9/13/F	20.0	22.6	11.3	12.6	−1.1
	9/17/F	20.0	32.0	16.0	16.0	1.0
	8/23/F	20.0	50.2	25.1	44.0	−1.8

tropic virus (9). It would appear from these results that detection of receptor activity is related to virus susceptibility of the cells from which the extracts originated.

Isolation of EBV Receptors by Chromatography on Immobilized EBV Column

Isolation and purification are prerequisite steps in studies to elucidate the structural properties of the EBV receptor activity. A direct approach to this end was attempted by chromatography of TS on a column of immobilized EBV. The virus preparation used to prepare the sepharose 4B-immobilized EBV was obtained from Pfizer Co. through the Special Virus-Cancer Program, U.S.A. It is a preparation partially purified by methods described by MAYYASI and TRAUL (14), and it contains 2.6×10^8 virus particles per ml. We did not further purify the virus as there is no suitable method of purification and because this virus is scarce due to the fact that there are no host cells which are completely permissive to infection with EBV (9).

Raji cell TS was applied to the EBV column prepared as described in the Materials and Methods. Both the extract and the column had been equilibrated in PBS. The column was developed with PBS containing 0.25 M NaCl and then with PBS-0.5 M NaCl. The fractions showing optical absorption were pooled, dialysed against HBSS and tested concurrently with the original extract for receptor activity. The results of this and two similar experiments using different batches of TS are shown in Table 6. The PBS-0.2 M NaCl eluates exhibited receptor activity but none of the PBS-0.5 M NaCl eluates tested caused a significant change in the effective concentration of EA particles.

Table 6. *Recovery of receptor activity in eluates from immobilized EBV column*[a]

Experiment	Virus control		PBS-0.25 M NaCl eluate		PBS-0.5 M NaCl eluate	
	EA particles $\times 10^{-5}$	Titer	Titer	Folds reduction in titer	Titer	Folds reduction in titer
1	18.6	9.3	5.9	1.6	7.8	1.2
2	27.0	13.5	10.4	1.3	12.7	1.1
3	38.0	19.0	9.2	2.1	17.8	1.1

[a] Pooled PBS-0.25 M NaCl and PBS-0.5 M NaCl eluates from the immobilized EBV column were tested for receptor activity. See text for detail

The PBS-0.25 M NaCl eluates upon electrophoresis in SDS polyacrylamide gel gave a stained pattern which is essentially similar to that of TS shown in Figure 2 B. This indicated that chromatography under the above conditions did not result in a qualitative purification of the receptor activity from the extract. Referring to the results shown in Table 4 however, it was considered possible that some of the polypeptides present in the eluates might have been co-isolated by virtue of their association with membranous structure although they have no inherent affinity for the immobilized virus. Therefore, extracts were first treated with the neutral detergent, NP40, before chromatography. Although this will abrogate receptor activity, this approach was considered worthwhile as it might allow separation

of substances having an inherent affinity for the immobilized EBV from those which do not.

TS was first exposed to NP40 and then chromatographed on an EBV column equilibrated in buffer containing the neutral detergent. The column was developed with PBS, followed with PBS-0.25 M NaCl and PBS-0.5 M NaCl. Figure 3 shows

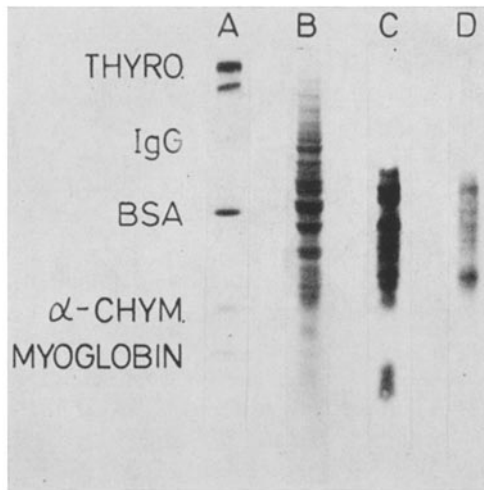

Fig. 2. Electrophoretic patterns of: *(A)*, a mixture of protein markers containing thyroglobulin (Thyro), rabbit immunoglobulin (IgG), bovine serum albumin (BSA), α-chymotrypsinogen (α-chym) and myoglobulin; and *(B)*, TS of Raji cells. TS was chromatographed in a column of immobilized EBV. The electrophoretic patterns of the resulting PBS-0.25 M NaCl and PBS-0.5 M NaCl eluates are shown in *(C)* and *(D)* respectively

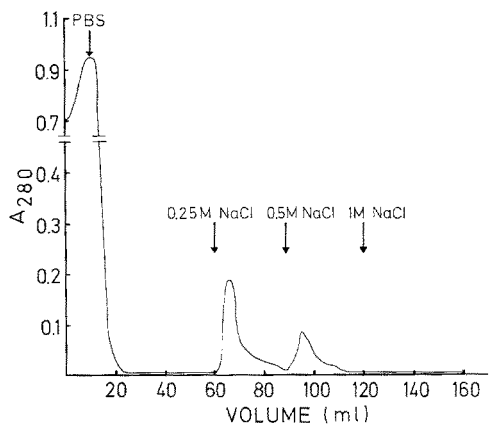

Fig. 3. Chromatography of TS on immobilized EBV columns. Two mg of TS protein in PBS containing 0.1 per cent NP40 was applied to an immobilized EBV column, 0.9×5 cm. The column was washed with PBS and developed by elution with PBS containing 0.25 M NaCl followed by PBS containing 0.5 M NaCl and 1.0 M NaCl. Effluent from column was monitored for optical absorption at 280 nm

a typical elution profile. As might be anticipated from the results shown in Table 4, the pooled eluates did not show detectable receptor activity. The electrophoretic patterns obtained with the eluates are shown in Figures 2C and D. The PBS-0.25 M NaCl eluate was found to be heterogeneous with respect to its protein content (Fig. 2C), although it was less heterogeneous than the original TS (Fig. 2B). The PBS-0.5 M NaCl eluate by contrast was substantially more homogeneous in its protein content (Fig. 2D). It is not possible by this approach, however, to associate receptor activity with the polypeptides that showed an apparent affinity for the immobilized virus.

Discussion

EBV receptor activity is here defined operationally as the activity which upon reaction with a virus preparation will cause a reduction in the effective concentration of EA inducing particles of that virus preparation. The activity detected in the Raji cell extract, TS, has satisfied this definition. It has been shown further that this activity might be associated with a membranous structure which is not sedimentable at $12{,}000 \times g$ for ½ hour, a step included in the preparation of TS, but is sedimentable at $100{,}000 \times g$ for 2 hours. Its association with membranous structure has probably rendered this activity resistant to digestion with trypsin. However, the extract has not been tested at higher concentrations of trypsin or against digestion with other proteolytic enzymes to ascertain if the receptor activity is associated with protein molecules. Sonication would be expected to disperse large membranous fragments to ones of smaller sizes but such treatment does not seem to affect the receptor activity associating with these structures. By contrast, treatment with neutral detergent which affects solubilization of membranous structures has led to abrogation of the receptor activity.

Interference of infectivity of EBV by the extracts might be alternatively interpreted as to indicate inactivation of the viruses by nonreceptor substances such as an enzyme. Although the present results do not permit an unequivocal decision, this possibility seems less likely: Catalytic activity of an enzyme might be expected to be more temperature dependent than was actually observed by us. Conversely, the temperature effects are more in harmony with receptor activity, as binding between viruses and their receptors occurs at 4° C. Moreover, although there are many membrane associated enzymic activities, in most instances such activities are often enhanced, stabilized or unaffected by the presence of neutral detergents (see WELLACH and WINSLER, 15). This is therefore not compatible with the observed susceptibility of the receptor activity to NP40. To account for the latter observation, it would seem possible that receptor activity might possibly depend on the topography of a multiple component site on the membraneous structure.

Direct isolation and purification of the receptor activity by affinity chromatography is confronted with two technical difficulties at present. Chromatography of the membranous structures, on the one hand, did not allow an appreciable degree of purification of this activity. This, as mentioned earlier is possibly due to co-isolation of substances which are not directly involved in receptor activity but are associated with the same receptor structure. On the other hand, dissociation of the membranous structure prior to chromatography abrogated its receptor

activity. Consequently, although there was indication of purification in terms of protein content, the resulting fractions lacked receptor activity. Therefore, it could not be ascertained by this approach alone whether component(s) directly involved in recpetor activity has (have) been purified from the other substance originally present in the extract. To circumvent these difficulties we are generating antibodies against the column isolates. Results of studies with the xenoantibodies will be reported later.

Acknowledgments

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