Concentration of Epstein–Barr Virus from Cell Culture Fluids with Polyethylene Glycol

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SUMMARY

Epstein–Barr virus (EBV) is usually obtained from supernatant fluids of cultures of primate lymphoblastoid cells. The isolation of small amounts of virus particles from large vol. of culture fluid offers particular problems because of the osmotic fragility of herpes viruses, and attempts to concentrate biologically active EBV by zonal centrifuging in solutions containing sucrose have been unsuccessful. It is shown here that EBV particles can be conveniently concentrated from culture fluids with polyethylene glycol. The biological activity of the concentrated particles is retained, as measured by a superinfection assay, and good yields are obtained from supernatant fluids containing as little as 2 × 10^2 EBV particles/ml.

Several primate lymphoblastoid cell lines spontaneously release small amounts of Epstein–Barr virus (EBV) into the tissue culture medium (Hinuma et al. 1967; Miller & Lipman, 1973). Because a complete lytic infection cycle has not been demonstrated for this virus, supernatant culture fluids from such spontaneous producer lines are presently the main source of EBV. Virus particles have previously been concentrated from the culture medium by either ultracentrifuging (Hinuma et al. 1967; Schulte-Holthausen & zur Hausen, 1970) or zonal centrifuging (Jensen, Buscheck & Riccardo, 1971). While the extended times required to sediment the virus in high capacity rotors make the ultracentrifuge impractical for processing large vol., the latter method has the disadvantage that > 90% of the biological activity of EBV is lost when the virus is sedimented in sucrose solutions (A. Adams, unpublished result).

‘Precipitation’ with polyethylene glycol (PEG) has been employed for concentrating a variety of bacterial (Yamamoto et al. 1970) and small animal viruses, including the enveloped vesicular stomatitis virus (McSharry & Benzinger, 1970). This method was found to be useful for the further purification of biologically active crude EBV concentrates in this laboratory (Jehn, Lindahl & Klein, 1972). I have, therefore, investigated the possibility of using PEG ‘precipitation’ as a convenient means of concentrating the virus directly from large vol. of culture medium.

The Burkitt lymphoma derived subline P3HR-1 (Hinuma & Grace, 1967) was the source of the infectious EBV. The line was propagated as a suspension culture in RPMI-1640 medium containing 10% foetal bovine serum. For virus production, cultures were adjusted to an initial concentration of 2 × 10^6 cells/ml and incubated, without further medium change, for 14 days at 33 °C in a humidified incubator with a 5% atmosphere of CO₂ (Hinuma et al. 1967). Cells and debris were then removed from the aged culture fluids by low speed sedimentation, solid, dry-heat sterilized NaCl (20 g/l) was added to the pooled supernatant fluids and polyethylene glycol 6000 (AB Kebo, Stockholm) was added as a 50% (w/v) sterile solution (autoclaved) in 0.5 M-NaCl. The PEG mixtures were left for at least one h at 0 ° to 4 °C before the sedimentable phase was collected. The virus was
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Fig. 1. Polyethylene glycol 6000 'precipitation' of EBV from the supernatant medium of P3HR-1 cells. Four ml samples of cell supernatant fluid containing 0.5 M-NaCl were mixed with various amounts of PEG. After 1 h at 0 °C, the suspensions were centrifuged for 10 min at 3000 g. The pellets were resuspended in 1 ml medium and the infectivity of several twofold dilutions of these suspensions tested. The PEG supernatant fractions were dialysed overnight against medium to remove the PEG prior to assaying for biological activity. The percentage of the total early antigen inducing particles recovered in the pellet (●●) and supernatant (○○) fractions are plotted as a function of PEG concentration.

collected by centrifuging at 7000 g for 15 min in the Sorvall GS3 rotor. The virus pellets were resuspended in 1 % of the initial vol. of serum-containing medium. Virus infectivity was monitored by quantitatively measuring the ability of appropriate virus dilutions to induce early antigen (EA) expression 48 h after infection of the superinfectable Raji cell line (Adams & Klein, 1973).

The effect of increasing PEG concentrations on the recovery of biologically active EBV from one P3HR-1 supernatant fluid is shown in Fig. 1. At PEG concentrations above 8 %, most of the infectious (EA-inducing) virus is recovered in the sedimentable phase, and only minor quantities of biologically active virus remain in the supernatant fluid. The 20 to 40 % loss in initial input infectivity thus appears to be due to inactivation of a fraction of the virus particles. We have been unable to find conditions for concentrating EBV that do not lead to some inactivation. A 40 to 60 % loss of infectivity has been found to occur when the virus is concentrated from culture supernatant fluids by conventional ultracentrifuging. With the PEG procedure the loss in initial infectivity occurs on addition of the reagents, and the yield of EA-inducing virus particles is subsequently not significantly altered if the virus is allowed to stand in the presence of 8 % PEG and high salt for up to one week at 4 °C. Thus, EBV can be conveniently concentrated from very large culture vol. by simply letting the fine PEG 'precipitate' settle for about 3 days and then carefully decanting and discarding most of the supernatant fluid prior to centrifuging. The presence of high salt concentration is essential for effective PEG 'precipitation' to occur (Yamamoto et al. 1970), but the yield of EBV was not greatly affected when the final NaCl concentration was varied between 0.45 and 0.60 M. The salt concentration of most tissue culture media is close to 0.15 M and the addition of 20 g/l NaCl employed here is sufficient to bring the final salt concentration within the appropriate range.

The P3HR-1 line is the best spontaneous producer of EBV. When grown for optimal virus production, 1 ml of unconcentrated cell-free supernatant fluid can induce EA
expression in $10^5$ to $10^6$ cells of a sensitive indicator line such as Raji. As approximately $10\%$ of the virus particles are enveloped and infectious (Hinuma et al. 1967), $\sim 10^6$ virus particles/ml should be present in the P3HR-1 culture medium. Other cell lines, including those most often used as the source of EBV for lymphocyte transformation studies, release several orders of magnitude less virus (Miller & Lipman, 1973). The PEG method, not being a true precipitation procedure but rather a type of two-phase separation technique, has been shown to be independent of virus concentration (Yamamoto et al. 1970) and should therefore be applicable for concentrating EBV from other producer lines. The lack of concentration dependence for EBV was demonstrated in a model experiment with the P3HR-1 derived virus. Four 400 ml samples, an undiluted cell supernatant fraction containing initially $1.5 \times 10^4$ EA-inducing particles/ml and three tenfold dilutions of this same supernatant fluid made in serum containing medium, were treated with $2\%$ NaCl and $8\%$ PEG. The total yield of EA-inducing particles in the four resulting virus pellets was $4.9 \times 10^7$, $5.3 \times 10^6$, $4.4 \times 10^6$, and $5.1 \times 10^4$, respectively. Thus, over a 1000-fold concentration range, $80\%$ of the initial virus present was recoverable in the sedimentable fraction.

One of our main reasons for concentrating EBV from large vol. has been to obtain sufficient quantities of virus DNA for nucleic acid hybridization studies. Because $8\%$ PEG ‘co-precipitates’ nearly all of the high mol. wt. DNA in the cell supernatant fluids (Yamamoto et al. 1970), it was of special interest to determine whether the non-virus DNA present in the aged culture medium could be subsequently removed. It is difficult to directly degrade the non-encapsulated cellular DNA contaminating the crude PEG concentrates, due to the presence of substances that inhibit the action of pancreatic DNase. However, by pelleting the PEG concentrated virus suspensions for 1 h at 75000 g in the Spinco SW 27 rotor and resuspending the pellets in a small vol. ($0.1\%$ of initial culture vol.) of phosphate-buffered saline solution (PBS) containing $5 \times 10^{-3}$ M-MgCl$_2$ and $2 \times 10^{-4}$ M-CaCl$_2$, it is possible to degrade at least $95\%$ of the non-virus DNA to acid-soluble material by incubation (for 1 h at $30\,^\circ$C) with $50\,\mu$g/ml crystalline pancreatic DNase I. No significant loss of EA-inducing activity was caused by the second centrifuging step or by the DNase treatment. Employing this two step concentration procedure, approximately $4\,\mu$g of virus DNA have regularly been obtained per 10 l of cell supernatant fluid.

It is concluded that the PEG method is suitable for the concentration of EBV. The yield of both infectious virus and virus DNA has always been equivalent to or greater than that obtained with the more awkward ultracentrifuge concentration procedures. Though only $10\%$ of the virus particles released by P3HR-1 cells are enveloped (Hinuma et al. 1967), the yield of virus DNA indicates that the biologically inactive nucleocapsids are also concentrated by this procedure. Because it is possible to concentrate the virus from quite dilute suspensions in medium containing serum (less than 200 EA-inducing particles per ml), the method should be applicable for recovering virus from all cell lines which release EBV into the culture medium.

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REFERENCES


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