Herpes Simplex Virus Type-1
and Human Lymphocytes: Virus Expression and the Response to
Infection of Adult and Foetal Cells

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SUMMARY

The growth of Herpes simplex virus type 1 (HSV-1) in human lymphocytes of
adult and foetal origin was studied. Virus DNA synthesis, antigen and particle pro-
duction and the yield of infectious progeny were determined in cultured lympho-
cytes with or without exposure to stimulating concentrations of the mitogens
phytohaemagglutinin and pokeweed mitogen. Separated sub-populations of cells
were examined and the conclusion reached that only the stimulated T-lymphoblast
was permissive for full virus expression.

Stimulation of cell DNA synthesis in response to infection was observed in
cultures of adult and foetal lymphocytes under conditions which were non-
permissive for virus growth. Morphological change and prolonged culture survival
were a feature of foetal lymphocytes exposed to u.v. irradiated HSV-1.

INTRODUCTION

A number of herpes group viruses are able to infect and replicate in peripheral blood
lymphocytes of the appropriate host species. Such viruses include Marek's disease virus of
fowl (Payne et al. 1974); the monkey viruses Herpes saimiri and Herpes ateles (Rabson et al.
1971; Deindhardt et al. 1973; Falk et al. 1974); the Epstein–Barr virus (Epstein et al. 1965;
Klein, 1973); murine and possibly human cytomegaloviruses (Joncas et al. 1975; Olding
et al. 1975; St Jeor & Weisser, 1977). For some of these, notably the Epstein–Barr virus and
Marek's disease virus, infection of host lymphocytes plays a dominant role in the pathology
of disease. Furthermore, a characteristic feature of herpes virus infection of lymphoid cells
is the diversity of consequences of such an event, which can vary from virus growth and
death of the host cell to minimum detectable expression of virus functions and permanent
survival of the infected cell as a transformed, immortal cell line.

Natural infection of leucocytes by Herpes simplex virus (HSV) has been described in
immunosuppressed patients suffering from disseminated lesions (Naraqi et al. 1976) and
the ability of HSV to grow in cultures of human lymphocytes has been the subject of several
studies (Nahmias et al. 1964; Gőnczöl et al. 1969; Bouroncle et al. 1970; Denman et al.
1974; Kirchner et al. 1977).

It has been consistently observed that whereas primary lymphocyte cultures and several
lymphoblastoid cell lines (Henle et al. 1969; Robey et al. 1976) support little or no virus
production, cultures of primary cells which have been exposed to phytohaemagglutinin
(PHA) are considerably more permissive.
The purpose of the present study was to compare the ability of primary human lymphocytes of adult and foetal origin to support HSV expression under different experimental conditions, to examine the reason for increased virus production in the presence of PHA and to determine how far HSV resembles other herpesviruses with regard to its interaction with lymphoid cells.

METHODS

Cells. Human lymphocytes, which were maintained in RPMI 1640 with HEPES buffer, supplemented with 10% foetal calf serum, were obtained from (a) the peripheral blood of adult volunteers with no detectable complement fixing or neutralizing antibody to HSV, and (b) blood withdrawn from placentae after delivery (foetal blood). BHK21 cells, maintained in Eagle’s minimal essential medium plus 10% foetal calf serum, were used for the preparation of high titre stocks of virus. RK13 cells, maintained in medium 199 plus 10% foetal calf serum, were used for virus titration assays. Primary kidney cells, obtained from young rabbits, were used to prepare virus for inoculation into adult rabbits for antibody production.

All tissue culture media contained 100 units/ml penicillin and 100 μg/ml streptomycin. All cells were maintained at 37 °C in an atmosphere of 5% CO₂ in air.

Virus. A primary isolate of Herpes simplex virus (HSV) was obtained from Dr J. Munro, Public Health Laboratory Services, University Hospital of Wales, Cardiff. The virus was isolated from an oral lesion, grown in a culture of human amnion cells and shown to be HSV type 1 by pock size on the chorioallantoic membrane of hens’ eggs (Lowry et al. 1970) and neutralization by specific antiserum (Plummer et al. 1970). This virus was plaque-purified three times on BHK21 cells and stocks grown on the same type of cell to a titre of > 10⁸ p.f.u./ml.

Preparation of lymphocyte cultures. Lymphocytes were purified from heparinized whole blood by a two-step process involving centrifugation on Ficoll (6.4%)–Hypaque (9.8%) followed by 2 h incubation of washed leucocytes from the gradient interface at 37 °C in plastic tissue culture bottles. After this incubation period non-adherent cells consisted of lymphocytes plus less than 2% polymorphonuclear leucocytes. The latter disappeared after 1 to 2 days in culture. Lymphocytes were washed and resuspended in RPMI 1640 plus 10% foetal calf serum at approx. 10⁶ cells/ml. Cells from different individual donors were not pooled.

Separation of B- and T-lymphocytes. The technique used was an adaptation of that described by Lay et al. (1971). A suspension of lymphocytes (10⁶ to 10⁷ cells/ml) in RPMI 1640 was added to an equal volume of 2% washed sheep erythrocytes and an equal volume of foetal calf serum.

The mixture was centrifuged at 850 rev/min for 5 min at room temperature, incubated at 37 °C for 10 min and then transferred to 4 °C for a further 30 min. At the end of this period two thirds of the supernatant fluid were removed and the cell pellet gently resuspended. E-rosettes could be seen on microscopic examination of the suspension and the proportion of lymphocytes which formed rosettes was determined.

The suspension was then centrifuged through Ficoll (6.4%)–Hypaque (9.8%). Non-rosetting cells, the B-lymphocyte enriched population, were taken from the gradient interface, washed and resuspended in RPMI. Rosetting cells, the T-lymphocyte enriched population (Wortis et al. 1973), sedimented with sheep erythrocytes. The latter were lysed by brief exposure to 0.81% cold ammonium chloride solution and the remaining white cells resuspended in RPMI after washing.
Mitogen stimulation of lymphocyte cultures. Phytohaemagglutinin (PHA) was obtained from Wellcome Research Laboratories, Beckenham, England. Used at a concentration of 1 µg/ml this mitogen produced a stimulation index

\[
\text{S.I.} = \frac{\text{ct/min incorporated by stimulated cells}}{\text{ct/min incorporated by unstimulated cells}},
\]

of from 30 to 120 in different experiments 3 days after addition to primary cultures of adult cells. Foetal cells treated identically had stimulation indices of 30 to 90.

Pokeweed mitogen, obtained from Sigma Chemical Company, St Louis, Missouri, U.S.A. was used at 10 µg/ml and after 3 days produced S.I. values of 20 to 30 for adult cells and 15 to 30 for cultures of foetal cells.

HSV titration assay. A virus plaque assay was used. RK13 monolayers, to which 0.1 ml samples of virus suspension were added for an adsorption period of 1 h at room temperature were overlaid with medium 199 plus 10% FCS and incubated for 40 h at 37°C. At the end of this period the cells were fixed, stained and examined microscopically for the presence of plaques. This technique is a modification of that already described (Westmoreland & Rapp, 1976).

Indirect immunofluorescence test for HSV antigens. Rabbit antiserum to HSV was derived from animals which had received repeated intramuscular inoculations of crude virus preparations derived from infected rabbit cells maintained in medium 199 plus 10% adult pre-immune rabbit serum. Antiserum used in indirect immunofluorescence tests was shown by complement fixation according to the method of Bradstreet & Taylor (1962) to have a high titre (> 1/64) of antibody to HSV. Fluorescein conjugated sheep anti-rabbit-immunoglobulin (SAR-FITC) was purchased from Gibco, Grand Island, New York, U.S.A.

Cytospun preparations of lymphocytes were fixed in acetone and stored at −20°C prior to testing. The presence of virus-specific antigens was detected by incubating fixed cells for 30 min at room temperature in immune rabbit antiserum diluted with an equal volume of phosphate buffered saline (PBS). Slides were washed three times in PBS and incubated for a further 30 min at room temperature in SAR-FITC diluted with an equal volume of PBS. The cells were then rinsed three times in PBS, mounted in 70% glycerol:30% PBS and examined for positive immunofluorescence using a Leitz Dialux microscope equipped for blue incident light fluorescence.

Analysis of DNA. The technique used was an adaptation of that described previously (Westmoreland & Rapp, 1976). Lymphocyte cultures at a concentration of 10⁶ cells/ml were maintained in medium containing 6³H-thymidine at 2.5 µCi/ml for the indicated labelling period. Total radioactive incorporation into DNA was assayed in sarkosyl-EDTA extracted material spotted on to filter paper and subjected to TCA precipitation at 4°C. Incorporation of ³H-thymidine into virus and cell-specific DNA was determined by isopycnic ultracentrifugation in neutral caesium chloride gradients consisting of 0.4 ml of sample and 4.6 ml caesium chloride solution of initial density 1.948 g/ml. The conditions employed (48 h spin at 32000 rev/min using Beckman L5-65 ultracentrifuge and type 50 angle rotor) produced a shallow gradient enabling wide separation of virus (1.72 g/ml) and cell (1.69 g/ml) DNA peaks. The gradient was fractionated using an ISCO model 273 fraction collector. Fractions (0.1 ml) spotted on to filter paper were subjected to 5% cold TCA extraction and TCA-insoluble radioactivity was assayed after ethanol washing and dehydration in acetone. Samples were counted in Permablend III/toluene liquid scintillant using a Packard Tri-Carb liquid scintillation spectrometer.

Electron microscopy. Specimens were prepared for electron microscope examination
using the technique described by Watkins & Sanger (1977). Thin sections of pelleted lymphocyte cultures were stained with uranyl acetate and lead citrate, and examined on a Philips EM 300.

**Infection procedure.** Adult or foetal lymphocytes were exposed to varying multiplicities of herpes simplex virus in 0.1 ml/10^6 cells final volume. After adsorption at 37 °C for 1 h, 10 ml PBS plus 10% newborn calf serum was added to each infection mixture and the cells pelleted by bench centrifugation. The infected cell pellet was resuspended at 10^6 cells/ml in RPMI 1640 plus 10% foetal calf serum and dispensed in 0.2 to 1.0 ml samples for incubation at 37 °C. Samples were removed at intervals, the cells disrupted by freeze-thawing or ultrasonication, and released virus titrated on RK13 cells.

Heat inactivation curves for HSV-1 at 37 °C were obtained from parallel experiments from which lymphocytes were omitted.

**RESULTS**

**Herpes simplex virus growth in cultured human lymphocytes**

The production of infectious progeny by HSV infected, unstimulated human lymphocytes was very limited. At low input multiplicities (0.1 to 0.5 p.f.u./cell) the detectable virus ‘yield’ was no greater than surviving inoculum incubated at 37 °C for the same period in the absence of cells; virus yields were greater at higher input multiplicities but in no experiment did they exceed the initial inoculum.

Foetal lymphocytes were found to be consistently more permissive for virus growth than adult cells, but the yield was very low compared with that obtained from permissive cells such as BHK21. Virus ‘growth’ under these conditions might have represented prolonged survival as described by St Jeor & Weisser (1977) for human cytomegalovirus in cultures of unstimulated human lymphocytes.
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81-72

8.1-71 ~

7.6 ~

1.69 ~

× 5 ~

3

2

1

Y-

II

II

II

10 20 30 40

Fraction number

Fig. 2. Caesium chloride gradient profile of radioactive DNA extracted from HSV-1 infected adult lymphocytes. Unstimulated cells were infected and incubated for 48 h in medium containing 2.5 μCi/ml 3H-thymidine prior to extraction of DNA. ○—○, Radioactivity profile obtained from 10^6 infected cells; ○—○, radioactivity profile obtained from 10^6 uninfected cells.

Herpes simplex virus adsorption to human lymphocytes

The ability of HSV to adsorb to human lymphocytes was determined by titrating virus in the supernatant after bench centrifugation of lymphocytes and virus incubated together as described in Methods. Supernatant unadsorbed virus was compared with the titre of samples from a similarly treated suspension of virus in PBS. As shown in Fig. 1, HSV adsorbed rapidly and efficiently to human adult lymphocytes. The same result was obtained using foetal lymphocytes suggesting that the failure of unstimulated lymphocytes to support growth of HSV was not due to a failure of the virus to adsorb.

DNA synthesis by unstimulated, infected human lymphocyte cultures

The DNA from infected and uninfected adult and foetal lymphocyte cultures was subjected to isopycnic ultracentrifugation under conditions which separate HSV DNA (1.72 g/ml) and cell DNA (1.69 g/ml) to determine whether infected lymphocyte cultures supported virus DNA synthesis, and whether exposure to HSV had any effect on cellular DNA synthesis. Infected and uninfected identically labelled cultures were treated in parallel so that direct comparison between their DNA synthesis could be made. A typical gradient profile is shown in Fig. 2. Virus-specific DNA could be detected in cultures of both adult and foetal cells labelled during 48 h p.i.

Somewhat surprisingly it was observed that the amount of cellular DNA synthesized by infected lymphocyte cultures was consistently greater than that synthesized by uninfected parallel cultures (Table 1 and 2). This discrepancy persisted for several days after infection and was most apparent in cultures infected at input multiplicities of 1 to 10 p.f.u./cell. At multiplicities less than 1 p.f.u./cell no reproducible differences were detected between DNA synthesis by infected and control cultures; cultures infected with HSV at an input multiplicity of more than 10 p.f.u./cell rapidly degenerated.
Fig. 3. Foetal lymphocyte cultures exposed to u.v. irradiated HSV-1 at an input multiplicity (calculated from non-irradiated virus titre) of 10 p.f.u./cell. (a) Phase contrast illuminated micrograph of uninfected foetal lymphocytes after 6 days in culture. (b) Similar micrograph of foetal lymphocytes exposed 6 days earlier to HSV-1 which had been irradiated for 10 min. (c) Phase contrast illuminated micrograph of cell clumps in a culture of foetal lymphocytes infected as (b). (d) Giemsa stained preparation of a single clump of foetal cells 8 weeks after exposure to 10 min u.v.-irradiated HSV-1. Enlarged blastoid cells are visible in the cluster. Isolated small lymphocytes are also shown.
Temporary stimulation of cell DNA synthesis was observed in HSV infected adult and foetal cells but not in cultures exposed to extracts from uninfected BHK21 cells or formalin-treated HSV. Viable HSV had the ability to stimulate cell DNA synthesis by lymphocytes from adult donors with no evidence of antiviral antibody and from foetal blood; thus it seems unlikely that stimulation of cell DNA synthesis represented an in vitro immunological response to virus antigens.

The effect of ultraviolet irradiated HSV-1 on human foetal lymphocytes

Within 48 h after infection foetal lymphocytes showed marked cell clumping compared with parallel cultures of uninfected cells. Cultures infected at an input multiplicity of 5 p.f.u./cell or greater degenerated after 5 to 6 days, in contrast to uninfected cultures of foetal lymphocytes which could be maintained in the laboratory for 3 to 6 weeks.

In an attempt to reduce the cytopathic properties of HSV-1, stock virus was subjected to u.v. irradiation at a distance of 20 cm from a Philips 15 W germicidal lamp. Samples of virus were irradiated for periods of 30 s to 1 h during which time the suspensions were shaken gently to ensure uniform irradiation. Virus infectivity (initial titre $10^8$ p.f.u./ml) was abolished by more than 12 min exposure to u.v. irradiation using the conditions described.

Foetal cells exposed to irradiated HSV at multiplicities (calculated from non-irradiated titre) between 1 and 10 p.f.u./cell, characteristically showed clumping 1 to 2 days after infection, although considerably fewer cell clusters were seen in cultures exposed to virus which had been irradiated for periods in excess of 15 to 20 min (Fig. 3). Moreover, exposure to u.v. inactivated HSV was associated with extended culture survival. (The medium from such cells was changed at weekly intervals when samples of lymphocytes were removed for pulse labelling but otherwise the cultures were undisturbed). The longest surviving cultures (5 months) were derived from cells exposed to 5 and 10 min u.v. irradiated virus. Although a number of such cultures have been passaged once, it has not proved possible to show indefinite proliferation of these cells.

Fig. 3(d) shows Giemsa stained cells from long term surviving cultures of foetal cells exposed to irradiated HSV-1. These cultures characteristically had a high proportion of blast-like cells, often persisting in clumps. Although infectious virus was not recovered from cultures infected with inoculum receiving more than 2 min irradiation, virus-specific antigens could be detected for several weeks in a small proportion of surviving cells in cultures exposed to 5 and 10 min irradiated HSV.

Growth of Herpes simplex type 1 in mitogen stimulated human lymphocytes

Cultures which had previously been exposed to stimulating concentrations of phytohaemagglutinin (PHA) or pokeweed mitogen (PWM) supported considerably more virus growth than unstimulated cultures or cells to which mitogen was added at the time of infection (Fig. 4a and 5a). Both adult and foetal lymphocytes showed increased permissiveness after mitogen stimulation, and PHA treated cultures consistently supported more virus growth than PWM treated cultures. Exposure to HSV greatly reduced the blastogenic response of lymphocytes to both mitogens (Table 1 and 2); moreover, the virus appeared selectively cytotoxic to pre-stimulated PHA lymphoblasts and, to a lesser degree, PWM lymphoblasts. In unstimulated cultures infection was associated with an observed increase rather than a decrease in cells with lymphoblastoid morphology.
Fig. 4. Growth of HSV-1 in unseparated and separated sub-populations of adult human lymphocytes, infected at an input multiplicity of 10 p.f.u./cell. Each point represents the virus yield from $10^5$ cells (initially cultured). (a) Unseparated lymphocytes; (b) B-enriched sub-population; (c) T-enriched sub-population. ●—●, Unstimulated cells; ■—■, PHA stimulated cells; ○—○, PWM stimulated cells. Mitogens were added to the cultures 3 days before infection.
Herpes simplex and human lymphocytes

Fig. 5. Growth of HSV-1 in unseparated and separated sub-populations of foetal human lymphocytes, infected at an input multiplicity of 10 p.f.u./cell. Each point represents the virus yield from 10^6 cells (initially cultured). (a) Unseparated lymphocytes; (b) B-enriched sub-population; (c) T-enriched sub-population. •—•, Unstimulated cells; ■—■, PHA stimulated cells; ○—○, PWM stimulated cells. Mitogens were added to the cultures 3 days before infection.
Table 1. Incorporation of $^3$H-thymidine into virus- and cell-specific DNA by human adult and foetal lymphocytes. (i) Mitogen added to fresh cultures at the time of infection

<table>
<thead>
<tr>
<th>Culture</th>
<th>Amount incorporated during 48 h experimental period (ct/min/cell x $10^{-8}$ (± s.d.))</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Adult lymphocytes</td>
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<tr>
<td></td>
<td>Foetal lymphocytes</td>
</tr>
<tr>
<td>Control (no mitogen)</td>
<td>1.69 g/ml ± 1.72 g/ml ±</td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.3 (0.1) -</td>
</tr>
<tr>
<td>+ HSV-1</td>
<td>3.5 (0.5) &lt; 0.1 -</td>
</tr>
<tr>
<td>PHA (1 μg/ml)</td>
<td>116.9 (8.9) -</td>
</tr>
<tr>
<td>Uninfected</td>
<td>6.6 (1.0) &lt; 0.1 -</td>
</tr>
<tr>
<td>+ HSV-1</td>
<td>48.9 (3.6) -</td>
</tr>
<tr>
<td>PWM (10 μg/ml)</td>
<td>10.8 (0.7) &lt; 0.1 -</td>
</tr>
<tr>
<td>Uninfected</td>
<td>161.3 (10.7) -</td>
</tr>
<tr>
<td>+ HSV-1</td>
<td>77.7 (9.0) -</td>
</tr>
<tr>
<td>+ HSV-1</td>
<td>5.7 (0.4) 2.2 (0.1) -</td>
</tr>
</tbody>
</table>

* Each culture incubated for 48 h labelling period after infection.
† Total radioactivity in peak at 1.69 g/ml assumed to be cell specific DNA. Total radioactivity in peak at 1.72 g/ml assumed to be HSV-1 specific DNA.

Table 2. Incorporation of $^3$H-thymidine into virus and cell specific DNA by human adult and foetal lymphocytes. (ii) Pre-stimulated cultures. Mitogen added 3 days before infection

<table>
<thead>
<tr>
<th>Culture</th>
<th>Amount incorporated during 48 h experimental period (ct/min/cell x $10^{-8}$ ± s.d.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult lymphocytes</td>
</tr>
<tr>
<td></td>
<td>Foetal lymphocytes</td>
</tr>
<tr>
<td>Control (no mitogen)</td>
<td>1.69 g/ml ± 1.72 g/ml ±</td>
</tr>
<tr>
<td>Uninfected</td>
<td>3.5 (0.8) -</td>
</tr>
<tr>
<td>+ HSV-1</td>
<td>6.7 (1.0) 0.3 (0.1) -</td>
</tr>
<tr>
<td>PHA (1 μg/ml)</td>
<td>161.3 (10.7) -</td>
</tr>
<tr>
<td>Uninfected</td>
<td>10.8 (0.7) 23.8 (3.2) -</td>
</tr>
<tr>
<td>+ HSV-1</td>
<td>77.7 (9.0) -</td>
</tr>
<tr>
<td>PWM (10 μg/ml)</td>
<td>5.7 (0.4) 2.2 (0.1) -</td>
</tr>
</tbody>
</table>

* Each culture incubated for 3 days before infection and 48 h labelling period.
† Total radioactivity in peak at 1.69 g/ml assumed to be cell specific DNA. Total radioactivity in peak at 1.72 g/ml assumed to be HSV-1 specific DNA.

DNA synthesis by mitogen treated, infected lymphocyte cultures

Equilibrium density gradient centrifugation was used to analyse the pattern of virus and cell-specific DNA synthesis in mitogen treated lymphocyte cultures. Tables 1 and 2 show data assembled from five batches of similar experiments in which unstimulated, pre-stimulated or simultaneously stimulated and infected adult and foetal lymphocyte cultures were examined, together with uninfected parallel cultures, for incorporation of $^3$H-thymidine into virus and cell-specific DNA.

From such experiments the following conclusions can be drawn. First, significant amounts of virus-specific DNA could only be detected in adult cells which had been pre-stimulated or cultured for 2 days prior to infection in the absence of mitogen. (Lymphocyte
DNA synthesis generally increased somewhat during *in vitro* culture, probably due to cell stimulation by foetal calf serum in the medium. This might account for the increased permissiveness to HSV of unstimulated lymphocytes after limited *in vitro* culture.) A small amount of virus-DNA was detected in unstimulated cultures, sevenfold more in PWM stimulated cultures and almost 100-fold more in PHA stimulated cultures.

Second, foetal cells were able to support small amounts of virus DNA synthesis without prior culture, possibly as a consequence of the greater metabolic activity of these cells compared with adult lymphocytes (reflected by the uptake of $^3$H-thymidine by uninfected fresh cultures). The amount of virus DNA synthesized by foetal cells was greater after *in vitro* culture and considerably increased by PWM and PHA pre-stimulation.

Third, the amount of cell-specific DNA synthesized by mitogen treated cultures of adult or foetal cells was significantly reduced by infection. This was observed in both pre-stimulated cultures and those to which mitogen and virus was added simultaneously, compared with uninfected parallel cultures. However, in each experiment the amount of cell-specific DNA synthesized by unstimulated infected adult and foetal cells exceeded the amount synthesized by uninfected cultures.

**HSV antigen production by mitogen treated human lymphocytes**

Virus-specific antigens were readily detected in infected lymphocyte cultures (Fig. 6). In most infected experimental cultures the majority of cells showed no evidence of virus antigen production (Table 3). Unfortunately, it has not proved possible to determine, by infectious centre assays, the proportion of antigen positive non-productive cells. It seems most probable, however, that in unstimulated cultures at least, where the yield of progeny virus/cell was much lower than the percentage of antigen positive cells, some non-productive cell supported virus antigen synthesis. This conclusion is supported by the electron microscope studies described below.
Table 3. *HSV-antigen synthesis by human lymphocytes*

<table>
<thead>
<tr>
<th></th>
<th>% Immunofluorescent cells (± s.d.)*</th>
<th>24 h p.i.</th>
<th>72 h p.i.</th>
</tr>
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<tbody>
<tr>
<td><strong>Adult lymphocytes</strong></td>
<td></td>
<td></td>
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<tr>
<td>Unstimulated cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Infected on Day 0</td>
<td>0.4 (0.1)</td>
<td>4.4 (1.3)</td>
<td></td>
</tr>
<tr>
<td>(b) Infected on Day 3</td>
<td>0.5 (0.1)</td>
<td>5.0 (0.7)</td>
<td></td>
</tr>
<tr>
<td>PHA treated cells</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(a) Infected on Day 0</td>
<td>0.6 (0.1)</td>
<td>4.3 (0.2)</td>
<td></td>
</tr>
<tr>
<td>(b) Infected on Day 3</td>
<td>1.0 (0.1)</td>
<td>7.2 (4.5)</td>
<td></td>
</tr>
<tr>
<td>PWM treated cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Infected on Day 0</td>
<td>0.6 (0.1)</td>
<td>2.6 (2.1)</td>
<td></td>
</tr>
<tr>
<td>(b) Infected on Day 3</td>
<td>0.6 (0.1)</td>
<td>4.5 (4.5)</td>
<td></td>
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<tr>
<td><strong>Foetal lymphocytes</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Unstimulated cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Infected on Day 0</td>
<td>0.9 (0.1)</td>
<td>16.3 (1.7)</td>
<td></td>
</tr>
<tr>
<td>(b) Infected on Day 3</td>
<td>1.2 (0.1)</td>
<td>17.1 (1.8)</td>
<td></td>
</tr>
<tr>
<td>PHA treated cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Infected on Day 0</td>
<td>3.8 (0.9)</td>
<td>42.7 (2.6)</td>
<td></td>
</tr>
<tr>
<td>(b) Infected on Day 3</td>
<td>8.9 (0.9)</td>
<td>73.9 (10.0)</td>
<td></td>
</tr>
<tr>
<td>PWM treated cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Infected on Day 0</td>
<td>0.7 (0.1)</td>
<td>32.6 (8.6)</td>
<td></td>
</tr>
<tr>
<td>(b) Infected on Day 3</td>
<td>3.7 (0.4)</td>
<td>62.3 (13.0)</td>
<td></td>
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</table>

* Data from four experimental cultures.

*HSV particle production by human lymphocytes*

The appearance of herpesvirus particles in infected lymphoblasts was detected by electron microscope examination of thin sections of infected cells. Both non-enveloped intranuclear particles and enveloped extranuclear virions were observed. (Fig. 7). Virus particles were not observed in cells with the morphology of small lymphocytes, only in enlarged blast-like cells with evidence (such as the presence of numbers of mitochondria) to suggest that their lymphoblast appearance preceded rather than was a consequence of infection. Margination of chromatin, the appearance of membrane ‘whorls’ and dense granules, were a characteristic feature of degenerating infected cells, similar to those described by Bouroncle and co-workers, (1970).

In general, virus particles were scattered and rather few in number, no more than about 20 being observed in any cell section. Regular arrays of intranuclear particles were not observed and many of the particles appeared to lack an electron dense core. The proportion of cells with nuclear particles in different cultures could not be ascertained with great accuracy from the small number of cells (approx. 50) examined from each experiment. However, in unstimulated cultures less than 10% of the cells contained virus particles. The largest number of particle containing cells was observed in cultures infected after pre-stimulation by PHA. In such cultures of adult lymphocytes 30 to 50% of the cells contained virus particles, and in cultures of foetal cells 70 to 90% of the cells had nuclear particles 3 days p.i.
Fig. 7. Electron micrographs of HSV-1 infected, PHA stimulated foetal lymphocytes. (a) Lobed nucleus with nucleocapsids and enveloped virion. A degenerating mitochondrion is also shown. (b) Degenerating cell showing cytoplasmic membrane 'whorls' and granules as well as virus particles and margination of nuclear chromatin. (c) Nuclear particles and cytoplasmic enveloped virions in degenerating lymphoblast. No normal cytoplasmic organelles remain.
Table 4. Separated populations of human adult and foetal lymphocytes – T-cell and B-cell markers, \(^{3}\text{H}\)-thymidine uptake and virus growth in sub-populations separated after 3 days in culture

<table>
<thead>
<tr>
<th>Cells</th>
<th>(%) E-R*</th>
<th>(%) EAC'-R*</th>
<th>(%) Surface</th>
<th>(^{3}\text{H})-TdT(_{4}) ct/min/10(^5) cells</th>
<th>Max p.f.u./10(^5) cells</th>
<th>(^{3}\text{H})-TdT(_{4}) ct/min/10(^5) cells</th>
<th>Max p.f.u./10(^5) cells</th>
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<tr>
<td>Unstimulated</td>
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</tr>
<tr>
<td>(a) Unseparated</td>
<td>38.8(\dagger)</td>
<td>19.1</td>
<td>14.2</td>
<td>1517</td>
<td>(3 \times 10^4)</td>
<td>3113</td>
<td>(1.0 \times 10^3)</td>
</tr>
<tr>
<td>(b) B-enriched</td>
<td>(&lt; 1)</td>
<td>45.5</td>
<td>16.3</td>
<td>734</td>
<td>(&lt; 10^4)</td>
<td>2003</td>
<td>(1.0 \times 10^4)</td>
</tr>
<tr>
<td>(c) T-enriched</td>
<td>67.3</td>
<td>8.2</td>
<td>(&lt; 1)</td>
<td>1049</td>
<td>(2 \times 10^4)</td>
<td>1003</td>
<td>(9 \times 10^4)</td>
</tr>
<tr>
<td>PHA stimulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Unseparated</td>
<td>46.1</td>
<td>17.8</td>
<td>5.0</td>
<td>47277</td>
<td>(3 \times 10^6)</td>
<td>85600</td>
<td>(2 \times 10^9)</td>
</tr>
<tr>
<td>(b) B-enriched</td>
<td>1.1</td>
<td>40.4</td>
<td>6.7</td>
<td>885</td>
<td>(&lt; 10^4)</td>
<td>3310</td>
<td>(&lt; 10^4)</td>
</tr>
<tr>
<td>(c) T-enriched</td>
<td>75.8</td>
<td>22.3(\S)</td>
<td>(&lt; 1)</td>
<td>49185</td>
<td>(8 \times 10^4)</td>
<td>108540</td>
<td>(6 \times 10^6)</td>
</tr>
<tr>
<td>PWM stimulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Unseparated</td>
<td>38.7</td>
<td>20.0</td>
<td>4.2</td>
<td>18770</td>
<td>(6 \times 10^4)</td>
<td>44533</td>
<td>(3 \times 10^4)</td>
</tr>
<tr>
<td>(b) B-enriched</td>
<td>(&lt; 1)</td>
<td>47.5</td>
<td>7.3</td>
<td>7908</td>
<td>(&lt; 10^4)</td>
<td>19597</td>
<td>(&lt; 10^4)</td>
</tr>
<tr>
<td>(c) T-enriched</td>
<td>70.9</td>
<td>7.8</td>
<td>(&lt; 1)</td>
<td>9394</td>
<td>(9 \times 10^4)</td>
<td>54742</td>
<td>(3 \times 10^5)</td>
</tr>
</tbody>
</table>

* E-R = E-rosettes; EAC'-R = EAC'-rosettes.
† Maximum titre assayed 3 days after infection at an input multiplicity of 10 p.f.u./cell.
‡ The proportion of E-R cells was reduced after 3 days culture in vitro.
§ The proportion of EAC' rosettes was invariably high in this sub-population, clumped lymphoblasts in particular seemed affected. It is probable that this was an artefact due to adhesive properties of PHA stimulated T-cells.

HSV growth in separated sub-populations of human lymphocytes

The conclusion drawn from the preceding data is that, even under the most permissive experimental conditions, not all the cells were able to support virus growth. Human lymphocyte cultures are a heterogeneous mixture of cells, and experiments were undertaken to determine whether virus growth could be shown to be restricted to any particular type of cell.

The success of PHA pre-stimulation in increasing virus production observed in this and previous studies suggested that the stimulated T-lymphoblast might be the most permissive cell. The absence of data from stimulated B-lymphoblasts had prevented any conclusion as to whether stimulation or T-cell origin, or both, were required for virus growth.

It was found that isolated B-lymphocyte enriched populations did not respond well to any mitogen tested. However, it was possible to separate pre-stimulated cultures into B-enriched and T-enriched populations by the E-rosetting technique and thus obtain a stimulated non-rosetting sub-population of cells from PWM treated cultures. PHA treated cells were separated into a stimulated E-rosetting population and a metabolically inactive non-E-rosetting population. Separated sub-populations of lymphocytes were also examined by membrane immunofluorescence (Raff et al. 1970) for the presence of surface human immunoglobulin and for the ability to form EAC' rosettes (Lay & Nussenzweig, 1968) with sheep erythrocytes plus antibody plus mouse complement. Both techniques distinguish B-lymphocytes from T-cells and both indicated significant separation of B- and T-cells by the E-rosetting method employed. The total number of cells with B-cell characteristics decreased during in vitro culture, with or without exposure to mitogens, but the consistent distribution of cells bearing B- and T-specific markers (Table 4) supports the view that
Herpes simplex and human lymphocytes

metabolically active B-enriched sub-populations from PWM stimulated cultures are indeed of B-cell origin.

It was found that good reproducibility could be obtained between different experimental populations of both adult and foetal lymphocytes provided that the cells were separated from blood samples within 2 h of collection. Table 4 shows results obtained from separated pre-stimulated or unstimulated cultures of lymphocytes, and the yield of HSV obtained from separated sub-populations. Lymphocytes separated in this way were infected under similar conditions and Fig. 4 and 5 compare the observed growth of HSV in stimulated and unstimulated, B-enriched, T-enriched and unseparated lymphocyte cultures. A significant yield of progeny virus was only obtained from cultures containing stimulated T-lymphoblasts. Unstimulated T-cells and stimulated B-lymphoblasts did not support virus growth.

DISCUSSION

The results of this study on the interaction between Herpes simplex virus type 1 and human lymphocytes of adult and foetal origin confirm the hypothesis (Kirchner et al. 1977) that full virus expression occurs in stimulated T-lymphoblasts and provide evidence that stimulated cells of B-lymphocyte origin are not permissive for virus growth. Moreover, it is probable that virus antigen synthesis and particle production were also confined to T-lymphoid cells. Full virus expression and progeny production required lymphocyte activation which in culture could be produced by the mitogens PHA and PWM; and also to some extent by in vitro culture in medium containing foetal calf serum. That this was a necessary but not sufficient condition was demonstrated by the failure of metabolically active cells in the B-lymphocyte enriched population to support virus growth. Small, unstimulated, lymphocytes of T- and B-cell origin, although able to adsorb HSV, did not support complete virus expression; unfortunately it was not possible, on the basis of data presented here, to deduce unequivocally whether or not such cells supported virus DNA synthesis but it seems probable that they did not do so.

Under conditions favourable for HSV growth, infected T-lymphoblasts degenerate; however, virus production by these cells was very inefficient compared with the yield from permissive cells such as BHK21 fibroblasts. The expression of HSV in cultured human lymphocytes was analogous to the productive expression of other viruses of the herpes group in cultured lymphocytes in so far as full virus expression appeared to be confined to a specific sub-population of cells. Nevertheless, cultures which did not support virus growth responded to infection. It was observed that HSV-1 had the ability to ‘switch on’ lymphocyte metabolism by a non-immunological mechanism which could only be detected under non-permissive conditions for virus growth and was sustained in cultures exposed to ultraviolet irradiated virus. The origin of those cells whose DNA synthesis is stimulated by HSV remains to be determined.

Some comment can be made about the significance of these observations. Previous reports have indicated that the herpesviruses which infect lymphoid cells can be divided into two groups, namely T-lymphotropic herpesviruses, including Marek’s disease virus (Payne et al. 1974), Herpes saimiri (Wallen et al. 1973) and Herpes atelis (Falk et al. 1975); and B-lymphotropic herpesviruses, including EBV (Jondal & Klein, 1973; Menezes et al. 1977), murine cytomegalovirus (Olding et al. 1975) and Herpes macaca (Graze & Royston, 1975). It would appear that Herpes simplex aligns with the T-lymphotropic group. The importance of this observation in vivo remains to be determined and may be clarified by an examination of virus growth in HSV-antigen stimulated immune lymphoblasts.
It is probable that the stage at which virus growth was blocked in unstimulated lymphocytes was post adsorption but prior to virus DNA synthesis and it is unlikely merely to represent the absence, at least in B-lymphocytes, of a cell component involved in DNA replication.

Further studies are in progress on the observed lymphocyte stimulation by active and partially ultraviolet inactivated Herpes simplex virus. In view of the well established ability of other herpesviruses to immortalize lymphocytes from an appropriate host, the observations described in this paper may indicate that Herpes simplex also has this potential.

This investigation was supported by a Medical Research Council Project Grant and carried out with the excellent technical assistance of Mrs J. Eady and Miss S. Evans. I should also like to thank Dr C. Sanger for carrying out the electron microscope examinations and Professor J. F. Watkins for his encouragement and helpful discussions.

REFERENCES


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