Multiple Interactions Between Murine Cytomegalovirus and Lymphoid Cells In Vitro

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SUMMARY

Spleen cultures from various strains of mice were infected in vitro with murine cytomegalovirus (MCMV). Infectious centres were established in a small proportion (not greater than 1%) of the cells. Virus could be rescued from these cells by co-cultivation with syngeneic or allogeneic fibroblasts, but the frequency of rescue could not be altered by incubation with cyclic nucleotide analogues, iododeoxyuridine, cortisol, or allogeneic spleen cells. In addition a smaller fraction of the cell population, possibly a sub-population of the infectious centres, replicated virus spontaneously. The presence of mitogens did not affect these interactions qualitatively or quantitatively. A third response to infection was an inhibition in DNA synthesis, which was suffered by unstimulated cultures and by cells transformed by concanavalin A and bacterial lipopolysaccharides, although overall cell viability was maintained. This response was also mediated by u.v.-inactivated virus.

INTRODUCTION

It is apparent that many herpes viruses, including cytomegaloviruses, can persist within lymphoid cells in a non-replicating state (Plummer, 1973; zur Hausen, 1975). Under certain conditions, virus gene expression is permitted, with the result that there is now recognized a wide spectrum of consequences as far as the virus is concerned, ranging from limited production of specific virus antigens to complete replication of the virus. The control mechanisms are not fully understood. Much less consideration has been given to the effects of latent virus genomes upon normal cellular functions. Such considerations are important, however, since lymphoid cells are engaged in a multitude of functions in the body. In addition, the presence of a potentially lytic virus within the lymphatic system of an animal has obvious relevance to pathological conditions, as is well illustrated by the consequences of immunosuppression of patients, which frequently results in dissemination of herpes viruses and ensuing tissue damage. The human cytomegalovirus is particularly notorious in this regard (Weller, 1971; Plummer, 1973).

The murine cytomegalovirus (MCMV) has been employed as a model for cytomegalovirus activation in chronically infected animals (Henson et al. 1972). These workers were able to reactivate or 'rescue' MCMV from spleen cells and lymph node cells of chronically infected mice, by co-cultivating these cells with mouse embryo cultures in vitro, although the frequency of reactivation was very low. We also obtained very low reactivation under similar conditions (unpublished results). Latent or chronic MCMV infection is also widespread in
nature, since immunosuppressive therapy of wild mice frequently yielded activation of the virus (Gardner et al. 1974).

In order to find a system more amenable to study, we decided to investigate the interactions between MCMV and spleen cells in vitro, with a view to determining the extent of permissiveness of various types of spleen cell toward MCMV and to attempt to obtain a latent infection, which we might then use to study the factors involved in reactivation. The spleen was chosen as the source of lymphoid cells partly for practical reasons, and also since MCMV had been reactivated previously from spleen cells (Henson et al. 1972).

METHODS

**Virus.** The Smith strain of MCMV, propagated at low multiplicity (0.01 p.f.u./cell) in mouse embryo cultures, was used in these studies.

**Mice.** The following strains of mice were used: C3H/HeDub; C3H/HeJ; SWR; DBA; BALB/c and random bred Swiss white from a local colony. No differences were observed between these strains regarding the results presented here.

**Cells.** Mouse embryo cells (derived from random bred Swiss white mice, except where indicated) were propagated in Dulbecco’s modified MEM (Gibco, Catalogue no. H-20), supplemented with 5% (v/v) foetal bovine serum; 20 μg/ml gentamycin and 0.25 μg/ml fungizone, in Petri dishes or roller bottles, and used after one or two passages.

Spleen cells were obtained by teasing the cells out of the bisected spleen into Hanks’ balanced salt solution (HBSS, without divalent cations). The cells were pelleted by centrifugation at 1000 g for 5 min, washed in HBSS, and resuspended in 0.16 M-NH₄Cl. The cells were pelleted, as before, and washed again in HBSS before suspending in culture medium. Total exposure time to NH₄Cl was less than 7 min.

Spleen cells were cultivated in RPMI 1640 with 10% (v/v) foetal calf serum and the other supplements, in tubes or microtitre wells, at a concentration of 2 to 5 × 10⁶ cells/ml. Cell viability was determined by counting trypan blue excluding cells in a haemocytometer.

**Infectivity assays.** MCMV was assayed by plaque formation on mouse embryo monolayers, usually employing the centrifugal method of assay (Osborn & Walker, 1968; Hudson, Misra & Mosmann, 1976b). Spleen cells were disrupted by three cycles of freezing and thawing.

Infectious centres were assayed by pelleting the cells, washing them twice and plating on to freshly confluent mouse embryo monolayers with or without a 0.5% (w/v) agarose overlay. The presence of an overlay was shown not to inhibit plaque formation. The overlay was added in the form of 2% (w/v) agarose 1 h after the spleen cells had been added, by which time the cells had settled on to the fibroblast monolayer. Plaques were counted 5 to 7 days after adding the overlay.

**Infection of spleen cells in vitro.** Cells were pelleted at low speed and resuspended in a small volume of medium containing MCMV. After 60 min incubation at 37 °C, the mixture was diluted tenfold and the cells pelleted gently, and then washed three times to remove unadsorbed virus. Additional washes were found to be unnecessary. The cells were then resuspended in fresh medium at the initial concentration. Unless indicated otherwise, the multiplicity of infection was 1.0 p.f.u./cell (which corresponds to ~ 30 centrifugal p.f.u./cell).

**Radioactive MCMV.** Virus was labelled with ³H-dThd and purified as described previously (Mosmann & Hudson, 1973). Briefly, the labelled extracellular virus was freed from traces of cellular debris by low speed centrifugation, and pelleted at high speed. The pellets
were resuspended in a small volume and treated with electrophoretically purified DNase (50 μg/ml) and pancreatic RNase (100 μg/ml) at 37 °C for 30 min. The virus was again pelleted and resuspended in PBS or medium, after rinsing to remove traces of soluble radioactivity.

Ultraviolet inactivation of MCMV. The virus, in MEM, was spread out in one or more culture dishes and exposed to a Sylvania 15 watt germicidal lamp at a distance of 15 cm for 7 min, to inactivate virus infectivity completely.

Isotope incorporation. Transformation of spleen cells by mitogens was monitored by incubating microtitre cultures, in quadruplicate, with ³H-dThd (2-5 μCi added to each 0-2 ml culture) for 4 or 16 h. At the end of this time, the cells were removed from their wells, and filtered on to fibreglass filters, which were then soaked in cold 10% (w/v) TCA for 30 min. The filters were then washed twice with 5% (w/v) TCA and once with ethanol before measuring radioactivity. Pulse labelling with ³H-Urd was performed in a similar manner. Radioactivity values are quoted as arithmetic means of the quadruplicate samples. Generally, and especially for the stimulated cultures with their higher incorporation levels, the replicate samples were within ± 25% of the mean values. For this reason statistical significance values are not quoted in the results. Simple inspection of the values and the reproducibility of observed changes was in most cases sufficient to convince us of the reality of an altered rate of isotope incorporation.

Electron microscopy. Negative staining of virus particles was performed with phosphotungstate, pH 6.4. For samples containing small numbers of particles, the virions were pelleted on to grids placed at the bottom of Beckman SW 50-1 tubes, by centrifuging at 25000 rev/min for 60 min in the SW 50-1 rotor.

Materials. Concanavalin A; lipopolysaccharide (E. coli and S. typhimurium); dibutyryl cyclic-AMP; dibutyryl cyclic-GMP; iododeoxyuridine (IdUrd); and pancreatic DNase (RNase-free) were all purchased from Sigma Chemical Co. ³H-dThd and ³H-Urd (40 to 60 Ci/mmol) were from Amersham-Searle or New England Nuclear.

RESULTS

Establishment of infectious centres

Whenever MCMV was added to spleen cultures derived from random-bred or inbred mice, virus infectivity decreased to a low or undetectable level over a period of two to three days. This happened whether or not the cells had been stimulated by the mitogens Con A (concanavalin A) or LPS (lipopolysaccharide), as illustrated in Fig. 1. This same result was also obtained if the mitogens were added after virus adsorption. The decay in infectivity of the MCMV was apparently due to thermal inactivation, since in other experiments it was found that MCMV lost infectivity at similar rates in medium alone, and in medium containing intact or disrupted spleen cells.

The number of infectious centres remained approximately constant so long as cell viability was maintained (usually 3 to 4 weeks).

Although virus infectivity disappeared to undetectable levels by five days post infection in the experiment shown in Fig. 1, infectious centres could be detected by plating samples of the washed cells on to monolayers of mouse embryo fibroblasts (ME cells), which support MCMV replication and give rise to plaques if an agarose overlay is attached. In the experiment shown (Fig. 1) approx. 0.01% of the cells registered as infectious centres and therefore at least this number of cells contained an intact virus genome. Evidently this number was not affected by the presence of mitogens.
MCMV infectivity in spleen cultures. Spleen cells from an adult Swiss mouse were divided into 8 equivalent cultures. They were incubated for two days with or without mitogen. Four cultures were then infected with MCMV, and the other four cultures were mock infected with medium. Duplicate samples were removed periodically for p.f.u. assay (by the centrifugal mode) and, on the sixth day after infection, for IC. All mock infected cultures were negative for p.f.u. and IC. ○, No mitogen; □, +Con A (2 μg/ml); ▲, +LPS (50 μg/ml); ■, +Con A and LPS; △, mock infected ± Con A, LPS.

MCMV replication in spleen cultures

In the majority of experiments, some virus replication did occur after the decay of the initial inoculum. However, this never amounted to more than 1 p.f.u./100 cells, which implies that only a small proportion of spleen cells supported virus growth. This did not appear to be related to the strain of mouse. Fig. 2 shows a typical response, and includes data for cultures infected before (Fig. 2a) and after (Fig. 2b) mitogen stimulation. The mitogens had no apparent significant effect, with the possible exception of Con A, which tended to decrease virus yields when added after virus infection. This could be due to an indirect effect of activated T-cells. In all cases infectious centres could also be detected throughout the cultivation period, but their numbers were not influenced by the mitogens, in agreement with the results presented in Fig. 1. Cultures have not been tested for their long-term capacity to support virus replication.

Infectious centres (IC) could be measured independently of p.f.u. in most samples, since the cells were normally washed to remove extracellular virions, and at most times tested IC were in excess of p.f.u. Furthermore p.f.u. are subject to centrifugal enhancement of infectivity (Hudson et al. 1976b), whereas IC are not. This is illustrated in Table 1, which compares the two parameters for MCMV-infected C3H-HeJ spleen cultures. Thus a significant contribution of free virions (p.f.u.) to IC is reflected by an increase in the centrifugal/standard ratio. The only time period that caused difficulty in assessment of IC was the time of maximum p.f.u. production.
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![Fig. 2. Replication of MCMV in spleen cultures. Infection before (a), and after (b), mitogens. Spleen cultures were established from SWR strain mice. One set of cultures was exposed to mitogens (or untreated) for 2 days before infecting with MCMV, followed by cultivation in medium without mitogens. The other set of cultures were infected with MCMV first and incubated with mitogens (or untreated) immediately after virus adsorption. Periodically, samples were removed for assay of p.f.u. (on disrupted cells plus medium) per ml as described in Methods. Initial viable cell concentrations ranged from 1-38 to 1-71 x 10^3/ml. ●●, No mitogen; ▲▲, Con A (5 µg/ml); ■■, LPS (40 µg/ml).](image)

Table 1. Comparison between infectious centres (IC) and infectious virions (p.f.u.)

<table>
<thead>
<tr>
<th>Mode of infection</th>
<th>IC/ml</th>
<th>p.f.u./ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (s)</td>
<td>6.7 x 10^9</td>
<td>3.3 x 10^9</td>
</tr>
<tr>
<td>Centrifugal (c)</td>
<td>1.67 x 10^4</td>
<td>8.65 x 10^3</td>
</tr>
<tr>
<td>Ratio c/s</td>
<td>2.5</td>
<td>26.2</td>
</tr>
</tbody>
</table>

The level of MCMV replication in spleen cultures varied between zero and 10^8 p.f.u./10^8 cells. This variation apparently reflected a difference between spleens of individual mice, since cultures from separate spleens compared at the same time gave quantitatively different responses to MCMV.

The results presented so far indicate that MCMV can establish infectious centres in a small and variable proportion of the spleen cells, and in addition, after at least one day of infection in vitro, another minority cell population can replicate the virus. At present we do not know if these two cell types are the same, but it is possible that some of the infectious centres are responsible for the eventual virus replication.

Nature of the infectious centres

The number of IC established by MCMV infection was proportional to input multiplicity, although even at 25 p.f.u./cell only about 1-0% of the cells could be activated by ME
monolayers to yield virus. This is shown in Table 2 for infected C3H/HeJ spleen cells at 2 h p.i. This number could represent the maximum number of cells harbouring an intact virus genome, or could simply reflect the inefficiency of the activation process. To test the latter possibility, spleen cells containing IC were incubated with various agents, known to affect lymphocyte function, either before or during the co-cultivation with ME cells. These agents included allogeneic spleen cells, cyclic nucleotide analogues, IdUrd (which promotes activation of EB virus from human lymphoblasts; Hampar, Derge & Showalter, 1974), and cortisol. Table 3 shows some of these results. In this experiment Swiss mouse spleen cultures were assayed for IC 15 days after infection, and the various samples (in duplicate) were added to the ME monolayers without an overlay. No significant difference was found, except that the plaques formed after IdUrd treatment were delayed until the reagent had decayed, since IdUrd blocks MCMV development (unpublished results). Similar results were obtained if an agarose overlay was added, and the reagents were added for 24 h prior to co-cultivation with ME cells. It is possible, therefore, that the number of IC detected normally represents all the cells containing an intact virus genome.

The virus activated from the IC was examined in the electron microscope, and was found to possess the typical herpes-like morphology, including the multicapsid virions characteristic of MCMV (Hudson, Misra & Mosmann, 1976a).

The origin of the ME cells used for reactivating MCMV was apparently unimportant,
**MCMV-lymphoid cell interactions**

Table 4. Reactivation of MCMV from spleen cells by syngeneic and allogeneic mouse embryo cells

<table>
<thead>
<tr>
<th>Source of infected spleen cells</th>
<th>ME cells</th>
<th>IC per 10⁶ spleen cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWR</td>
<td>Swiss</td>
<td>3.4 x 10⁶</td>
</tr>
<tr>
<td>SWR</td>
<td>SWR</td>
<td>3.4 x 10⁶</td>
</tr>
<tr>
<td>SWR</td>
<td>C3H</td>
<td>3.0 x 10⁶</td>
</tr>
<tr>
<td>C3H</td>
<td>Swiss</td>
<td>4.3 x 10⁶</td>
</tr>
<tr>
<td>C3H</td>
<td>SWR</td>
<td>6.4 x 10⁶</td>
</tr>
<tr>
<td>C3H</td>
<td>C3H</td>
<td>4.0 x 10⁶</td>
</tr>
</tbody>
</table>

Table 5. Reactivation of MCMV in the presence of DNase or antiserum

<table>
<thead>
<tr>
<th>Time after co-cultivation (h)</th>
<th>R(DNase)*</th>
<th>R(as)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>1.07</td>
<td>1.08</td>
</tr>
<tr>
<td>47</td>
<td>0.92</td>
<td>1.07</td>
</tr>
<tr>
<td>48</td>
<td>1.10</td>
<td>1.01</td>
</tr>
<tr>
<td>49</td>
<td>1.13</td>
<td>0.44</td>
</tr>
<tr>
<td>50</td>
<td>0.96</td>
<td>0.56</td>
</tr>
<tr>
<td>51</td>
<td>1.16</td>
<td>0.89</td>
</tr>
<tr>
<td>52</td>
<td>1.12</td>
<td>0.87</td>
</tr>
<tr>
<td>53</td>
<td>1.06</td>
<td>0.97</td>
</tr>
</tbody>
</table>

* R(DNase) = ratio: IC with pancreatic DNase (50 µg/ml) treatment/IC without DNase.
† R(as) = ratio: IC with antiserum (1/10 dilution of hyperimmune rabbit antiserum) treatment/IC without antiserum.

since both syngeneic and allogeneic ME cells could be used with equal efficiency, as shown in Table 4. This result, together with the others reported above, lends support for the concept that the cells harbouring latent MCMV genomes (i.e. the IC) may not be lymphocytes, but some other cell component of the spleen. Cell separation and identification techniques are necessary for further evaluation of this problem, however, and these are presently in progress.

**Mechanism of activation of MCMV from infectious centres**

Activation could result in replication of the virus within spleen cells, followed by spread of progeny virions to the ME cells, or in the transport of infectious DNA into the ME cells, where it would then replicate. If the former were true, then the number of plaques in the ME cells should be reduced by MCMV-antiserum but not by DNase, whereas the reverse should happen for the alternative mechanism. Such an experiment was performed by plating infected C3H/HeJ spleen cells (24 h p.i.) on to replicate ME monolayers, and at various times exposing duplicate mixtures to either antiserum, DNase, or medium alone, for 60 min prior to adding an agarose overlay. The overlay did not contain enzyme or antiserum. The results are shown in Table 5. At about 49 h after plating, there was a release of p.f.u., which was reduced by antiserum but not by DNase. Ratios did not deviate significantly from unity between 1 and 45 h and between 54 and 72 h after plating. Thus it appears that MCMV does replicate in the spleen cells harbouring the virus genome, and then emerges to infect the neighbouring ME cells. This result supports the concept that the virus can replicate occasionally in a small number of spleen cells, and that ME cell mediated reactivation enhances the event.
Table 6. dThd/Urd incorporation in MCMV-infected spleen cultures

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Sample</th>
<th>+ Con A (2 μg/ml)</th>
<th>+ LPS (50 μg/ml)</th>
<th>+ Con A</th>
</tr>
</thead>
<tbody>
<tr>
<td>dThd</td>
<td>Uninfected (c)</td>
<td>5197</td>
<td>117094</td>
<td>38472</td>
</tr>
<tr>
<td></td>
<td>MCMV (v)</td>
<td>1048</td>
<td>20662</td>
<td>6757</td>
</tr>
<tr>
<td>Ratio v/c</td>
<td>0.20</td>
<td>0.18</td>
<td>0.18</td>
<td>0.23</td>
</tr>
</tbody>
</table>

| Urd      | Uninfected (c) | 14749            | 50992           | 24979  |
|          | MCMV (v)     | 7618             | 28744           | 13302  |
| Ratio v/c | 0.52         | 0.56             | 0.53            | 0.58   |

Fig. 3. Effect of viable and u.v.-inactivated MCMV upon ³H-dThd incorporation in (a) 3T3 cells, (b) Con A stimulated spleen cells. Cultures of 3T3 cells, containing 6 × 10⁵ cells per 50 mm dish, and DBA mouse spleen cells, 2 × 10⁶ cells/ml (incubated for 48 h with 2 μg/ml Con A), were infected centrifugally with viable (50 c.p.f.u./cell) MCMV, or an equivalent amount of u.v.-inactivated MCMV. Periodically samples of 3T3 cells (in duplicate) and spleen cells (in quadruplicate) were taken and incubated with ³H-dThd for 4 h, followed by assay for TCA-insoluble ct/min. The time points indicate the middle of each 4 h labelling period. Values are expressed as ratios of ct/min incorporated by infected sample/ct/min incorporation by uninfected sample. ○—○, Viable MCMV; ■—■, u.v.-inactivated MCMV.

Virus suppression of macromolecule synthesis in spleen cells

The overall cell viability of spleen cultures, measured as trypan blue-excluding cells, was not affected by MCMV for at least one week after infection. Both infected and uninfected cultures showed a gradual decline in cell viability during this period. In contrast, MCMV inhibited DNA synthesis and, to a lesser extent, RNA synthesis, in untreated and mitogen-treated cultures, as shown in Table 6. Data are shown for DBA spleen cultures 24 h p.i. although similar results were obtained for other mouse strains and other times after infection. The degree of inhibition was similar in all cultures, which suggests that most of the cell types, including transformed B-cells and T-cells, were susceptible to MCMV. However, this suppression did not require virus gene expression since u.v.-inactivated MCMV was equally effective in inhibiting DNA synthesis in Con A-stimulated spleen cultures (Fig. 3b), and in LPS-stimulated and unstimulated cultures. This situation contrasts with the effect of MCMV upon DNA synthesis in mouse fibroblasts (Fig. 3a) where only infectious virus...
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**Table 7. Association of 3H-dThd labelled MCMV with spleen cells**

<table>
<thead>
<tr>
<th>Time after infection (h)</th>
<th>% cell-associated radioactivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8.5</td>
</tr>
<tr>
<td>24</td>
<td>3.2</td>
</tr>
<tr>
<td>48</td>
<td>3.3</td>
</tr>
<tr>
<td>72</td>
<td>4.4</td>
</tr>
<tr>
<td>96</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* The input virus contained 63000 ct/min.

can inhibit DNA synthesis. In the latter instance DNA synthesis is renewed from about 10 h p.i. due to virus DNA replication. These results suggest that MCMV only needs to interact in some way with spleen cells, perhaps at the cell surface, and only transiently, in order to inhibit cellular DNA synthesis, whereas in permissive fibroblasts virus gene expression is required. In accordance with this concept of a transient interaction between spleen cells and virus, it was found that only a small fraction, less than 10%, of radioactively labelled MCMV remained cell-associated after infection, as seen in Table 7. Furthermore, it was shown in other tests that the amount of cell associated radioactive virus was not affected by prior incubation of the cells with Con A.

**DISCUSSION**

The results described have indicated that MCMV interacts in several ways with mouse spleen cells. The virus inhibits DNA synthesis in the untreated spleen cell culture and also in those cells transformed by the mitogens Con A and LPS (T-cells and B-cells respectively). In addition, the virus establishes infectious centres in a small population of the cells, and in most spleen cultures a small number of cells eventually produce progeny virus several days after infection. However, the latter response was not always evident, although infectious centres were. Thus the virus-producing cells may be a different class of cells, or may be a sub-population of the infectious centres.

The inhibitory effect of MCMV has obvious relevance to *in vivo* infections, which show generalized immunosuppression (Howard & Najarian, 1974; Howard, Miller & Najarian, 1974). It is conceivable that the virus may interfere with many lymphoid cell functions, although cell viability was not noticeably affected in the experiments described here. It is interesting that the inhibition in DNA synthesis did not require virus gene expression, in contrast to the inhibitory effect of the virus in fibroblasts. A possible explanation for this is that only surface contact between virus and lymphoid cell is required. In fact the inhibition might be explicable simply in terms of competition between virus and mitogen for cell surface receptors. This possibility is being explored further. Nevertheless, this would not detract from the relevance of the effect to natural infections. It should also be pointed out that the inhibitory effect can also be shown with *in vivo* passaged virus (unpublished results), although our experiments are normally done with *in vitro* passaged virus for practical reasons. Comparative studies between the two types of virus are under way, to determine if they behave differently quantitatively.

Infectious centres are defined as cells containing rescuable virus genomes, and which give rise to plaques on fibroblast monolayers only when assayed as whole spleen cells, but which contain no detectable p.f.u. when assayed as disrupted cell preparations. It is possible that some may replicate virus spontaneously if left in liquid culture. The number of
infectious centres was small, in the range of 0.01 to 1.0% of the total cell population (depending upon the multiplicity of infection). This number could not be altered by prior incubation of the cells with mitogens, and was not increased by subsequent incubation with cyclic nucleotide analogues, IdUrd, or cortisol, or incubation with allogeneic spleen cells. These results support the possibility that the cells forming infectious centres are not lymphocytes but some other type of spleen cell present in relatively small numbers. Cell separation techniques, now in progress, should shed light on this problem. In this connection it is interesting that Olding, Jensen & Oldstone (1975) reported the rescue of MCMV from spleen cells infected in vivo, although no quantitative data were supplied. The cell type harbouring the virus possessed some properties like B-lymphocytes and required allogeneic fibroblasts for activation, in contrast to our studies reported here, in which allogeneic and syngeneic fibroblasts were equally efficient. Conceivably the two systems could represent different cell populations, although Henson et al. (1972) used syngeneic fibroblasts in their studies.

We originally commenced our studies with spleens from chronically infected mice, following the observations of Henson et al. (1972), but we abandoned this system because of the infrequency of reactivation, compared with the present in vitro system which provides us with a quantitatively more satisfying response.

The situation contrasts with herpes simplex virus, which shows mitogen dependent replication in human leukocyte cultures (Nahmias, Kibrick & Rosan, 1964; Kleinman et al. 1972), and B-cell mitogen dependent replication in mouse spleen cultures (Kirchner et al. 1976).

During the activation process, virus replicates within the spleen cells and then emerges, in a DNase-resistant and antiserum-sensitive form, to infect the fibroblasts. This shows that MCMV is capable of replicating in the spleen culture in the appropriate environment, and in fact in most experiments (though not all) some replication was detected in the absence of co-cultivation, although judging from the virus yields, the number of cells involved was probably much smaller than the number of infectious centres. It may be that few infectious centres replicate and release virus spontaneously whereas contact with fibroblasts is required for the optimal response. This would then concur with the results of histological studies on in vivo infected spleens, from which it was seen that MCMV could replicate in some mononuclear cells during the acute phase of infection (Osborn & Shahidi, 1973).

Further studies on the in vitro system described here should help in the understanding of with which cell types MCMV interacts and how the multiple features of infection are inter-related and controlled.

REFERENCES


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