J. gen. Virol. (1986), **67**, 2595–2604. *Printed in Great Britain*

Key words: *HCMV/replication/human bone marrow cells*

Human Cytomegalovirus Replicates in Primary Human Bone Marrow Cells

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(*Accepted 31 July 1986*)

SUMMARY

As an attempt to elucidate further the pathogenesis of human cytomegalovirus (HCMV) infection the replication of HCMV in primary human bone marrow cells (BMC) has been investigated. It was found that BMC held in culture in general were susceptible to HCMV infection. Compared to human embryonic lung cells, however, the replicative cycle of HCMV AD169 in BMC as determined by the analysis of viral protein and DNA synthesis was delayed and productive virus infection was restricted to a subset of BMC not exceeding 21% of the total cell population. Both of these phenomena may explain the short-term persistence of HCMV in BMC cultures which was observed over 3 months. By experiments with specifically enriched and depleted cell populations and by indirect double immunofluorescence experiments we found that both bone marrow fibroblasts and a subset of bone marrow stem cells supported productive virus infection. The finding that HCMV replicates in early stem cells of the human bone marrow may explain important aspects of the pathogenesis of HCMV infection including the presence of HCMV in peripheral blood leukocytes.

INTRODUCTION

Human cytomegalovirus (HCMV) has been frequently isolated from human leukocytes and their subsets as reported from different laboratories throughout the last 10 years (Harnden *et al.*, 1967; Fiala *et al.*, 1975; Rinaldo *et al.*, 1977; Macher *et al.*, 1983; summarized by Kirchner, 1983). Positive results of virus isolation were most frequently achieved by co-cultivation of human granulocytes with indicator cells, suggesting that the granulocyte cell fraction is one of the major virus reservoirs (Fiala *et al.*, 1975; Rinaldo *et al.*, 1980). Attempts to cultivate HCMV *in vitro* in primary human polymorphonuclear or mononuclear cells, however, were not successful and even infection of progenitor cell lines such as myeloblastoid lines did not lead to any appreciable virus replication (Rinaldo *et al.*, 1978; Einhorn & Öst, 1984). The recent finding of HCMV RNA in lymphocytes of naturally infected individuals (Schrier *et al.*, 1985) would suggest the possibility that HCMV does replicate in lymphocytes *in vivo*. These results, however, would not exclude the possibility that virus replication can also occur in early stem cells of the human bone marrow (Hirsch, 1984). The latter theory is supported by the finding that HCMV patients frequently show alterations of bone marrow cells (BMC) especially thrombocytopenia (Verdonck *et al.*, 1985), and that in generalized infections the presence of HCMV can be demonstrated in the bone marrow (Brunning, 1981; Bodem *et al.*, 1983). Furthermore, HCMV infection is the most frequent disease observed after bone marrow transplantation (Neimann *et al.*, 1977) and the reported T cell subset alterations after HCMV infection (Carney *et al.*, 1981) may well be explained by replication of the virus in early stem cells. Moreover, several reports on the replication of HCMV in different erythroleukaemic and lymphoblastoid cell lines *in vitro* suggest a susceptibility to HCMV of immature cells rather than of finally differentiated cell

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fractions (Joncas *et al.*, 1975; Furukawa,1979). For these reasons we investigated whether primary human BMC are susceptible to HCMV infection and if this replication involves primary stem cells.

METHODS

Virus and viral growth. For preparation of virus stocks human embryonic lung cells (HEL) WI-38 were used. Cells were held in Basal Medium Eagle (BME) containing 10% foetal calf serum (FCS), 100 units/ml penicillin, 100 μg/ml streptomycin and were grown at 37 °C in a humidified atmosphere containing 5% CO_2. Three days after subcultivation cells were infected with HCMV strain AD169 at a low m.o.i. (0·01 p.f.u./cell) to avoid generation of defective particles (DeMarchi & Kaplan, 1977) and supernatant virus was harvested 10 days after infection. As kindly tested by Dr W. Nicklas, German Cancer Research Center, Heidelberg, cells and virus were free of mycoplasma contamination. For mycoplasma testing, samples of virus and cell suspensions were cultured for 7 to 10 days in PPLO broth (Difco) containing 10% yeast lysate and 20% horse serum. Subsequently, portions of these cultures were grown on PPLO agar (Difco) supplemented with 20% horse serum and 10% yeast lysate. Petri dishes were microscopically examined for mycoplasma growth twice a week.

Virus titrations were performed on HEL cells with an overlay of 0·3% agarose (Sigma) in BME and 10% FCS for 10 days at 37 °C in a humidified atmosphere. Infectious centre assays were performed in a modification of a procedure developed for herpes simplex virus which takes into account the trypsin resistance of HCMV (Rapp, 1984). Thus, bone marrow cells were infected with HCMV AD169 at an m.o.i. of 5 p.f.u./cell. Following 2 h incubation at 37 °C cells were thoroughly washed and cultivated as outlined below. Five days after infection when the input virus was heat-inactivated, cells were washed again and mixed in serial dilutions with HEL indicator cells. Cells were seeded in Petri dishes and when attached overlaid with 0·3% agarose in BME and FCS. Cultures were monitored for the appearance of c.p.e. every second day and were re-fed weekly. Fourteen to 16 days after cultivation the cells were fixed with 3·5% formaldehyde and stained with 0·1% crystal violet. The method was shown to yield approximately 97% infectious centres on isogenous cells.

Bone marrow cells. Primary human BMC were isolated from sternal punch biopsies. The heparinized bone marrow biopsies were layered onto metrizoate/Ficoll gradients (Lymphoprep; Nyegaard, Oslo, Norway) to remove contaminating erythrocytes and centrifuged for 30 min at 400 *g*. Cells in the supernatant were isolated and washed twice and subsequently cultivated in a medium for long-term maintenance of BMC modified from Gartner & Kaplan (1980) at a density of 5×10^5 cells per ml. This medium consisted of RPMI 1640 supplemented with 12·5% heat-inactivated FCS, 12·5% heat-inactivated horse serum, 1 mM-L-glutamine, 10 μg/ml β-alanine, 100 IU/ml penicillin G and 100 μg/ml streptomycin. Cells were re-fed weekly by removal of the supernatant, centrifugation of the supernatant cells and readdition of these cells suspended in fresh growth medium to the adherent layer. The outgrowth of adherent cells was observed after 1 week of culture. To separate human bone marrow stem cells from differentiated cells and fibroblasts, a combination of plastic adherence and indirect rosetting with the monoclonal antibody OKT10 was used. OKT10 has earlier been shown to react with early human haematopoietic stem cells (Janossy *et al.*, 1981; Greaves, 1981; Crawford *et al.*, 1981). BMC purified as described above were first cultivated in growth medium for 7 days in plastic flasks (Falcon) to allow fibroblasts to attach to the solid phase. The remaining supernatant cells were then labelled with OKT10 and OKT10-positive cells were separated by indirect rosetting with goat anti-mouse IgG-coupled erythrocytes as previously described (Braun *et al.*, 1984). $OKT10^+$ cells and $OKT10^-$ cells were further cultivated in growth medium for 10 days and subsequently infected with HCMV AD169 at an m.o.i. of 2 p.f.u./cell. Virus titrations were performed using the supernatants of the cultures.

Antibodies and immunofluorescence techniques. As antibodies against HCMV late antigens a high titre ($\geqslant 1000$) human convalescent serum was used. Human serum was preabsorbed to non-infected HEL cells in order to avoid non-specific reactions with human BMC. The specificity of antibodies was proved by using mock-infected HEL cells, mock-infected BMC, and on HEL cells infected with HCMV at an m.o.i. of 0·1 p.f.u./cell for 48 h. OKT10 was used as an antibody specifically directed against bone marrow stem cells.

Indirect immunofluorescence on HCMV-infected BMC was performed as described previously (Schober *et al.*, 1984). In brief, acetone-fixed BMC were labelled with the respective antibody at 4 °C in 0·1 M-phosphate-buffered saline and 1% FCS to cover possible Fc-receptors. Subsequently, cells were thoroughly washed and incubated with a fluorescein isothiocyanate (FITC)-coupled goat anti-human IgG $F(ab')_2$ fragment (Medac, Hamburg, F.R.G.). Mock-infected BMC and HCMV-infected HEL cells served as controls for specificity.

SDS-polyacrylamide gel electrophoresis. SDS-PAGE was performed essentially as described by Thomas & Kornberg (1975). Approximately 3×10^5 mock-infected and HCMV-infected BMC and HEL cells (m.o.i. 3 p.f.u./cell) were washed three times in Tris-buffered saline (TBS; 0·05 M-Tris-HCl pH 7·4, 0·15 M-NaCl) and were boiled for 3 min in sample buffer (0·0625 M-Tris-HCl pH 6·8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0·001% bromophenol blue). 10% polyacrylamide gels were run as 0·75 mm slab gels. Internal molecular weight

standards were carbonic anhydrase (mol. wt. 29000), ovalbumin (mol. wt. 45000), albumin (mol. wt. 66000) and phosphorylase B (mol. wt. 97400), all purchased from New England Nuclear.

After fixation in 10% TCA gels were silver-stained according to Switzer *et al.* (1979) and Oakley *et al.* (1980).

Dot blot hybridization. Dot blot hybridizations were performed in a modified version of a procedure previously described (Brandsma & Miller, 1980; McDonough & Spector, 1983; Spector *et al.*, 1984). The DNA of approx. 10^6 HCMV-infected BMC was spotted on nitrocellulose filters pre-activated in 20 × SSC (SSC is 0·15 M-NaCl, 0·015 M-sodium citrate). Filters were subsequently soaked in 0·1 M-HCl for 2 min, then for a further two 15 min periods in 0·5 M-NaOH, neutralized twice in 1 M-TBS pH 7·4 (1 M-Tris–HCl pH 6·8, 0·6 M-NaCl) for 2 min, once in 0·5 M-Tris–HCl pH 7·4 and 1·2 M-NaCl for 7 min, and baked for 3 h at 80 °C in a vacuum oven. Prehybridization was for 3 h in 6 × SSC, 0·5% SSC, 0·5% SDS, 5 × Denhardt's solution and 100 μg/ml herring sperm DNA at 68 °C. Hybridization was under the same conditions with 0·002 M-EDTA and 80 ng/ml of ^{32}P-labelled nick-translated cloned HCMV DNA (sp. act. 10^8 c.p.m/μg). HCMV clones having no cross-hybridizing activity to the cellular genome (Rüger *et al.*, 1984) were kindly obtained from Drs B. Fleckenstein and R. Rüger, Institut für Klinische Virologie, Universität Erlangen, F.R.G. Cosmids used as probes in equimolar amounts were pCM3, pCM52, pCM104, pCM5018, pCM5009, pCM5004, pCM5003, pCM5002 and pRR3. Washing was for four 5 min periods in 2 × SSC, 0·1% SDS at room temperature and then for 3 h in 0·2 × SSC, 0·1% SDS at 68 °C. Subsequently filters were dried and exposed to Kodak XAR-5 films. Following autoradiography the single dots were cut out and radioactivity was counted in a liquid scintillation spectrophotometer.

RESULTS

When human BMC were cultivated under the conditions described above, after 3 to 5 days the outgrowth of fibroblasts and subsequently the formation of cobblestone areas of haematopoiesis was observed as described earlier by Gartner & Kaplan (1980). A typical picture of such a culture is given in Fig. 1 (*b*), in which different bone marrow stem cells and among these single fibroblast-like cells are shown. Primary BMC without any precultivation, however, did not show any adherent cells (Fig. 1 *a*). To prove in general the capacity of such primary human BMC cultures to replicate HCMV, freshly isolated BMC and BMC cultivated for 3 weeks, when cobblestone areas of haematopoiesis became visible, were infected with HCMV AD169 at an m.o.i. of 2 and were monitored for the appearance of a c.p.e. and virus production. As shown in Fig. 1 (*c*) typical c.p.e. with inclusion bodies developed in precultivated BMC from day 8 after infection and progeny virus appeared in supernatants from day 10 (Fig. 2). Virus titres in precultivated BMC were only 10% of titres obtained in HEL cells and in general did not exceed 2×10^5 p.f.u./ml. Furthermore, virus replication in BMC showed a delay of approx. 8 days as compared to that in HEL cells (Fig. 2). Freshly isolated BMC, however, did not show any virus replication upon infection with HCMV AD169. Thus, it appeared possible that the virus replication observed was not due to replication of HCMV AD169 in these cells, but might reflect the activation of latent virus from the bone marrow cells. This, however, seemed unlikely for two reasons. First, mock-infected control BMC did not show any virus replication (Fig. 2), and second the BMC we used were obtained from children under 5 years of age, who were HCMV-seronegative by ELISA (data not shown).

In order to determine whether the observed delay in the increase of virus titres in BMC was indeed due to a delayed infectious cycle of HCMV, we compared viral protein and DNA synthesis in infected BMC and HEL cells. Results of these studies are presented in Fig. 3. From SDS–PAGE analysis it was evident that 8 days after infection HEL cells express HCMV late proteins with relative molecular weights of 132K, 120K, 68K, 52K and 31K. These proteins probably correspond to the proteins gp132, gp120, vp68, gp55 and vp32 as described by Stinski (1977). From the relative intensity of the bands it is likely that the 68K protein represents the major capsid protein of HCMV, whereas the 52K protein might represent the major virion glycoprotein (Farrar & Oram, 1984; Nowak *et al.*, 1984). In BMC cells, however, expression of these proteins was not observed before 16 days after infection, whereas 8 days after infection a 72K protein was identified which might correspond to an early protein of HCMV (Stinski *et al.*, 1981). Corresponding results were obtained when viral DNA synthesis was monitored by a dot blot hybridization assay with cloned HCMV DNA fragments showing no cross-hybridizing activity to the cellular genome. Whereas HEL cells synthesized large amounts of viral DNA

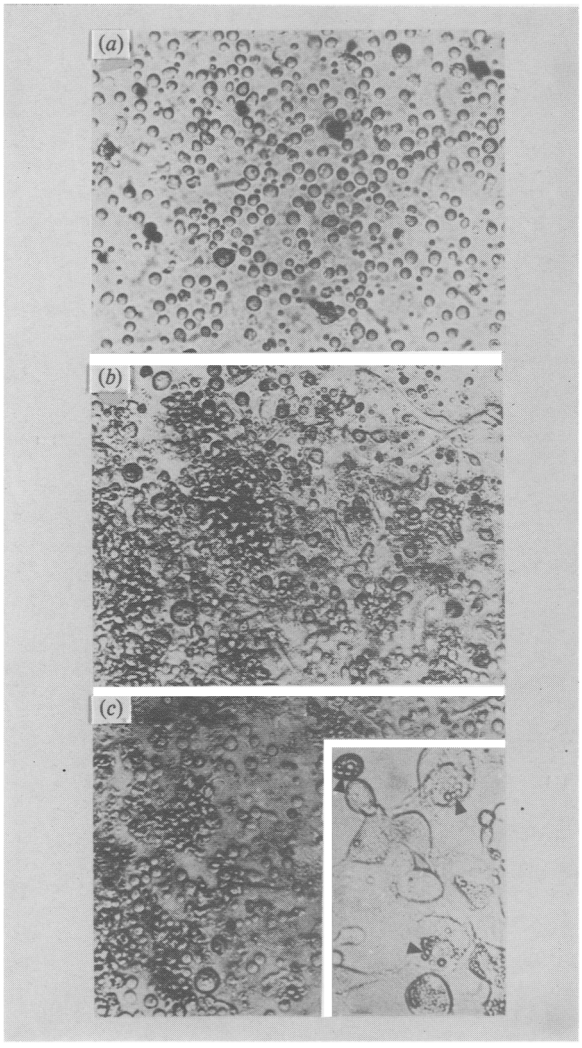

Fig. 1

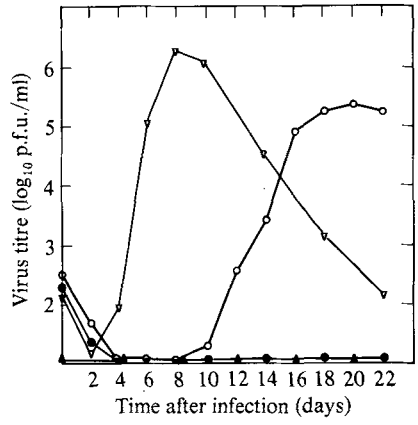

Fig. 2

Fig. 1. Microscopic image of primary human BMC and BMC infected with HCMV AD169. (*a*) Freshly isolated human BMC (× 320). (*b*) Human BMC after 3 weeks of cultivation (× 320). (*c*) Precultivated bone marrow cells 10 days after infection with HCMV AD169 (× 320). Inclusion bodies (inset) are marked by an arrowhead.

Fig. 2. Replication of HCMV AD169 in freshly isolated BMC, pre-cultivated BMC and in HEL cells. ●, Replication of HCMV AD169 in freshly isolated BMC. ○, Replication of HCMV AD169 in BMC pre-cultivated for 3 weeks. ▲, Results of virus titrations from supernatant of mock-infected pre-cultivated BMC. △, Replication of HCMV AD169 in HEL cells.

8 days after infection, this level was not observed in BMC until day 12 (Fig. 3*b*). Virtually no background hybridization was observed in these experiments when mock-infected HEL or BMC were tested. The relatively strong hybridization signal obtained in HCMV-infected HEL and BMC immediately after infection (zero time point) may thus indicate the presence of input virus. Taken together these data indicate that HCMV in BMC replicates more slowly than in HEL cells.

As described above, virus titres in BMC in general were lower than in HEL cells. This might indicate either a lower production of progeny virus per cell by BMC or the productive infection

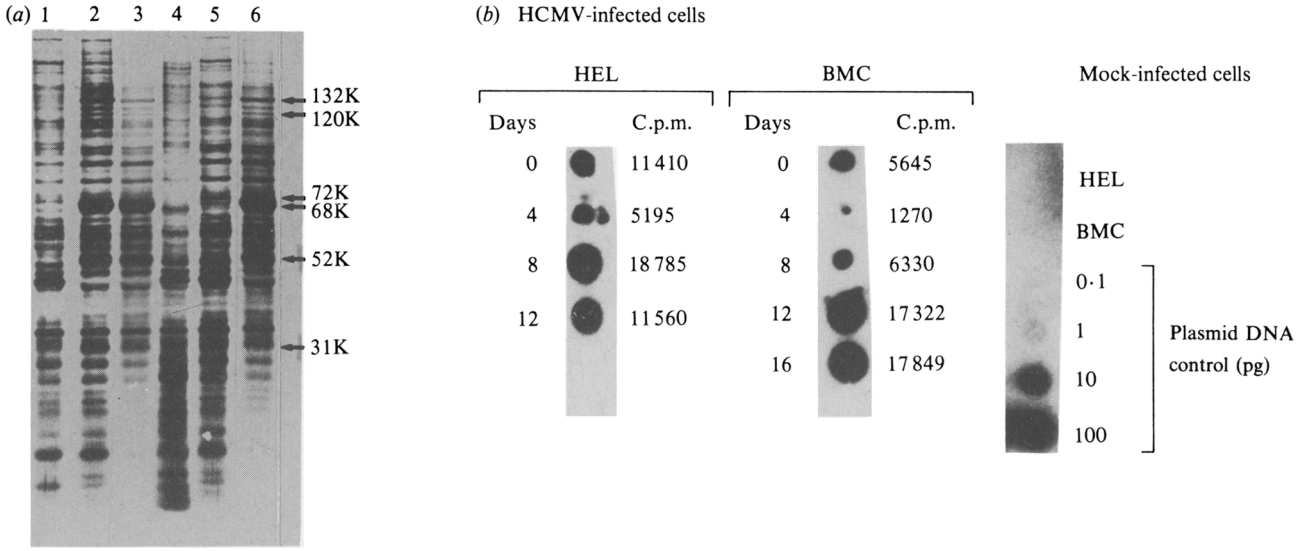

Fig. 3. Viral protein and DNA synthesis in pre-cultivated BMC and HEL cells. (*a*) SDS–PAGE analysis of proteins of HEL and BMC cells at different times after infection. Lane 1, mock-infected HEL cells; lane 2, HEL cells 8 days after infection with HCMV AD169; lane 3, HEL cells 16 days after infection with HCMV AD169; lane 4, mock-infected BMC; lane 5, BMC 8 days after infection with HCMV AD169; lane 6, BMC 16 days after infection with HCMV AD169. Major viral proteins and their relative mol. wt. are given at the right hand side of the figure. (*b*) Dot blot hybridization of DNA of HCMV-infected HEL and BMC cells with ^{32}P-labelled cloned DNA probes. Times at the left hand side indicate days after infection; on the right hand side c.p.m. of single dots as determined in a liquid scintillation counter are indicated.

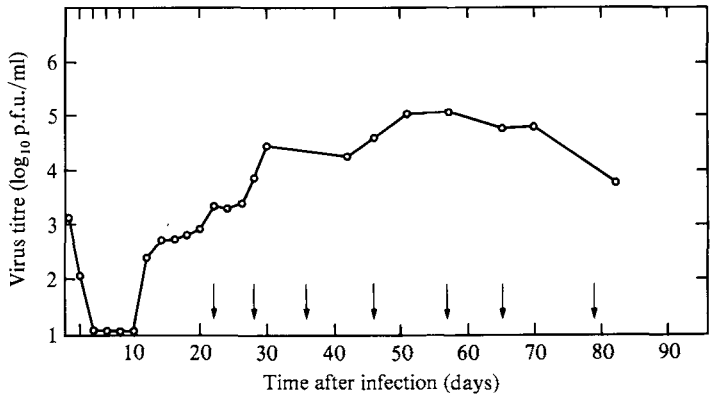

Fig. 4. Continuous production of infectious progeny virus by HCMV-infected BMC. Arrows indicate time points of subcultivation of the infected cells.

Table 1. *Results of infectious centre assays and indirect immunofluorescence tests on HCMVAD169-infected bone marrow cells*

Cell source	Purity	Infectious centres (%)† (range)	HCMV-antigen positive cells (% immunofluorescent)† 8 days p.i. (range)	16 days p.i. (range)
HEL	ND*	97 (95–98·5)	96 (94–98)	95 (94–98)
BMC (freshly isolated)	ND	⩽0·2 (⩽0·2)	⩽0·5 (⩽0·5)	⩽0·5 (⩽0·5)
BMC (3 weeks pre-cultivated)	ND	21 (16–27)	4 (2–8)	20 (18–26)
Bone marrow fibroblasts	1% OKT10$^+$	78 (75–83)	ND	84 (77–90)
OKT10$^+$ cells	95% OKT10$^+$	24 (17–29)	ND	45 (31–56)
OKT10$^-$ cells	2% OKT10$^+$	⩽0·2 (⩽0·2)	ND	⩽0·5 (⩽0·5)
OKT3$^+$ cells	95% OKT3$^+$	⩽0·2 (⩽0·2)	ND	⩽0·5 (⩽0·5)
(Peripheral blood T cells)	2% OKT10$^+$			

* ND, Not determined.
† Data summarize the results of three experiments.

of only a subset of BMC by HCMV. Thus in order to determine virus production by BMC and HEL cells, infectious centre assays and indirect immunofluorescence experiments on HCMV-infected BMC and HEL cells were performed. As presented in Table 1, infectious progeny virus was produced by more than 95% of HEL cells, but by only 21% of BMC. This result was further confirmed by indirect immunofluorescence experiments, in which at 8 days post-infection 96% of HEL cells showed a positive reaction with an HCMV-positive human convalescent serum, but only 4% of BMC. Sixteen days after infection, 95% of HEL cells were immunofluorescence-positive, but only 20% of BMC.

The fact that the replication of HCMV in BMC is restricted to a cell subset not exceeding 20% of the cells is in accordance with the finding that the replication of HCMV is slower in BMC than in HEL cells. It may also underline the persistence of HCMV infection in BMC which was observed over a period of 3 months (Fig. 4). This persistence was characterized by the continuous production of infectious progeny virus over the whole time, at titres of approximately 10^5 p.f.u./ml.

The observation that only a subset of BMC actively produced infectious progeny virus raised the question as to the nature of this subset. As described above, BMC cultures consisted of a variety of cell types. Among these are bone marrow stem cells, bone marrow fibroblasts and cellular elements of the peripheral blood. Thus, in an attempt to identify the HCMV-susceptible

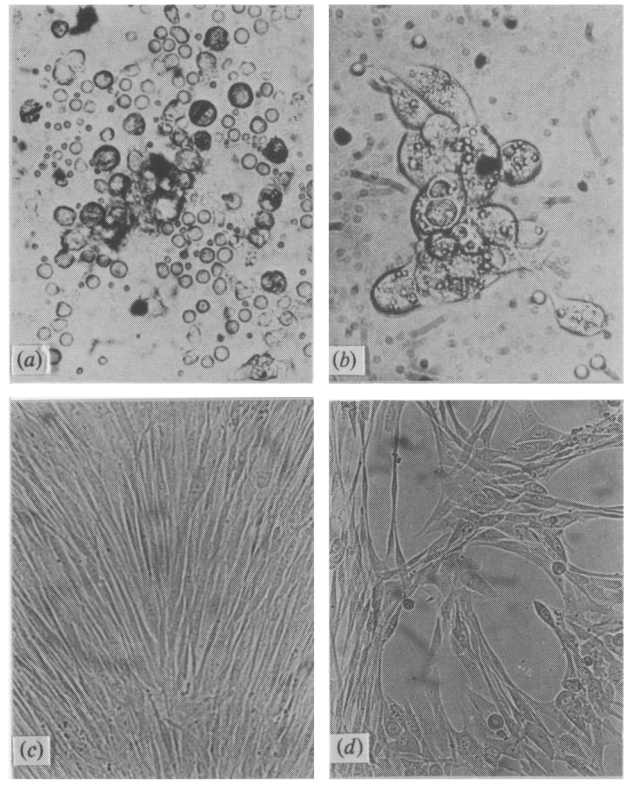

Fig. 5. Micrographs of preparatively separated bone marrow fibroblasts and OKT10$^+$ cells before and after infection with HCMV AD169. (*a*) Primary OKT10$^+$ BMC (× 250). (*b*) OKT10$^+$ BMC 10 days after infection with HCMV AD169 (× 480). (*c*) Primary human bone marrow fibroblasts (× 250). (*d*) Bone marrow fibroblasts 10 days after infection with HCMV AD169 (× 250).

cell subsets among these different cell populations, we separated these cellular subpopulations by a preparative technique combining the use of plastic adherence and indirect rosetting with the monoclonal antibody OKT10. This antibody was earlier described to recognize human bone marrow stem cells (Crawford *et al.*, 1981; Greaves, 1981). As a result of these experiments, we obtained three different cell cultures consisting of relatively pure bone marrow fibroblasts (Fig. 5*c*), OKT10$^+$ bone marrow stem cells (Fig. 5*a*) and OKT10$^-$ cells (not shown) as determined by cell morphology and indirect immunofluorescence (Table 1). Upon separate infection of these cell populations with HCMV AD169 a c.p.e. was observed in bone marrow fibroblasts and OKT10$^+$ cell cultures (Fig. 5*b*, *d*), but not in OKT10$^-$ cells. Consistent data were obtained when virus production was determined by plaque assay as shown in Fig. 6. Accordingly, infectious progeny virus was produced in bone marrow fibroblast and OKT10$^+$ cell cultures, the latter, however, producing lower amounts of virus. Again, OKT10$^-$ cells did not produce any measurable amounts of progeny virus. Replication of HCMV in fibroblasts and OKT10$^+$ cells appeared to be delayed in comparison to HEL cells. This was also reflected by immunofluorescence data and by the fact that in infectious centre assays bone marrow fibroblasts showed 78% positive cells, whereas OKT10$^+$ cells showed 24% infectious centres and OKT10$^-$ cells were virtually negative (Table 1). To further confirm the true stem cell nature of the OKT10$^+$ cells, we have added a further experiment in which we infected OKT10$^-$ and OKT3$^+$ peripheral blood T cells with HCMV. As had been expected, these cells did not produce any infectious progeny virus (Table 1) and were negative in immunofluorescence tests for HCMV proteins.

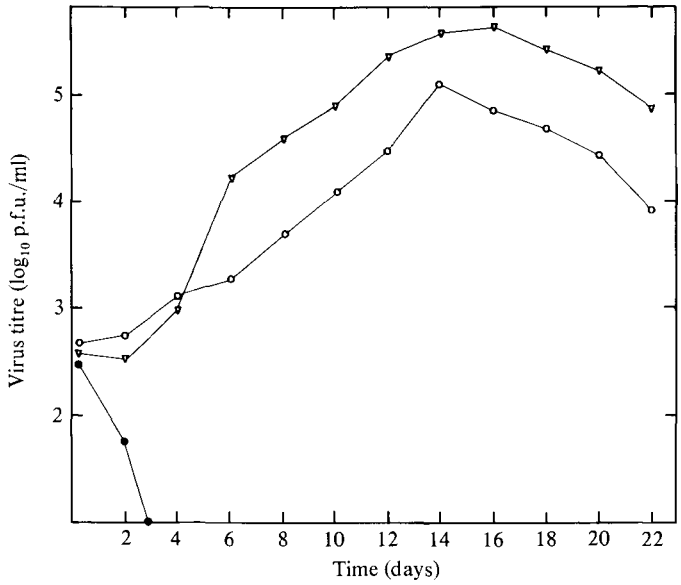

Fig. 6. Replication of HCMV AD169 in bone marrow cell subpopulations: △, in bone marrow fibroblasts; ○, in OKT10$^+$ cells; ●, in OKT10$^-$ cells.

DISCUSSION

The lymphotropic properties of HCMV have been recognized for a long time. It has, however, not been possible to demonstrate replication of HCMV in primary human leukocytes or any of their stem cells. The recent results of Einhorn & Öst (1984) show expression of HCMV early antigen in freshly isolated BMC and BMC held in culture for 6 days, but do not demonstrate any appreciable virus replication. In line with these results, we too could not demonstrate any replication of HCMV in freshly isolated BMC, but found appreciable virus replication with typical c.p.e. in BMC pre-cultivated for 3 weeks. This finding may indicate that in order to obtain virus replication in these cells a certain cellular differentiation step may be necessary, as recently described by Gönczöl *et al.* (1984) for the replication of HCMV in human embryonal carcinoma cells and by Daniels *et al.* (1978) for the replication of herpes simplex virus in human macrophages. Assuming the BMC to be a possible site of viral latency, it seemed possible that the observed titre rises were due to reactivation of latent virus by prolonged cultivation of BMC. This, however, was unlikely since we did not observe any titre rises in mock-infected BMC cultures and since the bone marrow donors were HCMV seronegative by ELISA. Furthermore, a typical HCMV c.p.e. with inclusion bodies was observed in the infected BMC.

It was observed that BMC in comparison to HEL cells produced lower amounts of infectious virus and showed some delay in the infectious cycle. This finding may be due to the fact that infection of BMC with HCMV involved primarily only a few susceptible cells producing virus and that during the time of the infectious cycle more and more cells reach a differentiation stage at which they become permissive for infection by the progeny virus from initially infected cells. The possibility of such a virus–cell interaction was also supported by the low percentage of infectious centres found in BMC and would result in a balance between outgrowing cells and virus-producing cells which may also explain our finding of a short-term persistence of the virus in such BMC cultures. A similar delay in virus replication and the establishment of a short-term persistence has been recently described for the replication of HCMV in human smooth muscle cells (Tumilowicz *et al.*, 1985).

The low percentage of virus-producing cells in BMC cultures, however, made it important to determine the nature of the viral target cell in BMC. By experiments with preparatively separated BMC subpopulations it was shown that HCMV replicated well in the fibroblast and

$OKT10^+$ cells, but not in the $OKT10^-$ cell fraction. This was documented by the demonstration of c.p.e., titre rises, infectious centre assays and indirect immunofluorescence. There were, however, contradictory results between these assays; for instance 24% of $OKT10^+$ cells were positive by infectious centre assay, but 45% were positive for immunofluorescence using an antiserum to HCMV late antigen. This might be explained if either some of the $OKT10^+$ cells and also of the bone marrow fibroblasts had non-specifically adsorbed viral particles to their surface, or if under infectious centre assay conditions they did not produce infectious particles. A further difference was observed in the percentage of infectious centres formed by unseparated BMC (21%), and formed by bone marrow fibroblasts (78%) and $OKT10^+$ cells (24%). In this context it should be noted that $OKT10^-$ cells were virtually negative in infectious centre assays, and thus the percentage of infectious centres formed in BMC results from the mixture of these three different cell populations. From the finding that peripheral blood T cells under these conditions do not show any appreciable virus replication (Table 1) but produce limited amounts of progeny virus after stimulation with interleukin-2 (Braun & Reiser, 1986), it might be concluded that a differentiation step to a more mature form of mononuclear cells is necessary to overcome the intrinsic resistance of such cells to HCMV infection. It can not be ruled out that there also exists a cell population abortively infected with HCMV. In this context, Rice *et al.* (1984) have shown that replication of HCMV in primary human lymphocytes and monocytes is restricted to the expression of immediate early gene products and that wild-type isolates of HCMV have a higher capacity to express such gene products than laboratory strains. Though we did not prove the ability of wild-type strains to replicate in BMC, it is likely that such strains may show replication characteristics different from the AD169 strain which has been adapted to HEL or HFF cells over many years.

In conclusion, we have shown that HCMV may replicate in primary human BMC under certain conditions and that this replication involves both bone marrow fibroblasts and $OKT10^+$ stem cells.

The authors appreciate the excellent technical assistance of I. Helmreich and M. Treiber and the expert editorial work of M. Kasamasch. This work was supported by the Deutsche Forschungsgemeinschaft, grant no. II B6-Br839/11.

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(Received 25 March 1986)