PATHOGENESIS OF CYTOMEGALOVIRUS INFECTION

I. Activation of Virus from Bone Marrow-Derived Lymphocytes by In Vitro Allogenic Reaction*

By LARS B. OLDING, FRED C. JENSEN, and MICHAEL B. A. OLDSTONE

(From the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037)

One of the most challenging problems in medicine concerns latent virus infections, in particular the specific identification of tissues that harbor these viruses, the ways viruses persist, and the mechanism(s) by which such agents are activated. Cytomegalovirus (CMV),[1] like other herpes viruses, can persist within an organism in a latent form despite the presence of a vigorous antiviral immune response (reviewed in references 1 and 2). Several epidemiologic surveys show that 40–80% of humans over the age of 40 have serologic evidence of prior CMV infection (1–3) and less than 1% of seropositive healthy individuals shed virus into their urine (4). CMV infection would be regarded as an ordinary acute infection associated with numerous subclinical attacks, complete recovery, and eradication of the virus except that reappearance of this virus is common. For example, pregnancy is often associated with active CMV infection (5), and this virus has been recovered from up to 28% of pregnant women (6, 7). Acute CMV infection occurs in nearly 90% of patients 1–2 mo after surgery for kidney transplantation (8). CMV has been implicated as the cause of a mononucleosis-like illness occurring in patients after open heart surgery, and available evidence suggests that this infection is apparently transmitted by blood transfusions (9–13). Both terminal leukemia and Hodgkin's disease in patients undergoing immunotherapy may also be associated with CMV infection (14). The frequent occurrence of CMV infection in patients undergoing pregnancy, renal transplantation, and blood transfusions suggests that this virus may be activated by means of immunologic reaction to foreign antigens.

Host-specific CMVs have been demonstrated in several species including guinea pig, rat, hamster, mouse, and man. The primary CMV infection in mice (MCMV) is very similar to that in man (15–16). Using the murine model, we studied whether MCMV was carried in lymphocytes taken from adult mice

*This is publication no. 898 from Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California 92037. This research was supported by a grant from The National Foundation-March of Dimes, no. I-364, and in part by U. S. Public Health Service grant AI-07007 and Expressen's Prenatal Research Fund (Stockholm, Sweden).

[1] *Abbreviations used in this paper:* Con A, concanavalin A; CMV, cytomegalovirus; CPE, cytopathic effects; FCS, fetal calf serum; FFU, focus-forming units; FITC, fluorescein isothiocyanate; LPS, lipopolysaccharide; MCMV, mouse CMV; MEC, mouse embryo cells; MuLV, murine leukemia virus; PBS, phosphate-buffered saline; PHA-P, phytohemagglutinin-P; PWM, pokeweed mitogen.

infected with MCMV in utero or at birth and whether virus could be activated in vitro by co-cultivation with antigenically foreign tissues.

Materials and Methods

Animals

Pregnant 2- to 3-mo old C3H/HeJ and SWR/J mice were obtained from Jackson Laboratories, Bar Harbor, Maine, and pregnant 2- to 3-mo old C3H/St, BALB/c, and Ha/ICR mice from the L. C. Strong Research Foundation, San Diego, Calif. Nude mice were originally obtained from Jacques Miller, Walter and Eliza Hall Research Institute, Melbourne, Australia. These mice have been inbred in our institute since 1971. The major *H-2* alleles of the mice are: C3H/HeJ and C3H/St mice, $H\text{-}2^{kk}$; BALB/c mice, $H\text{-}2^{dd}$; SWR/J mice, $H\text{-}2^{qq}$; nude mice off BALB/c, $H\text{-}2^{dd}$; nude mice off CBA, $H\text{-}2^{kk}$. The Ha/ICR mice are outbred.

Pregnant mice were inoculated intraperitoneally (i.p.) 3–5 days before delivery with a 1,000 tissue culture infective dose ($TCID_{50}$) of MCMV and nursed their own babies. Newborns were inoculated i.p. with 100 $TCID_{50}$ of the virus within 24 h of birth; C3H/St and Ha/ICR mice were nursed by their own mothers, C3H/HeJ and nude mice by Ha/ICR foster mothers. C3H/HeJ and C3H/St mice were sacrified at 2–3 mo, Ha/ICR mice at 5 mo, and nude mice at 2–4 wk of age.

Virus

MCMV was originally provided by Dr. Richard I. Carp, Department of Microbiology, New York State Institute for Research in Mental Retardation, Staten Island, N. Y., and a virus stock was maintained in our laboratory by passages on BALB/c or Ha/ICR mouse embryo cells (MEC), grown in minimal essential medium (MEM) with 10% fetal calf serum (FCS), glutamine, and antibiotics (growth media). Infected MEC were disrupted by sonication and mixed with the infected supernate. This pool was fortified with 10% sorbitol and frozen at −70°C in aliquots. Stock virus when diluted to 10^{-7}/0.2 ml inoculum caused cytopathology in confluent monolayers of BALB/c MEC.

Antibodies to MCMV

The presence of antibodies to MCMV was determined by an immunofluorescence technique with infected cells as a target and serum collected from axillary artery blood or plasma obtained from the orbital venous plexus. Monolayer cultures of BALB/c MEC were grown on cover slips and infected with MCMV. When such cultures showed cytopathic effects (CPE), the cover slips were washed in phosphate-buffered saline (PBS) and fixed in absolute ether plus 95% ethanol (1:1) for 10 min and in 95% ethanol for 20 min. The fixed cells were washed in PBS, incubated initially for 30 min with sera or plasma from the infected animals, washed in PBS, and then stained with rabbit antibody to mouse immunoglobulin G (IgG) conjugated to fluorescein isothiocyanate (FITC). Techniques for staining, raising specific antisera, conjugating to FITC, and testing immunologic specificities of the reagents have been published (17, 18). Only cultured cells infected with MCMV showed characteristic staining of intranuclear viral inclusions. An identical pattern was seen in cells infected with MCMV and stained with a monospecific rabbit antibody to MCMV conjugated with FITC, kindly provided by Dr. Berge Hampar, National Cancer Institute, Bethesda, Md. Infected cells treated with uninfected mouse serum or plasma and fluoresceinated antibody to murine IgG did not stain.

Lymphoid Cell Preparations

Immediately after exsanguination, the animals' thymuses and spleens were removed and single cell suspensions prepared by gently mincing with sharp scalpels. Tissue fragments and cell aggregates were removed by sedimentation at 1 *g* for 30 min at 4°C and/or by passage through a nylon mesh. Viability was determined by the trypan blue dye exclusion test.

To further purify lymphocytes, Ficoll-Hypaque gradient centrifugation was used (19). Macrophages were removed by suspending gradient purified cells in MEM with 5% heat-inactivated FCS, through a nylon wool column (Fenwall Laboratories, Morton Grove, Ill.). In a few experiments, macrophages were removed by repeated passages on glass surfaces. A neutral red dye test (dye

concentration of 1/6,000) showed 0.5% or less macrophage contamination. Thymus-derived (T) cells were purified by removing bone marrow-derived (B) cells with a nylon wool column (20), or by using a rabbit antibody to B cells with a complement (C) source (21). This antisera was kindly provided by Dr. Robert Blanden, Department of Microbiology, The John Curtin School of Medical Research, Canberra City, Australia. B-lymphoid cells were purified by killing T cells with anti-θ serum and C. The methodology for preparation and use of these various antisera and their specificities have been reported (21, 22). Briefly, anti-B sera inhibited the formation of antibody by plaque-forming cells but did not inhibit T-cell-dependent cytotoxicity, whereas anti-θ sera did not abrogate the formation of antibody by plaque-forming cells but did abolish T-cell killing in the assay of Brunner et al. (23). Further purity of each lymphoid population was judged by staining with FITC-conjugated antibody to θ-antigen. In none of the B-cell populations were cells with surface θ-antigen seen.

Spleen cells were disrupted by treatment for 15 s $\times$ 4 with 1 min rests at 1.3–1.5 A in a Raytheon model DF101 Sonic oscillator (Raytheon Data Systems Co., Norwood, Mass.). Cell disruption was confirmed microscopically.

Co-Cultivation Technique

Suspensions of either 1–5 $\times$ 10^6 lymphoid cells from whole spleens or purified B or T lymphocytes from infected animals were mixed with 1–2 $\times$ 10^5 MEC and planted in 60 $\times$ 15-mm plastic petri dishes. The primary MEC were prepared from histoincompatible embryos. Controls consisted of lymphocytes mixed with 1–5 $\times$ 10^5 MEC from isologous donors. In some experiments, suspensions of spleen or thymus cells from infected mice were cultured alone since such cells provide their own layer of fibroblasts. All cultures were run at least in triplicate and were nourished with growth media initially and every week thereafter for 4–7 wk. Nonadhering lymphoid cells were kept in the cultures by carefully collecting the supernatant fluid from the cultures, pelleting the cells by centrifugation at 400 *g* for 10 min, resuspending these cells in fresh growth media, and putting them back on the feeder layer. Incubation was at 37°C in 5% CO_2. Cultures were checked at least every other day for evidence of CPE.

Mitogenic Stimulation

Suspensions of 5 $\times$ 10^6 lymphocytes from the spleens of 6- to 8-wk old C_3H/St mice infected at birth with MCMV and mixed with 5 $\times$ 10^5 isologous MEC were cultured with various mitogens: lipopolysaccharide (LPS) (kindly provided by Dr. David C. Morrison, Scripps Clinic and Research Foundation, La Jolla, Calif., and prepared as described elsewhere [24]), 10 μg/ml medium; pokeweed mitogen (PWM) (Grand Island Biological Co., Grand Island, N. Y.), 50 μg/ml medium; concanavalin A (Con A) (Miles-Yeda Ltd., Rehovet, Israel, crystallized three times), 1, 5, and 10 μg/ml medium; and phytohemagglutinin-P (PHA-P) (Difco Laboratories, Detroit, Mich.), 50 μg/ml. The dosages selected for the various mitogens had given maximal stimulation as determined by earlier experiments with murine lymphocytes. An enriched medium was employed as described (25) except that Earle's balanced salt solution was used and nucleic acid precursors omitted. All cultures were incubated with 5% CO_2 in air at 37°C. The ability of various mitogens to initiate blast transformation was observed in concurrent cultures containing 5 $\times$ 10^6 lymphocytes (in 2 ml of growth media) from spleens of mice infected with MCMV. After 3 days of culture, 1.0 μCi of [*methyl*-^{3}H]thymidine (spe act 6.7 Ci/mmol, New England Nuclear Corp., Boston, Mass.) was added to each culture. 4 h later the cultures were placed in an ice bath; cells were washed three times in cold PBS, resuspended in bovine serum albumin (1 mg/ml) and precipitated with 20% TCA. The precipitates were collected on Whatman GF/A filters, dried, suspended in Aquasol (New England Nuclear Corp.) and counted in a Beckman LS-230 liquid scintillation counter (Beckman Instrument, Inc., Fullerton, Calif.). 5 $\times$ 10^5 lymphocytes from infected and uninfected mice cultured without mitogens were handled in an identical manner and served as controls.

Identification of MCMV

Immunofluorescence. Cells from cultures with CPE were fixed and stained on cover slips as described above by using a monospecific rabbit antibody to MCMV conjugated with FITC which had been previously absorbed with normal MEC.

Neutralization Test. Supernate and cells from cultures showing CPE were collected, sonicated, and layered in varying dilutions on monolayers prepared from BALB/c MEC in order to determine the number of focus-forming units (FFU) present. A volume of media containing 25–50 FFU of the virus was mixed with an equal volume of MEM containing serial twofold dilutions of monospecific rabbit antibody (heated at 56°C for 30 min) to MCMV and incubated at 37°C for 30 min. The mixture was absorbed on confluent monolayer cultures of BALB/c MEC for 60 min at 37°C, then removed, and the monolayer was overlaid with agar containing 2% FCS. Controls consisted of cultures incubated with material showing CPE but not treated with antiserum, as well as cultures incubated with antiserum alone. Quadruplicate cultures were used throughout. Cultures were again overlaid with agar on the 5th day and examined for CPE on the 10th day when the serum dilution showing a 50% reduction of CPE was determined.

Histologic and Electron Microscopic Studies. Cultured cells on cover slips were fixed in absolute methanol and stained by May-Grünewald and Giemsa stains. For electron microscopy, pellets of infected cells initially suspended in 0.025% trypsin and washed in FCS were fixed in 2.5% glutaraldehyde and examined by Professor Peter Lampert, Department of Pathology, University of California, Medical School, San Diego, Calif.

Results

Clinical State of Mice Infected with MCMV. Over 75% of mice delivered from mothers infected during pregnancy survived, yet only 50% of C3H/St and Ha/ICR mice and 10% of C3H/HeJ and nude mice infected at birth survived the 1st mo of life. Infectious virus was not found in the plasma from weanling or older C3H/St, C3H/HeJ, or Ha/ICR mice. In contrast, antibodies to MCMV were found in all the C3H/St and C3H/HeJ, and over 75% of Ha/ICR mice tested.

Recovery of MCMV from Lymphoid Cells of the Spleen. When suspensions of spleen cells from C3H/HeJ or Ha/ICR mice were co-cultivated with isologous MEC, no CPE appeared during 6–8 wk of culture. In contrast, when the spleen cells were co-cultured with allogenic MEC, CPE usually appeared during the 2nd to 3rd wk (Table I). Sometimes, CPE spread rapidly; within 3–4 days the changes had progressed from occasional megalic fibroblasts to involvement of virtually all of the cells (Fig. 1 A–C). On occasion, only one or two of the triplicate or quadruplicate cultures became infected during the observation period. At times, cultures had to be fed with fresh MEC to replace those fibroblasts destroyed by the lymphoid cells before CPE began. When lymphoid cells were sonicated before being cultured with isologous MEC, no infectious virus was released.

Lymphocyte Subclass Harboring Latent MCMV. Co-cultures of purified preparations of T-lymphoid cells from the thymus or the spleens of neonatally infected 3-mo old mice with allogenic MEC yielded no CPE over a period of 7 wk observation (Table II). In contrast, from the same lymphoid pools and under similar conditions MCMV was easily recovered from B lymphoid cell populations with CPE appearing by the 3rd wk. CPE did not occur when B-cell preparations were co-cultured with isologous MEC.

To ensure that MCMV could grow in B lymphocytes, we studied the effects of this virus in nude mice. Such mice have a congenital absence of thymus (T-less) and represent a convenient animal model for dissecting T-cell, B-cell function. Nude mice infected at birth with MCMV generally die within 3 wk of systemic MCMV disease. We took spleen cells from such infected mice 2 wk after instituting infection, and removed macrophages (passage on glass adhering surface or nylon wool) and theta-bearing cell contamination by treatment with

TABLE I

Recovery of MCMV from Spleen Lymphoid Cells of Adult Mice Which Had Been Infected In Utero or at Birth

Spleen cell donor				MCMV recovery* after co-culture with embryo cells‡ from:			
Strain	No. of mice (pool)	MCMV injection§	Age	C3H/HeJ ($H\text{-}2^{kk}$)‖	BALB/c ($H\text{-}2^{dd}$)‖	SWR/J ($H\text{-}2^{qq}$)‖	Ha/ICR
			mo				
C3H/HeJ	3	In utero	2	0/4¶	4/4	4/4	ND
C3H/HeJ	3	At birth	2	0/4	4/4	3/4	ND
Ha/ICR	3	In utero	5	ND	3/4	2/4	0/4

* Virus identified as MCMV by immunofluorescence and neutralization test (see Materials and Methods).

‡ 1–5 × 10^6 spleen cells co-cultured with 1–2 × 10^5 MEC in MEM containing 10% FCS. Cultures made in triplicate or quadruplicate.

§ Pregnant mice injected i.p. with 1,000 $TCID_{50}$ of MCMV, or newborn mice injected i.p. with 100 $TCID_{50}$ within 24 h of birth.

‖ Major histocompatibility complex (*H-2*).

¶ Number of cultures from which MCMV was recovered over the total number of cultures observed. Results are from one experiment. Three additional experiments gave similar results.

anti-θ serum and C. When the remaining lymphoid cells were co-cultured with BALB/c MEC, CPE occurred within 1 wk. This occurred with both intact cells and cells disrupted by sonication (Table III).

Activation of Virus by Mitogens. Lymphoid cells from the spleens of 6- to 8-wk old mice infected with MCMV at birth were mixed with various mitogens and co-cultured with isologous MEC. Varying doses of PWM, Con A, and PHA-P did not activate virus during a 7-wk old observation period. The inability to recover virus with these mitogens occurred despite active blastogenesis as measured by the incorporation of free [^{3}H]thymidine into [^{3}H]thymidine TCA precipitable counts in cultured cells. In contrast virus was obtained when these infected lymphoid cells were cultured with LPS and isologous MEC. 10 μg/ml of LPS added to 5 × 10^6 lymphoid cells resulted in the largest amount of [^{3}H]thymidine incorporation into TCA precipitable counts and to the recovery of virus.

There was no significant difference in the incorporation of [^{3}H]thymidine into TCA precipitable cell counts among mitogen-stimulated lymphoid cells from MCMV infected and uninfected mice. In a typical experiment, incubation of 5 × 10^6 lymphoid cells from infected mice with 10 μg LPS or 50 μg PWM resulted in an average of 11,492 and 9,159 TCA precipitable cpm, respectively. Incubation of similar numbers of lymphoid cells from uninfected mice with these mitogens led to 22,404 and 6,282 TCA precipitable cpm, respectively. Cells not stimulated with mitogens gave the following counts: infected cells, 563; uninfected cells, 1,437.

Identification of Recovered Virus. The virus in each culture showing CPE

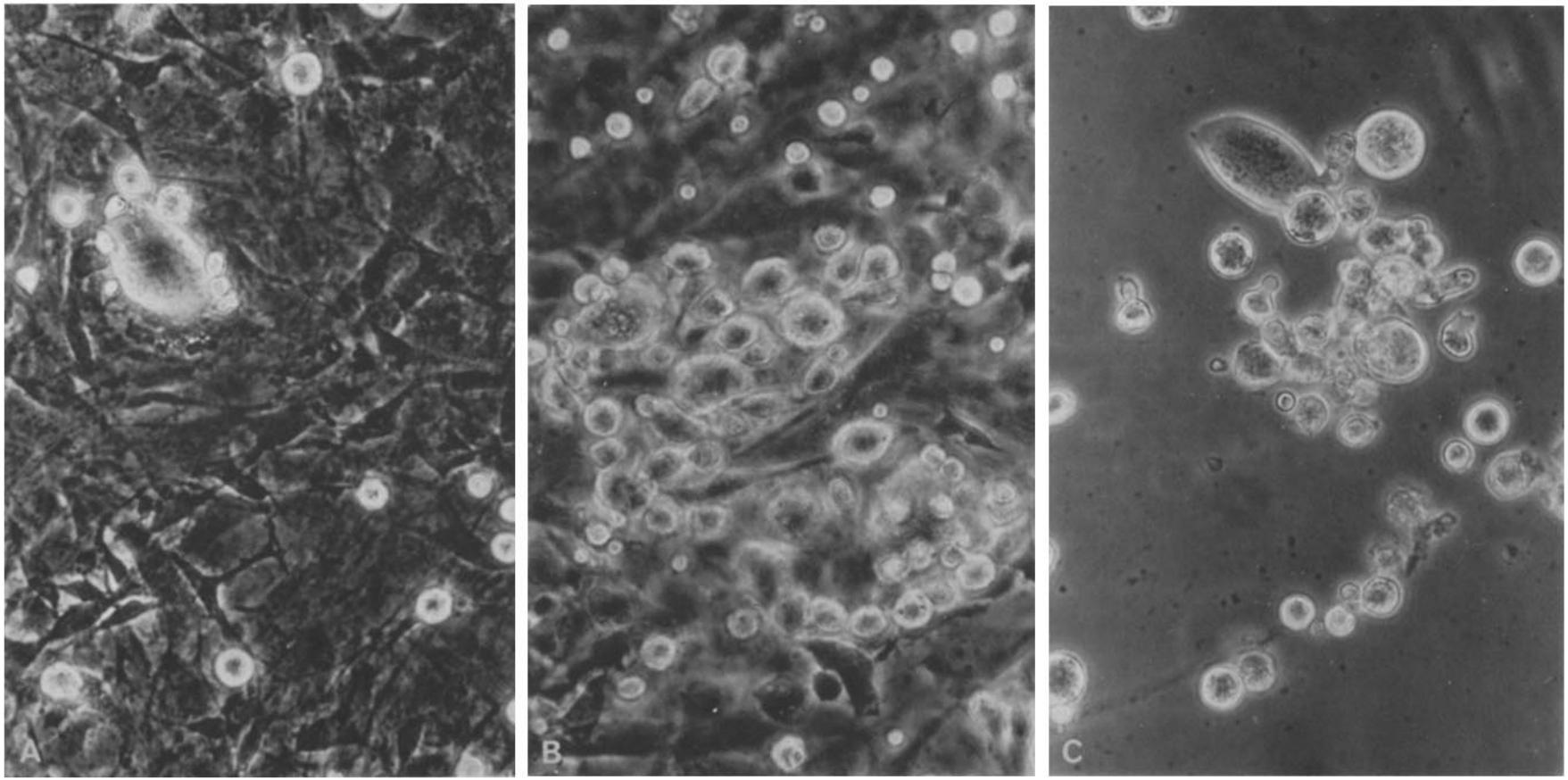

FIG. 1. Activation in vitro of MCMV from lymphocytes of spleens from adult mice after co-culture with allogenic MEC: (A) early CPE on 2nd wk of culture with megalic fibroblast surrounded by lymphocytes; (B) foci of megalic cells 2 days later; and (C) final stage when all cells were infected and most of them disrupted except for occasional clusters shown in the picture (phase contrast × 1,100).

TABLE II

*Recovery of MCMV From Purified Populations of B Lymphocytes of Adult Mice Which Had Been Infected at Birth**

Strain	No. of mice (pool)	Age	Tissue source	Lymphocyte preparation‡	MCMV recovery after co-culture with histoin-compatible embryo cells (BALB/c)
		mo			
C3H/St	4	3	Thymus	T	0/3§
C3H/St	4	3	Spleen	T	0/3
C3H/St	4	3	Spleen	B	2/3
C3H/St	4	3	Spleen	B + T	2/3

* Newborn mice injected i.p. with 100 $TCID_{50}$ of MCMV within 24 h of birth.
‡ Lymphocytes purified by Ficoll-Hypaque gradient centrifugation. Macrophages and B lymphocytes removed by passage through a nylon wool column. T lymphocytes killed by treatment with a congenic anti-θ antiserum and guinea pig C (see Materials and Methods).
§ Number of cultures from which MCMV was recovered over total number of cultures observed. Results are from one experiment. Additional experiments gave similar results.

TABLE III

*Recovery of MCMV from B Lymphocytes of Infected Nude Mice**

No. of mice (pool)	Days after infection	Tissue source	Lymphocyte preparation‡	MCMV recovery after co-culture with embryo cells
3	17	Spleen	Crude	2/3§
3	17	Spleen	B cells, alive	3/3
3	17	Spleen	B cells, sonicated	3/3

* Homozygous nude (n/n) mice from BALB/c background injected i.p. with 200 $TCID_{50}$ of MCMV.
‡ Lymphocytes were obtained by Ficoll-Hypaque gradient centrifugation. Purified lymphocytes deprived of macrophages were treated with a congenic anti-θ antiserum and guinea pig C.
§ Number of cultures from which MCMV was recovered over total number of cultures observed.

was positively identified as MCMV by the occurrence of characteristic intranuclear viral inclusions when stained by fluoresceinated specific antibody to MCMV (Fig. 2). Further, virus recovered from lymphocytes was specifically neutralized by antibodies to MCMV. In addition, electron microscopy of infected cells showed viral structures typical for the herpes virus group (Fig. 2), and light microscopy showed the characteristic nuclear inclusions usually associated with CMV (Fig. 2).

Discussion

Throughout life, virus remains in a latent state in the lymphocytes of many mice infected in utero or at birth with MCMV. Although this virus cannot be detected

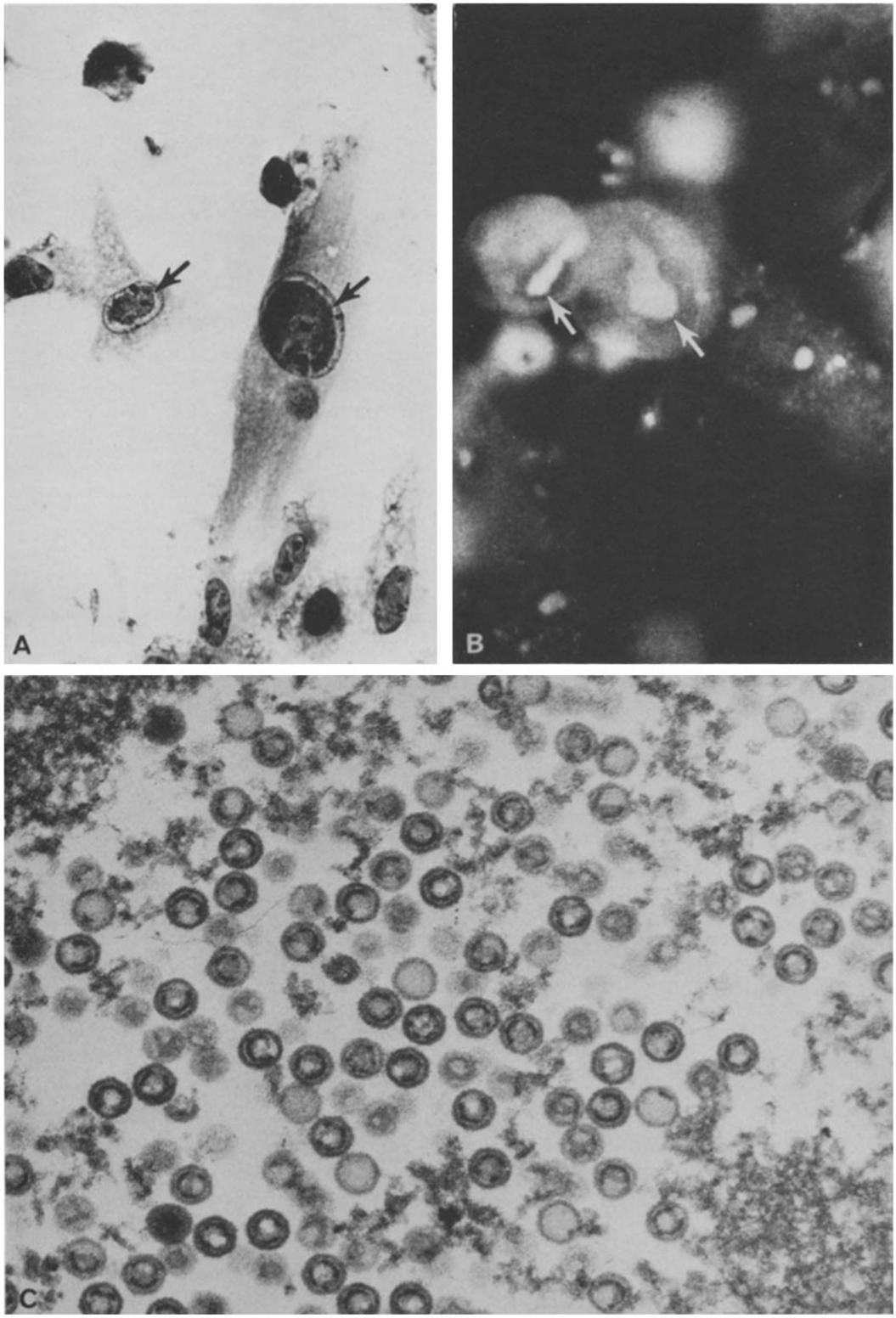

FIG. 2. Identification of MCMV as the agent activated from lymphocytes of adult mice after co-culture with allogenic MEC: (A) characteristic nuclear inclusions are seen in cells stained with May-Grünewald and Giemsa, × 900; (B) recovered virus identified as MCMV by immunofluorescence using a rabbit antibody to MCMV conjugated with FITC. Intranuclear viral inclusions specifically stained (arrows), × 1,000; and (C) ultrastructure of recovered virus showing icosahedral nucleocapsids typical for herpes viruses (glutaraldehyde fixation, ×50,000).

in sonicated lymphoid cells, it can be recovered once the lymphocytes are cultured with allogenic fibroblasts. MCMV can be isolated from purified populations of these animals' B cells and from spleen cells of T-less homologous nude mice (in which both glass adherent and contaminating theta-bearing cells have been removed). Purified T cells show no such virus. Thus CMV, which had lain dormant, was fully capable of becoming activated to replicate in B cells and could be recovered from them. Recently, both cultured human B-cell lines and peripheral B but not T cells have been reported to have receptors for Epstein-Barr virus, which like CMV is in the herpes virus group (26).

We are aware that techniques used to obtain subpopulations of lymphocytes have their limitations. For example, glass surfaces or nylon fibers may remove some lymphoblasts as well as macrophages; cells other than B or T cells comprise lymphoid populations, and theta or immunoglobulin present on a cell's surface may vary in amount depending on a cell's position in its growth cycle and hence escape separation with the reagents used. However, since no MCMV could be activated from 5×10^6 T lymphocytes, it seems unlikely that the virus recovered from the B-lymphocyte population received any contribution from T-cell contamination.

MCMV were first detected in murine lymphocyte cultures at different times, usually not until the 14th day and more often during the 3rd wk (range 2–5 wk). We could not be certain whether virus was released more quickly from purified B-cell populations than from heterologous B- and T-cell populations. Occasionally, only one or two of four cultures became positive. These results suggest that all lymphocytes may not harbor the virus, and that those having virus carry very little. In preliminary experiments in which [^{3}H]thymidine-labeled MCMV DNA was hybridized to lymphocytes, there was an average of less than one copy of viral DNA per B lymphocyte (Kingsbury, Olding, and Oldstone, unpublished data).

MCMV was recovered from lymphocytes after co-cultivation with allogenic fibroblasts. No virus was detected when these lymphocytes were disrupted before being cultured or when lymphocytes, intact or sonicated, were cultured under like conditions with isologous fibroblasts or those with similar *H-2* loci. Active blast transformation occurred after addition of several mitogens. However, only one, a B-lymphocyte mitogen, LPS, was adequate to activate MCMV. We conclude that (*a*) virus can be present in lymphocytes in a noninfectious form; (*b*) cell division is needed for virus activation; (*c*) allogenic stimulation leads to virus activation; and (*d*) the B-cell mitogen, LPS, activates virus. Factors other than those we tried may also be important in activating this virus.

Others have shown that murine leukemia virus (MuLV) can be activated both in vivo by means of a graft vs. host reaction and in vitro by using mixed lymphocyte cultures containing various anti-*H-2* cells (27–30). In contrast to our study, the mitogens they used were unable to activate MuLV from lymphocytes. Similar to our findings, co-cultivation with allogenic cells did release virus. In contrast to MCMV, MuLV is apparently released from T and not from B lymphocytes.

We found no evidence that persistence of MCMV was associated with any dysfunction of lymphocyte responses to various mitogens. Thus lymphoid cells from the spleens of adult mice which had been infected at birth with MCMV

responded with similar mitogenic indices as did uninfected age and sex matched controls to LPS, PWM, Con A, and PHA-P.

In several human diseases, CMV becomes activated (1, 2) by mechanisms that are not understood. Based on our observations that MCMV is activated from lymphocytes after allogenic stimulation in vitro, and on the known association of CMV activation and allogenic immunologic reaction in vivo (after human renal transplants, blood transfusions, and pregnancies), it seems likely that latent CMV can be activated from lymphocytes of the recipient or perhaps the donor during allogenic stimulation as previously intimated (32). Experiments to test this hypothesis in man and to activate MCMV in vivo are presently under way. Similar pathogenic mechanisms may, in part, explain the occurrence of infectious mononucleosis particularly in the post-transfusion syndrome. Our observations add additional evidence that viruses are carried in lymphocytes (reviewed in reference 31). If a parallel exists in other infectious diseases or in those chronic disorders believed to be associated with viral infection, then the techniques described here may be applicable to recovering viruses from those patients.

Finally, in addition to demonstrating that latent MCMV is carried and can be activated in B lymphocytes, our experiments with nude mice clearly showed that virus replicated in their B lymphocytes. Similar observations were made in other murine strains and rarely in a C3H/St mouse. In all these instances, virus was recovered rapidly and directly from lymphocytes without allogenic co-cultivation. Others have successfully recovered CMV from lymphoid tissues and peripheral blood cells (33–36).

Summary

After infection in utero or at birth with a cell culture adapted strain of mouse cytomegalovirus (MCMV), several mouse strains developed a latent virus infection in the presence of specific antiviral antibodies. Up to 5 mo after infection, MCMV could be activated and recovered from spleen lymphocytes of the infected animals that were co-cultivated with histoincompatible (*H-2* foreign) mouse embryo cells from uninfected animals. In contrast, co-cultivation of lymphoid cells from infected mice with mouse embryo cells from syngeneic, histocompatible (*H-2* similar) donors did not activate MCMV. Similarly, MCMV was not recovered from sonicated lymphoid cells. Virus was activated by treating viable lymphoid cells with lipopolysaccharide, a B-cell mitogen, but was not activated by a variety of other mitogens such as phytohemagglutinin, concanavalin A, or pokeweed mitogen. Subsequent purification of lymphoid cells from infected mice by a variety of techniques indicated that MCMV was harbored in the B-lymphocyte population.

We thank Professor Peter W. Lampert, University of California at San Diego, School of Medicine, and Dr. David Kingsbury, University of California at Irvine, School of Medicine, for their help in part of this work, Mr. Craig Crowley for excellent technical assistance, and Ms. Phyllis Minick for aid in manuscript preparation.

Received for publication 4 November 1974

Bibliography

1. Weller, T. H. 1971. The cytomegaloviruses: ubiquitous agents with protean clinical manifestations (Part one). *N. Engl. J. Med.* **285**:203.
2. Weller, T. H. 1971. The cytomegaloviruses: ubiquitous agents with protean clinical manifestations (Part two). *N. Engl. J. Med.* **285**:267.
3. Krech, U. H., M. Jung, and F. Jung. 1971. Cytomegalovirus Infections of Man. S. Karger AG., Basel, Switzerland.
4. Stern, H. 1968. Isolation of cytomegalovirus and clinical manifestations of infection at different ages. *Br. Med. J.* **1**:665.
5. Medearis, D. N., R. Montgomery, and L. Youngblood. 1970. Cytomegalovirus infection of the female genital tract. *Pediatr. Res.* **4**:461.
6. Reynolds, D. W., S. Stagno, T. S. Hosty, M. Tiller, and C. A. Alford. 1973. Maternal cytomegalovirus excretion and perinatal infection. *N. Engl. J. Med.* **289**:1.
7. Numazaki, Y., N. Yano, and T. Morizuka. 1970. Primary infection with human cytomegalovirus: virus isolation from healthy infants and pregnant women. *Am. J. Epidemiol.* **91**.410.
8. Craighead, J. E., J. B. Hanshaw, and C. B. Carpenter. 1967. Cytomegalovirus infection after renal allotransplantation. *JAMA (J. Am. Med. Assoc.)*. **201**:725.
9. Lang, D. J., E. M. Scolnick, and J. T. Willerson. 1968. Association of cytomegalovirus infection with the postperfusion syndrome. *N. Engl. J. Med.* **278**:1147.
10. Caul, E. O., S. K. R. Clarke, M. G. Mott, T. G. M. Perham, and R. S. E. Wilson. 1971. Cytomegalovirus infections after open heart surgery. *Lancet.* **1**:777.
11. Foster, K. M., and I. Jack. 1969. A prospective study of the role of cytomegalovirus in post-transfusion mononucleosis. *N. Engl. J. Med.* **280**:1311.
12. Prince, A. M., W. Szmuness, S. J. Millian, and D. S. David. 1971. A serologic study of cytomegalovirus infection following blood transfusion in tumor surgery *JAMA (J. Am. Med. Assoc.)*. **211**:1341.
13. Stevens, D. P., L. F. Barker, A. S. Ketcham, and H. M. Meyer. 1970. Asymptomatic cytomegalovirus infection following blood transfusion in tumor surgery. *JAMA (J. Am. Med. Assoc.)*. **211**:1341.
14. Duwall, C. P., A. R. Casazza, P. M. Grimley, P. P. Carbone, and W. P. Rowe. 1966. Recovery of cytomegalovirus from adults with neoplastic disease. *Ann. Intern. Med.* **64**:531.
15. Brodsky, I., and W. P. Rowe. 1958. Chronic subclinical infection with mouse salivary gland virus. *Proc. Soc. Exp. Biol. Med.* **19**:654.
16. Medearis, D. N. 1964. Mouse cytomegalovirus infection. II. Observations during prolonged infections. *Am. J. Hyg.* **80**:103.
17. Oldstone, M. B. A., and F. J. Dixon. 1968. Immunohistochemical study of allergic encephalomyelitis. *Am. J. Pathol.* **52**:251.
18. Oldstone, M. B. A., and F. J. Dixon. 1969. Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection. I. Relationship of antibody production to disease in neonatally infected mice. *J. Exp. Med.* **129**:483.
19. Mendelsohn, J., A. Skinner, and S. Kornfeld. 1971. The rapid induction by phytohemagglutinin of increased α-aminoisobutyric acid uptake by lymphocytes. *J. Clin. Invest.* **50**:818.
20. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3**:645.
21. Blanden, R. 1970. Mechanisms of recovery from a generalized viral infection: mouse pox. I. The effects of anti-thymocytic serum. *J. Exp. Med.* **132**:1035.

22. Oldstone, M. B. A. 1973. Thymus dependent (T) cell competence in chronic LCM virus infection. *In* Lymphocytic Choriomeningitis Virus and Other Arenaviruses. F. Lehmann-Grube, editor. Springer-Verlag KG., Berlin, West Germany. 185.
23. Brunner, K. T., J. Mauel, J. C. Cerottini, and B. Chapuis. 1968. Quantitative assay of the lytic action of immune lymphoid cells on ^{51}Cr-labeled allogeneic target cells in vitro; inhibition by isoantibody and by drugs. *Immunology*. **14**:181.
24. Morrison, D., and L. Leive. Two fractions of lipopolysaccharides from *E. coli* 0111:B4 as prepared by two extraction procedures. *J. Biol. Chem*. In press.
25. Click, R. E., L. Benck, and B. J. Alter. 1972. Immune responses in vitro. I. Culture conditions for antibody synthesis. *Cell. Immunol.* **3**:264.
26. Jondal, M., and G. Klein. 1973. Surface markers on human B and T lymphocytes. II. Presence of Epstein-Barr virus receptors on B lymphocytes. *J. Exp. Med.* **138**:1365.
27. Hirsch, M. S., S. M. Phillips, C. Solnik, P. H. Black, R. S. Schwartz, and C. B. Carpenter. 1972. Activation of leukemia viruses by graft versus host and mixed lymphocyte reactions in vitro. *Proc. Natl. Acad. Sci. U. S. A.* **69**:1069.
28. Hirsch, M. S., D. A. Ellis, P. H. Black, A. P. Monaco, and M. L. Wood. 1973. Leukemia virus activation during homograft rejection. *Science (Wash. D. C.)*. **180**:500.
29. Phillips, S. M., H. Gleichmann, M. S. Hirsch, P. Black, J. P. Merrill, R. S. Schwartz, and C. B. Carpenter. 1974. Cellular immunity in the mouse. IV. Altered thymic dependent lymphocyte reactivity in the chronic graft vs. host reaction and leukemia virus activation. *Cell. Immunol.* In press.
30. Phillips, S. M., M. S. Hirsch, J. Andre-Schwartz, C. Solnik, P. Black, R. Schwartz, J. P. Merrill, and C. B. Carpenter. 1974. Cellular immunity in the mouse. V. Further studies on leukemia virus activation in allogeneic reactions of mice: stimulatory parameters. *Cell. Immunol.* In press.
31. Wheelock, E. F., and S. T. Toy. 1973. Participation of lymphocytes in viral infections. *Adv. Immunol.* **16**:132.
32. Rowe, W. P., J. W. Hartley, S. Waterman, H. C. Turner, and R. J. Huebner. 1956. Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. *Proc. Soc. Exp. Biol. Med.* **92**:418.
33. Lang, D. J. 1972. Cytomegalovirus Infections in organ transplantation and post transfusion *Arch. Gesamte Virusforsch.* **37**:365.
34. Hardnen, P., T. R. Elsdale, D. E. Young, and A. Ross. 1967. The isolation of cytomegalovirus from peripheral blood. *Blood J. Hematol.* **30**:120.
35. Lang, D. J., and B. Noren. 1968. Cytomegaloviremia following congenital infection. *J. Pediatr.* **73**:812.
36. Diosi, P., E. Moldovan, and N. Tomescu. 1969. Latent cytomegalovirus infection in blood donors. *Br. Med. J.* **4**:660.