Growth Kinetics of Human Cytomegalovirus Are Altered in Monocyte-Derived Macrophages

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Stimulation of monocytes/macrophages with activated nonadherent cells allows productive nonlytic growth of human cytomegalovirus (HCMV), but the viral replication cycle is delayed relative to replication of HCMV in human fibroblasts. Analysis of infected monocyte-derived macrophage (MDM) mRNA for major immediate-early (MIE 86, 72, and 55) and late (pp65 and gB) gene expression by reverse transcription PCR indicates that transcription peaks at 3 and 7 days postinfection (dpi), respectively. In contrast, in human fibroblast controls, mRNA for MIE and late gene expression peaked at 5 and 48 h postinfection, respectively. Consistent with reverse transcription PCR experiments, double-label antibody experiments first detected MIE antigen expression at 12 h postinfection, peaking at 3 dpi, and late (pp65 or gB) antigen expression at 5 dpi, peaking at 7 dpi. MIE antigen was not detected between 3 and 7 dpi but reappeared and was coexpressed with pp65 in enlarged MDM nuclei at 7 dpi. After 7 dpi, macrophages with numerous vacuoles containing large amounts of pp65 and gB were observed in culture. These vacuoles were frequently seen at cellular contact points, suggesting that cell-to-cell transfer of virus was the major mode of viral transmission. Consistent with this observation, infectious virus was recovered from MDM cellular lysates but not culture supernatant. The delayed growth and compartmentalization of HCMV in macrophages may allow the cell to accommodate the viral replication cycle without cell lysis. In addition, the macrophage may function as a vehicle for cell-to-cell transmission of HCMV.

Epidemiological studies have demonstrated that peripheral blood mononuclear cells (PBMC) are an important source of human cytomegalovirus (HCMV). Although HCMV is occasionally isolated from peripheral blood samples of individuals during acute disease (4, 26), virus is rarely detected in PBMC from HCMV-seropositive asymptomatic patients. Viral expression in PBMC from the latter individuals is infrequent and limited to early events (23, 27, 38, 40). Interestingly, examination of biopsy tissue from organ transplant patients with HCMV disease reveals a high proportion of inflammatory cells which exhibit extensive viral expression in comparison to the frequency observed in peripheral blood (14). The above observations suggest that the state of cellular activation plays a significant role in induction of viral replication.

Assessment of separated cell populations from PBMC in natural or in vitro HCMV infections have identified monocytes as the predominant infected cell type (1, 7, 11, 12, 15, 17, 18, 20, 25, 30, 37). These cells are derived from myeloid progenitor cells in the bone marrow which are susceptible to HCMV infection in vitro (20). The myeloid progenitor cells are thought to be latent reservoirs of virus and induce HCMV replication upon cellular differentiation (20, 21).

A variety of different culture methods have been developed to study the growth of HCMV in primary monocyte-derived macrophages (MDM) (3, 4, 18, 27). In these systems, the ability of HCMV to replicate is dependent on the stage of cellular activation or differentiation. We have described a primary monocyte/macrophage culture system designed to facilitate monocyte differentiation into macrophages (16, 29). In this system, monocytes are cocultured with antigen- or mito-

gen-activated autologous nonadherent cells for a controlled period to allow for monocyte stimulation. Stimulated monocytes subsequently differentiate into several morphologically distinct macrophage phenotypes, including multinucleated giant cells. Macrophages derived by this method can be maintained in long-term culture without the addition of exogenous cytokines and are fully permissive for HCMV infection (16).

The vast majority of studies on HCMV gene expression have been performed in virally infected human fibroblasts (HF) which do not represent a naturally infected cell in the human host. During productive HCMV infection of these cells, the virus life cycle is regulated by sequential expression of the viral genome. Immediate-early (IE) genes are rapidly transcribed after infection, and expression of early and late gene products follows. In HF cells, IE gene expression peaks at 5 h postinfection (hpi), while early and late gene products peak at 24 and 48 hpi, respectively (8, 39). We have observed that the kinetics of viral production in MDM are considerably slower than in HF. To determine the basis of these delayed kinetics, we examined expression of representative IE and late viral genes in MDM. We found the expression of IE and late genes were dramatically delayed during productive infection and that virus appeared to be compartmentalized in vacuoles in the cytoplasm. These observations imply a unique adaptation of the virus to the cell which may facilitate a mechanism of trafficking and cellular survival.

MATERIALS AND METHODS

Isolation and culture of MDM. PBMC were isolated from the blood samples of HCMV-seronegative donors selected from a pool of donors at Oregon Health Sciences University. Heparinized whole blood was underlaid with Histopaque (Sigma Chemical Co., St. Louis, Mo.) and centrifuged at 1,500 rpm in a Sorvall RT6000B centrifuge for 25 min at room temperature. The PBMC band was collected, washed twice with sterile saline and once with serum-free medium, and resuspended at 4×10^7 cells per ml in Iscove's medium (GIBCO Laboratories,

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Grand Island, N.Y.) with 1.0% penicillin-streptomycin solution (GIBCO) and 10% pooled human serum (prepared from the Oregon Health Sciences University donor pool). Approximately 1.5×10^7 PBMC were plated per chamber onto Lab-Tek 2 chamber slides (Nunc, Inc., Naperville, Ill.), and approximately 5×10^7 PBMC were plated onto 60-mm-diameter Primaria culture dishes (Becton Dickinson, Lincoln Park, N.J.) and incubated at 37°C with 7% CO₂. Adherent cells were induced to differentiate by cocultivation with concanavalin A (5.0 µg/ml; Sigma)-treated nonadherent cells for 24 h. Subsequently, all nonadherent cells were removed and adherent MDM were cultured in complete 60/30 medium (60% AIM V medium and 30% Iscove's medium [GIBCO] supplemented with 10% human serum and 1% penicillin-streptomycin). The adherent cells were greater than 99% esterase positive at 72 h (16). MDM cultures were fed every 3 days with 50% fresh medium and 50% spent medium clarified by centrifugation. Replacement of this conditioned medium at each feeding was necessary for optimal MDM differentiation. Day 1 of differentiation is defined as the day after the initial PBMC isolation and stimulation with concanavalin A.

HCMV infection of MDM. Several recent isolates of HCMV were used to infect primary cultures of MDM. These isolates (I-G, PH, PO, and LY) were isolated from transplant patients with HCMV disease and passed through HF and frozen below passage 12 in liquid nitrogen (16, 25). Frozen samples from this stock were thawed and passed for three additional rounds through HF cells prior to MDM infections. Supernatants from HCMV-infected HF cells were used as the source of MDM inoculum, which was diluted 1:1 with MDM-conditioned medium. Briefly, MDM cultures containing approximately 5×10^6 cells per 60-mm-diameter culture dish or 3×10^6 cells per slide chamber were infected at 8 to 10 days postdifferentiation with infected HF supernatants containing approximately 10^8 PFU per dish. Virus inoculum was incubated with MDM for 2 h, removed with thorough washing, and replaced by conditioned MDM medium.

Immunofluorescence of HCMV IE and late antigen. HCMV-infected MDM grown on chamber slides were fixed in methanol and made permeable with 0.3% Triton X-100 in phosphate-buffered saline (PBS). Monolayers were blocked with 20% normal goat serum in PBS and incubated for 1 h at 37°C with a 1:100 dilution of one of the following antibodies raised against HCMV gene products: a polyclonal IE rabbit antibody generated against IE 72 using IE 72 recombinant protein, a monoclonal L antibody against pp65, or a monoclonal gB antibody against glycoprotein B (2). Binding of primary antibody was detected with fluorescein isothiocyanate- or tetramethyl rhodamine isocyanate-conjugated secondary antibodies raised in the appropriate species and visualized with an upright Leitz fluorescent microscope or a Leica Confocal Laser Scanning Microscope equipped with a Leitz Fluorovolt-FU Microscope and argon-krypton laser (CLSM AR/KR-Laser).

PCR of HCMV IE and late antigen. For RNA isolation, HCMV-infected MDM grown in 60-mm-diameter culture dishes were lysed directly by adding RNA STAT-60 (1.5 ml/dish) (TEL-TEST "B", Inc. Friendswood, Tex.). The RNA was then extracted with chloroform and precipitated with isopropanol. After a 75% ethanol rinse, the RNA was suspended in diethyl pyrocarbonate-treated water and used for PCR analysis. Reverse transcriptase PCR and cDNA amplification was performed with the GeneAmp ThermoStable rTth Reverse Transcriptase RNA PCR Kit (Perkin-Elmer Cetus, Norwalk, Conn.). For the reverse transcription (RT) reaction, a 3' oligonucleotide primer and 250 ng of total RNA were used along with 17 µl of master mixture (see Perkin-Elmer Cetus Protocol) at 70°C for 10 min. The resulting product was then transferred to another tube containing the 5' primer and 79 µl of master mixture (see Perkin-Elmer Cetus Protocol) for 2 min at 95°C for 1 cycle, then 1 min at 95°C, and 1 min at 60°C for 35 cycles, and finally, 7 min at 60°C for 1 cycle. All 5' oligonucleotides used for PCR were end-labeled with [γ -³²P]ATP by using polynucleotide kinase and purified by fast protein liquid chromatography. The RT-PCR products were separated on a 5% acrylamide gel in 1× TBE (Tris-borate-EDTA). IE primers amplified a 435-bp sequence of the MIE gene within exon 4 (IE-1/UL123) of this gene (5). The primers were primer MIE-4 (5'-CCAAGCGGCCTCTGATAACCAAGCC-3') and primer MIE-5 (5'-CAGCA CCATCCTCTCTCTCTGG-3') (9). We also used IE primers spanning the exon 6-7 junction (IE-2/UL122), which generate a 659-bp product from exon 5 and a 198-bp product from the exon 6-7 splice junction (5). The primers were 55/86-5' (5'-GCACACCAACGTGCAGACTCGGC-3') and 55/86-3' (5'-TG GCTGCCTCGATGGCCAGGCTC-3'). Late primers amplified a 401-bp sequence that corresponds to sequences in the 3' end of pp65 RNA that also codes for pp71 (therefore also amplifying pp71 mRNA) (28). The primers were pp65-3205 (5'-CCCGCTACCCGATCGTGTGCGAATCACCC-3') and pp65-3579 (5'-CCGGGGACAGTCCGGCTTGGGTGTCC-3'). Primers specific for gB, a late glycoprotein, were used to amplify a 360-bp fragment from the center of the RNA (6). The primers were gB5' (5'-AGCTGCGTGACCATCAACCAAA CC-3') and gB3' (5'-CTGCGAGTAAAGTTCCAGTACCC-3'). Finally, the primers specific for the β -actin control, used to determine the integrity of the RNA, were β actin-L (5'-CCTTCTGGGATGGAGTCTG-3') and β actin-R (5'-GGAGCAATGATCTTGTATCTTC-3').

FIG. 1. Growth of HCMV in differentiated macrophages. An HCMV one-step growth curve in infected MDM (A) and HF cells (B). MDM and HF cells were infected with recent HCMV isolates (I-G and PH) as described in Materials and Methods. (A) Macrophages infected with I-G (○) or PH (□) and supernatants infected with I-G (◇) or PH (△) were harvested at the indicated times, and viral titers were determined by plaque assay on HF cells. (B) HF infected with I-G (—○—) or PH (---□---) were harvested at the indicated times, and viral titers were determined by plaque assay on HF cells.

RESULTS

HCMV replication is delayed in MDM. MDM were cultured until the appearance of multinucleated giant cells which occurred between 6 and 10 days poststimulation, depending on the donor. Following multinucleated giant cell formation, MDM cultures were infected with recent isolates of HCMV (I-G and PH). HCMV-infected MDM culture lysates or supernatants were assayed for the production of cell-associated or

FIG. 2. PCR-RNA analysis of HCMV MIE and late (pp65/pp71; gB) gene expression in infected macrophages. Whole-cell RNA was extracted from HCMV-infected (I-G) MDM at 1, 3, 5, and 7 dpi. Oligonucleotide MIE primers were designed to detect the presence of exon 4 (A) (generating a 435-bp product), exon 5 (B) (generating a 659-bp product), and exon 6-7 splice junction (B) (generating a 198-bp product). The pp65/pp71 primers (C) were designed to generate a 401-bp product. The gB primers (D) were designed to generate a 360-bp product. Oligonucleotide primers generated to produce a 202-bp product from β -actin RNA were utilized to demonstrate the integrity of the macrophage RNA. Inf. and INF., infected; M, mock infected.

extracellular virus by plaque assay on susceptible fibroblast monolayers (Fig. 1). In MDM lysates, HCMV production for both I-G and PH was first detected at 5 days postinfection (dpi), peaking at 7 dpi, with continued high levels of virus production occurring over the next 12 days (Fig. 1A). Detection of HCMV after 5-dpi culture suggested a delayed virus replication cycle relative to the infectious cycle in HF cells. In control infections of HF cells with I-G and PH, HCMV production was detected as early as 2 dpi, with a peak at 3 dpi (Fig. 1B). The delayed appearance of virus in MDM was also observed with another recent HCMV isolate (PO), indicating that this observation was not strain dependent (data not shown). The kinetic growth differences observed between MDM and HF cells was not due to differential attachment, absorption, and nuclear localization of the viral genome in these cells (16; unpublished results). Unlike infected HF cells

in which virus is readily recovered from supernatants, HCMV was not detected in MDM culture supernatants (Fig. 1). This observation suggests that progeny virus remains primarily cell associated in MDM.

The kinetics of HCMV mRNA expression is altered in MDM. To determine if the delay in the appearance of HCMV in MDM is a result of altered kinetics of HCMV gene expression, the presence of mRNAs encoding proteins from the MIE region (UL122 and UL123), pp65/pp71 (UL82 and UL83), and gB (UL55) were analyzed by RT-PCR. Figure 2 represents the kinetics of mRNA expression for the HCMV isolate I-G in MDM. Similar results were also obtained for the HCMV isolate PH (data not shown). For this experiment, whole-cell RNA was extracted from HCMV-infected MDM at 1, 3, 5, and 7 dpi and from infected HF cells at 2, 6, 12, 18, 24, 37, 48, and 72 hpi. Since the MIE gene generates multiply spliced tran-

FIG. 3. Double-label immunofluorescence for the presence of MIE and pp65 gene products in HCMV-infected MDM. HCMV-infected (I-G) MDM were fixed at various intervals postinfection. Double-label immunofluorescence was performed with antibodies directed against IE 72 antigen (rhodamine) and pp65 antigen (fluorescein). (A) IE 72 was first detected at 12 hpi, peaking at 3 dpi (red nuclei). Magnification, $\times 93.7$. (B) Between 3 and 5 dpi, HCMV-infected macrophages with both IE 72 and pp65 antigens (yellow nuclei) were detected in cells. Magnification, $\times 93.7$. (C) By 5 dpi, only pp65 antigen (green nuclei) was observed. Magnification, $\times 46.9$. (D and E) Between 5 and 7 dpi, cells with large nuclei containing both IE 72 and pp65 antigens were observed. Magnifications, $\times 46.9$ (D) and $\times 93.7$ (E). (F) By 7 dpi, cells with large nuclei and numerous vacuoles containing pp65 were observed in infected macrophage cultures. Magnification, $\times 234.4$.

scripts with common and unique exons, oligonucleotide MIE primers were designed to detect expression of mRNAs encoding MIE exon 4, exon 5, and the exon 6-7 splice junction, generating RT-PCR products of 435, 659, and 198 bp, respectively (Fig. 2A and B). The exon 5 primers would also detect the presence of a late mRNA encoding a late 40-kDa protein (33). To detect expression from the genes encoding pp65 pp71 and gB, primers were utilized to generate products of 401 and 360 bp, respectively (Fig. 2C and 2D). Primers to β -actin RNA which span the exon 4 and 5 junction were used to demonstrate the integrity of the macrophage RNA as well as determine the presence of contaminating DNA.

Examination of RNA extracted from MDM by RT-PCR revealed a peak of MIE exon 4 and exon 6-7 splice junction products at 3 dpi (Fig. 2A and B, respectively). Interestingly, the exon 6-7 splice junction product which produces an mRNA encoding the IE 55-kDa protein is observed only when HF cells are treated with protein synthesis inhibitors (36; data not shown). Exon 5 signal increased through day 7, probably representing expression of not only IE 86 but also the L40 mRNA (Fig. 2B). RT-PCR analysis of pp65/pp71 and gB mRNA first detected expression of these genes at 5 dpi, with a peak at 7

dpi. In contrast to viral mRNA production in MDM, MIE mRNA peaked in HF cells at 5 hpi, while pp65/pp71 and gB peaked at 48 hpi (28, 36, 39; data not shown).

Expression of HCMV proteins is delayed in MDM. To detect expression of viral proteins, HCMV-infected MDM were fixed at various intervals postinfection for a period of 7 days and stained with virus-specific antibodies by double-label immunofluorescence. Figure 3 represents the immunostaining pattern of expression for the IE 72 and pp65 proteins with the HCMV isolate I-G. Similar IE and pp65 expression patterns were also observed with HCMV isolates PH, PO, and LY (data not shown). Consistent with mRNA expression patterns described above, IE 72 antigen was first detected at 12 hpi, with a maximum amount of cells expressing antigen (15%) at 3 dpi (Fig. 3A, red nuclei). Between 3 and 5 dpi, coexpression of both IE 72 and pp65 antigens was detected in HCMV-infected MDM (Fig. 3B, yellow nuclei). At 5 dpi, only pp65 antigen was observed in MDM cultures (Fig. 3C, green nuclei). Interestingly, if these latter infected MDM cultures were screened only for the presence of IE 72 antigen, virus would not have been detected in these cultures. After 7 dpi, IE 72 antigens reappeared in cultures coexpressed with pp65 antigen in large nu-

FIG. 4. Compartmentalization and spread of HCMV in MDM. (A and B) HCMV-infected (I-G) macrophages were examined at 14 dpi for the presence of IE 72 (green) and pp65 (red) antigens by double-label immunofluorescence (A) or the presence of gB (red) by single-label immunofluorescence. Magnifications, $\times 422$ (A) and $\times 268$ (B). (C) To illustrate cell-to-cell transmission of HCMV in MDM cultures, HCMV-infected (I-G) MDM were examined by double-label immunofluorescence with antibody directed against gB (red) or trans-Golgi (anti-mannosidase II) (22) (green). Magnification, $\times 422$. The confocal microscopy image was focused at the level of cell contact with the tissue culture slide.

clei (Fig. 3D and E, yellow nuclei). During this interval, MDM either expressing IE 72 or pp65 were also observed in foci adjacent to these coexpressing cells (Fig. 3D and E). Cells expressing single viral antigens probably represent secondary rounds of infection. At 7 dpi when infectious virus peaks in MDM, coexpression of IE 72 and pp65 was detected in macrophages with enlarged nuclei representing about 5% of infected cells (Fig. 3F). In these cells, distinct structures staining for the presence of pp65 antigen were observed closely associated with the cell nucleus. The temporal patterns of gB and gH expression were also examined by immunofluorescence and follow the same kinetics displayed by pp65 (data not shown).

In contrast to IE expression in MDM, by 6 hpi, 100% of HF cells demonstrated the presence of IE 72, which remained throughout the course of infection (data not shown). Similarly, accelerated expression of pp65 and gB gene products in HF cells was first detected at 24 hpi, with expression in all cells by 48 hpi. Therefore, the reduced kinetics of HCMV gene expression in MDM is not an inherent property of the virus isolate but is due to cell-specific differences. Expression of viral antigen thus correlates with the previous RNA data, demonstrating the delayed kinetics of HCMV infection in MDM compared with infection in HF cells. In addition, the temporal appearance and distribution of viral antigen in macrophages suggest a unique adaptation of HCMV to the macrophage.

Compartmentalization and transmission of HCMV in MDM. We next examined the distribution of HCMV antigens in MDM in the late stages of viral infection. Infected cells were analyzed for MIE, pp65, or gB antigen localization by double-label immunofluorescence. In the late stages of infection, HCMV-infected cells with large nuclei and numerous vacuoles containing pp65 (Fig. 4A) and gB (Fig. 4B) were clearly evident in infected MDM cultures. The appearance of pp65- and gB-containing vacuoles in MDM coincides with the recovery of infectious virus from these cultures. In the late stages of infection (14 dpi), a higher frequency (20%) of cells with pp65- and gB-containing vacuoles was observed in these cultures.

The lack of virus in the supernatant as well as the development of distinct HCMV foci in MDM suggested that the major mode of transmission in these cultures is by cell-cell contact and fusion events. Consistent with these observations, cells at

various stages of fusion were observed with viral antigens coating intracellular surfaces at junctions of contact (Fig. 4C).

DISCUSSION

In this report we have examined characteristics of HCMV replication in MDM. We have found that relative to HF cells, HCMV infection of MDM displays a significant delay in the production of virus that is independent of viral strain. Consistent with these observations, the kinetics of HCMV gene expression was dramatically retarded in representative IE and late gene classes at the steady-state RNA and protein levels. The delay in viral expression may be a unique adaptation of HCMV for persistence in MDM and may explain the lack of cytopathic effect observed in virus-infected cells (16).

The mechanism responsible for the delayed kinetics of viral gene expression is unknown. Since IE gene products are important regulators of subsequent gene expression (10, 31, 32, 34–36), the altered kinetics of IE protein production in MDM may also influence the delayed appearance of early and late viral proteins. Interestingly, IE antigen peaked at 3 dpi, disappeared at 5 dpi, and reappeared at 7 dpi in cells coexpressing pp65 or gB. These observations contrast IE expression in HF cells which demonstrate the continuous presence of IE gene products throughout the viral replication cycle. The differences in HCMV expression patterns in the two cell types may reflect the stability of IE gene products and the rapid rate of replication in HF cells compared with that in MDM. The apparent reappearance of MIE gene expression at 7 dpi in MDM may represent the detection of the L40 gene product which contains amino acid sequences colinear with the MIE gene (33).

Another explanation for the delayed growth of HCMV in MDM is that altered gene expression occurs through differential splicing events resulting in RNAs encoding proteins with different functions. The fact that the MIE exon 6-7 splice junction that generates the IE 55 gene product is naturally utilized in MDM supports this possibility. Previously, utilization of this splice junction was observed only in HCMV-infected HF cells treated with protein synthesis inhibitors (36). We have also observed a newly characterized spliced MIE gene product in MDM (data not shown) which is also observed in

HF cells only in the presence of cycloheximide (19). Since MIE isoformic proteins possess different transcriptional activation functions dependent on the presence of specific functional domains (34), generation of novel MIE products may accelerate or retard the growth of virus within the cell. In addition to the above possibilities, the presence of cellular inhibitors and/or absence or modification of cellular cofactors that interact with viral proteins may also play a role in HCMV growth kinetics.

HCMV infection of HF cells causes cellular fusion and lysis resulting in the release of virus in the supernatant. In contrast, virus is exclusively cell associated in MDM and persists in these cells without lytic events. Examination of MDM during late-stage infection when infectious HCMV is recovered from cell lysates revealed the presence of numerous vacuoles filled with virus. Similar observations in MDM were made with human immunodeficiency virus (HIV) (13). Ultrastructural analysis of HIV-infected MDM indicated that virus was exclusively associated with intracellular vacuoles which were not associated with the plasma membrane (24). They suggest that such HIV-sequestering vacuoles are derived from the Golgi complex. In addition, we have recently utilized double-label immunofluorescence with cellular and viral markers to demonstrate that HCMV-infected vacuoles are derived from the Golgi complex (data not shown). Therefore, it appears that both viruses utilize a common pathway for intracellular trafficking and storage in MDM. This adaptation may serve two purposes. First, storage of the virus in discrete cellular compartments may not only protect virus from cellular degradation but may also protect the cell from damage produced by viral proteins. Second, storage of virus in intracellular compartments that never reach the plasma membrane would allow the virus to escape detection and clearance by the immune system. The latter ominous situation would allow persistence and trafficking of virus to target tissues via a cellular vehicle while evading immune detection.

Elucidating the mechanisms of HCMV replication in MDM will be crucial in understanding viral persistence and trafficking in the human host. The current studies indicate that HCMV replication in MDM differs greatly from HCMV replication in HF cells. These observations underline the importance of examining viral replication in biologically relevant cell types.

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REFERENCES

- Alder, S. P. 1983. Transfusion-associated cytomegalovirus infections. *Rev. Infect. Dis.* 5:922-933.
- Britt, W. J., and L. G. Vugler. 1992. Oligomerization of the human cytomegalovirus major envelope glycoprotein complex gB (gp55-116). *J. Virol.* 66:6747-6754.
- Buchmeier, N. A., and N. R. Cooper. 1989. Suppression of monocyte functions by human cytomegalovirus. *Immunology* 66:278-283.
- Carney, W. P., and M. S. Hirsch. 1981. Mechanisms of immunosuppression in cytomegalovirus mononucleosis. II. Virus-monocyte interactions. *J. Infect. Dis.* 144:47-54.
- Chee, M. S., A. T. Bankier, S. Beck, R. Bohni, C. M. Brown, R. Cerny, T. Horsnell, C. A. Hutchison III, T. Kouzarides, J. A. Martignetti, E. Preddie, S. C. Satchwell, P. Tomlinson, K. M. Weston, and B. G. Barrell. 1990. Analysis of the protein coding content of the sequence of human cytomegalovirus strain AD169. *Curr. Top. Microbiol. Immunol.* 154:75-103.
- Cranage, M. P., T. Kouzarides, A. T. Bankier, S. Satchwell, K. Weston, P. Tomlinson, B. Barrell, H. Hart, S. E. Bell, and A. C. Minson. 1986. Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. *EMBO J.* 5:3057-3063.
- Dankner, W. M., J. A. McCutchan, D. D. Richman, K. Hirata, and S. A. Spector. 1990. Localization of human cytomegalovirus in peripheral blood leukocytes by in situ hybridization. *J. Infect. Dis.* 161:31-36.
- DeMarchi, J. M., C. A. Schmidt, and A. S. Kaplan. 1980. Pattern of transcription of human cytomegalovirus in permissively infected cells. *J. Virol.* 35:277-286.
- Demmler, G. J., G. J. Buffone, C. M. Schimbor, and R. A. May. 1988. Detection of cytomegalovirus in urine from newborns by using polymerase chain reaction DNA amplification. *J. Infect. Dis.* 158:1177-1184.
- Depto, A. S., and R. M. Stenberg. 1989. Regulated expression of the human cytomegalovirus pp65 gene: octamer sequence in the promoter is required for activation by viral gene products. *J. Virol.* 63:1232-1238.
- Diosi, P., E. Moldovan, and N. Tomeson. 1969. Latent cytomegalovirus infection in blood donors. *Br. Med. J.* 4:660-662.
- Einhorn, L., and A. Ost. 1984. Cytomegalovirus infection of human blood cells. *J. Infect. Dis.* 149:207-214.
- Gendelman, H. E., J. M. Orenstein, M. A. Martin, C. Ferrua, R. Mitra, T. Phipps, L. A. Wahl, H. C. Lane, A. S. Fauci, D. E. Burke, D. Skillman, and M. S. Meltzer. 1988. Efficient isolation and propagation of human immunodeficiency virus on rCSF-1 treated monocytes. *J. Exp. Med.* 167:1428-1441.
- Gnann, J. W., Jr., J. Ahlman, C. Svalander, L. Olding, M. B. A. Oldstone, and J. A. Nelson. 1988. Inflammatory cells in transplanted kidneys are infected by human cytomegalovirus. *Am. J. Pathol.* 132:239-249.
- Howell, C. L., M. J. Miller, and W. J. Martin. 1979. Comparison of rates of virus isolation from leukocyte populations separated from blood by conventional and Ficoll-Paque/Macrodex methods. *J. Clin. Microbiol.* 10:533-537.
- Ibanez, C. E., R. Schrier, P. Ghazal, C. A. Wiley, and J. A. Nelson. 1991. Human cytomegalovirus productively infects primary differentiated macrophages. *J. Virol.* 65:6581-6588.
- Jordan, M. C. 1983. Latent infection and the elusive cytomegalovirus. *Rev. Infect. Dis.* 5:205-215.
- Kapaski, K., and G. P. A. Rice. 1988. Cytomegalovirus infection of peripheral blood mononuclear cells: effects on interleukin-1 and -2 production and responsiveness. *J. Virol.* 62:3603-3607.
- Kerry, J. A., A. Sehgal, S. W. Barlow, V. J. Cavanaugh, K. Fish, J. A. Nelson, and R. M. Stenberg. 1995. Isolation and characterization of a low-abundance splice variant from the human cytomegalovirus major immediate-early gene region. *J. Virol.* 69:3868-3872.
- Maciejewski, J. P., E. E. Bruening, R. E. Donahue, R. E. Mocarski, R. E. Young, and S. C. St. Jeor. 1992. Infection of hematopoietic progenitor cells by human cytomegalovirus. *Blood* 80:170-178.
- Minton, E. J., C. Tysoe, J. H. Sinclair, and J. B. P. Sissons. 1994. Human cytomegalovirus infection of the monocyte/macrophage lineage in bone marrow. *J. Virol.* 68:4017-4021.
- Moremen, K. W., O. Touster, and P. W. Robbins. 1991. Novel purification of the catalytic domain of Golgi alpha-mannosidase II. Characterization and comparison with the intact enzyme. *J. Biol. Chem.* 266:16876-16885.
- Neiman, P., P. B. Wasserman, P. B. Wentworth, G. F. Kao, K. G. Lerner, R. Storb, C. D. Buckner, R. A. Clift, A. Fefer, L. Fass, H. Glucksberg, and E. D. Thomas. 1973. Interstitial pneumonia and cytomegalovirus infection as complications of human marrow transplantation. *Transplantation* 15:478-485.
- Orenstein, J. M., M. S. Meltzer, T. Phipps, and H. E. Gendelman. 1988. Cytoplasmic assembly and accumulation of human immunodeficiency virus types 1 and 2 in recombinant human colony-stimulating factor-1-treated human monocytes: an ultrastructural study. *J. Virol.* 62:2578-2586.
- Rice, G. P. A., R. D. Schrier, and M. B. A. Oldstone. 1984. Cytomegalovirus infects human lymphocytes and monocytes: virus expression is restricted to immediate-early gene products. *Proc. Natl. Acad. Sci. USA* 81:6134-6138.
- Rinaldo, C. R., Jr., P. H. Black, and M. S. Hirsch. 1977. Interaction of cytomegalovirus with leukocytes from patients with mononucleosis due to cytomegalovirus. *J. Infect. Dis.* 136:667-678.
- Rinaldo, C. R., Jr., W. P. Carney, W. P. Richter, P. H. Black, and M. S. Hirsch. 1980. Mechanisms of immunosuppression in cytomegaloviral mononucleosis. *J. Infect. Dis.* 141:488-495.
- Ruger, B., S. Klages, B. Walla, J. Albrecht, B. Fleckenstein, P. Tomlinson, and B. Barrell. 1987. Primary structure and transcription of the genes coding for the two virion phosphoproteins pp65 and pp71 of human cytomegalovirus. *J. Virol.* 61:446-453.
- Schrier, R. D., J. A. McCutchan, J. C. Venable, J. A. Nelson, and C. A. Wiley. 1990. T-cell-induced expression of human immunodeficiency virus in macrophages. *J. Virol.* 64:3280-3288.
- Soderberg, C., S. Larson, S. Bergstedt-Lindquist, and E. Moller. 1993. Definition of a subset of human peripheral blood mononuclear cells that are permissive to human cytomegalovirus infection. *J. Virol.* 67:3166-3175.
- Spector, D. H., K. M. Klucher, D. K. Rabert, and D. A. Wright. 1990. Human cytomegalovirus early gene expression. *Curr. Top. Microbiol. Immunol.* 154:21-45.
- Staprans, S. I., D. K. Rabert, and D. H. Spector. 1988. Identification of sequence requirements and *trans*-acting functions necessary for regulated expression of a human cytomegalovirus early gene. *J. Virol.* 62:3463-3473.
- Stenberg, R. M., A. S. Depto, J. Fortney, and J. A. Nelson. 1989. Regulated expression of early and late RNAs and proteins from the human cytomegalovirus immediate-early gene region. *J. Virol.* 63:2699-2708.
- Stenberg, R. M., J. Fortney, S. W. Barlow, B. P. Magrane, J. A. Nelson, and P. Ghazal. 1990. Promoter-specific *trans* activation and repression by human

- cytomegalovirus immediate-early proteins involves common and unique protein domains. *J. Virol.* **64**:1556–1565.
35. **Stenberg, R. M., D. R. Thomsen, and M. F. Stinski.** 1984. Structural analysis of the major immediate early gene of human cytomegalovirus. *J. Virol.* **49**:190–199.
36. **Stenberg, R. M., P. R. Witte, and M. F. Stinski.** 1985. Multiple spliced and unspliced transcripts from human cytomegalovirus immediate-early region 2 and evidence for a common initiation site within immediate-early region 1. *J. Virol.* **56**:665–675.
37. **Taylor-Wiedeman, J., J. G. Sissons, L. K. Borysiewicz, and J. H. Sinclair.** 1991. Monocytes are a major site of persistence of human cytomegalovirus in peripheral blood mononuclear cells. *J. Gen. Virol.* **72**:2059–2064.
38. **Thomas, E., R. Storb, R. A. Clift, A. Fefer, F. L. Johnson, P. E. Neimer, K. G. Lerner, H. Glucksberg, and C. D. Buckner.** 1975. Bone-marrow transplantation (first of two parts). *N. Engl. J. Med.* **292**:832–843.
39. **Wathen, M. W., D. R. Thomson, and M. F. Stinski.** 1981. Temporal regulation of human cytomegalovirus transcription at immediate early and early times after infection. *J. Virol.* **38**:446–459.
40. **Weller, T. H.** 1971. The cytomegaloviruses: ubiquitous agents with protean clinical manifestations. *N. Engl. J. Med.* **285**:203–214.