Human Cytomegalovirus Increases Constitutive Production of Interleukin-6 and Leukemia Inhibitory Factor by Bone Marrow Stromal Cells


Human cytomegalovirus (CMV) infection is often associated with myelosuppression and acute inflammatory reaction in immunocompromised patients. We have previously documented that CMV exposure of bone marrow (BM) stromal cells reduces the capacity of these cells to support hematopoiesis because of a decreased production of colony-stimulating factors. This study examines the potential role of CMV on constitutive and lipopolysaccharide (LPS)-stimulated production of cytokines involved in inflammatory reaction, interleukin-6 (IL-6) and leukemia inhibitory factor (LIF) by BM stromal cells. The release of IL-6 was already detectable 2 hours post CMV-infection (2.5-fold increase in production) and the cumulative production of IL-6 after 5 days of infection was 23 ± 1.2 ng/mL (ninefold increase in production). CMV was also able to induce a time-dependent production of LIF that was maximal 8 hours after CMV infection (2.5-fold increase in production). Concomitantly, there was no detectable release of granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage CSF (GM-CSF) by CMV-infected stromal cells. The similar IL-6 and LIF production was found to be dependent of viral replication. The experiments have shown that CMV is a potent inducer of IL-6 and LIF with differential effect on constitutive and LPS-stimulated cytokine production by stromal cells; we suggest that CMV induction of IL-6 and LIF during the first hours of infection could play a role in CMV-induced inflammatory reaction. Moreover, our results show that human CMV can disturb the balanced cytokine network involved in the regulation of hematopoiesis.

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MATERIALS AND METHODS

BM stromal cell cultures. After informed consent, BM from normal volunteer donors were collected by sternal aspiration and BM-mononuclear cells (BM-MNC) were isolated by layering on a Ficoll-Hypaque density gradient (International Medical Product, Brussels, Belgium). BM stromal layers were established as previously described.1

In brief, 5 × 10^6 BM-MNC were plated in 35-mm petri dishes containing 1 mL α-minimal essential medium (α-MEM) (GIBCO, Grand Island, NY) supplemented with 15% fetal calf serum (FCS) (Seralab, Sussex, UK) and 2 × 10^-6 mol/L methyl prednisolone (Upjohn, Kalamazoo, MI) at 37°C, 7.5% CO2 in humidified air.

The adhering cells were fed at weekly intervals by complete replacement of the medium until a confluent layer of fibroblasts, macrophages, and fat cells had formed in each dish. Confluence was usually achieved after 4 to 6 weeks of culture.

Preparation of “live” and inactivated CMV. The human CMV strain AD169 was obtained from American Type Culture Collection (ATCC; Rockville, MD) and propagated in HEL fibroblasts (ATCC). Virus was obtained from the culture fluid, clarified by centrifugation

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at 500g, and stored at −80°C until use. The titer of the virus was determined by plaque assay.

Inactivation of CMV was performed by incubating the viral stock at 70°C for 15 minutes or by UV treatment (254 nm wavelength/5 cm distance for 40 minutes on ice).

CMV isolates from two patients, nos. 7 and 15 were also studied. These isolates were propagated in HEL fibroblasts and used at passage two or three.

**Infection of BM stromal cells.** The stromal cells were infected with CMV at a multiplicity of infection (MOI) of 0.1 for 2 hours at 37°C. These stromal cells were then washed twice to remove residual viral particles.

The percentage of stromal cells infected after 36 and 120 hours was analyzed by flow cytometry using indirect immunofluorescent staining with anti-CMV monoclonal antibodies (MoAbs) E13 (CMV immediate-early antigen; Clonetec, Paris, France) and B219 (late antigen, Monosan; Sanbio, Uden, The Netherlands). Briefly, the stromal cells were trypsinized and washed in Iscove medium containing 10% FCS to inactivate the trypsin. The detached stromal cells were permeabilized with a solution containing Nonidet P40 (Sigma, St Louis, MO) sucrose 10%, FCS 1% in phosphate-buffered saline (PBS) for 5 minutes at room temperature. The cells were washed in PBS containing gelatine and incubated with the anti-CMV antibodies. Bound antibody was revealed by fluorescein isothiocyanate (FITC)-conjugated goat-antimouse IgG (Immunotech, Marseille, France).

**Preparation of conditioned media for cytokine studies.** In all experiments, conditioned media (CM) were prepared from 6-week-old stromal cells when these cells had grown to form a confluent layer. After a 2-hour CMV exposure, the stromal cells were washed, fed with fresh medium, and then cultured up to 5 days.

CM of CMV-infected or uninfected stromal layers were collected after various periods of incubation and stored at −80°C in small aliquots until future use.

The production of cytokines was also evaluated after incubation of stromal cells with bacterial lipopolysaccharide (LPS) (Sigma) at 20 μg/mL for 24 hours after 2-hour CMV-exposure.

**Measurement of cytokines in CM.** Cytokines were measured in CM using specific immunologic procedures (enzyme-linked immunosorbent assay) (ELISA): IL-6 (Eurogenetics, Tesselendero, Belgium) (sensitivity 5 pg/mL, range 10 to 1,000 pg/mL) and LIF (Eurogenetics) (sensitivity 25 pg/mL, range 25 to 1,000 pg/mL).

**Anti-CMV drugs.** Two anti-CMV drugs tested at the concentration of 10 μg/mL were investigated: (1) 9-(1,3-dihydroxy-2-propoxy-methyl) guanine (GCV, Ganciclovir) (Syntex, CA); and (2) (S)-1-(3-hydroxy-2-phosphonoethoxypropyl) cytosine [(S)-HPMPC] (Gilead Sciences, CA).

GCV exerts its action against CMV by selective inhibition of the viral DNA polymerase through its 5'-triphosphate derivative. Recently, it has been shown that (S)-HPMPC, a member of the acrylic nucleoside phosphate family, is a potent and selective inhibitor of CMV replication in vitro. The effect of these two drugs on cytokine modulation during CMV infection was added to our experiments to evaluate the effects of the viral replication on cytokine perturbation.

**CMV infection of stromal cells in presence of polymyxin B.** The production of IL-6 and LIF was also evaluated after incubation of stromal cells with CMV in the presence of polymyxin B sulfate (Sigma) at 10 μg/mL. This antibiotic forms inert nonpyrogenic complexes with bacterial endotoxin and thus can neutralize any contaminating endotoxin present in our experiments.

**Source of anti-CMV antibodies.** To rule out the possibility that proteins of nonviral origin could be responsible for the IL-6 and LIF release, the production of these cytokines was also evaluated in the presence of human CMV hyperimmune globulins (Biotest, Frankfurt, Germany). CMV-seronegative human serum was used as a source of nonspecific IgG.

**Statistical analysis.** Comparison of cytokine production by CMV-infected and uninfected stromal cells was performed using the Student’s paired t-test.

**RESULTS**

**CMV infection of stromal cells.** Using flow cytometry, we found that after 36 hours of infection 10% of stromal cells expressed immediate early antigen (IEA) recognized by E13, but these cells were negative for the expression of late antigen (LA). However, after 120 hours of infection, 26% of stromal cells were positive for IEA and 22% expressed LA. Moreover, these cells showed the morphologic features of CMV. As observed with AD169 strain, the two CMV isolates (nos. 7 and 15) were also able to induce IEA and LA expression in stromal cells. However, isolate no. 7 showed antigen expression restricted to few fibroblasts. In contrast, isolate no. 15 gave a pattern of infection similar to that of AD169 (fibroblasts, macrophages, and adipocytes).

**IL-6 and LIF production during CMV infection.** We have compared the levels of IL-6 and LIF present in the CM of unstimulated stromal cells to that of stromal cells infected with CMV.

As shown in Fig 1, CMV was a potent inducer of IL-6 and LIF and this release was time-dependent. Uninfected stromal cells produced low levels of IL-6 and LIF; the cumulative release after 5 days was 2.5 ± 1 ng/mL and 0.5 ± 0.2 ng/mL, respectively. However, when stromal cells were infected with CMV, IL-6 and LIF production was greatly increased. The release of IL-6 was already detectable 2 hours postinfection (1.5 ± 0.5 ng/mL, 2.5-fold increase in production, P < .03) and at 24 hours, this level was rapidly increased to 18 ± 5 ng/mL (fivefold increase in production, P < .04) and was maintained up to 5 days of culture (23 ± 1 ng/mL, P < .0001). These kinetic experiments have also shown that CMV was able to increase the amount of LIF produced by stromal cells. In contrast to IL-6, peak induction was achieved at 8 hours (2.5-fold increase in production, P < .005) and this level of production was also maintained up to approximately 5 days (1.0 ± 0.2 ng/mL, P < .05).

**CMV-induced release of IL-6 and LIF in the presence of polymyxin B.** It has been shown that endotoxin is a major stimulus for production of cytokines such as IL-6, by monocytes and macrophages. Although we have used endotoxin-free media, the release of IL-6 and LIF in this study could be caused by endotoxin contamination during our experiments. To rule out this possibility, stromal cells were infected with CMV in the presence of polymyxin B at a concentration of 10 μg/mL. The release of IL-6 and LIF was evaluated after 24 hours.

The experiments showed that polymyxin B was unable to block IL-6 (Fig 2A) and LIF (Fig 2B) production during CMV infection. However, polymyxin decreased the capacity of LPS to induce release of cytokines as expected because we previously showed that production of IL-6, GM-CSF, and G-CSF can be increased after addition of LPS to stromal.
cytokine release after CMV infection

Fig 1. CMV stimulates IL-6 and LIF release by BM stromal cells. Supernatants of uninfected (●) or CMV-infected stromal cells (●) were tested for IL-6 and LIF production at the indicated time points. Results from four experiments are expressed as mean ± SEM.

cells. Finally, the observation that CMV was unable to induce release of GM-CSF and G-CSF during infection (data not shown) reinforced our conclusion that there was no endotoxin responsible for cytokine induction in these experiments.

Induction of cytokine production by stromal cells exposed to infectious and inactivated CMV. As shown in Fig 3A, infectious CMV increased IL-6 production within 24 hours postexposure (28.2 ± 11 ng/mL vs 6.2 ± 2.3 ng/mL, P < .02), whereas heat-treated virus had no effect on the production of IL-6 (8.4 ± 3.0 ng/mL) (n = 4).

However, UV-inactivated CMV behaved similarly to live virus and thus led to the secretion of significant amount of IL-6 (28.6 ± 7.0 ng/mL; P < .04). The fact that IL-6 release occurred even when UV-inactivated (but not heat-inactivated) CMV was used, suggested that a thermodabile viral protein(s) was responsible for this increased secretion of IL-6 and that viral infection was not necessary for this phenomenon. Comparable results were obtained for LIF (Fig 3B); as previously shown, CMV was able to induce LIF release after 24 hours of incubation (1.3 ± 0.2 ng/mL vs 0.5 ± 0.1 ng/mL) and UV-inactivation of CMV did not affect this increased production of LIF (1.8 ± 0.8 ng/mL). However, no stimulation of LIF production was seen when virus was inactivated by heat (0.8 ± 0.4 ng/mL).

Effect of CMV on cytokine production by stromal cells after LPS stimulation. Because stromal cells secrete a low level of cytokines and need to be stimulated to produce a significant level of cytokines, we have studied cytokine secretion by uninfected or CMV-infected stromal cells after activation by LPS.

Supernatants from stromal cells were assayed for IL-6 and LIF content after 5 days of CMV infection. At day 4, stromal cells were washed and fed with fresh medium supplemented or not with LPS for the last 24 hours of incubation.

The basal production of 12 different uninfected stromal cells after 24 hours was 6.36 ± 0.69 ng/mL for IL-6 and 0.38 ± 0.12 ng/mL for LIF. This basal production of cytokines in the CM of CMV-infected stromal cells was significantly different: 4.5 ± 0.8 ng/mL for IL-6 (P < .05) (Fig 4A) and 0.12 ± 0.06 ng/mL for LIF (P < .001) (Fig 4B). On the other hand, LPS-treated stromal cells produced 54.4 ± 11.8
produced constitutively $3.1 \pm 0.9$ ng/mL and the two drugs had no effect on this cumulative production.

As previously described, the CMV infection of stromal cells induced the production of IL-6 and after 5 days, the cumulative level of this cytokine reached $12.5 \pm 2.6$ ng/mL ($P < .04$). Furthermore, this increased production of IL-6 by infected stromal cells was not modified by GCV and HPMPC ($12.2 \pm 2.3$ and $13.6 \pm 3.2$ ng/mL, respectively, $P < .04$). On the other hand, at day 5, in uninfected stromal cells, LPS stimulation increased IL-6 production up to $57.6 \pm 21.3$ ng/mL compared with $14.7 \pm 5.9$ ng/mL ($P < .02$) in CMV-infected stromal cells.

In contrast, when stromal cells were infected by CMV in the presence of anti-CMV drugs, the level of IL-6 after LPS challenge was not different between uninfected and CMV-infected stromal cells ($50.9 \pm 26.2$ ng/mL for GCV-treated stromal cells and $48.8 \pm 9.6$ ng/mL for HPMPC-treated stromal cells).

**Fig 3.** IL-6 (A) and LIF (B) production by stromal cells exposed to infectious and inactivated CMV. CM were tested for both these cytokines 24 hours after exposure. The vertical bars represent the mean ± SEM of four experiments.

ng/mL of IL-6 and $0.9 \pm 0.2$ ng/mL of LIF. Incubation of CMV-treated stromal cells with LPS dramatically decreased the cytokine production by these cells: $17 \pm 3$ ng/mL ($P < .001$) and $0.4 \pm 0.1$ ng/mL ($P < .0001$) for IL-6 and LIF, respectively.

Thus, after 5 days of CMV infection, stromal cells were unable to respond to LPS stimulus and produced significantly less IL-6 and LIF than uninfected controls. Moreover, kinetic experiments have shown that these stromal cells became unable to produce IL-6 under LPS stimulation, 72 hours after CMV infection (data not shown).

**Effect of GCV and HPMPC on cytokine production during CMV infection.** We have evaluated after 5 days the cumulative release and LPS-stimulated production of IL-6 by stromal cells infected or not by CMV; the same experiments were made also in the presence of GCV and HPMPC at $10 \mu$g/mL. As shown in Table 1, uninfected stromal cells

**Fig 4.** Effect of 5-day CMV infection on the production of cytokines by stromal cells 24 hours after incubation with LPS. Results from 12 experiments were expressed as mean ± SEM. (I), Uninfected stromal cells; (II), CMV-infected stromal cells.
Table 1. Effect of Two Anti-CMV Drugs (GCV and HPMPC) on Cumulative Release and LPS-Stimulated Production of IL-6 by Stromal Cells After 5-Day CMV Infection

<table>
<thead>
<tr>
<th>Drugs</th>
<th>LPS</th>
<th>Uninfected Stromal Cells</th>
<th>CMV-infected Stromal Cells</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>-</td>
<td>3.1 ± 0.9</td>
<td>12.5 ± 2.6</td>
<td>.04</td>
</tr>
<tr>
<td>+</td>
<td>57.8 ± 21.3</td>
<td>14.7 ± 5.9</td>
<td>.02</td>
<td></td>
</tr>
<tr>
<td>GCV</td>
<td>-</td>
<td>3.8 ± 1.0</td>
<td>12.2 ± 2.3</td>
<td>.04</td>
</tr>
<tr>
<td>+</td>
<td>52.6 ± 19.7</td>
<td>50.9 ± 26.2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>HPMPC</td>
<td>-</td>
<td>3.7 ± 1.1</td>
<td>13.6 ± 3.2</td>
<td>.04</td>
</tr>
<tr>
<td>+</td>
<td>53.5 ± 17.0</td>
<td>48.8 ± 9.6</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Results from four experiments are expressed as mean production (ng/mL) ± SEM.
* Paired Student's t-test.

Parallel analysis of LIF in these experiments showed the same results: GCV and HPMPC did not modify the cumulative release of LIF by CMV-infected stromal cells, but these drugs were able to restore the response of CMV-infected stromal cells to LPS (data not shown).

Effect of clinical CMV isolates on constitutive and LPS-stimulated production of IL-6 and LIF. As shown in Table 2, the clinical CMV isolates (nos. 7 and 15) were also able to induce IL-6 and LIF production by stromal cells. The cumulative release of IL-6 and LIF after 24 hours infection was, respectively, 11.6 ± 2 ng/mL (12-fold increase in production) and 1.25 ± 0.25 ng/mL (2-fold increase in production) for the isolate no. 7 and 16.8 ± 7.3 ng/mL (12-fold increase in production) and 1.2 ± 0.23 ng/mL (1.8-fold increase in production) for the isolate no. 15.

Moreover, the release of these cytokines induced by the clinical isolates was time-dependent as observed in stromal cells infected with AD169 strain (data not shown). We have also evaluated at day 5, the LPS-stimulated production of IL-6 and LIF by stromal cells infected or not by CMV isolates nos. 7 and 15. Incubation of CMV (isolate no. 15)-treated stromal cells with LPS dramatically decreased the cytokine production by the cells: 13 ± 7 ng/mL (10% ± 4% of control production) and 0.5 ± 0.3 ng/mL (42% of control production) for IL-6 and LIF, respectively. Surprisingly, isolate no. 7 had no effect on LPS-induced production of IL-6 and LIF: respectively, 141 ± 14 ng/mL (116% ± 3% of control production) and 1.15 ± 0.1 ng/mL (96% of control production).

DISCUSSION

Since hematopoiesis is regulated in vivo by interactions between hematopoietic progenitor cells, cells of the BM microenvironment, and cytokines released by these cells, the disturbance of stromal cell function such as growth factor production could result in myelosuppression. Recently, we have shown that CMV can replicate effectively in stromal cells and the consequence of this CMV infection in vitro included decreased capacity of stromal cells to sustain hematopoiesis, the perturbation of growth factor production, and finally the destruction of the stromal layer after ≥3 weeks.22

Our study was designed to investigate the effect of CMV infection on IL-6 and LIF production by stromal cells during 5 days because in our previous report, we have seen that CMV exposure of stromal cells reduced the capacity of these cells to support B1-CFC growth, primitive myeloid stem cells growing on a preformed stromal layer. This culture requires 5 days of incubation.27

Our results show that the laboratory strain AD169 of CMV, at an MOI of 0.1 (a titer able to inhibit significantly the B1-CFC growth), was able to induce a concomitant release of IL-6 and LIF; the release of these two cytokines was observed as early as 2 hours postinfection even when UV-inactivated CMV (but not heat-treated virus) was used, suggesting that this release was independent of viral infection and was caused by thermolabile viral protein(s). Moreover, the possibility that endotoxin could be responsible for this effect was completely ruled out by adjunction of polymyxin B in our experiments.

Diloo et al.24 noted an increase in IL-6 production from BM stromal cells infected with a combination of CMV AD169 and Davis strains.

It was also recently shown that increased messenger IL-6 levels and increased secretion of IL-6 protein were induced early after exposure of endothelial cells to live CMV (AD169) but not UV-inactivated virus.25 This study was designed to observe the effect of CMV infection of human endothelial cells as a model for examining perturbations of the marrow microenvironment. However, it has been reported that a very low amount of endothelial cells were found in the BM stromal layer.26 For this reason, this model may not be adequate to specifically study the BM microenvironment.

Other viruses have been shown to induce IL-6 production in monocyte/macrophages.27-29 Moreover, a recent study showed that infection of peripheral blood mononuclear cells with human T-lymphotropic viruses resulted in production of both LIF and IL-6.30 However, the mechanisms of cytokine induction by human immunodeficiency virus (HIV) are unclear at the present time.

Our experimental observations show that increased IL-6 and LIF secretion was not associated with viral infection and replication of the virus because UV inactivation of the virus and specific anti-CMV drugs were unable to prevent the CMV-induced release of IL-6 and LIF by the stromal

Table 2. Effect of Clinical Isolates of CMV on Cytokine Production by Stromal Cells

<table>
<thead>
<tr>
<th>Virus</th>
<th>IL-6 (ng/mL)</th>
<th>LIF (ng/mL)</th>
<th>IL-6 (ng/mL)</th>
<th>LIF (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.9 ± 0.5</td>
<td>0.70 ± 0.012</td>
<td>125 ± 28</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>AD169</td>
<td>10.2 ± 0.2</td>
<td>0.90 ± 0.06</td>
<td>22 ± 2</td>
<td>0.63 ± 1.14</td>
</tr>
<tr>
<td>No. 7</td>
<td>11.6 ± 2.0</td>
<td>1.26 ± 0.25</td>
<td>141 ± 14</td>
<td>1.15 ± 0.15</td>
</tr>
<tr>
<td>No. 15</td>
<td>16.8 ± 7.3</td>
<td>1.20 ± 0.23</td>
<td>13 ± 7</td>
<td>0.50 ± 0.35</td>
</tr>
</tbody>
</table>

Results represent mean ± SEM of 2 experiments.
* The release of cytokines was evaluated after 24 hours of CMV infection.
† LPS stimulated production of cytokines after 5 days of CMV infection.
cells. Thus, viral structural proteins appear to be involved in the stimulatory effect of CMV on IL-6 and LIF production. However, protein of nonviral origin induced by CMV in the HEL fibroblasts used to propagate the virus could be responsible for cytokine release. The fact that commercially available preparation containing anti-CMV polyclonal IgG could eliminate the IL-6 and LIF induction activity of CMV and that CMV-seronegative serum did not affect IL-6 and LIF secretion ruled out this hypothesis (data not shown). These results strongly suggest that viral proteins are responsible for this IL-6 and LIF release.

A recent report by Geist et al. showed that the CMV immediate early gene products upregulate expression of the tumor necrosis factor (TNF) gene and increase the amount of steady-state TNF mRNA and TNF protein. This elegant study corroborates previous observations of the effect of specific CMV viral protein on expression of cytokine gene as TNF or IL-1. On the other hand, the recombinant HIV-1 gp120 can induce a number of cytokines as IL-10, interferon-α (IFN-α), IFN-γ, IL-6, and IL-1 in resting PB mononuclear cells.

The possible involvement of viral proteins such as recombinant glycoprotein B (gB) in the CMV-mediated release of IL-6 and LIF is now under investigation in our laboratory. Indeed, the interaction of CMV envelope glycoproteins with the cellular membrane of stromal cells could be responsible for the increased production of IL-6 and LIF. Finally, our results suggest a role of IL-6 and LIF as important mediators of host responses during infection and their contribution in the inflammatory process observed during CMV infection.

On the other hand, we have also investigated whether CMV can perturbate the LPS-stimulated production of IL-6 and LIF. Several mechanisms have been proposed to explain the impaired hematopoiesis observed in CMV patients. However, the effect of CMV infection on cytokine production is still controversial. It has been shown that CMV infection of cells such as monocytes, BM stromal cells, and endothelial cells, can disturb the production of cytokines such as IL-1 and CSFs. In our present study, the LPS-stimulated production of IL-6 and LIF by CMV-infected stromal cells was dramatically decreased in comparison with the production by uninfected stromal cells and this perturbation occurred at late stages of infection (5 days postinfection). Moreover, the kinetic experiments showed that stromal cells became unable to respond to LPS stimulation 72 hours postinfection, this phenomenon being related to viral replication. Indeed, it appears that actively replicating virus in BM stromal cells is necessary to interfere with cytokine production: our observations that GCV and HPMPC restore the production of IL-6 and LIF by CMV-infected stromal cells confirm that the replication of the virus is involved in this mechanism.

Sredni et al. using a murine model, have shown that sublethal murine CMV infection led to a significant decrease in BM cellularity and in the production levels of IL-6 and CSF. This study also provided evidence for viewing myelosuppression of murine cytomegalovirus infected mice as an effect of the infection on stromal cells.

Reduced IL-6 and LIF production during prolonged infection of BM stromal cells might thus contribute to impaired hematopoiesis observed in CMV infected patients. IL-6 and LIF are known to play a role in the hematopoiesis acting synergistically with other CSFs to induce proliferation of hematopoietic progenitor cells. The lack of growth factor production by cells of the microenvironment could be responsible for the myelosuppression. This explanation is reinforced by our previous observation that a combination of various cytokines (IL-6, IL-3, G-CSF, GM-CSF) can restore the normal growth pattern of B1-CFC on CMV-infected stromal cells. Moreover, a correlation (r = .9) between the capacity of stromal cells to sustain B1-CFC growth and their production of IL-6 and LIF after CMV infection confirmed the link between decreased B1-CFC growth and disturbance of cytokine production during CMV infection.

The mechanism by which CMV interferes with IL-6 and LIF production is not completely understood and is now under investigation. The reduction in secretion of IL-6 and LIF during CMV infection was not a consequence of stromal cell damage as indicated by trypan blue exclusion. Indeed, CMV-infected cells were still able to produce other cytokines such as IL-10 and this production was similar to controls (personal data). The production of inhibitors such as TNF-α and transforming growth factor-β (TGF-β) could be involved in the decreased production of cytokines. Indeed, Michelson et al. have shown that CMV infection induces TGF-β protein production in human fibroblasts. This hypothesis is currently investigated in our laboratory. One other mechanism could be the repression of genes coding for growth factor production. Simmons et al. have studied this hypothesis and found a specific deficiency of G-CSF transcripts. Another study suggested that CMV infection of blood mononuclear cells results in a metabolic derangement of these cells, which become unable to produce and respond to IL-1 or IL-2. Thus, much remains to be determined about the mechanisms responsible for the increased constitutive production of IL-6 and LIF and for the impaired production of these cytokines after few days of infection.

It has been shown in numerous publications that CMV strain AD169 and clinical isolates can vary in their tropism. In contrast to AD169 strain, a proportion of clinical isolates can inhibit the growth of hematopoietic progenitors directly. For these reasons, we have also evaluated the effect of two clinical isolates on cytokine production by stromal cells. Our results agree with data reported by Simmons et al., which showed that various isolates of CMV can have different capabilities for cell infection and alteration of cellular gene expression. The difference between clinical isolates can perhaps explain the difference observed in CMV-infected patients in terms of myelosuppression.

In conclusion, we have shown that CMV is able to modulate cytokine production and disturbs the balanced cytokine network, which controls proliferation and differentiation of hematopoietic progenitors. The early release of IL-6 and LIF after CMV exposure could play a role in
the acute inflammatory reaction in CMV-infected patients, whereas delayed reduced IL-6 and LIF production during infection of BM stromal cells might thus contribute to myelosuppression observed in CMV-infected patients. These results suggest that impaired hematopoiesis secondary to CMV could be overcome in vivo by treatment with specific growth factors.

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Human cytomegalovirus increases constitutive production of interleukin-6 and leukemia inhibitory factor by bone marrow stromal cells

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