Human Cytomegalovirus-induced Immunosuppression

Relationship to and Prostaglandin E$_2$ in Human Monocytes

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Abstract

Cytomegalovirus (CMV) has been associated with immunosuppression. Previously CMV was reported to interfere with signal transduction pathways in T cells. In this report the mechanisms underlying CMV-mediated immunosuppression were examined. Supernatants of CMV (Strains C-87, AD-169)-infected primary human monocyte (MO) cultures inhibited mitogenic T cell proliferative responses by > 95%. The inhibitory activity was observed 24 h through day 7 postinfection. The infection of MO was associated with a sustained elevation of intracellular levels of cAMP and the release of arachidonic acid (AA) and its metabolite PGE$_2$ (activator of adenylate cyclase) in culture supernatants. The AA release was incidentally associated with TNF-α production. Monoclonal antibodies to TNF-α and pentoxyphylline (inhibitor of TNF synthesis) inhibited both AA and PGE$_2$ release. The release of AA required protein synthesis and occurred under conditions consistent with the expression of CMV immediate early genes. Treatment of MO cultures at time of infection with 100 μM indomethacin or 1 μg of TNF-α mAb abolished the CMV-induced T cell inhibitory activity of the supernatants by 100%. These data suggest that TNF dependent release of AA and PGE$_2$ contributes to CMV-induced immunosuppression. (*J. Clin. Invest.* 1996. 97: 2635–2641.) Key words: Viruses • cytokines • TNF-α • PGE$_2$ • immunosuppression

Introduction

Cytomegalovirus (CMV)[1] infection usually is mild and subclinical, however, in immunocompromised patients as in organ transplant recipients and HIV-infected patients it is associated with a number of syndromes that vary in severity and can be fatal. CMV tends to accentuate the state of immunosuppression in those patients (1–8).

Acute infection with CMV is associated with sustained general immunosuppression of the host as demonstrated in CMV mononucleosis with impaired cell-mediated immunity (8–9). The peripheral blood lymphocytes (PBLs) from these patients exhibit a diminished proliferative response to mitogens and herpes-virus antigens with a reversal of CD4/CD8 cell ratio. This has been attributed to an increase in CD8-positive cells (9). CMV-infected monocytes (MO) in vitro were reported to be more suppressive when compared to uninfected MO for autologous lymphocyte responses to concanavalin A (10). It has been suggested that in vivo MO may act as a reservoir for CMV replication and dissemination (11).

The mechanism(s) underlying CMV-mediated immunosuppression is unclear. CMV infection in vitro has been shown to affect cellular activation pathways of human fibroblasts. CMV was reported to induce the hydrolysis of phosphatidyl inositol 4,5 biphosphate (PIP$_2$), Ca^{2+} influx, and an increase in intracellular free Ca^{2+}, as well as increased cellular levels of cAMP and cGMP (12), and arachidonic acid (AA) metabolism (13, 14). More recently, it has been reported that CMV affects transmembrane signaling pathways in CD4-positive T-lymphocytes. CMV enhanced HIV replication in T cells via a cAMP and protein kinase C–dependent pathway (15).

CMV is a potential co-factor in HIV infection. CMV enhanced HIV replication in T cells and in mononuclear phagocytic cells (15–17). CMV-stimulated PBMC from CMV seropositive patients and enhanced HIV replication when co-cultured with acutely infected PBMC (18). The enhancement of HIV replication was associated with the release of TNF-α from the CMV stimulated PBMC. Moreover, CMV-induced enhancement of HIV replication was inhibited by the presence of TNF inhibitors.

In this report it is demonstrated that CMV infection of human MO in vitro was associated with enhanced TNF-α production that induced the release of AA and PGE$_2$. The latter inhibited T-cell activity, which might partially account for the immunosuppressive effects characteristic of CMV. The phase of CMV expression responsible for the induction of AA release was consistent with the conditions associated with CMV-immediate early gene expression.

Methods

Cells and viruses. PBMC were prepared from blood obtained from healthy donors by density sedimentation on Ficoll-Hypaque (Pharmacia Fine Chemicals, Inc., Piscataway, NJ) (19). MO were prepared by adherence of mononuclear cells to serum precoated plastic dishes for 1 h at 37°C as previously described (20). Adherent cells were removed from dishes by gently scraping with a plastic scraper and were resuspended at 0.5×10^6/ml in RPMI 1640 supplemented with 10% heat-inactivated FCS (Sigma Chemical Co., St. Louis, MO), 2 mM glutamine (K.C. Biologila, Lenexa, KS) and 100 U/ml penicillin (Squibb-Marsam, Inc., Cherry Hill, NJ) and 5 μg/ml gentamicin

1. *Abbreviations used in this paper:* Ag, antigen; CH, cycloheximide; CMV, cytomegalovirus; CS, culture supernatant; ID, intermediate early; Indo, indomethacin; L, late; MO, monocytes; PI, postinfection.

(Whittaker M.A. Bioproducts, Walkerville, MD). By cytochemical parameters including Wright's peroxidase and nonspecific esterase stains of cytospin preparations, the adherent cells were 95% MO. MO were cultured in triplicate at a density of 0.5×10^6 cells/well using 12-well plates. Cells were allowed to grow in culture for 24 h after aspiration of nonadherent cells. The culture media were ultrafiltered and endotoxin free. Also, endotoxin-free distilled water was used for ficoll preparation. The FCS had < 30 pg/ml of endotoxin by the *Limulus Amebocyte* Lysate assay (Sigma Chemical Co.).

Virus inactivation. The infectivity of CMV was inactivated by irradiation with ultraviolet (UV) light. Briefly the CMV stock was irradiated by putting 1.5-ml aliquots in 35-mm dishes and exposing them to 80 ergs/s/mm^2 for 10 min. The radiation source was a General Electric G8T5 "germicidal" bulb producing light at 254 nm. The dose rate was measured by a Black-ray UV intensity meter (Ultra-Violet Products, Inc., San Gabriel, CA) (21). The infectivity of the UV-treated CMV was determined by plaque assay and was found to be reduced by 96%.

HCMV-AD169 infection. MO were infected with HCMV-AD169 at an moi of 1–2 PFU/cell for 120 min. Mock infection was carried out using cell-free supernatants of noninfected human foreskin fibroblasts (HFF). The cells remained adherent to the plastic after infection. The virus-containing medium was then aspirated, and the cells were washed twice with PBS. The media was then added to the cells, and the cultures were maintained at 37°C for predetermined intervals. Titration of CMV was performed by plaque assay as previously described (22).

Determination of AA release. ^{3}H-AA (100 Ci/mmol; Dupont NEN, Boston, MA) was added to MO cultures at a concentration of 0.1 μCi/ml for 24 h. The cells were then washed three times with PBS and then 1 ml RPMI 1640 supplemented with 10% FCS, and L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml) was added to each well. The cells were then incubated at 37°C for 4 h, and the AA released in the culture supernatants was measured in aquasol cocktail using a β-scintillation counter.

Mononuclear cell blastogenesis assay. The PBMC were obtained from heparinized blood from normal blood donors by layering onto Ficoll-Hypaque gradients and separating them by density centrifugation. PBMC were washed three times and adjusted to a concentration of 2×10^6/ml in RPMI 1640 medium. PBMC (2×10^5 cells) were then incubated in quadruplicate in 96-well plates in 0.2 ml of RPMI 1640 supplemented with L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml), and 10% heat-inactivated FCS. Supernatants from uninfected and CMV-infected MO were subjected to ultracentrifugation, to precipitate the virus, at 100,000 g for 2 h at 4°C. The supernatants were passed through 0.22-μm filters then added to the cells (50 μl/well). 24 h before harvest, tritiated thymidine was added to each well (0.5 μCi/ml, 6.7 Ci/mmol; ICN Chemical and Radioisotope Division, Irvine, CA), and its uptake by the cells was determined as previously described (23).

Measurement of PGE$_2$. Sample purification was carried out using Amprep microcolumns (Amersham International PIC, Little Chulfont UK) as described (24). PGE$_2$ was then assayed in the purified samples by Amersham EIA kits using the conditions recommended by the manufacturer.

Measurement of TNF-α. TNF-α was assayed by EIA Kits from Immunogenetics Inc., Antwerp, Belgium using the conditions specified by the manufacturer.

Statistical analysis. The mean±the standard deviation of triplicate determinations was calculated and significance ($P < 0.05$) was assessed by Student's t test.

Results

CMV infection of primary human MO. CMV infectivity of MO was determined by flow cytometry. The cells were infected with HCMV strain AD169 at an moi of 2 PFU/ cell. At
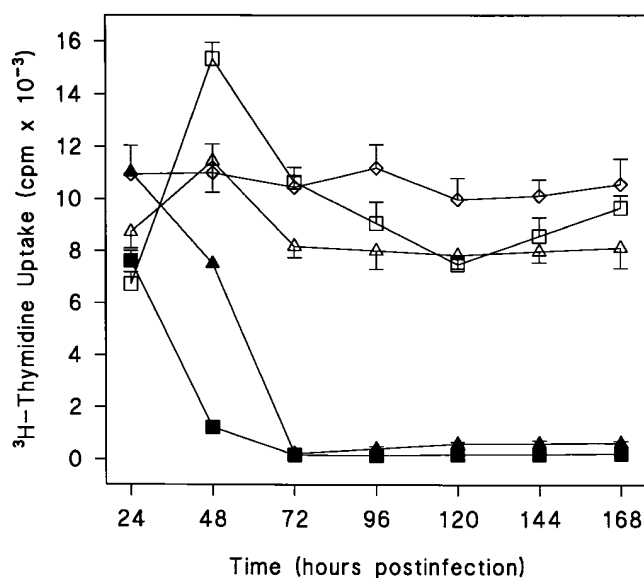

Figure 1. Effect of supernatants from CMV-infected MO cultures on PHA-induced proliferative response of PBMC from healthy donors. CS prepared at different times after CMV infection was added to PBMC in the presence or absence of PHA. [^{3}H]thymidine incorporation was determined 72 h after culture initiation. CS appeared to inhibit PHA-induced mitogenic response. CS prepared after CMV strains AD-169[■] or C-87[▲] infection or mock infection (◇) of MO cultures. Heat inactivation of CMV AD-169 (□) and C-87 (△), before challenge inhibited the induction of the CMV-induced immunosuppressive activity. Each datum point represents the mean of 3 samples ±SD, representative of three independent experiments.

selected time points postinfection (PI) the cells were analyzed for CMV immediate early (IE) and late (L) antigen (AG) expression. The percentages of MO that expressed IE Ag at 24, 72, and 144 h PI were 18, 9, and 26% and for L Ag were 0, 0, and 15%, respectively (data not shown). Furthermore, culture supernatants collected from CMV-infected MO cultures at days 7 and 14 PI were infectious to human fibroblasts and produced CMV-specific CPE with titers between 10^{-1} and 10^{-2} PFU/ml. These data indicate that MO are permissive to CMV infection and replication.

Effect of CMV-infected MO culture supernatant (CS) on PHA-induced proliferative response. MO were infected with viable or heat-inactivated (56°C for 1 h) CMV strain (AD 169 and C-87). The CS were collected daily through day 7 PI, and tested for their effect on PHA-induced proliferative responses of PBMC from healthy donors by measuring [^{3}H]thymidine uptake at 72 h after culture initiation. The thymidine uptake by CMV/CS-treated cells were significantly inhibited, compared to mock/CS-treated controls (Fig. 1). A marked enhancement in cellular proliferation, however, was observed if the CS were from heat-inactivated CMV-infected MO cultures. The effect of CMV/MO CS on the proliferation of PBMC in response to several mitogens, PHA, pokeweed mitogen and staphylococcus enterotoxin A was also tested. The addition of CS to PHA pokeweed mitogen, and staphylococcus enterotoxin A–activated PBMC resulted in 99, 98, and 99% inhibition of [^{3}H]thymidine uptake, respectively, when compared to CS from mock-treated control cells (data not shown). These findings indicate that the inhibitory effects mediated by viable

Table I. Effect of Supernatants from HCMV-Infected MO Cultures on IL-2–mediated Proliferative Response

Sample	[³H]Thymidine* uptake	Percent inhibition
	cpm	
Stimulated cells‡ + IL-2	29,679±2,153	—
Stimulated cells + IL-2§ + CS‖ (1:10)	1,474±356	93
Stimulated cells + IL-2 + CS (1:100)	18,657±1,196	10
Stimulated cells + IL-2 + CS (1:1,000)	20,208±1,432	2
Stimulated cells + medium	2,765±451	—
Stimulated cells + CS (1:10)	418±165	85
Stimulated cells + CS (1:100)	2,300±332	17
Stimulated cells + CS (1:1,000)	2,187±421	21

*0.5 μCi/ml added 24 h before measurement; ‡PBMC were stimulated with PHA (2.5 μg/ml); §10 U/ml; ‖100 ml/ml.

CMV could be due to the release of CMV-induced immuno-suppressive mediators in the CS or to inhibition of release of immunostimulatory factors by the infected cells.

Effect of CS from HCMV-infected MO on IL-2–dependent lymphocyte proliferation. The effect of CS from HCMV-infected MO on IL-2–dependent cellular proliferation was then examined. PBMC were incubated with PHA (2.5 μg/ml) for 48 h, PHA was then removed, and the cells were washed and further incubated for 72 h in the presence of IL-2 (10 U/ml) and/or CS from CMV-infected MO (100 μl/ml). IL-2–induced [³H]thymidine uptake was inhibited 93, 10, and 2% with 10, 100, and 1,000-fold dilutions of the culture supernatants, respectively (Table I). These findings indicate that CMV-induced soluble factor(s) in CS inhibit IL-2–dependent T cell proliferation.
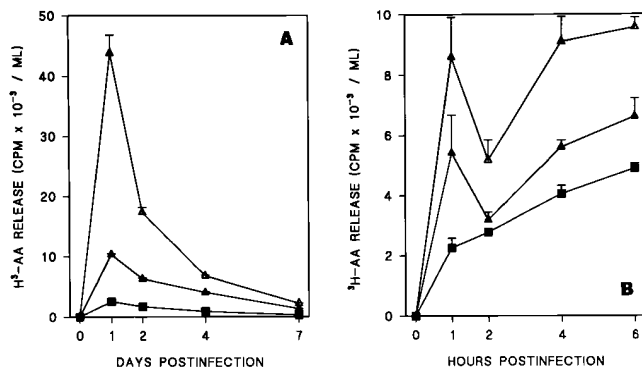

Figure 2. Effect of CMV infection on AA release in MO cultures. ³H-AA release was measured in the CS of infected and control MO cultures. MO cultures were mock infected (■), infected with viable (▲) or UV-irradiated CMV (Δ) at an moi of 2 PFU/cells or its equivalence. (*A*) The cells were radiolabeled with ³H-AA 24 h before harvest time, then they were processed for measurement of AA release. (*B*) The cells were radiolabeled for 24 h then were washed and infected for the indicated period of time. Each datum point is a mean of three samples ±SD, representative of three independent experiments.
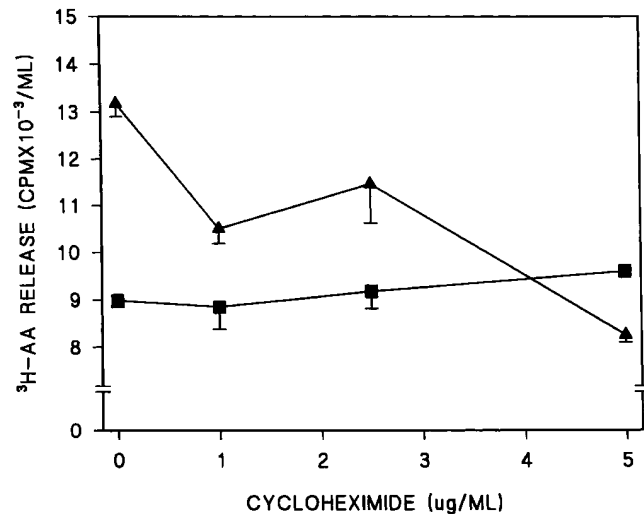

Figure 3. The effect of CH on CMV-induced AA release. 1-d-old MO were treated with CH after 2 h infection with CMV at an moi of 2 PFU/cell. AA release was measured 24 h PI. Each datum point is a mean of three samples ±SD, representative of two independent experiments. CMV-infected MO (▲); uninfected MO (□).

Effect of HCMV infection on intracellular levels of cAMP in human MO. Previously, it was shown that increasing intracellular levels of cAMP inhibited T cell activation (25, 26). Thus, whether there was an association between this phenomenon and the release of the immunosuppressive soluble factors was examined. Primary human MO were grown in vitro and infected with CMV strain AD 169 with an moi of 2 PFU/cell. CMV increased intracellular level of cAMP in the CMV-infected MO compared to the uninfected control cells through day 21 PI (data not shown). The peak of cAMP (∼ 4.0-fold) was observed 1 wk PI. These findings indicate that increased levels of cAMP in response to CMV infection of human MO might be required for CMV-induced pathogenesis.

Effect of HCMV infection on AA release in MO cultures. To test the hypothesis, whether the increase in intracellular levels of cAMP in HCMV-infected MO was due to AA released in response to CMV infection, ³H-AA release was measured in the CS of infected and control MO cultures. MO cultures were infected with viable or UV-irradiated CMV with an moi of 2 PFU/cell or its equivalence and at selected time intervals postinfection AA release was measured. At 24 h PI, viable and UV-irradiated CMV produced a 4- and 17-fold increase in AA release, respectively (Fig. 2 *A*). CMV also induced AA release early after infection. As shown in Fig. 3 *B* the pattern of AA release was biphasic with 2 peak activities at 1 and 6 h PI. Viable CMV enhanced AA release 2.3- and 1.4-fold while CMV-UV resulted in 3.8- and 2.0-fold increase at 1 and 6 h, respectively. These findings indicate that both viable and UV-irradiated CMV enhance the release of AA from MO. Interestingly, the peak of AA release preceded that of cAMP, which might indicate a cause-effect relationship between both events.

Effect of cycloheximide on CMV-induced AA release. The requirement for protein synthesis for the induction of AA was also evaluated. The MO cultures were incubated with culture medium containing nontoxic doses of the protein synthesis inhibitor cycloheximide (CH) and AA was measured 24 h PI. As shown in Fig. 3 CH inhibited the CMV-induced AA re-
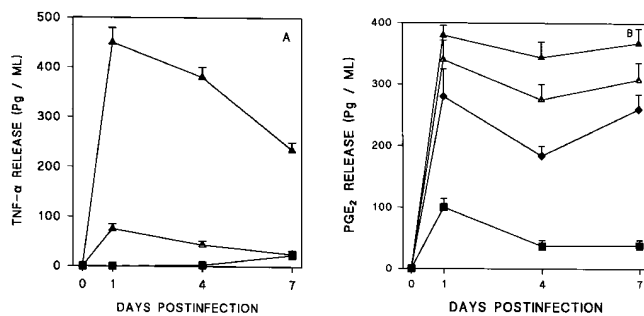
Figure 4. Relationship of CMV infection to TNF-α and PGE$_2$ release. (*A*) Effect of CMV infection on TNF-α release in MO cultures. 1-d-old MO were infected with CMV at an moi of 2 PFU/cell. At selected time intervals TNF-α was measured in presence or absence of pentoxyfylline (an inhibitor of TNF production). Mock-infected (■), CMV infected (▲) or CMV-infected pentoxyfylline (800 μM)-treated cells (Δ). (*B*) Effect of TNF-α inhibitors on CMV-induced release of PGE$_2$. MO cultures were infected as described above in the presence or absence of the appropriate treatments. Mock-infected cells (■), CMV-infected cells (▲), CMV-infected + and TNF-α mAb (Δ), CMV-infected cells + 800 μM pentoxyfylline (♦). Each datum point is a mean of triplicate samples ±SD, representative of two independent experiments.

lease in a dose-dependent manner. This indicated that protein synthesis was required to induce AA.

Effect of CMV infection on TNF-α release in MO cultures. HCMV has been shown to induce TNF-α secretion from PBMC and was also reported to activate phospholipase A$_2$ (PLA$_2$)in mononuclear phagocytes. Whether the observed increases in AA release in CMV-infected MO were related to
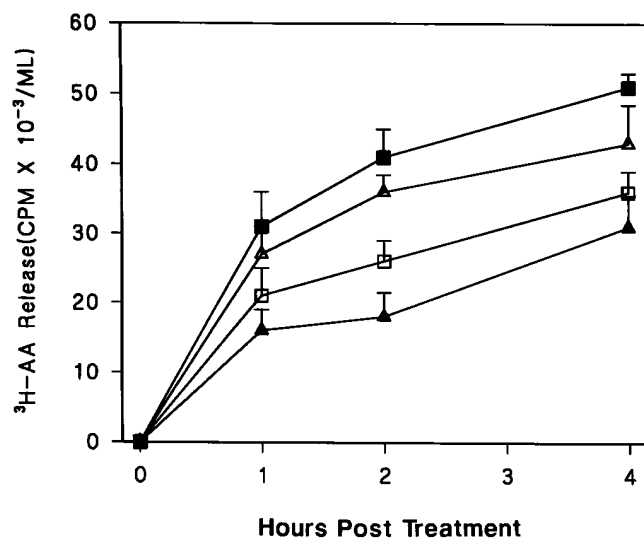

Figure 5. Time course for the effect of recombinant human TNF-α (0.1 μg/ml) and neutralizing mAb to TNF-α (0.1 μg/ml) on AA release in MO cultures. ³H-AA release was measured in supernatants at 1, 2, and 4 h after treatment. Control MO (□), TNF-treated MO (■), MO treated with anti TNF-α mAb (Δ), and MO treated with both TNF-α and anti TNF-α mAb (▲). Each datum point represents mean of three samples, representative of three independent experiments. The differences between TNF-treated samples and samples treated with mAb to TNF were statistically significant. At 2 h ($P < 0.007$) and 4 h ($P < 0.002$).

enhanced TNF release was examined. TNF-α was measured in the CS of CMV-infected MO at days 1, 4, and 7 PI. CMV-induced TNF release was maximal at 24 h PI followed by a progressive decline through day 7 PI (Fig. 4 *A*). However, when the infection was carried out in the presence of 800 μm pentoxifylline (an inhibitor of TNF synthesis), TNF release was inhibited approximately sixfold at day 1 PI compared to CMV alone. Furthermore, the peak of TNF release coincided with that of AA at day 1 PI, which might indicate the involvement of TNF-α in the AA release of CMV-infected MO.

Effect of TNF on AA release in MO cultures. To further elucidate the relationship between TNF and AA, the effect of TNF on AA release in uninfected MO was examined. MO were labeled with ³H-AA (1 μci/ml) for 24 h then washed and treated with recombinant TNF-α and/or anti–TNF-α mAb (0.1 μg/ml), and AA release was then measured 1, 2, and 4 h later. The results as depicted in Fig. 5 showed that TNF significantly enhanced AA release. Maximum enhancement was observed 2 h after TNF-α was added. Furthermore, incubation of the cells in the presence of both TNF-α and anti–TNF-α Ab was associated with a marked drop in AA release below the control values. These data suggest that steady state AA release in MO might be regulated by TNF-α.

Relationship of CMV-induced release of PGE$_2$ to TNF-α in MO cultures. AA is the parent compound of PGE$_2$ and the latter is known to inhibit T cell activation (27, 28). It was thus of interest to determine if there was a correlation between CMV-induced TNF-α production and PGE$_2$ release in human MO. MO were infected with 2 PFU/cell of CMV, and PGE$_2$ release was measured in CS at 1, 4, and 7 d PI by EIA. As shown in Fig. 4 *B*, HCMV enhanced PGE$_2$ release at day 1 PI 38-fold above the level in control cells. The virus-induced PGE$_2$ release was maintained at a near plateau level through day 7 PI. However, when the infection was carried out in presence of anti–TNF-mAb (0.1 μg/ml) or pentoxifylline (800 μm), 21 and 47% inhibition of the virus-induced PGE$_2$ release was observed at day 4 PI, respectively. These findings indicate that PGE$_2$ release in response to CMV infection is regulated via a TNF-dependent pathway.

Effect of anti–TNF-α Ab and indomethacin on CMV-mediated suppression of PHA-induced proliferative response of PBMC from healthy donors. The possibility that the HCMV-induced AA and PGE$_2$ release might have immunosuppressive effects on nearby uninfected cells was next examined. CMV-infected and uninfected MO cultures were grown in the presence or absence of either anti–TNFα mAb or indomethacin (Indo). The CS were then collected and tested for their effect on PHA-induced proliferative response of PBMC. CS from untreated CMV-infected cultures at days 1 and 7 PI inhibited PHA-induced [³H]thymidine uptake by 26 and 71%, respectively (Table II). Interestingly, anti–TNF-mAb (0.1 μg/ml) reversed 100 and 71% of the CMV/CS-induced inhibition at days 1 and 7, respectively. These findings indicate that the inhibition of cell proliferation might be mediated by TNF-α. Similarly, supernatants from indomethacin (Indo)-treated cultures reversed 100% of the HCMV-induced inhibition of [³H]thymidine uptake. The presence of Indo together with CMV/CS enhanced cellular proliferation by 145 and 83% above the control levels at days 1 and 7 PI, respectively (Table II). These data suggest that CMV-induced release of TNF-α contributes to CMV/CS-induced inhibition of PBMC proliferation via a PGE$_2$-dependent pathway.

Table II. Effect of TNF-mAb and Indo on CMV-induced Inhibition of PBMC Proliferative Response

	[³H]Thymidine uptate (cpm)			
	day 1	% change	day 7	% change
MO	9,053±2,323[§]		10,142±2,423[§]	
MO + anti–TNF-α	10,608±1,990	+17	11,396±1,562	+3
MO + Indo	9,817±863	+8	13,704±1,012	+35
MO + HCMV*	6,737±207	−26	2,976±612	−71
MO + HCMV + anti–TNF-α[‡]	9,465±1,673	+5	7,986±1,119	−21
MO + HCMV + Indo[‖]	22,201±1,285	+145	18,551±1,653	+83

*HCMV strain Ad-169 was used for infection at an moi of 2 PFU/cell. Supernatants from day 1 and day 7 were tested for their effect of PHA-induced proliferative response in the presence or absence of the appropriate treatment. PHA concentration was 2.5μg/ml. [‡]Anti–TNF-α-mAb concentration was 0.1 μg/ml. [‖]Indomethacin concentration was 100 μM. [§][³H]Thymidine uptake measured 72 h after culture initiation. (mean±SD), representative of three independent experiments.

The phase of CMV expression responsible for induction of AA. The release of AA and the induction of TNF-α and PGE$_2$ in CMV-infected MO occur at early times PI. To more precisely determine the phase of CMV-gene expression responsible for AA release, protein synthesis was blocked in infected cells with cycloheximide at −2 h and then released from the translational block in the presence or absence of functional transcriptional block (3′-deoxyadenosine) at 2 h PI. These conditions permit expression of CMV immediate early genes but not later genes when the CH block is released in the presence of 3′-deoxyadenosine (29). When the CH block is released in the absence of a transcriptional block, the early and late genes may then be expressed. Table III shows a summary of the data of a representative experiment. CMV-infected MO at 24 h PI had an enhanced AA release of 41% above the control. The presence of 3′-deoxyadenosine from 2 through 24 h PI after the release of CH block further enhanced the AA release to 71%. There was no significant difference in AA release between 3′-deoxyadenosine-treated (2–24 h PI) and untreated cultures after the release from the CH block. These data suggest that the AA release was the result of synthesis of mRNA in the absence of prior protein synthesis and consistent with the conditions associated with immediate early gene expression.

Discussion

The data presented in this report show that CMV infection of human MO was associated with the release of soluble factor(s) that inhibited T cell activity. The immunosuppressive activity was apparently regulated and mediated by TNF-α and PGE$_2$. Although the infection was confined to a limited number of cells, the culture supernatants were infectious 2 wk after infection. This is in agreement with other reports demonstrating IE and L CMV Ag expression and CMV replication in MO cultures (30). These observations would support the conclusion that MO are primarily involved in HCMV infection and CMV-induced pathogenesis and might serve as a reservoir for CMV replication and dissemination.

CMV was shown to affect transmembrane signaling pathways (12–14) including the cAMP-dependent protein kinase A pathway (15, 31). cAMP serves as a multifunctional relay for mitogenic stimuli acting as an intracellular signal for activation of eukaryotic cells. Recently, it was reported that CMV en-

Table III. Phase of CMV Gene Expression Responsible for Induction of AA Release

	Treatment			
CMV*	Cycloheximide[‡]	3′-Deoxyadenosine[§]	³H-AA[‖]	Change in AA release
			cpm/ml	%
−	—	—	10,005±345	—
+	—	—	14,142±566	41[¶]
+	−2–2 h	—	16,932±469	69**
+	−2–2 h	2–24 h	17,132±751	71**
+	−2–24 h	—	10,240±358	2
+	—	2–24 h	26,010±1,456	160
−	−2–24 h	—	10,688±561	6.5
−	—	2–24 h	20,907±911	109
−	22–2 h	2–24 h	11,885±504	19

*moi, 2 PFU/cell; [‡]10 μg/ml; [§]20 μg/ml; [‖]measured at 24 h PI, mean of three cultures processed separately±the standard deviation, representative of three independent experiments; [¶]statistically significant relative to the mock-infected control $P < 0.01$; **statistically significant relative to the mock-infected control $P < 0.001$.

hanced HIV replication in CD4+ T cells partially via a cAMP-dependent mechanism (15). The data from Figs. 2 and 3 show a correlation between the increase in intracellular levels of cAMP and the immunosuppressive activity of the CMV/MO CS. These observations indicate that cAMP might be involved in CMV-induced pathogenesis.

Since PGE_2 is known to activate adenylate cyclase, we sought to determine if AA release and PGE_2 synthesis are triggered by HCMV infection of MO. CMV infection enhanced AA and PGE_2 several fold in MO cultures (Figs. 3 and 5 *B*), which might explain the observed increase in intracellular levels of cAMP. The AA and PGE_2 release preceded the cAMP peak observed 1 wk PI, which might indicate a cause-effect relationship between both events. The kinetics of TNF-α release during CMV infection revealed that the peak of TNF-α release in culture supernatants coincided with that of AA and PGE_2, suggesting that the release of the latter might be TNF mediated. This hypothesis is supported by the fact that (*a*) anti–TNF-α and pentoxifylline reduced the release of PGE_2 from infected MO, (*b*) TNF-α was able to induce AA release when added to mock-infected MO, and (*c*) anti–TNF-α mAb resulted in a marked drop in AA release below the control values (Fig. 5). In several other cell systems (32–34) it has also been shown that TNF-α can activate PLA_2, the enzyme that cleaves AA from phospholipids. In the current study the induction of AA and PGE_2 required protein synthesis and was apparently triggered by immediate early CMV-genes (Table III).

CMV infection can interfere with the inflammatory function of MO, which may lead to the immunosuppressive activities associated with CMV. Data from the present study showed an enhancement of TNF-α release in HCMV-infected MO CS. The latter markedly inhibited the proliferative response of normal PBMC. The inhibition was reversed to approximately control levels when MO were grown in the presence of mAb to TNF-α (Table III). These findings are in agreement with others and support the concept that TNF-α is a key mediator in the pathogenesis of HCMV disease (18, 35). TNF-α has been identified as an important factor in the pathogenesis of AIDS. It was previously shown that CMV-activated PBMCs enhanced HIV replication via a TNF-α–dependent pathway (18). Down-regulation of TNF-α gene expression or neutralization of its activity may thus be beneficial in down-regulating both viruses and their concomitant pathology (35).

CMV may also influence HIV pathogenesis through superantigen induced activity. Recently it was reported that CMV-infected MO enhanced HIV replication in Vβ-12 T cells. This enhancement required direct cell to cell contact and viable virus, and it was mediated via a CMV gene product that possesses a superantigen-driven Vβ-12 selective property (36). This may affect the rate of HIV replication and consequently lymphocyte depletion leading to immunosuppression.

PGE_2 is primarily a suppressor of immune function as demonstrated in vitro by its ability to inhibit the production of immune regulatory lymphokines and T and B cell proliferation (28, 29, 37, 38). It is thus possible that the PGE_2 released in response to CMV infection contributed to the immunosuppressive activity of CMV. This is not inconceivable, particularly since CS-induced inhibition of T cell proliferation was associated with increased levels of PGE_2. Moreover, CS from indomethacin-treated cells markedly reversed the CMV-induced inhibition of PBMC proliferation to levels above the control values. Meanwhile, it is still possible that the CMV-mediated

inhibition of cellular proliferation was through a direct effect of AA released on T cells. It has been reported that AA inhibited IL-2 production in PBMC and Jurkat cells even at times of minimal or no PGE_2 release by the treated cultures (39). However, it is noteworthy to mention, that the exogenous addition of AA did not affect the PHA-induced proliferative response of PBMC (data not shown).

Taken altogether, the data presented in this report indicate that CMV-infection of peripheral blood MO induces the release of PGE_2 via a TNF-α-dependent pathway. The PGE_2 produced apparently contributes to CMV-mediated immunosuppression and might be involved in the pathogenesis of CMV disease. Understanding the mechanisms and the factors affecting CMV-induced pathogenesis might allow modulation of the impaired immune response in patients with CMV disease.

Acknowledgments

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