Modulation of Interleukin 1 Beta Gene Expression by the Immediate Early Genes of Human Cytomegalovirus

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Abstract

The immediate early (IE) genes of human cytomegalovirus (HCMV) can be expressed in monocytes/macrophages and are known to regulate other viral genes. The purpose of these studies was to determine if HCMV IE gene products also modulate expression of a monocyte/macrophage-derived gene, interleukin 1 (IL-1) beta. Steady-state cell-derived IL-1 beta mRNA was increased in lipopolysaccharide (LPS)-stimulated THP-1 cells when transfected with the HCMV IE1 + 2 genes, when compared to cells transfected with a control DNA. LPS-stimulated THP-1 cells also exhibited ~ 30-fold higher IL-1 CAT activity when cotransfected with IE1 + 2 than was observed for the same cells cotransfected with IL-1 CAT and a control plasmid containing the IE promoter alone. LPS increased IL-1 CAT activity in the absence of HCMV genes only twofold. IE1, by itself, increased IL-1 CAT activity in LPS-stimulated cells, whereas, IE2, by itself, caused no change in IL-1 CAT activity. These studies show that the IE1 gene of HCMV can regulate IL-1 beta gene expression. The observations further suggest that some of the inflammatory processes associated with HCMV infection may be due to an effect of HCMV IE genes on cell-derived genes, such as the IL-1 beta gene. (*J. Clin. Invest.* 1990. 85:1853–1857.) interleukin 1 • cytomegalovirus • immediate early genes

Introduction

Human cytomegalovirus (HCMV)[1] infection is an important cause of morbidity and mortality in immunocompromised hosts and usually results from activation of a latent infection (reviewed in references 1 and 2). Replication of HCMV is associated with sequential expression of three classes of viral genes: immediate early (IE), early, and late genes (3–5). Expression of HCMV IE genes can be detected in monocytes from HCMV-infected patients (6–8).

The IE genes of HCMV have been shown to regulate their own expression (9, 10) and the expression of early and late viral genes (9, 11–14). In addition, the HCMV IE genes have a positive effect on human immunodeficiency virus gene expression (15) and HCMV infection appears to facilitate HIV infection (16). These observations suggested that the IE genes of HCMV may also regulate cellular gene expression in monocytes/macrophages. This hypothesis is important to the pathogenesis of HCMV-related disease since the regulation of cellular genes by IE genes might play an important role in modulating the inflammatory processes that are seen in HCMV infection. To evaluate this hypothesis, we transfected plasmid constructs containing the IE genes of HCMV into a myelomonocytic cell line, THP-1, to evaluate the effects of HCMV IE gene products on interleukin 1 (IL-1) beta gene expression. IL-1 is a cytokine released from monocytes/macrophages that plays a major role in mediating a variety of inflammatory processes associated with various disease states (reviewed in references 17 and 18).

Methods

Reagents. Lipopolysaccharide (LPS; *Escherichia coli* 026:B6), formamide, Denhardt's solution, salmon testes DNA, acetyl CoA, diethyl pyrocarbonate, and guanidine isothiocyanate were obtained from Sigma Chemical Co. (St. Louis, MO). DEAE Dextran and *Escherichia coli* 23S and 16S rRNA were obtained from Pharmacia Five Chemicals (Uppsula, Sweden). Gene Screen Plus, ^{32}P-CTP, and ^{14}C-labeled chloramphenicol were obtained from New England Nuclear (Boston, MA).

Tissue culture. THP-1, a myelomonocytic cell line was obtained from American Type Culture Collection (Rockville, MD). The cells were maintained in suspension cultures in RPMI 1640 medium containing 10% FBS (HyClone Laboratories, Logan, UT), 4 mM L-glutamine, and 50 μg/ml gentamicin.

Plasmid constructs. The construction of plasmids pCAT760 containing the wild-type promoter-regulatory region of HCMV (Towne strain) major IE gene upstream from the procaryotic chloramphenicol acetyl transferase (CAT) gene, pLINK760 containing the promoter-regulatory region of the HCMV major IE gene alone, and plasmids containing the promoter-regulatory region of the HCMV major IE gene upstream of the bona fide IE1 (pCC), IE2 (pCSdlAcc), or IE1 + 2 (pCS) genes have been previously described (12, 19–20). The IL-1-CAT plasmid consists of the promoter region of the human pro IL-1 beta gene located between positions −1097 and +14 derived from the BDC-454 clone, described by Clark et al. (21, 22), inserted into plasmid pSV-CAT-3M (30).

Transfection. Transfections of THP-1 cells were performed using the DEAE transfection method (23). Cells were exposed to plasmids in a DEAE dextran solution for 60 min and then washed once in RPMI 1640 medium containing 1.5 U of heparin per milliliter and once in the same medium without heparin. The cells were then cultured in 100-mm plates at a concentration of 1×10^6 cells/ml in RPMI-1640 with 10% FBS. The cells remained either unstimulated for 48 h or were stimulated with LPS 24 h after dextran transfection and were harvested at various time points for RNA or at 24 h after LPS stimulation for the

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1. *Abbreviations used in this paper:* CAT, chloramphenicol acetyl transferase; HCMV, human cytomegalovirus; IE, immediate early; IL-1, interleukin 1; LPS, lipopolysaccharide.

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CAT assays. Transfection was optimized at 250 μg/ml of DEAE dextran and 1 μg/ml of plasmid.

CAT assay. CAT assays were performed as described by Gorman et al. (24). The acetylated derivatives were separated from the nonacetylated chloramphenicol by ascending chromatography using chloroform/methanol (95:5). The plates were exposed to Kodak XAR-2 film. Quantitation was performed on a thin layer chromatograph scanner from Radiomatic Instruments & Chemical Co. Inc. (Tampa, FL).

IL-1 mRNA. Whole cell RNA was isolated by the guanidine isothiocyanate CsCl method (25). The RNA was fractionated in a 1.5% denaturing agarose gel containing 2.2 M formaldehyde by the method of Lehrach et al. (26). *E. coli* 23S and 16S rRNA served as standards. The RNA was transferred to Gene Screen Plus as recommended by the manufacturer. Dot blot analysis was performed using a Gene Screen Plus membrane in a dot blot apparatus (Bio-Rad Laboratories, Richmond, CA) and was quantitated using twofold dilutions in H_2O starting from 10 μg in a solution of 50% formamide and 2.2% formaldehyde. A ^{32}P-labeled IL-1 beta probe was prepared using a random label primer kit from Bethesda Research Laboratories (Gaithersburg, MD). The IL-1 beta probe contained the coding region for amino acids number 1 to 139 of the complete 35-kD IL-1 beta precursor and was a kind gift of Dr. U. Gubler (27, 28). The Northern and dot blots were prepared as we previously described (29). Briefly, the blots were baked at 80°C for 2 h and then prehybridized in 46% formamide, 1 M NaCl, 10% Dextran, 0.05 M Tris, 1% SDS, 1× Denhardt's solution for 5 h and hybridized in the same solution with 10×10^6 cpm of labeled probe and 200 μg/ml of salmon sperm DNA. After hybridization, the blots were washed as suggested by the manufacturer.

Results

To evaluate the effects of the IE genes on endogenous IL-1 beta steady-state mRNA, THP-1 cells were transfected with pLink760 (the control plasmid) or pCS (IE1 + 2), and 24 h after transfection they were stimulated with LPS (10 μg/ml) for various periods of time. Northern blot analysis of whole cell RNA from THP-1 cells detected a band at 1.6 kb, which corresponds to the size of IL-1 beta mRNA (data not shown). No IL-1 mRNA was detected from Jurkat cells (a T cell line), which was used as a negative control. Dot blot analysis of whole cell RNA was used to quantitate differences between cells transfected with control or pCS (IE1 + 2) plasmids. An increase in steady-state IL-1 beta mRNA was detected at 3, 6, and 24 h after LPS stimulation in the cells transfected with the pCS (IE1 + 2) plasmid compared to cells transfected with the control plasmid (Fig. 1). The dot blots were washed and reprobed for actin mRNA, which showed equal amounts of steady-state mRNA at all time points in cells transfected with the control or pCS (IE1 + 2) plasmids (data not shown). No IL-1 beta mRNA was detected at the 0 h time point (i.e., after transfection but before stimulation with LPS) (data not shown). In addition, in control THP-1 cells transfected with pLink or PCS but not stimulated with LPS, no IL-1 beta mRNA was detected at the 3-, 6-, or 24-h time points (data not shown). We conclude that HCMV IE genes caused a significant increase in the IL-1 beta steady-state mRNA.

To evaluate the effects of HCMV IE1 and IE2 genes on IL-1 beta gene expression, THP-1 cells were first transfected with either the IL-1 CAT or pCAT760 plasmids alone. The IL-1 CAT was expressed at a low level in unstimulated cells and increased only twofold when the cells were stimulated with optimal amounts (10 μg/ml) of LPS for 24 h (data not shown). Expression from the HCMV major IE promoter using

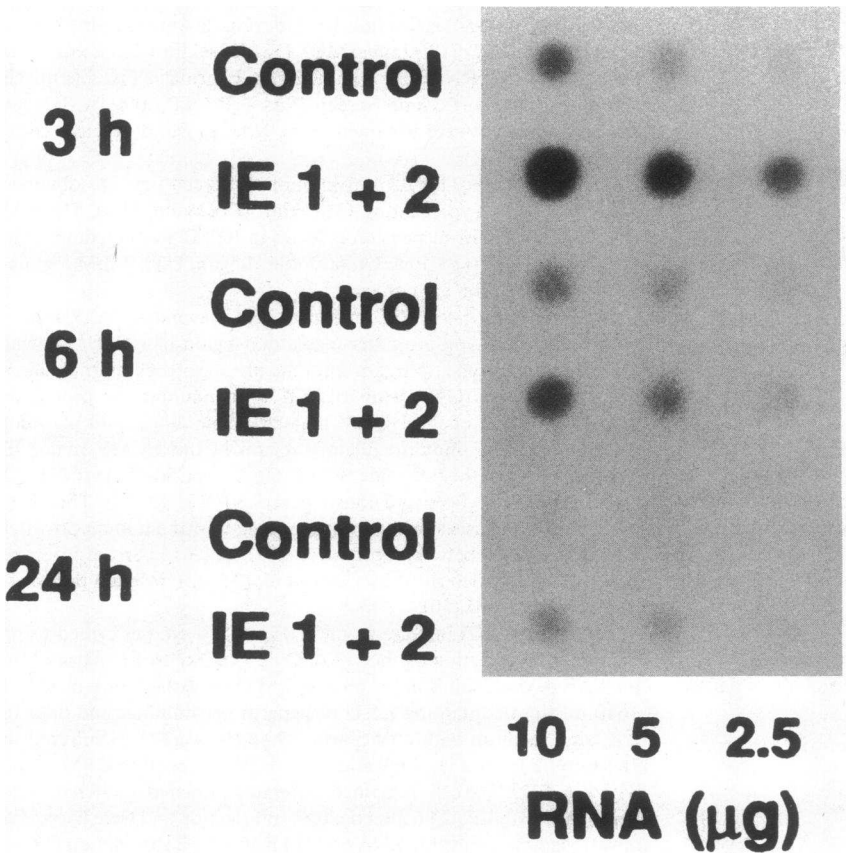

Figure 1. Dot blot analysis for IL-1 beta mRNA. THP-1 cells were transfected with pLink760 and pCS (1 μg/ml). 24 h after transfection, all cells were stimulated for 3, 6, or 24 h with LPS (10 μg/ml). The concentration of whole cell RNA is on the abscissa. The plasmids transfected into the cells and length of time they were stimulated with LPS is on the ordinate. No IL-1 beta mRNA was detected at the 0-h time point (i.e., after transfection but before stimulation with LPS) (data not shown). In addition, in control cells transfected with pLink or pCS but not stimulated with LPS, no IL-1 beta mRNA was detected at the 3-, 6-, or 24-h time points (data not shown).

pCAT760 also exhibited a low baseline level of expression and increased twofold with stimulation of the cells with LPS (10 μg/ml) (data not shown). IL-1 gene expression, as measured by CAT activity, showed a dose-response effect to increasing amounts of LPS in cells cotransfected with the IL-1 CAT and pCS (IE1 + 2) plasmids (Fig. 2 *A*). A dose-response effect on IL-1 gene expression was also noted with increasing amounts of the pCS (IE1 + 2) plasmid in LPS stimulated cells (Fig. 2 *B*). The optimum amount of LPS was 10 μg/ml and the optimum amount of pCS was 1 μg/ml for maximum expression of IL-1 CAT activity.

Cotransfection of the IL-1 CAT plasmid with plasmid constructs containing either pLink760 (the control plasmid), pCC (IE1), pCSdlAcc (IE2), or pCS (IE1 + 2) were performed to assess the effect of the individual HCMV IE genes. Cotransfection of the IL-1 CAT plasmid with pLink760 (control) resulted in a low level of expression which increased 1.5-fold when the cells were stimulated with LPS (10 μg/ml). Cotransfection of the IL-1 CAT plasmid with pCC (IE1) resulted in a 7.5-fold increase in CAT activity in unstimulated cells and a 15-fold increase in LPS-stimulated cells (Fig. 3). The plasmid, pCSdlAcc (IE2), had no effect on IL-1 CAT activity in unstimulated cells and LPS-stimulated cells. Cotransfection of the IL-1 CAT plasmid with pCS (IE1+2) showed a ninefold increase in unstimulated cells and a 33-fold increase in stimulated cells (Fig. 3).

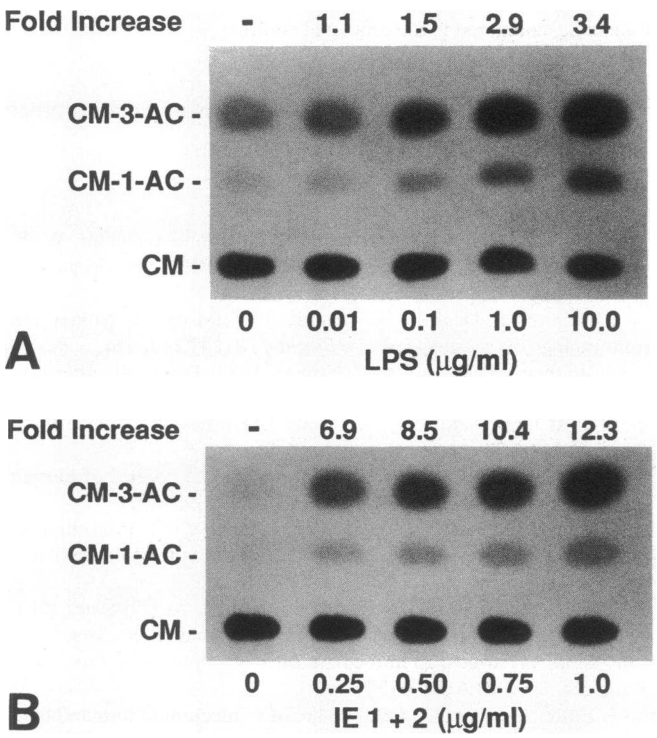

Figure 2. Effects of increasing amounts of LPS and HCMV IE1 + 2 gene products on expression of the IL-1 beta gene. (*A*) Effects of LPS. THP-1 cells (10^6/ml) were transfected using the DEAE dextran (250 μg/ml) method with IL-1 CAT (1 μg/ml) and pCS (1 μg/ml; IE1 + 2). 24 h after transfection, the cells were stimulated with increasing amounts of LPS for 24 h. CAT assays were performed as described in Methods. The ordinate shows chloramphenicol (*CM*) and the 1 and 3 acetylated forms of chloramphenicol (*CM-1-AC* and *CM-3-AC*). The concentrations of LPS are on the abscissa. IL-1 CAT activity is expressed as fold increase, compared to control. (*B*) Effects of IE1 + 2. THP-1 cells were transfected with IL-1 CAT (1 μg/ml) and increasing amounts of pCS (IE1+2). The total amounts of IE plasmids were maintained constant by adding to pCS the control plasmid (pLink760) to a total concentration of 1 μg/ml. 24 h after transfection, the cells were stimulated with LPS (10 μg/ml) for 24 h. CAT assays were performed as described in Methods. The amount of pCS (IE1 + 2) plasmid is on the abscissa. IL-1 CAT activity is expressed as fold increase, compared to control.

Discussion

These studies suggest that the HCMV IE gene products enhance expression from the IL-1 beta promoter and that the major contributor to enhanced expression is the IE1 gene product. The enhanced IL-1 beta gene expression was shown by quantitation of IL-1 beta mRNA and IL-1 CAT activity. The upregulation of the IL-1 beta gene does not appear to be a generalized activation of all cell genes since the amount of actin mRNA was not affected by the IE gene products. The effects of the IE gene products on IL-1 gene expression in this study are consistent with earlier studies which evaluated the effects of HCMV on cell protein synthesis. In this regard, HCMV infection, in contrast to other herpes virus infections, can cause an increase in host-cell DNA, RNA, and protein synthesis (31–34). Cells infected with HCMV show an initial decrease in protein synthesis followed by a very significant increase in protein synthesis. Since inactivated virus causes the initial decrease in protein synthesis but fails to induce protein synthesis at later time points, stimulation of protein synthesis appears to require a virally encoded protein.

The induction of IL-1 beta mRNA by LPS alone in THP-1 cells that was observed in this study is similar to that previously reported (35). THP-1 cells have been shown to produce similar amounts of IL-1 compared to blood monocytes when stimulated with LPS (36). IL-1 beta mRNA in THP-1 cells shows an initial increase with a peak at 3–4 h after stimulation and then a rapid decrease to a low steady-state (35). Although the IE gene products increased the relative amount of IL-1 mRNA, the pattern of the IL-1 steady-state RNA did not appear to be altered. The increased production of IL-1 mRNA in the presence of IE gene products is similar to what has been observed by Fenton et al. (35), when THP-1 cells are treated with LPS in the presence of cycloheximide. Although it is likely that HCMV IE gene products enhance the initiation of gene transcription, it is also possible that they block the effect of an LPS-induced repressor molecule, as has been postulated for the cycloheximide-induced superinduction of IL-1 beta mRNA (35). The fact that IE gene products also have a positive effect on IL-1 CAT expression renders unlikely a mechanism involving increased mRNA stability. The IE1 gene product positively regulates its own promoter (10); therefore, it is likely that the viral transactivator also positively regulates certain cellular promoter-regulatory regions. What these regulatory regions have in common for positive regulation by IE1 is presently unknown.

The IE gene products increased transcription from the IL-1 promoter independently of LPS stimulation (Fig. 3). The amount of transcription was markedly increased when the cells were stimulated with LPS. Although transcription from the IL-1 promoter could be induced by the IE gene products

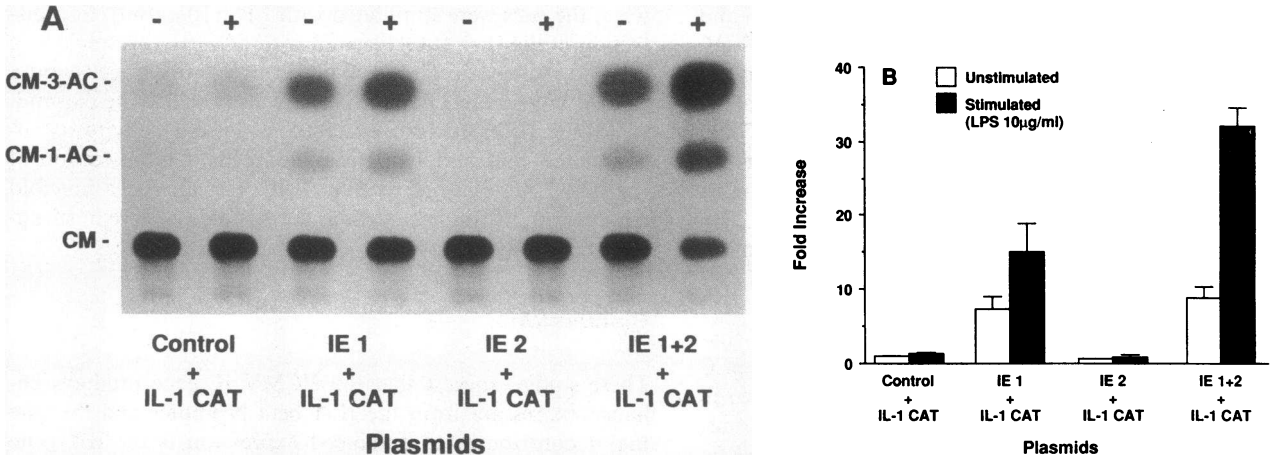

Figure 3. Effect of individual HCMV IE gene products on expression of the IL-1 beta gene. THP-1 cells were transfected, using the DEAE dextran (250 μg/ml) method (23) with IL-1 CAT (1 μg/ml) and 1 μg/ml of either pLink760 (control), pCC (IE1), pCSdlAcc (IE2), or pCS (IE1 + 2). The cells either remained unstimulated (−) or, 24 h after transfection, were stimulated (+) with LPS (10 μg/ml) for 24 h. CAT assays were performed as described in Methods. The IE plasmids are indicated on the abscissa. (*A*) A representative experiment. (*B*) A summary of three separate experiments. IL-1 CAT activity is expressed on the ordinate as fold increase, compared to unstimulated controls.

alone, IL-1 mRNA was detected only when the cells were also stimulated with LPS. This likely is explained by the presence of an AU-rich region in the 3′ untranslated region of the IL-1 mRNA (28, 45). For other genes, it is known that the presence of this AU rich region markedly affects mRNA stability and/or efficiency of translation (41–44). This observation suggests that it would be unlikely that there would be the same fold increase in IL-1 steady-state mRNA as the increase in IL-1 CAT activity. However, the IE gene products clearly increased the amounts of IL-1 steady-state mRNA produced in response to stimulation with LPS (Fig. 1).

Studies using whole virus to infect monocytes have noted a decrease in IL-1 activity (37, 38). However, the decreased IL-1 activity appears to be due to an inhibitor produced by the infected monocytes at late times after infection (35). Thus, it is not clear from these studies, whether the cells actually secreted less IL-1 protein. The assays used to measure IL-1 in these studies are also sensitive to inhibitors of IL-1.

This is the first demonstration that transfected HCMV IE genes can upregulate the cell-derived IL-1 beta gene. However, other viral regulatory genes, such as the HTLV I tat gene have been shown to regulate the cell-derived interleukin-2 (IL-2) and interleukin-2 receptor genes (39). The upregulation of the IL-2 gene by the tat gene products of HTLV I also required stimulation of the cells with a mitogen (39). Interestingly, the IE genes of HCMV also positively regulate the promoter of the human immunodeficiency virus (15). Furthermore, it has been demonstrated that there is a subset of patients with AIDS whose monocytes spontaneously release IL-1 (40). Finally, although the IE gene products do not upregulate all cell genes (i.e., the actin gene), they may upregulate the genes of other cytokines, such as tumor necrosis factor or interleukin-6, which have related control elements. These observations may explain some of the systemic manifestations of AIDS, such as fever, hypergammaglobulinemia, and the wasting syndrome (18). Furthermore, HIV-induced brain lesions are more severe when there is coinfection of the tissue with HCMV (16), which suggest that HCMV may play an important role in various inflammatory processes not only via direct viral infection of involved tissues but also by modulating other viral and cell-derived genes.

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