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Research Article

Cytomegalovirus (CMV) is an important cause of disease in the immunocompromised patient and CMV infection is associated with predominantly mononuclear inflammatory response. Since products of the CMV immediate early (IE) gene region are potent trans-activators, we used the monocyte cell line THP-1 and a transient transfection assay to determine if these viral proteins upregulate expression of the TNF gene. The IE genes of CMV upregulated TNF gene activity as judged by increases in promoter activity, steady state mRNA, and protein production. The presence or absence of the 3' untranslated region of the TNF gene did not affect gene expression induced by the IE gene products. These studies suggest that activation of TNF gene expression by the CMV IE gene products may, in part, account for the inflammatory response associated with CMV infections.

The Immediate Early Genes of Human Cytomegalovirus Upregulate Tumor Necrosis Factor-α Gene Expression

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Abstract

Cytomegalovirus (CMV) is an important cause of disease in the immunocompromised patient and CMV infection is associated with predominantly mononuclear inflammatory response. Since products of the CMV immediate early (IE) gene region are potent *trans*-activators, we used the monocyte cell line THP-1 and a transient transfection assay to determine if these viral proteins upregulate expression of the TNF gene. The IE genes of CMV upregulated TNF gene activity as judged by increases in promoter activity, steady state mRNA, and protein production. The presence or absence of the 3′ untranslated region of the TNF gene did not affect gene expression induced by the IE gene products. These studies suggest that activation of TNF gene expression by the CMV IE gene products may, in part, account for the inflammatory response associated with CMV infections. (*J. Clin. Invest.* 1994. 93:474–478.) Key words: Northern analysis • ELISA • CAT assay • transfection • untranslated region

Introduction

TNF is a cytokine with many biological effects (1). One of the important functions of TNF is to modulate inflammatory processes during various types of infection, especially bacterial infections (2–4). TNF has also been associated with the wasting syndromes that characterize chronic infections or malignancy (5), as well as with the lethal effects of Gram-negative septicemia and/or endotoxemia (6). In this regard, antibodies to TNF inhibit the lethal effects of endotoxin in animal models (7). Although it is clear that endotoxin derived from bacteria is an important trigger for TNF release, we postulated that viral proteins may also play a role in TNF production and release.

Cytomegalovirus (CMV)[1] is an important cause of pneumonia in immunocompromised patients (8, 9), and the expression of this virus in the lung also appears to trigger an inflammatory process in the lung (10, 11). After infection, CMV sequentially expresses its immediate early (IE), early, and late genes. The IE genes of CMV autoregulate their own expression, as well as the expression of both the early and late genes (12). The IE genes have also been shown to modulate the expression of other, heterologous, viral genes (13), including those of the human immunodeficiency virus (14) and adenovirus (15, 16). Previous studies from our laboratory have demonstrated that the CMV IE gene products can upregulate cell-derived genes, including IL-1 (17, 18) and IL-2, and IL-2R (19). Other immediate early cellular genes have also been shown to be sensitive to the presence of the CMV IE gene products (20, 21).

Since CMV infection of human monocytes and tissue macrophages can induce TNF production (22), we hypothesized that the CMV IE gene products, specifically, play an important role in the upregulation of TNF gene expression. Using a monocyte tissue culture system, we determined whether the CMV IE gene products could upregulate the TNF promoter and increase the amounts of steady state TNF mRNA and TNF protein. We found that the CMV IE gene products strongly upregulated expression of the TNF gene. These studies suggest that the inflammatory response seen in CMV infection may, in part, be due to the effect of the CMV IE gene products on TNF production.

Methods

Cell Culture. THP-1 cells (a monocytic cell line) were grown in suspension in RPMI 1640 (University of Iowa Cancer Center, Iowa City, IA) supplemented with 10% FCS (Sigma Chemical Co., St. Louis, MO), 2.0 mM L-glutamine (University of Iowa Cancer Center), and gentamicin (80 μg/ml) at 37°C in an atmosphere of 5% CO_2. Cells isolated from these cultures were transfected during the log phase of their growth.

Plasmids. Construction of all IE plasmids has been described previously (15, 23, 24). The plasmid, pLink760 (control) contains only the CMV IE promoter-regulatory region and consists of a 760-bp Sau3A-I fragment cloned into the bacterial vector pAT153. The plasmid pIE1 (pSVCC1) contains the CMV IE promoter-regulatory region upstream of the bona fide IE1 gene; it contains the 3.6-kb ClaI restriction fragment, which spans the IE1 gene as well as the CMV IE promoter-regulatory region cloned into the plasmid pSVOd. Plasmid pIE2 (pCSdlAcc) contains the CMV IE promoter-regulatory region upstream of the bona fide IE2 gene. Finally, pIE1 + 2 (pSVCS) contains the IE promoter-regulatory region upstream of both the IE1 and IE2 genes (25). The TNFCAT plasmids were constructed as previously described (26). 5′TNFCAT contains a 2.9-kb fragment, which includes the 5′TNF untranslated region and the CAT gene. 5′TNFCAT3′ is similar to 5′TNFCAT except that it contains a 1,021-bp fragment, which encompasses the 3′ untranslated region (UTR) of the TNF gene, located downstream from the CAT gene. Fig. 1 schematically displays these plasmids along with the 5′ and 3′ limits of each promoter. In addition, a series of plasmids that contain various deletions in IE1 or IE2 were used to determine the effect of the specific proteins in these interactions. These mutated plasmids have been described previously and have been shown to express protein (25).

Transfections. THP-1 cells were transfected via the DEAE-Dextran method as previously described (27). Briefly, the cells were washed and

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1. *Abbreviations used in this paper:* CMV, cytomegalovirus; IE, immediate early; UTR, untranslated region.

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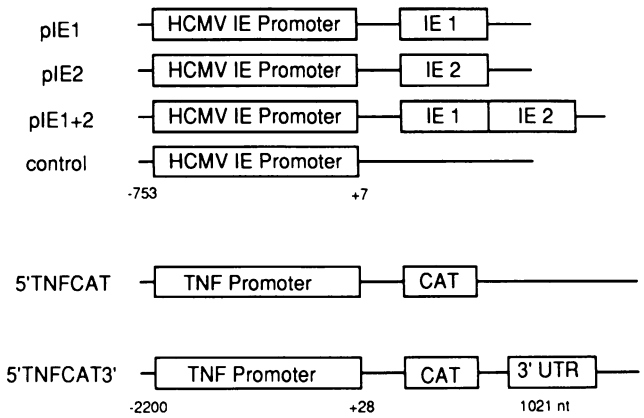

Figure 1. Schematic representation of the plasmid constructs used in these studies, along with the 5′ and 3′ extents of each promoter. Construction of the plasmids is described in Methods.

resuspended in serum-free medium at a density of 10×10^6 cells/ml. 1 ml of cells was added to 10 ml of serum free medium containing 100 μg/ml DEAE-Dextran (Pharmacia Fine Chemicals, Piscataway, NJ), 50 mM Tris, and 1 μg/ml of each plasmid DNA that was used. The cells were incubated in this medium for 20 min and then washed and resuspended in fresh medium containing 10% FCS for 48 h. For Northern blots, the cells were resuspended in fresh media for 24 h and then harvested at subsequent time intervals. In some experiments, LPS (10 μg/ml; Sigma Chemical Co.) was added at varying time intervals after transfection. Autoradiograms were quantitated using laser densitometry.

Differentiation. THP-1 cells were differentiated using a combination of hydrocortisone (HC; Sigma Chemical Co.) and phorbol 12-myristate 13-acetate (PMA; Sigma Chemical Co.). Cells were harvested and counted. 10^6 THP-1 cells were added to 4 ml RPMI with 10% FCS. The cultures were supplemented with hydrocortisone to a final concentration of 5×10^{-5} M. After 2 d the medium was changed and a similar amount of hydrocortisone was added. 3 d later the medium was again changed and supplemented with hydrocortisone. In addition, PMA was added to a final concentration of 2×10^{-8} M. Cells were then infected with the Towne CMV laboratory strain at a multiplicity of infection (MOI) of 10. Supernatants were harvested at various time points after infection.

CAT assays. Cell extracts were prepared and assayed as previously described (28). [^{14}C]Chloramphenicol (0.1 μCi; New England Nuclear, Wilmington, DE) was incubated with 10 mM acetyl coenzyme A and 100 ϕl of cell extract for 1 h at 37°C. The percentage of [^{14}C]-chloramphenicol that was converted to its acetylated derivatives was determined using ascending thin layer chromatography (TLC). Radioactivity was quantitated using an automated TLC scanner (Radiomatic, Tampa, FL).

Northern blot analysis. Total endogenous cellular RNA was isolated by using the guanidine isothiocyanate method (29). RNA was loaded onto a 1.5% agarose gel at a concentration of 10 μg RNA/lane. After electrophoresis, the RNA was transferred to a nylon membrane (Gene Screen Plus; New England Nuclear). Hybridization was performed at 60°C using a TNF cDNA probe (obtained from B. Beutler, University of Texas, Southwestern) labeled with [^{32}P]dCTP (New England Nuclear) in a nick translation system (Bethesda Research Laboratories, Gaithersburg, MD). Autoradiograms were developed after a 6-h exposure. Autoradiograms were quantitated using laser densitometry. Ethidium bromide staining of the gel was performed to ensure equal loading.

Protein determination. Endogenous TNF protein produced by transfected and infected THP-1 cells was assayed using a commercially available TNF ELISA (Endogen, Inc., Boston, MA). Supernatants were obtained from TNF cells transfected with either the control plasmid or pIE1 + 2. These supernatants were obtained at variable intervals after stimulation with LPS.

Results

Effects of the CMV IE gene products on expression of TNFCAT in THP-1 cells. To determine the effects of the CMV IE gene products on TNF gene expression, THP-1 cells were cotransfected with one of the TNFCAT constructs (i.e., 5′TNFCAT or 5′TNFCAT3′) and one of the CMV IE effector plasmids (i.e., control, pIE1, pIE2, or pIE1 + 2) (Fig. 1). For each transfection cells were stimulated for 24 h with LPS (10 μg/ml). Both IE1 and IE2 increased expression of the TNF promoter (Fig. 2). More CAT activity was noted when both the IE1 and IE2 gene products were present. No promoter activity was noted in the presence of the control plasmid. The presence of the TNF 3′ UTR markedly inhibited (by ~ 80%) the ability of LPS and the CMV IE gene products to upregulate the TNF promoter (Fig. 2). However, even in the presence of the 3′ UTR, the presence of the CMV IE gene products upregulated the TNF promoter to a greater degree than with stimulation alone (Table I).

Effect of mutant CMV IE gene products on expression of TNFCAT in THP-1 cells. To determine whether the effect of the CMV IE gene products was due to the CMV IE protein or to other aspects of the plasmid, THP-1 cells were cotransfected with the 5′TNFCAT plasmid and with one of the CMV IE1 or

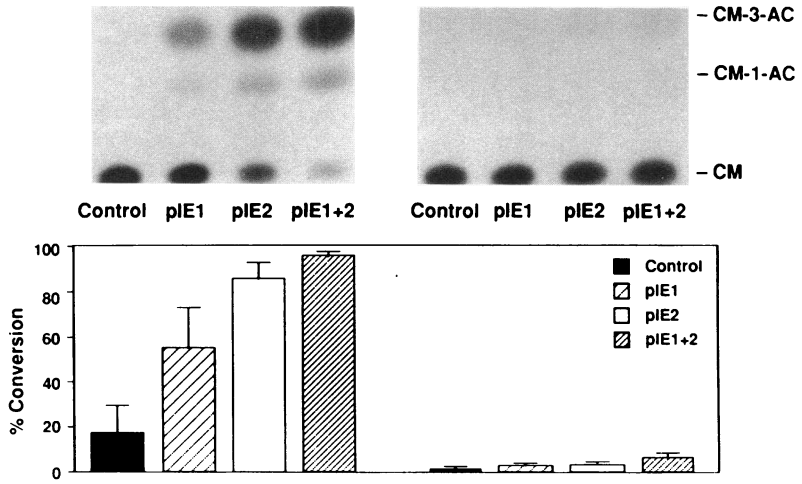

Figure 2. CMV IE gene products upregulate the TNF promoter. THP-1 cells were cotransfected with one of the CMV IE gene products and either 5′TNFCAT (*left*) or 5′TNFCAT3′ (*right*). Cells were stimulated 24 h after transfection with LPS (10 μg/ml) and harvested after an additional 24 h in culture. (*Top*) Representative experiment. (*Bottom*) Results from three separate experiments expressed as percent conversion of [^{14}C]chloramphenicol to its acetylated derivatives. Results are expressed as the mean + SEM.

Table I. The CMV IE Gene Products Upregulate the TNF Promoter Despite the Presence of the 3' UTR

	Percent conversion	Fold increase
Control	0.3	1
LPS alone	1.3	4.3
LPS and IE1 + 2	6.4	21

THP-1 cells were transfected and CAT assays were performed as described in Methods. All groups were transfected with the 5'TNFCAT3' plasmid. Control, cells also transfected with the control CMV plasmid and not stimulated with LPS; LPS alone, cells also transfected with the CMV control plasmid and stimulated with LPS; LPS and IE1 + 2, cells also transfected with the pIE1 + 2 plasmid and stimulated with LPS. Percent conversion represents conversion of [^{14}C]-chloramphenicol to its acetylated derivatives. Fold increase compares CAT activity in experimental groups of cells to control cells, which are assigned a fold increase of 1.

IE2 plasmids, which are known to produce defective proteins. For each transfection cells were stimulated as above. Mutations in any part of either IE1 or IE2 resulted in a marked decrease in TNF promoter activity as compared with the wild type IE1 or IE2 protein (Fig. 3). These data suggest that it is the CMV IE proteins, either IE1 or IE2, that are directly responsible for the increase in TNF promoter activity.

Effect of the CMV IE gene products on endogenous TNF gene expression in THP-1 cells. Since the ability of the CMV IE gene products to enhance expression of the TNF promoter was strongly modulated by the presence of the 3' UTR, we assessed the ability of the CMV IE gene products to effect steady state levels of endogenous TNF RNA. Whole cell RNA was obtained from THP-1 cells transfected with either the control plasmid or pIE1 + 2. Whole cell RNA was then detected by Northern blot analysis (30). The human TNF probe detected an RNA species of ~ 1.5 kb. The presence of the CMV IE gene products increased the amount of steady state TNF RNA (Fig. 4). The steady state level of TNF RNA increased 1.2-fold at 1 h, 3-fold at 3 h, and 7-fold at 5 h. By 24 h no significant differences were noted. These differences could not be accounted for on the basis of differences in loading as demonstrated by ethidium bromide staining, nor did the presence of the CMV IE gene products influence the expression of actin mRNA (data not shown). These data indicate that the CMV IE gene products increased the steady state levels of endogenous TNF RNA.

Effect of the CMV IE gene products on TNF protein production in THP-1 cells. Having shown that the CMV IE gene products upregulate expression of the TNF gene, we determined whether there was also an increase in TNF protein production. THP-1 cells were transfected with either the control plasmid or the plasmid containing pIE1 + 2. Cells were cultured for 24 h and then treated with LPS. Supernatants were harvested after varying time intervals and assayed for the presence of TNF protein production using a commercially available TNF ELISA.

TNF production peaked at 3 h after stimulation with LPS in the absence or presence of the CMV IE gene products. However, the amount of TNF produced in the presence of the IE gene products was significantly greater than that of the control cells (Fig. 5). Both the control cells and the cells transfected with the CMV IE gene products showed increased TNF production in response to increasing doses of LPS (Fig. 6). Again, the response was significantly higher in the cells containing the CMV IE gene products (Fig. 6).

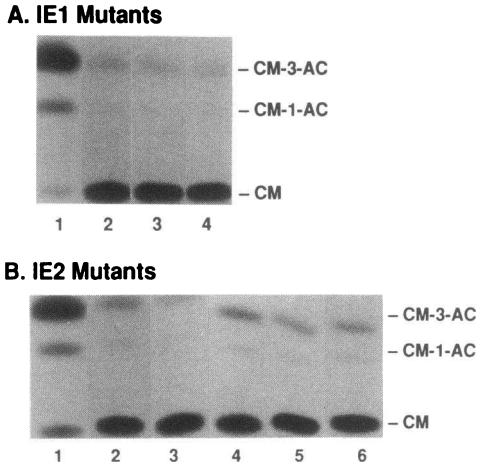

Figure 3. Mutations in the CMV IE gene products abolish upregulation of the TNF promoter. THP-1 cells were cotransfected with the 5'TNFCAT and with plasmids expressing mutant CMV IE proteins. Cells were stimulated 24 h after transfection with LPS (10 μg/ml) and harvested after an additional 24 h in culture. On the abscissa are the IE mutant plasmids and on the ordinate is conversion of unacetylated chloramphenicol (*CM*) to its acetylated derivatives (*CM-1-AC* and *CM-3-AC*). (*A*) A representative experiment where THP-1 cells were transfected with various pIE1 plasmids. Cells were transfected with either the wild type pIE1 (lane *1*) or with an IE1 plasmid with a 155-amino acid deletion in exon 4 (lane *2*), an IE1 plasmid with a 173-amino acid deletion in exon 4 (lane *3*), or an IE1 plasmid with a 143-amino acid deletion in exon 4 (lane *4*). These deletions are overlapping and cover the entire coding region for exon 4. (*B*) A representative experiment where THP-1 cells were transfected with various pIE2 plasmids. Cells were transfected with either the wild type pIE2 plasmid (lane *1*), or a plasmid that codes for a smaller IE2 protein (lane *2*), or plasmids with a 52-amino acid deletion in exon 3 (lane *3*), a 25-amino acid deletion in exon 5 (lane *4*), a 169-amino acid deletion in exon 2 (lane *5*) or a 290-amino acid deletion in exon 5 (lane *6*). The deletions in exon 5 are not overlapping.

To determine whether these effects were also seen with CMV infection, THP-1 cells were differentiated with hydrocortisone and PMA. Cell supernatants were harvested at various time points after infection. TNF production increased to a peak of 268 pg/10^6 cells at 24 h after infection in differentiated cells. Uninfected cells had no TNF activity despite differentiation (data not shown).

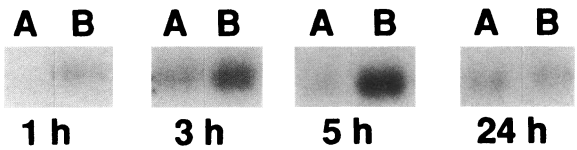

Figure 4. CMV IE gene products upregulate steady state TNF mRNA. THP-1 cells were transfected as described in Methods and allowed to incubate for 24 h. Cells were then harvested at varying time intervals and RNA was extracted as described in Methods. The time points on the abscissa represent intervals after the 24-h incubation period posttransfection. Equal amounts of RNA were loaded on to 1.5% agarose gels. Blots were transferred to a nylon membrane and probed with a ^{32}P-labeled TNF probe. Autoradiograms were obtained after a 6-h exposure.

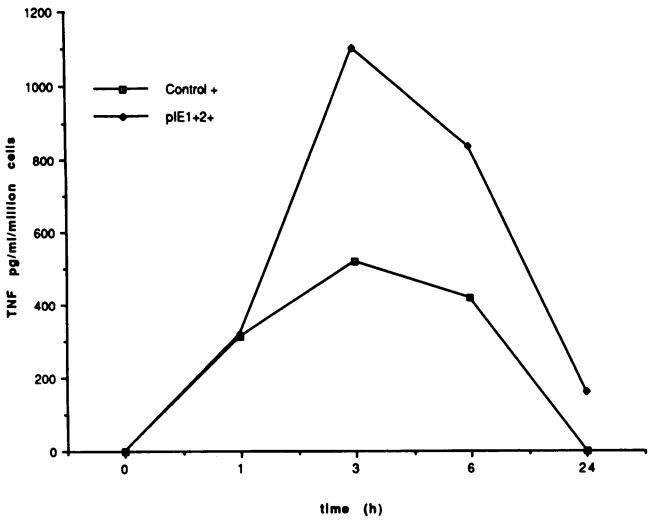

Figure 5. The presence of the CMV IE gene products upregulates TNF protein production over time. THP-1 cells were transfected as described in Methods. Cells were cultured for 24 h and then stimulated with LPS. Supernatants were harvested after varying time intervals after LPS exposure. On the abscissa is time (h) and on the ordinate is TNF production (pg/ml per 10^6 cells). Cells were transfected with the plasmid containing the CMV IE 1 + 2 genes (*filled diamonds*) or with the control plasmid (*open squares*). Shown is a representative study of three separate experiments that demonstrated the same effect.

Discussion

These studies demonstrate that the CMV IE gene products can enhance expression of the TNF gene. The effect of the IE gene products in THP-1 cells appeared to be due to the activity of both the IE1 and IE2 gene products. Mutations in the CMV IE1 or IE2 protein abolished this effect. Although the ability of the IE gene products to upregulate the TNF promoter was strongly modulated by the 3′ UTR, the presence of the CMV IE gene products resulted in increased amounts of steady state TNF mRNA and protein. Whether the CMV IE gene products directly increased mRNA production or increased message stability is not addressed by these studies. While stimulation with LPS also upregulates TNF protein production, the effect of LPS stimulation was much greater in the cells transfected with the CMV IE gene products, implying an additional effect from the presence of the CMV IE gene products. CMV infection of differentiated THP-1 cells also resulted in increased TNF production, confirming that THP-1 cells are a reasonable model for CMV infection of monocytes and macrophages.

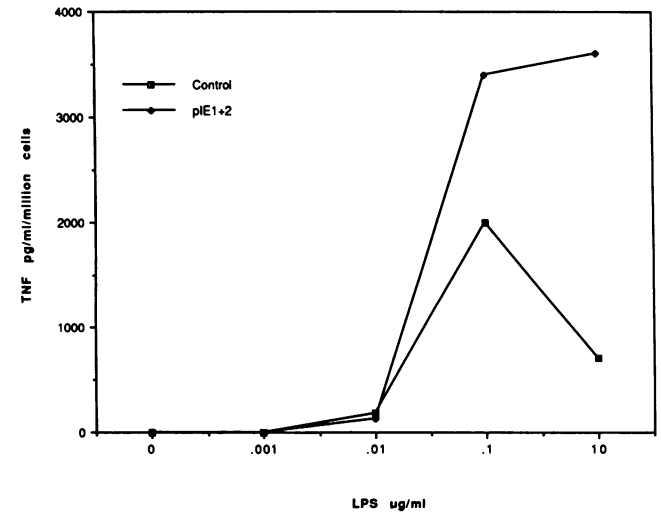

Figure 6. The presence of the CMV IE gene products upregulates TNF protein production over a range of LPS concentrations. THP-1 cells were transfected and harvested as noted in Methods. Various concentrations of LPS are noted on the abscissa. On the ordinate is TNF protein production (pg/ml per 10^6 cells). Cells were transfected with the plasmid containing the CMV IE 1 + 2 genes (*filled diamonds*) or with the control plasmid (*open squares*). Shown is a representative study of three separate experiments that demonstrated the same effect.

Regulation of transcription from the TNF gene has not been fully elucidated. Like most cytokine genes, the TNF promoter-regulatory region contains several elements that modulate production (31). A key feature of the promoter is the presence of four NF-κB motifs that appear important in the regulation of the promoter (32, 33). A cyclic AMP response element has been noted, along with SP-1 and AP-2 sites, within 130 bp of the transcriptional start site (34). In addition, TNF is one of a series of genes that is also controlled by a specific sequence in the 3′ UTR (35). This motif has also been noted in the interferon and IL-1 genes. This region appears to negatively affect RNA message stability and subsequently limit protein production. Although it is clear that the CMV IE gene products upregulate the TNF promoter, steady state RNA, and protein production, the exact mechanism of action of these viral gene products remains to be determined. Studies have demonstrated that IE1 may indirectly stimulate transcription from a region in the 5′ promoter of the IL-1 gene that binds a nonviral cellular factor (18, 36). However, a similar motif has not been noted in the TNF promoter.

A recent report by Smith et al. (22) noted an increase in TNF protein production from monocytes infected with CMV. In addition, infection of THP-1 cells with the laboratory strain of CMV AD169 resulted in an increase in TNF protein production (37). This study represents the first report of the effect of specific viral proteins on expression of the TNF gene.

Several other reports have demonstrated interactions between other infectious agents and the TNF gene. Becker et al. (38) demonstrated that macrophages infected with respiratory syncytial virus produced increased levels of TNF mRNA and protein. Although the mechanism for this finding was not defined, the effect was seen 1 h after infection. Goldfeld et al. (39) demonstrated upregulation of TNF in fibroblasts and monocytes using Sendai virus and LPS. Using promoter deletion plasmids, they were able to demonstrate that the NF-κB sites located in the TNF promoter are not necessary for viral induction of the TNF gene by Sendai virus. Other studies have shown that the interaction between HIV-1 and the CD4 receptor on mononuclear cells results in an increase in TNF production (40). Finally, it appears that cells previously infected with HIV-1 demonstrate augmented expression of TNF after infection with a second virus (41). This is particularly important in light of the current study with CMV, as CMV is a frequent copathogen in patients with HIV infection (42, 43). The relatively nonspecific response of TNF to infectious agents may provide a simple mechanism for immune control of an invading pathogen and a preliminary step toward immune surveillance.

In summary, patients with CMV infection display an inflammatory response marked by monocyte infiltration (10,

44). Recent studies strongly suggest that one part of this inflammatory process is the release of TNF (22). This study strongly suggests that the CMV IE gene products upregulate expression of the TNF gene during CMV infection.

Acknowledgments

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References

1. Remick, D. G., R. M. Streiter, M. K. Eskandari, D. T. Nguyen, M. A. Genord, C. L. Raiford, and S. L. Kunkel. 1990. Role of tumor necrosis factor-a in lipopolysaccharide-induced pathologic alterations. *Am. J. Pathol.* 136:49–60.

2. Beutler, B., D. Greenwald, J. D. Hulmes, M. Chang, Y.-C. E. Pan, J. Mathison, R. Ulevich, and A. Cerami. 1985. Identity of tumor necrosis factor and the macrophage-secreted factor cachectin. *Nature (Lond.)*. 316:552–554.

3. Beutler, B., and A. Cerami. 1986. Cachetin and tumour necrosis factor as two sides of the same biological coin. *Nature (Lond.)*. 320:584–587.

4. Beutler, B., and A. Cerami. 1988. Tumor necrosis factor, cachexia, shock and inflammation: a common mediator. *Annu. Rev. Biochem.* 57:505–518.

5. Tracey, K. J., H. Wei, K. R. Manogue, Y. Fong, D. G. Hesse, H. T. Nguyen, G. C. Kuo, B. Beutler, R. S. Cotran, A. Cerami, and S. F. Lowry. 1988. Cachectin/tumor necrosis factor induces cachexia, anemia, and inflammation. *J. Exp. Med.* 167:1211–1227.

6. Tracey, K. J., B. Beutler, S. F. Lowry, J. Merryweather, S. Wolpe, I. W. Milsark, R. J. Hariri, T. J. Fahey, A. Zentella, J. D. Albert, G. T. Shires, and A. Cerami. 1986. Shock and tissue injury induced by recombinant human cachetin. *Science (Wash. DC)*. 234:470–474.

7. Tracey, K. J., Y. Fong, D. G. Hesse, K. R. Manogue, A. T. Lee, G. C. Kuo, S. F. Lowry, and A. Cerami. 1987. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature (Lond.)*. 330:662–664.

8. Chatterjee, S. N., M. Fiala, J. Weiner, J. A. Stewart, B. Stacey, and N. Warner. 1978. Primary cytomegalovirus and opportunistic infections. Incidence in renal transplant recipients. *J. Am. Med. Assoc.* 240:2446–2449.

9. Peterson, P. K., P. McGlave, N. K. C. Ramsay, F. Rhame, E. Cohen, G. S. Perry, A. I. Goldman, and J. Kersey. 1983. A prospective study of infectious diseases following bone marrow transplantation: emergence of *Aspergillus* and *Cytomegalovirus* as the major causes of mortality. *Infect. Control (Thorofare)*. 4:81–89.

10. Meyers, J. D., N. Flourney, and E. D. Thomas. 1986. Risk factors for cytomegalovirus infection after human bone marrow transplantation. *J. Infect. Dis.* 153:478–488.

11. Cohen, J. I., and G. R. Corey. 1985. Cytomegalovirus infection in the normal host. *Medicine (Baltimore)*. 64:100–114.

12. Stinski, M. F. 1990. Cytomegalovirus and its replication. *In* Fields Virology. Vol. 2. Bernard N. Fields and David Knipe, editors. Raven Press, Ltd., New York. 1959–1980.

13. Pizzorno, M. C., P. O'Hare, L. Sha, R. L. LaFemina, and G. S. Hayward. 1988. *trans*-activation and autoregulation of gene expression by the immediate-early region 2 gene products of human cytomegalovirus. *J. Virol.* 62:1167–1179.

14. Davis, M. G., S. C. Kenney, J. Kamine, J. S. Pagano, and E.-S. Huang. 1987. Immediate-early gene region of human cytomegalovirus trans-activates the promoter of human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA.* 84:8642–8646.

15. Hermiston, T. W., C. L. Malone, P. R. Witte, and M. F. Stinski. 1987. Identification and characterization of the human cytomegalovirus immediate-early region 2 gene that stimulates gene expression from an inducible promoter. *J. Virol.* 61:3214–3221.

16. Tevethia, M. J., D. J. Spector, K. M. Leisure, and M. F. Stinski. 1987. Participation of two human cytomegalovirus immediate early gene regions in transcriptional activation of adenovirus promoters. *Virology.* 161:276–285.

17. Iwamoto, G. K., M. M. Monick, B. D. Clark, P. E. Auron, M. F. Stinski, and G. W. Hunninghake. 1990. Modulation of interleukin 1 beta expression by the immediate early genes of human cytomegalovirus. *J. Clin. Invest.* 85:1853–1857.

18. Hunninghake, G. W., B. G. Monks, L. J. Geist, M. M. Monick, M. S. Monroy, M. F. Stinski, A. C. Webb, J.-M. Dayer, P. E. Auron, and M. J. Fenton. 1992. The functional importance of a cap site-proximal region of the human prointerleukin 1b gene is defined by viral protein trans-activation. *Mol. Cell. Biol.* 12:3439–3448.

19. Geist, L. J., M. M. Monick, M. F. Stinski, and G. W. Hunninghake. 1991. The immediate early genes of human cytomegalovirus upregulate expression of the interleukin-2 and interleukin-2 receptor genes. *Am. J. Respir. Cell Mol. Biol.* 5:292–296.

20. Monick, M. M., L. J. Geist, M. F. Stinski, and G. W. Hunninghake. 1992. The immediate early genes of human cytomegalovirus upregulate expression of the cellular genes *myc* and *fos*. *Am. J. Respir. Cell Mol. Biol.* 7:251–256.

21. Hagemeier, C., S. M. Walker, P. J. G. Sissons, and J. H. Sinclair. 1992. The 72K IE1 and 80K IE2 proteins of human cytomegalovirus independently trans-activate the c-*fos*, c-*myc* and *hsp70* promoters via basal promoter elements. *J. Gen. Virol.* 73:2385–2393.

22. Smith, P. D., S. S. Saini, M. Raffeld, J. F. Manischewitz, and S. M. Wahl. 1992. Cytomegalovirus induction of tumor necrosis factor-a by human monocytes and mucosal macrophages. *J. Clin. Invest.* 90:1642–1648.

23. Stenberg, R. M., and M. F. Stinski. 1985. Autoregulation of the human cytomegalovirus major immediate early gene. *J. Virol.* 6:676–682.

24. Stinski, M. F., and T. J. Roehr. 1985. Activation of the major immediate early gene of human cytomegalovirus by cis-acting elements in the promoter-regulatory sequence and by virus specific trans-acting components. *J. Virol.* 55:431–441.

25. Malone, C. L., D. H. Vesole, and M. F. Stinski. 1990. Transactivation of a human cytomegalovirus early promoter by gene products from the immediate-early gene IE2 and augmentation by IE1: mutational analysis of the viral proteins. *J. Virol.* 64:1498–1506.

26. Beutler, B., and T. Brown. 1991. A CAT reporter construct allows ultrasensitive estimation of TNF synthesis and suggests that the TNF gene has been silenced in non-macrophage cell lines. *J. Clin. Invest.* 87:1336–1344.

27. Queen, C., and D. Baltimore. 1983. Immunoglobulin gene transcription is activated by downstream sequence elements. *Cell.* 33:741–748.

28. Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* 2:1044–1051.

29. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid fron sources enriched in ribonuclease. *Biochemistry.* 18:5294–5299.

30. Lehrach, D., D. Diamond, J. M. Woaney, and H. Boedtker. 1987. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. *Biochemistry.* 16:4743–4751.

31. Drouet, C., A. N. Shakhov, and C. V. Jongeneel. 1991. Enhancers and transcription factors controlling the inducibility of the tumor necrosis factor-a promoter in primary macrophages. *J. Immunol.* 147:1694–1700.

32. Collart, M. A., P. Baeuerle, and P. Vassalli. 1990. Regulation of tumor necrosis factor alpha transcription in macrophages: involvement of four -κB-like motifs and of constitutive and inducible forms of NF-κB. *Mol. Cell. Biol.* 10:1498–1506.

33. Shakhov, A. N., M. A. Collart, P. Vassalli, S. A. Nedospasov, and C. V. Jongeneel. „B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor a gene in primary macrophages. *J. Exp. Med.* 171:35–47.

34. Economou, J. S., K. Rhoades, R. Essner, W. H. McBride, J. C. Gasson, and D. L. Morton. 1989. Genetic analysis of the human tumor necrosis factor α/cachectin promoter region in a macrophage cell line. *J. Exp. Med.* 170:321–326.

35. Caput, D., B. Beutler, K. Hartog, R. Thayer, S. Brown-Shimer, and A. Cerami. 1986. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc. Natl. Acad. Sci. USA.* 83:1670–1674.

36. Crump, J. W., L. J. Geist, P. E. Auron, A. C. Webb, M. F. Stinski, and G. W. Hunninghake. 1992. The immediate early genes of human cytomegalovirus require only proximal promoter elements to upregulate expression of interleukin-1b. *Am. J. Respir. Cell Mol. Biol.* 6:674–677.

37. Turtinen, L. W., A. Assimacopoulos, and A. T. Haase. 1989. Increased monokines in cytomegalovirus infected myelomonocytic cell cultures. *Microb. Pathog.* 7:135–145.

38. Becker, S., S. Quay, and J. Soukup. 1991. Cytokine (tumor necrosis factor, IL-6, and IL-8) production by respiratory syncytial virus-infected human alveolar macrophages. *J. Immunol.* 147:4307–4312.

39. Goldfeld, A. E., C. Doyle, and T. Maniatis. 1990. Human tumor necrosis factor a gene regulation by virus and lipopolysaccharide. *Proc. Natl. Acad. Sci. USA.* 87:9769–9773.

40. Merrill, J. E., Y. Koyanagi, and I. S. Y. Chen. 1989. Interleukin-1 and tumor necrosis factor can be induced from mononuclear phagocytes by human immunodeficiency virus type 1 binding to the CD4 receptor. *J. Virol.* 63:4404–4408.

41. D'Addario, M., A. Roulston, M. A. Wainberg, and J. Hiscott. 1990. Coordinate enhancement of cytokine gene expression in human immunodeficiency virus type 1-infected promonocytic cells. *J. Virol.* 64:6080–6089.

42. Drew, W. L. Cytomegalovirus infection in patients with AIDS. *J. Infect. Dis.* 158:449–456.

43. Jacobson, M. A., and J. Mills, J. 1988. Serious cytomegalovirus disease in the acquired immunodeficiency syndrome (AIDS). *Ann. Int. Med.* 108:585–594.

44. Griffiths, P. D., and J. E. Grundy. 1987. Molecular biology and immunology of cytomegalovirus. *Biochem. J.* 241:313–324.