Tumour necrosis factor \( \alpha \) stimulates the activity of the human cytomegalovirus major immediate early enhancer/promoter in immature monocytic cells

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Both tumour necrosis factor \( \alpha \) (TNF-\( \alpha \)) and phorbol 12-myristate 13-acetate (PMA) stimulated human cytomegalovirus (HCMV) major immediate early (IE) enhancer/promoter activity in the HL-60 granulocyte/monocyte progenitor cell line when added to transfected cells. In U-937 monocytic cells, by contrast, TNF-\( \alpha \) had no stimulatory effect and the addition of PMA produced only marginal stimulation. In the mature THP-1 monocytic cell line and in differentiated HL-60 cells, addition of TNF-\( \alpha \) caused inhibition of the IE enhancer/promoter activity. The stimulating effect of PMA, as observed in the other cell lines, however, remained. Thus the effect of TNF-\( \alpha \) on the major IE enhancer/promoter activity is determined by the degree of differentiation of the infected cells. Unlike TNF-\( \alpha \) and PMA, the interleukins IL-1, IL-3, IL-6 as well as the cytokine GM-CSF were found to have no detectable influence on the activity of the IE enhancer/promoter activity which, likewise, was not affected by the presence of the modulator sequence. Since premonocytic cells are suggested to be sites of HCMV latency, the stimulation by TNF-\( \alpha \) could be of potential pathophysiological significance.

Human cytomegalovirus (HCMV) is the single most important infectious agent in patients under immunosuppressive therapy, particularly organ transplant recipients. Despite numerous investigations our understanding of the pathogenesis of HCMV infection is still incomplete. However, peripheral blood mononuclear cells (PBMCs), especially monocytes, are believed to play a central role in the pathogenesis of HCMV infection. It was suggested that monocytes may harbour the latent virus in healthy seropositive blood donors (Taylor-Wiedeman et al., 1991). During systemic HCMV infection in normal hosts both granulocytes and mononuclear leukocytes contain the virus (Salzman et al., 1988; Schrier et al., 1985). Furthermore, HCMV can infect bone marrow progenitor cells in vitro causing abortive or productive infection and interfere with normal haematopoiesis (Einhorn & Ost, 1984; Reiser et al., 1986; Simmons et al., 1990; Sing & Ruscetti, 1990). Very little is known about the regulation of HCMV gene expression in mononuclear cells and the factors precipitating reactivation of latent virus when immunosuppressive treatments are given.

Infection of promonocytic/monocytic cell lines such as HL-60, U-937 and THP-1 results in abortive infection (Rice et al., 1984). However, transition to productive infection may be achieved by stimulation of these cells with 12-O-tetradecanoylphorbol 13-acetate or phorbol 12-myristate 13-acetate (PMA) (Dudding & Garnett, 1987; Weinshenker et al., 1988). Unrestricted replication of HCMV in hydrocortisone-treated macrophages was recently reported (Lathey & Spector, 1991).

There is some evidence that HCMV expression in monocytic cells strongly depends on their stage of differentiation (Sinclair et al., 1992; Weinshenker et al., 1988). Sinclair et al. (1992) identified a cellular factor which inhibits HCMV expression in immature monocytic cells by binding to the HCMV immediate early (IE) modulator. It is quite possible that other cellular factors also influence HCMV expression.

Differentiation of bone marrow stem cells into monocytes/macrophages or granulocytes is triggered by several different cytokines. Monocytes/macrophages and granulocytes produce cytokines regulating the haematopoiesis of stem cells, but they and their haematopoietic precursors themselves are targets of cytokines produced by other cells in the bone marrow stroma or immune organs.

For human immunodeficiency virus type 1 (HIV-1) several different cytokines were found to be important mediators of virus replication in chronically infected promonocytic cells (Fauci, 1990). Since HCMV is a monotropic virus we studied several cytokines pertaining...
to monocytic cells with regard to their effect on the activity of the major IE promoter of HCMV.

The major IE promoter plays a central role in HCMV replication initiation by controlling HCMV IE protein expression. IE genes are the first to be transcribed to monocytic cells with regard to their effect on the replication initiation by controlling HCMV IE protein expression. The major IE promoter is strongly influenced by an upstream enhancer region with binding sites for various cellular transcription factors (Boshart et al., 1982; Boehringer Mannheim). HL-60 cells are closely related to early progenitor cells and have retained the ability to differentiate into granulocytes or monocyes/macrophages. As was shown by flow cytometry, HL-60 cells expressed CD34 and low levels of typical differentiation antigens such as adhesion molecules (CD11a, 11b, 11c, 18) and the monocytic marker CD14 on their surface (Table 1). The U-937 and THP-1 cells could be classified as being of the monocytic/macrophage lineage because they expressed typical monocytic differentiation markers. THP-1 cells seemed to be at a later differentiation stage than U-937 cells since they expressed a higher level of CD14 and other differentiation markers (Table 1).

Table 1. Phenotypic characterization of monocytic cell lines

<table>
<thead>
<tr>
<th>Marker (%) on cell line*</th>
<th>Marker</th>
<th>HL-60</th>
<th>U-937</th>
<th>THP-1</th>
<th>HL-60**†</th>
<th>HL-60***†</th>
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</thead>
<tbody>
<tr>
<td>CD34</td>
<td>15</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CD14</td>
<td>2</td>
<td>63</td>
<td>46‡</td>
<td>7</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>CD11a</td>
<td>3</td>
<td>98</td>
<td>47</td>
<td>3</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>CD11b</td>
<td>13</td>
<td>13</td>
<td>73</td>
<td>54</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>CD11c</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>CD18</td>
<td>5</td>
<td>29</td>
<td>52</td>
<td>41</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>CD71</td>
<td>94</td>
<td>73</td>
<td>89§</td>
<td>73§</td>
<td>99§</td>
<td></td>
</tr>
</tbody>
</table>

* The cell surface markers were determined by cytofluorometry using monoclonal antibodies as described in the text. Data given are mean averages of four independent experiments.
† For HL-60* and HL-60** see Fig. 3 legend.
‡ The density of CD14 antigen expression (mean fluorescence) was about three times higher on THP-1 cells as compared with HL-60** and U-937 cells.
§ The density of CD71 antigen expression was lower as compared with HL-60 and U-937 cells.

Cells were transfected by the DEAE-dextran method (Sambrook et al., 1989). Briefly, cells were transfected with 5 µg plasmid per 10⁷ cells in buffer containing 25 mM-Tris-HCl pH 7.4, 137 mM-NaCl, 5 mM-KCl, 0.7 mM-CaCl₂, 0.5 mM-MgCl₂, 0.6 mM-Na₂HPO₄ and 5 mg/ml DEAE-dextran (Pharmacia LKB Biotechnology) for 1 h at 37°C. Subsequently the cells were washed with RPMI medium. Samples of 10⁷ cells were cultured in RPMI-1640 medium (Biochrom) supplemented with FCS (Biochrom), selected for low endotoxin content, at a concentration of 10%, and the appropriate cytokine at 37 °C in a humidified atmosphere (5% CO₂) for 2 days. Transfection efficiencies for all the three cell lines were confirmed as all being equivalent in control experiments using ³²P-labelled plasmids. Cells from an identical transfection experiment without cytokine served as an appropriate control. The cells were then washed with PBS and disrupted by repeated freezing and thawing. After centrifugation, samples of the supernatants were assayed for protein (Bradford, 1976). Equal quantities of protein were used in the chloramphenicol acetyltransferase (CAT) assay as described by Gorman et al. (1982). Lysates were incubated with [¹⁴C]chloramphenicol (Amersham); the acetylated products were quantified after separation by thin-layer chromatography using a thin layer scintillator. Promoter activities were estimated relative to the conversion rate of [¹⁴C]chloramphenicol under the influence of cytokines and compared with the control samples. Tumour necrosis factor (TNF-α) and IL-3 receptor expression was determined with ¹²⁵I-labelled cytokines (Amersham). IL-1, IL-6 and GM-CSF receptors were detected using Fluorokine kits (Biermann) according to the supplier's recommendation. Phenotypic analysis of the cell lines
was performed using various monoclonal antibodies (Becton Dickinson).

Results are shown for HL-60, U-937 and THP-1 cells in Fig. 1 (a, b and c, respectively). All three cell lines were transfected with the constructs pRR55 or pMP32. In the HL-60 cell line, the IE enhancer/promoter activity with and without the modulator was significantly increased by recombinant TNF-α and PMA (Fig. 1 a). In the presence of TNF-α we measured a five- to sevenfold increase of gene expression after transfection of the cells with pRR55 or pMP32. Addition of PMA resulted in a seven- to ninefold increase in the level of CAT expression. Simultaneous application of both TNF-α and PMA did not result in any additional increase of CAT expression (data not shown). In U-937 cells transfected with either pRR55 or pMP32 no stimulatory effect of TNF-α was seen (Fig. 1 b). In contrast to this, PMA did stimulate the IE enhancer/promoter in the absence of the modulator. However, the degree of stimulation was lower than that found in HL-60 cells (by a factor of 2.5 to three) (Fig. 1 b). The stimulation by PMA was absent when the presence of the modulator was present, indicating that the presence of the modulator diminished the positive PMA effect on the HCMV IE enhancer/promoter in U-937 cells.

In the mature monocytic THP-1 cell line TNF-α induced an inhibition of the HCMV IE enhancer/promoter activity, irrespective of the presence or absence of the modulator (Fig. 1 c). The PMA effect, by contrast, varied according to whether or not the modulator sequence was present. Whereas PMA stimulated the IE enhancer/promoter activity in pRR55-transfected THP-1 cells (two- to threefold), the CAT activity measured in pMP32-transfected THP-1 cells was mainly unaffected by PMA (Fig. 1 c). When incubating pRR55-transfected THP-1 cells with both TNF-α and PMA the inhibitory effect of TNF-α was neutralized (data not shown).

The specificity of these TNF-α effects was confirmed by preincubation of TNF-α with the neutralizing monoclonal anti-TNF-α antibody Di62 (kindly provided by Dr T. Diamantstein, Berlin, Germany); the results demonstrated that antibodies to TNF-α can inhibit both the stimulating effect of TNF-α in HL-60 cells and its inhibitory effect in THP-1 cells (Fig. 1 a to c), whereas the irrelevant IgG1 mouse monoclonal antibody, TSH, had no influence (data not shown). The PMA effect in HL-60 cells was unlikely to be TNF-α-mediated because PMA did not induce TNF-α synthesis in HL-60 cells (Table 2) and antibody Di62 did not abolish the PMA effect (data not shown). In pMP32- and pRR55-transfected THP-1 cells Di62 increased the small PMA-mediated stimulation by a factor of two and five respectively, indicating that the slight PMA effect occurring in this cell line may result, at least partially, from the induction of large amounts of TNF-α which seem to counteract the PMA effect (data not shown).

**Table 2. Endogenous and PMA-induced synthesis of TNF-α in monocytic cells**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Stimulator</th>
<th>Synthesis of TNF-α (pg per ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-60*</td>
<td>PMA</td>
<td>62</td>
</tr>
<tr>
<td>U-937</td>
<td>PMA</td>
<td>56</td>
</tr>
<tr>
<td>THP-1</td>
<td>PMA</td>
<td>1269</td>
</tr>
<tr>
<td>HL-60*</td>
<td>PMA</td>
<td>230</td>
</tr>
<tr>
<td>HL-60*</td>
<td>PMA</td>
<td>1450</td>
</tr>
</tbody>
</table>

*Monocytic cells (10⁶ cells/ml) were cultured for 4 h in the presence or absence of PMA (10 ng/ml) and the supernatants were collected. Synthesis of cytokines was measured by ELISA. For definition of HL-60* see Fig. 3 legend. Data of one out of three representative experiments are shown.
Both the stimulatory effect of TNF-α in HL-60 cells and its inhibitory effect in THP-1 cells were clearly dependent on concentration (Fig. 2a, b).

We also studied the influence of interleukins IL-1, IL-3 and IL-6 as well as the cytokine GM-CSF on the expression of CAT under control of the HCMV IE enhancer/promoter in the presence or absence of the modulator in the three cell lines. Despite the presence of a sufficient number of receptors for these cytokines on all the three cell lines (not shown), neither GM-CSF nor any interleukin altered the HCMV enhancer/promoter activity even when given in excess (> 1 ng/ml) (data not shown). Furthermore, like TNF-α, IL-1β, IL-6 and GM-CSF inhibited U-937 and THP-1 cells, suggesting functional activity of the appropriate cytokine receptor. HL-60 cells were affected only by TNF-α, IL-6 and GM-CSF but not by IL-1β.

In summary, we were able to demonstrate that the cytokine TNF-α is involved in the regulation of HCMV IE enhancer/promoter activity in monocytic cells. In our experiments the effect of TNF-α depended strongly on the cell line studied. TNF-α-induced stimulation of the IE enhancer/promoter activity in immature, granulocyte/macrophage progenitor cell-related HL-60 cells contrasted with the inhibition of this activity observed in mature monocytes like THP-1. Therefore, the stimulating effect of TNF-α on the HCMV IE enhancer/promoter decreases with increasing cell differentiation.

This suggestion was confirmed by studying the effect of TNF-α on HL-60 cells induced to differentiate along the monocyte/macrophage lineage in vitro (see Table 1). Differentiated HL-60 cells were obtained either by cultivating cells in the presence of TNF-α prior to transfection (HL-60*) or by selection of a spontaneously differentiated subclone (HL-60**). As was expected in differentiated HL-60 cells the response of the HCMV IE enhancer/promoter to TNF-α and PMA was similar to that in THP-1 cells (Fig. 3a, b).

The differences between the cell lines do not seem to be due to variations in TNF-α responsiveness. HL-60, U-937 and THP-1 cells expressed similar amounts of TNF-α receptors on their surface (3575, 3002, 4603 receptors per cell, respectively) and their expression of adhesion molecules was increased by TNF-α (data not shown). Furthermore, endogenous TNF-α production was even lower in U-937 and THP-1 cells than in HL-60 cells (Table 2). In conclusion, it seems unlikely that the U-937 and THP-1 cells failed to respond to exogenous TNF-α because of either high endogenous TNF-α levels or the absence of receptors.

PMA affected the HCMV IE enhancer/promoter activity in a more complex manner than TNF-α did. Whereas PMA stimulated gene expression in all three cell lines transfected with pRR55, the level of stimulation decreased as the cells became more differentiated. When the IE enhancer/promoter was coupled to the modulator, significant stimulation by PMA occurred only in the immature cell line HL-60 (Fig. 1 a to c). Therefore, the stimulatory effect of PMA on the IE enhancer/promoter was counteracted by the modulator in more differentiated cells.

Both TNF-α and PMA are known to be inducers of the transcription factor NF-κB (Lenardo & Baltimore, 1989) and Sp-1 (Hamanaka et al., 1992). HCMV IE enhancer has four NF-κB binding sites and five Sp-1 binding sites (Cherrington & Mocarski, 1989). As our results confirm, TNF-α and PMA have distinct modes of action. Unlike the activation induced by TNF-α, PMA-induced activation of the transcription factor NF-κB...
involves the protein kinase C system (Meichle et al., 1990; Sen & Baltimore, 1986). TNF-α effects sphingomyelin hydrolysis generating ceramide, which in turn may act as an inducer of NF-κB. Sphingomyelin hydrolysis was observed both in HL-60 and U-937 cells (Kim et al., 1991).

Therefore, we propose that during differentiation of early progenitor cells into monocytes a factor accumulates which, in combination with TNF-α, may induce inhibition of HCMV enhancer/promoter activity in mature cells such as THP-1 and differentiated HL-60 cells. In vitro experiments have shown that TNF-α induces an antiviral state or antiviral cytotoxicity against HCMV-infected fibroblasts (Duncombe et al., 1990; Ito & O'Malley, 1987). The molecular mechanism of this antiviral action of TNF-α is unknown but if this effect in fibroblasts can be attributed to the inhibition of the IE enhancer/promoter as observed in our model, this should be of considerable interest.

Similar effects of TNF-α were described as occurring in HIV-1 replication. TNF-α stimulates the long terminal repeat (LTR) of HIV-1 in vitro and induces HIV-1 replication in latently infected immature mononuclear cells (e.g. U-937 and HL-60 cells), whereas in mature mononuclear cells (e.g. THP-1 cells and freshly isolated human monocytes) no stimulation by TNF-α of HIV-1 production was observed (Griffin et al., 1991; Mellors et al., 1991; Tadmori et al., 1991). This phenomenon was discussed as being a result of constitutive TNF-α expression in HIV-1-infected mature monocyteic cells (Griffin et al., 1991). As shown for the expression of HIV-1, the activity of the HCMV IE enhancer/promoter can be stimulated by PMA in both immature and mature cells. There are obvious similarities between the effects of TNF-α and PMA on the HIV-1 LTR and HCMV IE enhancer/promoter. In both systems the TNF-α effect correlated with the differentiation stage of infected or transfected cells. In this context, the stimulation of HIV-1 LTR and HCMV IE enhancer/promoter by TNF-α in immature, early progenitor-related cells seems of particular interest.

TNF-α, a cytokine produced by monocytes/macrophages and T lymphocytes, is thought to play an important role in the host response to inflammation and infection (Beutler & Cerami, 1987). Elevated levels of TNF-α have also been reported in serum taken from most patients after renal or bone marrow transplantation and from patients with septicemia (Segolchi et al., 1991). For transplant recipients, particularly bone marrow recipients, a high prevalence of systemic HCMV infection in the post-transplantation phase is well documented (Prösch et al., 1992; Rubin, 1990; Winston et al., 1990). Apart from primary infection or reinfecation of the patient through the graft or blood transfusions, reactivation of latent HCMV is thought to be the most important mode of infection. If HCMV is able to infect progenitor cells latently in vivo, increased TNF-α levels may mediate the reactivation of latent virus in these cells. This hypothesis was confirmed by our observation that approximately 80% of patients with documented septicaemia also developed active HCMV infection, as diagnosed by the presence of HCMV antigen and HCMV DNA in PBMCs (W. D. Döcke et al., unpublished).

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References


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