Repression of human cytomegalovirus major immediate early gene expression in a monocytic cell line


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We have previously shown that a major site of persistence of human cytomegalovirus (HCMV) in healthy carriers is in peripheral blood monocytes. However, monocytes are difficult to infect in vitro with HCMV, and HCMV gene expression cannot be reproducibly detected in peripheral blood cells of healthy carriers. Here we show that the monocytic cell line THP1 is non-permissive for HCMV infection due to a block in expression of the HCMV major immediate early (IE) promoter. This repression is correlated with the presence of a differentiation-specific cellular factor which binds to the imperfect dyad symmetry and the 21 bp enhancer repeats of the major IE promoter regulatory region and which has characteristics of MBF1, a factor which we have previously defined in HCMV non-permissive, undifferentiated teratocarcinoma cells. Both differentiation of THP1 cells into macrophages, which results in a decrease in this factor, or deletion of the factor's binding sites from the IE promoter/enhancer lifts this repression and permits expression from the major IE promoter.

Much evidence is accumulating to suggest that the state of differentiation of the host cell dictates the level of permissivity for human cytomegalovirus (HCMV) infection (Gonczol et al., 1984; Weinshenker et al., 1988). By polymerase chain reaction (PCR) we have recently shown that a major site of persistence of HCMV in healthy seropositive individuals is in the monocyte fraction of peripheral blood mononuclear cells (Taylor-Wiedeman et al., 1991). However, it is well established that it is difficult to infect peripheral blood monocytes in vitro or to detect HCMV antigen in monocytes of healthy carriers, either because too few monocytes carry the virus or because these cells do not express HCMV gene products. Recent work is now suggesting that terminal differentiation of monocytes to tissue macrophages is necessary in order for permissivity for HCMV infection (Ibanez et al., 1991; Lathey & Spector, 1991; St Jeor et al., 1991). Here we show that the monocytic cell line THP1, which is non-permissive for HCMV infection but becomes permissive upon phorbol myristate acetate (PMA) stimulation to macrophages (Weinshenker et al., 1988), is unable to express the HCMV major immediate early (IE) promoter. This repression is correlated with the presence of a differentiation-specific nuclear factor, which has the characteristics of a factor we have previously defined in undifferentiated human teratocarcinoma cells.

As with undifferentiated teratocarcinoma (T2) cells (Gonczol et al., 1984) the monocytic cell line THP1 is non-permissive for HCMV infection, but differentiation of THP1 cells into macrophages with PMA leads to productive infection (Weinshenker et al., 1988). Although, formally, these authors could not rule out that induction of permissivity by PMA was due to increased viral uncoating or penetration, they suggested that the lack of infection may have been due to regulation of viral gene expression as is seen for teratocarcinoma cells (Weinshenker et al., 1988). In undifferentiated T2 cells the lack of IE1 expression is due to the presence of the imperfect dyad symmetry and the 21 bp repeats of the major IE enhancer, which act as sites of negative regulation and bind a differentiation-specific negative regulator of IE1 expression, MBF1 (Shelbourn et al., 1989; Kothari et al., 1991). Consequently, we investigated whether the lack of IE1 expression in THP1 cells could also be due to these repressor sites.

Fig. 1 shows that repression of IE1 after infection of THP1 cells is reflected in the level of transient expression of major IE reporter chloramphenicol acetyltransferase (CAT) constructs. Electroporation (25 μF at 450 V followed by 960 μF at 450 V) of THP1 cells with 1 μg of pEScat, which contains a full-length major IE promoter, results in low levels of CAT expression. However, electroporation of THP1 cells with pIEPlcat, which lacks the major IE imperfect dyad symmetry and the 21 bp repeat motifs of the enhancer, results in high levels of CAT activity (see Fig. 1). In differentiated THP1 cells, the deleted or full-length major IE promoter constructs are expressed equally well (see Fig. 1). As with undifferentiated T2 cells (Kothari et al., 1991), we have
confirmed that this repression in THP1 cells is due, at least in part, to the 21 bp repeat motifs of the IE enhancer, as expression of pIEPlcat is repressed in THP1 cells if a single 21 bp repeat is cloned into pIEPlcat (Fig. 1).

As the repression of IE gene expression in undifferentiated T2 cells is correlated with the presence of MBF1 which decreases upon retinoic acid (RA)-induced differentiation, we asked whether a similar factor is also present in non-permissive THP1 cells. In Fig. 2, we show by mobility shift assays, using either the 21 bp repeat element or the 3' half of the imperfect dyad symmetry as probes, and nuclear extracts from undifferentiated THP1 cells, that THP1 cells do contain a nuclear factor that binds to the imperfect dyad symmetry and the 21 bp repeat, and which forms a complex with mobility similar to that of MBF1. As with RA-induced differentiation of T2 cells, PMA-induced differentiation of THP1 cells into macrophages also results in a decrease in this factor. The faster migrating complexes seen in Fig. 2 are also similar to those seen in T2 cells but do not appear to be correlated to changes in permissivity (Shelbourn et al., 1989).

Our results show that monocytic cells are unable to express the major IE promoter but this repression is lifted upon differentiation into a macrophage cell type by PMA. As with the teratocarcinoma cell system, we have also shown this lack of IE expression in THP1 cells is correlated with the presence of a differentiation-specific cellular factor which binds to the imperfect dyad symmetry and the 21 bp enhancer element of the IE1 promoter/regulatory region. However, we cannot rule out that the induced expression of IE1 upon differentiation of THP1 cells may be due to additional positive or negative factors. We believe that such differentiation-specific nuclear factors may play a pivotal role in the regulation of HCMV major IE expression and give an insight into the control of the class of genes expressed during HCMV persistence and reactivation. These data would suggest that monocytes, which are a major site of persistence of HCMV, are unable to express IE1 and differentiation of these cells into macrophages is necessary for IE expression and full viral production. This would be consistent with the known difficulty in infecting peripheral blood monocytes and the lack of HCMV expression in peripheral blood of healthy carriers, as well as with the permissivity for HCMV infection associated with differentiation of human peripheral blood monocytes into macrophages. We are at present determining the levels of HCMV gene expression in peripheral blood monocytes before and after PMA stimulation by reverse transcription followed by PCR.

Fig. 1. (a) THP1 cells or (b) PMA-stimulated cells were electroporated with 1 μg of pEScat (lane 1), pIEPlcat (lanes 2 and 3) or pIE21Acat (lane 4). CAT conversions (%) are 15, 45, 41 and 12 for (a) lanes 1 to 4 and 40, 33, 32 and 40 for (b) lanes 1 to 4 respectively. Control untransfected cell lanes are indicated by c. Panel (c) shows the deletion vectors with respect to the major IE promoter/regulatory region. All constructs have been described previously (Shelbourn et al., 1989; Kothari et al., 1991).

Fig. 2. (a) The 3' half of the imperfect dyad symmetry present in the major IE promoter/regulatory region or (b) the 21 bp repeat motif of the HCMV enhancer were used as probes for band shift experiments, as described previously (Shelbourn et al., 1989; Kothari et al., 1991), with nuclear extracts from undifferentiated THP1 cells (lanes 1, 3 and 5) or differentiated cells (lanes 2, 4 and 6). Lanes 1 and 2 contain no additional competitor, lanes 3 and 4 contain 1 μg of additional unlabelled (a) 3' competitor or (b) 21 bp competitor, and lanes 5 and 6 contain 1 μg of unlabelled non-specific poly(dI-dC). The arrowhead indicates the position of the MBF1-like complex.
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References


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