Journal of General Virology (1992), 73, 433–435. *Printed in Great Britain*

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

J. H. Sinclair,* J. Baillie, L. A. Bryant, J. A. Taylor-Wiedeman and J. G. P. Sissons

Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K.

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 pIEP1cat, 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

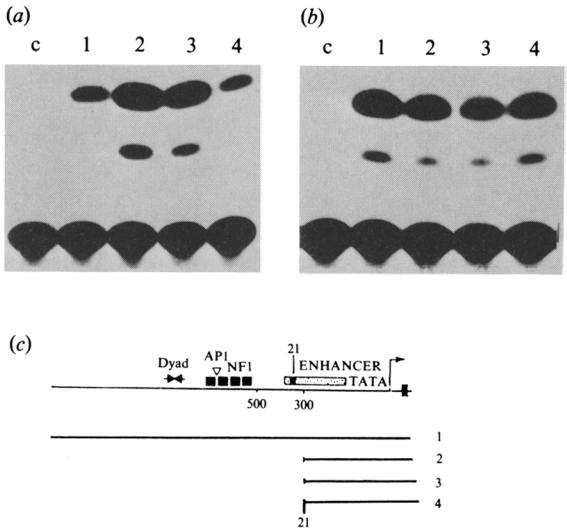

Fig. 1. (*a*) THP1 cells or (*b*) PMA-stimulated cells were electroporated with 1 μg of pEScat (lane 1), pIEP1cat (lanes 2 and 3) or pIE21Acat (lane 4). CAT conversions (%) are 15, 45, 41 and 12 for (*a*) lanes 1 to 4 and 40, 33, 32 and 40 for (*b*) lanes 1 to 4 respectively. Control untransfected cell 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).

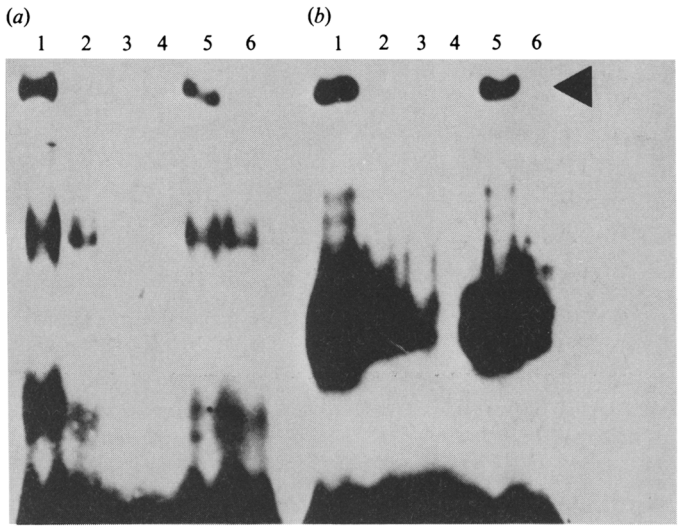

Fig. 2. (*a*) The 3′ half of the imperfect dyad symmetry present in the major IE promoter/regulatory region or (*b*) the 21 bp repeat motif of the HCMV enhancer were used as probes for band shift experiments, as described previously (Shelbourn *et al.*, 1989; Kothari *et al.*, 1991), with nuclear extracts 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.

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 pIEP1cat is repressed in THP1 cells if a single 21 bp repeat is cloned into pIEP1cat (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.

We would like to thank the Medical Research Council and The Wellcome Trust for financial support.

References

GONCZOL, E., ANDREWS, P. W. & PLOTKIN, S. A. (1984). Cytomegalovirus replicates in differentiated but not in undifferentiated human embryonal carcinoma cells. *Science* **224**, 159–161.

IBANEZ, C. E., SCHRIER, R., WILEY, C., GHAZAL, P. & NELSON, J. A. (1991). HCMV productivity infects primary differentiated macrophages. *Journal of Virology* **65**, 6581–6586.

KOTHARI, S. K., BAILLIE, J., SISSONS, J. G. P. & SINCLAIR, J. H. (1991). The 21 bp repeat element of the human cytomegalovirus major immediate early enhancer is a negative regulator of gene expression in undifferentiated cells. *Nucleic Acids Research* **19**, 1767–1771.

LATHEY, J. L. & SPECTOR, S. A. (1991). Unrestricted replication of human cytomegalovirus in hydrocortisone-treated macrophages. *Journal of Virology* **65**, 6371–6375.

ST JEOR, S., MACIEWJEWSKI, J. & YOUNG, N. (1991). Replication of human cytomegalovirus in macrophages and monocytes and the effect of cytokines on virus gene expression. *Exerpta Medica International Congress Series*, no. 978, pp. 303–306.

SHELBOURN, S. L., KOTHARI, S. K., SISSONS, J. G. P. & SINCLAIR, J. H. (1989). Repression of human cytomegalovirus gene expression associated with a novel immediate early region binding factor. *Nucleic Acids Research* **17**, 9165–9171.

TAYLOR-WIEDEMAN, J. A., SISSONS, J. P. G., BORYSIEWICZ, L. K. & SINCLAIR, J. H. (1991). Monocytes are a major site of persistence of human cytomegalovirus in peripheral blood mononuclear cells. *Journal of General Virology* **72**, 2059–2064.

WEINSHENKER, B. G., WILTON, S. & RICE, G. P. A. (1988). Phorbol ester-induced differentiation permits productive human cytomegalovirus infection in a monocytic cell line. *Journal of Immunology* **140**, 1625–1631.

(Received 22 July 1991; Accepted 11 October 1991)