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Development of a model for cytomegalovirus infection of oligodendrocytes

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The well-characterized human oligodendroglioma (HOG) cell line, cells of which resemble immature oligodendrocytes, was used to investigate the level of permissiveness to human cytomegalovirus (CMV) infection. Expression of CMV genes was incomplete following exposure of HOG cells to CMV, in contrast with results observed with the astroglioma cell line U373-MG (used as a positive control). However, treatment with phorbol 12-myristate 13-acetate (PMA) or with dibutryl cAMP (dbcAMP) plus the phosphatase inhibitor 1-isobutyl-3,3-methyl xanthine (IBMX) rendered the HOG cells fully permissive to CMV; down-regulation of HLA class I and production of virions were only observed under these conditions. In contrast to the findings seen with the HOG cell line, treatment of U373-MG cells with dbcAMP/IBMX or PMA did not interfere with CMV-induced down-regulation of HLA class I. However, these chemical stimulators reduced virus production in U373-MG cells by 30 and 70%, respectively.

Introduction

While immunocompetent individuals are not at risk of neurological consequences arising from human cytomegalovirus (CMV) infection, immunosuppressed individuals, neonates and developing foetuses can develop central nervous system pathology following CMV infection (Dorfman, 1973; Raine & Fields, 1973; Nakervis *et al.*, 1984; Patchell *et al.*, 1985; Guyotat *et al.*, 1987; Morgello *et al.*, 1987; Jacobson & Mills, 1988). Previous studies have found that human foetal astrocytes, as well as some astrocytoma and neuroblastoma cell lines, are fully permissive to CMV infection (Poland *et al.*, 1990, 1994). Immunohistological investigation of CMV involvement in AIDS encephalitis predominantly identifies CMV associated with microglial nodules or with multinucleate monocyte-like cells and, to a lesser extent, CMV infection of astrocytes and neurons (Wiley & Nelson, 1988; Schmidbauer *et al.*, 1989; Balluz *et al.*, 1996); however, two reports have provided evidence that indicates CMV infection of oligodendrocytes (Schmidbauer *et al.*, 1989; Power *et al.*, 1990).

Very few oligodendroglial cell lines exist. However, a report by Kashima *et al.* (1993) identified one oligodendroglioma cell line (the HOG cell line) from a panel of oligodendrogliomas, astrocytomas and neuroblastomas as the most representative of immature oligodendrocytes. Northern and Western blot analyses of the HOG cell line revealed expression of K7 keratin, but not glial fibrillary acidic protein or neurofilament proteins. The HOG cell line has also been reported to express the oligodendrocyte markers galactocerebroside, galactocerebroside sulphate and 2′,3′-cyclic nucleotide 3′-phosphodiesterase (Post & Dawson, 1992). Other investigators have reported that 24 h exposure of oligodendroglioma cells to 1 mM dibutryl cAMP (dbcAMP) is sufficient to induce cells to differentiate to a phenotype more closely resembling mature oligodendrocytes (Raible & McMorris, 1993).

We have utilized the HOG cell line as representative oligodendrocyte-like cells to investigate the permissiveness of oligodendrocytes to infection with CMV. Incomplete CMV gene expression was observed in unstimulated HOG cells and full permissiveness to CMV replication could be induced by pretreatment with either phorbol esters or dbcAMP/1-isobutyl-3,3-methyl xanthine (IBMX). Only when cells were permissively infected (i.e. under conditions where extracellular CMV was produced) were immunologically relevant cellular alterations such as down-regulation of HLA class I expression observed.

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Methods

■ **Materials.** dbcAMP, IBMX and phorbol-12-myristate-13-acetate (PMA) were purchased from Sigma.

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■ **Antibodies.** Murine monoclonal antibodies recognizing CD55 were gifts from W. F. Rosse (clone 1H4; Duke University Medical Center, Durham, NC, USA) or were raised in this laboratory (clone MBC1). W6/32 (anti-HLA class I) was obtained from the European Culture Collection (CAMR, Porton Down, UK), BRIC229 (anti-CD59) was from Bioproducts Laboratories, and anti-CD46 was from B. Loveland (clone E4.3; Austin Research Institute, Victoria, Australia). Fluorochrome-conjugated goat anti-mouse IgG antisera were purchased from Sigma. Antibody against the viral antigens immediate early 1 (IE1)/IE2 was a gift from J. H. Sinclair (Addenbrooke's Hospital, Cambridge, UK), anti-early antigen cocktail (CCH2) and anti-pp65 early antigen were purchased from Dako, and anti-glycoprotein B (anti-gB; viral envelope antigen) was obtained from Graham Farrar (CAMR).

■ **Cells.** The U373-MG cell line was obtained from the ATCC (Rockville, Md., USA). The HOG cell line was a gift from G. Dawson (University of Chicago School of Medicine, Chicago, Ill., USA). Primary human fibroblasts were isolated from neonatal foreskins by standard techniques. Briefly, human neonatal foreskins were collected from infants between the ages of 6 and 18 months. No samples were processed from neonates with abnormal cytogenics. The foreskins were cut in small pieces, incubated in 0·25% trypsin and 20 μg/ml DNase in PBS for 30 min at 37 °C and further dissociated by gentle pipetting. Fibroblasts were allowed to adhere and grow out from larger tissue pieces in plastic six-well dishes (Life Technologies). Pure cultures of fibroblasts were obtained by subsequent harvesting of single cells by trypsinization. All cells were cultured in Dulbecco's minimum essential medium (DMEM) with non-essential amino acids supplemented with 1 mM sodium pyruvate, Earle's basic salt solution, 100 U/ml penicillin, 100 μg/ml streptomycin and 5% foetal calf serum (FCS). In some cases, the medium for HOG cells was supplemented with 1 μg/ml PMA or 0·5 mM dbcAMP with 0·5 mM IBMX (final concentrations given).

■ **Virus infection.** The laboratory strain of human CMV used for these studies was AD169 obtained from the ATCC (Bethesda, Md., USA). Virus stocks were propagated and quantified on primary explant fibroblasts using standard procedures (Spiller *et al*., 1996*a*). UV light was used to inactivate CMV by exposing virus stocks to a short wavelength (312 nm) UV light source for 20 min. Conditions for inactivation were determined empirically; stocks were considered UV-inactivated when the CMV stock failed to cause cytopathic effects when incubated with fibroblasts and observed for 10–14 days. In all experiments, cells were exposed to virus for 1 hr at 37 °C and then washed to remove inoculum. U373-MG cells were infected with an m.o.i. of 10, which resulted in viral antigen expression in all cells, abundant cytomegaly and formation of many multinuclear syncytial giant cells. HOG cells were incubated with virus at an m.o.i. of 100, but under no conditions were any morphological changes observed which were indicative of infection; there was also no evidence of cytomegaly and no formation of syncytia.

Following a 1 h incubation with virus, inoculum was removed by washing with PBS and the cells were placed in medium containing either the appropriate stimulators or no stimulator (controls). Medium was replaced daily with fresh medium containing the stimulatory agents. CMV produced by infected HOG and U373-MG cells was quantified by plaque assay from cell supernatants at 3 and 4 days post-infection (p.i.). No PMA or dbcAMP/IBMX was added to the medium after 3 days p.i. to avoid interference with CMV quantification. Cell debris was cleared from the supernatant by centrifugation (800 ***g***) and, where required, the intracellular virus was separately assessed. Intracellular virus was released, after removing the supernatant and adding fresh cell medium, by harvesting the infected monolayer with a sterile cell scraper, snap freezing the cells in liquid nitrogen, thawing the cells once and mechanically releasing the virus by repeated passage through a 25-gauge needle. The resultant homogenate was allowed to incubate with fibroblasts to quantify virus; the same standard plaque assay was used to quantify extracellular virus in the cell supernatants.

■ **Flow cytometry studies.** Flow cytometry analysis was performed as previously described (Spiller *et al*., 1996*b*). Adherent cell lines were disaggregated by incubation with 15 mM EDTA at 4 °C and suspended in cold flow cytometry solution (1% BSA, 15 mM EDTA, 15 mM sodium azide in PBS). All procedures were carried out at 4 °C and all solutions contained 30 mM sodium azide to prevent endocytosis or antibody patching and capping. Cells were analysed on a Becton-Dickinson FACScan. Non-specific antibody binding was determined with monoclonal anti-factor H (clone OX23) as the primary antibody (5 μg/ml). One-way analysis of variance with post-hoc analysis using Fisher's least significant test and Bonferroni's correction for multiple comparisons was used to identify differences between groups.

■ **Northern blot analysis.** Total RNA was extracted from the astrocytoma cell line U373-MG and HOG cells using UltraSpec RNA isolation reagents (AMS Biotechnology). Total RNA (5 or 10 μg) was separated on a 1% formaldehyde–agarose gel. RNA from U373-MG cells was used as the positive control for permissive cells as previously documented (Koval *et al*., 1991) and RNA from mock-infected cells was included as a negative control to identify non-specific bands for viral probes. The RNA was transferred to non-charged nylon membranes (Nytran membranes, Amersham) and hybridized with ^{32}P-labelled RNA probes made using Redivue [^{32}P]CTP and Rediprime random labelling kits (Amersham). Specific cDNA probes for exon 4 of the IE1 gene (U122) and the entire early gene US11 were isolated from plasmids containing the genomic sequences obtained from Rudiger Ruger (Boehringer Mannheim, Penzberg, Germany) and Gavin Wilkinson (Department of Medicine, UWCM, Cardiff, UK), respectively. Standardization of gene expression was performed by comparison with the expression of the house-keeping gene GAPDH (probe obtained from David Llewellyn, Department of Medical Biochemistry, UWCM, Cardiff, UK). A specific probe for the delayed early gene gB (UL55) was generated by nested PCR using the following primers: outer, GAGGACAACGAAATCC-TGTTGGGCA and GTCGACGGTGGAGATACTGCTGAGG; inner, ACCACCGCACTGAGGAATGTCAG and TCAATCATGCGTTTG-AAGAGGTA. These primers were based on the genomic sequence of gB as previously described (Cranage *et al*., 1986) and purified CMV was used as the PCR target. A specific probe for the late gene gp64 was generated by PCR using the primers previously published by Bevan *et al*. (1991). Densitometry analysis of all gels was accomplished using the Bio-Rad flat bed scanner and Bio-Rad Molecular Analyst software. Gel images were printed using a Fargo Primera Pro Dye sublimation printer.

■ **Immunocytochemistry.** Cells (10 000) were seeded on acid-etched cover-slips and allowed to adhere overnight at 37 °C in cell culture medium which, in some cases, contained 1 μg/ml PMA; PMA remained throughout the infection unless otherwise stated. Cells were infected with CMV the next morning and the infection was allowed to proceed for 48 h prior to fixing and staining the cells for antigens of interest. Cells were washed twice in ice-cold PBS (pH 7·4) for 3 min, fixed for 10 min at room temperature with 2% paraformaldehyde in PBS and washed twice with ice-cold PBS (3 min) and twice with ice-cold PBS containing 1% BSA. Cells were then permeabilized with 0·01% Triton X-100 in PBS–1%

BSA for 3 min, washed twice more with PBS–1% BSA and stained with primary antibodies recognizing IE1/IE2, early antigens, gB or isotype matched controls (all used at final concentrations of 5 μg/ml). Unbound primary antibody was removed by washing three times in ice-cold PBS–1% BSA and cells were incubated with FITC-labelled goat anti-mouse antibody, washed twice with PBS–1% BSA, twice more with PBS, then mounted using Citifluor (UKC Chemical Laboratories), and examined using a Leica confocal microscope.

Results

Immunohistochemistry

HOG cells were incubated with an excess of CMV for 48 h prior to immunohistological analysis. No difference in the binding of the control antibody was observed between cells infected with CMV and/or incubated with 1 μg/ml PMA and cells that were mock-infected in the absence of PMA. An antibody which recognizes two separate early antigens (CCH2) stained the nuclei of about 40% of the HOG cells following exposure to CMV (Fig. 1). However, in the presence of PMA, both the intensity and the frequency (50–70%) of the nuclear staining increased for CMV-infected cells. Similar changes were observed in the expression of the major immediate early gene products IE1 and IE2 using an antibody which recognizes the third exon of both proteins (data not shown). Staining for the late viral gene product gB following infection was observed only in the presence of PMA (data not shown).

Northern blot analysis

Confirmation of the immunohistochemical findings was obtained through Northern blot analysis. RNA from mock-infected and CMV-infected HOG cells in the presence or absence of PMA was harvested at 1, 2, 3 and 5 days p.i. Control RNA was obtained from mock-infected and CMV-infected U373-MG astrocytoma cells. In the case of HOG cells, 10 μg per lane of total RNA was loaded onto gels as compared to 5 μg per lane for U373-MG cells due to the lower intensity of virus message in HOG cells. CMV infection of the U373-MG cell line was performed in the absence of PMA. The IE1 mRNA species (1·9 kb) was present at 1 day p.i. for the infected U373-MG cells and infected HOG cells treated with PMA, but not at day 1 p.i. in infected HOG cells in the absence of PMA (Fig. 2). The expression of IE1 was weakly detected on day 2 p.i. in HOG cells in the absence of PMA and was almost equivalent to expression observed in PMA-treated HOG cells by day 3 p.i. The level of IE1 expression in PMA-treated or untreated HOG cells then decreased by day 5 p.i. The expression of US11, a representative early gene, was strong in U373-MG cells by 1 day p.i. (1·5 kb, Fig. 2). In the absence of PMA, weak US11 expression was only seen at 2 and 3 days p.i. for HOG cells, while in the presence of PMA US11 expression was apparent by 1 day p.i., with levels peaking at day 3 p.i. and declining by day 5 (Fig. 2). In accordance with immunohistological analysis, low levels of transcripts for gB were seen in HOG cells only when PMA was present; levels reached a peak at day 3 p.i. and had started to decline by day 5 p.i. However, the gB levels were much higher in CMV-infected U373-MG cells and remained elevated throughout infection (Fig. 2). Only trace amounts of transcripts for the late gene gp64 were observed at 1 day p.i. for CMV-infected U373-MG cells, but the gp64 levels steadily increased after that point. Expression of gp64 in CMV-infected HOG cells was similar to that seen

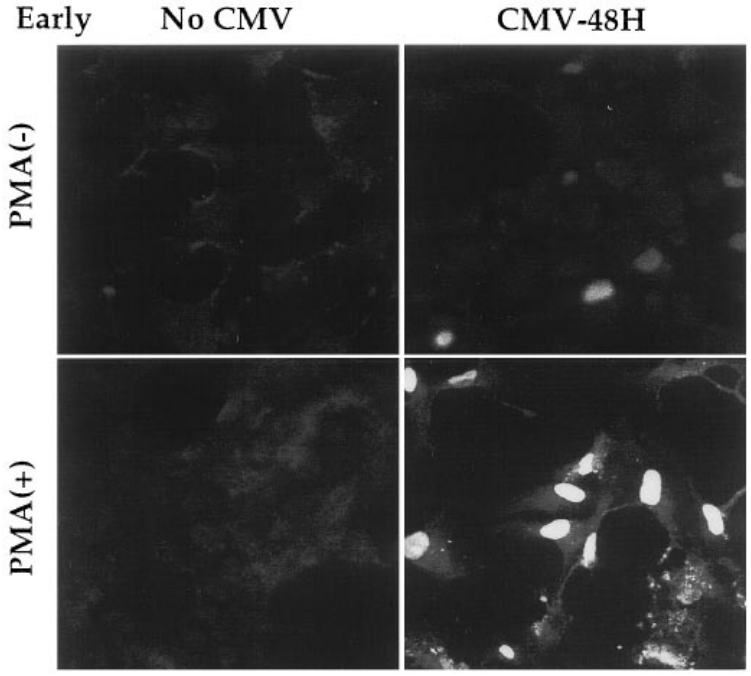

Fig. 1. Expression of CMV early genes in HOG cells at 48 h p.i. as assessed by indirect immunofluorescence using the CCH2 monoclonal antibody. Actual size of each of the four fields depicted is 250 × 250 μm (magnification × 200).

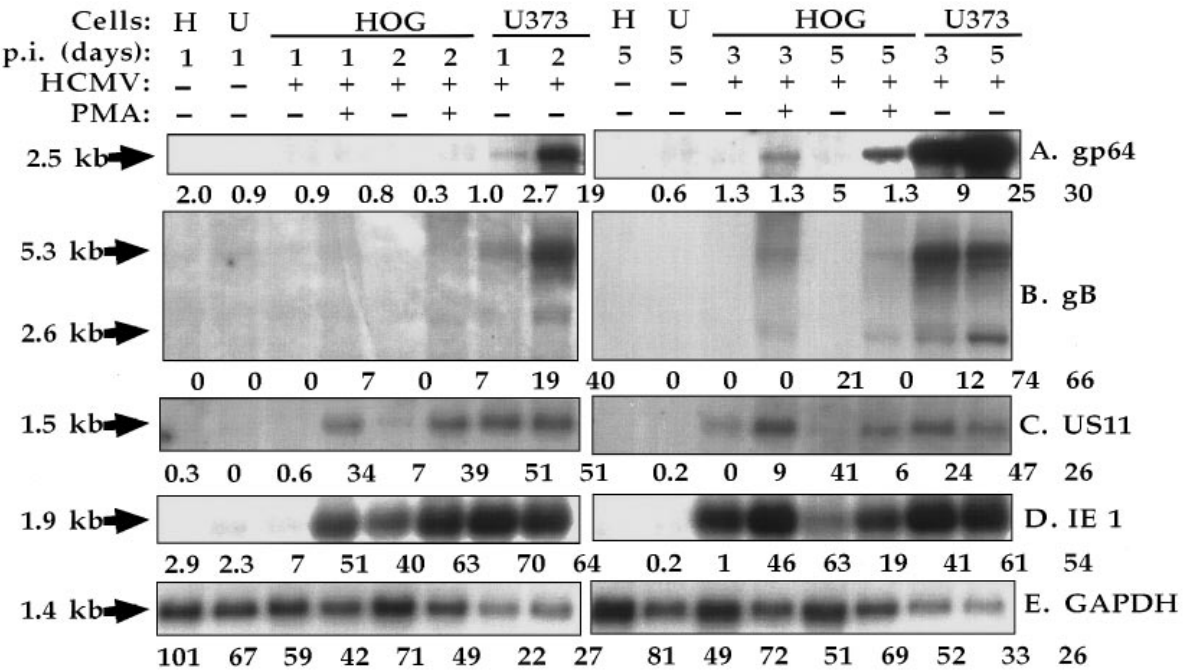

Fig. 2. Northern blot analysis for the presence of viral mRNA transcripts in mock-infected and CMV-infected U373-MG astrocytoma cells (U) and HOG cells (H) using specific probes against: (A) late CMV gene gp64; (B) delayed early CMV gene gB; (C) early CMV gene US11; (D) major IE1 gene; (E) host house-keeping gene GAPDH.

Table 1. Densitometry analysis of Northern blots

All densities are expressed in arbitrary units (AU) with background subtraction by the densitometry software. Sample loading variations were corrected for by standardizing amongst groups: band intensities were corrected by a factor which gave the same GAPDH values amongst all samples. Uncorrected GAPDH values were 41–101 AU for HOG cells and 21–66 AU for U373-MG cells, indicating reasonable consistency in loading. The IE1 gene, early gene US11, CMV envelope protein gB and late gene gp64 were examined at various days p.i. with CMV in the presence or absence of 1 μg/ml PMA.

Cell	PMA	CMV	Days p.i.	IE1	US11	gB	gp64
HOG	–	–	1	2·87	0·33	0·00	1·96
HOG	–	–	5	0·29	0·30	0·00	0·68
HOG	–	+	1	12·4	0·96	0·00	1·59
HOG	+	+	1	125	81·8	16·2	1·92
HOG	–	+	2	56·9	10·7	0·00	0·49
HOG	+	+	2	131	80·4	14·4	2·16
HOG	–	+	3	64·7	13·1	0·00	1·76
HOG	+	+	3	123	80·9	40·8	9·25
HOG	–	+	5	28·1	8·88	0·00	1·91
HOG	+	+	5	79·0	47·5	23·4	15·3
U373	–	–	1	3·48	0·00	0·08	1·41
U373	–	–	5	1·99	0·00	0·00	2·61
U373	–	+	1	329	238	88·8	12·5
U373	–	+	2	243	196	151	72·0
U373	–	+	3	190	145	228	76·0
U373	–	+	5	209	100	257	117

for gB in that expression was only observed in the presence of PMA. However, the amounts of gp64 mRNA were still increasing at day 5 in PMA-treated, CMV-infected HOG cells (Fig. 2). To allow quantitative comparison of Northern blots, densitometry analysis was performed and standardized for expression of the house-keeping gene GAPDH (Table 1).

Effect of differentiation of HOG cells with dbcAMP/IBMX

HOG cells incubated with PMA continued to divide, albeit at a reduced rate. In contrast, in HOG cells incubated with 50 mM dbcAMP plus 50 mM IBMX, cell growth was arrested within 48 h post-treatment and the cells formed long processes, adopting a morphology more characteristic of mature oligodendrocytes. These cells had a life span of 8 days after initial exposure to dbcAMP/IBMX, which was not influenced by CMV infection. The presence of either dbcAMP or IBMX alone was unable to arrest growth of HOG cells. CMV infection of these differentiated cells (at 24 h post-treatment with dbcAMP/IBMX) was fully permissive and expression of high levels of IE1, US11 and gB persisted until cell death at day 7 p.i. (Fig. 3); the time-course was similar to that following infection of U373-MG cells.

Production of extracellular CMV

No significant production of extracellular virus could be detected from untreated CMV-infected HOG cells, as com-

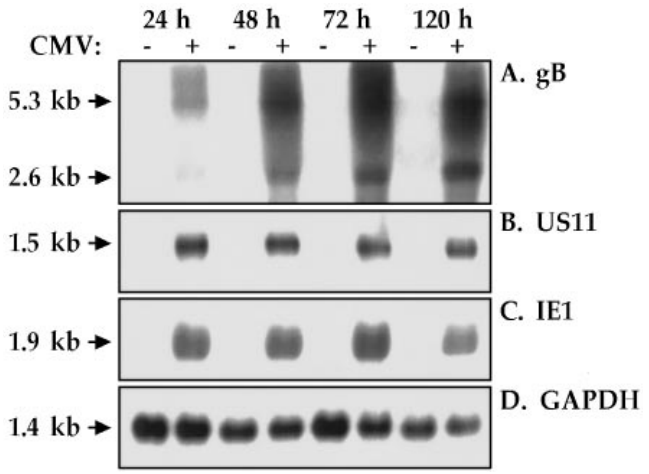

Fig. 3. Northern blot analysis for the presence of viral mRNA transcripts in mock-infected and CMV-infected HOG cells treated with 0·5 mM dbcAMP and 0·5 mM IBMX. Specific probes for (A) gB, (B) US11, (C) IE1 and (D) the host gene GAPDH were used to probe total RNA from the 1, 2, 3 and 5 day p.i. time-points.

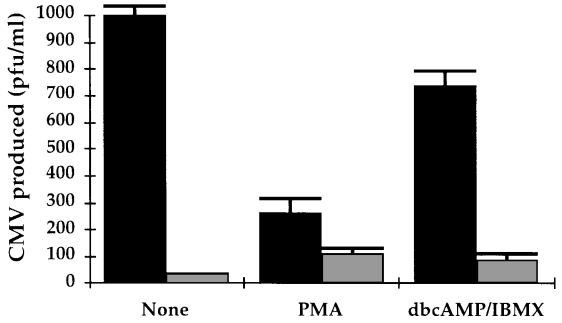

Fig. 4. CMV produced by infected U373 (black bars) and HOG cells (grey bars) at day 4 p.i. as determined by plaque-assay on fibroblasts. Results given represent supernatants from triplicate cultures of CMV-infected cells which were either untreated, treated with 1 μg/ml PMA or treated with 0·5 mM dbcAMP/0·5 mM IBMX up to day 3 p.i. Error bars represent 1 × SD for each condition measured in triplicate.

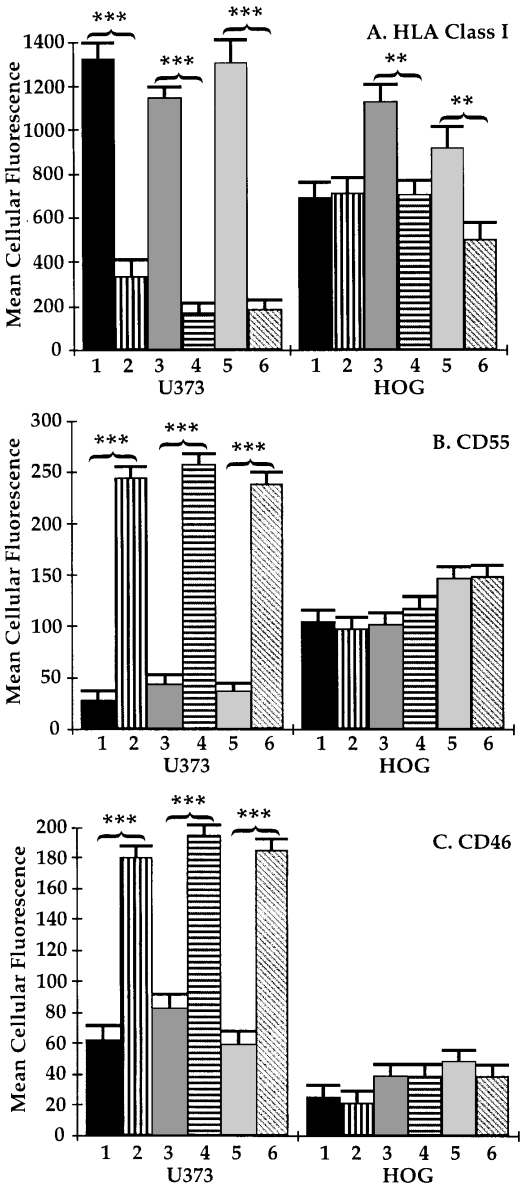

Fig. 5. Flow cytometry analysis of the cell surface expression of HLA class I (A), decay-accelerating factor (CD55; B), and membrane co-factor protein (CD46; C) at 4 days p.i. U373 cells are shown on the left-hand side and HOG cells on the right-hand side of each graph. Numbers correspond to the following treatments: (1) no CMV, no PMA, no dbcAMP/IBMX; (2) CMV-infected, no PMA, no dbcAMP/IBMX; (3) no CMV + 1 μg/ml PMA; (4) CMV-infected + 1 μg/ml PMA; (5) no CMV + 0·5 mM dbcAMP/0·5 mM IBMX; (6) CMV-infected + 0·5 mM dbcAMP/0·5 mM IBMX. Statistical analysis was performed on matched mock-infected and CMV-infected cells for the same condition (bracketed pairs of bars). ** and *** above brackets represent $P < 0{\cdot}01$ and $P < 0{\cdot}001$, respectively. Error bars represent 1 × SD for each condition measured in triplicate.

pared to 10^3 p.f.u. for the astrocytoma cell line at 3–4 days p.i. (Fig. 4). Treatment of CMV-infected HOG cells with phorbol esters or dbcAMP/IBMX resulted in the production of 10^2 p.f.u. at 3–4 days, a time-point chosen because it coincided with the peak expression of the CMV gB viral envelope gene mRNA expression (as shown in Fig. 2). Exposure of the astrocytoma cells to these agents had the opposite effect: dbcAMP/IBMX decreased virus production by 30% and PMA decreased it by 70% (Fig. 4). To address the concern that the CMV associated with the cells may be predominantly trapped within the cell, intracellular virus load was assessed separately following mechanical disruption to release the virus. The amounts of intracellular virus precisely mirrored those of extracellular virus from the same cells in both cell lines. HOG cells did not produce any intracellular virus in the absence of chemical stimulators (data not shown). There was consistently three times more virus inside the U373-MG cells than was released, but the trends in virus production following treatment with PMA or dbcAMP/IBMX were identical to the findings observed for the supernatants (data not shown).

Effect of CMV infection on host protein expression

CMV infection of HOG cells under normal conditions had a negligible effect on the expression of HLA class I. PMA increased the expression of HLA class I on mock-infected HOG cells and CMV infection of PMA-treated HOG cells resulted in a significant decrease in HLA class I expression as compared to the uninfected PMA-treated controls ($P < 0{\cdot}01$; Fig. 5). Treatment of uninfected cells with dbcAMP/IBMX increased the HLA class I expression of HOG cells, albeit to a lesser extent than PMA, and CMV infection of dbcAMP/IBMX-treated HOG cells caused a significant decrease in HLA class I expression compared with uninfected dbcAMP/IBMX-treated controls. Treatment of the mock-infected U373-MG cells with either dbcAMP/IBMX or PMA did not increase basal HLA class I expression. CMV infection markedly decreased HLA class I expression under all conditions. While the level of HLA-class I on CMV-infected U373-MG cells appeared to be consistently lower in the presence of either dbcAMP/IBMX or PMA, as compared to infected cells in the absence of these agents, this difference failed to reach statistical significance ($P = 0{\cdot}08$). We have previously shown that CMV infection of U373-MG cells causes marked up-regulation of the complement regulators MCP and DAF (Spiller *et al*., 1996*a*). No additive effect was observed for dbcAMP/IBMX or PMA on the up-regulation of DAF or MCP on CMV-infected U373-MG cells, and infection of HOG cells with CMV did not increase the expression of either DAF or MCP under any conditions (Fig. 5).

Discussion

Primary cultures of human oligodendrocytes are very difficult to obtain *in vitro* and usually require an associated layer of astrocytes in order to survive. Therefore, the acquisition of sufficient quantities of cells to be able to perform flow cytometry analysis, or the removal of astrocyte contamination to a degree sufficient for Northern blot analysis presents a considerable barrier to study. Oligodendrocyte cell lines represent a potential model for study. It has been shown that 24 h exposure of oligodendroglioma cells to 1 mM dbcAMP is sufficient to induce differentiation to a phenotype resembling mature oligodendrocytes (Raible & McMorris, 1993).

Many investigators have reported that cellular differentiation by the addition of chemicals or cytokines such as phorbol esters, DMSO, retinoic acid, hydrocortisone, concanavalin A, dimethylacetimide, sodium valproate, β-gonadotropin, nerve growth factor or dbcAMP is necessary to induce permissive CMV infection or to increase CMV gene expression and virus replication in primary cells and cell lines (Dutko & Oldstone, 1981; Tanaka *et al*., 1985, 1991; La Femina & Hayward, 1986; Weinshenker *et al*., 1988; Forbes *et al*., 1990; Ibanez *et al*., 1991; Turtinen & Seufzer, 1994; Kuntz-Simon & Obert, 1995). Here, we report that pretreatment of the oligodendrocyte cell line HOG with either PMA or dbcAMP/IBMX permitted infection with CMV as judged by the expression of the early and late CMV genes and release of extracellular infectious CMV particles. However, only dbcAMP in combination with the phosphatase inhibitor IBMX, which reduces degradation of dbcAMP, caused cellular differentiation and arrested growth of HOG cells. Similar methods have previously been used by Poland *et al*. (1994) to show that fully permissive CMV infection of a neuroblastoma cell line required differentiation by addition of dbcAMP, IBMX and nerve growth factor.

While addition of PMA caused terminal differentiation of the monocytic leukaemia cell line THP-1 with resultant induction of CMV replication (Weinshenker *et al*., 1988), PMA treatment of HOG cells did not alter the morphology or expression of oligodendrocyte markers. The only effect of PMA on HOG cells was a roughly twofold prolongation in the cell doubling time. However, PMA treatment increased the percentage of HOG cells infected by CMV and also increased expression of viral gene products (Fig. 1). Late CMV gene expression peaked at 3–5 days p.i. in PMA-treated HOG cells and then steadily decreased. The expression of CMV immediate early genes completely disappeared by 11–13 days p.i. In some experiments, HOG cells were exposed to CMV and PMA for 3 days, then the medium was replaced and the cells were passaged normally. Cells were then investigated for IE gene expression by both immunofluorescence and Northern blot analysis and no evidence of IE expression could be observed, with or without PMA, even though nested PCR analysis conducted on these cells still indicated that genomic gB was present at a low level in the cell population (data not shown). Whether this indicates that a large proportion of the cells carry the CMV genome but fail to re-express the genes, or that a very small proportion of the cells remain infected but escape detection by other techniques could not be determined. HOG cells differentiated with dbcAMP/IBMX expressed all viral genes until cell death at 8–9 days p.i.; cell death was independent of CMV infection as mock-infected cells also died at about the same time.

Both PMA and dbcAMP/IBMX treatment of CMV-infected HOG cells resulted in increased expression of the early and late CMV genes and the down-regulation of HLA class I on the surface of infected cells. HLA class I down-regulation is a well-established response to CMV infection, which probably confers an immunological advantage to infected cells in subverting the cellular immune response; it has been suggested that specific CMV genes are directly responsible for this phenomenon (Wiertz *et al*., 1996; Hengel *et al*., 1996; Ahn *et al*., 1996). Recently, we have reported that CMV infection of fibroblasts and astrocytes resulted in up-regulation of the complement regulators, decay-accelerating factor (CD55) and membrane co-factor protein (CD46), which enhance resistance of the infected cells to complement-mediated lysis (Spiller *et al*., 1996*a*). However, CMV infection

of HOG cells, either alone or in the presence of phorbol ester or dbcAMP/IBMX, did not cause up-regulation of complement inhibitor expression on these cells, suggesting that the mechanism for CD55 and CD46 up-regulation by CMV is complex, dependent on cell type, and probably not a direct effect of viral gene transactivation.

In summary, our findings demonstrate that unstimulated HOG cells may be infected by CMV, insofar as CMV enters the cells and expresses the immediate early genes. However, productive infection, as assessed by expression of late gene products and release of infectious viral particles, was only observed when the HOG cells were differentiated with dbcAMP/IBMX or were treated with PMA. Although PMA did not result in arrested growth of the HOG cells, it is likely that the cells were more differentiated than in the absence of PMA since a twofold extension in cell doubling time was seen under these conditions. These findings imply that while immature oligodendrocytes may not be fully permissive to CMV infection, terminal differentiation may not be a prerequisite for induction of CMV replication.

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